Waste-derived Mycelium Materials for Non-structural and Semi-structural Applications

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and, ethics procedures and guidelines have been followed.

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Abstract

Mycelium composites are materials that are produced by allowing natural fungal growth to bind lignocellulosic substrates into a single mass resembling any given mould geometry, typically possessing foam-like mechanical properties. Fungi are specifically used due to their growth characteristics, which constitute an expanding web-like structure of filaments comprising chitin-β-glucan cell walls and a heterotrophic growth process which digests and bonds substrates under ambient conditions. This cheap and environmentally sustainable bio fabrication method, which can be used to upcycle agricultural by-products and wastes into green materials, is experiencing increasing research interest and commercialisation globally. This thesis systematically explores biological optimisation of the manufacturing process, expansion to non-structural functional applications, specifically as thermally safer and cost-competitive alternatives to highly flammable petroleum- and natural gas-derived insulation and panelling materials, such as synthetic foams like extruded polystyrene and engineered woods containing flammable resorcinol- and polyvinyl acetate-based resins, and improvement of mycelium material mechanical properties.

Initially, biological optimisation of the manufacturing process was investigated, with the suitability of several fungal species and agricultural by-products assessed for use as matrix and filler phases in mycelium composites and for chitin-β-glucan polymer generation. While no inherent fungal characteristics, such as hyphal types, pathogenicity or association- or taxonomic-based classifications proved reliable predictors of growth performance, several high performing species were identified for use in the remaining research. Selected species included white-rot fungi for use as a composite binder and soil- and water-associated fungi with high cell wall concentrations of structural polymers, such as chitin and chitosan. Solid agricultural by-products were then assessed for their suitability as composite fillers. Rice hulls, sugarcane bagasse and wheat straw proved to be very poor nutrients, demonstrating a need for nutrient supplementation using wheat grains to achieve sufficient bonding within the biological composites. However, the sugarcane by-product blackstrap molasses was an exceptional nutrient for chitin-β-glucan polymer generation, outperforming even common laboratory nutrients, such as malt extract. Highly nutritious nutrients were associated with larger hyphal diameters and significant anastomosis, which generated pseudo-laminar sheets of mycelium.

A two-part investigation was then completed to produce fire resistant mycelium composite materials. The previously undocumented thermal degradation and fire reaction properties of the mycelium matrix phase were first investigated. Mycelial biomass exhibited a three-stage degradation process typical of biological materials, but superior fire reaction properties to other

competing thermoplastic polymers, such as polymethyl methacrylate and polylactic acid. The fibrous structure of mycelium was retained following pyrolysis, albeit with a reduction in its diameter and cell wall thickness, and mycelium exhibited certain flame-retardant properties, such as a high char residue and water vapour release. Mycelium composite materials were then produced utilising various combinations of agricultural and industrial by-products with a high silica content, such as rice hulls and glass fines, as the composite substrate filler phase. The composites produced exhibited outstanding fire safety properties, with lower average and peak heat release rates and longer estimated time to flash over than the extruded polystyrene foam and particleboard synthetic construction material references. They also released significantly less smoke and CO_2 and had very low raw material substrate costs. In addition to being low in cost the substrate materials were responsible for the improvements in fire performance with rice hulls yielding significant char and silica ash and composites containing glass fines exhibiting the best fire performance because of their significantly higher silica concentrations and low combustible material content.

Finally, investigations were completed into the improvement of the mechanical performance of mycelium through mild alkaline extraction and hot pressing to form nanopapers primarily comprising polysaccharides including fungal structural polymers, such as chitin and chitosan. The nanopapers produced exhibited much higher tensile strength than most existing mycelium materials, with comparable properties to paper and some plastics, but were weakened by inorganic Ca and organic lipid impurities within the nanopapers. Mycelium-derived nanopapers exhibited hydrophobic surface properties with high water advancing contact angles resulting from the presence of lipid residues within the nanopapers. These could be removed, and the surface properties subsequently tuned through HCl or H_2O_2 treatments.

These investigations have demonstrated that mycelium-derived materials have a range of useful functional properties and could be used as low-cost and environmentally sustainable alternatives to synthetic polymers in a range of non-structural and semi-structural applications.

Kurzfassung

Mycel-Verbundwerkstoffe sind Materialien, die hergestellt werden, in dem natürliches Pilzwachstum Lignocellulose-Substrate zu einer einzigen Masse bindet, die einer bestimmten Formgeometrie ähnelt und typischerweise schaumartige mechanische Eigenschaften aufweist. Pilze werden speziell aufgrund ihrer Wachstumseigenschaften verwendet, die eine expandierende netzartige Struktur von Filamenten aus Chitin-β-Glucan-Zellwänden und einen heterotrophen Wachstumsprozess darstellen, der Substrate unter Umgebungsbedingungen verdaut und bindet. Dieses günstige und umweltverträgliche Bio-Herstellungsmethode mit der landwirtschaftliche Nebenprodukte und Abfälle in umweltfreundliche Materialien umgewandelt werden können, erfährt weltweit ein zunehmendes Forschungsinteresse und eine zunehmende Vermarktung. Diese Dissertation untersucht systematisch die biologische Optimierung des Herstellungsprozesses und die Ausweitung auf nicht Strukturelle funktionale Anwendungen die insbesondere als thermisch sicherere und kostengünstige Alternativen für leicht entzündlichen Isolations- und Verkleidungsmaterialien sind. Beispiele dafür sind Erdöl- und Erdgasbasis, wie synthetische Schäume und extrudiertem Polystyrol sowohl Holzwerkstoffe, die entflammbare Harze auf Resorcin und Polyvinylacetatbasis enthalten und somit die Verbesserung der mechanischen Eigenschaften des Mycelmaterials unterstützt.

Zunächst wurde die biologische Optimierung des Herstellungsprozesses untersucht und die eignung mehrerer Pilzarten und landwirtschaftlicher Nebenprodukte für die Verwendung als Matrix- und Füllstoffphase in Mycel-Verbundwerkstoffen, die für die Erzeugung von Chitin-β-Glucan-Polymeren bewertet wurde. Es wurden keine inhärenten Pilzmerkmale wie: Hyphenarten und Pathogenität gefunden, die auf Assoziationen oder Taxonomien basierende Klassifizierungen, sich als zuverlässige Prädiktore für die Wachstumsleistung erwiesen haben. Es wurden mehrere leistungsstarke Arten für die Verwendung in den verbleibenden Untersuchungen identifiziert. Ausgewählte Arten schlossen Weißfäulepilze zur Verwendung als Verbundbindemittel und Boden sowie Wasserassozierte Pilze mit hohen Zellwandkonzentrationen von Strukturpolymeren wie Chitin und Chitosan ein. Anschließend wurden feste landwirtschaftliche Nebenprodukte auf ihre Eignung als Kompositfüllstoffe untersucht. Reishülsen, Zuckerrohrbagasse und Weizenstroh erwiesten sich als sehr schlechte Nährstoffe, was zeigt, dass eine Nahrungsergänzung mit Weizenkörnern erforderlich ist, um eine ausreichende Bindung innerhalb der biologischen Verbundstoffe zu erreichen. Das Zuckerrohrnebenprodukt Blackstrap Melasse war jedoch ein außergewöhnlicher Nährstoff für die Erzeugung von Chitin-β-Glucan-Polymeren und übertraf sogar übliche Labornährstoffe wie Malzextrakt. Hochnahrhafte Nährstoffe waren mit größeren

Hyphen-Durchmessern und einer signifikanten Anastomose verbunden, die pseudolaminare Myzelschichten erzeugte.

Des weiteren wurde eine zweiteilige Untersuchung durchgeführt, um feuerfeste Mycel-Verbundwerkstoffe herzustellen. Zuerst wurden die bisher nicht dokumentierten thermischen Abbau und Brandreaktionseigenschaften der Mycelmatrixphase untersucht. Mycelbiomasse zeigte einen für biologische Materialien typischen dreistufigen Abbauprozess, jedoch überlegene Brandreaktionseigenschaften gegenüber anderen konkurrierenden thermoplastischen Polymeren wie: Polymethylmethacrylat und Polymilchsäure. Die faserige Struktur des Mycels blieb nach der Pyrolyse erhalten, wenn auch mit einer Verringerung des Durchmessers und der Zellwandicke,- das Mycel zeigte bestimmte flammhemmende Eigenschaften das aussah wie ein hoher Verkohlungsrückstand sowie Wasserdampffreisetzung. Es wurden auch Mycel-Verbundwerkstoffe unter Verwendung verschiedener Kombinationen von landwirtschaftlichen und industriellen Nebenprodukten mit einem hohen Siliciumdioxidgehalt wie Reishülsen und Glasfeinstoffen als Füllstoffphase für das Verbundsubstrat hergestellt. Die hergestellten Verbundwerkstoffe zeigten hervorragende Brandschutzeigenschaften mit niedrigeren durchschnittlichen und maximalen Wärmeabgaberaten, einer längeren geschätzten Überschlagszeit auf als die Referenzen für extrudierten Polystyrolschaum sowie synthetische Spanplattenbaustoffe. Sie setzten auch deutlich weniger Rauch und CO_2 frei und hatten sehr niedrige Rohstoffsubstratkosten. Die Substratmaterialien waren nicht nur kostengünstig, sondern auch für die Verbesserung des Brandverhaltens bei Reishülsen verantwortlich, die signifikante Holzkohle- und Silica-Asche ergaben, und bei Verbundstoffen, die Glasfeinstoffe enthielten die aufgrund ihrer signifikant höheren Silica-Konzentrationen und des geringen Gehalts an brennbarem Material das beste Brandverhalten zeigten.

Schließlich wurden Untersuchungen zur Verbesserung der mechanischen Leistung von Mycel durch milde alkalische Extraktion und Heißpressen durchgeführt, um Nanopapiere zu bilden, die hauptsächlich Polysaccharide, einschließlich Pilzstrukturpolymere wie Chitin und Chitosan umfassen. Die hergestellten Nanopapiere zeigten eine viel höhere Zugfestigkeit als die meisten vorhandenen Myzelmaterialien mit vergleichbaren Eigenschaften wie Papier und einige Kunststoffe, wurden jedoch durch anorganische Ca und organische Lipidverunreinigungen in den Nanopapieren geschwächt. Von Mycel stammende Nanopapiere zeigten hydrophobe Oberflächeneigenschaften mit hohen Kontaktwinkeln, die auf das Vorhandensein von Lipidresten in den Nanopapieren zurückzuführen waren. Diese könnten entfernt und die Oberflächeneigenschaften anschließend durch HCl- oder H_2O_2-Behandlungen angepasst werden.

Diese Untersuchungen zeigten, dass von Mycel abgeleitete Materialien eine Reihe nützlicher funktioneller Eigenschaften aufweisen, und die als kostengünstige und umweltverträgliche Alternative zu synthetischen Polymeren in einer Reihe nicht struktureller und semi-struktureller Anwendungen eingesetzt werden können.

List of Publications

This doctoral thesis consists of an introduction to the research area and the following appended publications:

Publication A

Jones, M, Huynh, T, Dekiwadia, C, Daver, F & John, S 2017. 'Mycelium Composites: A Review of Engineering Characteristics and Growth Kinetics', *Journal of Bionanoscience*, vol. 11, no. 4, pp. 241-57.

Publication B

Jones, M, Mautner, A, Bismarck, A, John, S 2019. 'Engineered Mycelium Composite Construction Materials from Fungal Biorefineries: A Critical Review', *Materials and Design*. (submitted)

Publication C

Jones, M, Huynh, T & John, S 2018. 'Inherent Species Characteristic Influence and Growth Performance Assessment for Mycelium Composite Applications', *Advanced Materials Letters*, vol. 9, no. 1, pp. 71-80.

Publication D

Jones, M, Lawrie, A, Huynh, T, Morrison, P, Mautner, A, Bismarck, A & John, S 2018. 'Agricultural By-product Suitability for the Production of Chitinous Composites and Nanofibers Utilising Trametes versicolor and Polyporus brumalis Mycelial Growth', *Process Biochemistry*, no. 80, pp. 95-102.

Publication E

Jones, M, Bhat, T, Kandare, E, Thomas, A, Joseph, P, Dekiwadia, C, Yuen, R, John, S & Wang, C 2018. 'Thermal Degradation and Fire Reaction Properties of Fungal Mycelium and Mycelium Bio-Composites, *Scientific Reports*, vol. 8, p. 17583.

Publication F

Jones, M, Bhat, T, Huynh, T, Kandare, E, Yuen, R, Wang, C & John, S 2018. 'Waste-derived Low-cost Mycelium Composite Construction Materials with Improved Fire Safety', *Fire and Materials*, no. 42, pp. 816-825.

(Continued over page)

Publication G

Jones, M, Weiland, K, Kujundzic, M, Theiner, J, Kählig, H, Kontturi, E, John, S, Bismarck, A & Mautner, A 2019. 'Waste-derived Low-cost Mycelium Nanopapers with Tunable Mechanical and Surface Properties, *Biomacromolecules*, vol. 20, no. 9, pp. 3513-3523

Conference papers not included in the thesis:

Jones, M, Bhat, T, Wang, C, Moinuddin, K & John, S 2017. Thermal Degradation and Fire Reaction Properties of Mycelium Composites. In 21st International Conference on Composite Materials, Xi'an, China.

Jones, M, Huynh, T & John, S 2018. Inherent Hyphal Characteristic Growth Assessment for Mycelium Composite Applications. In Advanced Materials World Congress, Singapore.

Bhat, T, **Jones, M**, Kandare, E, Yuen, R, Wang, C & John, S 2018. Biomass and Waste-derived Sustainable Mycelium Composite Materials with Enhanced Fire Safety. In 18th European Conference on Composite Materials, Athens, Greece.

Jones, M, Lawrie, A, Huynh, T, Morrison, P, Mautner, A, Bismarck, A & John, S 2019. Mycelium-derived Fungal Chitin Nanofibres from Low-cost Sugarcane By-products. In 6th International Conference on Multifunctional, Hybrid and Nanomaterials, Sitges, Spain.

Jones, M, Weiland, K, Kujundzic, M, Mautner, A, Bismarck, A & John, S 2019. 'Sustainable Mycelium-derived Chitinous Thin Films'. In 22nd International Conference on Composite Materials, Melbourne, Australia.

Weiland, K, **Jones, M**, Fortea-Verdejo, M, Mautner, A, Bismarck, A & John, S. 'Waste-derived Nanocellulose-chitin Hybrid Materials with Tunable Physical Properties'. In 22nd International Conference on Composite Materials, Melbourne, Australia.

Jones, M, Weiland, K, Kujundzic, M, Mautner, A, Bismarck, A & John, S 2019. 'Chitin-chitosan Thin Films from Microbiologically Upcycled Agricultural By-products'. In 13th International Conference on the Mechanical Behaviour of Materials, Melbourne, Australia.

Media items not included in the thesis:

Huynh, T & **Jones, M** 2018, 'Scientists create new building material out of fungus, rice and glass', *The Conversation*, 20 June [Online]. Available at: https://theconversation .com/scientists-create-new-building-material-out-of-fungus-ice-and-glass-98153.

Huynh, T, **Jones, M** & John, S 2018. Interview in *Today Tonight*. [television broadcast] Channel 7, 15 and 26 November 2018.

Author Contributions

The author contributed to the appended publications included in this thesis as follows:

Publication A: I performed all data collection, interpretation, SEM, writing, manuscript preparation, submission and revisions. C.D. supervised SEM. All authors contributed to editing and proof reading.

Publication B: I performed all data collection, interpretation, writing, manuscript preparation, submission and revisions. All authors contributed to editing and proof reading.

Publication C: I performed all experimental design, sample preparation, experimental work, data analysis, writing, manuscript preparation and submission. All authors contributed to editing and proof reading. T.H. and S.J. supervised the work.

Publication D: I contributed to experimental design, performed all sample preparation, experimental work, data analysis, writing, manuscript preparation, submission and revisions. A.L. and T.H. contributed to experimental design. P.M. performed the UHPLC. All authors contributed to editing and proof reading. T.H., A.L. and S.J. supervised the work.

Publication E: I contributed to experimental design, prepared all samples, completed the SEM, EDS and XPS experimental work, hyphal diameter and TEM data analysis, contributed to writing and performed all revisions. T.B. performed the TGA-FTIR, GCMS and most of the writing. A.T. performed the PCFC. C.D. performed the TEM. T.B. and R.Y. performed the cone calorimetry. All authors contributed to editing and proof reading. T.B. and C.W. prepared and submitted the manuscript. C.W. and S.J. supervised the work.

Publication F: I contributed to experimental design, performed all sample preparation, SEM and EDS, contributed to cone calorimetry experimental work, performed all data analysis, writing, manuscript preparation, submission and revisions. T.B. performed the TGA. T.B. and R.Y. performed most of the cone calorimetry. All authors contributed to editing and proof reading. C.W. and S.J. supervised the work.

Publication G: I performed all experimental design and sample preparation, yield assessment, SEM, EDS, TGA and contact angle goniometry experimental work, performed all data analysis, writing manuscript preparation, submission and revisions. K.W. contributed to sample preparation and performed the tensile tests. M.K. contributed to sample preparation and FTIR and ssNMR data analysis. J.T. performed the elemental analysis and FTIR. H.K. performed the ssNMR. E.K. performed the sugar analysis. A.M. performed the iGC and zeta potential measurements. All authors contributed to editing and proof reading. A.M., A.B. and S.J. supervised the work.

List of Symbols

a_w	Water activity (-)
ε_f	Strain to failure (%)
E	Elastic modulus (GPa)
F	F-value (-)
G	Hyphal growth unit (µm)
µ	Growth rate per unit biomass (µgh/L)
μ_{max}	Maximum growth rate per unit biomass (µgh/L)
m	Sample mass (g)
n	Sample size (-)
p	p-value (-)
ρ	Envelope or skeletal density (kg/m^3)
r	Growth radius (mm)
σ	Standard deviation (-)
σ_{UTS}	Ultimate tensile strength (MPa)
t_{ig}	Time to ignition (s)
t_{fo}	Time to flashover (s)
Ø	Inoculum diameter (mm)
θ_A	Advancing contact angle (°)
x_0	Initial cell mass (µg)
x	Biomass (g)
X_{acetyl}	Fraction of acetylated monosaccharide units (%)
$\bar{y}$	Replicate set average (-)

List of Acronyms

ABS	Acrylonitrile butadiene styrene
ACS	American Chemical Society
AMU	Atomic mass unit
ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
ATR	Attenuated total reflection
BET	Brunauer–Emmett–Teller
CNMMN	Carbon-nitrogen modified Melin-Norkrans medium
COP	Carbon monoxide production
CO_2P	Carbon dioxide production
D.A.	Degree of acetylation
d.wt	Dry weight (biomass)
EDS	Energy dispersive x-ray spectroscopy
EDTA	Ethylenediaminetetraacetic acid
ERH	Equilibrium relative humidity
FTIR	Fourier transform infrared spectroscopy
GCMS	Gas chromatography–mass spectrometry
HPEAC	High performance anion exchange chromatography
HPLC	High-performance liquid chromatography
HRC	Heat release capacity
HRR	Heat release rate
IEP	Isoelectric point
IR	Infrared
iGC	Inverse gas chromatography
ISO	International Organization for Standardization
MDPE	Mid-density polyethylene
MEA	Malt extract agar
MLR	Mass loss rate
MS	Mass spectroscopy
OPM	Orbits per minute
ÖNORM	Austrian Standards International
PCFC	Pyrolysis combustion flow calorimetry
PFA	Polyfurfuryl alcohol resin
PHRR	Peak heat release rate

PLA	Polylactic acid
PMMA	Polymethyl methacrylate
PTFE	Polytetrafluoroethylene
RH	Relative humidity
RHR	Average heat release rate
rpm	Revolutions per minute
SBR	Styrene butadiene rubber
SD	Standard deviation
SE	Standard error
SEM	Scanning electron microscopy
ssNMR	Solid-state nuclear magnetic resonance
SRS	Sugar recovery standard
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
THR	Total heat release
TPHRR	Temperature at peak heat release rate
TSR	Total smoke release
TTI	Time to ignition
UHPLC	Ultra-high-performance liquid chromatography
UPLC	Ultra-performance liquid chromatography
UV	Ultraviolet
WM	Wet mass (biomass)
wt%	Percentage by weight
vol%	Percentage by volume
XPS	Extruded polystyrene
XPS	X-ray photon spectroscopy

Table of Contents

Part II – Appended Publications

Part I

Introduction

Chapter 1
Context and Background

1.1 A need for sustainability

Environmental sustainability is increasingly becoming a priority in many countries as the effects of climate change and the destruction and pollution of the environment from the seas to the skies across the globe becomes more apparent. In particular, a global shift from reusable to single-use products has resulted in massive increases in synthetic waste generation with approximately 54% (141 Mt) of all non-fiber plastics being disposed of in the same year they were produced (Geyer, Jambeck & Law 2017). There are currently three waste management options for plastic waste: recycling, thermal destruction, and disposal in landfill. Of the estimated 6.3 billion tons of plastic waste generated to date, only about 9% has been recycled because mixed plastics and used plastic contamination often make recycling commercially unappealing (Andrady 2015; Geyer, Jambeck & Law 2017).

The most common waste management strategy for plastic and general waste globally is disposal in landfill, which accounts for 79% of plastic waste management (Geyer, Jambeck & Law 2017). If current production and waste management trends continue, about 12 billion tons of plastic will be in landfills and the natural environment by 2050 (Geyer, Jambeck & Law 2017). Synthetic waste disposed in landfill releases toxic substances and greenhouse gases as it degrades, which alone contributes 75% of total greenhouse gases associated with waste management. 55% of which is methane which has 21-25 times the global warming potential of carbon dioxide (Allen Consulting Group 2009).

With global mitigation against climate change and environmental damage, there is a need for alternatives to synthetic materials. Regulations in Germany, the Netherlands, and the United Kingdom already require whole-life carbon assessment on construction projects and impose restrictions on materials with carbon-intensive supply chains (European Commission 2014; Green Construction Board 2013). It is expected that two-thirds of the $1.5 trillion global chemical industry could eventually be replaced by renewable resources with many global chemical giants already shifting their focus from petrochemical processes to life sciences (Mandel & Teige 2019; Mohanty, Misra & Drzal 2005).

1.2 Sustainable alternatives to synthetic plastics

Use of materials technologies such as bioplastics, which are plastics produced from renewable biomass sources, such as vegetable fats and oils, corn starch, straw, woodchips, sawdust and recycled food waste, are increasing as much as 20-30% a year (Vidal 2008) because they are more environmentally friendly than traditional synthetic plastics, such as polystyrene, polyethylene and polyurethane. However, bioplastic manufacturing still typically produces 20-70% of the carbon emissions associated with traditional plastic production processes, and some biodegradables have equivalent carbon intensity (UN Environment Programme 2014). They are at least 20% more expensive than traditional plastics to manufacture (Dell 2010) and many will not break down in landfill, requiring additional processing in anaerobic digesters to be composted (Vidal 2008). Bioplastics disposed of in landfill also produce methane if they anaerobically degrade due to the presence of moisture (Dell 2010). Development of new materials of greater economic viability and environmental sustainability is required to facilitate our transition to a more sustainable world, with the key requirements of these materials being low manufacturing costs and energy requirements, minimal carbon emissions and full biodegradability.

Mycelium composites are a type of novel, economical and environmentally sustainable materials that have attracted increasing academic and commercial interests over the past decade. Mycelium is the vegetative growth of filamentous fungi that bonds organic matter through a network of hyphal micro-filaments in a natural biological process that can be exploited to produce composite materials (Haneef et al. 2017; Holt et al. 2012; Islam et al. 2017; Jones, M; et al. 2017; Pelletier et al. 2013). These filaments act as a continuous fibrous phase called matrix (Pelletier et al. 2013; Thakur & Siṅgha 2013) that interfaces with a dispersed phase of partially digested organic particles or fibres that increase material volume (filler) (Thakur & Siṅgha 2013). This process is cost-effective because mycelium can grow on and bind agricultural and industrial waste materials that have little or no commercial value (DP CleanTech 2013; Sustainability Victoria 2014) and convert them into higher-value composite materials. Alternatively, the fibrillar (fibrous) and matrix (dispersed) polymers present in fungal cell walls can be chemically extracted for applications ranging from chitin nanofiber production to cosmetics, pharmaceuticals, water treatment and absorption of heavy metals (Di Mario et al. 2008).

1.3 Objectives, scope and research questions

The key incentives for the use of mycelium materials are their low cost and density in addition to their low energy manufacturing process and biodegradability (Abhijith, Ashok & Rejeesh 2018; Arifin & Yusuf 2013; Haneef et al. 2017). However, mycelium composites typically have

foam-like mechanical properties, attributed to the use of weak organic fillers and biological matrix (Appels et al. 2019), and a slow biological manufacturing process. This limits their current applications significantly, with their most widespread use being in packaging (Abhijith, Ashok & Rejeesh 2018; Dell Inc. 2016; Gosden 2016; Holt et al. 2012). Significant research into biological optimisation of the manufacturing process, improvement in mechanical performance of these materials and expansion to non-structural, functional applications is consequently necessary.

Despite the direct correlation between all manufacturing and material properties of mycelium composites and their constituents (species and substrate material), existing literature features arbitrary selection of fungal species (fibrous matrix phase of composite) and substrate materials (organic reinforcement phase of composite). There is also no literature on the thermal degradation properties of mycelium or the fire reaction and fire safety properties of mycelium or mycelium composites and minimal work on chemical or physical processing to improve the mechanical performance of these materials.

This project aims to produce thermally safer and cost-competitive alternatives to highly flammable synthetic polymers and engineered woods for applications including insulation, furniture and panelling. The specific research objectives include; biological optimisation of manufacturing (species selection and substrate biocompatibility assessment), investigation of the thermal degradation, fire reaction and fire safety properties of mycelium composites, and improvement of the primary mechanical properties through chemical and physical processing.

The following research questions will be addressed:

1. How do inherent species characteristics of fungi (e.g. hyphal types, pathogenicity, taxonomic- and association-based classifications) affect the growth performance (hyphal extension rate and growth density) of the fibrous matrix phase (mycelium) and hence manufacturing time of mycelium composites?
2. Which abundant Australian agricultural by-product (organic substrate filler phase) yields the highest mycelial (fibrous matrix phase) growth (associated with shortest manufacturing time and maximum interfacial bonding)?
3. How does mycelium thermally decompose and what are its thermal degradation properties?
4. How do the fire reaction and fire safety properties of mycelium composites compare with synthetic polymer foam, such as extruded polystyrene, and engineered wood, such as particleboard, and can their properties be improved through incorporation of industrial by-products, such as glass fines?

5. Can the mechanical properties of mycelium be improved by removing non-structural hyphal elements using chemical treatment?

1.4 Thesis methodology

Biological optimisation of mycelium composite manufacturing is addressed first, since maximising fungal growth is important to both accelerating the manufacturing process and achieving maximum interfacial bonding between the mycelium matrix phase and the organic residue filler phase. Fungal species and agricultural residue substrate selection is hence a key initial factor which will carry through to the rest of the research.

The mycelium matrix constituent is addressed first. Common mycological growth assessment techniques, such as hyphal extension rate analysis on solid media and growth density assessment as dry weight of fungi grown on liquid media are utilised to identify top performing species for use in the remaining research. This approach combines a two-dimensional metric with a three-dimensional metric, comprehensively describing fungal growth potential and omitting low-performing species at an early stage in development. With the estimated existence of some 1.5-5.1 million species of fungi a comprehensive assessment of all species is impossible. Instead potentially influential growth characteristics of species groups, such as hyphal types, pathogenicity and taxonomic- and association-based classifications are examined, with species selected as test candidates based on their possession of variations in these growth characteristics.

An evaluation of the suitability of common agricultural residues as nutrients for the two top performing fungal species is then completed. Agricultural residues are assessed since one of the main aims of the project is to produce low-cost materials derived from wastes or by-products. While fungal growth performance is simply assessed on laboratory solid and liquid media, evaluation of how well a given fungal species grows on an agricultural residue is significantly more complicated. This stems from the fact that unlike with solid and liquid laboratory media mycelial biomass is not easily separated from agricultural residue, which makes growth quantification challenging. Quantification is possible through the correlation between ergosterol, a sterol unique to fungi and some microscopic algae and protozoa, and fungal biomass. Colonised agricultural reside substrates are digested using alkaline chemical treatment and the ergosterol content of the liquid quantified using high-performance liquid chromatography (HPLC) in order to assess fungal growth on each respective residue. Scanning electron microscopy (SEM) is utilised to characterise hyphal network morphology on each substrate. Top performing agricultural residues are identified and retained for use in the remaining research.

With fungal species and agricultural residue selection complete composite materials can now be manufactured. However, with the main material property of interest being the thermal degradation properties and fire resistance of the materials, characterisation of the undocumented thermal degradation properties of the mycelium matrix phase is necessary. Changes to the physical structure, reduction in hyphal diameters and chemical composition following pyrolysis are investigated to gain an in-depth understanding of the thermal degradation and decomposition mechanisms. Parameters such as the onset of decomposition, residual char, evolved gases and heat release are measured in addition to the effect of incubation period (growth time) on mycelium composite fire properties.

Mycelium composite materials are then manufactured, consolidating the previous research completed regarding fungal species with high hyphal extension rates and growth density, most suitable substrates and the thermal degradation properties of mycelium. Thermally stable substrates and additives, such as rice hulls and glass fines, are used in conjunction with highly nutritious substrate constituents to produce fire resistant composite materials. The fire reaction and fire safety properties of these materials are then characterised using cone calorimetry and compared with commercial building materials, such as extruded polystyrene insulation foam and particle board. The cost of the composites is also assessed as a function of raw material costs and composition.

With significant literature documenting the low mechanical properties of mycelium composites, investigations are then completed into improving the tensile strength of mycelium-based materials. Low strength in mycelium composites results from the often low-strength agricultural residue utilised in these composites as filler, which is weakly bonded by a hyphal filament matrix, and the presence of non-structural hyphal elements, such as proteins, lipids and cytoplasm. As such, the use of these low-strength composite fillers is eliminated with them instead utilised solely as nutrient sources for fungal growth from which non-structural elements can be removed using alkaline chemical treatments. This process constitutes the conversion of agricultural biomass into high-strength natural polymers within fungal biomass, such as chitin, chitosan and glucan, which can be hot-pressed to produce homogenous nanopapers. Emphasis was on cost and environmental impact with only cheap agricultural by-products and natural fungal growth from high growth performance species used to obtain chitinous fungal biomass. The morphology, composition and molecular structure of fungal chitin-β-glucan nanopapers were then analysed in addition to their physical, mechanical and surface properties. Mycelium-derived nanopapers were also compared with nanopapers produced from common white button mushroom fruiting bodies.

1.5 Thesis outline

Part I: Introduction

Chapter 2: Literature Review

This chapter addresses the biological and engineering-specific aspects of mycelium composite production and examines historical and current applications of mycelium composites. Classification structures, physiology and physical characteristics of fungi are outlined in addition to optimal growth environments, growth modelling, fungal nutrition and metabolism. Current mycelium composite research and industrial applications are then addressed in addition to the expanding field of engineered mycelium materials, examining the influence of the continuous mycelium matrix and dispersed agricultural residue filler phase on mechanical performance, physical processing, resin infusion, sandwich composites, hybridisation, thermal conductivity, termite resistance, acoustic and water absorption properties of mycelium composites. A range of potential applications including superabsorbent, paper, textiles and automotive components are also detailed.

Chapter 3: Results and Discussion

This chapter provides a concise summary of the most important elements of the results and discussion of the appended publications. Biological manufacturing optimisation is completed through an investigation into the growth performance of fungal species based on potentially influential growth characteristics of species groups and the suitability of various agricultural residues assessed as composite fillers based on the ergosterol concentrations of the colonised substrates before the previously undocumented thermal degradation properties of mycelium are detailed. Mycelium composite materials utilising substrates with high silica contents are then produced based on the findings of the previous research and their fire reaction and safety properties characterised. Finally, investigations are completed into improving the tensile strength of mycelium materials through extraction and consolidation of chitin, chitosan and β-glucan polymers from mycelium using alkaline treatment and hot pressing and their physical and surface properties documented.

Chapter 4: Conclusions and Future Research

This chapter summarises all major findings and conclusions arising from this research project and identifies several research topics that require further investigation.

Part II: Appended Publications

The most important publications arising from this work are appended in the second part of this thesis. This comprises two review articles and five research articles.

Chapter 2

Literature Review

2.1 Introduction

Mycelium materials research spans multiple disciplines, incorporating elements of mycology, microbiology, chemistry and materials science. This chapter provides an overview of the essential elements from each field relevant to mycelium materials, addresses the biological and engineering-specific aspects of mycelium composite production and examines their historical and current applications. Classification structures, physiology and physical characteristics of fungi are outlined in addition to optimal growth environments, growth modelling, fungal nutrition and metabolism. Although generally characterised as polymer grade foams and used primarily for packaging and construction applications, the mechanical performance of mycelium composites varies and is governed by hyphal architecture, cell wall composition, composite constituents and growth kinetics which are discussed in detail. Current mycelium composites research and industrial applications are also addressed in addition to the expanding field of engineered mycelium materials, examining the influence of the continuous mycelium matrix and dispersed agricultural residue filler phases on mechanical performance, physical processing, resin infusion, sandwich composites, hybridisation, thermal conductivity, termite resistance, acoustic and water absorption properties of mycelium composites. A range of potential applications including superabsorbent, paper, textiles and automotive components are also detailed.

2.2 Fungal biology

Fungi are a group of diverse unicellular or multicellular spore-producing organisms which feed on organic matter. They include moulds, yeasts, mushrooms and toadstools (Stevenson 2010). The fungal kingdom is currently one of the least comprehensively studied and documented kingdoms with only approximately 80,000 to 120,000 species recorded (Webster & Weber 2007) of 1.5 million (Hawksworth 2001) to 5.1 million species (Blackwell 2011), although these figures have been suggested to be somewhat overestimated (Tedersoo et al. 2014).

Remarkably fungi are more closely related to animals than to any other biological kingdom (Baldauf 1993). Over 600 million years ago the two kingdoms shared a common ancestor **(Figure 2.1)** which through evolution developed a means of external digestion (Stamets 2005). This process occurs through secretion of enzymes such as cellulases, oxidases,

phosphatases, chitinases and proteases (Sinsabaugh 1994), which break down food sources, followed by absorption of the solubilised nutrients (Webster & Weber 2007).

Although fungi share the same basic genetic structures as plants and animals, their cellular biology differs greatly from other kingdoms (British Mycological Society 2005). The key differences are that while plants and animals comprise of cells organised into tissues and organs, fungi are generally filamentous (long thin cellular structure, hyphae based) and in contrast to plants and animals, their nuclear envelope, which comprises two lipid bilayer membranes surrounding the nucleus, remains intact during cell division (Raven & Johnson 1999).

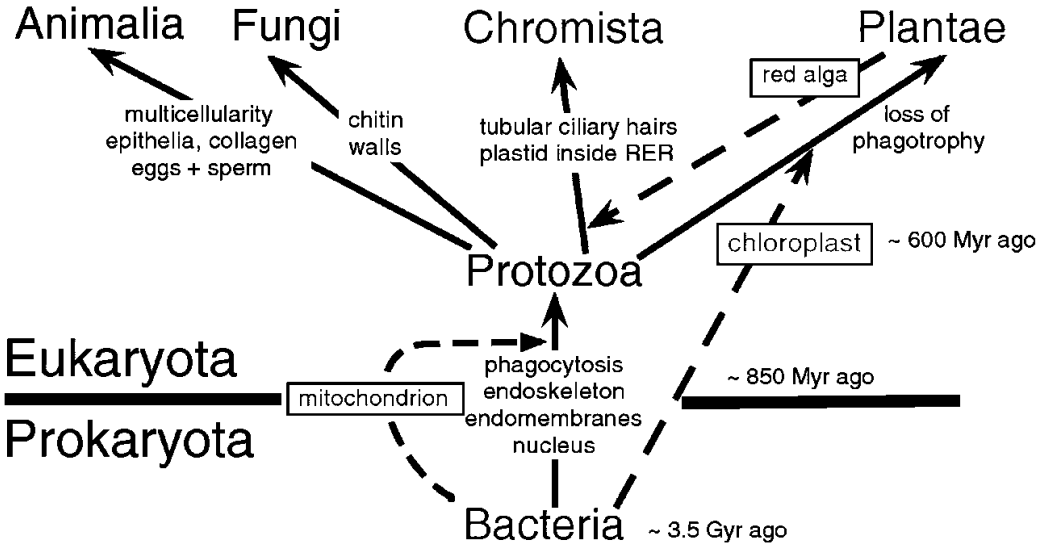

Figure 2.1. The six-kingdom (Animalia, Fungi, Chromista, Plantae, Protista, Bacteria) classification of life. Reproduced with permission from Cavalier-Smith (2010).

2.3 Fungal classification

The fungal kingdom comprises of a vast array of diverse species each exhibiting unique characteristics and similarities with genetically related species. Over the years, the scientific community has attempted to map these relationships, producing classification structures for fungi based on increasingly accurate methods of fungal identification and categorisation (Webster & Weber 2007).

2.3.1 Taxonomic classification of the fungal kingdom

Taxonomic classification involves the use of a phylogenetic tree which arranges fungi into hierarchal groups **(Figure 2.2)** and serves three main purposes. It provides a reference framework of recognisable features; it attempts to group together related organisms, and it

provides information about the characteristics of an identified species of interest (Webster & Weber 2007).

Early fungal taxonomic approaches were based on morphological and microscopic characteristics augmented by biochemical and ultrastructural features and were largely arbitrary due to taxonomists proposing widely differing classification schemes (i.e. species names and location within a phylogenetic tree) depending on which features they felt were most relevant (Weber 2009). Taxonomic methods used today are significantly more sophisticated with the use of multi-locus datasets, extensive taxon sampling, and exhaustive analytical processes as standard procedure (Hibbett et al. 2007).

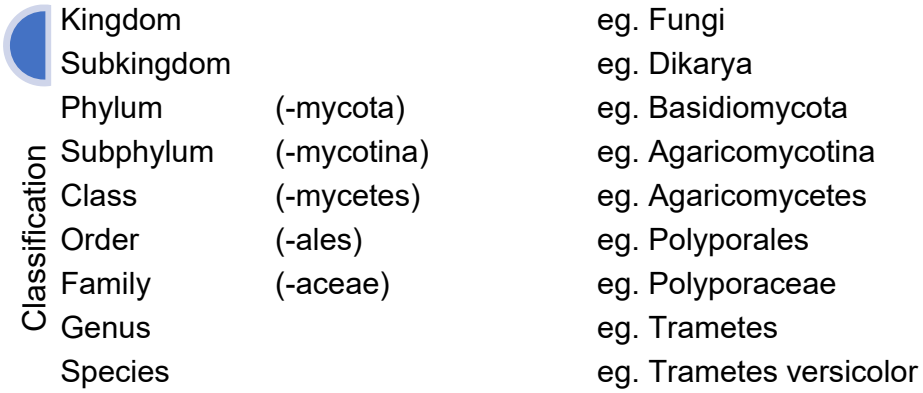

Figure 2.2. Phylogenetic tree of classification. Data from Pitt and Hocking (2009) and Stalpers et al. (2004). Reproduced with permission from Jones, M; et al. (2017).

The taxonomic system by Hibbett et al. (2007) **(Figure 2.3)** describes the current taxonomic classification of fungi. The classification accepts one kingdom, one subkingdom, seven phyla, ten subphyla, 35 classes, 12 subclasses and 129 orders. It is based largely (90%) on recently published literature with the remainder based on automatically typified teleomorphic names (Hibbett et al. 2007).

Most notably it rejects the traditionally recognised Zygomycota and Chytridiomycota phyla, which have long been recognised to be polyphyletic (containing organisms derived from more than one common evolutionary ancestor and are hence unable to be classified together). These phyla are reclassified into more appropriate groups with their previous association to the traditional phyla noted.

Taxonomic classification systems are especially useful from a materials science perspective because they characterise many properties exhibited by documented species and identify other related species with similar properties. This provides a scientific foundation for comparison of the mechanical performance of species known to exhibit similar or dissimilar characteristics.

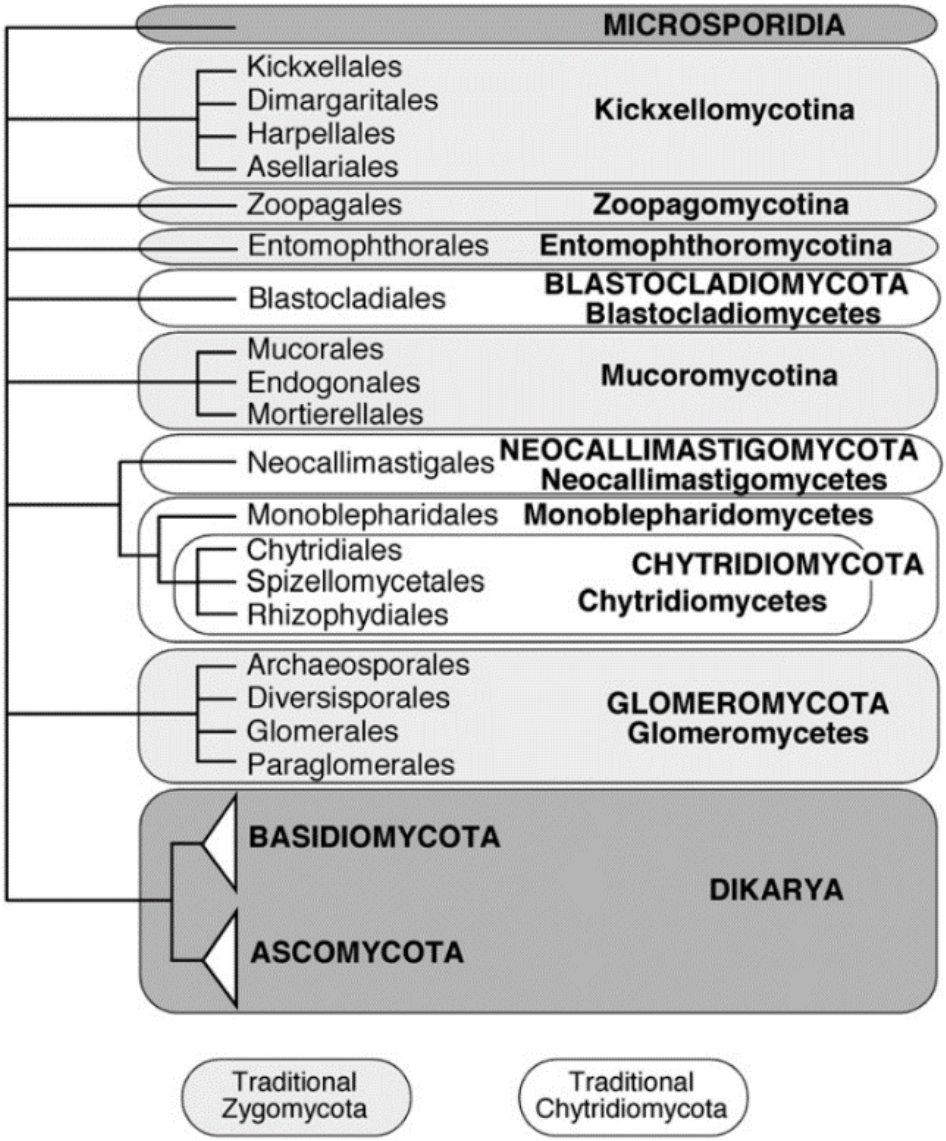

Figure 2.3. Phylogeny and classification of fungi. Basal fungi and Dikarya. Branch lengths are not proportional to genetic distances. Reproduced with permission from Hibbett et al. (2007).Substrate associations of fungi

Fungi sometimes exist independently in nature but in most cases, share an association with other organisms, including plants. In such cases, classification is based on the host and the respective benefits or threats with which the relationship presents each organism.

2.3.2.1 Saprophytic (saprotrophic) fungi

Saprophytic fungi use enzymes to digest organic matter into small molecules, which they then absorb as nutrients (Clegg & Mackean 1994). They are the most relevant group of fungi from a materials science perspective because they convert organic waste into mycelial mass. They comprise of three main groups based on their order of appearance during the digestion process (Stamets 2005).

a) Primary colonisers appear first in nature. They have high growth rates and rapidly expand, attach to, and decompose simple compounds.

b) Secondary colonisers rely on the primary colonisers to partially break down plant and animal tissue before digesting the more complex compounds.

c) Tertiary colonisers are found towards the end of the decomposition process, thriving in conditions created by primary and secondary colonisers and relying on highly complex microbial environments.

2.3.2.2 Pathogenic (parasitic) fungi

Pathogenic fungi endanger the host's health and cause diseases in organisms. Some pathogenic fungi behave like saprotrophic fungi, but most are microfungi, barely visible to the naked eye, inflicting cankers and lesions on the shoots and leaves of living plants (Stamets 2005). Many pathogens use specialised toxins, enzymatic degradation, subversions of cellular processes, mechanical forces, or a combination of these, to rapidly invade and colonise host material (Sexton & Howlett 2006). While animal pathogens typically only cause serious fungal infections in immunocompromised hosts (including humans), plant pathogens have mechanisms allowing invasion of even healthy hosts including mechanical penetration using appressoria (Sexton & Howlett 2006). These highly organised enlarged hyphal ends feature a narrow hyphal strand on the underside called a penetration peg which penetrates the epidermal cell wall and accelerates inoculation (Kavanagh 2005). Although some pathogenic fungi such as biotrophs are not suitable for mycelium composite applications because they must be in contact with a host plant to survive, non-obligate pathogens are more versatile and can grow and multiply on dead organic matter as well as on living host tissue (Kavanagh 2005).

2.3.2.3 Symbiotic fungi

Symbiotic fungi form associations with plants in a mutually beneficial relationship. They comprise of two main groups based on location within the host (Stamets 2005).

a) Mycorrhizal fungi commonly invade the roots of vascular plants intracellularly (endomycorrhizal) or extracellularly (ectomycorrhizal), with the mycelial growth of the fungus extending the plants nutrient absorption ability and zone. The fungus benefits from access to the plants secreted sugars (e.g. hexose).

b) Endophytic fungi grow within plants, threading their mycelia between the cell walls and enhancing growth ability and resistance to parasites and infections. The fungus also benefits from access to plant secreted sugars.

2.4 Optimal fungal growth environments and classifications

Fungi can also be classified by their optimal growth environment based on temperature, water activity and pH **(Table 2.1)**. Water activity (a_w) is the ratio of the vapour pressure of water in a material to the vapour pressure of pure water at the same temperature (Griffin, DH 1996). It is a decimal value, but when expressed as a percentage it gives equilibrium relative humidity (ERH) (Rockland 1987). Mesophilic or neutrophilic fungi experience optimal growth under ambient environmental conditions while extremophilic fungi thrive in extreme habitats (Singh 2012). Classifications systems that describe the affinity for optimum growth at certain environmental conditions are important to optimise mycelial growth for commercial bio-materials production.

Table 2.1. Extremophilic (low and high) and mesophilic (medium) fungal groups. Data from Burge (2006); Dix and Webster (1995); Griffin, DH (1996); Gross and Robbins (2000); Hassan et al. (2016); Horikoshi (1999); Karch (2008); Maheshwari, Ramesh, Bharadwaj and Bhat (2000). Optimal ranges discussed in 2.6.1.

Parameter	Low	Medium	High
Temperature (°C)	Psychrophilic (0 – 20)	Mesophilic (0 – 50)	Thermophilic (20 – 50)
Water activity (a_w)	Xerophilic (0 – 0.8)	Mesophilic (0.8 – 0.9)	Hydrophilic (0.9 – 1)
pH	Acidophilic (1 – 6)	Neutrophilic (7)	Alkaliphilic (8 – 11)

2.5 Phases and kinetics of fungal growth

The vegetative part of filamentous fungi comprises a network of fine white filaments (hyphae) known collectively as mycelium (Stevenson 2010) which spread upon or penetrate a substrate (Webster & Weber 2007).

A fungal spore inoculated on a nutrient rich medium will form a germ tube, which will experience exponential non-photosynthetic growth undergoing dichotomous (dividing into two sections) or lateral branching (Prosser, JI 1993) fuelled by digestion of carbon and nitrogen based feed stock (Carlile, MJ 1995). Extension occurs only in the apical (tip) region which has a hemi ellipsoidal shape with wall growth throughout the remainder of the extension zone taking the form of a circumferential extension which ceases when the hypha reaches its full width (Webster & Weber 2007).

Three typical growth phases will occur following inoculation of suitable media:

a) Lag phase – is a period of zero or low population growth as the inoculated cells grow accustomed to their new chemical and physical environment (Kavanagh 2005). Lag phase duration varies by species (Krauke & Sychrova 2010; Petersen, Frohne & Kennedy 2002) but exhibits an inverse relationship with growth rate. Faster-growing species consequently have shorter lag phases (González, Resnik & Vaamonde 1988).

b) Exponential phase – Under optimal conditions, exponential growth occurs with proportionate increases in biomass including cell number, dry weight and nucleic acid and protein content of the population (Carlile, M, Watkinson & Gooday 2001).

c) Stationary phase – if essential nutrients are exhausted, or toxic products accumulate the exponential phase of growth will end, and the fungal cells enter a period known as the stationary phase in which the specific growth rate returns to zero and biomass remains relatively constant (Carlile, M, Watkinson & Gooday 2001; Kavanagh 2005). This phase is thought to be the reason that fungi can survive for long periods without additional nutrients, but if this phase is maintained incessantly, cells may begin to die.

It is desirable to minimise the lag phase and ensure that optimal environmental conditions and abundant nutrients are available to maximise growth rate and yield and prevent growth entering the stationary phase prematurely.

2.5.1 Modelling of fungal growth kinetics

Growth during the exponential phase can be mathematically modelled using empirical growth equations, such as linear, exponential, logistic or two-phase models (Mitchell et al. 2004). Individual hyphae grow at a constant linear rate (Griffin, DH 1996), but the majority of colony based microbial growth is calculated using either logistic regression, two-phase or exponential models (Carlile, M, Watkinson & Gooday 2001; Mitchell et al. 2004).

The logistic and two-phase models are most commonly used to calculate growth in open systems (continuous culture) in which media is simultaneously supplied to and removed from the system to maintain a constant volume (e.g. bioreactors) (Mitchell et al. 2004). The exponential model is most commonly used to calculate growth in closed systems where no media is added or removed (batch culture growth) (Carlile, M, Watkinson & Gooday 2001; Griffin, DH 1996; Kavanagh 2005). This is the most appropriate model since materials science work completed to date, utilises sealed batches of moulds (Holt et al. 2012; Jiang, Walczyk & McIntyre 2014; Jiang et al. 2013; Lelivelt 2015; López Nava et al. 2016; Pelletier et al. 2013; Travaglini et al. 2013).

The exponential model is derived from the proportional relationship between population growth rate (dx/dt) and growth rate per unit biomass, commonly referred to as specific growth rate (μ) **(Equation 2.1)** (Carlile, M, Watkinson & Gooday 2001). The model itself **(Equation 2.6)** can be used to calculate biomass aspects, such as dry weight (Carlile, M, Watkinson & Gooday 2001). This is a parameter of interest because it has a correlation with mycelial density (Carlile, M, Watkinson & Gooday 2001).

Mycelial branching increases the number of growing-points over time giving an accelerating exponential growth rate pattern **(Figure 2.4a)** and logarithmic dry weight increase **(Figure 2.4b)** (Carlile, M, Watkinson & Gooday 2001; Griffin, DH 1996; Kavanagh 2005).

Equation 2.1.	$\frac{dx}{dt} = \mu_{max} x$	Population growth rate (dx/dt) is proportional to growth rate per unit biomass (μ) multiplied by population biomass (x)
Equation 2.2.	$\frac{dx}{x} = \mu_{max} dt$	Variables are separated and grouped
Equation 2.3.	$\int_{x_0}^{x} \frac{1}{x} dx = \int_{0}^{t} \mu_{max} dt$	Biomass is integrated between initial cell mass (x_0) and biomass, x, and time between zero and time, t
Equation 2.4.	$\ln\left(\frac{x}{x_0}\right) = \mu_{max} t$	Both sides are then exponentiated
Equation 2.5.	$\frac{x}{x_0} = e^{\mu_{max} t}$	A solution for biomass (x) is then computed
Equation 2.6.	$x = x_0 e^{\mu_{max} t}$	Exponential equation modelling biomass at any given time achieved

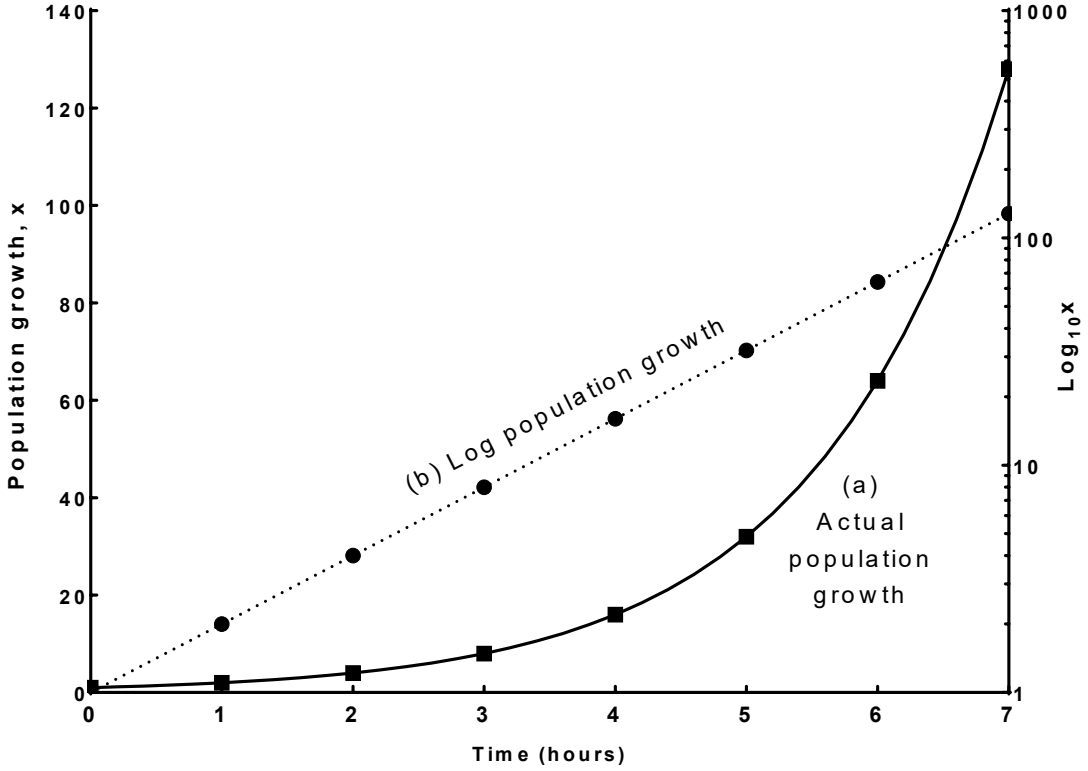

Figure 2.4. Exponential model showing (a) actual and (b) log population growth.X can represent any measure of population growth (cell number, dry weight, protein or nucleic acid content). Data from Carlile, M, Watkinson and Gooday (2001); Kavanagh (2005). Reproduced with permission from Jones, M; et al. (2017).

2.6 Exogenous factors affecting fungal growth

Exogenous factors such as environmental conditions and chemical nutrition have a significant influence on fungal growth, affecting the lag phase duration, exponential phase growth rate and dictating if or when the stationary phase is reached **(Table 2.2)**. These factors are especially relevant to mycelium material manufacturing time.

2.6.1 Effect of environmental conditions on fungal growth

2.6.1.1 Inoculation conditions

Inoculum describes any fungal biomass constituent that can be used to colonise new substrate material, such as spores, hyphal or fruiting body tissue in liquid or on a nutritious solid medium, such as grain or sawdust. Inoculation conditions have significant influence over both lag phase duration and exponential phase growth rate. Decreased inoculum density results in increased lag phase duration (Baert et al. 2008; González, Resnik & Vaamonde 1987; Gougouli et al.

2011; McGonigle, Hovius & Peterson 1999; McGonigle & Miller 1993; Samapundo et al. 2007; Sautour et al. 2003), decreased specific growth rate (Meyrath 1963; Wang, Zhong & Yu 1997) and decreased maximum yield (Meyrath 1963; Yang, F-C & Liau 1998; Zhang & Zhong 1997). This effect can be mitigated by the presence of trace elements, such as calcium, copper, iron, manganese, zinc, nickel and molybdenum, and higher sugar concentrations in smaller inocula (Meyrath 1963). Inoculation using cells vigorously growing on an identical medium results in the absence of a lag phase (Carlile, M, Watkinson & Gooday 2001) and is the optimal inoculation method. Ideal inoculation density is 10-32% inoculum to substrate ratio (by volume) depending on liquid or solid tissue inoculum used with an incubation time of 5-14 days depending on the substrate (McIntyre et al. 2012).

2.6.1.2 Effect of temperature on fungal growth

Temperature plays a similar role, with temperatures less than 10°C resulting in increased lag phase duration and reduced exponential phase growth rate in mesophilic species (Ji et al. 2007; Khokhar et al. 2010). Warm temperatures, exceeding 15°C, are especially important for initial growth rate which doubles with every 10°C increment temperature increase up to the point where the fungus starts to dehydrate or denature (Burge 2006). Growth will typically be inhibited by temperatures above 40°C, which inactivate enzymes (Chang 1992), disrupt hydrogen bonding and hydrophobic interactions, and lead to the denaturation of proteins and nucleic acids (Kavanagh 2005). Optimal temperatures are less than 10°C for psychrophiles, between 18-22°C for mesophiles and over 37°C for thermophiles but also depend on water availability and nutrients (Burge 2006).

2.6.1.3 Effect of water activity (a_w) on fungal growth

Water plays a crucial role in fungal metabolism with reduced water availability (<0.65 water activity) to cells adversely affecting fungal growth (Kavanagh 2005). Water activity is most important during the lag phase (Ji et al. 2007) with the adverse effects of lower temperatures significantly exacerbated by low water activity levels (Pardo et al. 2005). Water activity has only a slight effect on growth rate post lag phase (Pardo et al. 2005). Optimal water activity levels are 0.6 – 0.8 for xerophilic fungi (dry loving – primary colonisers), 0.8-0.9 for slightly xerophilic (secondary colonisers) and >0.9 for hydrophilic (water loving – tertiary colonisers) (Kung'u 2016).

2.6.1.4 Effect of pH on fungal growth

pH is important because most fungi are acidophilic and grow well in the pH range of 4 to 6. This acidic environment provides the hydrogen content required for optimal fungal growth (Kavanagh 2005). pH levels outside these ranges (less than 3 or greater than 8) will adversely

affect growth (Kavanagh 2005) and increase lag phase duration (Chipeta, du Preez & Christopher 2008).

Table 2.2. Influence of environmental parameters on the lag and exponential phases. Data from Carlile, M, Watkinson and Gooday (2001); Griffin, DH (1996); Kavanagh (2005); Kung'u (2016); McIntyre et al. (2012).

Parameter	Lag phase	Exponential phase	Optimum
↑ Inoculum density	↓	↑ Growth rate ↑ Maximum yield	Identical medium 10 – 32% ratio
↑ Temperature	↓	↑ Growth rate	Psychrophiles, 0 – 17°C Mesophiles, 15 – 40°C Thermophiles, 37 – 50°C
↑ Water activity	↓	↑ Growth rate	Xerophiles, 0.6 – 0.8 a_w Mesophiles, 0.8 – 0.9 a_w Hydrophiles, 0.9 – 1.0 a_w
Extreme pH	↑	↓ Growth rate	pH 4 to 6

2.6.2 Fungal chemical physiology and nutrition

Fungal cells rely on macronutrients (carbon, nitrogen, oxygen, sulphur, phosphorus, potassium, magnesium) in millimolar concentrations and micronutrients (hydrogen, calcium, copper, iron, manganese, zinc, nickel and molybdenum) in micromolar concentrations to support their cellular functions (Garraway & Evans 1984; Griffin, DH 1996; Kavanagh 2005). Optimising fungal nutrition is challenging since every chemical found in living organisms in addition to many manufactured, and inorganic materials can potentially support fungal growth. Nutritional studies struggle with availability of media lacking certain elements which are necessary to analyse fungal response to graded elemental quantities (Griffin, DH 1996). Exhaustive studies and universally applicable rules are unavailable, but the roles of key macronutrients are well documented **(Table 2.3)**.

2.6.2.1 The role and sources of carbon in fungal growth

Fungi are chemoorganotrophs, meaning that they oxidise chemical bonds in organic compounds to attain energy and carbon to support cellular function. Sugars in particular ranging from simple hexoses (e.g. glucose) to polysaccharides (e.g. cellulose, starch, and lignin) are common carbon sources that support growth (Kavanagh 2005). Carbon source suitability for any given species is heavily influenced by natural evolutionary factors.

Table 2.3. Source and function of macronutrients. Adapted from Kavanagh (2005).

Element	Sources	Functions
Carbon	Carbohydrates	Energy source Structural element
Magnesium	Mg^{2+} salts	Supports enzyme activity, cell structure, organelle structure
Nitrogen	NO_3-, NO_2-, NH_4+, amines, amides	Structural element Supports proteins and enzyme production and function
Oxygen	Air, O_2	Supports ergosterol synthesis, unsaturated fatty acid synthesis, respiratory enzymes, oxidative enzymes
Phosphorus	Phosphates	Supports biosynthesis of nucleic acids, phospholipids, glycophosphates
Potassium	K^+ salts	Supports ionic balance, enzyme activity
Sulphur	Sulphates, methionine	Source of sulphydryl amino acids, vitamins

Mesophilic microflora are succeeded by thermophilic microflora in nature and as such, mesophiles thrive first as the temperature increases, consuming the simpler carbon sources (sugars, amino acids, and organic acids) and leaving only polysaccharide constituents of biomass (cellulose and hemicelluloses) available to thermophiles. As such, mesophiles are better suited to simpler sugars while thermophilic fungi are well adapted for polysaccharide utilisation (Maheshwari, R. 2016). The same is true with respect to primary, secondary and tertiary colonisers with the faster growing primary colonisers rapidly consuming available simple sugars and leaving only the more complex sugars available to the secondary and tertiary colonisers. This has led to natural affinities within these groups for these different carbon sources (Kung'u 2016; Stamets 2005).

Glucose is the most widely utilisable simple sugar and occurs naturally as the repeating unit in cellulose, starches, and other carbohydrates. It can be utilised as a sole carbon and energy source but has an inhibitory effect on utilisation of other carbon sources (Canevascini et al. 1979; Griffin, DH 1996). Fructose, mannose and galactose are also widely utilisable simple sugars (Griffin, DH 1996; Sistrom & Machlis 1955). More complex sugars such as cellulose and cellobiose induce higher levels of cellulase (enzyme) activity than glucose alone (more

than 10 times higher) (Canevascini et al. 1979) resulting in more rapid substrate decomposition.

2.6.2.2 The role and sources of nitrogen in fungal growth

Fungi are non-diazotrophic (unable to fix atmospheric nitrogen N_2) and must be supplied with fixed organic (amines, amides, ammonium salts, amino acids) or inorganic nitrogen-containing compounds (ammonia (NH_3), ammonium (NH_4^+), nitric acid, urea) (Kavanagh 2005). Fungal amino acids, nucleic acids, cell wall polysaccharides, phospholipids, and vitamins can be synthesised from these sources of inorganic nitrogen through anabolic or catabolic (breakdown of organic nitrogen compounds and nitrate reduction to ammonia) reactions (Griffin, DH 1996). Nitrogen availability may be a growth limiting factor in nature (Kavanagh 2005).

Nitrate is the most commonly available form of nitrogen in soil and is widely utilisable by fungi, excluding some Chytridiomycetes, Oomycetes, and Basidiomycetes. Ammonium is the only more reduced form of nitrogen that is widely utilisable (Griffin, DH 1996) with most species growing well on ammonium (Boddy, Marchant & Read 1988; Bouras et al. 2016; Finlay, Frostegård & Sonnerfeldt 1992; France & Reid 1984). Urea, amino acids, and other organic nitrogen compounds are utilisable to varying degrees dependent on fungal species and specific compound used. A mixture of amino acids (e.g. casein hydrolysate) supports greater and more rapid growth than any single amino acid (Griffin, DH 1996).

2.6.2.3 The role of oxygen in fungal growth

Most fungi are obligate aerobes and require oxygen. Different species respond to oxygen availability in different ways however generally, growth in obligate aerobes is markedly reduced if oxygen partial pressure drops below normal atmospheric levels (Kavanagh 2005). As per Dalton's law of partial pressures, the atmospheric pressure of air (101.3kPa at sea level) is the sum of its constituents (oxygen and nitrogen) and water vapour pressure (6.3kPa at 37°C) (Peacock 1998). Since oxygen makes up 21% of dry air, the inspired normal atmospheric oxygen pressure is $0.21 \times (101.3 - 6.3) = 19.95$ kPa (minimum oxygen pressure for optimal growth in obligate aerobes).

2.6.2.4 The role of other macronutrients in fungal growth

The influence of other macronutrients on fungal growth is less well documented. Phosphorus (Repeèkienë 2001) and potassium (Anamika 2015; Nagadesi & Arya 2013) in conjunction with nitrogen have been found to improve growth rate and yield. Sulphur (Amich et al. 2013) and phosphorus (Kavanagh 2005) availability can be growth limiting factors. However, magnesium

can be omitted without adversely affecting cellulolytic activity (the hydrolysing of cellulose) (Siu & Sinden 1951).

2.7 Hyphal architecture of fungi

Chemical nutrition also affects mycelial density within the hyphal network. High concentrations of carbon can increase branching and decrease hyphal extension rate (Trinci & Collinge 1975) as can increased oxygen uptake (Lysek 1984). Increased sulphur and nitrogen concentration also yield a greater number of branches per millimetre of hypha (Larpent 1966).

An inversely proportional relationship is present between the degree of branching and hyphal extension rates due to the increased utilisation of substrate and production of inhibitory staling compounds, such as aldehydes, as hyphal density increases meaning the hyphal extension rate is insufficient to allow growth into new areas of substrate (Prosser, JI 1993). Increased growth rate results in branch formation at increased proximity to the hyphal tip (Griffin, D, Timberlake & Cheney 1974).

Cytoplasmic vesicles influence branching in septate hyphae (Webster & Weber 2007) with the accumulation of vesicles behind septa leading to lateral branch formation. This occurs during growth with vesicles produced in distal hyphal regions and transported to the tip where they fuse with existing walls and membranes to give hyphal extension (Prosser, JI & Trinci 1979; Trinci & Collinge 1975).

2.7.1 Hyphal types of the Basidiomycota

Inherent biological characteristics also influence mycelial density, an especially good example being the mono-, di- and tri- mitic hyphal networks of the Basidiomycota (Pegler 1996). Mycelium materials to date have almost exclusively utilised basidiomycetes which can be constructed of up to three distinct hyphal types (Corner 1953). The three main hyphal types are generative, binding (also known as ligative) and skeletal hyphae **(Table 2.4) (Figure 2.5)**.

Further hyphal types have since been identified (Clémençon & Emmett 2004; Pegler 1996), however many of these are intermediates between the three principle types discussed above or function in the same way. They are known as sarco-, skeleto-ligative, arboriform and gloeoplerous hyphae (Webster & Weber 2007).

The number of different hyphal types present in a species is described using the mitic system. Monomitic species comprise of only generative hyphae; dimitic species comprise of two hyphal types (usually generative and skeletal) and trimitic species comprise of all three principle hyphal types (Webster & Weber 2007).

Table 2.4. Hyphal types of the Basidiomycota. Data from Webster and Weber (2007) and Breitenbach and Kränzlin (1986).

Parameter	Generative	Binding	Skeletal
Wall thickness	Thin	Thick	Thick
Internal structure	Hollow, usually contain cytoplasm	Often solid	Often solid
Origin	Always present	Generative hyphae	Generative hyphae
Growth	Growth platform for other hyphae	Limited growth, weave between other hyphae	Spread laterally
Branching	Moderately branched	Highly branched	Unbranched, or very sparsely branched
Septa	Yes	No	No

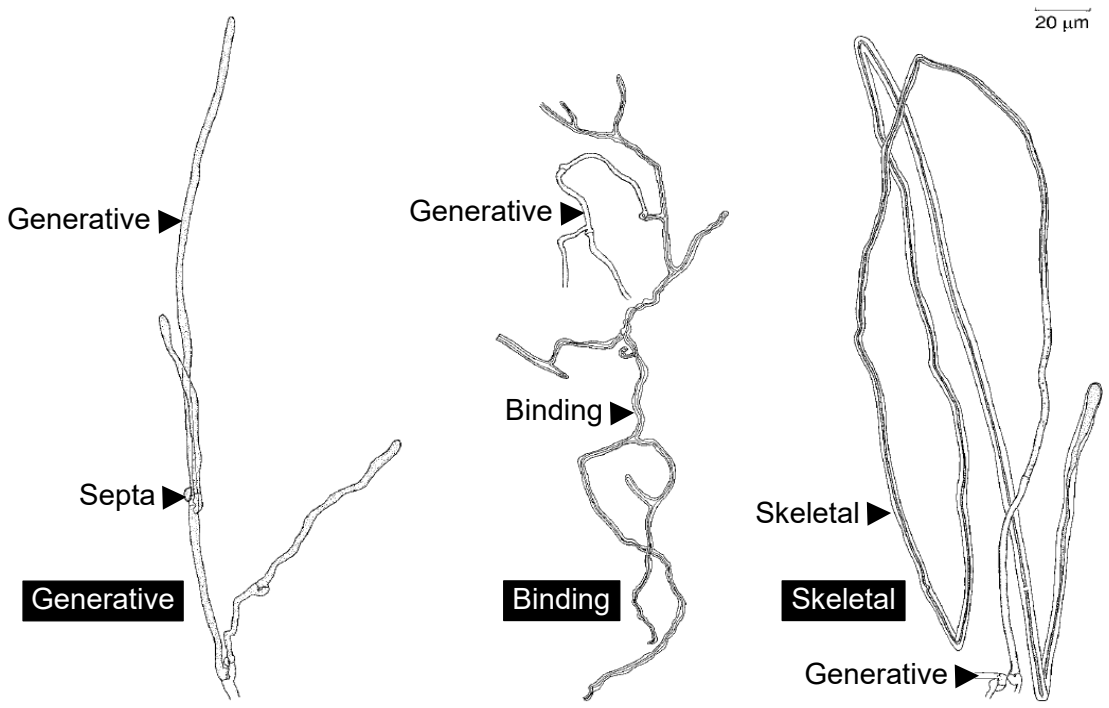

Figure 2.5. Representations of generative, binding and skeletal hyphal types from the fruiting body of *Trametes versicolor*. Reproduced with permission from Webster and Weber (2007).

2.8 Mycelium materials and their applications

Fungi are a natural and renewable source of valuable structural polymers, such as chitin and chitosan, as opposed to cellulose which is the main structural polymer in plant cell walls **(Table 2.5, Figure 2.6)** and can subsequently be used to produce materials. Chitin is a linear macromolecule composed of N-acetylglucosamine units and is also the main component of most insect and other arthropod exoskeletons (Rinaudo 2007). It is strong with a nanofibril tensile strength of ~1.6-3.0 GPa (Bamba et al. 2017) resulting from the rigid, hydrogen bonded chains of the macromolecules (Webster & Weber 2007).

Fungal cell walls are present in hyphae, which form a mycelium of hyphal filaments or fruiting bodies and comprise a thick and complex fibrous network of chitin, other polysaccharides, such as glucans, manno-proteins, chitosan, polyglucuronic acid or cellulose, and smaller quantities of proteins and glycoproteins (Bartnicki-Garcia 1968; Wessels et al. 1990). These components result in mycelium exhibiting mechanical properties typical of lignocellulosic materials, such as wood and cork **(Figure 2.7)**. However, mycelium composites comprising a fibrous matrix phase of mycelium grown through a dispersed filler phase of agricultural residue have lower densities and elastic moduli than pure mycelium and are generally classified as foams **(Figure 2.7)**. This is due to the amount of air contained within and between the often porous and loosely packed filler (Holt et al. 2012).

Table 2.5 Major fibrillar and matrix polymers of fungal groups. Data from Kavanagh (2005).

Taxonomic group	Fibrillar polymers	Matrix polymers
Ascomycetes	Chitin, β(1,3), β(1,6)-glucans	α(1,3)-glucan, galactomannoproteins
Basidiomycetes	Chitin, β(1,3), β(1,6)-glucans	α(1,3)-glucan, xylomannoproteins
Chytridiomycetes	Chitin, glucan	Glucan
Zygomycetes	Chitin, chitosan	Polyglucuronic acid

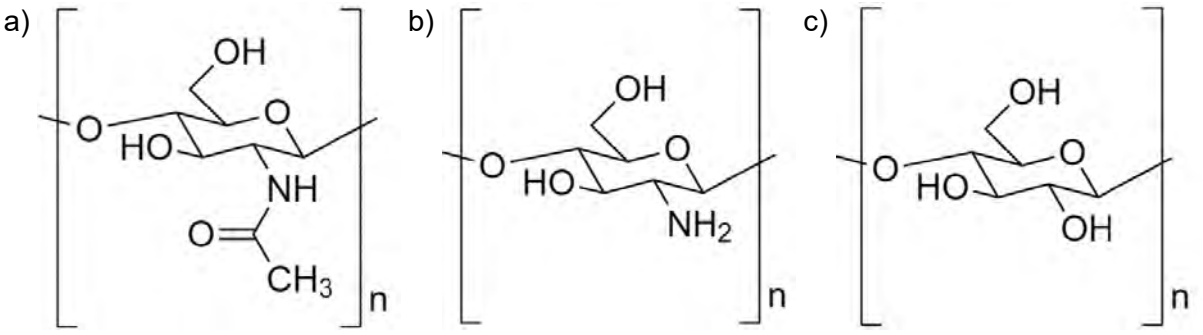

Figure 2.6. Molecular structures of (a) chitin, (b) chitosan and (c) cellulose.

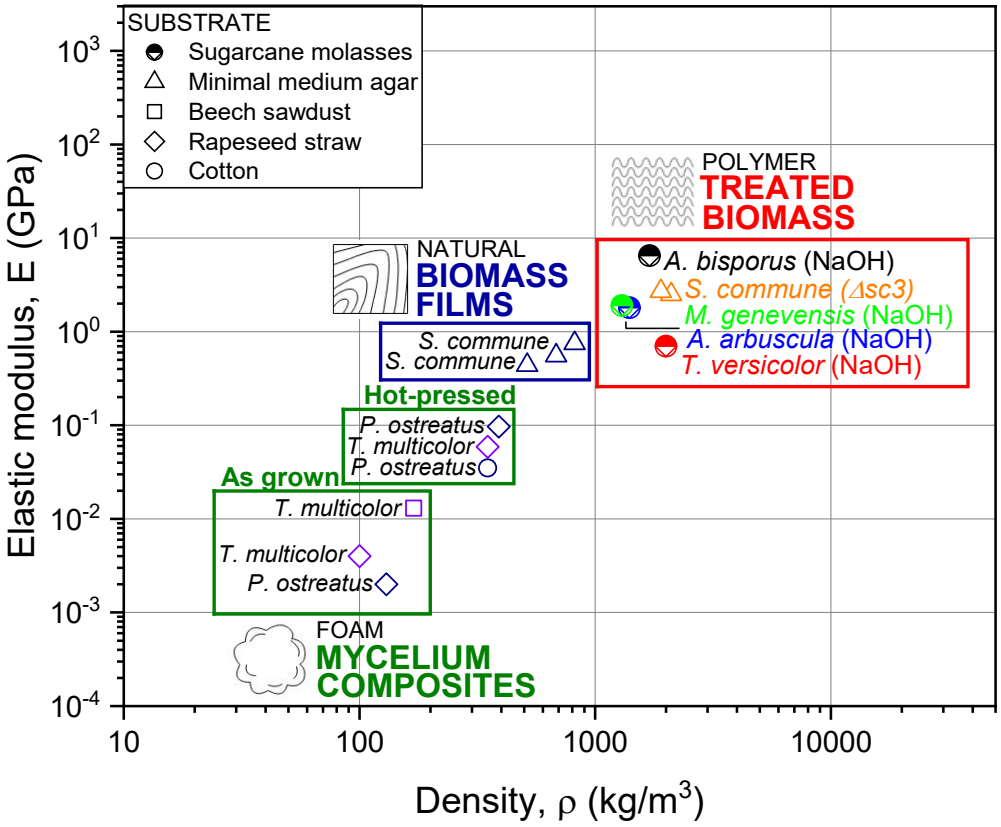

Figure 2.7. Elastic modulus (GPa) and density (kg/m^3) of as grown and hot-pressed mycelium composites grown on various substrates, natural mycelial biomass films, genetically modified (Δsc3) and NaOH treated biomass. Data from Appels et al. (2019); Appels et al. (2018); Ashby, Shercliff and Cebon (2018); Jones, M. et al. (2019). Reproduced with permission from Jones, M. et al. (2019).

2.8.1 Mycelium composites as packaging foams

Mycelium foam is a viable alternative to polystyrene foam (Holt et al. 2012; López Nava et al. 2016). Density can surpass desirable limits for light-weight foam applications depending on the substrate used by up to 20 times **(Figure 2.8)** (Holt et al. 2012), which can be problematic for packaging applications where mass-based freight costs can be significant. Compressive and flexural strength are lower than expanded polystyrene **(Figure 2.8)** but are within acceptable limits. Compressive strength is especially important for packaging applications because the primary requirement is to protect the contents from damage.

Modulus of elasticity, dimensional stability, degradation rate, flame retardance characteristics and thermal conductivity are also generally acceptable (Holt et al. 2012), although water absorption can sometimes be unacceptably high for packaging applications (114-278%) (López Nava et al. 2016).

Mycelium based foam packaging is currently produced by Ecovative Design **(Figure 2.9a)** and Sealed Air (Gunther 2013) and used by Dell to cushion large computer servers during shipping **(Figure 2.9b)** (Dell Inc. 2016). IKEA is also looking at adopting the packaging for their products (Gosden 2016).

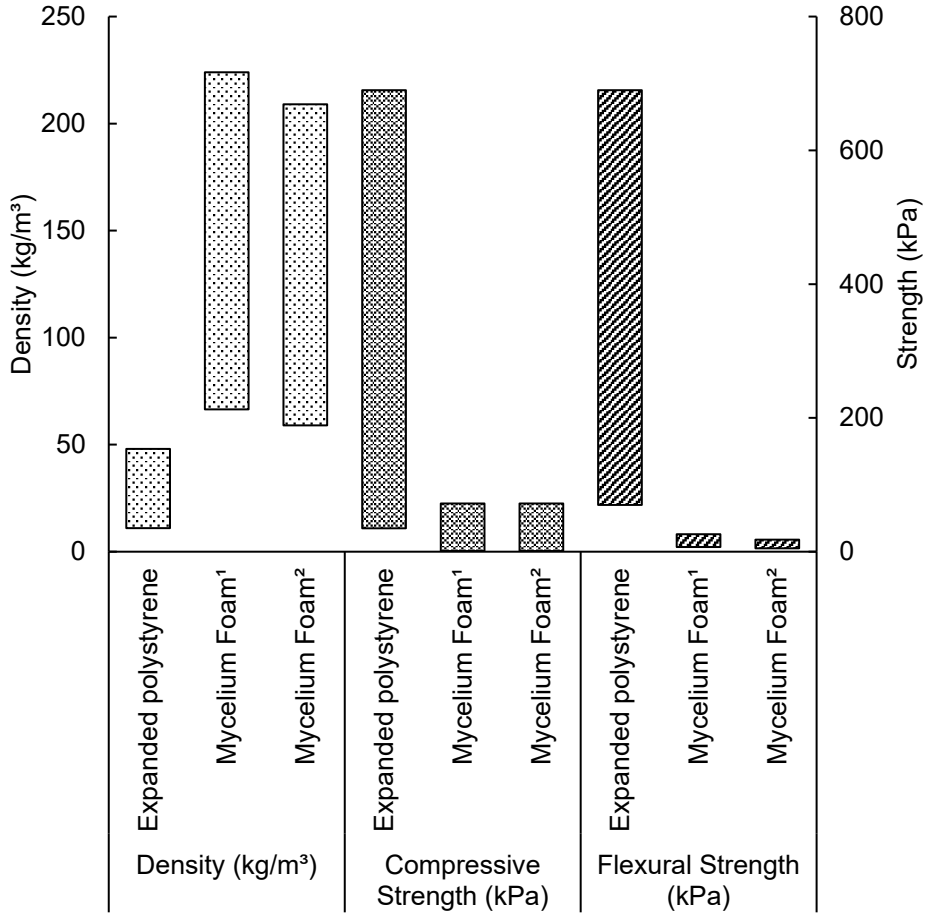

Figure 2.8. Material property range comparison between mycelium-based foams [1]*Ganoderma sp.* grown on cotton biomass, [2]*Pleurotus sp.* on wheat biomass and traditional polystyrene foams. Data from ASTM International (2004); Holt et al. (2012); López Nava et al. (2016). Reproduced with permission from Jones, M; et al. (2017).

Mycelium composites are manufactured using a low-energy, natural manufacturing process, which sequesters carbon and is one of the key advantages of these materials **(Figure 2.10)**. Raw material is required as a precursor and can realistically constitute any material that can sustain fungal growth, such as carbohydrates (Jones, M; et al. 2017; Kavanagh 2005). Low-cost lignocellulosic agricultural or forestry by-products or wastes are commonly used as fibrous substrates, such as straw, or particulate substrates, such as sawdust, to keep the cost of mycelium composites low and to facilitate waste upcycling and circular economy (Camere & Karana 2018; Jones, M et al. 2018; Pelletier et al. 2013). However, usage of these cheap,

low-grade materials as substrates, while keeping costs low and environmental sustainability high, has the unfortunate side effect of limiting fungal growth and hence compromises the material properties of the composite. Although this compromise is acceptable for production of foam-like mycelium composites, higher grade and more expensive substrates such as nutritious wheat grains and saw dusts are sometimes used when mechanical properties are a priority (Elsacker et al. 2019; Travaglini et al. 2013; Xing et al. 2018).

a) b)

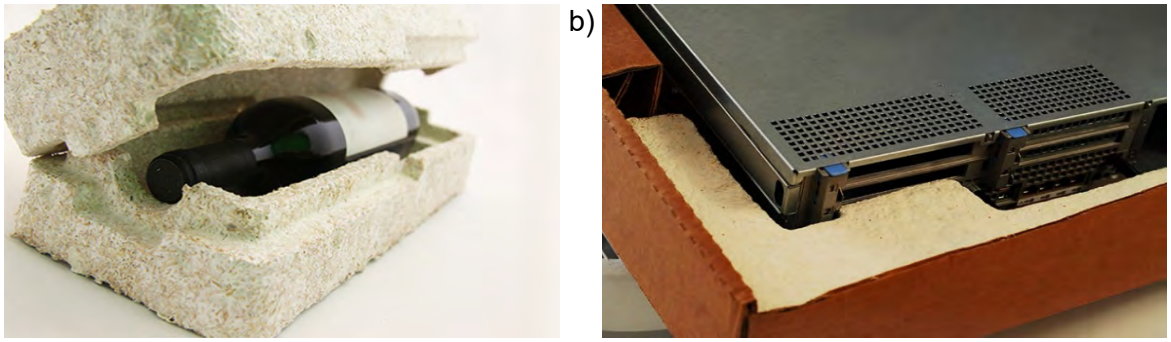

Figure 2.9. Mycelium packaging foams used for (a) wine bottle packaging and (b) Computer packaging for Dell. Reproduced with permission from Ecovative Design (2016).

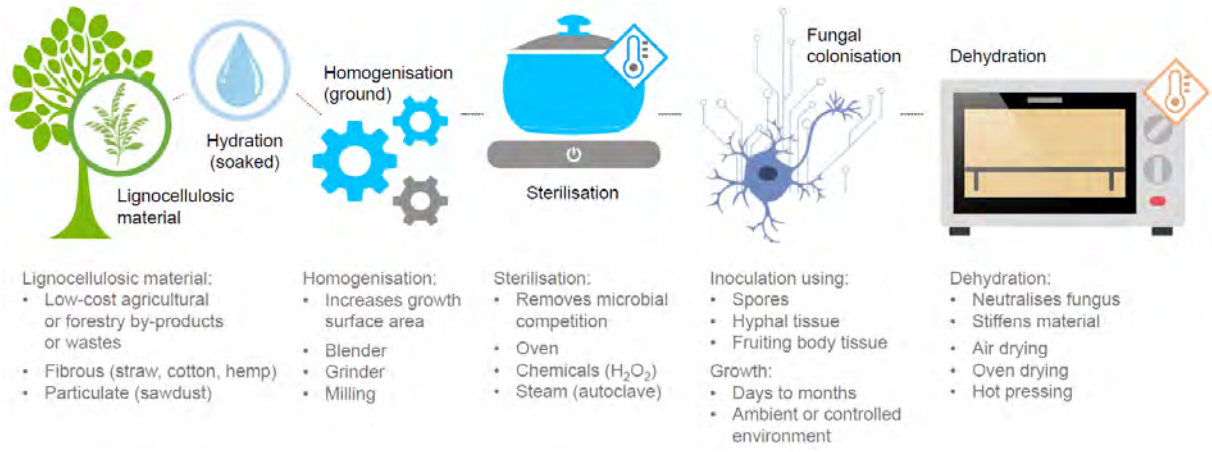

Figure 2.10. Mycelium composite manufacturing process detailing the key stages, the purpose and the possible variations in the processes utilised during each stage.

Irrespective of the grade of the material, substrates are first soaked in water to hydrate them, since moisture is very important to fungal growth, with the duration of this stage varying from substrate to substrate (Elsacker et al. 2019). Substrates such as rice hulls absorb very little moisture, making the duration of soaking less important than for inoculation media, such as wheat grains which swell considerably and require soaking durations of at least 48 h (Jones, M et al. 2018). Hydrated raw material is then homogenised to increase the growth surface area, which can be completed using low-energy mechanical processes, achievable using a kitchen blender, or grinding or milling depending on requirements and manufacturing scale

(Elsacker et al. 2019). Macerated raw material is then sterilised to remove the microbial competition of existing bacteria and fungi already present in the material. This can be completed using high temperature conditions in an oven, which has the disadvantage of drying out the substrate, or a pressure cooker or autoclave, which keeps the substrate hydrated and is hence preferred. Chemicals such as hydrogen peroxide (H_2O_2) can also be used to sterilise the substrate, but while less energy intensive than other sterilisation methods are less effective, resulting in higher contamination rates (Lelivelt 2015).

Composite assembly itself is completed using the natural and biological fungal growth process, which binds the lignocellulosic material into 3D geometries mirroring the mould the substrate is packed into (Holt et al. 2012; Jones, M; et al. 2017). The lignocellulosic substrate is inoculated by introducing and evenly dispersing 10-32 wt% of any element of fungal biomass, such as spores in a liquid solution or hyphal or fruiting body tissue grown on a nutrient rich substrate, such as wheat grains, to the lignocellulosic material contained within the mould (Jones, M; et al. 2017; McIntyre et al. 2012). Spores have the advantage of being very easily and evenly dispersed throughout the substrate and provide many initial growth points, but require a nutrient-rich substrate, initially struggling to grow on low-grade materials. Grain- or sawdust-based inocula mitigate this problem by supplying a nutrient-rich substrate to support initial growth, which can then spread to lower-grade substrates, but provide fewer initial growth points and are more difficult to evenly disperse (Jones, M et al. 2018).

Following inoculation, moulds can be stored under ambient conditions or in a temperature-controlled environment at ~25-27°C for a growth period of days to months depending on the fungal species and substrate used and the degree of bonding desired (Griffin, DH 1996; Jones, M; et al. 2017). Ambient conditions are obviously cheaper and more energy efficient to maintain but will result in slower growth than environments of elevated temperature. Following the growth period, the composite materials can be removed from the moulds and hot-pressed, oven or air dried to dehydrate the material and neutralise the fungus. This simultaneously ensures that it cannot grow further or spread while stiffening the composite material (McIntyre et al. 2012). Hot-pressing and oven drying are favoured by industry as they are the fastest dehydration processes, with hot-pressing also consolidating the material and resulting in higher mechanical properties. Fully processed mycelium composite materials are fully biodegradable and comprise ~95 wt% lignocellulosic material bound using ~5 wt% fungal mycelium for nutrient rich substrates (estimated based on an ergosterol concentration of ~870 ppm, corresponding to 50 mg of biomass for every 1 g wheat grains grown over 7 d) (Jones, M et al. 2019).

2.8.2 Mycelium composites as construction materials

Foam-like physical and mechanical properties make mycelium composites suitable for non-structural construction applications including insulation materials and door cores **(Figure 2.11a).** Mycelium-based acoustic insulation foams are already commercially available in Europe and the United States **(Figure 2.11b)**, with significant advances also being made in the development of mycelium-based thermal insulation foams and highly flexible polymer-like materials **(Figure 2.11c)**. Introduction of a soy-based resin within mycelium composites can extend their use to semi-structural applications, such as panelling, flooring, cabinetry and other furnishings **(Figure 2.11d)**. However, despite the vast potential of these materials, which have been commercially available for over a decade, their adoption has been slow. Dell uses mycelium foams for packaging of business servers and IKEA has also expressed interest in adopting mycelium-based packaging (Dell Inc. 2016; Gosden 2016).

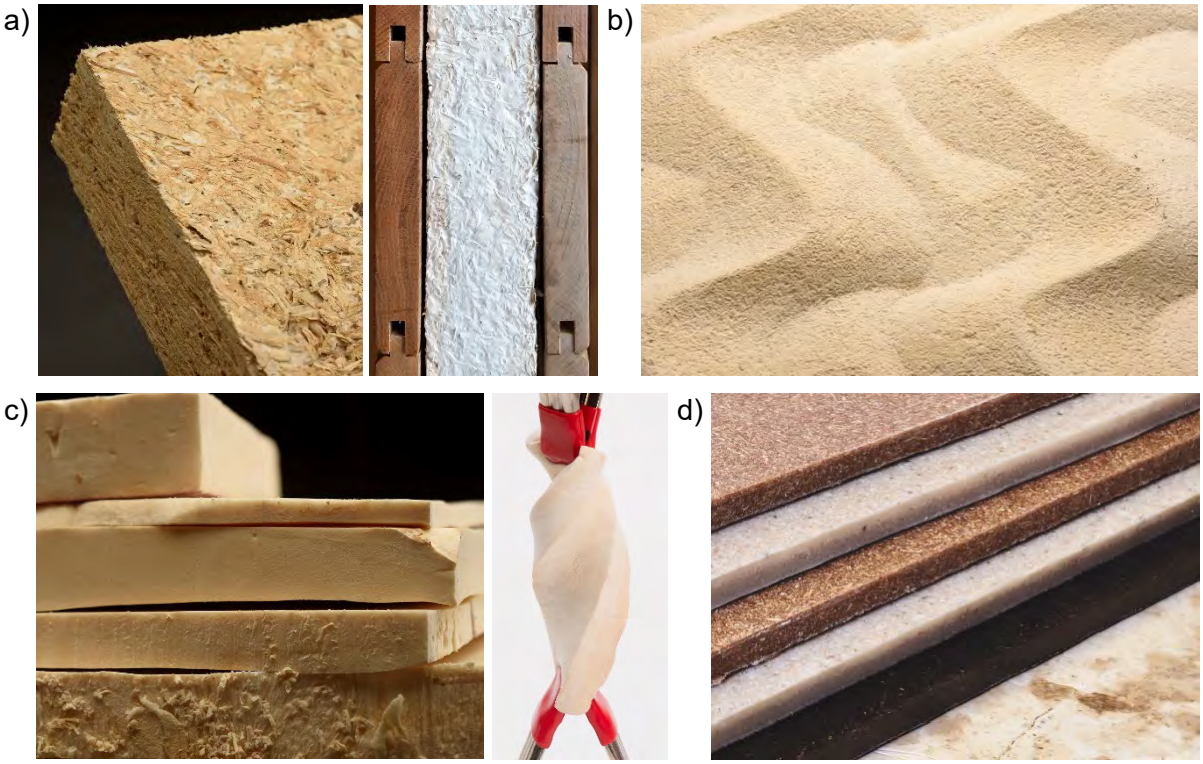

Figure 2.11. Commercial mycelium composite construction materials as a) particleboard replacements for wall panelling and door cores, b) acoustic foams, c) flexible insulation foams and d) bio-resin infused laminate flooring. Images courtesy of Ecovative Design LLC (Green Island, USA).

Nevertheless, for the most part mycelium materials remain a predominantly underutilised niche product favoured by a select group of artists and designers, used to produce everything from furnishings such as chairs and lampshades to artistic structures, such as Philip Ross' "Mycotectural Alpha" tea house and the 12 m "Hy-Fi" organic compostable tower, comprising

over 10,000 bricks, showcased by the New York Museum of Modern Art in 2014 (Austin 2013; Fisher 2010; Jones, M; et al. 2017; Rajagopal 2014; Superflux 2014). This underutilisation could be the result of a patent monopoly on mycelium materials resulting in a lack of industrial commercial viability, a lack of trust in this new material platform for applications beyond packaging or a lack of awareness among the general public and industry as to the existence of this material. Interest is however growing in mycelium materials with companies now active in the United States, Italy, Indonesia, the Netherlands and research spanning the United States, Italy, Belgium, the Netherlands, Australia, Austria and Switzerland.

2.8.3 Mycelium composites in architectural structures

Mycelium composite bricks have been used for the construction of architectural structures since 2009, when *Ganoderma lucidum* and sawdust were used to produce the 500 brick "Mycotectural Alpha" teahouse **(Figure 2.12a)** (Fisher 2010) commissioned by Düsseldorf Kunsthalle for the 25th anniversary of their "Eat Art" exhibition where it was displayed before being boiled and served to museum guests as a herbal tea (MycoWorks 2014a).

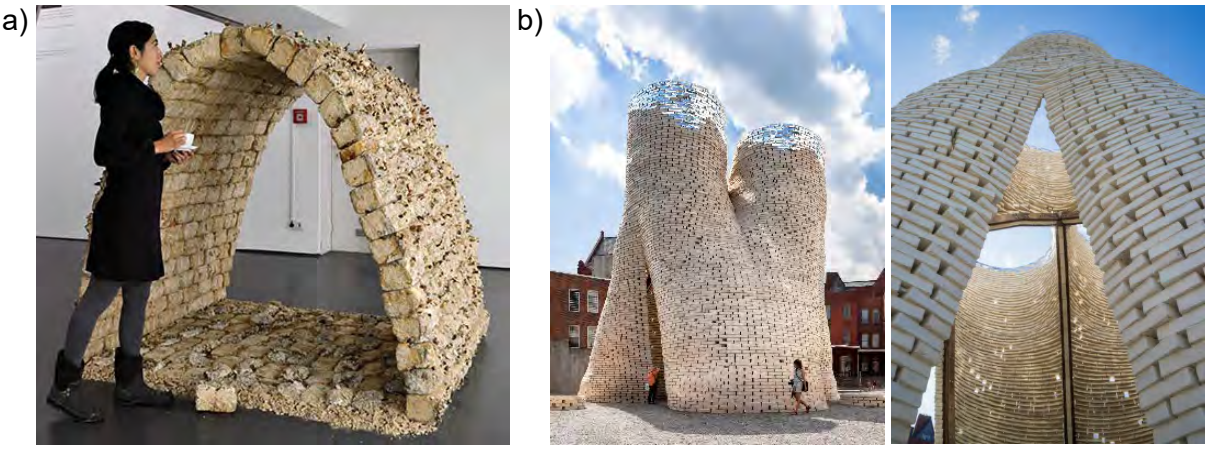

Figure 2.12. Examples of mycelium-based construction materials. (a) Mycotectural Alpha teahouse, (b) Hy-Fi organic compostable tower (c) Myco board. Reproduced with permission from LafargeHolcim Foundation, Zurich, Switzerland and Kris Graves Photography, Ecovative Design (2016); MycoWorks (2014b).

The largest mycelium structure produced was the "Hy-Fi" organic compostable tower constructed in 2014 which won the New York Museum of Modern Art's Young Architects Program that year **(Figure 2.12b)**. It exceeded 12 m in height and comprised more than 10,000 bricks produced from shredded corn stalks and an undisclosed species of fungi (Rajagopal 2014). A range of other similar mycelium composite brick-based structures also exist in museums and galleries across the globe (Austin 2013; Bulatov 2009; Superflux 2014) providing examples of innovative industrial design and architecture.

2.8.4 Other potential applications of mycelium composites

Although mycelium packaging foams, construction and architectural materials are the best-known examples of mycelium materials (Everett-Green 2014; Fisher 2010; Gosden 2016; Gunther 2013; Watson 2016) many other potential applications, have been proposed.

Mycelium materials are as geometrically versatile as plastics and are viable for the manufacture of products with simple to complex design geometry and uniqueness. Mycelial growth will digest organic feedstocks irrespective of arrangement with remarkable precision. Simple shapes **(Figure 2.13a)** are easily achieved using basic moulds (Fondazione PLART 2014). More complex geometries can be produced using 3D printed moulds, which can also be incorporated into the structure through the use of digestible polylactic acid or potato starch external scaffolding **(Figure 2.13b)** (Klarenbeek 2014).

a) b)

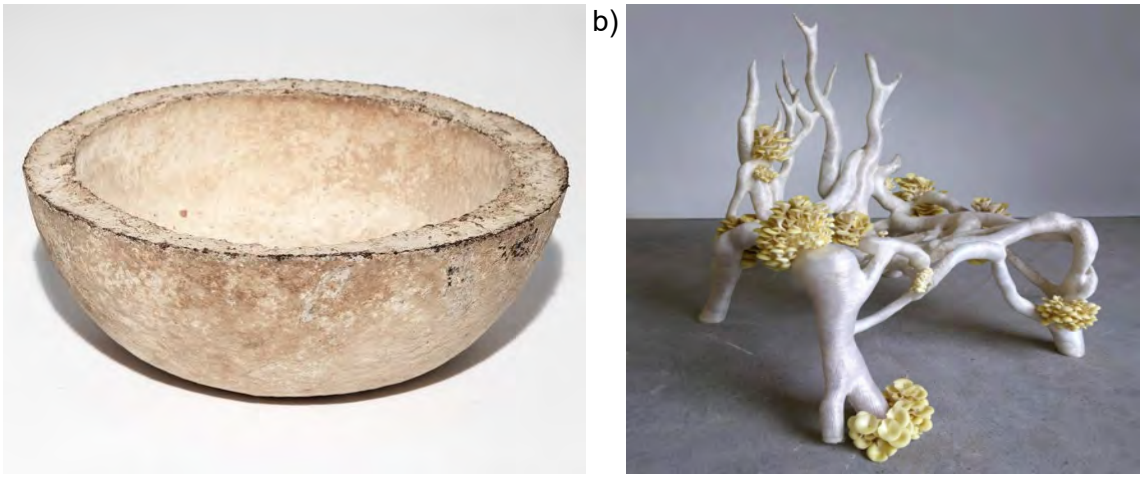

Figure 2.13. Complex geometries achievable with mycelium materials. (a) Mycelium bowl produced using a simple mould, (b) mycelium chair with external scaffold printed from potato starch and interior comprising of mycelium. Reproduced with permission from Maurizio Montalti and Eric Klarenbeek (2017).

Ford also filed several patents for production of complex vehicle parts from mycelium, which can be mass produced using injection moulding (shaping of heated media through its injection into a mould) **(Figure 2.14a)**. Pins, hinges or fasteners can be incorporated into parts seamlessly via mycelial growth **(Figure 2.14b)** and parts comprising of both structural and foam sections with density variation between outer and inner sections achieved using different species and substrate blends (Kalisz & Rocco 2011, 2012a, 2012b, 2012c; Kalisz et al. 2012; Rocco & Kalisz 2012a, 2012b, 2012c). However, the foam-like mechanical properties of mycelium make the potential of mycelium composites for automotive components other than insulation questionable.

Paper sheets can also be produced from Mucorales (Zygomycota) mycelial pulp plasticized with a small amount of bleached jack pine kraft fibres (7%). This combination exhibits a bursting strength only slightly less than paper sheets comprising entirely of wood fibres and much better fire resistance. They also have a comparable tensile strength to traditional paper, high gloss, good printing characteristics and approximately four times more stretch (Conkey, Van Horn & Shema 1957). Zygomycota hyphae also exhibit an absorption ability comparable to many commercial superabsorbent and inhibit bacterial growth, odour formation, and fungal yeast growth. The porous absorbent structures are suitable for applications such as wound and hygiene products or filtering aids (Edebo 2002).

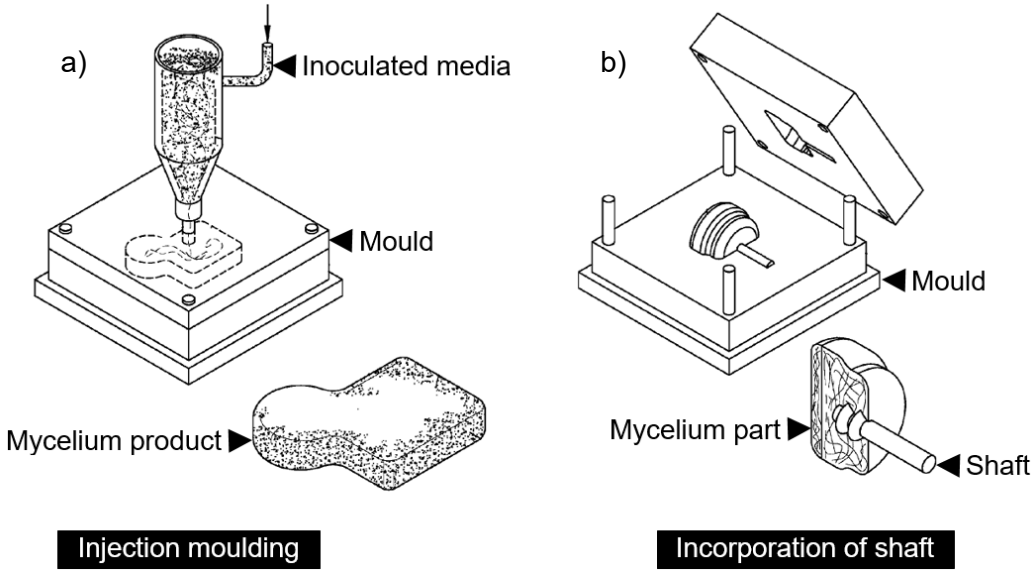

Figure 2.14. Production of complex vehicle parts using mycelium. (a) Injection moulding of a vehicle part comprised of mycelium, (b) incorporation of a shaft in part via mycelial growth. Representation from U.S. Patent 8,227,225 Rocco and Kalisz (2012c). Reproduced with permission from Jones, M; et al. (2017).

2.9 Engineering mycelium composite properties

2.9.1 Influence of the mycelium binder on composite mechanical performance

The mycelium constituent of mycelium composites is often blamed for their limited mechanical performance (Jiang et al. 2019; Travaglini et al. 2013). However, recent studies investigating chitin-glucan extracts derived from mycelium have found the matrix phase to be quite strong (up to 25 MPa tensile strength) (Jones, M. et al. 2019), suggesting that insufficient fungal growth density limiting matrix quantity and matrix-filler interface are more likely to be responsible for limited mechanical performance. The species of fungus utilised as the matrix phase to bind dispersed agricultural filler into mycelium composites affects growth density and degree of interfacial bonding at the mycelium-substrate interface, which varies significantly by

species and substrate (Jones, M, Huynh & John 2018), and does appear to affect the mechanical properties of the material.

The mycelial matrix network structure also affects the mechanical properties of mycelium composites. Although the tensile properties of fungal hyphae used in fermentation have been studied, with estimated hyphal ultimate tensile strengths of up to 24 MPa and elastic moduli of up to 140 MPa, the mechanical properties of wood-rot fungi hyphae are not well characterised (Li, ZJ et al. 2002; Stocks, Stuart M. & Thomas, Colin R. 2001; Stocks, S. M. & Thomas, C. R. 2001). Generative hyphae alone (monomitic hyphal systems), which are hollow and contain cytoplasm, are suggested to provide limited mechanical performance, with binding hyphae (dimitic and trimitic hyphal systems) responsible for material strength (Bayer & McIntyre 2012, 2015). Although there is no literature confirming this, it is true that mycelium composites utilising trimitic species, such as *T. versicolor* or *multicolor* exhibit higher tensile (0.04 MPa) and flexural strengths (0.22 MPa) than monomitic species, such as *P. ostreatus* (0.01 MPa tensile strength, 0.06 MPa flexural strength) when grown on rapeseed straw (Appels et al. 2019). *T. versicolor* also has a higher compressive strength than *P. ostreatus* when grown on hemp (0.26 MPa compared with 0.19 MPa) (Lelivelt 2015). However, the fact that the presence of structural polymers, such as chitin and chitosan, is limited to the thin hyphal cell wall, which also contains polysaccharides (e.g. galactose, mannose and fucose), phosphate, proteins, lipids and mineral salts (Bartnicki-Garcia 1968; Jones, M. et al. 2019) makes the importance of the hyphal structure questionable, with mycelial biomass (matrix) quantity likely to more greatly influence mechanical performance.

2.9.2 Influence of the substrate filler on composite mechanical performance

The physical and mechanical properties of as-grown mycelium composites are often dependent on the substrate, which acts as the dispersed filler phase of the composite material. As-grown composites typically have a density ranging from 60-300 kg/m^3, with composites containing an agricultural by-product filler phase, such as bast fibers or straw, having lower densities (60-130 kg/m^3) than composites containing forestry by-product substrates, such as sawdust (87-300 kg/m^3) **(Figure 2.15a)**. Only limited data is available on the mechanical properties of mycelium composites for the various groups of substrates.

Tensile properties are among the best characterised material properties of mycelium composites. Reported tensile properties vary significantly between studies for sawdust substrates (0.05-0.18 MPa) but sawdust does appear to be associated with higher tensile strengths than straw substrates (0.01-0.04 MPa) **(Figure 2.15b)**. However, the tensile properties of as-grown sawdust-based mycelium composites do not correlate with the mechanical properties of the substrates themselves. Clear, straight grained Beech wood

sections have a similar or higher tension perpendicular to grain strength (5-7 MPa) than red oak (5.5 MPa) (Buschow et al. 2001; Green, Winandy & Kretschmann 1999), while as-grown composites using a beech sawdust substrate have much lower tensile strength (0.05 MPa) than composites with a red oak sawdust substrate filler (0.18 MPa). This indicates that the tensile properties of as-grown mycelium composites are more heavily influenced by failure of the mycelium matrix than the dispersed substrate filler and that substrates must be nutrient rich, rather than strong, to establish a dense mycelium network and maximise mycelium composite tensile properties. Some lower-grade substrate materials, such as agricultural by-products and wastes, which are attractive due to their low cost, typically lack optimal fungal nutrients including easily utilisable simple sugars (e.g. fructose, glucose, sucrose) and instead contain more complex carbon sources (e.g. cellulose and lignin) (Faruk et al. 2012). While white rot fungi are suitable for these lignocellulosic substrates, some agricultural by-products, like rice hulls, also contain large quantities of minerals, such as silica, which limit fungal growth (Jones, M et al. 2019). Reduced fungal growth on these less easily utilised substrates compromises interfacial bonding between hyphae and organic matter and adversely affects the tensile strength of the mycelium matrix phase (He et al. 2014; Jones, M et al. 2019; Travaglini et al. 2013).

Unfortunately, inconsistent and limited data is available concerning the compressive properties of mycelium composites. Elsacker et al. (2019) found that the compressive moduli of as-grown composites utilising fibrous hemp and flax hurd substrates were higher than those of particulate pine shavings (0.64 and 0.73 MPa compared to 0.14 MPa, respectively), however their study only tested to 70-80% strain and subsequently did not assess compressive strength. Conversely, Ghazvinian et al. (2019) assessed the compressive strength of mycelium composites grown on a white oak sawdust and a wheat straw substrate, finding that the sawdust particulate substrate had a much higher compressive strength than the fibrous straw (1.1 MPa compared to 0.17 MPa, respectively), but did not assess stiffness **(Figure 2.15c)**. Only Travaglini et al. (2013) assessed both compressive modulus (1 MPa) and strength (0.49 MPa) of mycelium composites with a red oak sawdust substrate. Despite significant gaps in the characterisation of mycelium composites under compressive loading conditions, it seems likely that particulate substrates, such as sawdust, provide higher compressive properties to the composite than fibrous substrates such as straw. The compressive properties of porous materials are strongly correlated with their porosity and pore size, with increased porosity associated with reduced mechanical performance (Ashby, Shercliff & Cebon 2018; Xia et al. 2013). This suggests that the compressive performance of as-grown composites would depend on the compressive properties and porosity of the filler, the composite itself and the degree to which the fungus digests the filler, increasing its porosity

in the process (Kavanagh 2005). However, the compressive properties of as-grown composites have been found to be largely independent of the particle size of the filler phase (Islam et al. 2018).

Particle geometry also had no significant effect on the flexural strength of mycelium composites, which when subjected to bending experience a maximum tensile stress at one surface, to zero at the midplane, to a maximum compressive stress at the opposite surface (Roylance 2000). Although fibrous geometries should improve the tensile properties of the surfaces if aligned in the loading direction, and hence the flexural properties of the composite overall (Chand & Fahim 2008), the significant fungal growth on air exposed surfaces likely results in enzymatic fiber degradation and damage, compromising the beneficial effects of the fibers present (Choudhury 2017). Air transmission is critical for fungal growth with mycelial density highest at the air exposed surfaces and lowest in the core, where depending on the porosity of the filler there could be limited or even no growth unless the filler is artificially aerated (Jones, M; et al. 2018; Webster & Weber 2007). The lack of improvement in the flexural properties of mycelium composites incorporating fibrous surfaces was supported by the poor flexural properties of cotton fiber-based composites (1 MPa and 0.05 MPa, respectively), although fibrous straw-based composites did exhibit better flexural stiffness (1-3 MPa) and strength (0.06-0.22 MPa) **(Figure 2.15d)**. Conversely, a particulate Beech sawdust substrate resulted in much higher flexural modulus (9 MPa) and strength (0.29 MPa), which was most likely the result of its nutrient composition promoting the formation of a dense, continuous matrix phase on the air exposed surface of the composite. The importance of the substrate nutrient profile to composite flexural properties is supported by results obtained by Tudryn et al. (2018), who found that increased nutrition at homogenization increased specific flexural stress and specific flexural modulus, due to the presence of a larger, more continuous hyphal matrix.

In general, the value of any given substrate in reinforcing the composite appears to be more heavily governed by the nutrient profile of the substrate with more nutritious substrates promoting more fungal growth and bonding, since failure always occurs in the mycelium matrix rather than the substrate filler irrespective of loading condition. This unfortunately makes cheap, low-grade agricultural and forestry residues often only suitable for the manufacture of foam-like mycelium composites, unless further processing techniques, such as hot or cold pressing, resin infusion or hybridisation are utilised to improve mechanical performance (Jones, M et al. 2019).

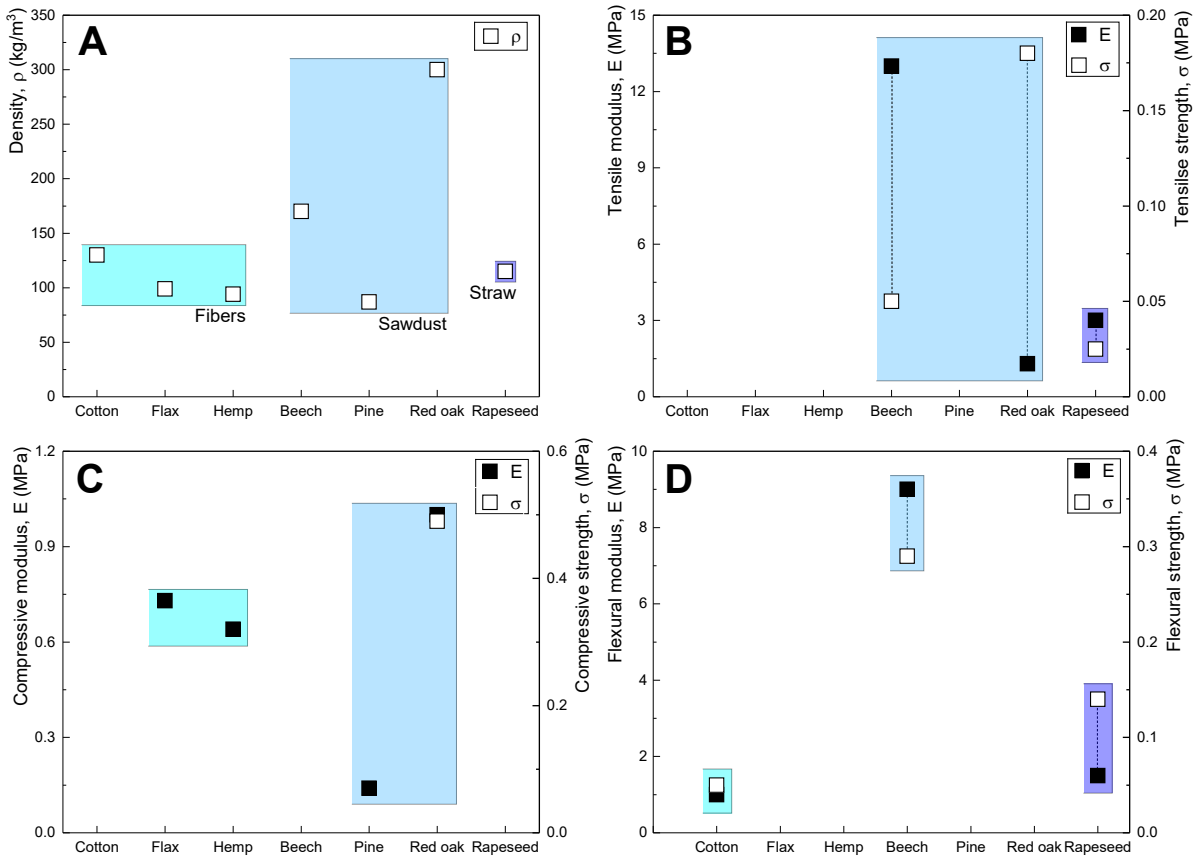

Figure 2.15. Ranges for a) density, b) tensile, c) compressive and d) flexural material properties of as-grown mycelium composites comprising fiber- (cotton, flax, hemp), wood sawdust- (beech, pine, red oak) and straw-based (rapeseed) dispersed filler phases (substrates). Data from (Appels et al. 2019; Elsacker et al. 2019; Travaglini et al. 2013)

2.9.3 Hot and cold pressing to improve mycelium composite mechanical properties

The mechanical properties of mycelium composites can be significantly improved using physical processing, such as cold or hot pressing. This is expected since pressing consolidates composite materials, reduces the porosity of the material and increases the material density in general (Dai, Yu & Zhou 2007). Pressing also helps to reorientate fibers horizontally in the plane of the panel (Butterfield et al. 1992) and panel thickness reduction during pressing results in considerable and intimate fiber contact between the walls of the fibers at points of overlap (Carvalho & Costa 1998). In mycelium composites produced using *P. ostreatus* grown on rapeseed straw, cold pressing was associated with a significant improvement in tensile strength (0.01 MPa to 0.03 MPa) and a higher elastic modulus (2 MPa to 9 MPa) (Appels et al. 2019). It also significantly improved the flexural properties of the composites with higher flexural strengths (0.06 MPa to 0.21 MPa) and moduli (1 MPa to 15 MPa) achieved post cold pressing (Appels et al. 2019). Even greater improvements in

mechanical performance could be achieved through hot pressing. The main mechanisms associated with hot pressing are the phase change (evaporation) of water, compaction and stress relaxation of the material via conduction and convection and mass transfer occurring as a result of gaseous and bound water diffusion and hydrodynamic flow of gaseous and liquid water (Carvalho & Costa 1998). This occurs via diffusion of steam through the network or voids in fibers, diffusion of water through cellular walls or as water or steam flow through cell membranes and voids (Stamm 1964). Temperature, gas pressure and moisture content all influence the heat and mass transfer through the thickness, impacting plasticization and compaction of the material (Carvalho & Costa 1998). Tensile properties of hot-pressed *T. multicolor* and *P. ostreatus* composites grown on rapeseed straw were significantly higher than as-grown samples, with strength increases of 0.04 MPa to 0.15 MPa and 0.01 to 0.24 MPa, respectively, and elastic moduli increases of 4 MPa to 59 MPa and 2 MPa to 97 MPa, respectively (Appels et al. 2019). Hot pressing also improved the flexural strength of *T. multicolor* and *P. ostreatus* composites grown on rapeseed straw (0.22 MPa to 0.86 MPa and 0.06-0.87 MPa, respectively) and the flexural moduli of the composites (3 MPa to 80 MPa and 1 MPa to 72 MPa, respectively) (Appels et al. 2019). Both cold and hot pressing were associated with significant reductions in the strain to failure of the samples, resulting from the reduced moisture content of the composites following pressing, which would otherwise act as a plasticiser (Sombatsompop & Chaochanchaikul 2004). Cold pressing of *P. ostreatus* grown on rapeseed straw reduced their strain to failure (2.8% to 0.8%), while hot pressing of *P. ostreatus* and *T. multicolor* grown on rapeseed straw was associated with lower strain to failure (2.8% to 0.7% and 4.7% to 0.9%, respectively) (Appels et al. 2019).

2.9.4 Resin infused mycelium composites and sandwich structures

Mycelium composites are being increasingly used as low-density cores bonded between two thin laminate facings called skins in sandwich structures (Jiang et al. 2019; Jiang et al. 2017; Wong, Arumugasamy & Mustapha 2019). Skins can be any sheet material, from metals such as aluminium (Wong, Arumugasamy & Mustapha 2019), to natural materials such as woven jute, flax or cellulose (Jiang et al. 2017). These skins provide resistance against in-plane and lateral bending loads, while the mycelium core holds the skins in place and resists shear loads (Allen 2013; Kim & Christensen 2000; Vinson 2018). The improvement in mechanical performance that a sandwich structure provides is subsequently dependent on the loading conditions. Several recent studies have examined the use of mycelium composites in sandwich structures but any significant improvement in mechanical performance has yet to be reported, making the value of mycelium sandwich composites debatable. Wong, Arumugasamy and Mustapha (2019) recently reported unsurprisingly that a sandwich

structure comprising a mycelium composite sandwiched between aluminium alloy laminates had no better compressive properties than a normal mycelium composite and while skins provide varying degrees of improvement to the flexural strength of sandwich structures with a mycelium composite core, similar results can be achieved using simpler methods. For example, mycelium composite sandwich structures comprising jute, flax or cellulose textile reinforcement skins have effective flexural moduli of 4.6-6.5 MPa (Jiang et al. 2017), with similar performance achievable by simply varying the substrate of the mycelium composite itself (flexural moduli of 1-9 MPa) or hot-pressing (flexural moduli of 34-80 MPa) (Appels et al. 2019).

The most significant improvement in the mechanical performance of sandwich structures with mycelium composite core and a woven jute, flax or cellulose skin is associated with resin infusion. This is also hardly surprising or even novel since the use of a resin infusion in a mycelium composite effectively replaces the mycelium matrix with a stronger resin one. The difference between a resin-infused mycelium composite and a natural composite comprising resin and agricultural residue or fibers is then unclear as is the sustainability of such a composite, which lacks a natural biological manufacturing process. Jiang et al. (2019) reported that soy-based resin infused over 30-120 s saturates the entire material and is responsible for an improvement in core and skin shear yield and ultimate stress and sandwich flexural strength. Core shear yield stress and ultimate strength were highest for resin-infused samples reinforced with flax skins (up to 128.9 yield and 135.3 kPa ultimate stress) (Jiang et al. 2019). This was due to the increased mycelial growth on these skins, since the nutrient profile of flax stimulates more fungal growth than jute or cellulose, facilitating greater branching networks and interfacial bonding. The resin infusion unsurprisingly provided a significant improvement compared to flax sandwich composites lacking resin (core shear yield and ultimate stresses of up to 29.5 kPa and 38.7 kPa, respectively) (Jiang et al. 2017). The most common failure mode of the sandwich structures was tensile failure of the core material (mycelium-bound agricultural waste), indicating that this was still the weakest part of the structure. Effective flexural strengths of up to 30 MPa for resin-infused flax reinforced sandwich structures were achieved, which are significantly higher than flax-reinforced sandwich structures lacking resin (up to 6 MPa) (Jiang et al. 2017) and low-density polyethylene (LDPE) (14 MPa) but lower than acrylonitrile butadiene styrene (ABS) (75 MPa) (MatWeb 2018). The sandwich structures (410 kg/m^3) also had lower densities than LDPE (920 kg/m^3) and ABS (1100 kg/m^3) and were suggested as potential replacements for LDPE and ABS interior panels in automotive and sports products.

2.9.5 Hybridisation of mycelium composites to improve mechanical performance

The mechanical properties of mycelium composites, comprising a network of fungal mycelium grown through a substrate, can be improved through hybridisation with small quantities of synthetic rubbers, such as styrene-butadiene rubber, or natural fibers, such as cellulose nanofibrils. While these improvements are arguably predictable when hybridising a weak mycelium composite with stronger synthetic or natural polymers, the small volume fractions required to do so, and the thresholds associated with mechanical property improvement are interesting. Styrene-butadiene rubber negligibly affects fungal growth performance in quantities up to 5 vol% with only a slight delay in germination and no effect on the growth rate (He et al. 2014). Larger volumes of the latex hinder growth (8 vol%) or kill the fungus (10 vol%) since the latex reduces the void volume within the composite, hindering the oxygen transmission and absorption required for fungal growth (He et al. 2014; Kavanagh 2005). Mycelium composites produced using cotton seed hulls and *P. ostreatus* had a compressive strength of 177 kPa, which could be almost doubled with the addition of 5 vol% styrene-butadiene rubber (343 kPa) (He et al. 2014). This is due to the void volume reduction and volume density increase (181 kg/m^3 to 225 kg/m^3) associated with the inclusion of the latex (He et al. 2014). Even smaller quantities of nanocellulose can be used to improve mechanical performance with increases in flexural strength (1.5 MPa to 3.5 MPa) and modulus (220 MPa to 575 MPa) of hybrid materials produced by cold and hot pressing wood particles with mycelium growing on them hybridised with 2.5 wt% nanocellulose (Sun, W et al. 2019). Notably, further increases in nanocellulose content did not provide any significant improvement in mechanical performance suggesting a low threshold nanocellulose density required for improvement of adhesion of particles and subsequent flexural properties (Theng et al. 2015). These improvements in mechanical performance at low nanocellulose concentrations could make hybridisation using nanocellulose a viable method for improving the mechanical performance of mycelium composites. However, in some cases, such as hybridisation using latex, the small improvement in mechanical performance attained post hybridisation may well be offset by the additional costs, processing and reduced environmental sustainability associated with a latex-mycelium composite material.

2.9.6 Thermal conductivity properties of mycelium composites for insulation applications

Mycelium composites containing high-performance natural insulators such as straw and hemp fibers bound using mycelial growth have both low densities (57-99 kg/m^3) and thermal conductivities (0.04-0.08 W/m·K) **(Figure 2.16)**. This makes them excellent insulation materials, able to compete with conventional commercial thermal insulation products, such as

glass wool (57 kg/m^3, 0.04 W/m·K) and extruded polystyrene (XPS, 34 kg/m^3, 0.03 W/m·K) (Papadopoulos 2005) in addition to other natural insulators including sheep wool (18 kg/m^3, 0.05 W/m·K) and kenaf (105 kg/m^3, 0.04 W/m·K) (Asdrubali, D'Alessandro & Schiavoni 2015).

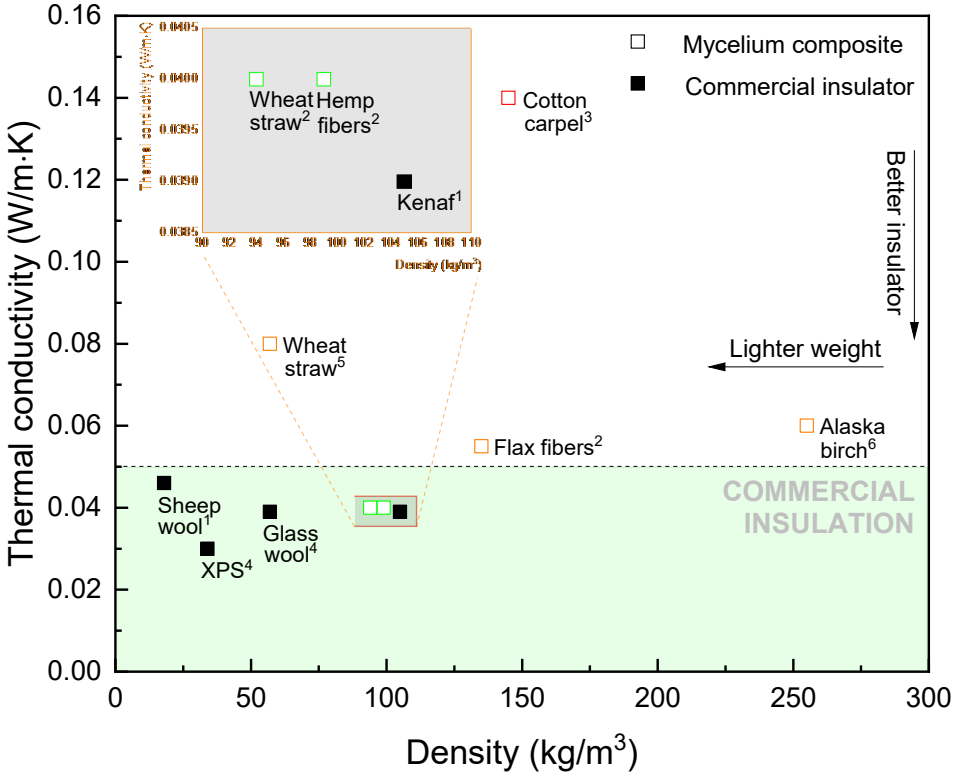

Figure 2.16. Density (kg/m^3) and thermal conductivity (W/m·K) of mycelium composites produced using various substrates (coloured square markers, colours: green = low thermal conductivity, orange = medium thermal conductivity, red = high thermal conductivity) and commercial insulation materials, such as glass wool, sheep wool, XPS foam and kenaf (black solid square markers). Data from [1]Asdrubali, D'Alessandro and Schiavoni (2015), [2]Elsacker et al. (2019), [3]Holt et al. (2012), [4]Papadopoulos (2005), [5]Xing et al. (2018) and [6]Yang, Z et al. (2017). Density and thermal conductivity values are averages based on the available data sets.

Lower thermal conductivities are associated with better insulation materials and are primarily influenced by material density and to a lesser extent moisture content (Collet & Prétot 2014; Jerman et al. 2013; Uysal et al. 2004). For example, a 67% increase in density will result in a 54% increase in thermal conductivity in hemp concretes (a bio-composite material comprising hemp shive and lime), while a 90% increase in relative humidity (completely dry to 90% RH) will only result in a thermal conductivity rise of 15-20% (Collet & Prétot 2014). The strong correlation between material density and thermal conductivity is the result of the presence of large quantities of dry air, which has a very low thermal conductivity (26.2×10^{-3} W/m·K at 0.1

MPa, 300 K) (Kadoya, Matsunaga & Nagashima 1985), present in low density materials. These large quantities of air mean that low density materials are often excellent thermal insulators.

Straw and hemp are well-established natural thermal insulation materials, which derive their useful insulation properties from their porous structure and the low bulk density of the bundled fibers, leading to trapping of a large amount of air between the fibres in the insulation (Kymäläinen & Sjöberg 2008; Wall et al. 2012). Their thermal insulation properties vary primarily based on the density of the pack, moisture content and fiber type (Bainbridge 1986). Mycelium composites utilising a wheat straw filler have reported thermal conductivities of 0.04 W/m·K (Elsacker et al. 2019) and 0.08 W/m·K (Xing et al. 2018), respectively, although the former value seems questionable given that it is associated with a higher density composite than the latter (94 kg/m^3 compared to 57 kg/m^3) and is significantly lower than the conductivity of straw bales themselves (0.07-0.08 W/m·K) (Pruteanu 2010). Hemp fiber-based mycelium composites were also reported to have thermal conductivities (0.04 W/m·K) (Elsacker et al. 2019) significantly lower than hemp concretes (0.1 W/m·K) (Collet & Prétot 2014). Even mycelium composites produced using substrates exhibiting poorer insulation properties, such as those incorporating a cotton carpel substrate (0.10-0.18 W/m·K) (Holt et al. 2012) have thermal conductivity values comparable with gypsum (0.17 W/m·K), high density hardboard (0.15 W/m·K), plywood (0.12 W/m·K), and both hardwoods (0.16 W/m·K) and softwoods (0.12 W/m·K) (Bergman et al. 2011). This makes mycelium composites a viable low-cost and environmentally sustainable alternative to conventional commercial building insulation materials.

2.9.7 Acoustic properties of mycelium and its composites for noise absorption

Mycelium itself is an excellent acoustic absorber, exhibiting strong inherent low frequency absorption (< 1500 Hz) and outperforming cork and commercial ceiling tiles in road noise attenuation (Pelletier et al. 2019). This non-typical property means that mycelium foam can be used in conjunction with other materials to improve their low frequency absorption properties. Alternatively, mycelium composite comprising mycelium-bound agricultural residue can also provide broader range acoustic absorption with 70-75% absorption or better achievable for perceived road noise (Pelletier et al. 2013). A-weighted decibels express the relative loudness of sounds in air as perceived by the human ear, with the magnitude of low frequency sounds reduced to correlate with the lessened sensitivity of human ears at low frequencies (<1000 Hz), while higher frequency sounds are left uncorrected (St. Pierre, Maguire & Automotive 2004). This allows interpretation of the perceived loudness of domestic noises, such as dogs barking (500-1500 Hz), human speech (85-255 Hz) and street noise (700-1300 Hz) to humans

(Feinberg et al. 2005; Owren, Berkowitz & Bachorowski 2007; Pongrácz, Molnár & Miklósi 2006; Sandberg 2003).

Acoustic absorbers are typically fibrous, porous or reactive resonators with examples including nonwovens, fibrous glass, mineral wools, felt and foams (Bell & Bell 1994; Seddeq 2009). Absorbers convert the mechanical motion of air molecules travelling in sound waves into low-grade heat, which prevents sound accumulation in enclosed spaces and reduces reflected noise strength (Bell & Bell 1994). All mycelium composites tested were associated with lower perceptual road noise (45.5-60 dBa) than traditional reference absorbers, such as commercial ceiling tiles (61 dBa), urethane foam board (64 dBa) and plywood (65 dBa) **(Figure 2.17a, b)**. The best individual fillers for acoustic absorption were rice straw (52 dBa), hemp pith (53 dBa), flax shive (53.5), sorghum fiber (54 dBa) and switchgrass (55 dBa) **(Figure 2.17a)**. However, even better acoustic absorption could be achieved through mixtures of fillers (50-50 wt%) with the best combinations being rice straw-sorghum fiber (45.5 dBa), rice straw-cotton bur fiber (47 dBa) and sorghum fiber-switchgrass (47 dBa) **(Figure 2.17b)**.

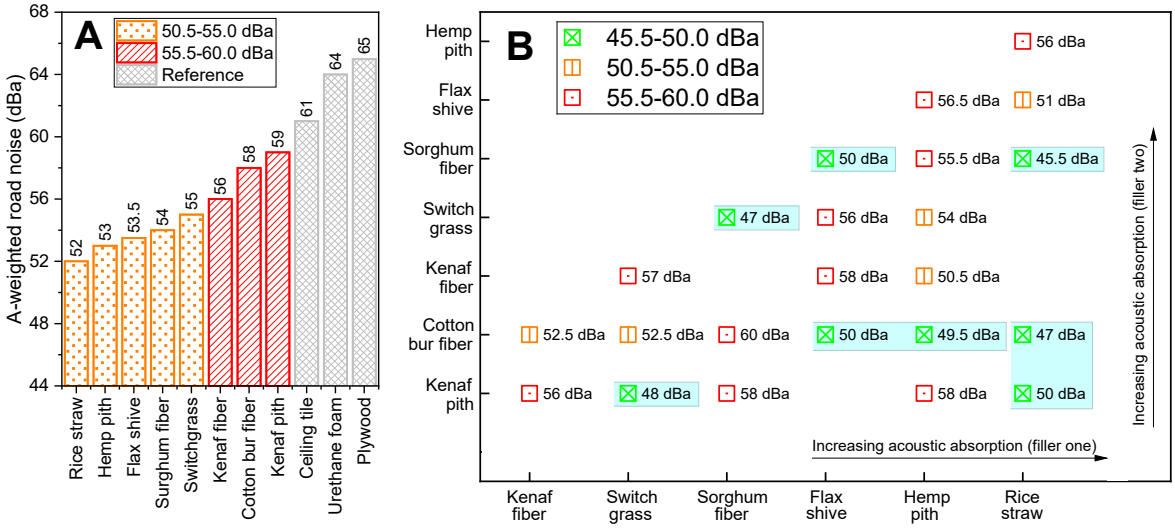

Figure 2.17. A-weighted perceptual road noise for mycelium composites comprising a) individual substrates compared to traditional acoustic absorbers and b) 50-50 wt% mixtures of selected fillers. Colours: green cross: 45.5-50.0 dBa, orange line: 50.5-55.0 dBa, red dot: 55.5-60.0 dBa, grey: traditional reference absorbers. Data is based on an integrated A-weighted response with typical road noise excitation (1000 Hz) rounded to the nearest 0.5 dBa from Pelletier et al. (2013).

The excellent acoustic absorption properties of mycelium composites can be attributed to their porous, fibrous nature. Impedance and propagation constants used to describe the acoustic properties of materials are greatly influenced by the air flow resistance of a material, with higher airflow resistance associated with greater acoustic absorption (Ren & Jacobsen 1993).

The fibers in mycelium composites act as frictional elements, resisting acoustic wave motion and decreasing its amplitude as the sound waves attempt to move through the tortuous passages of the material and are converted to heat in the process (Hemond 1983). Thin fibers provide better acoustic absorption since they can move more easily and the greater number of fibers per unit volume results in more tortuous paths and greater air flow resistance (Koizumi, Tsujiuchi & Adachi 2002; Sun, F, Banks-Lee & Peng 1993). Surface pore concentration and geometry are also important with porosity necessary for sound waves to enter the material and tortuosity required for efficient damping (Seddeq 2009). Porosity and airflow resistance affect the height and width of sound wave peaks, while tortuosity influences the high frequency acoustic properties of porous materials (Seddeq 2009). Less dense, more open structures absorb low frequency sound in nonwoven fibrous materials (500 Hz), while denser structures are better for frequencies higher than 2000 Hz (Koizumi, Tsujiuchi & Adachi 2002). Compression of a material causes a reduction in acoustic absorption, resulting primarily from the reduction in thickness (Castagnede et al. 2000), and as such mycelium composites being utilised as acoustic absorbers should not be hot or cold pressed.

2.9.8 Water absorption properties of mycelium composites

One of the largest problems limiting the use of mycelium composites in materials science applications is their tendency to absorb large quantities of water quickly. Mycelium composites are typically hydroscopic, increasing in weight by ~40-580 wt% when in contact with water for 48-192 h (Appels et al. 2019; Elsacker et al. 2019; Holt et al. 2012; López Nava et al. 2016; Sun, W et al. 2019). The strong water absorption affinity of mycelium composites is the result of their typically cellulosic filler constituents, which contain numerous accessible hydroxyl groups (Zabihzadeh 2009), and the hydrophilic porous mycelium matrix and biologically-derived filler phases, which promote wicking (Chung, Suidan & Venosa 2011; Li, MM et al. 2013; Wei, Liang & McDonald 2015). Air dried mycelium composites incorporating a fibrous substrate of rapeseed straw or cotton bur fiber take up ~530-550 wt% moisture within 48 h when in contact with water **(Figure 2.18a)**. Although such a massive water uptake may seem a major problem some construction applications of mycelium composites, such as acoustic or thermal insulation, are fortunately for internal or dry locations not exposed to the weather, mitigating this otherwise significant problem. The most rapid weight increase occurs within the first 3 h, with an increase of ~220 wt% for both rapeseed straw- and cotton bur fiber-based composites **(Figure 2.18b)**. Water uptake then continues at a reduced rate for up to 48 h, before slowing and then stopping as the material reaches saturation (~580 wt%) **(Figure 2.18a)**. Rapeseed straw contains large quantities of cellulose (48.5 wt%) and pentosans (17 wt%) (Housseinpour et al. 2010), while cotton bur fibers predominantly comprise cellulose (98 wt% with <0.5 wt% pentosan) (Pigman 2012). Pentosans are water soluble polymers

composed of pentoses and are known to increase the amount of water absorbed by bread, while the hydroxyl groups in cellulose attract water molecules (Michniewicz, Biliaderis & Bushuk 1992; Zabihzadeh 2009). In contrast, mycelium composites comprising a particulate substrate, such as beech sawdust, are much less susceptible to water uptake with a weight increase of 23 wt% over 3 h contact with water, which slowly increases to 43 wt% over 192 h **(Figure 2.18a)**. Beech sawdust contains 26 wt% hydrophobic lignin in addition to its 48 wt% cellulose (Ruxanda, Alice Teacă & Spiridon 2008), which in conjunction with its higher material density and the smaller void content of the fine particulate filler, is likely to account for its reduced water uptake.

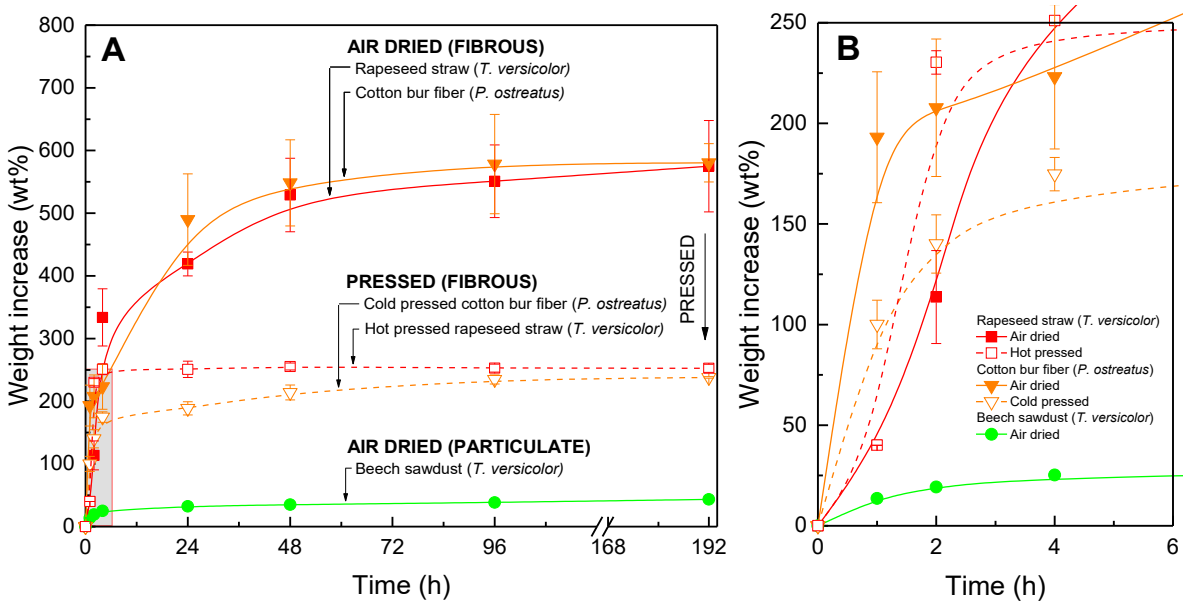

Figure 2.18. Weight increase (wt%) of air dried (solid lines) and hot and cold pressed (dotted lines) fibrous (*P. ostreatus* on cotton bur, orange, *T. versicolor* on rapeseed straw, red) and particulate (*T. versicolor* on beech sawdust, green) mycelium composite materials resulting from continuous contact with a water surface over (a) 192 h with (b) the most rapid absorption period (0-6 h) magnified. Data from Appels et al. (2019).

Hot or cold pressed mycelium composites also experience less than half the water uptake of air-dried composites (~250 wt% compared to ~580 wt%) **(Figure 2.18a)**. This is most likely because pressed materials have smaller void volumes, which impedes capillary action and hence water uptake (Dai, Yu & Zhou 2007). Cold pressed mycelium composites are slightly less absorbent (214 wt% after 48 h, 238 wt% after 192 h) than hot pressed composites (247 wt% after 48 h, 252 wt% after 192 h), achieving saturation faster than the drier hot-pressed composites since they are initially more hydrated. Heat treatment of lignocellulosic polysaccharide components, such as the depolymerisation of hemicelluloses at temperatures above 160°C, can reduce water absorption due to the reduced number of free hydroxyl groups

present (Boonstra & Tjeerdsma 2006; Hong 1984). However, since hot pressing primarily heat treats the mycelium-rich surfaces it is likely that any improvement in water absorption properties based on depolymerisation of hemicelluloses would only be realised through more uniform temperature application affecting the lignocellulosic core, such as oven drying. In addition to using particulate fillers and pressing, many bio-based coatings, such as polyfurfuryl alcohol resin (PFA), have also shown promise in reducing water absorption in natural fiber composites (Mokhothu & John 2017) and could be applied to mycelium composites to improve their water resistance.

2.9.9 Termite resistance of mycelium composites

Termites are a significant threat to residential and commercial buildings in many countries around the world with annual global estimates of structural damage to buildings from termites running into the billions of dollars (Logan & Buckley 1991). They are most prolific in Africa, Asia, South America and Australia but are also prominent in North America where they cause in excess of $US 100 million of damage each year to houses and businesses in New Orleans alone (Guillebeau, Hinkle & Roberts 2008). Mycelium composites have no termite resistant properties of their own, comprising completely biological and predominantly lignocellulosic material. However, termite resistance of mycelium composites can be improved through substrate selection and application of natural or commercial termiticides (Bajwa et al. 2017). Hemp-based mycelium composites have high termite-resistance, exhibiting high termite mortality rates (directly related to efficacy or repellence by termite treatments) and low mass losses resulting from termite infestation over 4 weeks (16-53 wt%). Kenaf-based composites exhibit moderate to complete termite mortality but are associated with the highest mass losses of any untreated mycelium composite (43-62 wt%). Corn-based composites have low termite resistance with slight to moderate termite mortality and 42-43% mass loss. The most effective natural termiticides are guayule resin (flavonoid, cinnamic, terpenoids, and p-anisic acid bioactive compounds) (Bultman, Chen & Schloman 1998) and vetiver oil (α- and β-vetivone bioactive compounds) (Zhu et al. 2001). A single coating of these oils provides complete termite mortality and are associated with mass losses of 18-28 wt% and 16-27 wt%, respectively, for treated mycelium composites. This mass loss is significantly less than untreated composites (42-62 wt%) and an untreated southern yellow pine (*Pinus taeda*) reference sample (80 wt%). Commercial borax termiticide provides less termite protection than the natural oils with 28-40 wt% mass loss resulting from termite infestation. The fungal species *Daedaleopsis confragosa*, *Ganoderma resinaceum* and *T. versicolor* have no significantly different effects on termite repellence or mass loss for mycelium composites. Other degradation parameters of mycelium composites, such as mould and weathering resistance remain undocumented.

2.10 Conclusions

Plastic waste management problems and increasingly stringent international regulations on embodied carbon makes the replacement of many traditionally synthetic materials with more environmentally responsible materials necessary. Mycelium composites utilise biological growth rather than expensive energy intensive manufacturing processes, require only low-cost organic waste as feedstock, can grow to fill complex geometries and have no end of life disposal costs since they are inherently biodegradable. This makes them economically and environmentally viable alternatives to many synthetic materials. The mechanical properties of mycelium are derived from the presence of structural polymers such as chitin, which is present in their cell walls, and endows mycelium with mechanical properties resembling natural materials (e.g. wood and cork). Conversely, mycelium composites, which contain porous and loosely packed filler, exhibit foam-like mechanical properties. Current applications of mycelium composites are restricted to packaging and some architectural and construction applications. However, mycelium composite research completed to date, while limited in quantity, details significant variation in performance and material potential attributed to yet unexplored biological variables and processing techniques. This in conjunction with recently demonstrated viability across a variety of applications suggests further investigation in this research area is warranted to unleash the full potential of mycelium as a material of the future.

Chapter 3

Summary of Publications: Results and Discussion

3.1 Publications A and B: introduction to the research area

Mycelium materials have attracted increasing academic and commercial interest over the past decade based on their economical and environmentally sustainable nature in addition to their low density, low thermal conductivity, high acoustic absorption and hydrophobic surface properties (Abhijith, Ashok & Rejeesh 2018; Appels et al. 2019; Haneef et al. 2017; Holt et al. 2012; Islam et al. 2017; Karana et al. 2018; Pelletier et al. 2013; Travaglini et al. 2013). Mycelium is the vegetative growth of filamentous fungi that bonds organic matter through a network of hyphal micro-filaments in a natural biological process that can be exploited to produce materials. These filaments contain fungal chitin, a renewable, easily isolated and abundant alternative to crustacean chitin, with a rigid structure that is associated with more pliable branched β-glucan or chitosan. This combination provides a native nanocomposite architecture of both strong and tough fiber networks, which can be extracted to obtain polymers or used as a natural binder in composite materials (Hassainia, Satha & Boufi 2018; Nawawi, W 2016; Nawawi, WanLee, et al. 2019). Mycelium materials are very cost-effective because mycelium can grow on and bind agricultural and industrial waste materials, e.g. rice hulls, sugarcane bagasse, wheat, barley straw and glass fines, that have little or no commercial value (DP CleanTech 2013; Sustainability Victoria 2014) and convert them into high-value composite materials or polymer extracts for multiple applications. The key incentives for the use of mycelium materials are their low cost, low environmental impact and carbon footprint, low density, reduced energy consumption and most importantly, their biodegradability (Abhijith, Ashok & Rejeesh 2018; Arifin & Yusuf 2013; Haneef et al. 2017). Furthermore, the wide variety of utilizable substrates coupled with technological improvements in processing means that manufacturers can customize mycelium materials to meet specific non-structural, semi-structural and functional requirements through tuning of properties such as fire resistance, tensile strength and wettability. The key challenges associated with working with this material class are their slow and undocumented biological manufacturing process, the unknown properties of the mycelium binder and the poor mechanical properties associated with mycelium materials incorporating waste- or by-product-based fillers. These challenges will be addressed in this thesis.

3.2 Publication C: manufacturing optimisation based on mycelium growth characteristic influence on hyphal extension and growth density

The extended manufacturing time associated with the mycelium matrix, the natural binder utilised in mycelium composites or polymer extracts, is derived from their slow biological growth and is a key limitation of mycelium materials when competing with rapidly producible synthetic materials. Growth density is also important as it is associated with the composite matrix volume fraction, interfacial bonding and chitin-glucan or chitin-chitosan structural polymer ratio and yields.

Historically, species selection in literature concerning mycelium composites has been almost completely random. Common, easily available species used in commercial mushroom cultivation are almost exclusively used, despite the presence of up to 5.1 million different species with significantly different growth rates, densities and structural polymers (Blackwell 2011; Kavanagh 2005). Uninformed arbitrary species selection compromises the mycelium matrix and will affect composite manufacturing time and material properties.

Hyphal characteristics vary significantly by species, which is the most influential growth performance factor in conjunction with environmental conditions and chemical nutrition. This study established a methodology for assessing the growth performance of fungi for mycelium material applications and investigated the effect of inherent species characteristics such as hyphal type, pathogenicity and taxonomic and association-based classification systems on hyphal extension rate and growth density. This was completed on groups of commonly used and non-traditional species, since there are too many fungi in existence to assess individually. Top performing species could then be identified and retained for use in the remaining research.

Daily radial growth measurement on solid media was an economical, efficient and effective method for assessing the hyphal extension rate of fungi. Trends in individual hyphal extension acceleration and deceleration were visible over as little as 7 days, and growth performance comparison between fungi was also possible for this period. This two-dimensional metric, combined with a three-dimensional growth density assessment derived from fungi grown in liquid media, comprehensively described fungal growth potential and could be used to delimit low-performing species at an early stage in development and help identify high-performing species.

Growth performance varied significantly and arbitrarily meaning that for optimal species selection, fungi should ideally be assessed on an individual basis to establish their suitability for composite manufacturing applications. Hyphal growth involves cell wall extension and

biosynthesis of wall components utilising chitin synthase isozymes with different kinetic parameters (K_M values) that vary in type and number by species (Carlile, M, Watkinson & Gooday 2001). Hyphal extension rate is also related to hyphal extension zone and colony peripheral growth zone dimensions which vary not only by species but by strain (Carlile, M, Watkinson & Gooday 2001).

Hyphal extension rate and branching were inversely related for basidiomycetes in this study, with dimitic species containing sparsely branched or unbranched skeletal hyphae exhibiting much larger hyphal extension rates than dimitic species containing highly branched binding hyphae **(Figure 3.1)**. However, an inverse relationship between growth density and branching of basidiomycetes was absent. Hyphal extension rate and branching are also inversely related for ascomycetes (Robinson & Park 1966) and the hyphal growth unit (G), which is a property of the mycelium that is mathematically linked to other hyphal and colony growth parameters (Kotov & Reshetnikov 1990), increases as branching becomes sparser (Prosser, J 1995). The effect of branching on dry weight (growth density) for basidiomycetes has not been extensively studied, but branching is known to increase the surface area of colonies and mediate hyphal fusion events, which aid nutrient assimilation and exchange between hyphae of the same colony (Harris 2008).

Pathogenic fungi, which rapidly invade and colonise host material utilising a combination of specialised toxins, cellular process subversion and mechanical force (Kavanagh 2005; Sexton & Howlett 2006) absent in the less aggressive saprotrophic species, were expected to exhibit improved growth performance. However, no consistent or significant growth performance improvement was present in these fungi. Pathogenic species are inherently more dangerous than non-pathogenic species with their infectious nature making them more difficult to render inert and hence safe materials harder to produce. Significant risks are also present during their manufacture for either plants or animals (including humans) exposed to the manufacturing process. As such, with no significant growth performance improvement present among these species, they should be excluded from mycelium composite manufacturing.

It is not known how variation in the substrate and environmental conditions would affect the growth performance of the fungi investigated as this varies on a species-specific basis. However, water-, animal- and soil- associated fungi provided no notable growth performance improvement and since most composite substrates are starch, cellulose or lignin-based (Haneef et al. 2017; Holt et al. 2012; López Nava et al. 2016; Pelletier et al. 2013; Travaglini et al. 2013), wood-associated fungi would be enzymatically best suited to digest these complex carbon sources.

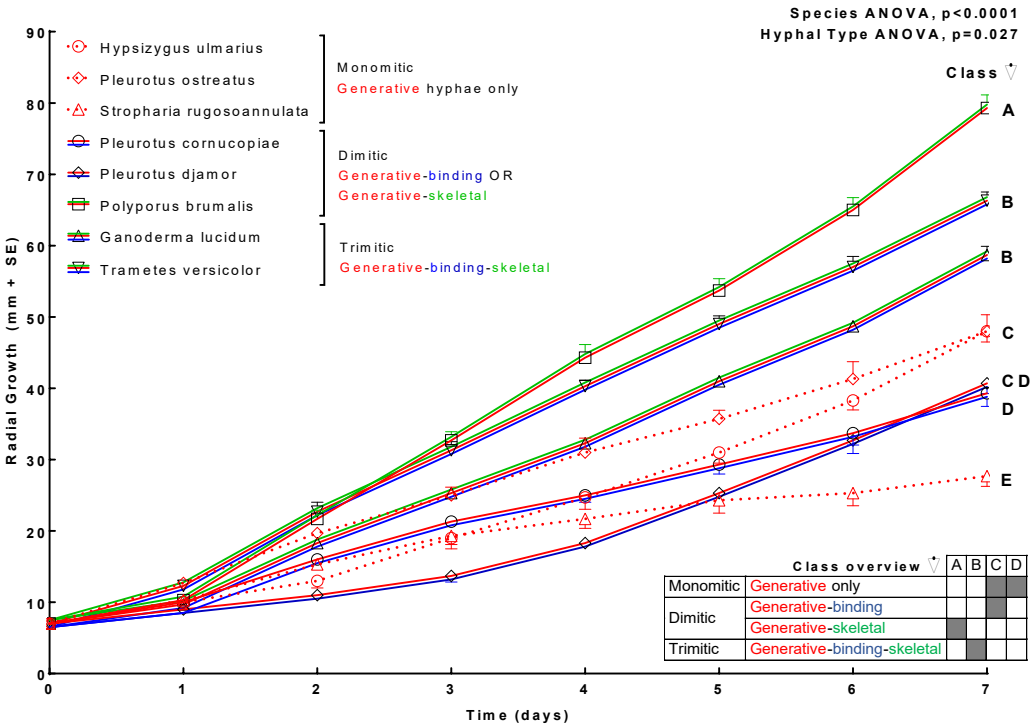

Figure 3.1. Hyphal extension rate measured as radial growth (mm + SE) over 7 days for monomitic (generative only, red dotted), dimitic generative-binding (red-blue), dimitic generative-skeletal (red-green) and trimitic (generative-binding-skeletal hyphae, red-blue-green) fungi. Error bars indicate standard error within triplicate sets. Class categories were letters of comparison based on Tukey's family error rate at p≤0.05 for species dependent ANOVA. Reproduced with permission from Jones, M, Huynh and John (2018).

T. versicolour and *P. brumalis* were the highest performing wood-associated fungi, achieving growth performance within the top 20% of species overall **(Figure 3.2)**. This supports their use in mycelium composites applications over other traditionally popular species including *G. lucidum* (Haneef et al. 2017; Holt et al. 2012; Travaglini et al. 2013) and *P. ostreatus* (Haneef et al. 2017; He et al. 2014; López Nava et al. 2016). *A. arbuscula* and *M. genevensis* were other high performing species associated with water and soil, respectively. Although not suitable for mycelium composite applications utilising lignocellulosic substrates, these species would provide excellent growth properties for chitin-β-glucan or chitin-chitosan polymer extract production. *S. vasiformis* and *B. cinerea* were excluded due to their pathogenic nature.

This study found the assessment of hyphal extension rate measured as radial growth and growth density measured as dry weight to be a simple, effective and resource conservative method for evaluating the viability of fungi for mycelium composite manufacturing. Hyphal types present, pathogenicity and traditional classification structures did not significantly affect growth with fungal growth performance highly variable both in terms of hyphal extension rate and growth density. However, growth performance can be rapidly and inexpensively

determined through methods such as those outlined in this study. This makes initial growth performance screening prior to more expensive and complicated testing possible. *T. versicolour* and *P. brumalis* were the most suitable species for mycelium composite applications based on growth performance and enzymatic compatibility with typical mycelium composite substrates. This supports their use over other traditionally used species such as *P. ostreatus* and *G. lucidum*. Other high performing species included *A. arbuscula* and *M. genevensis*, which could be used in fungal structural polymer generation.

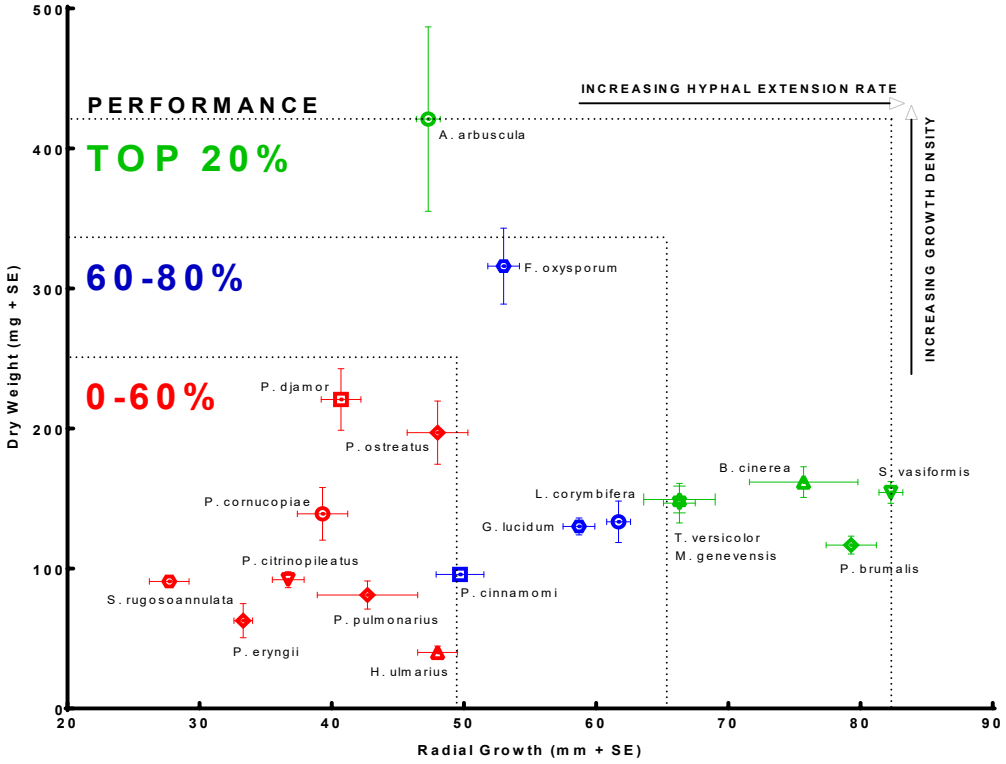

Figure 3.2. Hyphal extension (7-day radial growth in mm) versus growth density (14-day dry weight in mg) for all fungi assessed grouped by percentage performance compared with the highest performing species (low 0-60% - red, moderate 60-80% - blue, top 20% - green). Reproduced with permission from Jones, M, Huynh and John (2018).

3.3 Publication D: manufacturing optimisation based on an assessment of by-product-derived substrate suitability and microfiber viability

Agricultural by-products derived from cotton, flax, hemp, rice, sorghum and wheat are often used as substrates in mycelium composites (Camere & Karana 2018; He et al. 2014; Holt et al. 2012; López Nava et al. 2016; Pelletier et al. 2013), with only a few exceptions (Haneef et al. 2017; Pelletier et al. 2013; Travaglini et al. 2013). Growing fungi on such digestible materials can enhance the structural properties of such fillers, thus resulting in mycelial composites. The economical nature of these materials makes mycelium composites cost-

competitive with polymer materials and circumvents polymer associated waste disposal and pollution issues (Camere & Karana 2018; Haneef et al. 2017; Travaglini et al. 2013) and reduces issues associated with competition for land use for food production (Harvey & Pilgrim 2011; Rathmann, Szklo & Schaeffer 2010).

However, agricultural by-products typically lack optimal fungal nutrients, such as easily utilisable simple sugars, such as fructose, glucose, sucrose, and instead contain more complex carbon sources, such as cellulose and lignin (Faruk et al. 2012). Less easily utilised substrates reduce fungal growth and interfacial bonding between hyphae and organic matter (He et al. 2014; Travaglini et al. 2013). This adversely affects the mechanical properties of the resulting mycelium composites (Travaglini et al. 2013), decreases growth rates and results in an undesirably protracted manufacturing period.

The suitability of agricultural by-products for fungal growth is also relevant to the production of fungal-derived chitin. Waste from the marine food industry, such as crustacean shells (shrimp, crab and krill), is currently the major source of industrial chitin (Arbia et al. 2013; Hassainia, Satha & Boufi 2018; Ifuku 2014; Razak et al. 2018). However, crustacean-derived chitin is limited in supply by seasonal and regional variation and requires aggressive acid and alkaline treatments for purification and demineralisation to remove calcium carbonate, proteins, lipids and pigments. It also contains the allergenic protein ‘tropomyosin’ (Di Mario et al. 2008; Hassainia, Satha & Boufi 2018). Fungi offer a renewable, easily isolated and abundant alternative to crustacean chitin that can be rapidly produced on a large scale utilising heterotrophic growth on inexpensive agricultural by-products (Di Mario et al. 2008; Hassainia, Satha & Boufi 2018; Koza, Norton & van Leeuwen 2017; Liao et al. 2008; Nawawi, WanJones, et al. 2019; Razak et al. 2018). Fungal chitin also does not require demineralisation during extraction, however, the rigid chitin structure is associated with more pliable branched β-glucan, yielding a native nanocomposite architecture that can provide both strong and tough fiber networks when extracted (Hassainia, Satha & Boufi 2018; Nawawi, W 2016; Nawawi, WanLee, et al. 2019).

Determining the biocompatibility of substrates for fungal growth is vital for the production of mycelium composites and mycelium-derived chitins - but has not been previously assessed. This study aimed to determine the suitability of common (Australian) agricultural by-products, such as wheat straw, sugarcane bagasse, rice hulls and blackstrap molasses, as substrates to produce mycelium composites and mycelium-derived chitin; evaluate how the by-products compare with traditionally used nutrient-rich substrates, such as wheat grain and malt extract; and establish a methodology for assessing substrate suitability. Assessment of fungal growth was achieved through UHPLC-based quantification of ergosterol, a sterol unique to fungi and

some microscopic algae and protozoa (Mille-Lindblom, von Wachenfeldt & Tranvik 2004). This methodology can be used in future studies to select optimal substrates to ensure maximum fungal growth, bonding and growth rate. It also aimed to evaluate hyphal fusion, sheet formation and hyphal diameter metrics of the fungi grown on each substrate.

The solid agricultural by-products, such as wheat straw, rice hulls and sugarcane bagasse, alone lacked the nutrients and hyphal growth required to fully bond the fibrous fillers into mycelium composites **(Figure 3.3)**. Their similar ergosterol contents were supported by the similarity in their hyphal density, which was poor compared to the wheat grains, where hyphal fusion (anastomosis) resulted in pseudo-laminar sheet formation. Formation of these sheets is common for highly nutritious substrates due to the correlation between increased hyphal fusion and mature colony growth (Read et al. 2010). Consequently, the solid agricultural by-products assessed cannot be used as the sole constituents of mycelium composites. They would have to be supplemented with a more nutrient-rich substrate, such as wheat grains prior to use.

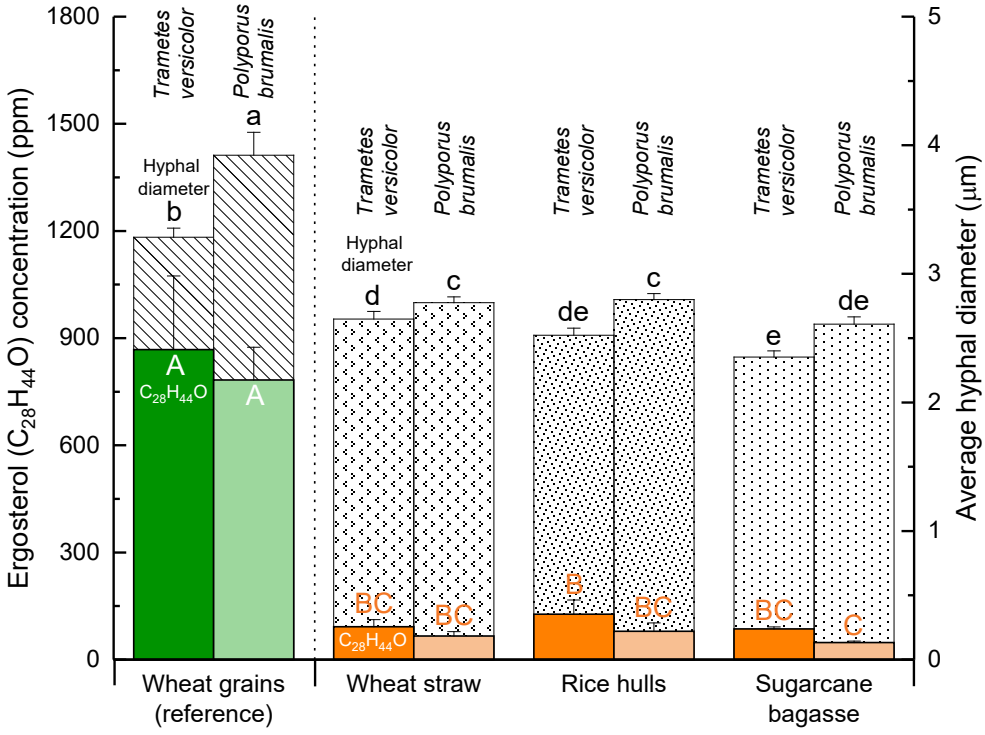

Figure 3.3. Ergosterol concentration (coloured, ppm) and average hyphal diameter (black-white shaded, µm) for *T. versicolor* and *P. brumalis* grown on wheat grain reference medium (green) and solid agricultural by-products (orange). Error bars indicate standard error within sets of four replicates. Non-normal data was log transformed prior to class categorisation. Class categories were letters of comparison based on Tukey's family error rate at $p \leq 0.05$ for sample-specific ANOVA. Reproduced with permission from Jones, M et al. (2019).

Liquid substrates resulted in significantly greater ergosterol production and hyphal growth than solid substrates, with fungi growing on the blackstrap molasses sugarcane by-product outperforming the malt extract reference standard **(Figure 3.4 Figure 3.5)**. Ergosterol production by *T. versicolor* was almost four times greater on blackstrap molasses than on malt extract, whereas that of *P. brumalis* only doubled. The large growth yield of *T. versicolor* on blackstrap molasses illustrates the viability of mycelial biomass as a source for structural polymers, such as chitin-glucan complex. Common mushroom (*A. bisporus*) constituents (whole fruiting body, stipe, pileus) have been utilised as a source of fungal chitin (Nawawi, W 2016; Nawawi, WanLee, et al. 2019). However, mushrooms take 14-21 d to grow and their use in materials science directly competes with food supply. *T. versicolor* mycelial biomass production on blackstrap molasses in this study is much more rapid (7 d) than mushrooms and is industrially scalable using bioreactors and continuous culture techniques (Kavanagh 2005) making it a viable source of natural polymers for materials science applications.

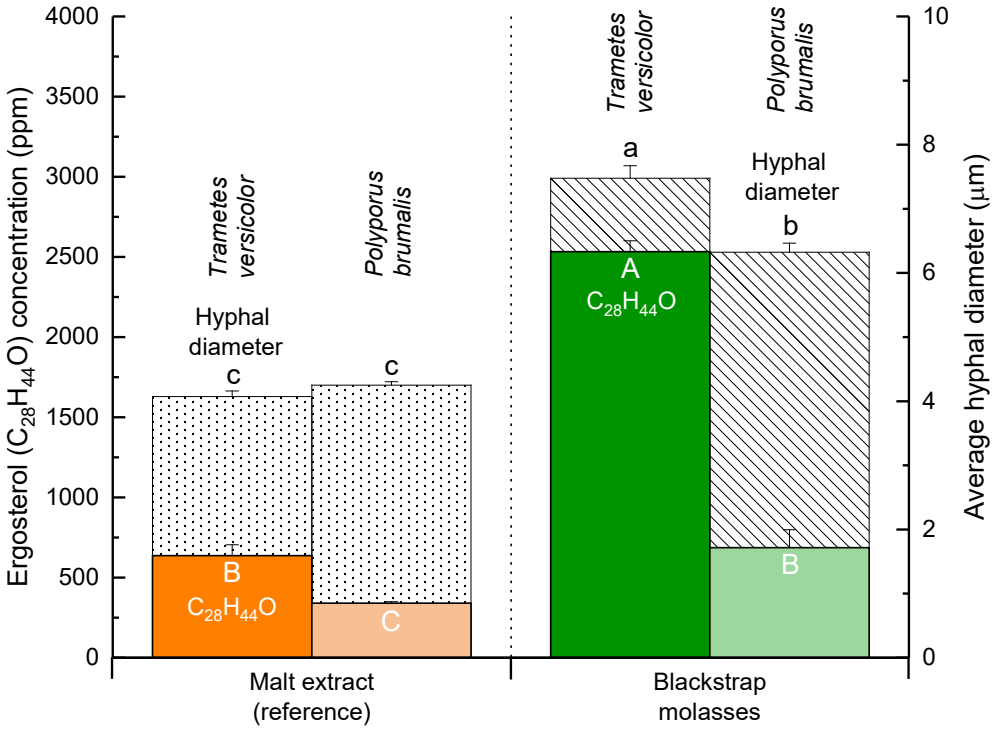

Figure 3.4. Ergosterol concentration (coloured, ppm) and average hyphal diameter (black-white shaded, µm) for *T. versicolor* and *P. brumalis* growing on malt extract reference medium (orange) and blackstrap molasses liquid by-product (green). Error bars indicate standard error within sets of four replicates. Non-normal data was log transformed prior to class categorisation. Class categories were letters of comparison based on Tukey's family error rate at $p \leq 0.05$ for sample-specific ANOVA. Reproduced with permission from Jones, M et al. (2019).

Solid agricultural by-products such as rice hulls, sugarcane bagasse and wheat straw resulted in insufficient growth to be considered as sole substrate constituents for mycelium composites. They should instead be partly supplemented with more nutrient-rich substrates. However, the liquid agricultural by-product blackstrap molasses resulted in very high biomass yields, outperforming even the commonly used laboratory nutrient malt extract. Such large biomass yields for fungi grown on inexpensive agricultural by-products offer a cheap, renewable, easily isolated and abundant alternative to crustacean chitin (Jones, M. et al. 2019; Nawawi, WanJones, et al. 2019).

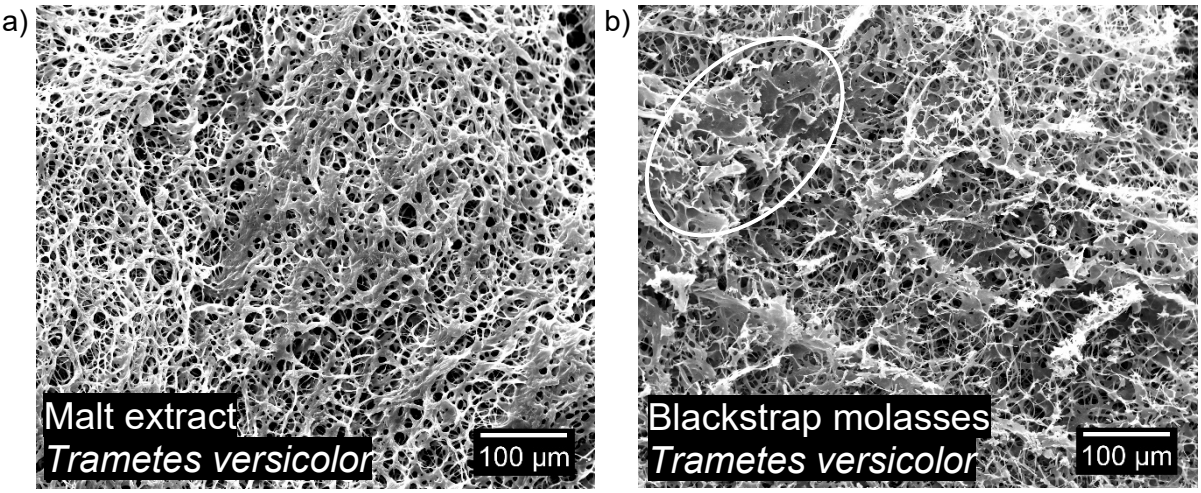

Figure 3.5. SE micrographs of mycelium biomass for *T. versicolor* and *P. brumalis* grown on malt extract and blackstrap molasses. Hyphal fusion and pseudo-laminar sheet formation and are highlighted using white ovals. Reproduced with permission from Jones, M et al. (2019).

3.4 Publication E: thermal degradation properties of mycelium

Many of the potential applications of mycelium-based composites are intended for high fire prone environments (e.g. packaging and building insulation) (Abhijith, Ashok & Rejeesh 2018; Holt et al. 2012; Xing et al. 2018). However, while the thermal degradation and fire properties of most agricultural materials used as mycelium composite fillers are known, very little is known about the thermal degradation and fire properties of mycelium itself as a binder and its composites. To meet the stringent fire safety regulations, it is imperative to characterize the fire properties of mycelium and mycelium composites. The heat, smoke and gases released by burning composites can also make fire-fighting extremely hazardous and increase the likelihood of serious injury and death (Mouritz, AP & Gibson 2007). Large quantities of organic matter present in mycelium composites can act as a fuel source and may escalate the fire risk by shortening the ignition time, increasing heat release and other fire risk factors such as flame spread and smoke toxicity, although these are yet to be quantified. These issues and the effect of incubation period (growth time), which controls the relative mycelial mass, on the fire

properties of mycelium composites need to be thoroughly quantified to enable wide practical applications.

This study investigated the thermal degradation properties and subsequent changes in the morphological and chemical structure of *T. versicolor* mycelium. Changes to the physical structure, reduction in hyphal diameters and chemical composition following pyrolysis were investigated to gain an in-depth understanding of the thermal degradation and decomposition mechanisms. Parameters such as the onset of decomposition, residual char, evolved gases and heat release rate were measured in addition to the effect of incubation period (growth time) on mycelium composite fire properties.

Morphological changes in mycelium were investigated pre- and post-pyrolysis using SEM and TEM. The fibrous mycelial network structure was retained after pyrolysis albeit with a substantial reduction in the hyphal diameter (10-30%) and cell wall thickness (66%) **(Figure 3.6)**. The retention of this fibrous structure is likely due to the presence of chitin in the cell walls of mycelium, which possesses excellent thermal stability and flame-retardant properties (Moussout et al. 2016; Pan et al. 2015). This shows that hyphal diameters could be tuned using thermal treatment to produce micro-sized carbon fibres that can then be used to toughen polymer-matrix composites for structural and non-structural engineering applications.

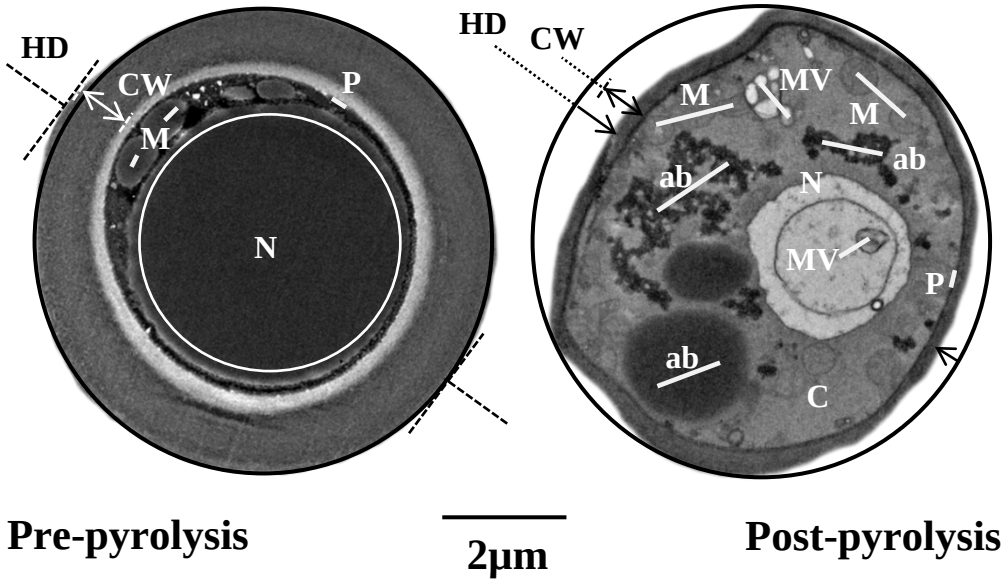

Figure 3.6. TEM transverse section micrographs detailing the ultrastructural, hyphal diameter and cell wall thickness changes in *T. versicolor* hypha pre- and post-pyrolysis (up to 600°C in nitrogen). Abbreviations: ab, accumulation body; C, cytoplasm; CW, cell wall; CWR, cell wall reduction; HD, hyphal diameter; HDR, hyphal diameter reduction; M, mitochondria; MV, multivesicular; N, nucleus; P, plasmalemma. Reproduced with permission from Jones, M; et al. (2018).

The TGA mass loss - temperature profiles exhibited three distinct stages with no significant differences between the thermal degradation characteristics of mycelium grown for 6, 12 and 18 d **(Figure 3.7)**. FTIR and Gas chromatography–mass spectrometry (GCMS) analysis identified complex thermal degradation patterns accompanied by the release of multiple flammable and non-flammable gaseous products. The residual mass was found to correlate with other studies performed on similar species of mycelium (Haneef et al. 2017). The relatively higher residue yield for mycelium compared to most thermoplastic (Cho et al. 2015; Fox et al. 2013; Renneckar et al. 2004) and thermoset (Braun et al. 2006; Cardona, Rogers & Van Erp 2007; Guo et al. 2007; Yee & Stephens 1996) polymers implies a potentially lower tendency to form smoke and toxic volatiles during thermal decomposition and combustion, thereby suggesting improved fire safety for the former.

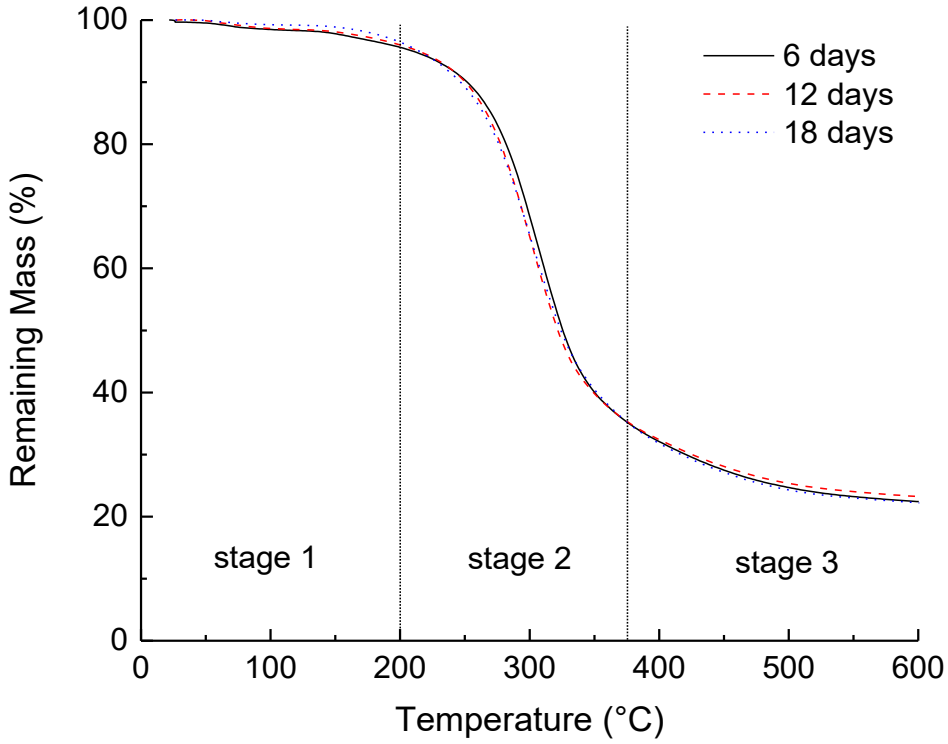

Figure 3.7. TGA-mass loss temperature curve of mycelium grown for 6, 12 and 18 days between 25-600°C under nitrogen. Reproduced with permission from Jones, M; et al. (2018).

Pyrolysis combustion flow calorimetry (PCFC) also showed that mycelium was less combustible and had improved fire safety over PMMA and PLA. Peak and total heat release values for mycelium were significantly lower when compared to both PMMA and PLA, as was the average heat release capacity of mycelium (Bhat et al. 2015; Fox et al. 2013). This indicated superior resistance to flaming combustion, which may be attributed to the higher residual char produced of mycelium in comparison to PMMA and PLA. Char acts as a thermal

insulator and inhibits oxygen migration at the solid/gas phase interface thereby limiting the flaming combustion process.

Growth time did not make any significant difference to the fire reaction properties of the mycelium composites. Cone calorimetry heat release rate (HRR) profiles of mycelium-wheat grain composites did not vary significantly with increasing growth periods, although the peak heat release rate, which is considered a critical property controlling the maximum temperature and flame spread rate (Mouritz, A, Mathys & Gibson 2006; Mouritz, AP & Gibson 2007) was marginally higher for the samples with no growth relative to that of composites grown for 6, 12 and 18 d. This observation suggested that the addition of mycelium resulted in marginal improvements to the fire reaction properties of the wheat grain substrate. However, the increase in thickness of the mycelium coating was not sufficient to cause significant variation in the fire reaction properties of the composite. This observation might point to the existence of a low threshold mycelial density required to enable effective fire retardation in mycelium composites.

The thermal degradation and fire safety of mycelium and mycelium-wheat grain composites have been characterised using various experimental techniques. Thermogravimetric analysis revealed that the growth time has no discernible effect on the thermal degradation characteristics of mycelium. FTIR and GCMS analysis facilitated the identification of complex thermal degradation patterns accompanied by the release of multiple flammable and non-flammable gaseous products. The fibrous structure of mycelium is retained following pyrolysis, albeit with a reduction in its diameter. The fire reaction properties of mycelium have found to be superior to other competing thermoplastic polymers (PMMA and PLA) due to its tendency to form relatively higher char yields. The presence of mycelium is responsible for an improvement in the fire reaction properties of wheat grains. However, beyond 6 days, the growth time was found to have no significant effect on the fire reaction properties of mycelium-wheat grain composites. Mycelium was found to possess certain flame-retardant properties (e.g. high char residue and release of water vapour) and could be used as an economical, sustainable and fire-safer alternative to synthetic polymers for binding matrices.

3.5 Publication F: mycelium composites with improved fire safety produced from agricultural and industrial wastes

The diverse range of useful properties of mycelium composites makes them suitable as low-cost and sustainable alternatives to widely used, highly flammable synthetic polymers (e.g. plastics including insulation foams) and resin-based engineered woods (e.g. particleboard). These traditional construction materials have been identified as the main cause of severe and fatal fire incidents worldwide (Cho et al. 2015; Miao et al. 2014; Mouritz, AP & Gibson 2007;

Xu, Jin & Jiang 2017) due to significant heat release and the toxic fumes generated during combustion (Cho et al. 2015; Miao et al. 2014). Modern houses constructed from unprotected engineered woods collapse over 3 times faster than older wood-based constructions (Izydorek et al. 2008). Plastic foams are often major contributors to fires involving rapid flame spread, which generate high volumes of smoke and toxic gases (Melville 1986) including carbon monoxide and hydrogen cyanide (Stec & Hull 2011).

This study aimed to produce predominantly waste-based, biologically manufactured materials with improved fire safety using a high performing fungal species and low-cost wastes with high silica contents, such as rice hulls and glass fines, to promote fire retardancy, since mycelium was found to have limited fire resistance of its own. This is the first study into the growth process of mycelium composites using industrial (glass fines) and agricultural (rice hulls) wastes, the thermal degradation properties of mycelium grown on different substrates, and the fire safety of the resultant composites.

T. versicolor was utilised due to its established high growth performance, in conjunction with rice hull and glass fine substrates. Rice hulls are considered a low-grade agricultural by-product. They have limited use in animal bedding, boiler fuel and as a filler for building materials, glass production and road construction but are largely discarded as waste (Defonseka 2014). Glass fines are typically used in asphalt, abrasive blasting, road aggregates, brick making, water filtration and insulation batts (Sustainability Victoria 2014) but are considered a waste material and mostly discarded (Alex Fraser Group 2014). These substrates were supplemented with wheat grains since neither substrate has sufficient nutrient content alone to facilitate adequate mycelial growth to bind the composite together.

Mycelium composite samples with varying rice hull and glass fine content were produced. Rice hulls could be used for up to 75 wt% of the mycelium composite, supplemented by 25 wt% inoculated wheat grains, without significantly compromising mycelial growth or interfacial bonding. Hyphae also readily spread on glass fines, although their high inorganic content limited growth to a weak surface spread that had to be supplemented by at least 50 wt% organic material (i.e. wheat grains or rice hulls) to facilitate sufficient growth to properly bond the constituents and hold the composites together.

The overall fire performance of all samples relative to volume-specific cost was assessed to establish how compositional variation affected the viability of mycelium composites as alternatives to typical synthetic construction materials **(Figure 3.8)**. Time to ignition (t_{ig}) and time to flashover (t_{fo}) (blue) were plotted with average (RHR_{180}) and peak heat release rates (PHRR) (green) to identify the safest and cheapest materials (left-hand quadrants). All

mycelium composites were significantly cheaper and safer than the synthetic construction materials considered.

Rice hull- and wheat grain-based mycelium composites incorporating 50 wt% glass fines (25RH50GF and 25WG50GF respectively) had the longest times to flashover overall.

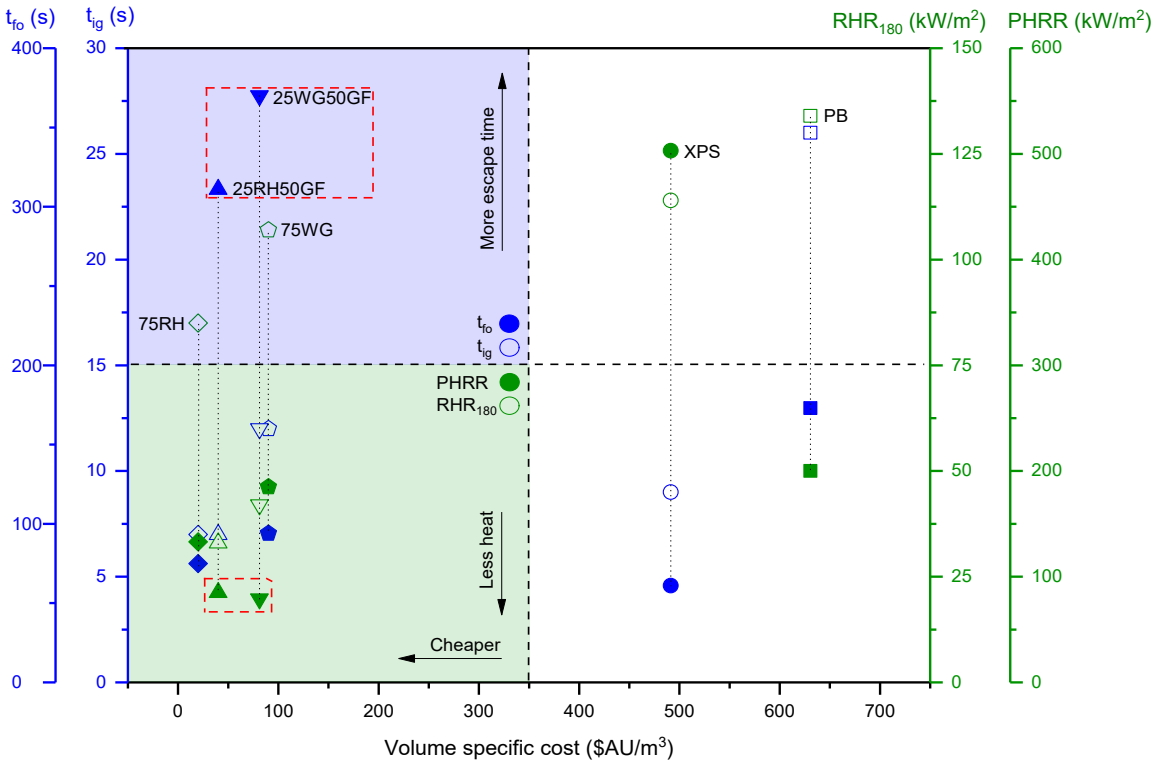

Figure 3.8. Overview of the cost of mycelium composites and synthetic construction materials with respect to fire performance. The safest and cheapest materials were identified in the left-hand quadrants. Materials associated with more escape time have longer times to ignition (t_{ig}, s) and flashover (t_{fo}, s) (upper quadrants). Materials associated with less heat generation have lower average (RHR_{180}) and peak (PHRR) heat release rates (kW/m^2) (lower quadrants). Abbreviations: 75RH = 75 wt% rice hulls, 75WG = 75 wt% wheat grains, 25RH50GF = 25 wt% rice hulls + 50 wt% glass fines, 25WG50GF = 25 wt% wheat grains + 50 wt% glass fines, XPS = ClimaFoam® extruded polystyrene (XPS) foam, PB = STRUCTAflor® particleboard. Reproduced with permission from Jones, M et al. (2018).

Flashover is the near-simultaneous ignition of all exposed materials in an enclosed area and is a common and very dangerous occurrence in residential and building fires (Liu & Chow 2014). Fires that reach flashover are approximately ten times more dangerous than fires that do not, and as such steps should be taken to prevent flashover (Clarke 1997; Liu & Chow 2014). These compositions also had the lowest average and peak heat release rates. Heat release rate (HRR) is commonly accepted as the most important fire reaction property due to

its role in fire growth and spread (Babrauskas & Fires 1997; Babrauskas & Peacock 1992). Heat released from burning material provides additional thermal energy to fires and strongly influences their behaviour (Mouritz, AP & Gibson 2007) and reaction properties including surface flame spread, smoke generation and carbon monoxide emission (Mouritz, A, Mathys & Gibson 2006; Sorathia, Divisjón & Lyon 1997). The average heat release rate (RHR_{180}) is considered the most appropriate variable for predicting full-scale fire properties (Brown, Fawell & Mathys 1994) while the peak heat release rate (PHRR), is considered a critical property controlling maximum temperature and flame spread rate (Mouritz, AP & Gibson 2007).

The lower heat release of rice hull based mycelium composites is attributable to significantly higher residue following decomposition of the rice hulls comprising of amorphous carbon-based char (approx. 20 wt%) and embedded silica (approx. 20 wt%) during combustion (Zhao et al. 2009). Char is derived from organic rice hull constituents, especially lignin. Lignin decomposes into aromatic fragments which are the principal constituents from which char is formed. The formation of char is known to increase flame retardancy by acting as a thermal insulation barrier due to its low thermal conductivity (Mouritz, AP & Gibson 2007) and to reduce smoke due to the ability of char to impede the release of ultra-small fragments of fibre into the smoke plume (Gilwee 1975; Parker & Kourtides 1983). In the presence of oxygen (on air exposed surfaces) char will oxidize leaving inert silica behind as the main constituent of the surface residue. Progressive accumulation of these silica layers results in the formation of a silica-ash layer which acts as a thermal barrier, preventing oxygen flow to the composite core. This lack of oxygen flow prevents further oxidation, which insulates the virgin materials in a shielding effect similar to fire retardancy improvements noted in organic polymers due to combustion of silicones (Hshieh 1998).

While heat release and flash over are important fire safety parameters, the majority of fire-related fatalities occur due to exposure to toxic gases rather than burns, generalised trauma or other causes (Babrauskas et al. 1992; Mouritz, AP & Gibson 2007). Short-term exposure to smoke consisting of small fragments of fibre and ultra-fine carbon particles is not considered a serious health hazard to humans but is an important safety concern because dense smoke can reduce visibility, cause disorientation and hinder firefighting efforts (Mouritz, AP & Gibson 2007). Carbon monoxide (CO) is generally considered the greatest individual hazard, and even very low levels can cause incapacitation and death (e.g. 1500 ppm will cause death within an hour) (Hirschler 1987). In contrast, carbon dioxide (CO_2) concentration must be more than 60 times higher (100,000 ppm) to cause death over the same period (Mouritz, AP & Gibson 2007).

Cost-specific total smoke release (TSR, black), CO (COP_{180}, red) and CO_2 (CO_2P_{180}, orange) production were also plotted (safest and cheapest materials associated with green quadrant) **(Figure 3.9)**. All mycelium composites produced less smoke than the synthetic construction materials. Compositions of 75 wt% rice hulls (75RH) had the lowest CO production overall, a low CO_2 production and were 24-31 times cheaper than extruded polystyrene and particleboard. Compositions of 25 wt% wheat grains + 50 wt% glass fines also had very low CO and CO_2 production.

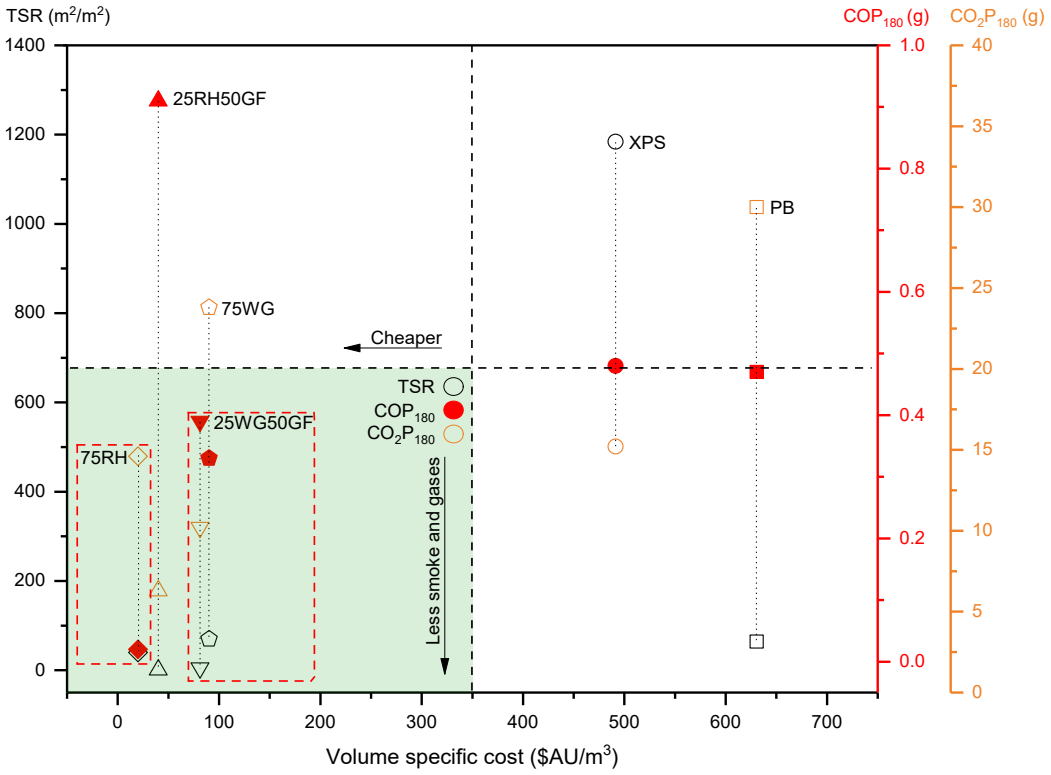

Figure 3.9. Overview of the cost of mycelium composites and synthetic construction materials with respect to smoke and gas release. Safer and more cost-effective materials were identified in the green quadrant. Safer materials have lower total smoke release (TSR), CO (COP_{180}) and CO_2 (CO_2P_{180}) production. Abbreviations: 75RH = 75 wt% rice hulls, 75WG = 75 wt% wheat grains, 25RH50GF = 25 wt% rice hulls + 50 wt% glass fines, 25WG50GF = 25 wt% wheat grains + 50 wt% glass fines, XPS = ClimaFoam® extruded polystyrene (XPS) foam, PB = STRUCTAflor® particleboard. Reproduced with permission from Jones, M et al. (2018).

This study has found mycelium composites to be an economical, sustainable and thermally safer alternative to synthetic construction materials. In particular, mycelium composites had much lower average and peak heat release rates and longer estimated time to flash over than the synthetic construction materials considered. They also released significantly less smoke and CO_2. Rice hulls yielded significant char and silica ash residues, which improved fire

performance. However, composites containing glass fines exhibited the best fire performance due to their significantly higher silica concentrations and low combustible material content. Increased concentrations of glass fines increased volume-specific cost but reduced mass-specific and density-specific costs. Overall, mycelium composites were very economical and exhibited far better fire safety parameters than the traditional extruded polystyrene and particleboard construction materials tested. Their use in civil construction would enable better fire safety in buildings.

3.6 Publication G: mycelium materials produced from extracted chitin, chitosan and β-glucan with tuneable mechanical and surface properties

Despite the range of useful functional applications for mycelium-derived composites, they typically exhibit mechanical properties resembling foams and natural materials. This results from the often low-strength agricultural waste or by-products utilised in these composites as filler, which are weakly bonded by a hyphal filament matrix, and the presence of non-structural hyphal elements, such as proteins, lipids and cytoplasm (Appels et al. 2019; Kavanagh 2005). However, mechanical performance can be improved by eliminating the use of these low-strength wastes and by-products as composite fillers, instead utilising them solely as nutrient sources for fungal growth and then removing non-structural elements from the isolated mycelium using chemical treatments. This process constitutes the conversion of agricultural biomass into valuable natural polymers within fungal biomass, such as combinations of chitin, chitosan and glucan.

Mycelium-derived chitin offers a cheap, renewable, easily isolated and abundant alternative to more expensive, seasonally and regionally limited, allergenic crustacean chitin (Di Mario et al. 2008; Hassainia, Satha & Boufi 2018). The fungal chitin structure is also associated with more pliable branched β-glucan or chitosan, providing a native nanocomposite architecture that is both strong and tough (Nawawi, WanLee, et al. 2019). Chitin derived from mycelium is also more viable than fungal chitin derived from edible mushrooms, which takes much longer to grow, is more expensive and directly competes with food supply.

This study aimed to produce waste-derived mycelium nanopapers with improved mechanical properties compared to existing mycelium materials. Emphasis was on cost and environmental impact with only cheap agricultural by-products and natural fungal growth from high growth performance species, such as *A. arbuscula*, *M. genevensis* and *T. versicolor*, used to obtain chitinous fungal biomass Structural polymers, such as chitin and chitosan were then extracted from this fungal biomass using simple and environmentally sustainable alkaline treatment, followed by vacuum filtration and hot-pressing to produce homogenous nanopapers. The morphology, composition and molecular structure of the nanopapers were

then analysed in addition to their physical, mechanical and surface properties. Mycelium-derived nanopapers were also compared with nanopapers produced from common white button mushroom (*A. bisporus*) fruiting bodies.

ATR-FTIR spectroscopy confirmed the presence of chitin in the samples. Papers derived from *A. bisporus* fruiting bodies had significantly higher N and glucosamine contents than those derived from mycelium (*A. arbuscula*, *M. genevensis* and *T. versicolor*), which indicated the presence of a significantly higher fraction containing a combination of chitin and chitosan in these papers. *A. arbuscula* and *M. genevensis* still displayed signals indicating the presence of chitin, but the significantly reduced intensity and lower fractions of acetylated monosaccharide units suggested a lower chitin content. This suggested the dominance of non-chitin polysaccharides and a higher degree of deacetylation in these extracts. *T. versicolor* papers had very low N concentrations with sugar analysis revealing that glucose was the prevalent sugar in these papers, most likely associated with large concentrations of glucan and a low glucosamine content. Chitosan was also present in all nanopapers, with solid state nuclear magnetic resonance (ssNMR) spectroscopy indicating that all papers were at least partially deacetylated as confirmed by reduced -CH_3 and C=O signals and lower fractions of acetylated monosaccharide units in these samples.

All mycelium-derived nanopapers only treated using NaOH contained large quantities of inorganics. Ca was especially prevalent in mycelium-derived samples, most likely derived from the molasses growth medium. Fungi growing in Ca rich environments typically contain Ca biomineralized in hyphae as calcite ($CaCO_3$) and calcium oxalate (Burford, Hillier & Gadd 2006). Biomineralization of hyphal filaments was visible using EDS point analysis and elemental composition mapping and ssNMR results, which displayed a C=O peak associated with carbonate or oxalate in all mycelium-derived papers, most prevalently in *T. versicolor*. ssNMR also indicated the presence of lipid residues in *A. arbuscula* and *M. genevensis*, through a -CH_2 peak. However, it should be noted that lipid residues are not uncommon in chitin (Reid et al. 2012). *A. arbuscula* papers were subsequently treated with H_2O_2 and HCl to remove the organic and inorganic impurities. Both H_2O_2 and HCl treatments were effective in removing all inorganic Ca salts from the samples, with only trace quantities of Ca remaining after treatment. Lipid residue concentrations also decreased, indicated by significant reductions in the ssNMR -CH_2 peaks and slightly more negative ζ-potentials between pH 5-10 resulting from greater chitin-based charge availability.

Despite variations in tensile performance, mycelium-derived nanopapers produced in this study matched or significantly outperformed all currently known mycelium-derived materials. Historically, mycelium composites have been characterised exclusively as foams, with low

densities and elastic moduli, despite physical processing such as hot pressing **(Figure 3.10)**. The mycelium-derived nanopapers exhibited higher ultimate tensile strengths than mycelium composites (Appels et al. 2019; Haneef et al. 2017) and mycelial biomass grown in controlled environments (Appels et al. 2018). They also exhibited similar tensile performance to several advanced mycelium materials utilising genetic modification of the SC3 hydrophobin (Δsc3) gene and controlled growth environments to produce schizophyllan rather than glucan linked chitin (Appels et al. 2018). However, the nanopapers produced in this study have the advantage of being able to be grown in any environment and being universally applicable to all fungal biomass rather than species and strain specific genetic modification. They were characterised as polymers, based on their density and elastic moduli which were also similar to or higher than existing mycelium composites, the mechanical properties of mycelial biomass and Δsc3 mycelium materials (Appels et al. 2019; Appels et al. 2018). Overall, mycelium-derived nanopaper were comparable to commercial copy paper and some plastics (Crompton 2012).

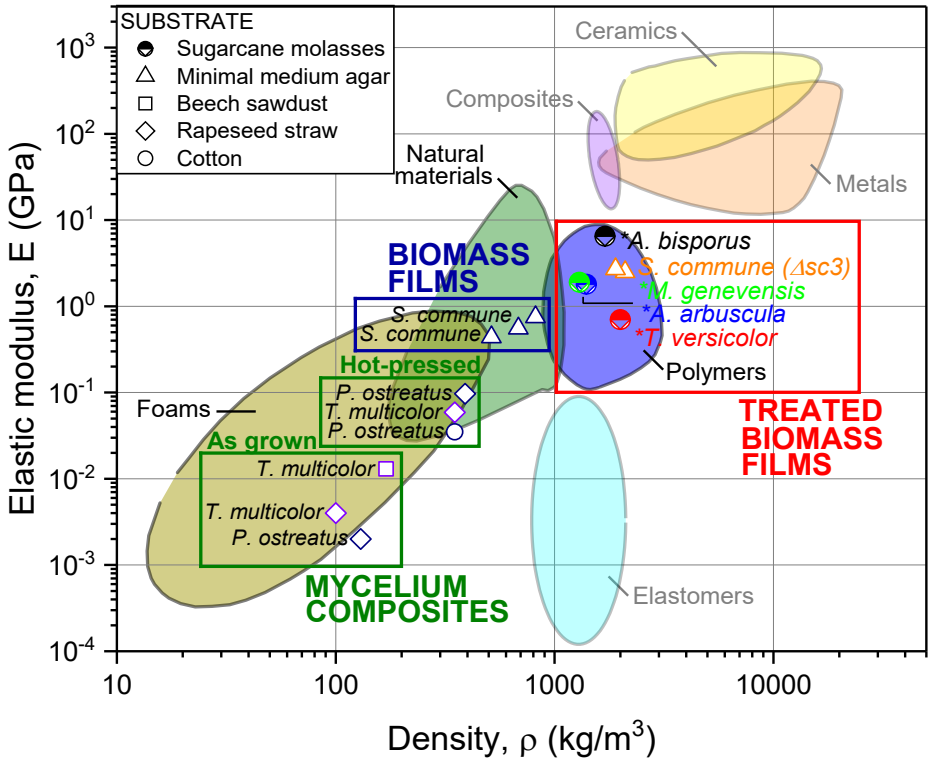

Figure 3.10. Ashby chart of elastic modulus (GPa) and density (kg/m3) of mycelium materials. The new chemically treated and hot-pressed nanopapers derived from mycelium and fruiting bodies produced in this study (*) are compared to existing as grown and hot-pressed mycelium composite materials, mycelial biomass and genetically modified Δsc3 biomass arranged by species and substrate. Data from Appels et al. (2019); Appels et al. (2018); (Ashby, Shercliff & Cebon 2018).

Mycelium-derived nanopapers (*A. arbuscula* and *M. genevensis*) were hydrophobic (contact angle > 90°), with high advancing water contact angles compared to *A. bisporus* fruiting body derived nanopapers and cellulose nanopapers **(Figure 3.11)**. The hydrophobicity of mycelium-derived materials has previously been noted (Haneef et al. 2017). Chitin and chitosan theoretically exhibit slightly higher surface energy than other polysaccharides, such as cellulose and starch, due to the presence of amino and amide moieties (Cunha, Ana Gisela & Gandini 2010). However, non-polar impurities have been noted to be responsible for lower polar surface energy components in less pure chitin (Cunha, Ana G. et al. 2008). Non-polar impurities responsible for the aroma of fungi, such as alcohols and acid derivatives and in particular lipid residues, are probably responsible for the hydrophobicity and low surface energies of mycelium-derived nanopapers (Wekesa et al. 2016). Hydrophobic properties could make mycelium-derived nanopapers useful for applications including coatings. *T. versicolor* mycelium-derived nanopapers did not support stable droplets, instead absorbing them on contact and were subsequently associated with advancing water contact angles of 0°.

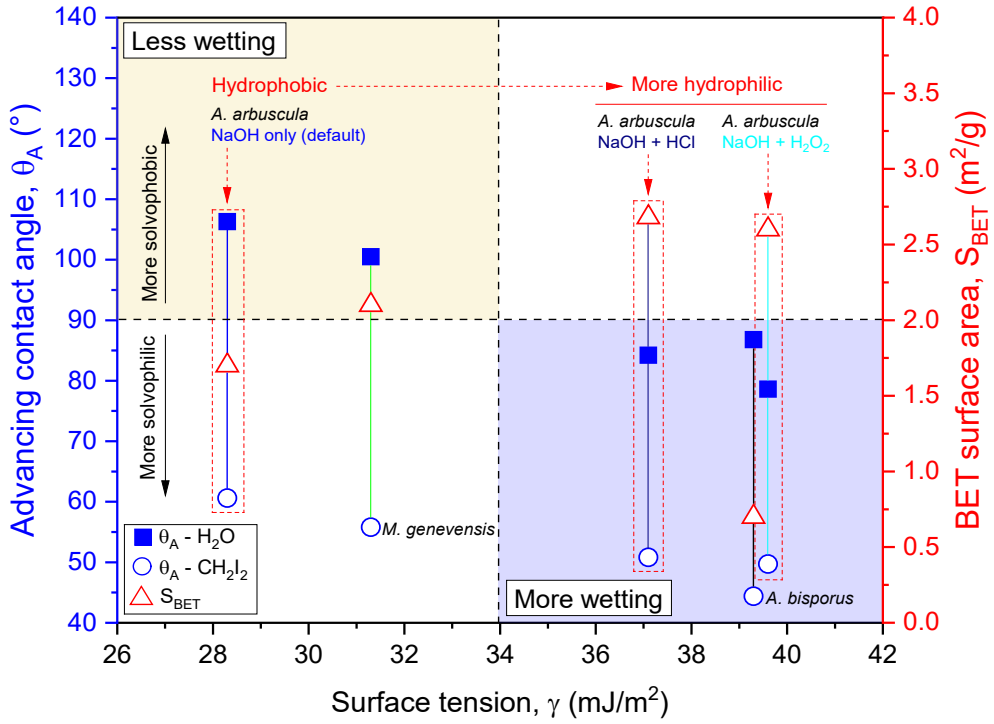

Figure 3.11. Advancing contact angle (blue markers, °), surface tension (mJ/m^2) and BET surface area (hollow red markers, m^2/g) measurements for *A. arbuscula* (blue connecting line) and *M. genevensis* (green connecting line) NaOH treated mycelium-derived nanopapers. *A. bisporus* fruiting body derived nanopapers (black connecting line) and *A. arbuscula* NaOH and H_2O_2 (cyan connecting line) or HCl (navy connecting line) treated nanopapers are also displayed. *T. versicolor* has been omitted from this figure as it was unable to be tested. Reproduced with permission from Jones, M. et al. (2019).

A. arbuscula mycelium-derived nanopapers treated with H_2O_2 and HCl were more hydrophilic than papers only treated with NaOH. The advancing water contact angles of these papers were significantly lower than NaOH only treated papers. The increased hydrophilicity of H_2O_2 and HCl treated papers most likely resulted from the removal of lipid residue present in *A. arbuscula* nanopapers only treated using NaOH. This reduction in lipid residue is also likely responsible for the higher BET surface areas of H_2O_2 or HCl treated samples. The more fibrous surface morphology of H_2O_2 and HCl treated papers, stripped of Ca salts, coupled with their lower water contact angles and higher surface tensions could potentially make these papers suitable for use as membranes in filtration applications.

All mycelium- and fruiting body derived nanopapers, except for *T. versicolor*, exhibited three stage thermal degradation typical of mycelium with char residues of ~20-23 wt% in a nitrogen atmosphere. *A. bisporus* fruiting body derived papers fully thermally decomposed in an air atmosphere, however *A. arbuscula* and *M. genevensis* mycelium-derived papers had an inorganic residue of ~8-9 wt%, attributable to their Ca content. *T. versicolor* mycelium derived nanopapers exhibited a multi-stage thermal degradation process up to 800°C and a final inorganic residue of ~34 wt% under air and nitrogen atmospheres, supporting the significant biomineralization of this species and the lower organic content of these nanopapers compared to the other mycelium-derived nanopapers. H_2O_2 and HCl treated nanopapers fully decomposed in an air atmosphere, with negligible inorganic char present above 600°C. This verified the effectiveness of H_2O_2 and HCl in removing inorganic impurities in mycelium-derived nanopapers grown on Ca rich substrates, such as sugarcane molasses.

Fungal growth provides a low-cost method for on-demand generation of natural nanofibrils, such as chitin and chitosan, from agricultural wastes and by-products. These nanofibrils were obtained via mild alkaline extraction of a common mushroom reference and various species of fungal mycelium grown on the sugarcane by-product molasses and hot pressed to produce nanopapers. Mycelium-derived nanopapers were more hydrophobic than pure chitin and other natural polysaccharides, such as cellulose and starch, resulting from the presence of lipid residues within the nanopapers. Mycelium-derived polymer extract yields were competitive with crustacean chitin and nanopapers produced from the extracts exhibited much higher tensile strength than most existing mycelium materials, with comparable properties to paper and some plastics. Further hydrogen peroxide or hydrochloric acid treatments removed organic and inorganic impurities rendering the mycelium-derived nanopapers hydrophilic. Nanopapers derived from common mushrooms were hydrophilic, contained fewer lipid residues and inorganic contaminants than those derived from mycelium and had higher tensile properties. These variations in surface morphology, wettability and mechanical properties highlight the customisable properties of these cheap and environmentally sustainable

materials making them potentially suitable for a wide range of applications, including coatings, membranes, packaging and paper.

Chapter 4

Conclusions and Suggestions for Future Research

This work constitutes a significant contribution to the emerging field of mycelium-derived materials. It takes a structured approach, with initial optimisation of the biological manufacturing process of both the fungal biomass, used as binder in a mycelium composite or as source of biological polymers, and the growth medium utilised as either mycelium composite filler or solely as a nutrient source. It then investigates the thermal degradation and fire safety of mycelium as a binder and mycelium composites utilising high silica substrates for construction applications requiring no or limited structural properties and some degree of fire resistance. Finally, chemical and physical processing techniques are investigated to tune the mechanical performance and surface properties of planar materials produced from mycelium-derived polymers. These investigations have demonstrated that mycelium-derived materials could potentially be utilised as a replacement for synthetic polymers in a range of non-structural and semi-structural applications.

4.1 Research question 1: How do inherent species characteristics of fungi (e.g. hyphal types, pathogenicity, taxonomic- and association-based classifications) affect the growth performance (hyphal extension rate and growth density) of the fibrous matrix phase (mycelium) and hence manufacturing time of mycelium composites?

Investigations into the optimisation of the mycelial growth demonstrated that inherent growth characteristics, such as hyphal types, pathogenicity and taxonomic- and association-based classifications were not reliable predictors of hyphal extension rate or growth density. A correlation existed between the presence of skeletal hyphae and increased hyphal extension rates, but no similar correlation was present for growth density. Pathogenic species provided no significant improvement in growth performance compared to non-pathogenic species and should not be used due to the health risks associated with these fungi. Species hyphal extension rates and growth densities varied significantly across and within all taxonomic- and association-based classifications. Despite these variations, association-based classifications remain useful in preliminary selection of species-substrate combinations, such as white rot wood-associated fungi for lignocellulosic substrates and water moulds for liquid nutrient solutions, although some species will be suitable for multiple substrate types. The investigation

found the assessment of hyphal extension rate measured as radial growth and growth density measured as dry weight to be a simple, effective and resource conservative method for initial evaluation of the viability of fungi for mycelium material manufacturing. *T. versicolour* and *P. brumalis* were identified as the most suitable species for mycelium composite applications based on growth performance and enzymatic compatibility with typical lignocellulosic mycelium composite substrates. This supports their use over other traditionally used species such as *P. ostreatus* and *G. lucidum*, which had poorer growth performance. Other high performing species included *A. arbuscula* and *M. genevensis*, which could be used in fungal structural polymer generation.

4.2 Research question 2: Which abundant Australian agricultural by-product (organic substrate filler phase) yields the highest mycelial (fibrous matrix phase) growth

Investigations were then conducted into the suitability of a range of abundant and low-cost agricultural by-products as substrate for production of mycelium composite or as a nutrient source for biomass and polymer generation. The solid agricultural by-products assessed, such as rice hulls, sugarcane bagasse and wheat straw, resulted in insufficient growth quantity and spread to be considered as sole substrate constituents for mycelium composites. More nutrient rich substrates, such as wheat grains, must be used to supplement the nutrient deficiencies of these substrates in order to achieve sufficient fungal growth and bonding of filler constituents. Liquid agricultural by-products assessed, such as blackstrap molasses, on the other hand resulted in very high biomass and anastomosis (hyphal fusion) resulting in the formation of pseudo-laminar sheets and significant fungal biomass. Fungal growth on blackstrap molasses outperformed even the highly nutritious and commonly used laboratory nutrient malt extract. The large biomass yields for fungi grown on inexpensive agricultural liquid by-products, such as blackstrap molasses demonstrated the viability of fungi as a cheap, renewable, easily isolated and abundant alternative to crustacean chitin, which could be used to produce renewable polymer materials. *T. versicolor* outperformed *P. brumalis* on all substrates and was selected for use in mycelium composite production in this research.

4.3 Research question 3: How does mycelium thermally decompose and what are its thermal degradation properties?

The high fire risk of the intended applications for mycelium composites prompted investigation into the completely undocumented thermal degradation and fire reaction properties of *T. versicolor* mycelium binder. Thermogravimetric analysis revealed that mycelium exhibits a three-stage degradation process typical of bio-based materials and that the growth time had no discernible effect on the thermal degradation characteristics of the mycelium. FTIR and

GCMS analysis identified complex thermal degradation patterns accompanied by the release of multiple flammable and non-flammable gaseous products. The fibrous structure of mycelium was retained following pyrolysis, albeit with a reduction in its diameter and cell wall thickness. Overall, the fire reaction properties of mycelium itself as a binder were found to be superior to other competing thermoplastic polymers (PMMA and PLA) due to its tendency to form relatively higher char yields but typical of bio-based materials. The presence of mycelium was also responsible for an improvement in the fire reaction properties of wheat grains. However, beyond 6 days, the growth time had no significant effect on the fire reaction properties of mycelium-wheat grain composites. Mycelium did possess certain flame-retardant properties (e.g. high char residue and release of water vapour), however the use of high silica substrates for mycelium composite filler plays a greater role in the fire resistance of mycelium composites.

4.4 Research question 4: How do the fire reaction and fire safety properties of mycelium composites compare with synthetic foam, such as polystyrene, and engineered wood, such as particleboard, and can their properties be improved through incorporation of industrial by-products, such as glass fines?

Fire resistant mycelium composites were produced using *T. versicolor* mycelial growth and high silica substrates, such as rice hulls and glass fines. Inclusion of a small quantity of wheat grains was necessary to achieve sufficient fungal growth to bind the composites together. The mycelium composites had much lower average and peak heat release rates and longer estimated time to flash over than synthetic construction materials, such as extruded polystyrene (XPS) foam and particleboard. They also released significantly less smoke and CO_2. Rice hulls yielded significant char and silica ash which improved fire performance, but composites containing glass fines exhibited the best fire performance due to their significantly higher silica concentrations and low combustible material content. Increased concentrations of glass fines increased volume-specific cost but reduced mass-specific and density-specific costs. Overall, mycelium composites were very economical and exhibited far better fire safety parameters than the traditional construction materials tested. Their use in civil construction would enable better fire safety in buildings.

4.5 Research question 5: Can the mechanical properties of mycelium be improved by removing non-structural hyphal elements using chemical treatment?

Despite the range of useful functional applications for mycelium-derived materials, their mechanical properties are typically limited to resembling foams or natural materials. This prompted investigation into methods to improve the mechanical performance of these materials to enable further applications. The mechanical performance of mycelium materials is limited by the low-strength agricultural residue composite filler, weak hyphal filament matrix, and non-structural hyphal elements they contain, but can be improved by using fungal growth as a low-cost method for on-demand generation of natural nanofibrils, such as chitin and chitosan, from agricultural wastes and by-products, rather than as a binder. These nanofibrils were obtained via mild alkaline extraction of a common mushroom reference and various species of fungal mycelium grown on the sugarcane by-product molasses and hot pressed to produce nanopapers. The species *A. arbuscula*, *M. genevensis* and *T. versicolor* mycelium were selected based their structural polymers and previously assessed growth performance and their polymer extracts characterised using ATR-FTIR, ^{13}C ssNMR, elemental and sugar analysis. Mycelium-derived polymer extract yields were competitive with crustacean chitin and nanopapers produced from the extracts exhibited much higher tensile strength than most existing mycelium materials, with comparable properties to paper and some plastics. Mycelium-derived nanopapers were more hydrophobic than pure chitin and other natural polysaccharides, such as cellulose and starch, resulting from the presence of lipid residues within the nanopapers. Further hydrogen peroxide or hydrochloric acid treatments removed organic and inorganic impurities rendering the mycelium-derived nanopapers hydrophilic. Nanopapers derived from common mushrooms were hydrophilic, contained fewer lipid residues and inorganic contaminants than those derived from mycelium and had higher tensile performance. These variations in surface morphology, wettability and mechanical performance make them potentially suitable for a wide range of applications, including coatings, membranes, packaging and paper.

4.6 Suggestions for future research

Literature comprehensively investigating the characteristics and applications of mycelium and mycelium composites is still very much lacking. Recent studies have demonstrated the significant potential of mycelium and mycelium composites, specifically in the areas of thermal and acoustic insulation with fire resistant properties. Now that these areas of promise have been identified they should be comprehensively investigated, both academically and

commercially, with the aim of producing insulation materials for widespread use in construction.

Significant research opportunities also still exist in the biological optimisation of manufacturing for insulation and packaging foams produced from mycelium, since protracted manufacturing durations remain one of the largest problems restricting the widespread use of mycelium composites as a replacement for these foams. Biological optimisation of manufacturing could include species selection or genetic modification, environmental condition control, such as temperature, humidity, oxygen availability in addition to variations in substrates, nutrients and additives. These factors could also be investigated with the aim of improving mycelium composite mechanical properties, which while not critical for insulation or packaging foam applications should be improved.

More broadly, one important material property across all possible applications for mycelium composites is water absorption. The tendency of mycelium composites to rapidly absorb very large quantities of water is a concern for any application that involves exposure to moisture. Consequently, there are significant research opportunities in reducing the water uptake of mycelium composites through substrate selection or modification, physical processing like hot or cold pressing and coatings. On the other hand, the water absorption characteristics of mycelium composites also present research potential as superabsorbent or in the areas of membrane science or filtration. Neat mycelium itself has also been found in several recent studies to have hydrophobic surface properties with potential research into its use in coatings possible.

Notably despite the environmental sustainability of mycelium composites being one of their key advantages over other materials there has also been no life cycle assessment work or investigation of suitable waste streams for mycelium composite production, such as urban wood and food waste, completed. This data is critical from both an academic and commercial perspective if mycelium composites are to be seriously considered for widespread use as a replacement for synthetic foams. Investment in research and commercialisation of mycelium composites is unlikely to occur until their benefit to the environment has been proven. For mainstream use of mycelium composites, research into factors affecting public health, such as the biosafety of the composites and barrier properties, such as water vapour and oxygen transmission, if used for food packaging, must also be investigated. The use of mycelium as an alternative to leather for textile applications is also an area experiencing significant current research interest with commercialisation imminent.

Other applications of particular promise for mycelium stem from the chitin, chitosan and β-glucan polymers it contains. Extraction of these polymers for use in 3D printed structures,

reinforcement for polymer nanocomposites, production of films, sheets and nanopapers opens new doors in the replacement of synthetic polymers across these applications, which can in turn expand the use of mycelium into the realms of most products traditionally made from synthetic polymers, including sports equipment, printed circuit boards and most other consumer products.

References

Abhijith, R, Ashok, A & Rejeesh, CR 2018, 'Sustainable packaging applications from mycelium to substitute polystyrene: a review', *Materials Today: Proceedings*, vol. 5, no. 1, pp. 2139-45.

Alex Fraser Group 2014, *Leading the way in glass recycling*, viewed 12 March 2018 <http://www.alexfraser.com.au/article/News/Leading_the_way_in_glass_recycling>.

Allen Consulting Group 2009, *National Waste Policy Regulatory Impact Statement* viewed 2 March 2017 <https://www.environment.gov.au/system/files/resources/326a2f81-17b0-4bb1-b380-b2e3b20a8941/files/nwp-ris.pdf>.

Allen, HG 2013, *Analysis and Design of Structural Sandwich Panels*, Pergamon Press, Oxford, U.K.

Amich, J, Schafferer, L, Haas, H & Krappmann, S 2013, 'Regulation of Sulphur Assimilation Is Essential for Virulence and Affects Iron Homeostasis of the Human-Pathogenic Mould Aspergillus fumigatus', *PLoS Pathogens*, vol. 9, no. 8, p. e1003573.

Anamika 2015, 'Study on Nutritional Requirements of Nematophagous Fungi in Terms of Carbon and Nitrogen Sources', *Journal of Agricultural Science*, vol. 7, no. 6, p. 227.

Andrady, AL 2015, *Plastics and Environmental Sustainability: Fact and Fiction*, John Wiley & Sons, Hoboken, NJ.

Appels, F, Camere, S, Montalti, M, Karana, E, Jansen, KM, Dijksterhuis, J, Krijgsheld, P & Wösten, HA 2019, 'Fabrication factors influencing mechanical, moisture-and water-related properties of mycelium-based composites', *Materials & Design*, vol. 161, no. 1, pp. 64-71.

Appels, F, Dijksterhuis, J, Lukasiewicz, CE, Jansen, KMB, Wösten, HAB & Krijgsheld, P 2018, 'Hydrophobin gene deletion and environmental growth conditions impact mechanical properties of mycelium by affecting the density of the material', *Scientific Reports*, vol. 8, no. 1, p. 4703.

Arbia, W, Arbia, L, Adour, L & Amrane, A 2013, 'Chitin extraction from crustacean shells using biological methods–a review', *Food Technology and Biotechnology*, vol. 51, no. 1, pp. 12-25.

Arifin, YH & Yusuf, Y 2013, 'Mycelium Fibers as New Resource for Environmental Sustainability', *Procedia Engineering*, vol. 53, no. 1, pp. 504-8.

Asdrubali, F, D'Alessandro, F & Schiavoni, S 2015, 'A review of unconventional sustainable building insulation materials', *Sustainable Materials and Technologies*, vol. 4, no. 1, pp. 1-17.

Ashby, MF, Shercliff, H & Cebon, D 2018, *Materials: Engineering, Science, Processing and Design*, Butterworth-Heinemann, Oxford, U.K.

ASTM International 2004, *Standard Specification for Rigid, Cellular Polystyrene Thermal Insulation,* ASTM C578–04, ASTM International, West Conshohocken, PA.

Austin, J 2013, *Mycotectural Polyominoes*, viewed 17 June 2016 <http://www.zero1.org/exhibitions/about-pp>.

Babrauskas, V & Fires, Q 1997, 'Why was the fire so big HHR: the role of heat release rate in described fires', *Fire & Arson Investigator*, vol. 47, no. 1, pp. 54-7.

Babrauskas, V, Levin, BC, Gann, RG, Paabo, M, Harris, RH, Peacock, RD & Yusa, S 1992, 'Toxic potency measurement for fire hazard analysis', *Fire Technology*, vol. 28, no. 2, pp. 163-7.

Babrauskas, V & Peacock, RD 1992, 'Heat release rate: the single most important variable in fire hazard', *Fire Safety Journal*, vol. 18, no. 3, pp. 255-72.

Baert, K, Devlieghere, F, Bo, L, Debevere, J & De Meulenaer, B 2008, 'The effect of inoculum size on the growth of Penicillium expansum in apples', *Food Microbiology*, vol. 25, no. 1, pp. 212-7.

Bainbridge, DA 1986, 'High performance low cost buildings of straw', *Agriculture, Ecosystems & Environment*, vol. 16, no. 3, pp. 281-4.

Bajwa, DS, Holt, GA, Bajwa, SG, Duke, SE & McIntyre, G 2017, 'Enhancement of termite (Reticulitermes flavipes L.) resistance in mycelium reinforced biofiber-composites', *Industrial Crops and Products*, vol. 107, pp. 420-6.

Baldauf, S, Palmer, J 1993, 'Animals and fungi are each other's closest relatives: Congruent evidence from multiple proteins', *Proceedings of the National Academy of Sciences*, vol. 90, no. 1, pp. 11558-62.

Bamba, Y, Ogawa, Y, Saito, T, Berglund, LA & Isogai, A 2017, 'Estimating the Strength of Single Chitin Nanofibrils via Sonication-Induced Fragmentation', *Biomacromolecules*, vol. 18, no. 12, pp. 4405-10.

Bartnicki-Garcia, S 1968, 'Cell Wall Chemistry, Morphogenesis, and Taxonomy of Fungi', *Annual Review of Microbiology*, vol. 22, no. 1, pp. 87-108.

Bayer, E & McIntyre, G 2012, *Substrate Composition and Method for Growing Mycological Materials*, U.S. Patent 20,120,315,687.

---- 2015, *Method for Growing Mycological Materials*, U.S. Patent 20,150,247,115.

Bell, LH & Bell, DH 1994, *Industrial Noise Control: Fundamentals and Applications*, Marcel Dekker, NY

Bergman, TL, Incropera, FP, DeWitt, DP & Lavine, AS 2011, *Fundamentals of Heat and Mass Transfer*, John Wiley & Sons, Hoboken, NJ

Bhat, T, Chevali, V, Liu, X, Feih, S & Mouritz, A 2015, 'Fire structural resistance of basalt fibre composite', *Composites Part A: Applied Science and Manufacturing*, vol. 71, no. 1, pp. 107-15.

Blackwell, M 2011, 'The Fungi: 1, 2, 3 … 5.1 million species?', *American Journal of Botany*, vol. 98, no. 3, pp. 426-38.

Boddy, L, Marchant, R & Read, DJ 1988, 'Nitrogen, Phosphorus and Sulphur Utilisation by Fungi', *Symposium of the British Mycological Society*, University of Birmingham, U.K., April, 1988.

Boonstra, MJ & Tjeerdsma, B 2006, 'Chemical analysis of heat treated softwoods', *Holz als Roh-und Werkstoff*, vol. 64, no. 3, p. 204.

Bouras, N, Holtz, MD, Aboukhaddour, R & Strelkov, SE 2016, 'Influence of nitrogen sources on growth and mycotoxin production by isolates of Pyrenophora tritici-repentis from wheat', *The Crop Journal*, vol. 4, no. 2, pp. 119-28.

Braun, U, Balabanovich, AI, Schartel, B, Knoll, U, Artner, J, Ciesielski, M, Döring, M, Perez, R, Sandler, JK & Altstädt, V 2006, 'Influence of the oxidation state of phosphorus on the decomposition and fire behaviour of flame-retarded epoxy resin composites', *Polymer*, vol. 47, no. 26, pp. 8495-508.

Breitenbach, J & Kränzlin, F 1986, *Fungi of Switzerland: Non-gilled fungi: Heterobasidiomycetes, Aphyllophorales, Gasteromycetes*, Verlag Mykologia, Luzern, Switzerland.

British Mycological Society 2005, *TG02: Fungal Cells*, viewed 5 May 2017 <https://www.academia.edu/34445716/TG02_Fungal_Cells_A_Teachers_Guide_to>.

Brown, J, Fawell, P & Mathys, Z 1994, 'Fire-hazard assessment of extended-chain polyethylene and aramid composites by cone calorimetry', *Fire and Materials*, vol. 18, no. 3, pp. 167-72.

Bulatov, D 2009, *Evolution Haute Couture: Art and Science in the Post-Biological Age*, Moscow Biennale, viewed 17th June 2016 <http://3rd.moscowbiennale.ru/en/program/special_projects/evolution.html>.

Bultman, JD, Chen, S-L & Schloman, WW 1998, 'Anti-termitic efficacy of the resin and rubber in fractionator overheads from a guayule extraction process', *Industrial Crops and Products*, vol. 8, no. 2, pp. 133-43.

Burford, EP, Hillier, S & Gadd, GM 2006, 'Biomineralization of fungal hyphae with calcite (CaCO3) and calcium oxalate mono-and dihydrate in carboniferous limestone microcosms', *Geomicrobiology Journal*, vol. 23, no. 8, pp. 599-611.

Burge, H 2006, 'How does heat affect fungi?', *The Environmental Report*, vol. 4, no. 3, p. 1.

Buschow, KJ, Cahn, RW, Flemings, MC, Ilschner, B, Kramer, EJ & Mahajan, S 2001, *Encyclopedia of Materials*, Elsevier, Amsterdam, Netherlands.

Butterfield, B, Chapman, K, Christie, L & Dickson, A 1992, 'Ultrastructural characteristics of failure surfaces in medium density fiberboard', *Forest Products Journal*, vol. 42, no. 6, p. 55.

Camere, S & Karana, E 2018, 'Fabricating materials from living organisms: An emerging design practice', *Journal of Cleaner Production*, vol. 186, no. 1, pp. 570-84.

Canevascini, G, Coudray, M-R, Southgate, R & Meier, H 1979, 'Induction and catabolite repression of cellulase synthesis in the thermophilic fungus Sporotrichum thermophile', *Microbiology*, vol. 110, no. 2, pp. 291-303.

Cardona, F, Rogers, D & Van Erp, G 2007, 'Investigation by TGA—FTIR of the Effect of Styrene Content and Thickness on the UV-curing of Vinylester Resins', *Journal of Thermoplastic Composite Materials*, vol. 20, no. 6, pp. 601-15.

Carlile, M, Watkinson, S & Gooday, G 2001, 'Fungal Cells and Vegetative Growth', in *The Fungi*, Academic Press, London, U.K.

Carlile, MJ 1995, 'The Success of the Hypha and Mycelium', in *The Growing Fungus*, Springer, Berlin, Germany.

Carvalho, LMH & Costa, CAV 1998, 'Modelling and simulation of the hot-pressing process in the production of medium density fiberboard (MDF)', *Chemical Engineering Communications*, vol. 170, no. 1, pp. 1-21.

Castagnede, B, Aknine, A, Brouard, B & Tarnow, V 2000, 'Effects of compression on the sound absorption of fibrous materials', *Applied Acoustics*, vol. 61, no. 2, pp. 173-82.

Chand, N & Fahim, M 2008, *Tribology of Natural Fiber Polymer Composites*, Woodhead Publishing, Cambridge, U.K.

Chang, A 1992, *Genetics and Breeding of Edible Mushrooms*, CRC Press, Boca Raton, U.S.A.

Chipeta, ZA, du Preez, JC & Christopher, L 2008, 'Effect of cultivation pH and agitation rate on growth and xylanase production by Aspergillus oryzae in spent sulphite liquor', *Journal of Industrial Microbiology and Biotechnology*, vol. 35, no. 6, pp. 587-94.

Cho, JH, Vasagar, V, Shanmuganathan, K, Jones, AR, Nazarenko, S & Ellison, CJ 2015, 'Bioinspired catecholic flame retardant nanocoating for flexible polyurethane foams', *Chemistry of Materials*, vol. 27, no. 19, pp. 6784-90.

Choudhury, AKR 2017, *Principles of Textile Finishing*, Woodhead Publishing, Cambridge, U.K.

Chung, S, Suidan, MT & Venosa, AD 2011, 'Partially acetylated sugarcane bagasse for wicking oil from contaminated wetlands', *Chemical Engineering & Technology*, vol. 34, no. 12, pp. 1989-96.

Clarke, FB 1997, 'Physiological effects of smoke: Managing escape', *ASHRAE journal*, vol. 39, no. 3, p. 47.

Clegg, CJ & Mackean, DG 1994, *Advanced Biology: Principles and Applications*, John Murray, London, U.K.

Clémençon, H & Emmett, V 2004, *Cytology and Plectology of the Hymenomycetes*, Schweizerbart Science Publishers, Stuttgart, Germany.

Collet, F & Prétot, S 2014, 'Thermal conductivity of hemp concretes: Variation with formulation, density and water content', *Construction and Building Materials*, vol. 65, no. 1, pp. 612-9.

Conkey, WH, Van Horn, WM & Shema, BFS, W.H. 1957, *Sheets comprising filaments of fungi*, U.S. Patent 2,811,442.

Corner, EJH 1953, 'The construction of polypores', in *Phytomorphology*, vol. 1. Introduction to Polyporus sulphureus, P. squamosus, P. betulinus and Polystictus microcyclus.

Crompton, TR 2012, *Physical testing of plastics*, Smithers Rapra Technology Ltd., Shropshire, U.K.

Cunha, AG, Fernandes, SCM, Freire, CSR, Silvestre, AJD, Neto, CP & Gandini, A 2008, 'What Is the Real Value of Chitosan's Surface Energy?', *Biomacromolecules*, vol. 9, no. 2, pp. 610-4.

Cunha, AG & Gandini, A 2010, 'Turning polysaccharides into hydrophobic materials: a critical review. Part 2. Hemicelluloses, chitin/chitosan, starch, pectin and alginates', *Cellulose*, vol. 17, no. 6, pp. 1045-65.

Dai, C, Yu, C & Zhou, X 2007, 'Heat and mass transfer in wood composite panels during hot pressing. Part II. Modeling void formation and mat permeability', *Wood and Fiber Science*, vol. 37, no. 2, pp. 242-57.

Defonseka, C 2014, *Introduction to Polymeric Composites with Rice Hulls*, Smithers Information Ltd., OH.

Dell Inc. 2016, *Green Packaging & Shipping*, viewed 21 July 2016 <http://www.dell.com/learn/us/en/uscorp1/corp-comm/mushroom-packaging>.

Dell, K 2010, *The Promise and Pitfalls of Bioplastic*, viewed 7 June 2016 <http://content.time.com/time/magazine/article/0,9171,1983894,00.html>.

Di Mario, F, Rapana, P, Tomati, U & Galli, E 2008, 'Chitin and chitosan from Basidiomycetes', *International Journal of Biological Macromolecules*, vol. 43, no. 1, pp. 8-12.

Dix, NJ & Webster, J 1995, 'Fungi of Extreme Environments', in *Fungal Ecology*, Springer, Berlin, Germany.

DP CleanTech 2013, *Understanding rice husk as a biomass fuel*, viewed 14 February 2017 <http://www.dpcleantech.com/files/Understanding-rice-husk-as-a-biomass-fuel-EN-V1-2013.9.4.pdf>.

Ecovative Design 2016, *Press Kit*, viewed 12 May 2016 <http://www.ecovativedesign.com/press-kit>.

Edebo, L 2002, *Porous structure comprising fungi cell walls*, U.S. Patent 6,423,337.

Elsacker, E, Vandelook, S, Brancart, J, Peeters, E & De Laet, L 2019, 'Mechanical, physical and chemical characterisation of mycelium-based composites with different types of lignocellulosic substrates', *PLOS ONE*, vol. 4, no. 7, p. e0213954.

European Commission 2014, *Communication from the commission to the European Parliament, the Council, the European Economic and Social Committee of the Regions on Resource Efficiency Opportunities in the Building Sector*, viewed 12 March 2016 <http://ec.europa.eu/environment/eussd/pdf/SustainableBuildingsCommunication.pdf>.

Everett-Green, R 2014, *A growth industry: How mushrooms are being used to create furniture*, viewed 15 February 2016 <https://www.theglobeandmail.com/life/home-and-garden/design/a-growth-industry-how-mushrooms-are-being-used-to-create-furniture/article17573415/>.

Faruk, O, Bledzki, AK, Fink, H-P & Sain, M 2012, 'Biocomposites reinforced with natural fibers: 2000–2010', *Progress in Polymer Science*, vol. 37, no. 11, pp. 1552-96.

Feinberg, DR, Jones, BC, Little, AC, Burt, DM & Perrett, DI 2005, 'Manipulations of fundamental and formant frequencies influence the attractiveness of human male voices', *Animal Behaviour*, vol. 69, no. 3, pp. 561-8.

Finlay, R, Frostegård, Å & Sonnerfeldt, AM 1992, 'Utilization of organic and inorganic nitrogen sources by ectomycorrhizal fungi in pure culture and in symbiosis with Pinus contorta Dougl. ex Loud', *New Phytologist*, vol. 120, no. 1, pp. 105-15.

Fisher, A 2010, *Industrial-Strength Fungus*, viewed 12 March 2016 <http://content.time.com/time/magazine/article/0,9171,1957474,00.html>.

Fondazione PLART 2014, *The Future of Plastic*, viewed 22 July 2016 <http://www.fondazioneplart.it/en-us/mostre/the_future_of_plastic.aspx>.

Fox, DM, Lee, J, Citro, CJ & Novy, M 2013, 'Flame retarded poly (lactic acid) using POSS-modified cellulose. 1. Thermal and combustion properties of intumescing composites', *Polymer Degradation and Stability*, vol. 98, no. 2, pp. 590-6.

France, RC & Reid, CP 1984, 'Pure culture growth of ectomycorrhizal fungi on inorganic nitrogen sources', *Microbial Ecology*, vol. 10, no. 3, pp. 187-95.

Garraway, MO & Evans, RC 1984, *Fungal Nutrition and Physiology*, John Wiley & Sons, Hoboken, NJ.

Geyer, R, Jambeck, JR & Law, KL 2017, 'Production, use, and fate of all plastics ever made', *Science Advances*, vol. 3, no. 7, p. e1700782.

Ghazvinian, A, Farrokhsiar, P, Vieira, F, Pecchia, J & Gursoy, B 2019, 'Mycelium-Based Bio-Composites For Architecture: Assessing the Effects of Cultivation Factors on Compressive Strength', *The eCAADe and SIGraDi Conference*, University of Porto, Portugal, 11-13 September 2019.

Gilwee, W 1975, 'Fire retardant materials in aviation', *4th Annual Plastics Symposium*, 29 May 1975.

González, HHL, Resnik, SL & Vaamonde, G 1987, 'Influence of inoculum size on growth rate and lag phase of fungi isolated from Argentine corn', *International Journal of Food Microbiology*, vol. 4, no. 2, pp. 111-7.

---- 1988, 'Influence of temperature on growth rate and lag phase of fungi isolated from Argentine corn', *International Journal of Food Microbiology*, vol. 6, no. 2, pp. 179-83.

Gosden, E 2016, *Ikea plans mushroom-based packaging as eco-friendly replacement for polystyrene*, viewed 12 May 2016 <http://www.telegraph.co.uk/news/earth/businessandecology/recycling/12172439/Ikea-plans-mushroom-based-packaging-as-eco-friendly-replacement-for-polystyrene.html>.

Gougouli, M, Kalantzi, K, Beletsiotis, E & Koutsoumanis, KP 2011, 'Development and application of predictive models for fungal growth as tools to improve quality control in yogurt production', *Food Microbiology*, vol. 28, no. 8, pp. 1453-62.

Green Construction Board 2013, *Low carbon routemap for the UK built environment*, viewed 23 May 2016 <https://www.greenconstructionboard.org/otherdocs/Routemap%20final%20report%2005032013.pdf>.

Green, DW, Winandy, JE & Kretschmann, DE 1999, *Mechanical properties of wood*, Wood handbook: wood as an engineering material, USDA Forest Service, WI.

Griffin, D, Timberlake, W & Cheney, J 1974, 'Regulation of macromolecular synthesis, colony development, and specific growth rate of Achlya bisexualis during balanced growth', *Microbiology*, vol. 80, no. 2, pp. 381-8.

Griffin, DH 1996, *Fungal Physiology*, John Wiley & Sons, Hoboken, NJ.

Gross, S & Robbins, EI 2000, 'Acidophilic and acid-tolerant fungi and yeasts', *Hydrobiologia*, vol. 433, no. 1, pp. 91-109.

Guillebeau, LP, Hinkle, N & Roberts, P 2008, *Summary of losses from insect damage and cost of control in Georgia 2006*, viewed 1 October 2019 <https://pdfs.semanticscholar.org/f531/e6dc905fd2a1bb7efe82b80a87fea0ab5aa5.pdf?_ga=2.35011745.1050984241.1570712157-1414794384.1566368534>.

Gunther, M 2013, *Can mushrooms replace plastic?*, viewed 12 May 2016 <http://www.theguardian.com/sustainable-business/mushrooms-new-plastic-ecovative>.

Guo, Z, Liang, X, Pereira, T, Scaffaro, R & Hahn, HT 2007, 'CuO nanoparticle filled vinyl-ester resin nanocomposites: Fabrication, characterization and property analysis', *Composites Science and Technology*, vol. 67, no. 10, pp. 2036-44.

Haneef, M, Ceseracciu, L, Canale, C, Bayer, IS, Heredia-Guerrero, JA & Athanassiou, A 2017, 'Advanced Materials From Fungal Mycelium: Fabrication and Tuning of Physical Properties', *Scientific Reports*, vol. 7, no. 1, p. 41292.

Harris, SD 2008, 'Branching of fungal hyphae: regulation, mechanisms and comparison with other branching systems', *Mycologia*, vol. 100, no. 6, pp. 823-32.

Harvey, M & Pilgrim, S 2011, 'The new competition for land: Food, energy, and climate change', *Food Policy*, vol. 36, no. 1, pp. S40-S51.

Hassainia, A, Satha, H & Boufi, S 2018, 'Chitin from Agaricus bisporus: Extraction and characterization', *International Journal of Biological Macromolecules*, vol. 117, no. 1, pp. 1334-42.

Hassan, N, Rafiq, M, Hayat, M, Shah, AA & Hasan, F 2016, 'Psychrophilic and psychrotrophic fungi: a comprehensive review', *Reviews in Environmental Science and Bio/Technology*, vol. 15, no. 2, pp. 147-72.

Hawksworth, DL 2001, 'The magnitude of fungal diversity: the 1.5 million species estimate revisited', *Mycological Research*, vol. 105, no. 12, pp. 1422-32.

He, J, Cheng, CM, Su, DG & Zhong, MF 2014, 'Study on the Mechanical Properties of the Latex-Mycelium Composite', *Applied Mechanics and Materials*, vol. 507, no. 1, pp. 415-20.

Hemond, CJ 1983, *Engineering Acoustics and Noise Control*, Prentice Hall, NJ.

Hibbett, DS, Binder, M, Bischoff, JF, Blackwell, M, Cannon, PF, Eriksson, OE, Huhndorf, S, James, T, Kirk, PM, Lücking, R, Thorsten Lumbsch, H, Lutzoni, F, Matheny, PB, McLaughlin, DJ, Powell, MJ, Redhead, S, Schoch, CL, Spatafora, JW, Stalpers, JA, Vilgalys, R, Aime, MC, Aptroot, A, Bauer, R, Begerow, D, Benny, GL, Castlebury, LA, Crous, PW, Dai, Y-C, Gams, W, Geiser, DM, Griffith, GW, Gueidan, C, Hawksworth, DL, Hestmark, G, Hosaka, K, Humber, RA, Hyde, KD, Ironside, JE, Kõljalg, U, Kurtzman, CP, Larsson, K-H, Lichtwardt, R, Longcore, J, Miądlikowska, J, Miller, A, Moncalvo, J-M, Mozley-Standridge, S, Oberwinkler, F, Parmasto, E, Reeb, V, Rogers, JD, Roux, C, Ryvarden, L, Sampaio, JP, Schüßler, A, Sugiyama, J, Thorn, RG, Tibell, L, Untereiner, WA, Walker, C, Wang, Z, Weir, A, Weiss, M, White, MM, Winka, K, Yao, Y-J & Zhang, N 2007, 'A higher-level phylogenetic classification of the Fungi', *Mycological Research*, vol. 111, no. 5, pp. 509-47.

Hirschler, MM 1987, 'Fire hazard and toxic potency of the smoke from burning materials', *Journal of Fire Sciences*, vol. 5, no. 5, pp. 289-307.

Holt, G, Mcintyre, G, Flagg, D, Bayer, E, Wanjura, J & Pelletier, M 2012, 'Fungal mycelium and cotton plant materials in the manufacture of biodegradable molded packaging material: Evaluation study of select blends of cotton byproducts', *Journal of Biobased Materials and Bioenergy*, vol. 6, no. 4, pp. 431-9.

Hong, B-W 1984, 'Dynamic viscoelasticity of hot pressed wood', *Journal of the Korean Wood Science and Technology*, vol. 12, no. 4, pp. 3-10.

Horikoshi, K 1999, 'Alkaliphiles: Some Applications of Their Products for Biotechnology', *Microbiology and Molecular Biology Reviews*, vol. 63, no. 4, pp. 735-50.

Housseinpour, R, Latibari, AJ, Farnood, R, Fatehi, P & Sepiddehdam, SJ 2010, 'Fiber morphology and chemical composition of rapeseed (Brassica napus) stems', *IAWA Journal*, vol. 31, no. 4, pp. 457-64.

Hshieh, FY 1998, 'Shielding effects of silica-ash layer on the combustion of silicones and their possible applications on the fire retardancy of organic polymers', *Fire and Materials*, vol. 22, no. 2, pp. 69-76.

Ifuku, S 2014, 'Chitin and chitosan nanofibers: Preparation and chemical modifications', *Molecules*, vol. 19, no. 11, pp. 18367-80.

Islam, M, Tudryn, G, Bucinell, R, Schadler, L & Picu, R 2017, 'Morphology and mechanics of fungal mycelium', *Scientific Reports*, vol. 7, no. 1, p. 13070.

---- 2018, 'Mechanical behavior of mycelium-based particulate composites', *Journal of Materials Science*, vol. 53, no. 24, pp. 16371-82.

Izydorek, MS, Samuels, MD, Zeeveld, PA & Smyser, JP 2008, *Report on structural stability of engineered lumber in fire conditions*, viewed 25 May 2018 <https://ulfirefightersafety.org/docs/NC9140-20090512-Report-Independent.pdf>.

Jerman, M, Keppert, M, Výborný, J & Černý, R 2013, 'Hygric, thermal and durability properties of autoclaved aerated concrete', *Construction and Building Materials*, vol. 41, no. 1, pp. 352-9.

Ji, Y, Zhu, K, Qian, H & Zhou, H 2007, 'Effect of water activity and temperature on growth of Penicillium citreoviride and Penicillium citrinum on MiGao (rice cake)', *Canadian Journal of Microbiology*, vol. 53, no. 2, pp. 231-6.

Jiang, L, Walczyk, D & McIntyre, G 2014, 'A New Process for Manufacturing Biocomposite Laminate and Sandwich Parts using Mycelium as a Binder', *American Society for Composites 29th Technical Conference*, Charlotte, NC, August 10-15 2014.

Jiang, L, Walczyk, D, McIntyre, G, Bucinell, R & Li, B 2019, 'Bioresin infused then cured mycelium-based sandwich-structure biocomposites: Resin transfer molding (RTM) process, flexural properties, and simulation', *Journal of Cleaner Production*, vol. 207, no. 1, pp. 123-35.

Jiang, L, Walczyk, D, McIntyre, G, Bucinell, R & Tudryn, G 2017, 'Manufacturing of biocomposite sandwich structures using mycelium-bound cores and preforms', *Journal of Manufacturing Processes*, vol. 28, no. 1, pp. 50-9.

Jiang, L, Walczyk, D, Mooney, L & Putney, S 2013, 'Manufacturing of Mycelium-based Biocomposites', *International SAMPE Technical Conference*, Covina, CA, October 29-31 2013.

Jones, M, Bhat, T, Huynh, T, Kandare, E, Yuen, R, Wang, C & John, S 2018, 'Waste-derived low-cost mycelium composite construction materials with improved fire safety', *Fire and Materials*, vol. 42, no. 7, pp. 816-25.

Jones, M, Bhat, T, Kandare, E, Thomas, A, Joseph, P, Dekiwadia, C, Yuen, R, John, S, Ma, J & Wang, C 2018, 'Thermal Degradation and Fire Properties of Fungal Mycelium and Mycelium-Biomass Composite Materials', *Scientific Reports*, vol. 8, no. 1, p. 17583.

Jones, M, Huynh, T, Dekiwadia, C, Daver, F & John, S 2017, 'Mycelium Composites: A Review of Engineering Characteristics and Growth Kinetics', *Journal of Bionanoscience*, vol. 11, no. 4, pp. 241-57.

Jones, M, Huynh, T & John, S, 2018, 'Inherent Species Characteristic Influence and Growth Performance Assessment for Mycelium Composite Applications', *Advanced Materials Letters*, vol. 9, no. 1, pp. 71-80.

Jones, M, Lawrie, A, Huynh, T, Morrison, P, Mautner, A, Bismarck, A & John, S 2019, 'Agricultural By-product Suitability for the Production of Chitinous Composites and Nanofibers', *Process Biochemistry*, vol. 80, pp. 95-102.

Jones, M, Weiland, K, Kujundzic, M, Theiner, J, Kahlig, H, Kontturi, E, John, S, Bismarck, A & Mautner, A 2019, 'Waste-Derived Low-Cost Mycelium Nanopapers with Tunable Mechanical and Surface Properties', *Biomacromolecules*, vol. 20, no. 9, pp. 3513-23.

Kadoya, K, Matsunaga, N & Nagashima, A 1985, 'Viscosity and thermal conductivity of dry air in the gaseous phase', *Journal of Physical and Chemical Reference Data*, vol. 14, no. 4, pp. 947-70.

Kalisz, RE & Rocco, CA 2011, *Cut sheet stock mycelium and method*, U.S. Patent 20,110,266,831.

---- 2012a, *Method of making a hardened elongate structure from mycelium*, U.S. Patent 8,298,809.

---- 2012b, *Method of making foamed mycelium structure*, U.S. Patent 8,227,233.

---- 2012c, *Method of making molded part comprising mycelium coupled to mechanical device*, U.S. Patent 8,227,224.

Kalisz, RE, Rocco, CA, Tengler, ECJ & Petrella-Lovasik, RL 2012, *Injection molded mycelium and method*, U.S. Patent 8,313,939.

Karana, E, Blauwhoff, D, Hultink, E & Camere, S 2018, 'When the material grows: A case study on designing (with) mycelium-based materials', *International Journal of Design*, vol. 12, no. 2, pp. 119-36.

Karch, C 2008, 'Water and Fungi', *The Environmental Reporter*, vol. 6, no. 2, p. 1.

Kavanagh, K 2005, *Fungi: Biology and Applications*, John Wiley & Sons, Hoboken, NJ.

Khokhar, Z-u, Athar, MA, Syed, Q-u-A, Baig, S, Nadeem, M, Sher, MG, Ali, S & Islam, I 2010, 'Studies on the development of the value added food by barley with Rhizopus oligosporus in koji fermentation', *Science International*, vol. 22, no. 3, p. 226.

Kim, B & Christensen, RM 2000, 'Basic two-dimensional core types for sandwich structures', *International Journal of Mechanical Sciences*, vol. 42, no. 4, pp. 657-76.

Klarenbeek, E 2014, *Designer of the Unusual*, viewed 2 May 2016 <http://ericklarenbeek.com/>.

Koizumi, T, Tsujiuchi, N & Adachi, A 2002, 'The development of sound absorbing materials using natural bamboo fibers', *WIT Transactions on The Built Environment*, vol. 59, no. 1, p. 1.

Kotov, V & Reshetnikov, SV 1990, 'A stochastic model for early mycelial growth', *Mycological Research*, vol. 94, no. 5, pp. 577-86.

Koza, CR, Norton, GA & van Leeuwen, JH 2017, 'Dewatering investigations on fungal biomass grown in thin stillage from a dry-mill corn ethanol plant', *Biomass and Bioenergy*, vol. 97, no. 1, pp. 65-9.

Krauke, Y & Sychrova, H 2010, 'Four Pathogenic Candida Species Differ in Salt Tolerance', *Current Microbiology*, vol. 61, no. 4, pp. 335-9.

Kung'u, J 2016, *The Phases of Fungal Growth in Indoor Environment*, viewed 25 August 2016 <http://www.moldbacteria.com/mold/the-phases-of-fungal-growth-in-indoor-environment.html>.

Kymäläinen, H-R & Sjöberg, A-M 2008, 'Flax and hemp fibres as raw materials for thermal insulations', *Building and Environment*, vol. 43, no. 7, pp. 1261-9.

Larpent, JP 1966, 'Caracteres et determinismes des correlations d'inhibition dans le mycelium jeune de quelques champignons', *Annales des Sciences Naturelles*, vol. 7, no. 1, p. 130.

Lelivelt, RJJ 2015, 'The mechanical possibilities of mycelium materials', Master thesis, Eindhoven University of Technology, Eindhoven, Netherlands.

Li, MM, Pan, HC, Huang, SL & Scholz, M 2013, 'Controlled experimental study on removing diesel oil spillages using agricultural waste products', *Chemical Engineering & Technology*, vol. 36, no. 4, pp. 673-80.

Li, ZJ, Shukla, V, Wenger, K, Fordyce, A, Pedersen, A & Marten, M 2002, 'Estimation of hyphal tensile strength in production-scaleAspergillus oryzae fungal fermentations', *Biotechnology and Bioengineering*, vol. 77, no. 1, pp. 601-13.

Liao, W, Liu, Y, Frear, C & Chen, S 2008, 'Co-production of fumaric acid and chitin from a nitrogen-rich lignocellulosic material–dairy manure–using a pelletized filamentous fungus Rhizopus oryzae ATCC 20344', *Bioresource Technology*, vol. 99, no. 13, pp. 5859-66.

Liu, J & Chow, W 2014, 'Study on flashover in small residential units with an open kitchen by nonlinear dynamics', *10th International Conference on Heat Transfer, Fluid Mechanics and Thermodynamics*, Orlando, FL, 14-16 January 2014.

Logan, J & Buckley, D 1991, *Subterranean termite control in buildings*, The Chemistry of Wood Preservation, Elsevier, Amsterdam, Netherlands.

López Nava, JA, Méndez González, J, Ruelas Chacón, X & Nájera Luna, JA 2016, 'Assessment of Edible Fungi and Films Bio-Based Material Simulating Expanded Polystyrene', *Materials and Manufacturing Processes*, vol. 31, no. 8, pp. 1085-90.

Lysek, G 1984, 'The Ecology and Physiology of the Fungal Mycelium', *Symposium of the British Mycological Society*, Bath University, 11-15 April 1983.

Maheshwari, R 2016, *Fungi: Experimental Methods in Biology*, CRC Press, Boca Raton, FL.

Maheshwari, R, Bharadwaj, G & Bhat, MK 2000, 'Thermophilic Fungi: Their Physiology and Enzymes', *Microbiology and Molecular Biology Reviews*, vol. 64, no. 3, pp. 461-88.

Mandel, TF & Teige, PJ 2019, *The Context for a Transition to a Renewable Resource Base*, Renewable Natural Resources: A Management Handbook For The Eighties, CRC Press, Boca Raton, FL.

MatWeb 2018, *Flexural Strength Testing of Plastics*, viewed 30 April 2019 <http://www.matweb.com/reference/flexuralstrength.aspx>.

McGonigle, TP, Hovius, JP & Peterson, RL 1999, 'Arbuscular mycorrhizae of American ginseng (Panax quinquefolius) in cultivated field plots: plant age affects the development of a colonization lag phase', *Canadian Journal of Botany*, vol. 77, no. 7, pp. 1028-34.

McGonigle, TP & Miller, MH 1993, 'Mycorrhizal Development and Phosphorus Absorption in Maize under Conventional and Reduced Tillage', *Soil Science Society of America Journal*, vol. 57, no. 4, pp. 1002-6.

McIntyre, G, Poetzsch, A, Van Hook, S & Flagg, D 2012, *Method for producing a composite material*, U.S. Patent 20,120,225,471.

Melville, H 1986, 'Introduction', in *Fire and Cellular Polymers*, Springer, Berlin, Germany.

Meyrath, J 1963, 'Influence of the size of inoculum on various growth phases in Aspergillus oryzae', *Antonie Van Leeuwenhoek*, vol. 29, no. 1.

Miao, Z, Xingna, L, Zhen, C, Ji, W & Wenhua, S 2014, 'Experimental Study of the Heat Flux Effect on Combustion Characteristics of Commonly Exterior Thermal Insulation Materials', *Procedia Engineering*, vol. 84, no. 1, pp. 578-85.

Michniewicz, J, Biliaderis, G & Bushuk, W 1992, 'Effect of added pentosans on some properties of wheat bread', *Food Chemistry*, vol. 43, no. 4, pp. 251-7.

Mille-Lindblom, C, von Wachenfeldt, E & Tranvik, LJ 2004, 'Ergosterol as a measure of living fungal biomass: persistence in environmental samples after fungal death', *Journal of Microbiological Methods*, vol. 59, no. 2, pp. 253-62.

Mitchell, DA, von Meien, OF, Krieger, N & Dalsenter, FDH 2004, 'A review of recent developments in modeling of microbial growth kinetics and intraparticle phenomena in solid-state fermentation', *Biochemical Engineering Journal*, vol. 17, no. 1, pp. 15-26.

Mohanty, AK, Misra, M & Drzal, LT 2005, *Natural Fibers, Biopolymers, and Biocomposites*, CRC Press, Boca Raton, FL.

Mokhothu, TH & John, MJ 2017, 'Bio-based coatings for reducing water sorption in natural fibre reinforced composites', *Scientific Reports*, vol. 7, no. 1, p. 13335.

Mouritz, A, Mathys, Z & Gibson, A 2006, 'Heat release of polymer composites in fire', *Composites Part A: Applied Science and Manufacturing*, vol. 37, no. 7, pp. 1040-54.

Mouritz, AP & Gibson, AG 2007, *Fire properties of polymer composite materials*, Springer, Berlin, Germany.

Moussout, H, Ahlafi, H, Aazza, M & Bourakhouadar, M 2016, 'Kinetics and mechanism of the thermal degradation of biopolymers chitin and chitosan using thermogravimetric analysis', *Polymer Degradation and Stability*, vol. 130, no. 1, pp. 1-9.

MycoWorks 2014a, *Mycotecture*, viewed 17 June 2016 <http://www.mycoworks.com/portfolio/mycotecture/>.

---- 2014b, *Portfolio*, viewed 2 May 2016 <http://www.mycoworks.com/>.

Nagadesi, P & Arya, A 2013, 'Effect of carbon and nitrogen on the growth of lignicolous fungi from Rathanmahal Wildlife Sanctuary, Gujarat, India', *International Journal of Science and Nature*, vol. 4, no. 2, pp. 237-45.

Nawawi, W 2016, 'Renewable Chitin Based Nanomaterials from Fungi', Doctor of Philosophy thesis, Imperial College London, London, U.K.

Nawawi, W, Jones, M, Murphy, RJ, Lee, K-Y, Kontturi, E & Bismarck, A 2019, 'Nanomaterials derived from fungal sources - is it the new hype?', *Biomacromolecules*, <10.1021/acs.biomac.9b01141>.

Nawawi, W, Lee, K-Y, Kontturi, E, Murphy, R & Bismarck, A 2019, 'Chitin nanopaper from mushroom extract: natural composite of nanofibres and glucan from a single bio-based source', *ACS Sustainable Chemistry & Engineering*, vol. 7, no. 7, pp. 6492-6.

Owren, MJ, Berkowitz, M & Bachorowski, J-A 2007, 'Listeners judge talker sex more efficiently from male than from female vowels', *Perception & Psychophysics*, vol. 69, no. 6, pp. 930-41.

Pan, H, Wang, W, Pan, Y, Song, L, Hu, Y & Liew, KM 2015, 'Formation of self-extinguishing flame retardant biobased coating on cotton fabrics via Layer-by-Layer assembly of chitin derivatives', *Carbohydrate Polymers*, vol. 115, no. 1, pp. 516-24.

Papadopoulos, AM 2005, 'State of the art in thermal insulation materials and aims for future developments', *Energy and Buildings*, vol. 37, no. 1, pp. 77-86.

Pardo, E, Lagunas, U, Sanchis, V, Ramos, AJ & Marín, S 2005, 'Influence of water activity and temperature on conidial germination and mycelial growth of ochratoxigenic isolates of Aspergillus ochraceus on grape juice synthetic medium. Predictive models', *Journal of the Science of Food and Agriculture*, vol. 85, no. 10, pp. 1681-6.

Parker, J & Kourtides, D 1983, 'New fireworthy composites for use in transportation vehicles', *Journal of Fire Sciences*, vol. 1, no. 6, pp. 432-58.

Peacock, AJ 1998, 'Oxygen at high altitude', *British Medical Journal*, vol. 317, no. 7165, pp. 1063-6.

Pegler, DN 1996, 'Hyphal analysis of basidiomata', *Mycological Research*, vol. 100, no. 2, pp. 129-42.

Pelletier, MG, Holt, GA, Wanjura, JD, Bayer, E & McIntyre, G 2013, 'An evaluation study of mycelium based acoustic absorbers grown on agricultural by-product substrates', *Industrial Crops and Products*, vol. 51, no. 1, pp. 480-5.

Pelletier, MG, Holt, GA, Wanjura, JD, Greetham, L, McIntyre, G, Bayer, E & Kaplan-Bie, J 2019, 'Acoustic evaluation of mycological biopolymer, an all-natural closed cell foam alternative', *Industrial Crops and Products*, vol. 139, no. 1, p. 111533.

Petersen, SO, Frohne, PS & Kennedy, AC 2002, 'Dynamics of a Soil Microbial Community under Spring Wheat', *Soil Science Society of America Journal*, vol. 66, no. 3, pp. 826-33.

Pigman, W 2012, *The Carbohydrates: Chemistry, Biochemistry, Physiology*, Elsevier, Amsterdam, Netherlands.

Pitt, JI & Hocking, AD 2009, 'Naming and Classifying Fungi', in *Fungi and Food Spoilage*, Springer, Berlin, Germany.

Pongrácz, P, Molnár, C & Miklósi, Á 2006, 'Acoustic parameters of dog barks carry emotional information for humans', *Applied Animal Behaviour Science*, vol. 100, no. 3, pp. 228-40.

Prosser, J 1995, 'Kinetics of filamentous growth and branching', in *The Growing Fungus*, Springer, Berlin, Germany.

Prosser, JI 1993, 'Growth kinetics of mycelial colonies and aggregates of ascomycetes', *Mycological Research*, vol. 97, no. 5, pp. 513-28.

Prosser, JI & Trinci, AP 1979, 'A model for hyphal growth and branching', *Journal of General Microbiology*, vol. 111, no. 1, pp. 153-64.

Pruteanu, M 2010, 'Investigations regarding the thermal conductivity of straw', *Buletinul Institutului Politehnic din Iasi. Sectia Constructii, Arhitectura*, vol. 56, no. 3, p. 9.

Rajagopal, A 2014, *Behind The Living's "100% Organic" Pavilion for MoMA PS1*, viewed 5 March 2016 <https://www.metropolismag.com/technology/behind-livings-100-organic-pavilion-moma-ps1/>.

Rathmann, R, Szklo, A & Schaeffer, R 2010, 'Land use competition for production of food and liquid biofuels: An analysis of the arguments in the current debate', *Renewable Energy*, vol. 35, no. 1, pp. 14-22.

Raven, PH & Johnson, GB 1999, *Biology*, McGraw-Hill, New York City, NY.

Razak, MA, Pinjari, AB, Begum, PS & Viswanath, B 2018, 'Biotechnological Production of Fungal Biopolymers Chitin and Chitosan: Their Potential Biomedical and Industrial Applications', *Current Biotechnology*, vol. 7, no. 3, pp. 214-30.

Read, ND, Fleißner, A, Roca, MG & Glass, NL 2010, 'Hyphal fusion', in *Cellular and Molecular Biology of Filamentous Fungi*, ASM Press, Sterling, VA.

Reid, DG, Mason, MJ, Chan, BK & Duer, MJ 2012, 'Characterization of the phosphatic mineral of the barnacle Ibla cumingi at atomic level by solid-state nuclear magnetic resonance: comparison with other phosphatic biominerals', *Journal of the Royal Society Interface*, vol. 9, no. 72, pp. 1510-6.

Ren, M & Jacobsen, F 1993, 'A method of measuring the dynamic flow resistance and reactance of porous materials', *Applied Acoustics*, vol. 39, no. 4, pp. 265-76.

Renneckar, S, Zink-Sharp, AG, Ward, TC & Glasser, WG 2004, 'Compositional analysis of thermoplastic wood composites by TGA', *Journal of Applied Polymer Science*, vol. 93, no. 3, pp. 1484-92.

Repeèkienë, J 2001, 'Influence of phosphorous fertilizers on fungial growth and oil hydrocarbon degradation', *Bone*, vol. 1, no. 16, p. 1.

Rinaudo, M 2007, 'Chitin and Chitosan—General Properties and Applications', *ChemInform*, vol. 38, no. 7, pp. 603-32.

Robinson, P & Park, D 1966, 'Volatile inhibitors of spore germination produced by fungi', *Transactions of the British Mycological Society*, vol. 49, no. 4, pp. 639-49.

Rocco, CA & Kalisz, RE 2012a, *Mycelium structure with self-attaching coverstock and method*, U.S. Patent 8,298,810.

---- 2012b, *Mycelium structures containing nanocomposite materials and method*, U.S. Patent 8,283,153.

---- 2012c, *Plasticized mycelium composite and method*, U.S. Patent 8,227,225.

Rockland, L 1987, *Water Activity: Theory and Applications to Food*, Taylor & Francis, Oxford, U.K.

Roylance, D 2000, *Stresses in beams*, Massachusetts Institute of Technology, Cambridge, MA.

Ruxanda, B, Alice Teacă, C & Spiridon, I 2008, 'Chemical modification of beech wood: Effect on thermal stability', *Bioresources*, vol. 3, no. 3, pp. 789-800.

Samapundo, S, Devlieghere, F, De Meulenaer, B & Debevere, J 2007, 'Growth kinetics of cultures from single spores of Aspergillus flavus and Fusarium verticillioides on yellow dent corn meal', *Food Microbiology*, vol. 24, no. 4, pp. 336-45.

Sandberg, U 2003, 'The multi-coincidence peak around 1000 Hz in tyre/road noise spectra', *Euronoise*, Naples, Italy, 19-21 May 2003.

Sautour, M, Dantigny, P, Guilhem, MC & Bensoussan, M 2003, 'Influence of inoculum preparation on the growth of Penicillium chrysogenum', *Journal of Applied Microbiology*, vol. 95, no. 5, pp. 1034-8.

Seddeq, HS 2009, 'Factors influencing acoustic performance of sound absorptive materials', *Australian Journal of Basic and Applied Sciences*, vol. 3, no. 4, pp. 4610-7.

Sexton, AC & Howlett, BJ 2006, 'Parallels in fungal pathogenesis on plant and animal hosts', *Eukaryotic Cell*, vol. 5, no. 12, pp. 1941-9.

Singh, OV 2012, *Extremophiles: Sustainable Resources and Biotechnological Implications*, John Wiley & Sons, Hoboken, NJ.

Sinsabaugh, RS 1994, 'Enzymic analysis of microbial pattern and process', *Biology and Fertility of Soils*, vol. 17, no. 1, pp. 69-74.

Sistrom, DE & Machlis, L 1955, 'The effect of D-glucose on the utilization of D-mannose and D-fructose by a filamentous fungus', *Journal of Bacteriology*, vol. 70, no. 1, pp. 50-5.

Siu, RGH & Sinden, JW 1951, 'Effects of pH, Temperature, and Mineral Nutrition on Cellulolytic Fungi', *American Journal of Botany*, vol. 38, no. 4, pp. 284-90.

Sombatsompop, N & Chaochanchaikul, K 2004, 'Effect of moisture content on mechanical properties, thermal and structural stability and extrudate texture of poly(vinyl chloride)/wood sawdust composites', *Polymer International*, vol. 53, no. 9, pp. 1210-8.

Sorathia, U, Divisjón, C & Lyon, R 1997, 'A review of fire test methods and criteria for composites', *SAMPE Journal*, vol. 33, no. 4, pp. 23-31.

St. Pierre, R, Maguire, DJ & Automotive, CS 2004, 'The impact of A-weighting sound pressure level measurements during the evaluation of noise exposure', *NOISE-CON*, Baltimore, MD, 12-14 July 2004.

Stalpers, JA, Cock, AWAMd, Robert, V & Stegehuis, G 2004, *MycoBank : fungal databases, nomenclature and species banks*, viewed 12 March 2017 <http://www.mycobank.org/>.

Stamets, P 2005, *Mycelium Running: How Mushrooms Can Help Save the World*, Ten Speed Press, Berkeley, CA.

Stamm, AJ 1964, *Wood and Cellulose Science*, Ronald Press Co., NY.

Stec, AA & Hull, TR 2011, 'Assessment of the fire toxicity of building insulation materials', *Energy and Buildings*, vol. 43, no. 2, pp. 498-506.

Stevenson, A 2010, *Oxford Dictionary of English*, Oxford University Press, Oxford, U.K.

Stocks, SM & Thomas, CR 2001, 'Strength of mid-logarithmic and stationary phase Saccharopolyspora erythraea hyphae during a batch fermentation in defined nitrate-limited medium', *Biotechnology and Bioengineering*, vol. 73, no. 5, pp. 370-8.

Stocks, SM & Thomas, CR 2001, 'Viability, strength, and fragmentation of Saccharopolyspora erythraea in submerged fermentation', *Biotechnology and Bioengineering*, vol. 75, no. 6, pp. 702-9.

Sun, F, Banks-Lee, P & Peng, H 1993, 'Sound absorption in an anisotropic periodically layered fluid-saturated porous medium', *Applied Acoustics*, vol. 39, no. 1, pp. 65-76.

Sun, W, Tajvidi, M, Hunt, CG, McIntyre, G & Gardner, DJ 2019, 'Fully Bio-Based Hybrid Composites Made of Wood, Fungal Mycelium and Cellulose Nanofibrils', *Scientific Reports*, vol. 9, no. 1, p. 3766.

Superflux 2014, *Mycotecture (Phil Ross)*, viewed 18 July 2016 <http://designandviolence.moma.org/mycotecture-phil-ross/>.

Sustainability Victoria 2014, *Market summary – recycled glass*, viewed 18 March 2017 <https://www.sustainability.vic.gov.au/-/media/SV/Publications/Government/Waste-and-resource-recovery/Recycled-materials-in-pavement/Market-Analysis-Glass-Sept-2014-PDF.pdf?la=en>.

Tedersoo, L, Bahram, M, Põlme, S, Kõljalg, U, Yorou, NS, Wijesundera, R, Ruiz, LV, Vasco-Palacios, AM, Thu, PQ, Suija, A, Smith, ME, Sharp, C, Saluveer, E, Saitta, A, Rosas, M, Riit, T, Ratkowsky, D, Pritsch, K, Põldmaa, K, Piepenbring, M, Phosri, C, Peterson, M, Parts, K, Pärtel, K, Otsing, E, Nouhra, E, Njouonkou, AL, Nilsson, RH, Morgado, LN, Mayor, J, May, TW, Majuakim, L, Lodge, DJ, Lee, SS, Larsson, K-H, Kohout, P, Hosaka, K, Hiiesalu, I, Henkel, TW, Harend, H, Guo, L-d, Greslebin, A, Grelet, G, Geml, J, Gates, G, Dunstan, W, Dunk, C, Drenkhan, R, Dearnaley, J, De Kesel, A, Dang, T, Chen, X, Buegger, F, Brearley, FQ, Bonito, G, Anslan, S, Abell, S & Abarenkov, K 2014, 'Global diversity and geography of soil fungi', *Science*, vol. 346, no. 6213, pp. 1256688-.

Thakur, VK & Siṅgha, AS 2013, *Biomass-based Biocomposites*, Smithers Rapra Technology Ltd., Shropshire, U.K.

Theng, D, Arbat, G, Delgado-Aguilar, M, Vilaseca, F, Ngo, B & Mutjé, P 2015, 'All-lignocellulosic fiberboard from corn biomass and cellulose nanofibers', *Industrial Crops and Products*, vol. 76, no. 1, pp. 166-73.

Travaglini, S, Noble, J, Ross, PG & Dharan, CKH 2013, 'Mycology Matrix Composites', *28th Annual Technical Conference of the American Society for Composites*, State College, PA.

Trinci, AP & Collinge, AJ 1975, 'Hyphal wall growth in Neurospora crassa and Geotrichum candidum', *Journal of General Microbiology*, vol. 91, no. 2, pp. 355-61.

Tudryn, GJ, Smith, LC, Freitag, J, Bucinell, R & Schadler, LS 2018, 'Processing and morphology impacts on mechanical properties of fungal based biopolymer composites', *Journal of Polymers and the Environment*, vol. 26, no. 4, pp. 1473-83.

UN Environment Programme 2014, *Innovation abounds in plastic substitutes, but it's behaviour change that will save our seas*, viewed 22 March 2016 <https://www.unenvironment.org/news-and-stories/story/innovation-abounds-plastic-substitutes-its-behaviour-change-will-save-our.>.

Uysal, H, Demirboğa, R, Şahin, R & Gül, R 2004, 'The effects of different cement dosages, slumps, and pumice aggregate ratios on the thermal conductivity and density of concrete', *Cement and concrete research*, vol. 34, no. 5, pp. 845-8.

Vidal, J 2008, *Sustainable bio-plastic can damage the environment*, viewed 11 February 2016 <https://www.theguardian.com/environment/2008/apr/26/waste.pollution>.

Vinson, J 2018, *The behavior of sandwich structures of isotropic and composite materials*, Routledge, Oxford, U.K.

Wall, K, Walker, P, Gross, C, White, C & Mander, T 2012, 'Development and testing of a prototype straw bale house', *Proceedings of the Institution of Civil Engineers-Construction Materials*, vol. 165, no. 6, pp. 377-84.

Wang, H-Q, Zhong, J-J & Yu, J-T 1997, 'Enhanced production of taxol in suspension cultures of Taxus chinensis by controlling inoculum size', *Biotechnology Letters*, vol. 19, no. 4, pp. 353-6.

Watson, B 2016, *Fungus not formaldehyde*, viewed 12 May 2016 <http://www.theguardian.com/sustainable-business/2016/apr/10/environmental-engineering-biomimicry-tapping-into-nature-business-water-soil-drought>.

Weber, R 2009, 'Recent Developments in the Molecular Taxonomy of Fungi', in *Physiology and Genetics: Selected Basic and Applied Aspects*, Springer, Berlin, Germany.

Webster, J & Weber, R 2007, *Introduction to Fungi*, Cambridge University Press, Cambrige, U.K.

Wei, L, Liang, S & McDonald, AG 2015, 'Thermophysical properties and biodegradation behavior of green composites made from polyhydroxybutyrate and potato peel waste fermentation residue', *Industrial Crops and Products*, vol. 69, no. 1, pp. 91-103.

Wekesa, N, Lilechi, D, Sigot, A, Cheruiyot, J, Kamau, R & Kisiangani, P 2016, 'Volatile and Non-polar Chemical Constituents of Cultivated Oyster Mushroom Pleurotus ostreatus', *International Journal of Pharmacognosy and Phytochemical Research*, vol. 8, no. 3, pp. 477-9.

Wessels, JGH, Mol, PC, Sietsma, JH & Vermeulen, CA 1990, 'Wall Structure, Wall Growth, and Fungal Cell Morphogenesis', in *Biochemistry of Cell Walls and Membranes in Fungi*, Springer, Berlin, Germany.

Wong, YJ, Arumugasamy, SK & Mustapha, KB 2019, 'Development of a computational predictive model for the nonlinear in-plane compressive response of sandwich panels with bio-foam', *Composite Structures*, vol. 212, no. 1, pp. 423-33.

Xia, XC, Chen, XW, Zhang, Z, Chen, X, Zhao, WM, Liao, B & Hur, B 2013, 'Effects of porosity and pore size on the compressive properties of closed-cell Mg alloy foam', *Journal of Magnesium and Alloys*, vol. 1, no. 4, pp. 330-5.

Xing, Y, Brewer, M, El-Gharabawy, H, Griffith, G & Jones, P 2018, 'Growing and testing mycelium bricks as building insulation materials', *IOP Conference Series: Earth and Environmental Science*, vol. 121, no. 2, p. 022032.

Xu, Q, Jin, C & Jiang, Y 2017, 'Compare the flammability of two extruded polystyrene foams with micro-scale combustion calorimeter and cone calorimeter tests', *Journal of Thermal Analysis and Calorimetry*, vol. 127, no. 3, pp. 2359–66.

Yang, F-C & Liau, C-B 1998, 'The influence of environmental conditions on polysaccharide formation by Ganoderma lucidum in submerged cultures', *Process Biochemistry*, vol. 33, no. 5, pp. 547-53.

Yang, Z, Zhang, F, Still, B, White, M & Amstislavski, P 2017, 'Physical and mechanical properties of fungal mycelium-based biofoam', *Journal of Materials in Civil Engineering*, vol. 29, no. 7, p. 04017030.

Yee, R & Stephens, T 1996, 'A TGA technique for determining graphite fiber content in epoxy composites', *Thermochimica Acta*, vol. 272, no. 1, pp. 191-9.

Zabihzadeh, SM 2009, 'Water uptake and flexural properties of natural filler/HDPE composites', *Bioresources*, vol. 5, no. 1, pp. 316-232.

Zhang, Y-H & Zhong, J-J 1997, 'Hyperproduction of ginseng saponin and polysaccharide by high density cultivation of Panax notoginseng cells', *Enzyme and Microbial Technology*, vol. 21, no. 1, pp. 59-63.

Zhao, Q, Zhang, B, Quan, H, Yam, RCM, Yuen, RKK & Li, RKY 2009, 'Flame retardancy of rice husk-filled high-density polyethylene ecocomposites', *Composites Science and Technology*, vol. 69, no. 15, pp. 2675-81.

Zhu, BC, Henderson, G, Chen, F, Fei, H & Laine, RA 2001, 'Evaluation of vetiver oil and seven insect-active essential oils against the Formosan subterranean termite', *Journal of Chemical Ecology*, vol. 27, no. 8, pp. 1617-25.

Part II

Appended Publications

Publication A

Literature Review

Fungal biology, fungal classification, growth phases and kinetics, exogenous factors, hyphal architecture, cell wall composition, composite constituents, material characterisation and applications.

Mycelium Composites: A Review of Engineering Characteristics and Growth Kinetics

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Mycelium composites comprise of networks of filamentous hyphae, utilising biological growth rather than expensive energy intensive manufacturing processes to convert low-cost organic wastes into economically viable and environmentally friendly materials. Although generally characterised as polymer grade foams and used primarily for limited packaging and construction applications, the mechanical performance of these materials varies significantly and is governed by hyphal architecture, cell wall composition, composite constituents and growth kinetics which are in turn influenced by inherent and exogenous factors. A range of potential applications have been proposed including acoustic dampers, super absorbents, paper, textiles, structural and electronic parts. Limited research, inconclusive data and the proposed applications and feasibility suggest that further investigation is warranted.

Keywords: composite, mycelium, growth kinetics, hyphal architecture, cell wall composition, engineering applications

1. INTRODUCTION

Synthetic materials are often derived from non-renewable resources (e.g. petroleum and natural gas based reinforcement) or harvested from limited natural resources (e.g. most balsa wood cores are harvested in tropical South American regions such as Ecuador) which results in price volatility due to supply fluctuations.[1] This is especially prevalent in the plastics industry which uses feedstocks such as crude oil, natural gas or naphtha derivatives to produce everything from polyethylene packaging and insulation to polypropylene car bumpers.[2] Feedstock cost is responsible for 50-70% of the end product's market price and as such variations in the two are closely correlated.[2] Many monomers such as propylene are only a few refining steps from crude oil itself and have a price correlation as high as 90%. Even resins, with a less direct linkage in terms of manufacture such as polystyrene have oil price correlations as high as 86%.[3]

The end of life options for synthetic materials are also both economically and environmentally negative. Waste management in Australia is a $6.9 billion per annum industry[4] with about 48% of waste generated in Australia disposed of in landfill.[5] In 2010-2011, Australians produced 2.2 million tonnes of synthetic plastic waste, only 14% of which was recycled because the value of plastics is generally too low to justify recycling.[6] Disposal of synthetic waste in landfill releases toxic substances and greenhouse gases which alone contribute 75% of total greenhouse gases associated with waste management. 55% of this contributed gas is methane which has 21-25 times the global warming potential of carbon dioxide.[7]

With diminishing petroleum fuel resources, increasing fuel prices and global mitigation against climate change and environmental damage, there is a need for alternatives to synthetic materials. Regulations in Germany, the Netherlands, and the United Kingdom already require whole-life carbon assessment on many projects and impose restrictions on materials with carbon-intensive supply chains.[8,9] It is expected that two-thirds of the $1.5 trillion global chemical industry could eventually be replaced by renewable resources with many global chemical giants already shifting their focus from petrochemical processes to life sciences.[10]

Materials science technologies such as bioplastics, biofilms[11] and photocatalytics[12] are increasing as much as 20-30% a year[13] because they are more environmentally friendly than conventional materials. However, bioplastic manufacturing still typically produces 20-70% of the carbon emissions associated with traditional processes, and some biodegradables have equivalent carbon intensity.[14] They are at least 20% more expensive than traditional plastics to manufacture[15] and many will not break down in landfill, requiring additional processing in anaerobic digesters to be composted.[13] Bioplastics disposed of in landfill also produce

methane if they anaerobically degrade due to the presence of moisture.[15]

Materials of greater economic viability and environmental sustainability are required to facilitate our transition to a more sustainable world. Cost competitive materials with low energy manufacturing processes, minimal carbon emissions and full biodegradability are possible and the key to their development depends on our ability to leverage natural biological materials and by-products.

2. FUNGAL BIOLOGY

Fungi are a group of diverse unicellular, multicellular or syntactical spore-producing organisms which feed on organic matter. They include moulds, yeasts, mushrooms and toadstools.[16] The fungal kingdom is currently one of the least comprehensively studied and documented kingdoms with only approximately 80,000 to 120,000 species recorded[17] of 1.5 million[18] to 5.1 million species[19], although these figures have been suggested to be somewhat overestimated.[20]

Remarkably fungi are more closely related to animals than to any other biological kingdom.[21] Over 600 million years ago the two kingdoms shared a common ancestor (Fig. 1) which through evolution developed a means of external digestion.[22] This process occurs through secretion of enzymes such as cellulases, oxidases, phosphatases, chitinases and proteases[23], which break down food sources, followed by absorption of the solubilised nutrients.[17]

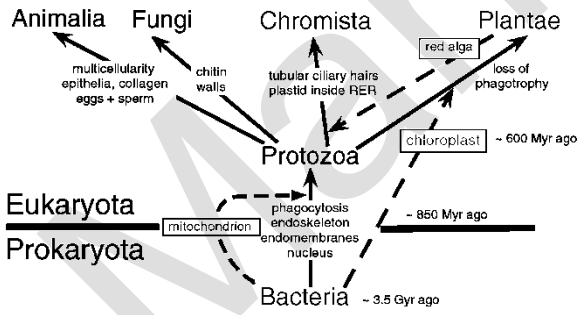

Fig. 1. The six-kingdom, two-empire classification of life. Reproduced from [24], T. Cavalier-Smith, *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 1537, 111 **(2010).** © 2010, The Royal Society.

Although fungi share the same basic genetic structures as plants and animals, their cellular biology differs greatly from other kingdoms.[25] The key differences are that while plants and animals comprise of cells organised into tissues and organs, fungi are generally filamentous (long thin cellular structure, hyphae based) and in contrast to plants and animals, their nuclear envelope remains intact during cell division.[26]

3. FUNGAL CLASSIFICATION

The fungal kingdom comprises of a vast array of diverse species each exhibiting unique characteristics and similarities with genetically related species. Over the years, the scientific community has attempted to map these relationships, producing classification structures for fungi based on increasingly accurate methods of fungal identification and categorisation.[17]

3.1. Taxonomic Classification

Taxonomic classification involves the use of a phylogenetic tree which arranges fungi into hierarchal groups (Fig. 2) and serves three main purposes. It provides a reference framework of recognisable features; it attempts to group together related organisms, and it provides information about the characteristics of an identified species of interest.[17]

Classification		
Kingdom		eg. Fungi
Subkingdom		eg. Dikarya
Phylum	(-mycota)	eg. Basidiomycota
Subphylum	(-mycotina)	eg. Agaricomycotina
Class	(-mycetes)	eg. Agaricomycetes
Order	(-ales)	eg. Polyporales
Family	(-aceae)	eg. Polyporaceae
Genus		eg. Trametes
Species		eg. Trametes versicolor

Fig. 2. Phylogenetic tree for classification. Compiled from literature.[27,28]

Early fungal taxonomic approaches were based on morphological and microscopic characteristics augmented by biochemical and ultrastructural features and were largely arbitrary due to taxonomists proposing widely differing classification schemes (i.e. species names and location within a phylogenetic tree) depending on which features they felt were most relevant.[29] Taxonomic methods used today are significantly more sophisticated with the use of multi-locus datasets, extensive taxon sampling, and exhaustive analytical processes as standard procedure.[30]

The taxonomic system by Hibbett et al. (2007)[30] (Fig. 3) describes the current taxonomic classification of fungi. The classification accepts one kingdom, one subkingdom, seven phyla, ten subphyla, 35 classes, 12 subclasses and 129 orders. It is based largely (90%) on recent validly published literature with the remainder based on automatically typified teleomorphic names.[30]

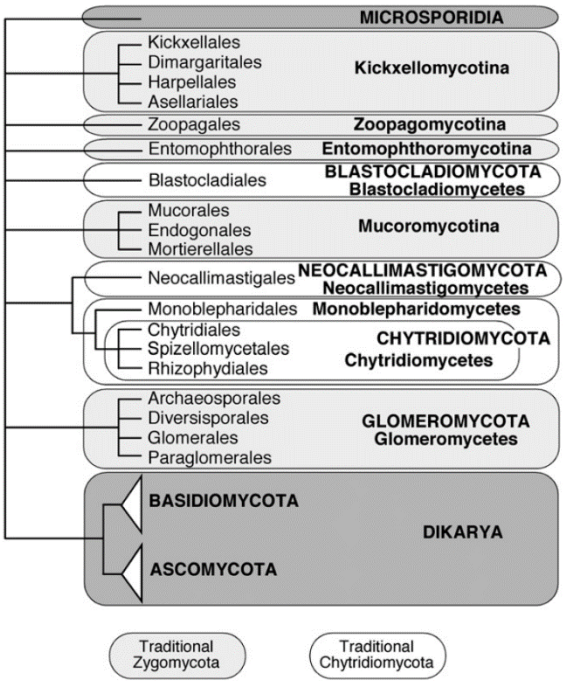

Fig. 3. Phylogeny and classification of fungi. Basal fungi and Dikarya. Branch lengths are not proportional to genetic distances. Reproduced with permission from [30], D.S. Hibbett, et al., *Mycol. Res.* 5, 509 **(2007)**. © 2007, Elsevier.

Most notably it rejects the traditionally recognised Zygomycota and Chytridiomycota phyla which have long been recognised to be polyphyletic (containing organisms derived from more than one common evolutionary ancestor and are hence unable to be classified together). These phyla are reclassified into more appropriate groups with their previous association to the traditional phyla noted.

Taxonomic classification systems are especially useful from a materials science perspective because they characterise many properties exhibited by documented species and identify other related species with similar properties. This provides a scientific foundation for comparison of the mechanical performance of species known to exhibit similar or dissimilar characteristics.

3.2. Fungal Associations

Fungi sometimes exist independently in nature but in most cases, share an association with other organisms, including plants. In such cases, classification is based on the host and the respective benefits or threats with which the relationship presents each organism.

Saprophytic (saprotrophic) fungi use enzymes to digest organic matter into molecules small enough to be absorbed by other organisms.[31] They are the most relevant group of fungi from a materials science perspective because they convert organic waste into mycelial mass. They comprise of three main groups based on their order of appearance during the digestion process.[22]

a) Primary colonisers occur first in nature. They have high growth rates and rapidly expand, attach to, and decompose simple compounds.

b) Secondary colonisers rely on the primary colonisers to partially break down plant and animal tissue before digesting the more complex compounds.

c) Tertiary colonisers are found towards the end of the decomposition process, thriving in conditions created by primary and secondary colonisers and relying on highly complex microbial environments.

Pathogenic (parasitic) fungi endanger the host's health and cause diseases in organisms. Some pathogenic fungi behave like saprotrophic fungi, but most are microfungi, barely visible to the naked eye, inflicting cankers and lesions on the shoots and leaves of living trees.[22]

Symbiotic fungi form associations with plants in a mutually beneficial relationship. They comprise of two main groups based on location within the host.[22]

a) Mycorrhizal fungi commonly invade the roots of vascular plants intracellularly (endomycorrhizal) or extracellularly (ectomycorrhizal), with the mycelial growth of the fungus extending the plants nutrient absorption ability and zone. The fungus benefits from access to the plants secreted sugars (e.g. hexose).

b) Endophytic fungi grow elsewhere within plants, threading their mycelia between the cell walls and enhancing growth ability and resistance to parasites and infections. The fungus also benefits from access to plant secreted sugars.

3.3. Optimal Growth Environment

Fungi can also be classified by their optimal growth environment based on temperature, water activity and pH (Table I). Mesophilic or neutrophilic fungi experience optimal growth under normal environmental conditions while extremophilic fungi thrive in abnormal or extreme habitats.[32]

Classifications systems that describe the affinity for optimum growth at certain environmental conditions are important to optimise mycelial growth for commercial bio-materials production.

Table I. Extremophilic (low and high) and mesophilic (medium) fungal groups. Compiled from literature.[33-40] Optimal ranges discussed in 5.1.

Parameter	Low	Medium	High
Temperature (°C)	Psychrophilic (0-20)	Mesophilic (0-50)	Thermophilic (20-50)
Water activity (a_w) (defined in 5.1)	Xerophilic (0-0.8)	Mesophilic (0.8-0.9)	Hydrophilic (0.9-1)
pH	Acidophilic (1-6)	Neutrophilic (7)	Alkaliphilic (8-11)

4. GROWTH PHASES AND KINETICS

The vegetative part of filamentous fungi comprises a network of fine white filaments (hyphae) known collectively as mycelium[16] which spread upon or penetrate a substrate.[17] A fungal spore inoculated on a nutrient rich medium will form a germ tube which for a period will experience exponential non-photosynthetic growth undergoing dichotomous (dividing into two sections) or lateral branching[41] fuelled by digestion of carbon and nitrogen based feed stock.[42] Extension occurs only in the apical (tip) region which has a hemiellipsoidal shape with wall growth throughout the remainder of the extension zone taking the form of a circumferential extension which ceases when the hypha reaches its full width.[17]

Three typical growth phases will occur following inoculation of suitable media:

a) Lag phase – is a period of zero or low population growth as the inoculated cells grow accustomed to their new chemical and physical environment.[43] Lag phase duration varies by species[44, 45] but exhibits an inverse relationship with growth rate (i.e. faster-growing species have shorter lag phases).[46]

b) Exponential phase – Under optimal conditions, exponential growth occurs with proportionate increases in biomass including cell number, dry weight and nucleic acid and protein content of the population.[47]

c) Stationary phase – if essential nutrients are exhausted, or toxic products accumulate the exponential phase of growth will end, and the fungal cells enter a period known as the stationary phase in which the specific growth rate returns to zero and biomass remains relatively constant.[43,47] This phase is thought to be the reason that fungi can survive for long periods without additional nutrients, but if this phase is maintained incessantly, cells may begin to die.[48]

It is desirable to minimise the lag phase and ensure that optimal environmental conditions and abundant nutrients are available to maximise growth rate and yield and prevent growth entering the stationary phase prematurely.

4.1. Modelling Growth Kinetics

Growth during the exponential phase can be mathematically modelled using empirical growth equations (linear, exponential, logistic, two phase).[49] Individual hyphae grow at a constant linear rate,[40] but the majority of colony based microbial growth is calculated using either logistic, two-phase or exponential models.[47,49]

The logistic and two-phase models are most commonly used to calculate growth in open systems (continuous culture) in which media is simultaneously supplied to and removed from the system to maintain constant volume (e.g. bioreactors).[49]

The exponential model is most commonly used to calculate growth in closed systems where no media is added or removed (batch culture growth).[40,43,47] This is the most appropriate model since materials science work completed to date, utilises sealed batches of moulds.[1,50-55]

The exponential model is derived from the proportional relationship between population growth rate (dx/dt) and growth rate per unit biomass, commonly referred to as specific growth rate (μ) (eqn. 1).[47]

The model itself (eqn. 6) can be used to calculate biomass aspects such as dry weight.[47] This is a parameter of interest because it has a correlation with mycelial density.[47]

Eqn. 1	$\frac{dx}{dt} = \mu_{max} x$	Population growth rate (dx/dt) is proportional to growth rate per unit biomass (μ) multiplied by population biomass (x)
Eqn. 2	$\frac{dx}{x} = \mu_{max} dt$	Variables are separated and grouped
Eqn. 3	$\int_{x_0}^{x} \frac{1}{x} dx = \int_{0}^{t} \mu_{max} dt$	Biomass is integrated between initial cell mass (x_0) and biomass, x, and time between zero and time, t
Eqn. 4	$\ln\left(\frac{x}{x_0}\right) = \mu_{max} t$	Both sides are then exponentiated
Eqn. 5	$\frac{x}{x_0} = e^{\mu_{max} t}$	A solution for biomass (x) is then computed
Eqn. 6	$x = x_0 e^{\mu_{max} t}$	Exponential equation modelling biomass at any given time achieved

Mycelial branching increases the number of growing-points over time giving an accelerating exponential growth rate pattern (Fig. 4a) and linear logarithm dry weight increase (Fig. 4b).[40,43,47]

Increased mycelial branching yields higher mycelial density which is of relevance to mycelium composite evaluation.

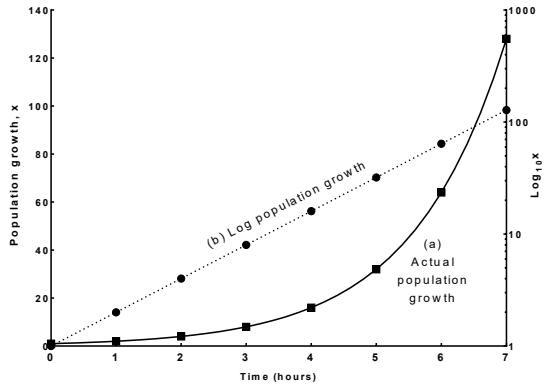

Fig. 4. Exponential model showing (a) actual and (b) log population growth. X can represent any measure of population growth (cell number, dry weight, protein or nucleic acid content). Compiled based on literature.[43,47]

5. EXOGENOUS FACTORS

Exogenous factors such as environmental conditions (Table II) and chemical nutrition have a significant influence on fungal growth, affecting the lag phase duration, exponential phase growth rate and dictating if or when the stationary phase is reached. These factors are especially relevant to mycelium material manufacturing time.

Table II. Relationship between environmental parameters, lag phase, exponential phase and optimum values. Compiled from literature.[40,43,47,56,57]

Environmental parameter	Lag phase	Exponential phase	Optimum
↑ Inoculum density	↓	↑ Growth rate ↑ Maximum yield	Identical medium 10-32% ratio
↑ Temperature	↓	↑ Growth rate	Psychrophiles, 0-17°C Mesophiles, 15-40°C Thermophiles 37-50°C
↑ Water activity	↓	↑ Growth rate	Xerophiles, 0.6-0.8 a_w Mesophiles, 0.8-0.9 a_w Hydrophiles, 0.9-1.0 a_w
Extreme pH	↑	↓ Growth rate	pH 4 to 6

5.1. Environmental Conditions

Inoculation conditions have significant influence over both lag phase duration and exponential phase growth rate. Decreased inoculum density results in increased lag phase duration,[58-64] decreased specific growth rate[65,66] and decreased maximum yield.[65,67,68] This effect can be mitigated by the presence of trace elements and higher sugar concentrations in smaller inocula.[65] Inoculation using cells vigorously growing on an identical medium results in the absence of a lag phase[47] and is the optimal inoculation method. Ideal inoculation density is 10-32% inoculum to substrate ratio (by volume) depending on liquid or solid tissue inoculum used with an incubation time of 5-14 days depending on materials involved.[56]

Temperature plays a similar role, with decreased temperature resulting in increased lag phase duration and reduced exponential phase growth rate.[69,70] High temperatures are especially important for initial growth rate which doubles with every 10°C increment temperature increase.[34] However, growth will be inhibited by extreme temperatures which inactivate enzymes,[71] disrupts hydrogen bonding and hydrophobic interactions, and leads to the denaturation of proteins and nucleic acids.[43] Optimal temperatures are less than 10°C for psychrophiles, between 18-22°C for mesophiles and over 37°C for thermophiles but also depend on water availability and nutrients.[34]

Water activity (a_w) is the ratio of the vapour pressure of water in a material to the vapour pressure of pure water at the same temperature.[40] It is a decimal value, but when expressed as a percentage it gives equilibrium relative humidity (ERH).[72] Water plays a crucial role in fungal metabolism with reduced water availability (<0.65 water activity) to cells adversely affecting fungal growth.[43] Water activity is most important during the lag phase[69] with the adverse effects of lower temperatures significantly exacerbated by low water activity levels.[73] Water activity has only a slight effect on growth rate post lag phase.[73] Optimal water activity levels are 0.6-0.8 for xerophilic fungi (dry loving – primary colonisers), 0.8-0.9 for slightly xerophilic (secondary colonisers) and >0.9 for hydrophilic (water loving – tertiary colonisers).[57]

pH is important because most fungi are acidophilic and grow well in the pH range of 4 to 6. This acidic environment provides the hydrogen content required for optimal fungal growth.[43] pH levels outside these ranges (less than 3 or greater than 8) will adversely affect growth[43] and increase lag phase duration.[74]

5.2. Chemical Physiology and Nutrition

Fungal cells rely on macronutrients (carbon, nitrogen, oxygen, sulphur, phosphorus, potassium, magnesium) in millimolar concentrations and micronutrients (hydrogen, calcium, copper, iron, manganese, zinc, nickel and molybdenum) in micromolar concentrations to support their cellular functions.[40,43,75] Optimising fungal nutrition is challenging since every chemical found in living organisms in addition to many manufactured, and inorganic materials can potentially support fungal growth. Nutritional studies struggle with availability of media lacking certain elements which are necessary to analyse fungal response to graded elemental quantities.[40] Exhaustive studies and universally applicable rules are unavailable, but

the roles of key macronutrients are well documented (Table III).

Table III. Source and function of macronutrients. Adapted from Kavanagh (2005).[41]

Element	Sources	Functions
Carbon	Carbohydrates	Energy source Structural element
Magnesium	Mg^{2+} salts	Supports enzyme activity, cell structure, organelle structure
Nitrogen	NO_3-, NO_2-, NH_4+, amines, amides	Structural element Supports proteins and enzyme production and function
Oxygen	Air, O_2	Supports ergosterol synthesis, unsaturated fatty acid synthesis, respiratory enzymes, oxidative enzymes
Phosphorus	Phosphates	Supports biosynthesis of nucleic acids, phospholipids, glycophosphates
Potassium	K^+ salts	Supports ionic balance, enzyme activity
Sulphur	Sulphates, methionine	Source of sulphydryl amino acids, vitamins

Carbon Fungi are chemoorganotrophs, meaning that they oxidise chemical bonds in organic compounds to attain energy and carbon to support cellular function. Sugars in particular ranging from simple hexoses (e.g. glucose) to polysaccharides (e.g. cellulose, starch, and lignin) are common carbon sources that support growth.[43] Carbon source suitability for any given species is heavily influenced by natural evolutionary factors.

Mesophilic microflora are succeeded by thermophilic microflora in nature and as such, mesophiles thrive first as the temperature increases, consuming the simpler carbon sources (sugars, amino acids, and organic acids) and leaving only polysaccharide constituents of biomass (cellulose and hemicelluloses) available to thermophiles. As such, mesophiles are better suited to simpler sugars while thermophilic fungi are well adapted for polysaccharide utilisation.[76]

The similar is true with respect to primary, secondary and tertiary colonisers with the faster growing primary colonisers rapidly consuming available simple sugars and leaving only the more complex sugars available to the secondary and tertiary colonisers. This has led to natural affinities within these groups for these different carbon types.[22,57]

Glucose is the most widely utilisable simple sugar, occurring naturally in cellulose, starches, and other carbohydrates. It can be utilised as a sole carbon and energy source but has an inhibitory effect on utilisation of other carbon sources.[40,77] Fructose, mannose and galactose are also widely utilisable simple sugars.[40,78] More complex sugars such as cellulose and cellobiose induce higher levels of cellulase (enzyme) activity than glucose alone (more than 10 times higher)[77] resulting in more rapid substrate decomposition.

Nitrogen Fungi are non-diazotrophic (unable to fix atmospheric nitrogen N_2) and must be supplied with fixed organic (amines, amides, ammonium salts, amino acids) or inorganic nitrogen-containing compounds (ammonia (NH_3), ammonium (NH_4^+), nitric acid, urea).[43] Fungal amino acids, nucleic acids, cell wall polysaccharides, phospholipids, and vitamins can be synthesised from these sources of inorganic nitrogen through anabolic or catabolic (breakdown of organic nitrogen compounds and nitrate reduction to ammonia) reactions.[40] Nitrogen availability may be a growth limiting factor in nature.[43]

Nitrate is the most commonly available form of nitrogen in soil and is widely utilisable by fungi, excluding some Chytridiomycetes, Oomycetes, and Basidiomycetes. Ammonium is the only more reduced form of nitrogen that is widely utilisable[40] with most species growing well on ammonium.[79-82] Urea, amino acids, and other organic nitrogen compounds are utilisable to varying degrees dependent on fungal species and specific compound used. A mixture of amino acids (e.g. casein hydrolysate) supports greater and more rapid growth than any single amino acid.[40]

Oxygen Most fungi are obligate aerobes and require oxygen. Different species respond to oxygen availability in different ways however generally, growth in obligate aerobes is markedly reduced if oxygen partial pressure drops below normal atmospheric levels.[43] The atmospheric pressure of air (101.3kPa at sea level) is the sum of its constituents (oxygen and nitrogen) and water vapour pressure (6.3kPa at 37°C).[83] Since oxygen makes up 21% of dry air, the inspired normal atmospheric oxygen pressure is 0.21 x (101.3 – 6.3) = 19.95kPa (minimum oxygen pressure for optimal growth in obligate aerobes).

The influence of other macronutrients on fungal growth is less well documented. Phosphorus[84] and potassium[85,86] in conjunction with nitrogen have been found to improve growth rate and yield. Sulphur[87] and phosphorus[43] availability can be growth limiting factors. However, magnesium can be omitted without adversely affecting cellulolytic activity.[88]

6. HYPHAL ARCHITECTURE

Chemical nutrition also affects mycelial density within the hyphal network. High concentrations of carbon can increase branching and decrease hyphal extension rate[89] as can increased oxygen uptake.[90] Increased sulphur and nitrogen concentration also yield a greater number of branches per millimetre of hypha.[91]

An inversely proportional relationship is present between the degree of branching and hyphal extension rates due to the increased utilisation of substrate and production of inhibitory staling compounds as hyphal density

increases meaning the hyphal extension rate is insufficient to allow growth into new areas of substrate.[41] Increased growth rate results in branch formation at increased proximity to the hyphal tip.[92]

Cytoplasmic vesicles influence branching in septate hyphae[17] with the accumulation of vesicles behind septa leading to lateral branch formation. This occurs during growth with vesicles produced in distal hyphal regions and transported to the tip where they fuse with existing walls and membranes to give hyphal extension.[89,93]

6.1. Hyphal Types of the Basidiomycota

Inherent biological characteristics also influence mycelial density, an especially good example being the mono-, di- and tri- mitic hyphal networks of the Basidiomycota.[94] Mycelium materials to date have almost exclusively utilised basidiomycetes which can be constructed of up to three distinct hyphal types.[95] The three main hyphal types are generative, binding (also known as ligative) and skeletal hyphae (Table IV) (Fig. 5).

Table IV. Hyphal types of the Basidiomycota. Compiled from literature.[17,96]

Parameter	Generative	Binding	Skeletal
Wall thickness	Thin	Thick	Thick
Internal structure	Hollow, usually contain cytoplasm	Often solid	Often solid
Origin	Always present	Generative hyphae	Generative hyphae
Growth	Growth platform for other hyphae	Limited growth, weave between other hyphae	Spread laterally
Branching	Branched	Highly branched	Unbranched, or very sparsely branched
Septa	Yes	No	No

Further hyphal types have since been identified,[94,97] however many of these are intermediates between the three principle types discussed above or function in the same way. They are known as sarco-, skeleto-ligative, arboriform and gloeoplerous hyphae.[17]

The number of different hyphal types present in a species is described using the mitic system. Monomitic species comprise of only generative hyphae; dimitic species comprise of two hyphal types (usually generative and skeletal) and trimitic species comprise of all three principle hyphal types.[17]

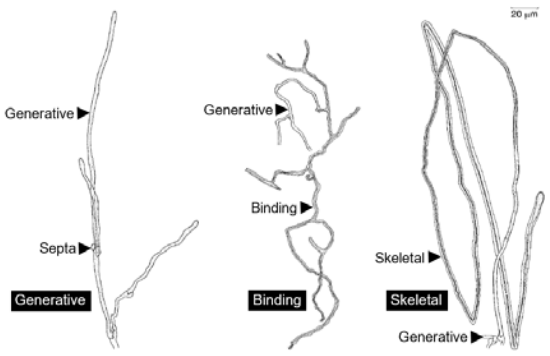

Fig. 5. Analysis of hyphal types from the fruiting body of *Trametes versicolor*. Reproduced with permission from [17], J. Webster & R.W.S Weber, Introduction to Fungi, Cambridge University Press, Cambridge, U.K. **(2007)**. © 2007, Cambridge University Press.

It is generally accepted that complex hyphal systems (e.g. trimitic) are more advanced forms than less complex hyphal systems (e.g. monomitic),[94,98,99] however network complexity can vary greatly irrespective of the number of hyphal types present, with some monomitic species (Fig. 6a) exhibiting a similar degree of branching to trimitic species (Fig. 6b).

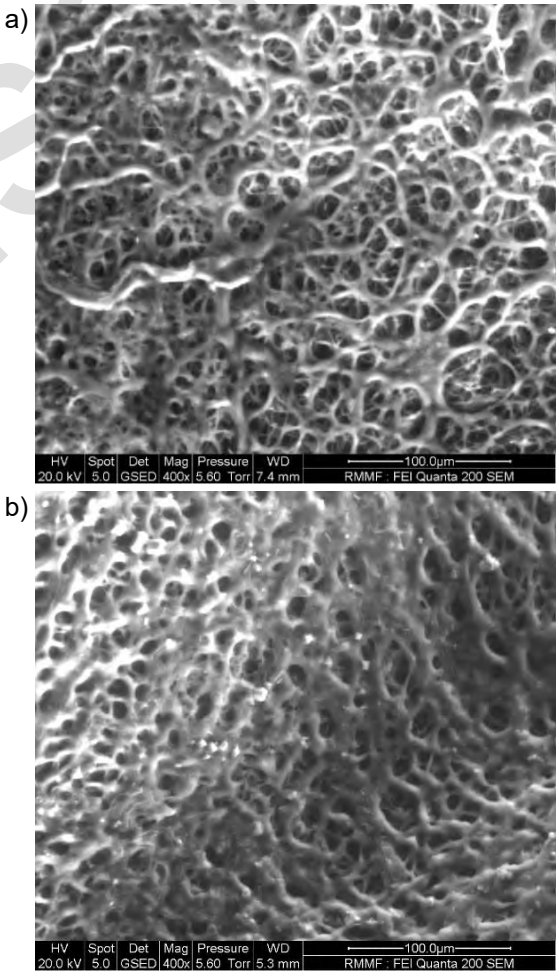

Fig. 6. Micrographs of strongly branched hyphal networks of (a) *Pleurotus eryngii* (monomitic) and (b) *Ganoderma sp.* (trimitic). Specimens grown on wheat grain. Produced at the RMIT Microscopy and Microanalysis Facility using Environmental Scanning Electron Microscopy.

Although there are no studies available that directly evaluate the effect of hyphal architecture on mechanical performance, there is circumstantial evidence indicating a correlation between the two.[52,100] The morphology of each species depends on the hyphal system present with the thickness of the hyphal walls and amount of water contained within their cells responsible for specific qualities of the fruiting bodies.[101] Soft and fleshy fruiting bodies are typical of species with simple hyphal systems (e.g. agarics) while tougher leathery or woody fruiting bodies are typical of species with complex hyphal systems (e.g. polyporales).[95]

Generative hyphae alone (monomitic hyphal systems) are suggested to provide limited mechanical performance, with binding hyphae (dimitic and trimitic hyphal systems) primarily responsible for material strength,[100,102] based on limited data. For example, *Trametes versicolor* (trimitic) has a higher compressive strength than *Pleurotus ostreatus* (monomitic) on the same growth medium (hemp).[52]

7. CELL WALL COMPOSITION

Cell wall composition is especially relevant to mycelium composites because it defines both cellular strength and shape in fungi. The cell wall normally comprises of a thick and complex fibrous network comprising of different polysaccharides (chitin, glucans, manno-proteins, chitosan, polyglucuronic acid or cellulose) and smaller quantities of proteins and glycoproteins.[103,104]

The fibrillar (fibrous) and matrix (dispersed) polymers present in a species vary significantly for different taxonomic groupings (Table V). This is important from a mechanical performance perspective because the structural properties of natural fibrillar and matrix polymers vary (Table VI).

Table V. Major fibrillar and matrix polymers of fungal groups. Adapted from Kavanagh (2005).[43]

Taxonomic group	Fibrillar polymers	Matrix polymers
Ascomycetes	Chitin, β(1,3), β(1,6)-glucans	α(1,3)-glucan, galactomannoproteins
Basidiomycetes	Chitin, β(1,3), β(1,6)-glucans	α(1,3)-glucan, xylomannoproteins
Chytridiomycetes	Chitin, glucan	Glucan
Zygomycetes	Chitin, chitosan	Polyglucuronic acid

One of the most important components of most fungal cell walls is chitin which is a white, hard, inelastic, nitrogenous polysaccharide that is also the main component of the exoskeleton of most insects and other arthropods.[105] Chitin is a linear polymer of the acetylated amino sugar N-acetylglucosamine which is very strong and has a tensile strength significantly greater than many synthetic materials such as carbon fibres and steel (Table VI) due to hydrogen bonding along the chains which give them rigidity.[17]

Table VI. Tensile strength of selected natural and artificial fibrous materials. Adapted from Ruiz-Herrera (2016).[106]

Material	Type	Tensile strength (MPa)
Chitin	Natural (Polysaccharide)	4000
Boron fibres	Synthetic (Synthetic fibre)	3400
Steel	Synthetic (Metallic alloy)	2800
Carbon fibres	Synthetic (Synthetic fibre)	1900-2600
Glass fibres	Synthetic (Synthetic fibre)	1500-2000
Cellulose	Natural (Polysaccharide)	900
Collagen	Natural (Protein)	100

The chitin content of fungal cell walls can vary based on a number of factors. Chitin content fluctuates inherently between and within taxonomic groups (Table VII) from trace amounts (~0.5%) up to 45%.[107] Physiological factors such as growth medium nutrient content and growth temperature can also result in variations in relative chitin levels in cell walls for some species.[108]

Table VII. Dry weight of polysaccharides in cell wall by taxonomic class. Adapted from literature.[28,40]

Phylum (Stalpers 2004)	Genus	Dry weight of wall (%) chitin
Oomycetes	Phytophthora	0
Saccharomyces	Saccharomyces	±1
Agaricomycetes	Schizophyllum	5
Mucormycotina (subdivision)	Mucor	9
Oomycetes	Leptomitus	14
Agaricomycetes	Coprinus	33
Sordariomycetes	Fusarium	39
Blastocladiomycetes	Allomyces	58

8. COMPOSITE CONSTITUENTS

Mycelium based materials are usually composites, only a select few materials utilise pure mycelial sheets, and even they are usually plasticised to increase flexibility.[109,110] Composites are materials which contain two or more chemically distinct phases (continuous and dispersed) which are separated by an interface or boundary on the microscopic level.[111]

The continuous phase is known as the matrix. This is interfaced with fibres which are the principal load-carrying constituents. A dispersed phase surrounds the fibres holding them in position and acting as a load transfer medium between the phases. The fibre is referred to as reinforcement to improve mechanical properties or as a filler to increase material volume.[112]

Composites are highly valued due to the unique combinations of material properties they exhibit when their multiple constituents are combined. Examples include combinations of stiffness, strength, weight, high-temperature performance, corrosion resistance, hardness or

conductivity not otherwise possible as single entities.[113]

The compressive strength of mycelium composites varies greatly depending on their constituents. Two mycelium composites using the genus *Ganoderma* achieved very different compressive strength results based on differing substrate materials. Cotton plant based mycelium composites achieved compressive strengths between 1 and 72 kPa,[50] while a red oak based mycelium composite achieved 490 kPa[55], almost seven times stronger. Tensile performance also has a correlation with growth medium, with *Ganoderma lucidum* and *Pleurotus ostreatus* exhibiting a higher Young's modulus (12-28 MPa vs. 4-17 MPa) and lower elongation (4-14% vs. 9-33%) when grown on cellulose rather than potato dextrose, although tensile strength was similar (both ~0.7-1.1 MPa).[11]

Additives improve the compressive strength of mycelium materials. A study utilising a *Pleurotus sp.* and cotton seed hulls found that mycelial density was increased (181-231 kg/m^3) by the presence of small concentrations (5%) of carboxylated styrene butadiene rubber (SBR) latex and silane coupling agent. The compressive strength was more than doubled (177-422.1 kPa) by the addition of latex.[114]

Small concentrations of other compounds can also significantly affect the properties of mycelium composites. Dry mycelium alone is quite brittle, but when plasticised with small concentrations of cellulose (e.g. incorporation of 7% bleached jack pine kraft fibres), sheet flexibility is sufficiently increased to allow paper-like flexure.[110]

Mixing of metal salts with other substrate constituents makes manipulation of electrical conductivity within mycelium based materials possible. Metal salts are not digested but rather adhere to carboxyl and phosphoryl functional groups on the exterior of the fungal cell wall.[115] Fungal cell walls are dielectric and inherent electrical insulators as they contain chitin, chitosan, and glucans. Precise modification of resistivity is therefore based on metal salt concentration in the growth medium.[116] Hyphae treated with Fe_3O_4 and N-TiO_2 can be used for absorption and photocatalysis[12] and it is possible that electrical circuits could be produced from mycelium sheets by treating specific tissues to vary in resistance.[116]

9. MATERIAL CHARACTERISATION AND APPLICATIONS

Studies conducted to date have generally characterised mycelium materials as polymer foam grade materials[52,55] with densities ranging from 59[53] to 318 kg/m^3.[55]

Compressive strength far exceeds tensile strength by almost three times (Fig. 7) for *Ganoderma lucidum* (a trimitic member of the polyporales) growing on *Quercus kelloggii* (Red Oak) which has an average material density and strength comparable to polymer foams, specifically polystyrene expanded foam.[55] The specimens in this study were denatured and tested before the chitin skin could fully form resulting in brittle, frangible specimens that fractured at boundaries between areas of mycelial growth and undigested woodchips. This suggested that the specimens may have been tested prematurely and the tensile and compressive strengths underestimated.

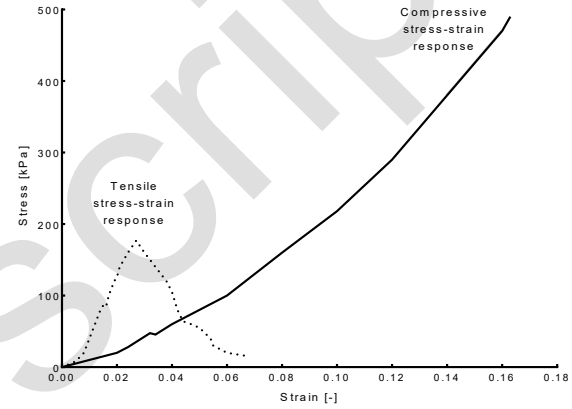

Fig. 7. Tensile and compressive stress-strain responses for mycelium test specimen of *Ganoderma lucidum* growing on Red Oak. Compiled based on Travaglini et al. (2013).[55]

Mycelium materials have been manufactured for a diverse range of applications including paper, textiles, foams (for packaging, acoustics, and medical applications), vehicle parts and electronics, with the number of patents lodged indicative of application feasibility (Fig. 8).

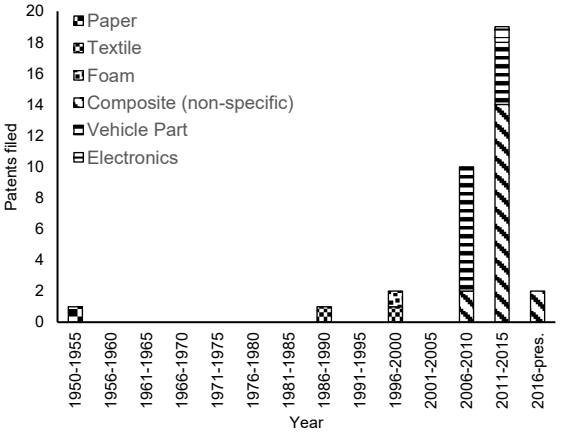

Fig. 8. Mycelium material patent applications from 1954 to present based on industrial uses. Compiled based on U.S and international patents.[65,98,100,108,113-142]

9.1. Mycelium Foams

Mycelium foam is a viable alternative to polystyrene foam.[50,53] Density can surpass desirable limits for light-weight foam applications depending on the substrate used by up to 20 times (Fig. 9)[50] which can be problematic for packaging applications where mass-based freight costs can be significant.

Compressive and flexural strength are lower than synthetic expanded polystyrene (Fig. 9) but are within acceptable limits. Compressive strength is especially important for packaging applications because the primary requirement is to protect the contents from damage.

Modulus of elasticity, dimensional stability, degradation rate, flame retardance characteristics and thermal conductivity are also generally acceptable,[50] although water absorption can sometimes be unacceptably high for packaging applications(114-278%).[53] Liquid absorbance ability can be desirable in other applications discussed in 9.3.

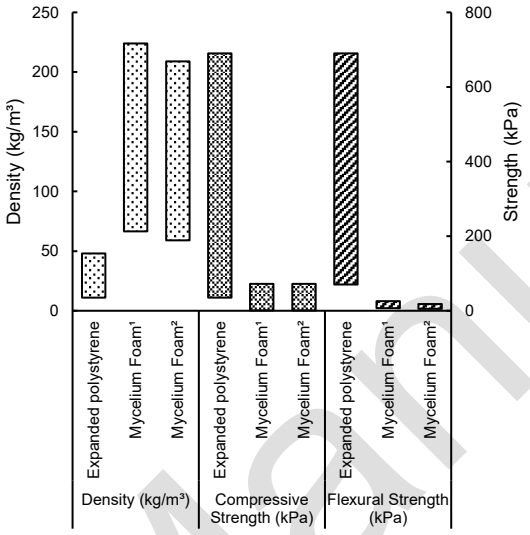

Fig. 9. Material property range comparison between mycelium based foams [1]Ganoderma sp. on cotton biomass, [2]Pleurotus sp. on wheat biomass and traditional polystyrene foams. Compiled from literature.[53,145,50]

Mycelium based foam packaging is currently produced by Ecovative Design (Fig. 10a) and Sealed Air[146] and used by Dell to cushion large computer servers during shipping (Fig. 10b).[147] IKEA is also looking at adopting the packaging for their products.[148]

Mycelium foams have many advantages over traditional synthetic alternatives. They utilise biological growth to convert low-cost organic waste into biodegradable mycelium foam (Fig. 11a). This natural process is both environmentally sustainable and has low manufacturing and end of life disposal costs.[51] Expanded polystyrene is produced from petroleum fuels and natural gas using energy and carbon intensive chemical processes (Fig. 11b). Expensive and dangerous chemicals are necessary including pentane (evolving flammable vapour)[149] and formaldehyde (toxic and corrosive).[150]

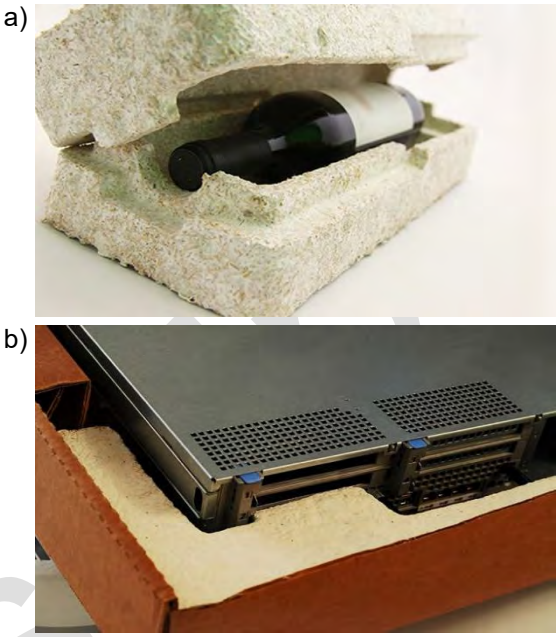

Fig. 10. Mycelium packaging foams used for (a) wine bottle packaging and (b) Computer packaging for Dell. Reproduced from [151], Ecovative Design press kit **(2016)**. © 2016.

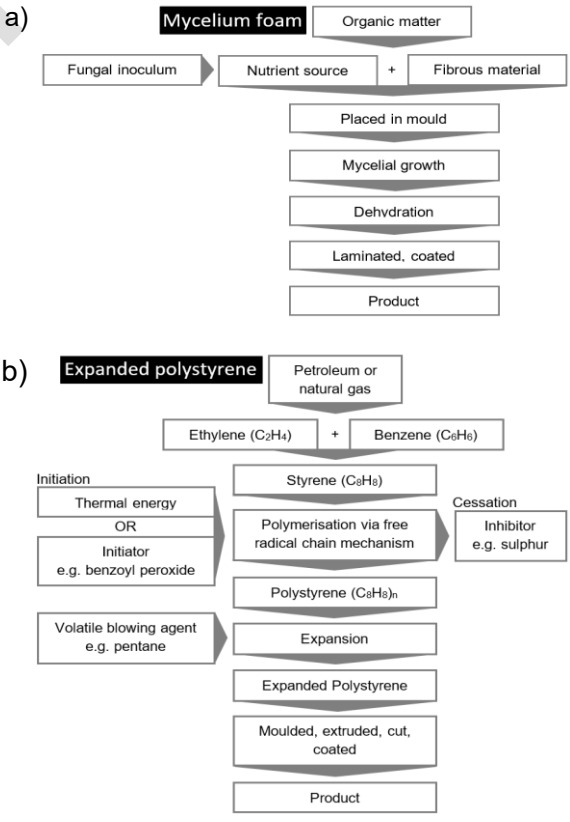

Fig. 11. Material production process comparison of (a) mycelium foam and (b) expanded polystyrene. Compiled from literature.[149,152-154]

9.2. Construction Materials

Mycelium bricks have been used for the construction of architectural structures since 2009, when *Ganoderma lucidum* and sawdust were used to produce the 500 brick "Mycotectural Alpha" teahouse (Fig. 12a)[155] commissioned by Düsseldorf Kunsthalle for the 25th anniversary of their "Eat Art" exhibition where it was displayed before being boiled and served to museum guests as a herbal tea.[156]

Fig. 12. Examples of mycelium based construction materials. (a) Mycotectural Alpha teahouse, (b) Hy-Fi organic compostable tower (c) MycoBoard™. Reproduced with permission from [157] MycoWorks, Portfolio **(2014)**. © 2014, LafargeHolcim Foundation, "The Living" **(2014)**. © 2014, Kris Graves, Portfolio **(2014)**. © 2014, [151], Ecovative Design, Press Kit **(2016)**. © 2016.

The largest mycelium structure produced was the "Hy-Fi" organic compostable tower (Fig. 12b) constructed in 2014 which won the New York Museum of Modern Art's Young Architects Program that year. It exceeded 12 m in height and comprised of more than 10,000 bricks produced from shredded corn stalks and an undisclosed species of fungi.[158]

A range of other similar brick based structures also exist in museums and galleries across the globe[159-162] showcasing mycelium's construction potential and providing examples of innovative industrial design and architecture.

Since mycelium bricks have been used primarily for art and design applications, very little is known about their engineering characteristics. However, *Ganoderma lucidum* on red oak biomass yields low density and low compressive strength in comparison to traditional solid building bricks made from clay or shale (Table VIII).

Table VIII. Material comparison between mycelium based and traditional clay or shale bricks. Compiled from literature.[55,163,164]

Property	Unit	Clay or shale brick	Mycelium brick[1]
Density	kg/m^3	~1900	318
Compressive strength	MPa	8.6-17.2	0.5

Note: [1]*Ganoderma lucidum* on red oak biomass

High compressive strength (8.6-17.2 MPa) is the key physical requirement of building bricks. Low water absorption is also required for severe weathering (20%) and moderate weathering (25%) grade bricks but not for negligible weathering grade bricks (no limit).[164]

The compressive strength achieved by *Ganoderma lucidum* on red oak is over 17 times below standard for a negligible weathering brick, but almost six times lighter than clay or shale building brick. This is the sole study examining the mechanical properties of mycelium bricks.

Material properties can be enhanced by the control of feed-stocks, mycelium strain and the addition of natural additives to promote bond strength at interfaces.[11,55]

Mycelium particleboard branded MycoBoard™ (Fig. 12c) has been used in applications such as work surfaces, moulded furniture components, seatbacks, architectural panels, door cores and cabinetry.[165] It offers an environmentally sustainable alternative to particle board, plywood, and fibreboard traditionally produced from pressed and extruded wood chips and synthetic resin.

Information regarding how the product is made and its composition is not publicly available due to probable intellectual property restrictions, but loose fibres of hemp and wood are combined with a bio-resin, some portion of which is converted into mycelium via fungal growth.[165]

The product compares well in both flexural strength and modulus of elasticity with traditional particle board products (Table IX).

Table IX. Material comparison of mycelium based and traditional particleboard. Compiled from literature.[165,166]

Property	Unit	Standard particle board	MycoBoard™
Density	kg/m^3	600-700	801
Flexural strength	MPa	14-18	15
Modulus of elasticity	MPa	2400-2800	2640

Bond strength of bio-resin and mycelial growth would thus appear to be equivalent to that achievable using synthetic resin since the substitution of these bonding media is the primary difference between MycoBoard™ and traditional particleboard.

9.3. Theoretical Applications

Although mycelium foams and construction materials are the best-known examples of mycelium materials[146,148,150,155,167] many other potential applications, have been proposed.

Mycelium materials are as geometrically versatile as plastics and are viable for the manufacture of products with simple to complex design geometry and uniqueness. Mycelial growth will digest organic feedstocks irrespective of arrangement with remarkable precision.

Simple shapes (Fig. 13a) are easily achieved using basic moulds.[168] More complex geometries can be produced using 3D printed moulds[169] which can also be incorporated into the structure through the use of digestible bioplastic or potato starch external scaffolding (Fig. 13b).[170]

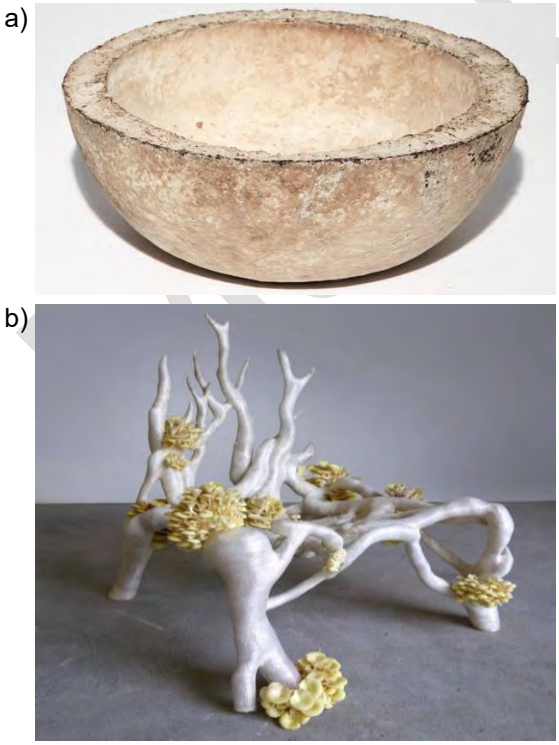

Fig. 13. Complex geometries achievable with mycelium materials. (a) Mycelium bowl produced using a simple mould, (b) mycelium chair with external scaffold printed from potato starch and interior comprising of mycelium. Reproduced with permission from Maurizio Montalti **(2017)**. © 2017, Eric Klarenbeek **(2017)**. © 2017.

Complex vehicle parts can also be mass produced using injection moulding (shaping of heated media through its injection into a mould). Pins, hinges or fasteners can be incorporated into parts seamlessly via mycelial growth and parts comprising of both structural and foam sections with density variation between outer and inner sections achieved using different species and substrate blends.[130-133,135-138]

Paper sheets can also be produced from Mucorales (Zygomycota) mycelial pulp plasticized with a small amount of bleached jack pine kraft fibres (7%). This combination exhibits a bursting strength only slightly less than paper sheets comprising entirely of wood fibres and much better fire resistance. They also have a comparable tensile strength to traditional paper, high gloss, good printing characteristics and approximately four times more stretch.[110] Textiles can be produced in a similar way from mycelial pulp. However, no information is available on their properties or performance.[118,126]

Basidiomycetes growing on agricultural by-products (rice straw, hemp pith, kenaf fibre, switchgrass, sorghum fibre, cotton bur fibre, flax shive) show promising acoustic absorption properties for automotive applications, damping dominant road noise frequencies between 500 Hz to 1600 Hz (large trucks) and 800 Hz to 1600 Hz (consumer grade automobiles). Even low performing substrate blends yield better than 70-75% absorption at the peak frequency of interest (1000 Hz) and exhibit superior acoustic properties to both standard construction grade materials and fibre based ceiling tiles.[54]

Zygomycota hyphae exhibit an absorption ability comparable to many commercial super absorbents and inhibit bacterial growth, odour formation, and fungal yeast growth. The porous absorbent structures are suitable for applications such as wound and hygiene products or filtering aids.[119]

10. CONCLUSIONS

Depleting petroleum reserves coupled with waste management problems and increasingly stringent international regulations on embodied carbon makes the replacement of many traditionally synthetic materials with more environmentally responsible materials necessary.

Mycelium composites utilise biological growth rather than expensive energy intensive manufacturing processes, require only low-cost organic waste as feedstock, can grow to fill complex geometries and have no end of life disposal costs since they are inherently

biodegradable. This makes them economically and environmentally viable alternatives to many synthetic materials.

The fungal kingdom consists of a very extensive range of species with greatly varying morphologies and properties. Strong circumstantial evidence links hyphal architecture with compressive strength. Cell wall polymer type and concentration are also likely to influence mechanical performance as the cell wall defines cellular shape and strength. Other composite constituents such as substrate feedstock and additives also strongly influence material properties including compressive strength, flexibility, and electrical conductivity.

Chemical nutrition and exogenous factors such as inoculum density, temperature, water activity and pH can impact mycelial density, cell wall polymer concentrations and growth rate of mycelium composites and as such are also likely to be factors of significance in research concerning this material type.

Mycelium composites are generally characterised as polymer foam grade materials, but most studies concede that mechanical performance can be improved through control of a number of inherent and exogenous biological factors.

Current applications of mycelium composites are restricted to packaging and limited construction applications. However, a wide range of applications have been proposed for this material type including acoustic dampers, super absorbents, paper, textiles, and vehicle and electronic parts.

Limited mycelium composite research details significant variation in performance and material potential attributed to as yet unexplored biological variables. This in conjunction with recently demonstrated viability across a variety of applications suggests further investigation in this research area is warranted to unleash the full potential of mycelium as a material of the future.

References

1. L. Jiang, D. Walczyk. and G. McIntyre., A New Process for Manufacturing Biocomposite Laminate and Sandwich Parts using Mycelium as a Binder. *Proceedings of ASC 2014* **(2014)**, August 10-15, Charlotte, NC.

2. N. Peksa, The Price Movement of Plastics, Spend Matters Network, Buckinghamshire, U.K. **(2012)**.

3. T. Langan, Price Wise: Managing oil price risk in resin prices, Plastics Today **(2011)**.

4. Australian Bureau of Statistics, Report No 8226.0 Electricity, Gas, Water and Waste Services 2006-07 **(2007)**.

5. Hyder Consulting, Waste and Recycling in Australia, Department of the Environment Water Heritage and the Arts **(2009)**.

6. Department of Sustainability, Water, Population and Communities, Waste generation and resource recovery in Australia - Reporting period 2010/11 **(2014)**.

7. Allen Consulting Group, National Waste Policy Regulatory Impact Statement **(2009)**.

8. Green Construction Board, Low carbon routemap for the UK built environment **(2013)**.

9. European Commission, Communication from the commission to the European Parliament, the Council, the European Economic and Social Committee of the Regions on Resource Efficiency Opportunities in the Building Sector **(2014)**.

10. A.K Mohanty, M. Misra and L.T. Drzal, Natural Fibers, Biopolymers, and Biocomposites, CRC Press, Boca Raton, FL **(2005)**.

11. M. Haneef, L. Ceseracciu, C. Canale, I.S Bayer, J.A. Heredia-Guerrero and A. Athanassiou, Advanced Materials From Fungal Mycelium: Fabrication and Tuning of Physical Properties, *Sci. Rep.*, 7, 41292 **(2017)**.

12. Y. Lian, X. Bai, X. Li, Z. Gao, Z. Hu and G. Hu, Novel fungal hyphae/Fe3O4 and N-TiO2/NG composite for adsorption and photocatalysis, *RSC Adv.*, 12, 6842-8 **(2017)**.

13. J. Vidal, Sustainable bio-plastic can damage the environment, The Guardian **(2008)**.

14. UNEP, Conventional vs. Biodegradable Plastics, Emerging Issue Update, 2 **(2014)**.

15. K. Dell, The Promise and Pitfalls of Bioplastic, Time Magazine **(2010)**.

16. A. Stevenson, Oxford Dictionary of English, OUP Oxford, Oxford, U.K. **(2010)**.

17. J. Webster and R. Weber, Introduction to Fungi, Cambridge University Press, Cambridge, U.K. **(2007)**.

18. D.L Hawksworth, The magnitude of fungal diversity: the 1.5 million species estimate revisited, *Mycol. Res.*, 12, 1422-32 **(2001)**.

19. M. Blackwell, The Fungi: 1, 2, 3 … 5.1 million species?, *Am. J. Bot.*, 3, 426-38 **(2011)**.

20. L. Tedersoo, M. Bahram, S. Põlme, U. Kõljalg, N.S. Yorou, R. Wijesundera, L.V. Ruiz, A.M. Vasco-Palacios, P.Q. Thu, A. Suija, M.E. Smith, C. Sharp, E. Saluveer, A. Saitta, M. Rosas, T. Riit, D. Ratkowsky, K. Pritsch, K. Põldmaa, M. Piepenbring, C. Phosri, M. Peterson, K. Parts, K. Pärtel, E. Otsing, E. Nouhra, A.L. Njouonkou, R.H. Nilsson, L.N. Morgado, J. Mayor, T.W. May, L. Majuakim, D.J. Lodge, S.S. Lee, K.-H. Larsson, P. Kohout, K. Hosaka, I. Hiiesalu, T.W. Henkel, H. Harend, L.-d. Guo, A. Greslebin, G. Grelet, J. Geml, G. Gates, W. Dunstan, C. Dunk, R. Drenkhan, J. Dearnaley, A. De Kesel, T. Dang, X. Chen, F. Buegger, F.Q. Brearley, G. Bonito, S. Anslan, S. Abell and K. Abarenkov, Global diversity and geography of soil fungi, *Science*, 346, 1256688 **(2014)**.

21. S. Baldauf, and J. Palmer, Proc. Natl. Acad. Sci. USA, 90, 11558 **(1993).**

22. P. Stamets, Mycelium Running: How Mushrooms Can Help Save the World, Ten Speed Press, Berkeley, CA **(2005)**.

23. R.S. Sinsabaugh, Enzymic analysis of microbial pattern and process, *Biol. Fertil. Soils*, 1, 69-74 **(1994)**.

24. T. Cavalier-Smith, Deep phylogeny, ancestral groups and the four ages of life, *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 1537, 111-32 **(2010)**.

25. British Mycological Society, TG02: Fungal Cells, A Teacher's Guide to Fungal Cells **(2005)**.

26. P.H. Raven and G.B Johnson, Biology, WCB/McGraw-Hill, New York City, NY **(1999)**.

27. J.I. Pitt and A.D. Hocking, Naming and Classifying Fungi, Fungi and Food Spoilage, Springer US, Boston, MA **(2009)**, pp. 11-7.

28. P.W. Crous, W. Gams, J.A. Stalpers, V. Robert and G. Stegehuis, Stud. Mycol. 50, 19 **(2004)**.

29. R.W.S Weber, Recent Developments in the Molecular Taxonomy of Fungi, Physiology and Genetics: Selected Basic and Applied Aspects, Springer, Heidelberg, Germany **(2009)**, pp. 1-15.

30. D.S. Hibbett, M. Binder, J.F. Bischoff, M. Blackwell, P.F. Cannon, O.E. Eriksson, S. Huhndorf, T. James, P.M. Kirk, R. Lücking, H. Thorsten Lumbsch, F. Lutzoni, P.B. Matheny, D.J. McLaughlin, M.J. Powell, S. Redhead, C.L. Schoch, J.W. Spatafora, J.A. Stalpers, R. Vilgalys, M.C. Aime, A. Aptroot, R. Bauer, D. Begerow, G.L. Benny, L.A. Castlebury, P.W. Crous, Y.-C. Dai, W. Gams, D.M. Geiser, G.W. Griffith., C. Gueidan, D.L. Hawksworth, G. Hestmark, K. Hosaka, R.A. Humber, K.D. Hyde, J.E. Ironside, U. Kõljalg, C.P. Kurtzman, K.-H. Larsson, R. Lichtwardt, J. Longcore, J. Miądlikowska, A. Miller, J.-M. Moncalvo, S. Mozley-Standridge, F. Oberwinkler, E. Parmasto, V. Reeb, J.D. Rogers, C. Roux, L. Ryvarden, J.P. Sampaio, A. Schüßler, J. Sugiyama, R.G. Thorn, L. Tibell, W.A Untereiner, C. Walker, Z. Wang, A. Weir, M. Weiss, M.M. White, K. Winka, Y.-J. Yao and N.

Zhang, A higher-level phylogenetic classification of the Fungi, *Mycol. Res.*, 5, 509-47 **(2007)**.
31. C.J. Clegg and D.G. Mackean, Advanced biology: principles and applications, Hodder Education, London, U.K. **(1994)**.
32. O.V Singh, Extremophiles: Sustainable Resources and Biotechnological Implications, Wiley, Hoboken, NJ **(2012)**.
33. N. Hassan, M. Rafiq, M. Hayat, A.A, Shah and F. Hasan, Psychrophilic and psychrotrophic fungi: a comprehensive review, *Reviews in Environmental Science and Bio/Technology*, 2, 147-72 **(2016)**.
34. H. Burge, How does heat affect fungi?, *The Environmental Report*, 4, 3 **(2006)**.
35. C. Karch, Water and Fungi, The Environmental Reporter, 6, 2 **(2008)**.
36. K. Horikoshi, Alkaliphiles: Some Applications of Their Products for Biotechnology, *Microbiology and Molecular Biology Reviews*, 4, 735-50 **(1999)**.
37. R. Maheshwari, G. Bharadwaj and M.K. Bhat, Thermophilic Fungi: Their Physiology and Enzymes, *Microbiology and Molecular Biology Reviews*, 3, 461-88 **(2000)**.
38. N.J**.** Dix and J. Webster, Fungi of Extreme Environments, Fungal Ecology, Springer, Dordrecht, Netherlands **(1995)**, p. 322-40.
39. S. Gross and E.I. Robbins, Acidophilic and acid-tolerant fungi and yeasts, *Hydrobiologia*, 1, 91-109 **(2000)**.
40. D.H. Griffin, Fungal Physiology, Wiley, Hoboken, NJ **(1996)**.
41. J.I. Prosser, Growth kinetics of mycelial colonies and aggregates of ascomycetes, *Mycol. Res.*, 5, 513-28 **(1993)**.
42. M.J. Carlile, The Success of the Hypha and Mycelium, The Growing Fungus, Springer, Dordrecht, Netherlands **(1995)**, p. 3-19.
43. Kavanagh K., Fungi: Biology and Applications, Wiley, Hoboken, NJ **(2005)**.
44. Y. Krauke and H. Sychrova, Four Pathogenic Candida Species Differ in Salt Tolerance, *Curr. Microbiol.*, 4, 335-9 **(2010)**.
45. S.O. Petersen, P.S. Frohne and A.C. Kennedy, Dynamics of a Soil Microbial Community under Spring Wheat, *Soil Sci. Soc. Am. J.*, 3, 826-33 **(2002)**.
46. H.H.L González, S.L. Resnik and G. Vaamonde, Influence of temperature on growth rate and lag phase of fungi isolated from Argentine corn, *Int. J. Food Microbiol.*, 2, 179-83 **(1988)**.
47. M.J Carlile, S.C. Watkinson and G.W. Gooday, The Fungi, Academic Press, London, U.K. **(2001)**, p. 85-184.
48. K. Ashrafi, Protein N-Myristoylation, Stationary Phase Survival and Aging, Washington University, Washington, WA **(1999)**.
49. D.A Mitchell, O.F von Meien, N. Krieger and F.D.H Dalsenter, A review of recent developments in modeling of microbial growth kinetics and intraparticle phenomena in solid-state fermentation, *Biochem. Eng. J.*, 1, 15-26 **(2004)**.
50. G. Holt, G. Mcintyre, D. Flagg, E. Bayer, J. Wanjura and M. Pelletier, Fungal mycelium and cotton plant materials in the manufacture of biodegradable molded packaging material: Evaluation study of select blends of cotton byproducts, *J.Biobased Mater. Bio.*, 4, 431-9 **(2012)**.
51. L. Jiang, D. Walczyk, L. Mooney and S. Putney, Manufacturing of Mycelium-based Biocomposites, SAMPE, Covina, CA **(2013)**, p. 1944-55.
52. R.J.J Lelivelt, The mechanical possibilities of mycelium materials, Eindhoven University of Technology, Eindhoven, Netherlands **(2015)**.
53. J.A. López, González J., X. Chacón and J.A. Luna J., Assessment of Edible Fungi and Films Bio-Based Material Simulating Expanded Polystyrene, *Mater. Man. Process.*, 8, 1085-90 **(2016)**.
54. M.G. Pelletier, G.A. Holt, J.D. Wanjura, E. Bayer and G. McIntyre, An evaluation study of mycelium based acoustic absorbers grown on agricultural by-product substrates, *Ind. Crop. Prod.*, 51, 480 **(2013)**.
55. S. Travaglini, J. Noble, P.G. Ross and C.K.H Dharan, Mycology Matrix Composites, *ASC - 28th Technical Conference*, Pennsylvania, U.S.A **(2013)**.
56. G. McIntyre, A. Poetzsch, S. van Hook and D. Flagg, Method for producing a composite material, U.S. Patent 20,120,225,471, Sep 6 **(2012)**.
57. J. Kung'u, The Phases of Fungal Growth in Indoor Environment, MBL Labs, Mississauga, Canada **(2016)**.
58. K. Baert, F. Devlieghere, L. Bo, J. Debevere and B. De Meulenaer, The effect of inoculum size on the growth of Penicillium expansum in apples, *Food Microbiol.*, 1, 212-7 **(2008)**.
59. M. Gougouli, K. Kalantzi, E. Beletsiotis and K.P. Koutsoumanis, Development and application of predictive models for fungal growth as tools to improve quality control in yogurt production, *Food Microbiol.*, 8, 1453-62 **(2011)**.
60. T.P. McGonigle, J.P. Hovius and R.L. Peterson, Arbuscular mycorrhizae of American ginseng (Panax quinquefolius) in cultivated field plots: plant age affects the development of a colonization lag phase, *Can. J. Bot.*, 7, 1028-34 **(1999)**.
61. T.P. McGonigle and M.H. Miller, Mycorrhizal Development and Phosphorus Absorption in Maize under Conventional and Reduced Tillage, *Soil Sci. Soc. Am. J.*, 4, 1002-6 **(1993)**.
62. M. Sautour, P. Dantigny, M.C. Guilhem and M. Bensoussan, Influence of inoculum preparation on the growth of Penicillium chrysogenum, *J. Appl. Microbiol.*, 5, 1034-8 **(2003)**.
63. H.H.L González, S.L. Resnik and G. Vaamonde, Influence of inoculum size on growth rate and lag phase of fungi isolated from Argentine corn, *Int. J. Food Microbiol.*, 2, 111-7 **(1987)**.
64. S. Samapundo, F. Devlieghere, B. De Meulenaer and J. Debevere, Growth kinetics of cultures from single spores of Aspergillus flavus and Fusarium verticillioides on yellow dent corn meal, *Food Microbiol.*, 4, 336-45 **(2007)**.
65. J. Meyrath, Influence of the size of inoculum on various growth phases in Aspergillus oryzae, Antonie van Leeuwenhoek, 1, 57-78 **(1963)**.
66. H.Q. Wang, J.J Zhong. and J.T. Yu, Enhanced production of taxol in suspension cultures of Taxus chinensis by controlling inoculum size, *Biotechnol. Lett.*, 4, 353-6 **(1997)**.
67. F.C Yang and C.B Liau, The influence of environmental conditions on polysaccharide formation by Ganoderma lucidum in submerged cultures, *Process Biochem.*, 5, 547-53 **(1998)**.
68. Y.H. Zhang and J.J Zhong, Hyperproduction of ginseng saponin and polysaccharide by high density cultivation of Panax notoginseng cells, *Enzyme Microb. Tech.*, 1, 59-63 **(1997)**.
69. Y. Ji, K. Zhu, H. Qian and H. Zhou, Effect of water activity and temperature on growth of Penicillium citreoviride and Penicillium citrinum on MiGao (rice cake), *Can. J. Microbiol.*, 2, 231-6 **(2007)**.
70. Z.U. Khokhar, M.A. Athar, Q.U. Syed, S. Baig, M. Nadeem, M.G. Sher, S. Ali and I. Islam, Studies on the development of the value added food by barley with Rhizopus oligosporus in koji fermentation, *Science International*, 3,6-8 **(2010)**.
71. A. Chang, Genetics and breeding of edible mushrooms, CRC Press, Boca Raton, FL **(1992)**.
72. L. Rockland, Water Activity: Theory and Applications to Food, Taylor & Francis, Abingdon, U.K. **(1987)**.
73. E. Pardo, U. Lagunas, V. Sanchis, A.J. Ramos. and S. Marín, Influence of water activity and temperature on conidial germination and mycelial growth of ochratoxigenic isolates of Aspergillus ochraceus on grape juice synthetic medium. Predictive models, *J. Sci. Food Agr.*, 10, 1681-6 **(2005)**.
74. Z.A Chipeta, J.C. Preez and L. Christopher, Effect of cultivation pH and agitation rate on growth and xylanase production by Aspergillus oryzae in spent sulphite liquor, *J. Ind. Microbiol. Biotech.*, 6, 587-94 **(2008)**.
75. M.O. Garraway and R.C. Evans, Fungal nutrition and physiology, Wiley, Hoboken, NJ **(1984)**.
76. R. Maheshwari, Fungi: Experimental Methods In Biology, CRC Press, Boca Raton, FL **(2016)**.
77. G. Canevascini, M.R. Coudray, R. Southgate and H. Meier, Induction and catabolite repression of cellulase synthesis in the thermophilic fungus Sporotrichum thermophile, *Microbiology*, 2, 291-303 **(1979)**.
78. D.E. Sistrom and L. Machlis, The effect of D-glucose on the utilization of D-mannose and D-fructose by a filamentous fungus, *J, Bacteriol.*, 1, 50-5 **(1955)**.
79. R.C France and C.P. Reid, Pure culture growth of ectomycorrhizal fungi on inorganic nitrogen sources, *Microbial Ecol.*, 3, 187-95 **(1984)**.
80. N. Bouras, M.D. Holtz, R. Aboukhaddour and S.E. Strelkov, Influence of nitrogen sources on growth and mycotoxin production by isolates of Pyrenophora tritici-repentis from wheat, *The Crop Journal*, 2, 119-28 **(2016)**.
81. R. Finlay, A. Frostegård and A.M. Sonnerfeldt, Utilization of organic and inorganic nitrogen sources by ectomycorrhizal fungi in pure culture and in symbiosis with Pinus contorta Dougl. ex Loud, *New Phytol.*, 1, 105-15 **(1992)**.

82. L. Boddy, R. Marchant and D.J. Read, Nitrogen, Phosphorus and Sulphur Utilisation by Fungi, Cambridge University Press, Cambridge, U.K. **(1989)**.
83. A.J. Peacock, Oxygen at high altitude, *Brit. Med. J.*, 7165, 1063-6 **(1998)**.
84. J. Repeèkienë, Influence of phosphorous fertilizers on fungial growth and oil hydrocarbon degradation, *Bone*, 16, 1 **(2001)**.
85. P. Nagadesi and A. Arya, Effect of carbon and nitrogen on the growth of lignicolous fungi from Rathanmahal Wildlife Sanctuary, Gujarat, India, *Int. J. Sci. Nat.*, 2, 237-45 **(2013)**.
86. A., Anamika, Study on Nutritional Requirements of Nematophagous Fungi in Terms of Carbon and Nitrogen Sources, *J. Agr. Sci.*, 6, 227 **(2015)**.
87. J. Amich, L. Schafferer, H. Haas and S. Krappmann, Regulation of Sulphur Assimilation Is Essential for Virulence and Affects Iron Homeostasis of the Human-Pathogenic Mould Aspergillus fumigatus, *PLOS Pathogens*, 9, e1003573 **(2013)**.
88. R.G.H Siu and J.W. Sinden, Effects of pH, Temperature, and Mineral Nutrition on Cellulolytic Fungi, *Am. J. Bot.*, 4, 284-90 **(1951)**.
89. A.P. Trinci and A.J. Collinge, Hyphal wall growth in Neurospora crassa and Geotrichum candidum, *J. Gen. Microbiol.*, 2, 355-61 **(1975)**.
90. G. Lysek, The Ecology and Physiology of the Fungal Mycelium, Cambridge University Press, Cambridge, U.K. **(1984)**.
91. J.P. Larpent, Caracteres et determinismes des correlations d'inhibition dans le mycelium jeune de quelques champignons, *Ann. Sci. Nat. Bot. Biol.*, 1, 130 **(1966)**.
92. D. Griffin, W. Timberlake and J. Cheney, Regulation of macromolecular synthesis, colony development, and specific growth rate of Achlya bisexualis during balanced growth, *Microbiology*, 2, 381-8 **(1974)**.
93. J.I. Prosser and A.P. Trinci, A model for hyphal growth and branching, *J. Gen. Microbiol.*, 1, 153-64 **(1979)**.
94. D.N. Pegler, Hyphal analysis of basidiomata, *Mycol. Res.*, 2, 129-42 **(1996)**.
95. E.J.H. Corner, The construction of polypores. *Phytomorphology* **(1953)**, 3,152.
96. J. Breitenbach and F. Kränzlin, Fungi of Switzerland: Non-gilled fungi: Heterobasidiomycetes, Aphyllophorales, Gasteromycetes, Verlag Mykologia, Lucerne, Switzerland **(1986)**.
97. H. Clémençon and V. Emmett, Cytology and Plectology of the Hymenomycetes, Schweizerbart Science Publishers, Stuttgart, Germany **(2004)**.
98. K.S. Ko and H.S. Jung, Molecular phylogeny of Trametes and related genera, *Antonie van Leeuwenhoek*, 3, 191-9 **(1999)**.
99. L. Ryvarden, Genera of polypores: Nomenclature and taxonomy, *Synopsis Fungorum*, 5, 1 **(1991)**.
100. E. Bayer and G. McIntyre, Substrate Composition and Method for Growing Mycological Materials, U.S. Patent 20,120,315,687, 13 Dec **(2012)**.
101. A. Ames, A Consideration of Structure in Relation to Genera of the Polyporaceae, *Annales Mycologici*, 11, 2, 209-253 **(1913)**.
102. E. Bayer and G. McIntyre, Method for Growing Mycological Materials, U.S. Patent 20,150,247,115, Sep 3 **(2015)**.
103. S. Bartnicki-Garcia, Cell Wall Chemistry, Morphogenesis, and Taxonomy of Fungi, *Annu. Rev. Microbiol.*, 1, 87-108 **(1968)**.
104. J.G.H. Wessels, P.C. Mol, J.H. Sietsma and C.A. Vermeulen, Wall Structure, Wall Growth, and Fungal Cell Morphogenesis, Biochemistry of Cell Walls and Membranes in Fungi, Springer, Heidelberg, Germany **(1990)**, p. 81-95.
105. M. Rinaudo, Chitin and Chitosan - General Properties and Applications, *ChemInform*, 7, 603-32 **(2007)**.
106. J. Ruiz-Herrera, Fungal Cell Wall: Structure, Synthesis, and Assembly, CRC Press, Boca Raton, FL **(2016)**.
107. G.A.F Roberts, Structure of Chitin and Chitosan. Chitin Chemistry, Macmillan Education, London, U.K. **(1992)**, p. 1-53.
108. K. Nilsson and J. Bjurman, Chitin as an indicator of the biomass of two wood-decay fungi in relation to temperature, incubation time, and media composition, *Can. J. Microbiol.*, 6, 575-81 **(1998)**.
109. R. Mazur, Mechanical Properties of Sheets Comprised of Mycelium - A Paper Engineering Perspective, State University of New York, NY **(2015)**.
110. W.H. Conkey, W.M. van Horn, B.F. Shema and W.H. Shockley, Sheets comprising filaments of fungi, U.S. Patent 2,811,442 Apr 12 **(1957)**.
111. F.L. Matthews and R.D. Rawlings, Composite Materials: Engineering and Science, CRC Press, Boca Raton, FL **(1999)**.
112. V.K. Thakur and A.S. Siṅgha, Biomass-based Biocomposites, Smithers Rapra Technology, Shrewsbury, U.K. **(2013)**.
113. D.R. Askeland, P.P Fulay and W.J. Wright, The Science and Engineering of Materials, Cengage Learning, Melbourne, Australia **(2010)**.
114. J. He, C.M. Cheng, D.G. Su and M.F. Zhong, Study on the Mechanical Properties of the Latex-Mycelium Composite, Applied Mechanics and Materials, Trans Tech Publications Inc., Zurich, Switzerland **(2014)**, pp. 415-20.
115. G. McIntyre, G. Tudryn, J. Betts and J. Winiski, Stiff Mycelium Bound Part and Method of Producing Stiff Mycelium Bound Parts, U.S. Patent 20,150,038,619, Feb 5 **(2015)**.
116. E. Bayer and G. McIntyre, Method of growing electrically conductive tissue, U.S. Patent 9,253,889, Feb 2 **(2016)**.
117. S. Yamanaka and R. Kikuchi, Complex of fibers and fungi and a process for preparation thereof, U.S. Patent 5,074,959, Dec 24 **(1991)**.
118. W.J.A. Dschida, Fungal cell wall production and utilization as a raw resource for textiles, U.S. Patent 5,854,056, Nov 28 **(1998)**.
119. L. Edebo, Porous structure comprising fungi cell walls, U.S. Patent 6,423,337, Jul 23 **(2002)**.
120. E. Bayer, S. Araldi and G. McIntyre, Method for Making Dehydrated Mycelium Elements and Product Made Thereby, U.S. Patent 20,130,280,791, Oct 24 **(2013)**.
121. E. Bayer and G. McIntyre, Method for producing rapidly renewable chitinous material using fungal fruiting bodies and product made thereby, U.S. Patent 8,001,719, Aug 23 **(2011)**.
122. E. Bayer and G. McIntyre, Self-supporting Composite Material Made With Mycelia Cells and Method of Making Same, U.S. Patent 20,130,295,368, Nov 7 **(2013)**.
123. E. Bayer, G. McIntyre and B.L. Swersey, Method for producing grown materials and products made thereby, U.S. Patent 20,080,145,577, Jun 9 **(2008)**.
124. E. Bayer, G. McIntyre and B.L. Swersey, Fabricated Product, U.S. Patent 20,140,069,004, Mar 13 **(2014)**.
125. E. Bayer, G. McIntyre and B.L. Swersey, Panel comprising composite of discrete particles and network of interconnected mycelia cells, U.S. Patent 8,999,687, Apr 7 **(2015)**.
126. G. McIntyre, E. Bayer and D. Flagg, Method of Producing a Chitinous Polymer Derived from Fungal Mycelium, U.S. Patent 20,120,227,899, Sep 13 **(2012)**.
127. G. McIntyre, E. Bayer and A. Palazzolo, Method of Growing Mycological Biomaterials, U.S. Patent 20,130,263,500, Oct 10 **(2013)**.
128. G. McIntyre, E. Bayer and C. Scully, Composite Material for Absorbing and Remediating Contaminants and Method of Making Same, U.S. Patent 20,130,095,559, Apr 18 **(2013)**.
129. G. McIntyre, J.D. Betts, G. Tudryn and L. Mooney, Method of Manufacturing a Stiff Engineered Composite, U.S. Patent 20,150,101,509, Apr 16 **(2015)**.
130. R.E. Kalisz and C.A Rocco, Method of making molded part comprising mycelium coupled to mechanical device, U.S. Patent 8,227,224, Jul 24 **(2012)**.
131. R.E Kalisz and C.A Rocco, Method of making foamed mycelium structure, U.S. Patent 8,227,233, Jul 24 **(2012)**.
132. R.E. Kalisz and C.A. Rocco, Method of making a hardened elongate structure from mycelium, U.S. Patent 8,298,809, Oct 30 **(2012)**.
133. R.E. Kalisz and C.A. Rocco, Cut sheet stock mycelium and method, U.S. Patent 20,110,266,831, Nov 3 **(2011)**.
134. R.E. Kalisz and C.A. Rocco, Hardened mycelium structure and method, U.S. Patent 20,130,202,855, Aug 8 **(2013)**.
135. R.E. Kalisz, C.A. Rocco, E.C.J Tengler and R.L Petrella-Lovasik, Injection molded mycelium and method, U.S. Patent 8,313,939, Nov 20 **(2012)**.
136. C.A Rocco and R.E. Kalisz, Plasticized mycelium composite and method, U.S. Patent 8,227,225, Jul 24 **(2012)**.
137. C.A Rocco and R.E. Kalisz, Mycelium structures containing nanocomposite materials and method, U.S. Patent 8,283,153, Oct 9 **(2012)**.
138. C.A. Rocco and R.E. Kalisz, Mycelium structure with self-attaching coverstock and method, U.S. Patent 8,298,810, Oct 30 **(2012)**.

139. P. Ross, Method for Producing Fungus Structures, U.S. Patent 20,120,135,504, May 31 **(2012)**.
140. L. Laboutiere, E. Lemiere and A. Mechineau, Process for manufacturing a composite material based on natural fibres seeded with mycelium and part obtained with such a process, French Patent WO2014195641A, Dec 11 **(2014)**.
141. D.D. Schaak and M.J Lucht, Biofilm Treatment of Composite Materials Containing Mycelium, US20150376565, **(2015)**.
142. G.R. McIntyre, J.D. Betts, G. Tudryn and L. Mooney, Method of Manufacturing a Stiff Engineered Composite, U.S. Patent 15,258,685, Sep. 7 **(2016)**.
143. E. Bayer and G.R. McIntyre, Composite Material, U.S Patent 20,170,000,040, Sep. 15 **(2017)**.
144. J. Winiski, S. van Hook, M. Lucht and G. McIntyre, Process for Solid-state Cultivation of Mycelium on a Lignocellulose Substrate, U.S Patent 20,160,264,926, May 9 **(2016)**.
145. ASTM International, ASTM C578–04, Standard Specification for Rigid, Cellular Polystyrene Thermal Insulation, West Conshohocken, PA **(2004)**.
146. M. Gunther, Can mushrooms replace plastic?, The Guardian **(2013)**.
147. Dell, Green Packaging & Shipping, **(2016)**.
148. E. Gosden, Ikea plans mushroom-based packaging as eco-friendly replacement for polystyrene, The Telegraph **(2016)**.
149. Department of Justice and Attorney-General, Compliance program report – expandable polystyrene bead manufacturing **(2013)**.
150. B. Watson, Fungus not formaldehyde, The Guardian **(2016)**.
151. Ecovative Design, Press Kit, **(2016)**.
152. E. Bayer and G. McIntyre, Fabricated Panel, U.S. Patent 20,130,224,840, Aug 29 **(2013)**.
153. United States Environmental Protection Agency, Organic Chemical Process Industry, Emissions Factors & AP 42 **(1991)**.
154. Washington State Department of Ecology, Polystyrene Manufacture, Washington State Air Toxic Sources and Emission Estimation Methods **(1998)**.
155. A. Fisher, Industrial-Strength Fungus, Time Magazine **(2010)**.
156. MycoWorks, Mycotecture **(2014)**.
157. MycoWorks, Portfolio, **(2014)**.
158. A. Rajagopal, Behind The Living's "100% Organic" Pavilion for MoMA PS1, Metropolis **(2014)**.
159. D. Bulatov, Evolution Haute Couture: Art and Science in the Post-Biological Age, Moscow Biennale **(2009)**.
160. R. Debatty, C. Evans, P. Garcia, A. Grover, New Art/Science Affinities, Carnegie Mellon University, Pittsburgh, PA **(2011)**.
161. J. Austin, Mycotectural Polyominoes, Zero1 **(2013)**.
162. Superflux, Mycotecture (Phil Ross), New York Museum of Modern Art, **(2014)**.
163. International Association for Earthquake Engineering. Buildings in Fired-Brick and other Masonry Units. Guidelines for Earthquake Resistant Non-engineered Construction **(2004)**.
164. ASTM International, ASTM C62-13a Standard Specification for Building Brick (Solid Masonry Units Made From Clay or Shale), West Conshohocken, PA **(2013)**.
165. Ecovative Design, MycoBoard, **(2016)**.
166. Engineered Wood Products Association of Australasia, Facts about particleboard and MDF **(2008)**, p. 4.
167. R. Everett-Green, A growth industry: How mushrooms are being used to create furniture, The Globe and Mail **(2014)**.
168. Fondazione PLART, The Future of Plastic **(2014)**.
169. E.M González Growing Architecture through Mycelium and Agricultural Waste, Columbia Graduate School of Architecture, New York City, NY **(2010)**.
170. E. Klarenbeek, Designers of the Unusual **(2014)**.

Reviewer comments:

Summary of Reviewers' Comments (Remarks to the Author)

The manuscript falls within the scope of the journal.

The manuscript should be formatted to the journal standard. Authors may combine all the word files in the main manuscript, references and figures for example.

Introduction section should be refined nicely, focusing current trend in this topic.

All the sections are concisely discussed.

As a reviewer, I would suggest the authors to focus on current trends in the field mycelium composites. The references may be updated to recent years if appropriate.

The manuscript should be formatted to the standard format of the journal.

Have your manuscript edited in correct scientific English with help of English native speaker or commercial English-editing services.

The authors are encouraged to cite a few references which are published in the "Journal of Biomaterials and Tissue Engineering" (http://www.aspbs.com/jbt.html) and the "Journal of Bionanoscience" (http://www.aspbs.com/jbns/), if appropriate.

Reviewer query 1: The manuscript falls within the scope of the journal.

Authors' response:
The authors thank the reviewers for their feedback.

Reviewer query 2: The manuscript should be formatted to the journal standard. Authors may combine all the word files in the main manuscript, references and figures for example.

Authors' response:
Completed. The manuscript was formatted as closely as possible to the "sample manuscript format for authors" PDF available from www.aspbs.com/job.html.

Reviewer query 3: Introduction section should be refined nicely, focusing current trend in this topic.

Authors' response:
The introduction has been reviewed. It is concise and 2 additional references from 2017 have been added to complement the existing 5 references from within the last 3 years and 8 references from within the last 5 years. More recent statistics are not available since updated government reports have not yet been released.

Reviewer query 4: All the sections are concisely discussed.

Authors' response:
The authors thank the reviewers for their feedback.

Reviewer query 5: As a reviewer, I would suggest the authors to focus on current trends in the field mycelium composites. The references may be updated to recent years if appropriate.

Authors' response:
Completed. Current trends are represented by 3 references from 2017, 14 references from 2016 and 8 references from 2015. Overall, the article contains 70 references published within the last 5 years.

Reviewer query 6: The manuscript should be formatted to the standard format of the journal.

Authors' response:
Completed, as previously detailed.

Reviewer query 7: Have your manuscript edited in correct scientific English with help of English native speaker or commercial English-editing services.

Authors' response:
The first author speaks English as a first language. The manuscript has been proof read by 6 post-doctoral academics who completed their degrees in English speaking countries. It has also been checked for over 250 writing errors using commercial writing software (Grammarly Premium).

Reviewer query 8: The authors are encouraged to cite a few references which are published in the "Journal of Biomaterials and Tissue Engineering" (http://www.aspbs.com/jbt.html) and the "Journal of Bionanoscience" (http://www.aspbs.com/jbns/), if appropriate.

Authors' response:
Advanced searches completed on 19 March, 2017 using Google Scholar for the keywords "mycelium", "composite", "biocomposite", "growth kinetics" and "hypha" in the journals "Journal of Biomaterials and Tissue Engineering" and "Journal of Bionanoscience" yielded no results relevant to the article in question.

Publication B

Literature Review

Mycelium materials for construction applications, influence of the mycelium binder and substrate filler on composite mechanical performance, hot and cold pressing to improve mechanical performance, resin infusion and sandwich structures, hybridisation, thermal conductivity and acoustic properties for insulation applications, thermal degradation and fire safety properties, water absorption, termite resistance.

Engineered Mycelium Composite Construction Materials from Fungal Biorefineries

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Abstract

Mycelium composites are an emerging class of cheap and environmentally sustainable materials experiencing increasing research interest and commercialisation in Europe and the United States for construction applications. These materials utilise natural fungal growth as a low energy bio-fabrication method to upcycle abundant agricultural by-products and wastes into more sustainable alternatives to energy intensive synthetic construction materials. Mycelium composites have customisable material properties based on their composition and manufacturing process and can replace foams, timber and plastics for applications, such as insulation, door cores, panelling, flooring, cabinetry and other furnishings. Due to their low thermal conductivity, high acoustic absorption and fire safety properties outperforming traditional construction materials, such as synthetic foams and engineered woods, they show particular promise as thermal and acoustic insulation foams. However, limitations stemming from their typically foam-like mechanical properties, high water absorption and many gaps in material property documentation make the targeted use of mycelium composites for specific, suitable applications necessary. Nonetheless, useful material properties in addition to the low costs, simplicity of manufacture and environmental sustainability of mycelium composites suggest that they will play a significant role in the future of green construction.

Keywords: Fungal mycelium, mechanical performance, insulation properties, fire safety, water and termite resistance

1. Introduction

Significant pressure has been applied to the construction industry over the past decade, as the supply of traditional construction materials, such as cement, bricks, timber, cladding and partitioning materials, has struggled to keep up with an ever increasing global population (Madurwar, Ralegaonkar & Mandavgane 2013; Pheng & Hou 2019). Production of these conventional construction materials consumes energy, limited natural resources and pollutes our air, land and water (Madurwar, Ralegaonkar & Mandavgane 2013). Up to 36% of the lifetime energy demands of a typical dwelling can be attributed to the harvest or extraction of

primary materials, manufacture, transport and construction of the building (Sartori & Hestnes 2007). Low energy buildings, while using less energy during occupation, are even less environmentally sustainable to build (up to 46% of the lifetime energy demands of the dwelling can be attributed to the construction of the building), due to the energy required to manufacture the increased levels of insulation, higher density materials and additional technologies they utilise (Monahan & Powell 2011; Thormark 2002).

The rapidly growing global population has also resulted in growing food demand and increased agricultural output, leading to the generation of agricultural by-products and wastes, such as sugarcane bagasse, rice husks, cotton stalks, straw and stover. (Bhuvaneshwari, Hettiarachchi & Meegoda 2019). The combined biomass residue generation of India and south east Asia alone is as much as 1 billion tons every year (Bhuvaneshwari, Hettiarachchi & Meegoda 2019; Tun et al. 2019). Low-grade agricultural by-products and wastes have limited applications with their primary use being fertiliser, animal bedding and fillers for building materials and road construction but they are largely discarded as waste or burned, generating carbon dioxide and other greenhouse gases (Defonseka 2014).

The vegetative growth of filamentous fungi (mycelium) has attracted increasing academic and commercial interests over the past decade as a new form of low energy bio-fabrication and waste upcycling (Holt et al. 2012; Jones, M; et al. 2017; Pelletier et al. 2013). Mycelium bonds organic matter through a network of hyphal micro-filaments in a natural biological process able to be exploited to produce both low-value materials, such as packaging and higher-value composite materials (Haneef et al. 2017; Holt et al. 2012; Islam et al. 2017; Jones, M; et al. 2017; Pelletier et al. 2013) from problematic agricultural and industrial waste materials with little or no commercial value (DP CleanTech 2013; Sustainability Victoria 2014). Mycelium acts as a continuous fibrous phase called the matrix (Pelletier et al. 2013; Thakur & Siṅgha 2013) that interfaces with a dispersed phase of partially digested agricultural residue (substrate filler) as it grows (Thakur & Siṅgha 2013).

Mycelium-derived materials have several key advantages over traditional synthetic materials including their low cost, density and energy consumption in addition to their biodegradability and low environmental impact and carbon footprint (Abhijith, Ashok & Rejeesh 2018; Arifin & Yusuf 2013; Haneef et al. 2017). A wide variety of utilisable substrates coupled with controlled processing techniques (e.g. growth environment, hot pressing) allow mycelium-derived materials to meet specific structural and functional requirements including fire resistance and thermal and acoustic insulation (Haneef et al. 2017; Holt et al. 2012; Jones, M; et al. 2017; Pelletier et al. 2013). This not only permits their use as waste-derived environmentally friendly alternatives to synthetic planar materials (e.g. plastic films and sheets) (Haneef et al. 2017),

and larger low density objects (e.g. synthetic foams and plastics) (Holt et al. 2012; López Nava et al. 2016; Pelletier et al. 2013; Travaglini et al. 2013) but also as semi-structural materials (e.g. panelling, flooring, furniture, decking) (Abrams 2014; Islam et al. 2017; Jiang et al. 2016; Jiang et al. 2017), paving the way for new possibilities in environmentally sustainable construction. However, several factors limit the current application and usage of mycelium materials, primarily stemming from their typically foam-like mechanical properties, high water absorption and many gaps in material property documentation. These limitations make further research and development of these materials necessary in addition to targeted usage in specific, suitable applications.

2. Fungal biopolymers and composite manufacturing

Fungi are a natural and renewable source of valuable structural polymers, such as chitin and chitosan, as opposed to cellulose which is the main structural polymer in plant cell walls **(Figure 1)**. Chitin is a linear macromolecule composed of N-acetylglucosamine units and is also the main component of most insect and other arthropod exoskeletons (Rinaudo 2007). It is strong with a nanofibril tensile strength of ~1.6-3.0 GPa (Bamba et al. 2017) resulting from a high dipole moment and hydrogen bonding between the chains of the macromolecules (Webster & Weber 2007).

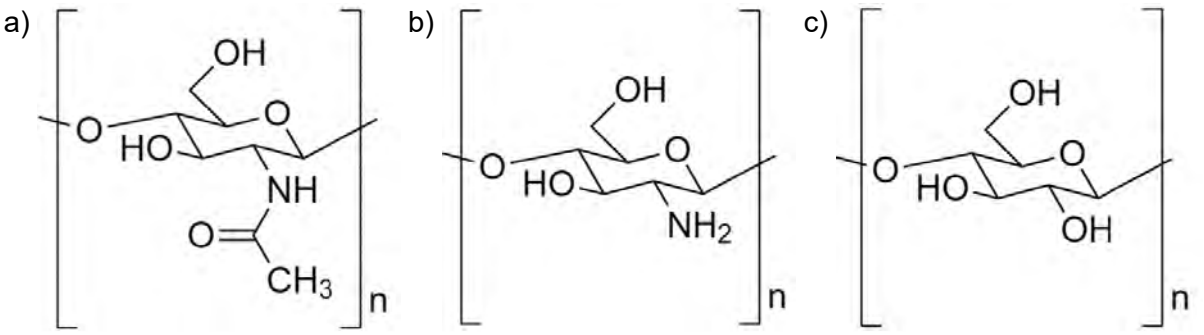

Figure 1. Molecular structures of (a) chitin, (b) chitosan and (c) cellulose.

Fungal cell walls are present in hyphae, which form a mycelium (collective noun) of hyphal filaments, comprising a thick and complex fibrous network of chitin, other polysaccharides, such as glucans, manno-proteins, chitosan, polyglucuronic acid or cellulose, and smaller quantities of proteins and glycoproteins (Bartnicki-Garcia 1968; Wessels et al. 1990). These components result in mycelium exhibiting mechanical properties typical of lignocellulosic materials, such as wood and cork (Appels et al. 2018). However, mycelium composites comprising a fibrous matrix phase of mycelium grown through a dispersed substrate filler phase of agricultural residue have lower densities and elastic moduli than pure mycelium and are generally classified as foams (Appels et al. 2019; Ashby, Shercliff & Cebon 2018). This is

due to the amount of air contained within and between the often porous and loosely packed substrate filler (Holt et al. 2012).

Mycelium composites are manufactured using a low-energy, natural manufacturing process, which sequesters carbon and is one of the key advantages of these materials **(Figure 2)**. Raw material is required as a precursor and can realistically constitute any material that can sustain fungal growth, such as carbohydrates (Jones, M; et al. 2017; Kavanagh 2005). Low-cost lignocellulosic agricultural or forestry by-products or wastes are commonly used as fibrous substrates, such as straw, or particulate substrates, such as sawdust, to keep the cost of mycelium composites low and to facilitate waste upcycling and circular economy (Camere & Karana 2018; Jones, M et al. 2018; Pelletier et al. 2013). However, usage of these cheap, low-grade materials as substrates, while keeping costs low and environmental sustainability high, has the unfortunate side effect of limiting fungal growth and hence compromises the material properties of the composite. Although this compromise is acceptable for production of foam-like mycelium composites, higher grade and more expensive substrates such as nutritious wheat grains and saw dusts are sometimes used when mechanical properties are a priority (Elsacker et al. 2019; Travaglini et al. 2013; Xing et al. 2018).

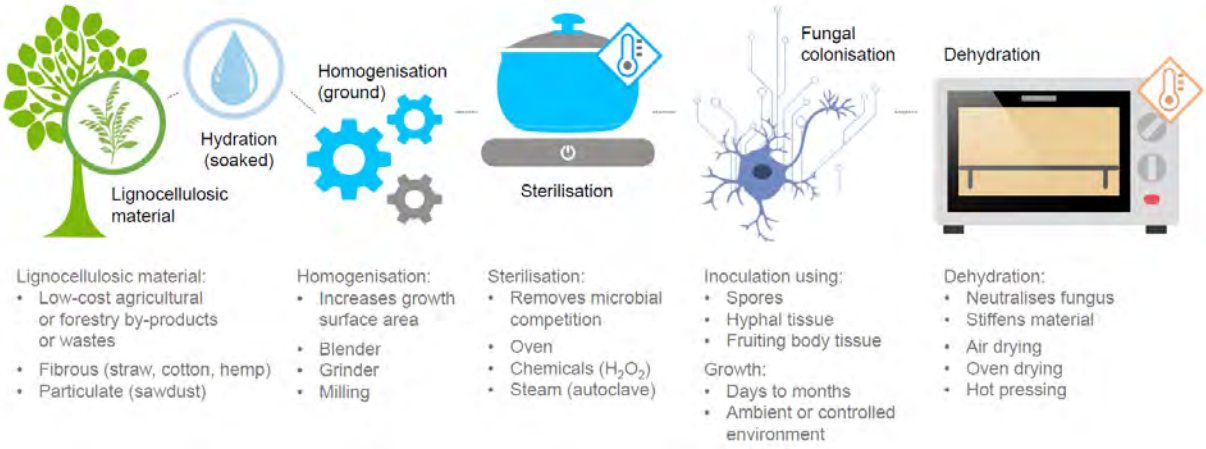

Figure 2. Mycelium composite manufacturing process detailing the key stages, the purpose and the possible variations in the processes utilised during each stage.

Irrespective of the grade of the material, substrates are first soaked in water to hydrate them, since moisture is very important to fungal growth, with the duration of this stage varying from substrate to substrate (Elsacker et al. 2019). Substrates such as rice hulls absorb very little moisture, making the duration of soaking less important than for inoculation media, such as wheat grains which swell considerably and require soaking durations of at least 48 h (Jones, M et al. 2018). Hydrated raw material is then homogenised to increase the growth surface area, which can be completed using low-energy mechanical processes, achievable using a kitchen blender, or grinding or milling depending on requirements and manufacturing scale

(Elsacker et al. 2019). Macerated raw material is then sterilised to remove the microbial competition of existing bacteria and fungi already present in the material. This can be completed using high temperature conditions in an oven, which has the disadvantage of drying out the substrate, or a pressure cooker or autoclave, which keeps the substrate hydrated and is hence preferred. Chemicals such as hydrogen peroxide (H_2O_2) can also be used to sterilise the substrate, but while less energy intensive than other sterilisation methods are less effective, resulting in higher contamination rates (Lelivelt 2015).

Composite assembly itself is completed using the natural and biological fungal growth process, which binds the lignocellulosic material into 3D geometries mirroring the mould the substrate is packed into (Holt et al. 2012; Jones, M; et al. 2017). The lignocellulosic substrate is inoculated by introducing and evenly dispersing 10-32 wt% of any element of fungal biomass, such as spores in a liquid solution or hyphal or fruiting body tissue grown on a nutrient rich substrate, such as wheat grains, to the lignocellulosic material contained within the mould (Jones, M; et al. 2017; McIntyre et al. 2012). Spores have the advantage of being very easily and evenly dispersed throughout the substrate and provide many initial growth points, but require a nutrient-rich substrate, initially struggling to grow on low-grade materials. Grain- or sawdust-based inocula mitigate this problem by supplying a nutrient-rich substrate to support initial growth, which can then spread to lower-grade substrates, but provide fewer initial growth points and are more difficult to evenly disperse (Jones, M et al. 2018).

Following inoculation, moulds can be stored under ambient conditions or in a temperature-controlled environment at ~25-27°C for a growth period of days to months depending on the fungal species and substrate used and the degree of bonding desired (Griffin 1996; Jones, M; et al. 2017). Ambient conditions are obviously cheaper and more energy efficient to maintain but will result in slower growth than environments of elevated temperature. Following the growth period, the composite materials can be removed from the moulds and hot-pressed, oven or air dried to dehydrate the material and neutralise the fungus. This simultaneously ensures that it cannot grow further or spread while stiffening the composite material (McIntyre et al. 2012). Hot-pressing and oven drying are favoured by industry as they are the fastest dehydration processes, with hot-pressing also consolidating the material and resulting in higher mechanical properties. Fully processed mycelium composite materials are fully biodegradable and comprise ~95 wt% lignocellulosic material bound using ~5 wt% fungal mycelium for nutrient rich substrates (estimated based on an ergosterol concentration of ~870 ppm, corresponding to 50 mg of biomass for every 1 g wheat grains grown over 7 d) (Jones, M et al. 2019).

Foam-like physical and mechanical properties make mycelium composites suitable for non-structural construction applications including insulation materials and door cores **(Figure 3a)**. Mycelium-based acoustic insulation foams are already commercially available in Europe and the United States **(Figure 3b),** with significant advances also being made in the development of mycelium-based thermal insulation foams and highly flexible polymer-like materials **(Figure 3c)**. Introduction of a soy-based resin within mycelium composites can extend their use to semi-structural applications, such as panelling, flooring, cabinetry and other furnishings **(Figure 3d)**.

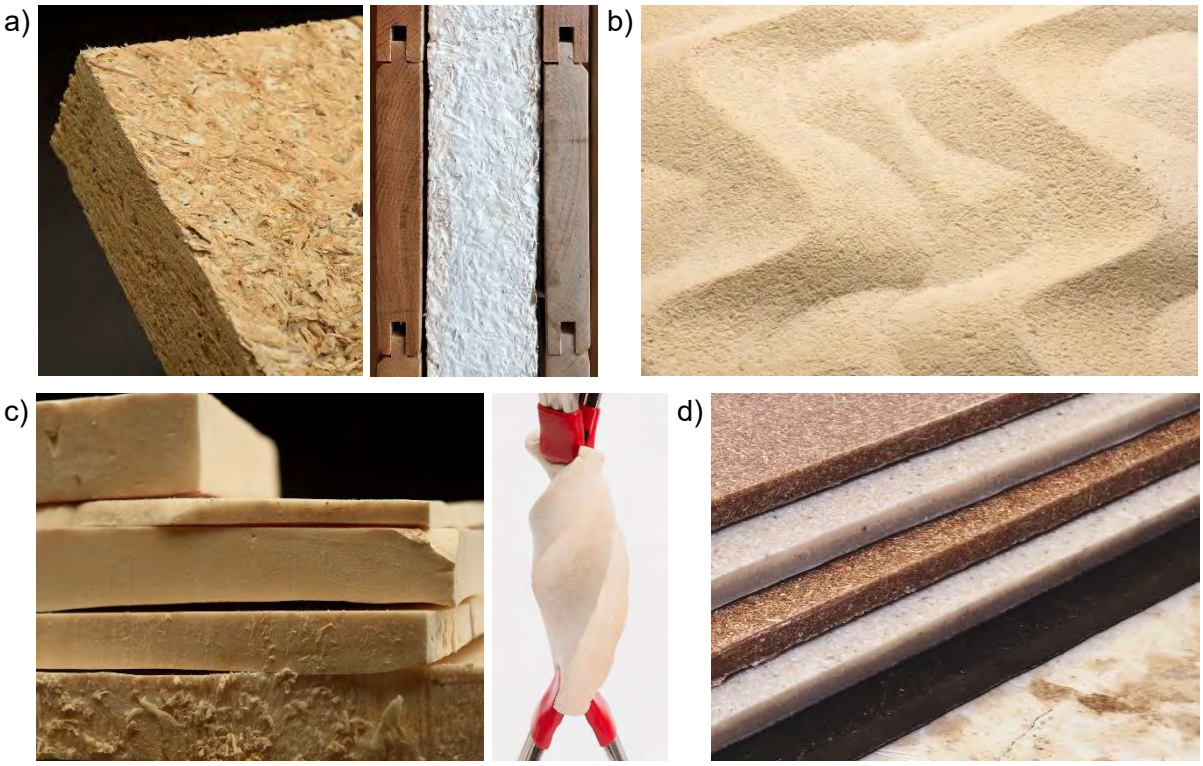

Figure 3. Commercial mycelium composite construction materials as a) particleboard replacements for wall panelling and door cores, b) acoustic foams, c) flexible insulation foams and d) resin infused laminate flooring. Images courtesy of Ecovative Design LLC (Green Island, USA).

However, despite the vast potential of these materials, which have been commercially available for over a decade, their adoption has been slow. Dell uses mycelium foams for packaging of business servers and IKEA has also expressed interest in adopting mycelium-based packaging (Dell 2016; Gosden 2016). Nevertheless, for the most part mycelium materials remain a predominantly underutilised niche product favoured by a select group of artists and designers, used to produce everything from furnishings such as chairs and lampshades to artistic structures, such as Philip Ross' "Mycotectural Alpha" tea house and the 12 m "Hy-Fi" organic compostable tower, comprising over 10,000 bricks, showcased by the

New York Museum of Modern Art in 2014 (Austin 2013; Fisher 2010; Jones, M; et al. 2017; Rajagopal 2014; Superflux 2014). This underutilisation could be the result of a patent monopoly on mycelium materials resulting in a lack of industrial commercial viability, a lack of trust in this new material platform for applications beyond packaging or a lack of awareness among the general public and industry as to the existence of this material. Interest is however growing in mycelium materials with companies now active in the United States, Italy, Indonesia, the Netherlands and research spanning the United States, Italy, Belgium, the Netherlands, Australia, Austria and Switzerland.

3. Engineering of mycelium composite material properties

3.1 Influence of the mycelium binder on composite mechanical performance

The mycelium constituent of mycelium composites is often blamed for their limited mechanical performance (Jiang et al. 2019; Travaglini et al. 2013). However, recent studies investigating chitin-glucan extracts derived from mycelium have found the matrix phase to be quite strong (up to 25 MPa tensile strength) (Jones, M. et al. 2019), suggesting that insufficient fungal growth density limiting matrix quantity and matrix-filler interface are more likely to be responsible for limited mechanical performance. The species of fungus utilised as the matrix phase to bind dispersed agricultural filler into mycelium composites affects growth density and degree of interfacial bonding at the mycelium-substrate interface, which varies significantly by species and substrate (Jones, Huynh & John 2018), and does appear to affect the mechanical properties of the material. How well a fungal species grows on any given substrate is influenced by natural evolutionary factors. In nature, mesophilic (optimal growth at moderate temperatures) microflora are succeeded by thermophilic (optimal growth at high temperatures) microflora. Mesophiles accordingly thrive first with rising temperature, consuming the simpler carbon sources (sugars, amino acids, and organic acids) and leaving only polysaccharide constituents of biomass (cellulose and hemicelluloses) available for thermophiles. Many similar examples exist in nature, such as faster growing primary colonisers rapidly consuming available simple sugars and leaving only the more complex sugars available to the secondary and tertiary colonisers. This has led to natural affinities within these groups for these different carbon sources (Kung'u 2016; Stamets 2005), which significantly affects how well a fungal species will grow on any given substrate. Since most mycelium composites are grown on lignocellulosic agricultural by-products and wastes, typically lacking optimal fungal nutrients, such as easily utilisable simple sugars (e.g. fructose, glucose and sucrose), white rot fungi, which degrade both cellulose and lignin (e.g. *Trametes*, *Ganoderma* and *Pleurotus* genera, phylum Basidiomycota), are typically used (Appels et al. 2019; Elsacker et al. 2019; Haneef et al. 2017; Holt et al. 2012; Jones, M et al. 2018).

The mycelial matrix network structure also affects the mechanical properties of mycelium composites. A good example is the mono-, di- and tri- mitic hyphal networks exhibited by basidiomycetes (Pegler 1996). Hyphal networks of basidiomycetes can comprise up to three distinct hyphal types, generative, binding and skeletal, with key differences in cell wall thickness, internal structure and branching characteristics (Corner 1953; Jones, M; et al. 2017). The number of different hyphal types present in a species is described using the mitic system. Monomitic species comprise only generative hyphae, dimitic species comprise two hyphal types (usually generative and skeletal) and trimitic species comprise of all three principle hyphal types (Webster & Weber 2007). Generative hyphae are thin walled, hollow and branched while skeletal hyphae are thick walled, often solid and sparsely branched or unbranched. Binding (ligative) hyphae are also thick walled, often solid and highly branched. It is generally accepted that complex hyphal systems (e.g. trimitic) are more advanced forms than less complex hyphal systems (e.g. monomitic) (Ko & Jung 1999; Pegler 1996; Ryvarden 1991), with the wall thickness of the hyphal system and amount of water contained within their cells responsible for specific qualities of the biomass (Ames 1913). Although the tensile properties of fungal hyphae used in fermentation have been studied, with estimated hyphal ultimate tensile strengths of up to 24 MPa and elastic moduli of up to 140 MPa, the mechanical properties of wood-rot fungi hyphae are not well characterised (Li, ZJ et al. 2002; Stocks, Stuart M. & Thomas, Colin R. 2001; Stocks, S. M. & Thomas, C. R. 2001). Generative hyphae alone (monomitic hyphal systems), which are hollow and contain cytoplasm, are suggested to provide limited mechanical performance, with binding hyphae (dimitic and trimitic hyphal systems) responsible for material strength (Bayer & McIntyre 2012, 2015). Although there is no literature confirming this, it is true that mycelium composites utilising trimitic species, such as *T. versicolor* or *multicolor* exhibit higher tensile (0.04 MPa) and flexural strengths (0.22 MPa) than monomitic species, such as *P. ostreatus* (0.01 MPa tensile strength, 0.06 MPa flexural strength) when grown on rapeseed straw (Appels et al. 2019). *T. versicolor* also has a higher compressive strength than *P. ostreatus* when grown on hemp (0.26 MPa compared with 0.19 MPa) (Lelivelt 2015). However, the fact that the presence of structural polymers, such as chitin and chitosan, is limited to the thin hyphal cell wall, which also contains polysaccharides (e.g. galactose, mannose and fucose), phosphate, proteins, lipids and mineral salts (Bartnicki-Garcia 1968; Jones, M. et al. 2019) makes the importance of the hyphal structure questionable, with mycelial biomass (matrix) quantity likely to more greatly influence mechanical performance.

3.2 Influence of the substrate filler on composite mechanical performance

The physical and mechanical properties of as-grown mycelium composites are often dependent on the substrate, which acts as the dispersed filler phase of the composite material.

As-grown composites typically have a density ranging from 60-300 kg/m^3, with composites containing an agricultural by-product filler phase, such as bast fibers or straw, having lower densities (60-130 kg/m^3) than composites containing forestry by-product substrates, such as sawdust (87-300 kg/m^3) **(Table 1)**. Only limited data is available on the mechanical properties of mycelium composites for the various groups of substrates.

Tensile properties are among the best characterised material properties of mycelium composites. Reported tensile properties vary significantly between studies for sawdust substrates (0.05-0.18 MPa) but sawdust does appear to be associated with higher tensile strengths than straw substrates (0.01-0.04 MPa) **(Table 1)**. However, the tensile properties of as-grown sawdust-based mycelium composites do not correlate with the mechanical properties of the substrates themselves. Clear, straight grained Beech wood sections have a similar or higher tension perpendicular to grain strength (5-7 MPa) than red oak (5.5 MPa) (Buschow et al. 2001; Green, Winandy & Kretschmann 1999), while as-grown composites using a beech sawdust substrate have much lower tensile strength (0.05 MPa) than composites with a red oak sawdust substrate filler (0.18 MPa). This indicates that the tensile properties of as-grown mycelium composites are more heavily influenced by failure of the mycelium matrix than the dispersed substrate filler and that substrates must be nutrient rich, rather than strong, to establish a dense mycelium network and maximise mycelium composite tensile properties. Some lower-grade substrate materials, such as agricultural by-products and wastes, which are attractive due to their low cost, typically lack optimal fungal nutrients including easily utilisable simple sugars (e.g. fructose, glucose, sucrose) and instead contain more complex carbon sources (e.g. cellulose and lignin) (Faruk et al. 2012). While white rot fungi are suitable for these lignocellulosic substrates, some agricultural by-products, like rice hulls, also contain large quantities of minerals, such as silica, which limit fungal growth (Jones, M et al. 2019). Reduced fungal growth on these less easily utilised substrates compromises interfacial bonding between hyphae and organic matter and adversely affects the tensile strength of the mycelium matrix phase (He et al. 2014; Jones, M et al. 2019; Travaglini et al. 2013).

Unfortunately, inconsistent and limited data is available concerning the compressive properties of mycelium composites. Elsacker et al. (2019) found that the compressive moduli of as-grown composites utilising fibrous hemp and flax hurd substrates were higher than those of particulate pine shavings (0.64 and 0.73 MPa compared to 0.14 MPa, respectively), however their study only tested to 70-80% strain and subsequently did not assess compressive strength. Conversely, Ghazvinian et al. (2019) assessed the compressive strength of mycelium composites grown on an unknown sawdust and an unknown straw substrate, finding that the sawdust particulate substrate had a much higher compressive

strength than the fibrous straw (1.1 MPa compared to 0.17 MPa, respectively), but did not assess stiffness **(Table 1)**. Only Travaglini et al. (2013) assessed both compressive modulus (1 MPa) and strength (0.49 MPa) of mycelium composites with a red oak sawdust substrate. Despite significant gaps in the characterisation of mycelium composites under compressive loading conditions, it seems likely that particulate substrates, such as sawdust, provide higher compressive properties to the composite than fibrous substrates such as straw. The compressive properties of porous materials are strongly correlated with their porosity and pore size, with increased porosity associated with reduced mechanical performance (Ashby, Shercliff & Cebon 2018; Xia et al. 2013). This suggests that the compressive performance of as-grown composites would depend on the compressive properties and porosity of the filler, the composite itself and the degree to which the fungus digests the filler, increasing its porosity in the process (Kavanagh 2005). However, the compressive properties of as-grown composites have been found to be largely independent of the particle size of the filler phase (Islam et al. 2018).

Particle geometry also had no significant effect on the flexural strength of mycelium composites, which when subjected to bending experience a maximum tensile stress at one surface, to zero at the midplane, to a maximum compressive stress at the opposite surface (Roylance 2000). Although fibrous geometries should improve the tensile properties of the surfaces if aligned in the loading direction, and hence the flexural properties of the composite overall (Chand & Fahim 2008), the significant fungal growth on air exposed surfaces likely results in enzymatic fiber degradation and damage, compromising the beneficial effects of the fibers present (Choudhury 2017). Air transmission is critical for fungal growth with mycelial density highest at the air exposed surfaces and lowest in the core, where depending on the porosity of the filler there could be limited or even no growth unless the filler is artificially aerated (Jones, M; et al. 2018; Webster & Weber 2007). The lack of improvement in the flexural properties of mycelium composites incorporating fibrous surfaces was supported by the poor flexural properties of cotton fiber-based composites (1 MPa and 0.05 MPa, respectively), although fibrous straw-based composites did exhibit better flexural stiffness (1-3 MPa) and strength (0.06-0.22 MPa) **(Table 1)**. Conversely, a particulate Beech sawdust substrate resulted in much higher flexural modulus (9 MPa) and strength (0.29 MPa), which was most likely the result of its nutrient composition promoting the formation of a dense, continuous matrix phase on the air exposed surface of the composite. The importance of the substrate nutrient profile to composite flexural properties is supported by results obtained by Tudryn et al. (2018), who found that increased nutrition at homogenization increased specific flexural stress and specific flexural modulus, due to the presence of a larger, more continuous hyphal matrix.

In general, the value of any given substrate in reinforcing the composite appears to be more heavily governed by the nutrient profile of the substrate with more nutritious substrates promoting more fungal growth and bonding, since failure always occurs in the mycelium matrix rather than the substrate filler irrespective of loading condition. This unfortunately makes cheap, low-grade agricultural and forestry residues often only suitable for the manufacture of foam-like mycelium composites, unless further processing techniques, such as hot or cold pressing, resin infusion or hybridisation are utilised to improve mechanical performance (Jones, M et al. 2019).

Table 1. Density, tensile, compressive and flexural material properties of as-grown mycelium composites comprising fibrous and particulate dispersed agricultural filler substrates. Data from [1]Appels et al. (2019), [2]Travaglini et al. (2013), [3]Elsacker et al. (2019) and [4]Ghazvinian et al. (2019).

Loading	**Substrate type**	**Substrate**	**$\rho_{envelope}$ (kg/m^3)**	**E MPa**	**$\sigma_{ultimate}$ MPa**
Tension	Fibrous	Rapeseed straw[1]	115	3.0	0.025
	Particulate	Beech sawdust[1]	170	13.0	0.05
		Red oak sawdust[2]	300	1.30	0.18
Compression	Fibrous	Flax hurd[3]	99	0.73	-
		Hemp hurd[3]	94	0.64	-
		Unknown straw[4]	192	-	0.17
	Particulate	Pine shavings[3]	87	0.14	-
		Red oak sawdust[2]	300	1.0	0.49
		Unknown sawdust[4]	552	-	1.1
Flexure	Fibrous	Cotton fibers[1]	130	1.0	0.05
		Rapeseed straw[1]	115	1.5	0.14
	Particulate	Beech sawdust[1]	170	9.0	0.29

3.3 Hot and cold pressing to improve mycelium composite mechanical properties

The mechanical properties of mycelium composites can be significantly improved using physical processing, such as cold or hot pressing. This is expected since pressing consolidates composite materials, reduces the porosity of the material and increases the material density in general (Dai, Yu & Zhou 2007). Pressing also helps to reorientate fibers horizontally in the plane of the panel (Butterfield et al. 1992) and panel thickness reduction during pressing results in considerable and intimate fiber contact between the walls of the fibers at points of overlap (Carvalho & Costa 1998). In mycelium composites produced using

P. ostreatus grown on rapeseed straw, cold pressing was associated with a significant improvement in tensile strength (0.01 MPa to 0.03 MPa) and a higher elastic modulus (2 MPa to 9 MPa) (Appels et al. 2019). It also significantly improved the flexural properties of the composites with higher flexural strengths (0.06 MPa to 0.21 MPa) and moduli (1 MPa to 15 MPa) achieved post cold pressing (Appels et al. 2019). Even greater improvements in mechanical performance could be achieved through hot pressing. The main mechanisms associated with hot pressing are the phase change (evaporation) of water, compaction and stress relaxation of the material via conduction and convection and mass transfer occurring as a result of gaseous and bound water diffusion and hydrodynamic flow of gaseous and liquid water (Carvalho & Costa 1998). This occurs via diffusion of steam through the network or voids in fibers, diffusion of water through cellular walls or as water or steam flow through cell membranes and voids (Stamm 1964). Temperature, gas pressure and moisture content all influence the heat and mass transfer through the thickness, impacting plasticization and compaction of the material (Carvalho & Costa 1998). Tensile properties of hot-pressed *T. multicolor* and *P. ostreatus* composites grown on rapeseed straw were significantly higher than as-grown samples, with strength increases of 0.04 MPa to 0.15 MPa and 0.01 to 0.24 MPa, respectively, and elastic moduli increases of 4 MPa to 59 MPa and 2 MPa to 97 MPa, respectively (Appels et al. 2019). Hot pressing also improved the flexural strength of *T. multicolor* and *P. ostreatus* composites grown on rapeseed straw (0.22 MPa to 0.86 MPa and 0.06-0.87 MPa, respectively) and the flexural moduli of the composites (3 MPa to 80 MPa and 1 MPa to 72 MPa, respectively) (Appels et al. 2019). Both cold and hot pressing were associated with significant reductions in the strain to failure of the samples, resulting from the reduced moisture content of the composites following pressing, which would otherwise act as a plasticiser (Sombatsompop & Chaochanchaikul 2004). Cold pressing of *P. ostreatus* grown on rapeseed straw reduced their strain to failure (2.8% to 0.8%), while hot pressing of *P. ostreatus* and *T. multicolor* grown on rapeseed straw was associated with lower strain to failure (2.8% to 0.7% and 4.7% to 0.9%, respectively) (Appels et al. 2019).

3.4 Resin infused mycelium composites and sandwich structures

Mycelium composites are being increasingly used as low-density cores bonded between two thin laminate facings called skins in sandwich structures (Jiang et al. 2019; Jiang et al. 2017; Wong, Arumugasamy & Mustapha 2019). Skins can be any sheet material, from metals such as aluminium (Wong, Arumugasamy & Mustapha 2019), to natural materials such as woven jute, flax or cellulose (Jiang et al. 2017). These skins provide resistance against in-plane and lateral bending loads, while the mycelium core holds the skins in place and resists shear loads (Allen 2013; Kim & Christensen 2000; Vinson 2018). The improvement in mechanical performance that a sandwich structure provides is subsequently dependent on the loading

conditions. Several recent studies have examined the use of mycelium composites in sandwich structures but any significant improvement in mechanical performance has yet to be reported, making the value of mycelium sandwich composites debatable. Wong, Arumugasamy and Mustapha (2019) recently reported unsurprisingly that a sandwich structure comprising a mycelium composite sandwiched between aluminium alloy laminates had no better compressive properties than a normal mycelium composite and while skins provide varying degrees of improvement to the flexural strength of sandwich structures with a mycelium composite core, similar results can be achieved using simpler methods. For example, mycelium composite sandwich structures comprising jute, flax or cellulose textile reinforcement skins have effective flexural moduli of 4.6-6.5 MPa (Jiang et al. 2017), with similar performance achievable by simply varying the substrate of the mycelium composite itself (flexural moduli of 1-9 MPa) or hot-pressing (flexural moduli of 34-80 MPa) (Appels et al. 2019).

The most significant improvement in the mechanical performance of sandwich structures with mycelium composite core and a woven jute, flax or cellulose skin is associated with resin infusion. This is also hardly surprising or even novel since the use of a resin infusion in a mycelium composite effectively replaces the mycelium matrix with a stronger resin one. The difference between a resin-infused mycelium composite and a natural composite comprising resin and agricultural residue or fibers is then unclear as is the sustainability of such a composite, which lacks a natural biological manufacturing process. Jiang et al. (2019) reported that soy-based resin infused over 30-120 s saturates the entire material and is responsible for an improvement in core and skin shear yield and ultimate stress and sandwich flexural strength. Core shear yield stress and ultimate strength were highest for resin-infused samples reinforced with flax skins (up to 128.9 yield and 135.3 kPa ultimate stress) (Jiang et al. 2019). This was due to the increased mycelial growth on these skins, since the nutrient profile of flax stimulates more fungal growth than jute or cellulose, facilitating greater branching networks and interfacial bonding. The resin infusion unsurprisingly provided a significant improvement compared to flax sandwich composites lacking resin (core shear yield and ultimate stresses of up to 29.5 kPa and 38.7 kPa, respectively) (Jiang et al. 2017). The most common failure mode of the sandwich structures was tensile failure of the core material (mycelium-bound agricultural waste), indicating that this was still the weakest part of the structure. Effective flexural strengths of up to 30 MPa for resin-infused flax reinforced sandwich structures were achieved, which are significantly higher than flax-reinforced sandwich structures lacking resin (up to 6 MPa) (Jiang et al. 2017) and low-density polyethylene (LDPE) (14 MPa) but lower than acrylonitrile butadiene styrene (ABS) (75 MPa) (MatWeb 2018). The sandwich structures (410 kg/m^3) also had lower densities than LDPE (920 kg/m^3) and ABS (1100 kg/m^3) and were

suggested as potential replacements for LDPE and ABS interior panels in automotive and sports products.

3.5 Hybridisation of mycelium composites to improve mechanical performance

The mechanical properties of mycelium composites, comprising a network of fungal mycelium grown through a substrate, can be improved through hybridisation with small quantities of synthetic rubbers, such as styrene-butadiene rubber, or natural fibers, such as cellulose nanofibrils. While these improvements are arguably predictable when hybridising a weak mycelium composite with stronger synthetic or natural polymers, the small volume fractions required to do so, and the thresholds associated with mechanical property improvement are interesting. Styrene-butadiene rubber negligibly affects fungal growth performance in quantities up to 5 vol% with only a slight delay in germination and no effect on the growth rate (He et al. 2014). Larger volumes of the latex hinder growth (8 vol%) or kill the fungus (10 vol%) since the latex reduces the void volume within the composite, hindering the oxygen transmission and absorption required for fungal growth (He et al. 2014; Kavanagh 2005). Mycelium composites produced using cotton seed hulls and *P. ostreatus* had a compressive strength of 177 kPa, which could be almost doubled with the addition of 5 vol% styrene-butadiene rubber (343 kPa) (He et al. 2014). This is due to the void volume reduction and volume density increase (181 kg/m^3 to 225 kg/m^3) associated with the inclusion of the latex (He et al. 2014). Even smaller quantities of nanocellulose can be used to improve mechanical performance with increases in flexural strength (1.5 MPa to 3.5 MPa) and modulus (220 MPa to 575 MPa) of hybrid materials produced by cold and hot pressing wood particles with mycelium growing on them hybridised with 2.5 wt% nanocellulose (Sun, W et al. 2019; Weiland et al. 2019). Notably, further increases in nanocellulose content did not provide any significant improvement in mechanical performance suggesting a low threshold nanocellulose density required for improvement of adhesion of particles and subsequent flexural properties (Theng et al. 2015). These improvements in mechanical performance at low nanocellulose concentrations could make hybridisation using nanocellulose a viable method for improving the mechanical performance of mycelium composites. However, in some cases, such as hybridisation using latex, the small improvement in mechanical performance attained post hybridisation may well be offset by the additional costs, processing and reduced environmental sustainability associated with a latex-mycelium composite material.

3.6 Thermal conductivity properties of mycelium composites for insulation applications

Mycelium composites containing high-performance natural insulators such as straw and hemp fibers bound using mycelial growth have both low densities (57-99 kg/m^3) and thermal

conductivities (0.04-0.08 W/m·K) **(Figure 4)**. This makes them excellent insulation materials, able to compete with conventional commercial thermal insulation products, such as glass wool (57 kg/m^3, 0.04 W/m · K) and extruded polystyrene (XPS, 34 kg/m^3, 0.03 W/m · K) (Papadopoulos 2005) in addition to other natural insulators including sheep wool (18 kg/m^3, 0.05 W/m·K) and kenaf (105 kg/m^3, 0.04 W/m·K) (Asdrubali, D'Alessandro & Schiavoni 2015).

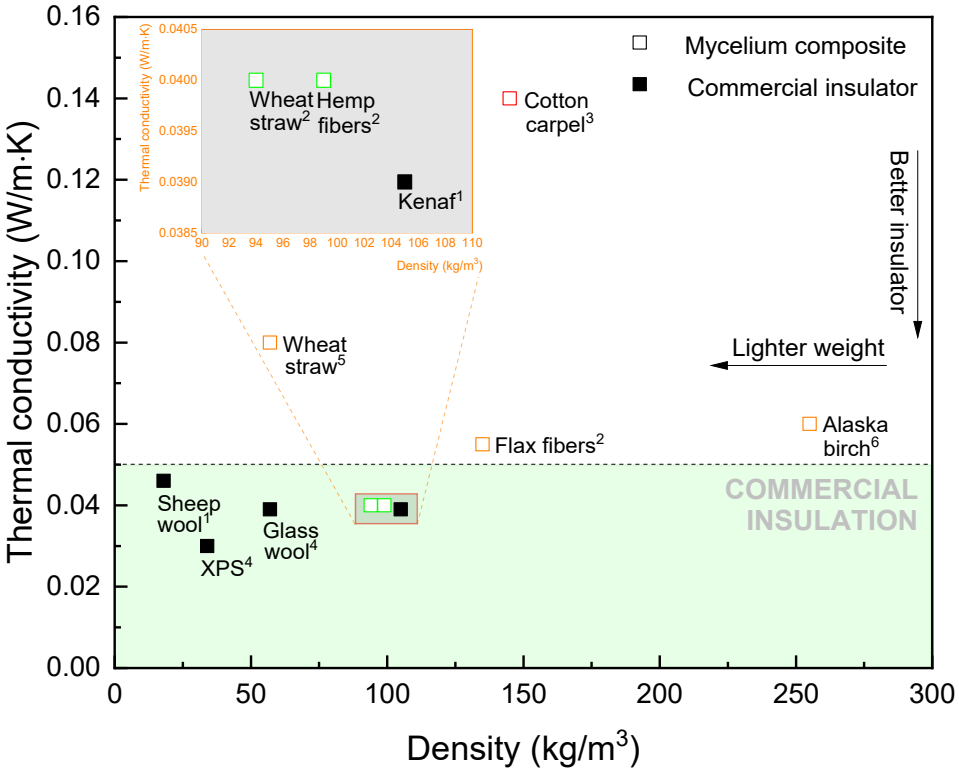

Figure 4. Density (kg/m^3) and thermal conductivity (W/m·K) of mycelium composites produced using various substrates (coloured square markers, colours: green = low thermal conductivity, orange = medium thermal conductivity, red = high thermal conductivity) and commercial insulation materials, such as glass wool, sheep wool, XPS foam and kenaf (black solid square markers). Data from [1]Asdrubali, D'Alessandro and Schiavoni (2015), [2]Elsacker et al. (2019), [3]Holt et al. (2012), [4]Papadopoulos (2005), [5]Xing et al. (2018) and [6]Yang, Z et al. (2017). Density and thermal conductivity values are averages based on the available data sets.

Lower thermal conductivities are associated with better insulation materials and are primarily influenced by material density and to a lesser extent moisture content (Collet & Prétot 2014; Jerman et al. 2013; Uysal et al. 2004). For example, a 67% increase in density will result in a 54% increase in thermal conductivity in hemp concretes (a bio-composite material comprising hemp shive and lime), while a 90% increase in relative humidity (completely dry to 90% RH) will only result in a thermal conductivity rise of 15-20% (Collet & Prétot 2014). The strong correlation between material density and thermal conductivity is the result of the presence of

large quantities of dry air, which has a very low thermal conductivity (26.2×10^{-3} W/m·K at 0.1 MPa, 300 K) (Kadoya, Matsunaga & Nagashima 1985), present in low density materials. These large quantities of air mean that low density materials are often excellent thermal insulators.

Straw and hemp are well-established natural thermal insulation materials, which derive their useful insulation properties from their porous structure and the low bulk density of the bundled fibers, leading to trapping of a large amount of air between the fibres in the insulation (Kymäläinen & Sjöberg 2008; Wall et al. 2012). Their thermal insulation properties vary primarily based on the density of the pack, moisture content and fiber type (Bainbridge 1986). Mycelium composites utilising a wheat straw filler have reported thermal conductivities of 0.04 W/m·K (Elsacker et al. 2019) and 0.08 W/m·K (Xing et al. 2018), respectively, although the former value seems questionable given that it is associated with a higher density composite than the latter (94 kg/m^3 compared to 57 kg/m^3) and is significantly lower than the conductivity of straw bales themselves (0.07-0.08 W/m·K) (Pruteanu 2010). Hemp fiber-based mycelium composites were also reported to have thermal conductivities (0.04 W/m·K) (Elsacker et al. 2019) significantly lower than hemp concretes (0.1 W/m·K) (Collet & Prétot 2014). Even mycelium composites produced using substrates exhibiting poorer insulation properties, such as those incorporating a cotton carpel substrate (0.10-0.18 W/m·K) (Holt et al. 2012) have thermal conductivity values comparable with gypsum (0.17 W/m·K), high density hardboard (0.15 W/m·K), plywood (0.12 W/m·K), and both hardwoods (0.16 W/m·K) and softwoods (0.12 W/m·K) (Bergman et al. 2011). This makes mycelium composites a viable low-cost and environmentally sustainable alternative to conventional commercial building insulation materials.

3.7 Acoustic properties of mycelium and its composites for noise adsorption

Mycelium itself is an excellent acoustic absorber, exhibiting strong inherent low frequency absorption (< 1500 Hz) and outperforming cork and commercial ceiling tiles in road noise attenuation (Pelletier et al. 2019). This non-typical property means that mycelium foam can be used in conjunction with other materials to improve their low frequency absorption properties. Alternatively, mycelium composite comprising mycelium-bound agricultural residue can also provide broader range acoustic absorption with 70-75% absorption or better achievable for perceived road noise (Pelletier et al. 2013). A-weighted decibels express the relative loudness of sounds in air as perceived by the human ear, with the magnitude of low frequency sounds reduced to correlate with the lessened sensitivity of human ears at low frequencies (<1000 Hz), while higher frequency sounds are left uncorrected (Pierre Jr, Maguire & Automotive 2004). This allows interpretation of the perceived loudness of domestic noises, such as dogs

barking (500-1500 Hz), human speech (85-255 Hz) and street noise (700-1300 Hz) to humans (Feinberg et al. 2005; Owren, Berkowitz & Bachorowski 2007; Pongrácz, Molnár & Miklósi 2006; Sandberg 2003).

Acoustic absorbers are typically fibrous, porous or reactive resonators with examples including nonwovens, fibrous glass, mineral wools, felt and foams (Bell & Bell 1994; Seddeq 2009). Absorbers convert the mechanical motion of air molecules travelling in sound waves into low-grade heat, which prevents sound accumulation in enclosed spaces and reduces reflected noise strength (Bell & Bell 1994). All mycelium composites tested were associated with lower perceptual road noise (45.5-60 dBa) than traditional reference absorbers, such as commercial ceiling tiles (61 dBa), urethane foam board (64 dBa) and plywood (65 dBa) **(Figure 5a, b)**. The best individual fillers for acoustic absorption were rice straw (52 dBa), hemp pith (53 dBa), flax shive (53.5), sorghum fiber (54 dBa) and switchgrass (55 dBa) **(Figure 5a)**. However, even better acoustic absorption could be achieved through mixtures of fillers (50-50 wt%) with the best combinations being rice straw-sorghum fiber (45.5 dBa), rice straw-cotton bur fiber (47 dBa) and sorghum fiber-switchgrass (47 dBa) **(Figure 5b)**.

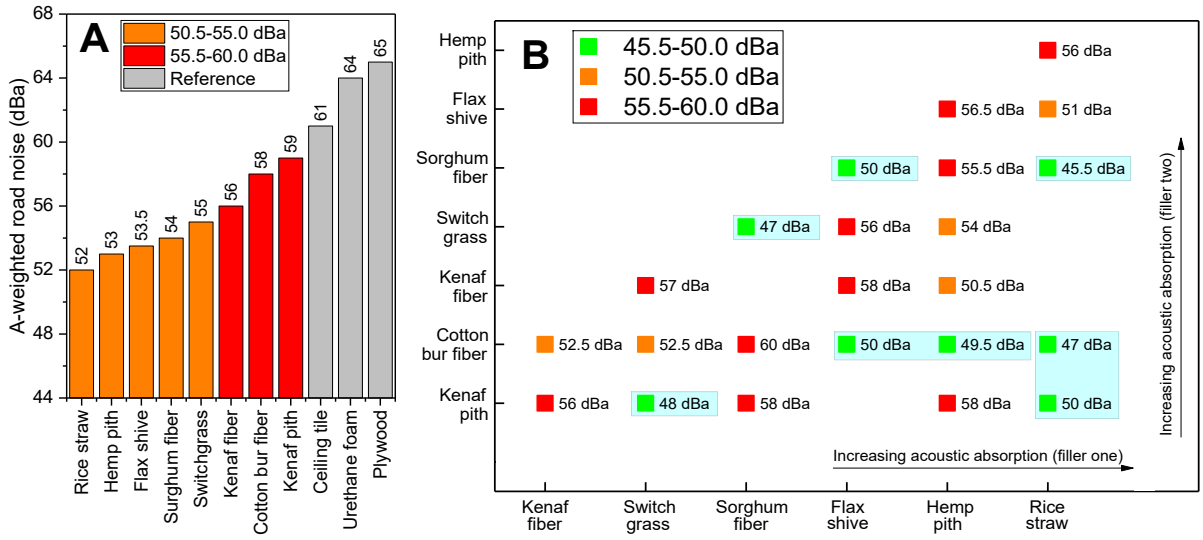

Figure 5. A-weighted perceptual road noise for mycelium composites comprising a) individual substrates compared to traditional acoustic absorbers and b) 50-50 wt% mixtures of selected fillers. Colours: green: 45.5-50.0 dBa, orange: 50.5-55.0 dBa, red: 55.5-60.0 dBa, grey: traditional reference absorbers. Data is based on an integrated A-weighted response with typical road noise excitation (1000 Hz) rounded to the nearest 0.5 dBa from Pelletier et al. (2013).

The excellent acoustic absorption properties of mycelium composites can be attributed to their porous, fibrous nature. Impedance and propagation constants used to describe the acoustic properties of materials are greatly influenced by the air flow resistance of a material, with

higher airflow resistance associated with greater acoustic absorption (Ren & Jacobsen 1993). The fibers in mycelium composites act as frictional elements, resisting acoustic wave motion and decreasing its amplitude as the sound waves attempt to move through the tortuous passages of the material and are converted to heat in the process (Hemond 1983). Thin fibers provide better acoustic absorption since they can move more easily and the greater number of fibers per unit volume results in more tortuous paths and greater air flow resistance (Koizumi, Tsujiuchi & Adachi 2002; Sun, F, Banks-Lee & Peng 1993). Surface pore concentration and geometry are also important with porosity necessary for sound waves to enter the material and tortuosity required for efficient damping (Seddeq 2009). Porosity and airflow resistance affect the height and width of sound wave peaks, while tortuosity influences the high frequency acoustic properties of porous materials (Seddeq 2009). Less dense, more open structures absorb low frequency sound in nonwoven fibrous materials (500 Hz), while denser structures are better for frequencies higher than 2000 Hz (Koizumi, Tsujiuchi & Adachi 2002). Compression of a material causes a reduction in acoustic absorption, resulting primarily from the reduction in thickness (Castagnede et al. 2000), and as such mycelium composites being utilised as acoustic absorbers should not be hot or cold pressed.

3.8 Thermal degradation and fire safety properties of mycelium and its composites

Mycelium itself has no notable or useful fire retardant properties, typically exhibiting a three-stage thermal degradation process, with degradation and fire reaction properties typical for cellulosic and other biologically derived materials (Haneef et al. 2017; Jones, M et al. 2017; Yang, H et al. 2007). Initially, free and chemically bonded water evaporates between 25-200°C (~5 wt%) (Jones, M; et al. 2018). This is followed by a much larger mass loss between 200-375°C, with onset of decomposition at ~280-290°C (Haneef et al. 2017; Jones, M et al. 2018; Jones, M; et al. 2018). This larger mass loss results from the degradation of organic constituents, such as proteins and polysaccharides (~70 wt%) and is associated with water vapour release (Jones, M; et al. 2018). The release of water vapour during combustion is the only true fire retardant property of mycelium, making mycelium thermally no better as a binder than any other natural polymer (Jones, M; et al. 2018). Although hyphal constituents, such as chitosan and hydrophobins (cysteine-rich proteins that form a hydrophobic coating), have been found to improve fire retardancy in fabrics, they do not occur in sufficient quantities to provide fire retardancy properties in mycelium (Alongi et al. 2014; Costes et al. 2017; Hu et al. 2013; Jones, M; et al. 2018). Hydrophobins in particular have been reported to promote char formation by favouring dehydration rather than depolymerisation of polysaccharides (Alongi et al. 2014), but genetically modified *Schizophyllum commune* mycelial biomass lacking its hydrophobin gene has actually been reported to have higher char yields (32 wt% average)

than wild type *S. commune* biomass (27 wt% average) (Appels et al. 2018). Approximately 20-30 wt% carbonaceous char is typically formed at 450-600°C for mycelial biomass pyrolyzed in a nitrogen atmosphere (Appels et al. 2018; Haneef et al. 2017; Jones, M; et al. 2018).

Although mycelium itself does not have significant fire-retardant properties, mycelium composites incorporating substrates or fillers that are rich in natural phenolic polymers, such as lignin, and naturally occurring or synthetically produced silica (SiO_2) can exhibit significantly improved thermal degradation, fire reaction and safety properties (Jones, M et al. 2018). This is not entirely surprising since the filler phases constitute the bulk of the material anyway and if inflammable or difficult to burn will lend their properties to the composite. Rice hulls contain 25-30 wt% lignin (Ismail & Waliuddin 1996) and 15-20 wt% silica, which is biosynthesized through the polymerization of silicic acid and distributed in the hulls as hydrated grains (Bansal, Ahmad & Sastry 2006). Glass fines comprise primarily of silica (SiO_2), but can contain up to 30 wt% organic surface matter, which is sufficient for mycelial growth to bind to as opposed to uncontaminated glass which mycelium cannot grow on (Jones, M et al. 2018). Both rice hull and glass fines are considered waste materials and are available in large quantities globally at low cost (Alex Fraser Group 2014; Defonseka 2014; Food and Agriculture Organization of the United Nations 2017; Prasad, Maiti & Venugopal 2001).

Mycelium composites containing large quantities of rice hulls (75 wt%) have lower average and peak heat release rates (107 kW/m^2 and 185 kW/m^2, respectively) compared to synthetic foams, such as extruded polystyrene (XPS, 114 kW/m^2 and 503 kW/m^2, respectively) and engineered woods, such as particleboard (134 kW/m^2 and 200 kW/m^2, respectively) **(Table 2)**. Since both extruded polystyrene and engineered wood resins, such as resorcinol- and polyvinyl acetate-based resins, are derived from crude oil this is hardly a surprise and the logic of the widespread use of synthetic materials that have not been treated to improve their thermal stability in fire prone applications, such as construction, is questionable. Heat released from burning material provides additional thermal energy to fires and strongly influences their behaviour (Mouritz, AP & Gibson 2007) and reaction properties including surface flame spread, smoke generation and carbon monoxide emission (Mouritz, A, Mathys & Gibson 2006; Sorathia, Divisjón & Lyon 1997). Heat release rate (HRR) is subsequently considered the most important fire reaction property due to its role in fire growth and spread (Babrauskas & Fires 1997; Babrauskas & Peacock 1992), with the average value (RHR_{180}) indicating full-scale fire performance (Brown, Fawell & Mathys 1994) and the peak value (pHRR) suggesting maximum temperature and flame spread rate (Mouritz, AP & Gibson 2007).

The lower heat release rates associated with rice hull-based mycelium composites are attributable to the higher charring rice hulls (~20 wt% carbonaceous char residue and ~20

wt% embedded silica) (Jones, M et al. 2017; Zhao et al. 2009), rather than the mycelium, which only represents ~5 wt% of the composite and yields less char (~20-30 wt%) (Jones, M; et al. 2018; Jones, M et al. 2019). Char is derived from organic constituents of rice hulls, especially aromatic compounds such as lignin, which decomposes into aromatic fragments that form char (Gosselink 2011). Char formation and oxidation on air exposed surfaces increases flame retardancy, acting as a thermal insulation barrier due to its low thermal conductivity (Mouritz, AP & Gibson 2007) and reducing smoke by impeding fiber fragment release and preventing oxidation (Gilwee 1975; Hshieh 1998).

Addition of glass fines within the substrate of the composite further improves the fire reaction and safety properties of mycelium composites which is logical, since it significantly increases the silica (inflammable) content of the material. Mycelium composites incorporating 50 wt% glass fines have much longer times to flashover (311-370 s) than synthetic materials, such as extruded polystyrene (XPS) (61 s) and particleboard (173 s) **(Table 2)**. Flashover is the near-simultaneous ignition of all exposed materials in an enclosed area and is a common and very dangerous occurrence in residential and building fires (Liu & Chow 2014). Fires that reach flashover are approximately ten times more dangerous than fires that do not (Clarke 1997; Liu & Chow 2014). Composites incorporating large quantities of glass fines (50 wt%) also have very low average (33-42 kW/m^2) and peak (79-85 kW/m^2) heat release rates compared to synthetic construction materials, such as XPS (114 kW/m^2 average and 503 kW/m^2 peak) and particle board (134 kW/m^2 average and 200 kW/m^2 peak) **(Table 2)**.

Table 1. Summary of cone calorimetry performance and fire safety parameters. Data from Jones, M et al. (2018).

Type	Sample	Time		Heat release rate		Gas release		
		Ignition, t_{ig} (s)	Flashover, t_{fo} (s)	Average, RHR_{180} (kW/m^2)	Peak, pHRR (kW/m^2)	Smoke, TSR (m^2/m^2)	CO, COP_{180} (g)	CO_2, CO_2P_{180} (g)
Synthetic	ClimaFoam® extruded polystyrene (XPS) foam	9	61	114	503	1184	0.48	15.2
	STRUCTAflor® particleboard	26	173	134	200	64	0.47	30.0
Mycelium composite†	75 wt% wheat grains	12	94	107	185	70	0.33	23.8
	75 wt% rice hulls	7	75	85	133	40	0.02	14.6
	25 wt% wheat grains + 50 wt% glass fines	12	370	42	79	5	0.39	10.2
	25 wt% rice hulls + 50 wt% glass fines	7	311	33	85	0.9	0.91	6.3

t_{ig} = time to ignition, RHR_{180} = average heat release rate from ignition to 180 s after ignition, pHRR = peak heat release rate, t_{fo} = estimated time to flashover in room fire test (ASTM International 2017), TSR = total smoke release, COP_{180} = carbon monoxide produced from ignition to 180 s after ignition, CO_2P_{180} = carbon dioxide produced from ignition to 180 s after ignition. † inoculated using 25 wt% wheat grain inoculum.

However, despite the dangers associated with heat release and flashover, most fire-related fatalities are caused by toxic gases rather than burns, generalised trauma or other causes (Babrauskas et al. 1992; Mouritz, AP & Gibson 2007). Carbon monoxide (CO) causes incapacitation and death at very low concentrations (e.g. 1500 ppm will cause death within an

hour) and is considered the greatest individual hazard (Hirschler 1987). In contrast, carbon dioxide (CO_2) concentration must be more than 60 times higher (100,000 ppm) to cause death over the same period (Mouritz, AP & Gibson 2007). Rice hull-based mycelium composites have up to much lower CO emission (0.02 g) than particleboard (0.47 g) and XPS (0.48 g), in addition to lower CO_2 emission (14.6 g compared to 15.2 g for XPS and 30.0 g for particleboard) **(Table 2)**. Wheat grain- and rice hull-based mycelium composites incorporating 50 wt% glass fines also emit much less smoke (0.9-5 m^2/m^2) than traditional construction materials, such as particleboard (64 m^2/m^2) and XPS (1184 m^2/m^2) **(Table 2)**. Short-term exposure to smoke consisting of small fragments of fibre and ultra-fine carbon particles is not considered a serious health hazard to humans but is an important safety concern because dense smoke can reduce visibility, cause disorientation and hinder firefighting efforts (Mouritz, AP & Gibson 2007).

3.9 Water absorption properties of mycelium composites

One of the largest problems limiting the use of mycelium composites in materials science applications is their tendency to absorb large quantities of water quickly. Mycelium composites are typically hydroscopic, increasing in weight by ~40-580 wt% when in contact with water for 48-192 h (Appels et al. 2019; Elsacker et al. 2019; Holt et al. 2012; López Nava et al. 2016; Sun, W et al. 2019). The strong water absorption affinity of mycelium composites is the result of their typically cellulosic filler constituents, which contain numerous accessible hydroxyl groups (Zabihzadeh 2009), and the hydrophilic porous mycelium matrix and biologically-derived filler phases, which promote wicking (Chung, Suidan & Venosa 2011; Li, MM et al. 2013; Wei, Liang & McDonald 2015). Air dried mycelium composites incorporating a fibrous substrate of rapeseed straw or cotton bur fiber take up ~530-550 wt% moisture within 48 h when in contact with water **(Figure 7a)**. Although such a massive water uptake may seem a major problem some construction applications of mycelium composites, such as acoustic or thermal insulation, are fortunately for internal or dry locations not exposed to the weather, mitigating this otherwise significant problem. The most rapid weight increase occurs within the first 3 h, with an increase of ~220 wt% for both rapeseed straw- and cotton bur fiber-based composites **(Figure 6b)**. Water uptake then continues at a reduced rate for up to 48 h, before slowing and then stopping as the material reaches saturation (~580 wt%) **(Figure 6a)**. Rapeseed straw contains large quantities of cellulose (48.5 wt%) and pentosans (17 wt%) (Housseinpour et al. 2010), while cotton bur fibers predominantly comprise cellulose (98 wt% with <0.5 wt% pentosan) (Pigman 2012). Pentosans are water soluble polymers composed of pentoses and are known to increase the amount of water absorbed by bread, while the hydroxyl groups in cellulose attract water molecules (Michniewicz, Biliaderis & Bushuk 1992; Zabihzadeh 2009). In contrast, mycelium composites comprising a particulate substrate, such

as beech sawdust, are much less susceptible to water uptake with a weight increase of 23 wt% over 3 h contact with water, which slowly increases to 43 wt% over 192 h **(Figure 6a)**. Beech sawdust contains 26 wt% hydrophobic lignin in addition to its 48 wt% cellulose (Ruxanda, Alice Teacă & Spiridon 2008), which in conjunction with its higher material density and the smaller void content of the fine particulate filler, is likely to account for its reduced water uptake.

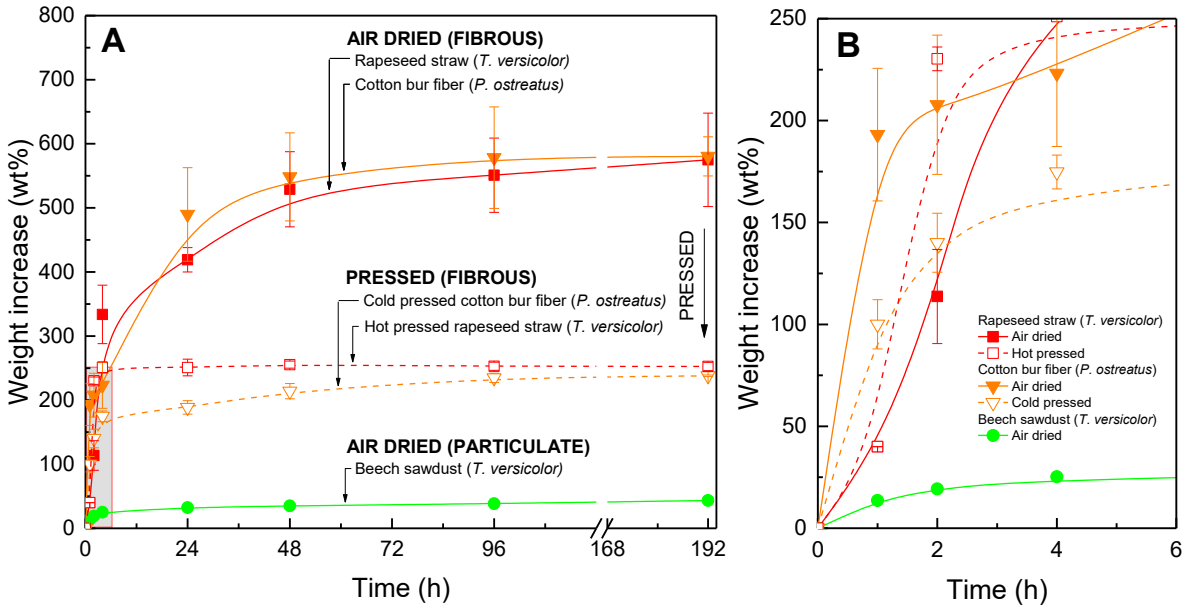

Figure 6. Weight increase (wt%) of air dried (solid lines) and hot and cold pressed (dotted lines) fibrous (*P. ostreatus* on cotton bur, orange, *T. versicolor* on rapeseed straw, red) and particulate (*T. versicolor* on beech sawdust, green) mycelium composite materials resulting from continuous contact with a water surface over (a) 192 h with (b) the most rapid absorption period (0-6 h) magnified. Data from Appels et al. (2019).

Hot or cold pressed mycelium composites also experience less than half the water uptake of air-dried composites (~250 wt% compared to ~580 wt%) **(Figure 6a)**. This is most likely because pressed materials have smaller void volumes, which impedes capillary action and hence water uptake (Dai, Yu & Zhou 2007). Cold pressed mycelium composites are slightly less absorbent (214 wt% after 48 h, 238 wt% after 192 h) than hot pressed composites (247 wt% after 48 h, 252 wt% after 192 h), achieving saturation faster than the drier hot-pressed composites since they are initially more hydrated. Heat treatment of lignocellulosic polysaccharide components, such as the depolymerisation of hemicelluloses at temperatures above 160°C, can reduce water absorption due to the reduced number of free hydroxyl groups present (Boonstra & Tjeerdsma 2006; Hong 1984). However, since hot pressing primarily heat treats the mycelium-rich surfaces it is likely that any improvement in water absorption properties based on depolymerisation of hemicelluloses would only be realised through more uniform temperature application affecting the lignocellulosic core, such as oven drying. In

addition to using particulate fillers and pressing, many bio-based coatings, such as polyfurfuryl alcohol resin (PFA), have also shown promise in reducing water absorption in natural fiber composites (Mokhothu & John 2017) and could be applied to mycelium composites to improve their water resistance.

3.10 Termite resistance of mycelium composites

Mycelium composites have no termite resistant properties of their own, comprising completely biological and predominantly lignocellulosic material. However, termite resistance of mycelium composites can be improved through substrate selection and application of natural or commercial termiticides (Bajwa et al. 2017). Hemp-based mycelium composites have high termite-resistance, exhibiting high termite mortality rates (directly related to efficacy or repellence by termite treatments) and low mass losses resulting from termite infestation over 4 weeks (16-53 wt%). Kenaf-based composites exhibit moderate to complete termite mortality but are associated with the highest mass losses of any untreated mycelium composite (43-62 wt%). Corn-based composites have low termite resistance with slight to moderate termite mortality and 42-43% mass loss. The most effective natural termiticides are guayule resin (flavonoid, cinnamic, terpenoids, and p-anisic acid bioactive compounds) (Bultman, Chen & Schloman 1998) and vetiver oil (α- and β-vetivone bioactive compounds) (Zhu et al. 2001). A single coating of these oils provides complete termite mortality and are associated with mass losses of 18-28 wt% and 16-27 wt%, respectively, for treated mycelium composites. This mass loss is significantly less than untreated composites (42-62 wt%) and an untreated southern yellow pine (*Pinus taeda*) reference sample (80 wt%). Commercial borax termiticide provides less termite protection than the natural oils with 28-40 wt% mass loss resulting from termite infestation. The fungal species *Daedaleopsis confragosa*, *Ganoderma resinaceum* and *T. versicolor* have no significantly different effects on termite repellence or mass loss for mycelium composites. Other degradation parameters of mycelium composites, such as mould and weathering resistance remain undocumented.

4. Conclusion

Mycelial growth provides a unique low energy bio-fabrication method to upcycle abundant agricultural by-products and wastes into cheap and environmentally sustainable alternatives to energy intensive synthetic construction materials for applications, such as acoustic and thermal insulation, door cores, panelling, flooring, cabinetry and other furnishings. Acoustic and thermal insulation materials are typically highly porous and low in density, trapping air and attenuating sound waves, while door cores, panelling, flooring and cabinetry require scratch resistance (hardness), high flexural strengths and stiffness. Mycelium composites exhibit foam-like mechanical properties, which can be improved to resemble natural materials (e.g.

wood and cork) and plastics (e.g. polyethylene, acrylonitrile butadiene styrene) through fungal species (continuous phase) and dispersed agricultural residue filler selection, physical processing (e.g. hot and cold pressing), resin infusion and hybridisation with materials, such as latex and cellulose. Mycelium composites are particularly well suited for thermal and acoustic insulation applications, exhibiting similar or lower thermal conductivities than commercial thermal insulation materials and 70-75% acoustic absorption or better, outperforming traditional ceiling tiles, urethane foam and plywood. They also exhibit better fire reaction and fire safety properties than traditional construction materials such as extruded polystyrene and particleboard and good termite resistance utilising natural termiticides. However, their typically foam-like mechanical properties, high water absorption and many gaps in material property documentation currently limit the application and usage of mycelium materials with further research and development of these materials necessary, in addition to targeted usage in specific, suitable applications. Nonetheless, the growing trends in the research and commercialisation of mycelium composite materials and their useful material properties makes them an effective, cheap and environmentally sustainable technology emerging with the potential to significantly contribute to the future of green construction.

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Conflicts of interest

None.

References

Abhijith, R, Ashok, A & Rejeesh, CR 2018, 'Sustainable packaging applications from mycelium to substitute polystyrene: a review', *Materials Today: Proceedings*, vol. 5, no. 1, Part 2, pp. 2139-45.

Abrams, M 2014, *Construction Materials Made from 'Shrooms*, The American Society of Mechanical Engineers (ASME), viewed 08 February 2018, <http://www.asme.org/engineering-topics/articles/construction-and-building/construction-materials-made-from-shrooms>.

Alex Fraser Group 2014, *Leading the way in glass recycling*, <http://www.alexfraser.com.au/article/News/Leading_the_way_in_glass_recycling>.

Allen, HG 2013, *Analysis and design of structural sandwich panels: the commonwealth and international library: structures and solid body mechanics division*, Elsevier.

Alongi, J, Carletto, RA, Bosco, F, Carosio, F, Di Blasio, A, Cuttica, F, Antonucci, V, Giordano, M & Malucelli, G 2014, 'Caseins and hydrophobins as novel green flame retardants for cotton fabrics', *Polymer Degradation and Stability*, vol. 99, pp. 111-7.

Ames, A 1913, *A Consideration of Structure in Relation to Genera of the Polyporaceae*.

Appels, F, Camere, S, Montalti, M, Karana, E, Jansen, KM, Dijksterhuis, J, Krijgsheld, P & Wösten, HA 2019, 'Fabrication factors influencing mechanical, moisture-and water-

related properties of mycelium-based composites', *Materials & Design*, vol. 161, pp. 64-71.
Appels, F, Dijksterhuis, J, Lukasiewicz, CE, Jansen, KMB, Wösten, HAB & Krijgsheld, P 2018, 'Hydrophobin gene deletion and environmental growth conditions impact mechanical properties of mycelium by affecting the density of the material', *Scientific Reports*, vol. 8, no. 1, p. 4703.
Arifin, YH & Yusuf, Y 2013, 'Mycelium Fibers as New Resource for Environmental Sustainability', *Procedia Engineering*, vol. 53, pp. 504-8.
Asdrubali, F, D'Alessandro, F & Schiavoni, S 2015, 'A review of unconventional sustainable building insulation materials', *Sustainable Materials and Technologies*, vol. 4, pp. 1-17.
Ashby, MF, Shercliff, H & Cebon, D 2018, *Materials: engineering, science, processing and design*, Butterworth-Heinemann.
ASTM International 2017, *Standard Test Method for Heat and Visible Smoke Release Rates for Materials and Products Using an Oxygen Consumption Calorimeter*, E1354-17.
Austin, J 2013, *Mycotectural Polyominoes*, viewed 17.06.2016 <http://www.zero1.org/exhibitions/about-pp>.
Babrauskas, V & Fires, Q 1997, 'Why was the fire so big', *HHR: the role of heat release rate in described fires. Fire Arson Investig*, vol. 47, pp. 54-7.
Babrauskas, V, Levin, BC, Gann, RG, Paabo, M, Harris, RH, Peacock, RD & Yusa, S 1992, 'Toxic potency measurement for fire hazard analysis', *Fire technology*, vol. 28, no. 2, pp. 163-7.
Babrauskas, V & Peacock, RD 1992, 'Heat release rate: the single most important variable in fire hazard', *Fire safety journal*, vol. 18, no. 3, pp. 255-72.
Bainbridge, DA 1986, 'High performance low cost buildings of straw', *Agriculture, Ecosystems & Environment*, vol. 16, no. 3, pp. 281-4.
Bajwa, DS, Holt, GA, Bajwa, SG, Duke, SE & McIntyre, G 2017, 'Enhancement of termite (Reticulitermes flavipes L.) resistance in mycelium reinforced biofiber-composites', *Industrial Crops and Products*, vol. 107, pp. 420-6.
Bamba, Y, Ogawa, Y, Saito, T, Berglund, LA & Isogai, A 2017, 'Estimating the Strength of Single Chitin Nanofibrils via Sonication-Induced Fragmentation', *Biomacromolecules*, vol. 18, no. 12, pp. 4405-10.
Bansal, V, Ahmad, A & Sastry, M 2006, 'Fungus-mediated biotransformation of amorphous silica in rice husk to nanocrystalline silica', *Journal of the American Chemical Society*, vol. 128, no. 43, pp. 14059-66.
Bartnicki-Garcia, S 1968, 'Cell Wall Chemistry, Morphogenesis, and Taxonomy of Fungi', *Annual Review of Microbiology*, vol. 22, no. 1, pp. 87-108.
Bayer, E & McIntyre, G 2012, *Substrate Composition and Method for Growing Mycological Materials*, U.S. Patent 20,120,315,687.
---- 2015, *Method for Growing Mycological Materials*, U.S. Patent 20,150,247,115.
Bell, LH & Bell, DH 1994, *Industrial noise control: Fundamentals and applications*, Marcel Dekker New York.
Bergman, TL, Incropera, FP, DeWitt, DP & Lavine, AS 2011, *Fundamentals of heat and mass transfer*, John Wiley & Sons.
Bhuvaneshwari, S, Hettiarachchi, H & Meegoda, JN 2019, 'Crop Residue Burning in India: Policy Challenges and Potential Solutions', *International journal of environmental research and public health*, vol. 16, no. 5, p. 832.
Boonstra, MJ & Tjeerdsma, B 2006, 'Chemical analysis of heat treated softwoods', *Holz als Roh-und Werkstoff*, vol. 64, no. 3, p. 204.
Brown, J, Fawell, P & Mathys, Z 1994, 'Fire-hazard assessment of extended-chain polyethylene and aramid composites by cone calorimetry', *Fire and materials*, vol. 18, no. 3, pp. 167-72.
Bultman, JD, Chen, S-L & Schloman, WW 1998, 'Anti-termitic efficacy of the resin and rubber in fractionator overheads from a guayule extraction process', *Industrial Crops and Products*, vol. 8, no. 2, pp. 133-43.

Buschow, KJ, Cahn, RW, Flemings, MC, Ilschner, B, Kramer, EJ & Mahajan, S 2001, 'Encyclopedia of materials', *Science and technology*, vol. 1, p. 11.
Butterfield, B, Chapman, K, Christie, L & Dickson, A 1992, 'Ultrastructural characteristics of failure surfaces in medium density fiberboard', *Forest products journal (USA)*.
Camere, S & Karana, E 2018, 'Fabricating materials from living organisms: An emerging design practice', *Journal of Cleaner Production*, vol. 186, pp. 570-84.
Carvalho, LMH & Costa, CAV 1998, 'MODELING AND SIMULATION OF THE HOT-PRESSING PROCESS IN THE PRODUCTION OF MEDIUM DENSITY FIBERBOARD (MDF)', *Chemical Engineering Communications*, vol. 170, no. 1, pp. 1-21.
Castagnede, B, Aknine, A, Brouard, B & Tarnow, V 2000, 'Effects of compression on the sound absorption of fibrous materials', *Applied Acoustics*, vol. 61, no. 2, pp. 173-82.
Chand, N & Fahim, M 2008, Tribology of Natural Fiber Polymer Composites, Woodhead Publishing.
Choudhury, AKR 2017, *Principles of textile finishing*, Woodhead Publishing.
Chung, S, Suidan, MT & Venosa, AD 2011, 'Partially acetylated sugarcane bagasse for wicking oil from contaminated wetlands', *Chemical Engineering & Technology*, vol. 34, no. 12, pp. 1989-96.
Clarke, FB 1997, 'Physiological effects of smoke: Managing escape', *ASHRAE journal*, vol. 39, no. 3, p. 47.
Collet, F & Prétot, S 2014, 'Thermal conductivity of hemp concretes: Variation with formulation, density and water content', *Construction and building materials*, vol. 65, pp. 612-9.
Corner, EJH 1953, 'The construction of polypores', in *Phytomorphology*, vol. 1. Introduction to Polyporus sulphureus, P. squamosus, P. betulinus and Polystictus microcyclus, pp. 152 - 69.
Costes, L, Laoutid, F, Brohez, S & Dubois, P 2017, 'Bio-based flame retardants: When nature meets fire protection', *Materials Science and Engineering: R: Reports*, vol. 117, pp. 1-25.
Dai, C, Yu, C & Zhou, X 2007, 'Heat and mass transfer in wood composite panels during hot pressing. Part II. Modeling void formation and mat permeability', *Wood and Fiber Science*, vol. 37, no. 2, pp. 242-57.
Defonseka, C 2014, *Introduction to Polymeric Composites with Rice Hulls*, Smithers Information Limited.
Dell 2016, *Green Packaging & Shipping*, viewed 21.07.2016 <http://www.dell.com/learn/us/en/uscorp1/corp-comm/mushroom-packaging>.
DP CleanTech 2013, *Understanding rice husk as a biomass fuel*.
El-Tahlawy, K 2008, 'Chitosan phosphate: A new way for production of eco-friendly flame-retardant cotton textiles', *Journal of the Textile Institute*, vol. 99, no. 3, pp. 185-91.
Elsacker, E, Vandelook, S, Brancart, J, Peeters, E & De Laet, L 2019, 'Mechanical, physical and chemical characterisation of mycelium-based composites with different types of lignocellulosic substrates', *PLOS ONE*.
Faruk, O, Bledzki, AK, Fink, H-P & Sain, M 2012, 'Biocomposites reinforced with natural fibers: 2000–2010', *Progress in polymer science*, vol. 37, no. 11, pp. 1552-96.
Feinberg, DR, Jones, BC, Little, AC, Burt, DM & Perrett, DI 2005, 'Manipulations of fundamental and formant frequencies influence the attractiveness of human male voices', *Animal behaviour*, vol. 69, no. 3, pp. 561-8.
Fisher, A 2010, 'Industrial-Strength Fungus', *Time Magazine*.
Food and Agriculture Organization of the United Nations 2017, *Rice Market Monitor*.
Ghazvinian, A, Farrokhsiar, P, Vieira, F, Pecchia, J & Gursoy, B 2019, *Mycelium-Based Bio-Composites For Architecture: Assessing the Effects of Cultivation Factors on Compressive Strength*.
Gilwee, W 1975, 'Fire retardant materials in aviation', paper presented to Proceedings of the 4th Annual Plastics Symposium.

Gosden, E 2016, 'Ikea plans mushroom-based packaging as eco-friendly replacement for polystyrene', *The Telegraph*, viewed 12/5/2016, <http://www.telegraph.co.uk/news/earth/businessandecology/recycling/12172439/Ikea-plans-mushroom-based-packaging-as-eco-friendly-replacement-for-polystyrene.html>.
Gosselink, RJA 2011, 'Lignin as a renewable aromatic resource for the chemical industry', University of Twente.
Green, DW, Winandy, JE & Kretschmann, DE 1999, 'Mechanical properties of wood', *Wood handbook: wood as an engineering material. Madison, WI: USDA Forest Service, Forest Products Laboratory, 1999. General technical report FPL; GTR-113: Pages 4.1-4.45*, vol. 113.
Griffin, DH 1996, *Fungal Physiology*, Wiley, Hoboken, NJ.
Haneef, M, Ceseracciu, L, Canale, C, Bayer, IS, Heredia-Guerrero, JA & Athanassiou, A 2017, 'Advanced Materials From Fungal Mycelium: Fabrication and Tuning of Physical Properties', *Scientific Reports*, vol. 7, p. 41292.
He, J, Cheng, CM, Su, DG & Zhong, MF 2014, 'Study on the Mechanical Properties of the Latex-Mycelium Composite', *Applied Mechanics and Materials*, vol. 507, no. 1, pp. 415-20.
Hemond, CJ 1983, *Engineering acoustics and noise control*, Prentice Hall.
Hirschler, MM 1987, 'Fire hazard and toxic potency of the smoke from burning materials', *Journal of fire sciences*, vol. 5, no. 5, pp. 289-307.
Holt, G, Mcintyre, G, Flagg, D, Bayer, E, Wanjura, J & Pelletier, M 2012, 'Fungal mycelium and cotton plant materials in the manufacture of biodegradable molded packaging material: Evaluation study of select blends of cotton byproducts', *Journal of Biobased Materials and Bioenergy*, vol. 6, no. 4, pp. 431-9.
Hong, B-W 1984, 'Dynamic viscoelasticity of hot pressed wood', *Journal of the Korean Wood Science and Technology*, vol. 12, no. 4, pp. 3-10.
Housseinpour, R, Latibari, AJ, Farnood, R, Fatehi, P & Sepiddehdam, SJ 2010, 'Fiber morphology and chemical composition of rapeseed (Brassica napus) stems', *IAWA journal*, vol. 31, no. 4, pp. 457-64.
Hshieh, FY 1998, 'Shielding effects of silica-ash layer on the combustion of silicones and their possible applications on the fire retardancy of organic polymers', *Fire and materials*, vol. 22, no. 2, pp. 69-76.
Hu, S, Song, L, Pan, H & Hu, Y 2013, 'Effect of a novel chitosan-based flame retardant on thermal and flammability properties of polyvinyl alcohol', *Journal of Thermal Analysis and Calorimetry*, vol. 112, no. 2, pp. 859-64.
Islam, M, Tudryn, G, Bucinell, R, Schadler, L & Picu, R 2017, 'Morphology and mechanics of fungal mycelium', *Scientific Reports*, vol. 7, no. 1, p. 13070.
---- 2018, 'Mechanical behavior of mycelium-based particulate composites', *Journal of materials science*, vol. 53, no. 24, pp. 16371-82.
Ismail, MS & Waliuddin, A 1996, 'Effect of rice husk ash on high strength concrete', *Construction and building materials*, vol. 10, no. 7, pp. 521-6.
Jerman, M, Keppert, M, Výborný, J & Černý, R 2013, 'Hygric, thermal and durability properties of autoclaved aerated concrete', *Construction and building materials*, vol. 41, pp. 352-9.
Jiang, L, Walczyk, D, McIntyre, G & Bucinell, R 2016, 'A new approach to manufacturing biocomposite sandwich structures: mycelium-based cores', paper presented to ASME 2016 11th International Manufacturing Science and Engineering Conference.
Jiang, L, Walczyk, D, McIntyre, G, Bucinell, R & Li, B 2019, 'Bioresin infused then cured mycelium-based sandwich-structure biocomposites: Resin transfer molding (RTM) process, flexural properties, and simulation', *Journal of Cleaner Production*, vol. 207, pp. 123-35.
Jiang, L, Walczyk, D, McIntyre, G, Bucinell, R & Tudryn, G 2017, 'Manufacturing of biocomposite sandwich structures using mycelium-bound cores and preforms', *Journal of Manufacturing Processes*, vol. 28, pp. 50-9.

Jones, M, Bhat, T, Huynh, T, Kandare, E, Yuen, R, Wang, C & John, S 2018, 'Waste-derived low-cost mycelium composite construction materials with improved fire safety', *Fire and materials*, vol. 42, no. 7, pp. 816-25.
Jones, M, Bhat, T, Kandare, E, Thomas, A, Joseph, P, Dekiwadia, C, Yuen, R, John, S, Ma, J & Wang, C 2018, 'Thermal Degradation and Fire Properties of Fungal Mycelium and Mycelium-Biomass Composite Materials', *Scientific Reports*, vol. 8, no. 1, p. 17583.
Jones, M, Bhat, T, Wang, C, Moinuddin, K & John, S 2017, 'Thermal Degradation and Fire Reaction Properties of Mycelium Composites', paper presented to 21st International Conference on Composite Materials, Xi'an, China.
Jones, M, Huynh, T, Dekiwadia, C, Daver, F & John, S 2017, 'Mycelium Composites: A Review of Engineering Characteristics and Growth Kinetics', *Journal of Bionanoscience*, vol. 11, no. 4, pp. 241-57.
Jones, M, Huynh, T & John, S,

2018, 'Inherent Species Characteristic Influence and Growth Performance Assessment for Mycelium Composite Applications', *Advanced Materials Letters*, vol. 9, no. 1, pp. 71-80.
Jones, M, Lawrie, A, Huynh, T, Morrison, P, Mautner, A, Bismarck, A & John, S 2019, 'Agricultural By-product Suitability for the Production of Chitinous Composites and Nanofibers', *Process Biochemistry*.
Jones, M, Weiland, K, Kujundzic, M, Theiner, J, Kahlig, H, Kontturi, E, John, S, Bismarck, A & Mautner, A 2019, 'Waste-Derived Low-Cost Mycelium Nanopapers with Tunable Mechanical and Surface Properties', *Biomacromolecules*.
Kadoya, K, Matsunaga, N & Nagashima, A 1985, 'Viscosity and thermal conductivity of dry air in the gaseous phase', *Journal of physical and chemical reference data*, vol. 14, no. 4, pp. 947-70.
Kavanagh, K 2005, *Fungi: Biology and Applications*, Wiley, Hoboken, NJ.
Kim, B & Christensen, RM 2000, 'Basic two-dimensional core types for sandwich structures', *International Journal of Mechanical Sciences*, vol. 42, no. 4, pp. 657-76.
Ko, KS & Jung, HS 1999, 'Molecular phylogeny of Trametes and related genera', *Antonie Van Leeuwenhoek*, vol. 75, no. 3, pp. 191-9.
Koizumi, T, Tsujiuchi, N & Adachi, A 2002, 'The development of sound absorbing materials using natural bamboo fibers', *WIT Transactions on The Built Environment*, vol. 59.
Kung'u, J 2016, *The Phases of Fungal Growth in Indoor Environment*, MBL, viewed 25/08/2016 <http://www.moldbacteria.com/mold/the-phases-of-fungal-growth-in-indoor-environment.html>.
Kymäläinen, H-R & Sjöberg, A-M 2008, 'Flax and hemp fibres as raw materials for thermal insulations', *Building and environment*, vol. 43, no. 7, pp. 1261-9.
Lelivelt, RJJ 2015, 'The mechanical possibilities of mycelium materials'.
Li, MM, Pan, HC, Huang, SL & Scholz, M 2013, 'Controlled experimental study on removing diesel oil spillages using agricultural waste products', *Chemical Engineering & Technology*, vol. 36, no. 4, pp. 673-80.
Li, ZJ, Shukla, V, Wenger, K, Fordyce, A, Pedersen, A & Marten, M 2002, 'Estimation of hyphal tensile strength in production-scaleAspergillus oryzae fungal fermentations', *Biotechnology and Bioengineering*, vol. 77, pp. 601-13.
Liu, J & Chow, W 2014, 'Study on flashover in small residential units with an open kitchen by nonlinear dynamics'.
López Nava, JA, Méndez González, J, Ruelas Chacón, X & Nájera Luna, JA 2016, 'Assessment of Edible Fungi and Films Bio-Based Material Simulating Expanded Polystyrene', *Materials and Manufacturing Processes*, vol. 31, no. 8, pp. 1085-90.
Madurwar, MV, Ralegaonkar, RV & Mandavgane, SA 2013, 'Application of agro-waste for sustainable construction materials: A review', *Construction and building materials*, vol. 38, pp. 872-8.
MatWeb 2018, *Flexural Strength Testing of Plastics*, viewed 30 April 2019 <http://www.matweb.com/reference/flexuralstrength.aspx>.

McIntyre, G, Poetzsch, A, Van Hook, S & Flagg, D 2012, *Method for producing a composite material*, U.S. Patent 20,120,225,471.
Michniewicz, J, Biliaderis, G & Bushuk, W 1992, 'Effect of added pentosans on some properties of wheat bread', *Food Chemistry*, vol. 43, no. 4, pp. 251-7.
Mokhothu, TH & John, MJ 2017, 'Bio-based coatings for reducing water sorption in natural fibre reinforced composites', *Scientific Reports*, vol. 7, no. 1, p. 13335.
Monahan, J & Powell, JC 2011, 'An embodied carbon and energy analysis of modern methods of construction in housing: A case study using a lifecycle assessment framework', *Energy and Buildings*, vol. 43, no. 1, pp. 179-88.
Mouritz, A, Mathys, Z & Gibson, A 2006, 'Heat release of polymer composites in fire', *Composites Part A: Applied science and manufacturing*, vol. 37, no. 7, pp. 1040-54.
Mouritz, AP & Gibson, AG 2007, *Fire properties of polymer composite materials*, vol. 143, Springer Science & Business Media.
Owren, MJ, Berkowitz, M & Bachorowski, J-A 2007, 'Listeners judge talker sex more efficiently from male than from female vowels', *Perception & Psychophysics*, vol. 69, no. 6, pp. 930-41.
Papadopoulos, AM 2005, 'State of the art in thermal insulation materials and aims for future developments', *Energy and Buildings*, vol. 37, no. 1, pp. 77-86.
Pegler, DN 1996, 'Hyphal analysis of basidiomata', *Mycological Research*, vol. 100, no. 2, pp. 129-42.
Pelletier, MG, Holt, GA, Wanjura, JD, Bayer, E & McIntyre, G 2013, 'An evaluation study of mycelium based acoustic absorbers grown on agricultural by-product substrates', *Industrial Crops and Products*, vol. 51, no. 1, pp. 480-5.
Pelletier, MG, Holt, GA, Wanjura, JD, Greetham, L, McIntyre, G, Bayer, E & Kaplan-Bie, J 2019, 'Acoustic evaluation of mycological biopolymer, an all-natural closed cell foam alternative', *Industrial Crops and Products*, vol. 139, p. 111533.
Pheng, LS & Hou, LS 2019, 'The Economy and the Construction Industry', in *Construction Quality and the Economy*, Springer, pp. 21-54.
Pierre Jr, RLS, Maguire, DJ & Automotive, CS 2004, 'The impact of A-weighting sound pressure level measurements during the evaluation of noise exposure', paper presented to Conference NOISE-CON.
Pigman, W 2012, *The Carbohydrates: Chemistry and Biochemistry Physiology*, Elsevier.
Pongrácz, P, Molnár, C & Miklósi, Á 2006, 'Acoustic parameters of dog barks carry emotional information for humans', *Applied Animal Behaviour Science*, vol. 100, no. 3-4, pp. 228-40.
Prasad, CS, Maiti, KN & Venugopal, R 2001, 'Effect of rice husk ash in whiteware compositions', *Ceramics International*, vol. 27, no. 6, pp. 629-35.
Pruteanu, M 2010, 'Investigations regarding the thermal conductivity of straw', *Buletinul Institutului Politehnic din Iasi. Sectia Constructii, Arhitectura*, vol. 56, no. 3, p. 9.
Rajagopal, A 2014, 'Behind The Living's "100% Organic" Pavilion for MoMA PS1', *Metropolis*.
Ren, M & Jacobsen, F 1993, 'A method of measuring the dynamic flow resistance and reactance of porous materials', *Applied Acoustics*, vol. 39, no. 4, pp. 265-76.
Rinaudo, M 2007, 'Chitin and Chitosan—General Properties and Applications', *ChemInform*, vol. 38, no. 7, pp. 603-32.
Roylance, D 2000, *Stresses in beams*, Massachusetts Institute of Technology, Cambridge, USA.
Ruxanda, B, Alice Teacă, C & Spiridon, I 2008, *Chemical modification of beech wood: Effect on thermal stability*, vol. 3.
Ryvarden, L 1991, 'Genera of polypores: Nomenclature and taxonomy', *Synopsis Fungorum*, vol. 5, pp. 1-363.
Sandberg, U 2003, 'The multi-coincidence peak around 1000 Hz in tyre/road noise spectra', paper presented to Euronoise Conference, paper ID.
Sartori, I & Hestnes, AG 2007, 'Energy use in the life cycle of conventional and low-energy buildings: A review article', *Energy and Buildings*, vol. 39, no. 3, pp. 249-57.

Seddeq, HS 2009, 'Factors influencing acoustic performance of sound absorptive materials', *Australian Journal of Basic and Applied Sciences*, vol. 3, no. 4, pp. 4610-7.
Sombatsompop, N & Chaochanchaikul, K 2004, 'Effect of moisture content on mechanical properties, thermal and structural stability and extrudate texture of poly(vinyl chloride)/wood sawdust composites', *Polymer International*, vol. 53, no. 9, pp. 1210-8.
Sorathia, U, Divisjón, C & Lyon, R 1997, 'A review of fire test methods and criteria for composites', *SAMPE journal*, vol. 33, no. 4, pp. 23-31.
Stamets, P 2005, *Mycelium Running: How Mushrooms Can Help Save the World*, Ten Speed Press.
Stamm, AJ 1964, 'Wood and cellulose science', *Wood and cellulose science.*
Stocks, SM & Thomas, CR 2001, 'Strength of mid-logarithmic and stationary phase Saccharopolyspora erythraea hyphae during a batch fermentation in defined nitrate-limited medium', *Biotechnology and Bioengineering*, vol. 73, no. 5, pp. 370-8.
Stocks, SM & Thomas, CR 2001, 'Viability, strength, and fragmentation of Saccharopolyspora erythraea in submerged fermentation', *Biotechnology and Bioengineering*, vol. 75, no. 6, pp. 702-9.
Sun, F, Banks-Lee, P & Peng, H 1993, 'Sound absorption in an anisotropic periodically layered fluid-saturated porous medium', *Applied Acoustics*, vol. 39, no. 1-2, pp. 65-76.
Sun, W, Tajvidi, M, Hunt, CG, McIntyre, G & Gardner, DJ 2019, 'Fully Bio-Based Hybrid Composites Made of Wood, Fungal Mycelium and Cellulose Nanofibrils', *Scientific Reports*, vol. 9, no. 1, p. 3766.
Superflux 2014, *Mycotecture (Phil Ross)*, New York Museum of Modern Art,, viewed 18.07.2016 <http://designandviolence.moma.org/mycotecture-phil-ross/>.
Sustainability Victoria 2014, *Market summary – recycled glass*.
Thakur, VK & Siṅgha, AS 2013, *Biomass-based Biocomposites*, Smithers Rapra Technology Ltd., Shropshire, U.K.
Theng, D, Arbat, G, Delgado-Aguilar, M, Vilaseca, F, Ngo, B & Mutjé, P 2015, 'All-lignocellulosic fiberboard from corn biomass and cellulose nanofibers', *Industrial Crops and Products*, vol. 76, pp. 166-73.
Thormark, C 2002, 'A low energy building in a life cycle—its embodied energy, energy need for operation and recycling potential', *Building and environment*, vol. 37, no. 4, pp. 429-35.
Travaglini, S, Noble, J, Ross, PG & Dharan, CKH 2013, 'Mycology Matrix Composites', paper presented to 28th ASC Technical Conference, State College, PA.
Tudryn, GJ, Smith, LC, Freitag, J, Bucinell, R & Schadler, LS 2018, 'Processing and morphology impacts on mechanical properties of fungal based biopolymer composites', *Journal of Polymers and the Environment*, vol. 26, no. 4, pp. 1473-83.
Tun, MM, Juchelkova, D, Win, MM, Thu, AM & Puchor, T 2019, 'Biomass Energy: An Overview of Biomass Sources, Energy Potential, and Management in Southeast Asian Countries', *Resources*, vol. 8, no. 2, p. 81.
Uysal, H, Demirboğa, R, Şahin, R & Gül, R 2004, 'The effects of different cement dosages, slumps, and pumice aggregate ratios on the thermal conductivity and density of concrete', *Cement and concrete research*, vol. 34, no. 5, pp. 845-8.
Vinson, J 2018, *The behavior of sandwich structures of isotropic and composite materials*, Routledge.
Wall, K, Walker, P, Gross, C, White, C & Mander, T 2012, 'Development and testing of a prototype straw bale house', *Proceedings of the Institution of Civil Engineers-Construction Materials*, vol. 165, no. 6, pp. 377-84.
Webster, J & Weber, R 2007, *Introduction to Fungi*, Cambridge University Press.
Wei, L, Liang, S & McDonald, AG 2015, 'Thermophysical properties and biodegradation behavior of green composites made from polyhydroxybutyrate and potato peel waste fermentation residue', *Industrial Crops and Products*, vol. 69, pp. 91-103.

Weiland, K, Jones, M, Fortea-Verdejo, M, Mautner, A, Bismarck, A & John, S 2019, 'Waste-derived Nanocellulose-Chitin Hybrid Materials with Tunable Physical Properties', paper presented to 22nd International Conference on Composite Materials, Melbourne, Australia.

Wessels, JGH, Mol, PC, Sietsma, JH & Vermeulen, CA 1990, 'Wall Structure, Wall Growth, and Fungal Cell Morphogenesis', in PJ Kuhn, APJ Trinci, MJ Jung, MW Goosey & LG Copping (eds), *Biochemistry of Cell Walls and Membranes in Fungi*, Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 81-95.

Wong, YJ, Arumugasamy, SK & Mustapha, KB 2019, 'Development of a computational predictive model for the nonlinear in-plane compressive response of sandwich panels with bio-foam', *Composite Structures*, vol. 212, pp. 423-33.

Xia, XC, Chen, XW, Zhang, Z, Chen, X, Zhao, WM, Liao, B & Hur, B 2013, 'Effects of porosity and pore size on the compressive properties of closed-cell Mg alloy foam', *Journal of Magnesium and Alloys*, vol. 1, no. 4, pp. 330-5.

Xing, Y, Brewer, M, El-Gharabawy, H, Griffith, G & Jones, P 2018, 'Growing and testing mycelium bricks as building insulation materials', paper presented to IOP Conference Series: Earth and Environmental Science.

Yang, H, Yan, R, Chen, H, Lee, DH & Zheng, C 2007, 'Characteristics of hemicellulose, cellulose and lignin pyrolysis', *Fuel*, vol. 86, no. 12-13, pp. 1781-8.

Yang, Z, Zhang, F, Still, B, White, M & Amstislavski, P 2017, 'Physical and mechanical properties of fungal mycelium-based biofoam', *Journal of Materials in Civil Engineering*, vol. 29, no. 7, p. 04017030.

Zabihzadeh, SM 2009, 'Water uptake and flexural properties of natural filler/HDPE composites', *BioResources*, vol. 5, no. 1, pp. 316-232.

Zhao, Q, Zhang, B, Quan, H, Yam, RCM, Yuen, RKK & Li, RKY 2009, 'Flame retardancy of rice husk-filled high-density polyethylene ecocomposites', *Composites Science and Technology*, vol. 69, no. 15, pp. 2675-81.

Zhu, BC, Henderson, G, Chen, F, Fei, H & Laine, RA 2001, 'Evaluation of vetiver oil and seven insect-active essential oils against the Formosan subterranean termite', *Journal of Chemical Ecology*, vol. 27, no. 8, pp. 1617-25.

Publication C

Addresses Research Question 1:

How do inherent species characteristics of fungi (e.g. hyphal types, pathogenicity, taxonomic- and association based classifications) affect the growth performance (hyphal extension rate and growth density) of the fibrous matrix phase (mycelium) and hence manufacturing time of mycelium composites?

Inherent species characteristic influence and growth performance assessment for mycelium composite applications

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Abstract

Composite materials produced using mycelial growth attract commercial and academic interest due to their economic, environmentally sustainable and green manufacturing process. However, their manufacture via slow biological growth affects the larger scale production viability of these materials, which must compete with rapidly producible synthetic materials. Hyphal characteristics vary significantly by species, which is the most influential growth performance factor in conjunction with environmental conditions and chemical nutrition. This study assessed the effect of potential growth predictors such as hyphal type, pathogenicity, taxonomic and association based classification systems on hyphal extension rate and growth density for commonly used and non-traditional species. It provides a simple, low-cost process for screening species by growth performance prior to more application-dependent mechanical evaluation. This facilitates more efficient and accurate species selection for composite manufacturing applications. Trimitic and dimitic species containing skeletal hyphae exhibited higher hyphal extension rates than species containing generative-binding or purely generative hyphae but no other parameters investigated in this study were good predictors for growth performance with significant species-specific variation present instead. However, the methodology used to test growth performance did prove effective and could be used on a case by case basis for growth screening in mycelium composite applications. Copyright © 2018 VBRI Press.

Keywords: Inherent species characteristics, species selection methodology, growth performance assessment, mycelium, composite.

Introduction

Increasing government and public environmental awareness have driven both academic and commercial interest in mycelium composites over the past decade (Jones et al. 2017). Mycelium is the vegetative growth of filamentous fungi that bonds organic matter through a network of hyphal micro-filaments to produce economical and environmentally sustainable biocomposites (Jiang et al. 2014). This natural biological growth acts as a low energy manufacturing process enabling the production of environmentally friendly alternatives to synthetic planar materials (e.g. plastic films and sheets) (Haneef et al. 2017), and larger low density (light weight) objects (e.g. synthetic foams and plastics) (Holt et al. 2012; López Nava et al. 2016; Pelletier et al. 2013; Travaglini et al. 2013).

For mycelium composites to compete with traditional synthetic materials, they must exhibit comparable material properties and be rapidly producible. Although several recent studies have investigated the material properties (Haneef et al. 2017; Holt et al. 2012; López Nava et al. 2016; Pelletier et al. 2013; Travaglini et al. 2013) and manufacturing procedures (Jiang et al. 2016a; Jiang et al. 2016b) of mycelium composites, inherent factors affecting production rate have not yet been addressed. Production rate is particularly important because manufacturing via natural biological growth is inherently slow compared to traditional manufacturing processes and limits the large-scale viability of mycelium composite production.

Fungal species used, in conjunction with environmental conditions and chemical nutrition, is the most influential growth performance parameter (Kavanagh 2005) and is hence important to the production rate. Existing mycelium composites research has exclusively utilised easily sourced basidiomycetes from the *Pleurotus* and *Ganoderma* genera (Haneef et al. 2017; Holt et al. 2012; López Nava et al. 2016; Pelletier et al. 2013; Travaglini et al. 2013). With 80,000 to 120,000 documented species (Webster & Weber 2007), of some 1.5 million (Hawksworth 2001) to 5.1 million fungal species (Blackwell 2011) estimated to be in existence, such limited and arbitrary selection is too random. However, the existence of such a large number of species makes species selection very challenging.

Basidiomycetes contain up to three distinct hyphal types, namely generative, binding (also known as ligative) and skeletal hyphae (Corner 1953). The number of different hyphal types present in a species is described using the mitic system. Monomitic species comprise of

only generative hyphae; dimitic species comprise of two hyphal types, and trimitic species comprise of all three principle hyphal types (Webster & Weber 2007). Each hyphal type exhibits different degrees of branching. Skeletal hyphae are unbranched or very sparsely branched, generative hyphae are moderately branched, and binding hyphae are highly branched (Breitenbach & Kränzlin 1986; Webster & Weber 2007). Degrees of branching and hyphal extension rates are inversely proportional in ascomycetes due to increased utilisation of substrate and inhibitory staling compounds produced (e.g. aldehydes) (Robinson & Park 1966) as hyphal density increases meaning the hyphal extension rate is insufficient to allow growth into new areas of the substrate (Prosser 1993). The hyphal growth unit (G), which is a property of the mycelium that is mathematically linked to other hyphal and colony growth parameters (Kotov & Reshetnikov 1990), also increases as branching becomes sparser (Prosser 1995). As such, hyphal types present may potentially be a growth performance predictor that could assist in species selection to increase mycelium composite production rates.

Pathogenicity, which describes an organism's ability to attack and infect a host, could also potentially be used to assist species selection and increase production rates. Many pathogens use specialised toxins, enzymatic degradation, subversions of cellular processes, mechanical forces, or a combination of these, to rapidly invade and colonise host material (Kavanagh 2005; Sexton & Howlett 2006). While animal pathogens typically only cause serious fungal infections in immunocompromised hosts, plant pathogens have mechanisms allowing invasion of even healthy hosts including mechanical penetration using appressoria. Appressoria are highly organised enlarged hyphal ends that feature a narrow hyphal strand on the underside known as a penetration peg which penetrates the epidermal cell wall and accelerates inoculation (Howard & Valent 1996; Kavanagh 2005; Mendgen et al. 1996; Sexton & Howlett 2006). Although some pathogenic fungi such as biotrophs are not suitable for mycelium composite applications because they must be in contact with a host plant to survive, non-obligate pathogens are more versatile and can grow and multiply on dead organic matter as well as on living host tissue (Kavanagh 2005). The virulent and aggressive nature of some species allowing them to compete with a living host could make pathogenicity a growth performance indicator able to assist mycelium composite species selection.

Existing fungal taxonomic and association based classification structures could also potentially be used in growth performance predictions to aid mycelium composite species selection. Established taxonomic classifications provide a reference framework of recognisable features, related organisms and useful information about the characteristics of a given species (Webster & Weber 2007). Fungal associations can also be used to describe the preferred growth environment and host-organism relationship shared by groups of fungi (Stamets 2005). Since these pre-existing, well-developed classifications grouped organisms based on morphological, biochemical and ecological similarities, growth performance similarities could also exist, allowing for the prediction of growth performance of multiple species within the same group based on the performance of other group members.

This study investigated simple and resource conservative methods for assessing the viability of fungal species for use in mycelium composite manufacturing applications. It aimed to address these potentially

Table 1. Selected species and associated variables. Compiled from [1]Breitenbach and Kränzlin (1986, 1991, 1995); [2]Stalpers et al. (2004) and Wu et al. (2013).

Identification*	Hyphal Type (mitic)†[1]			Pathogenic‡[2]		Association[2]	Species Variability	Source
	Mono-	Di-	Tri-	Yes	No			
Allomyces arbuscula [Bl]						Water		RMIT
Botrytis cinerea [A]				P				RMIT
Fusarium oxysporum [A]				P		Soil		RMIT
Ganoderma lucidum [Ba]			GBS		x			NGMS
Hypsizygus ulmarius [Ba]	G				x			NGMS
Lichtheimia corymbifera [M]				A		Animal		RMIT
Mucor genevensis [M]						Soil		RMIT
Phytophthora cinnamomi [O]				P		Water		RMIT
Pleurotus citrinopileatus [Ba]							x	NGMS
Pleurotus cornucopiae [Ba]		GB					x	NGMS
Pleurotus djamor [Ba]		GB					x	NGMS
Pleurotus eryngii [Ba]							x	NGMS
Pleurotus ostreatus [Ba]	G				x	Wood	x	NGMS
Pleurotus pulmonarius [Ba]							x	NGMS
Polyporus brumalis [Ba]		GS			x			RMIT
Saksenaea vasiformis [M]				A		Animal		RMIT
Stropharia rugosoannulata [Ba]	G				x			NGMS
Trametes versicolor [Ba]			GBS		x	Wood		NGMS

*Species and phylum, A = Ascomycota, Ba = Basidiomycota, Bl = Blastocladiomycota, M = Mucoromycota, O = Oomycota (Chromista).
†G = generative hyphae (monomitic), GB = generative and binding hyphae (dimitic), GS = generative and skeletal hyphae (dimitic), GBS = generative, binding and skeletal hyphae (trimitic).
‡P = pathogenic to living plants, A = pathogenic to animals or humans.

influential growth factors; determine if they affect hyphal extension rate or growth density, and compare the growth performance of commonly used and non-traditional fungi. Growth performance assessment is more time efficient and cheaper than material property testing and can be used to delimit low-performing species at an early stage in development and identify high-performing species.

Materials and experimental methodology

Fungal cultures

Species selection was systematically based on 1) hyphal type [mono-, di- and trimitic] 2) pathogenicity [pathogenic and non-pathogenic to living plants or animals and humans], 3) fungal association [animal, soil, water, wood] 4) species variability [*Pleurotus* genus] **(Table 1).** Initial selection was based on availability from RMIT culture collections and commercial sources.

Isolates from the RMIT University microbiology culture collection (Bundoora, Australia) were stored cultures under oil on nutrient agar slopes, which were subcultured onto fresh sterile malt extract agar (Neogen, Michigan) plates and their identity verified by technicians. Inverted plates were incubated at 22°C in darkness for 7 days.

Other isolates were purchased from New Generation Mushroom Supplies (Melbourne, Australia) (NGMS). Samples were supplied as mycelial masses growing on wheat grain sealed in plastic bags with filter patches. These isolates were subcultured onto malt extract agar plates and incubated as before.

Media preparation and inoculation

Solid media (hyphal extension rate measured as radial growth)

Malt extract agar (Neogen, Michigan) was prepared as per instructions and autoclaved at 121°C for 15 minutes. Molten agar was poured aseptically into 90 mm petri dishes and allowed to solidify. Isolate cultures were cut into inoculum disks (Ø7 mm) using the blunt base of a sterilised 1.0 mL pipette tip. New pipette tips were used for each species. One single inoculum disk was placed on the edge of each petri dish in contact with the dish wall. Each experiment was conducted with triplicate dishes containing a single inoculum. Triplicate dishes were individually parafilmed, sealed in groups in zip lock bags and incubated at 25°C in darkness for 7 days.

Liquid media (growth density measured as dry weight)

Malt extract liquid media was prepared by mixing malt extract (Morgan's Brewing Co., Yatala) with Milli-Q® water (1 g/10 mL) and autoclaved as described above. The liquid was dispensed as 100 mL aliquots into 250 mL glass jars using a sterile syringe. Inoculum disks were prepared as previously described and suspended in the liquid media. Each experiment was conducted with triplicate jars containing a single inoculum and incubated at 22°C in darkness for 14 days on a Paton Scientific OP3422 orbital shaker at 100 orbits per minute (OPM).

Growth measurements

Hyphal extension rate

Daily radial growth (mm) was measured from the centre of the inoculum disk to the tip of the of the longest hypha. This was completed at the same time each day for consistency for 7 days. This growth period was selected based on preliminary trials which showed the faster species filling a 90-mm petri dish in this time **(Fig. 1a).**

Growth density

End-point dry weight was measured after 14 days of growth. This growth period was selected based on preliminary trials showing this to be the optimal time required for the slower species to have significant enough mass to weigh. The liquid media and mycelial masses were vacuum filtered using grade 1 chromatography paper and an EMD Millipore XX104710 filtration device. Masses were then dried for 48 hours in a 50°C oven and weighed using an OHAUS Explorer analytical balance **(Fig.1b)**.

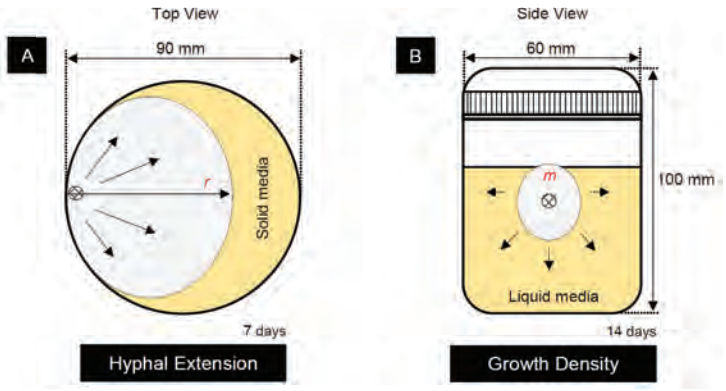

Fig. 1. Mycelial growth measured as a) hyphal extension on agar solid media plates measured radially from the petri dish wall (*r*, mm) over 7 days and b) growth density from broth liquid media measured as 14-day dry weight (*m*, mg). Inoculum disc (cross-hatched, 7mm in diameter) used to inoculate both solid and liquid media.

Data processing and statistical analysis

End-point radial growth and dry weight were statistically analysed in Microsoft Excel and graphed using GraphPad Prism (version 7.02). Data was checked for normality using Kolmogorov-Smirnov test on Minitab (version 16.2) where normal data was $p \geq 0.15$. For data that was normal, ANOVA (Analysis of Variance) was performed, and significant differences were considered at $p \leq 0.05$. Class categories for normal data were generated using letters of comparison based on Tukey's family error rate. Data that was not normal was transformed until normal where indicated, and ANOVA compared. For non-parametric data, Kruskal-Wallis test was conducted where significant differences were considered at $p \leq 0.05$. Class categories for non-parametric data were generated using group membership based on k-means clustering.

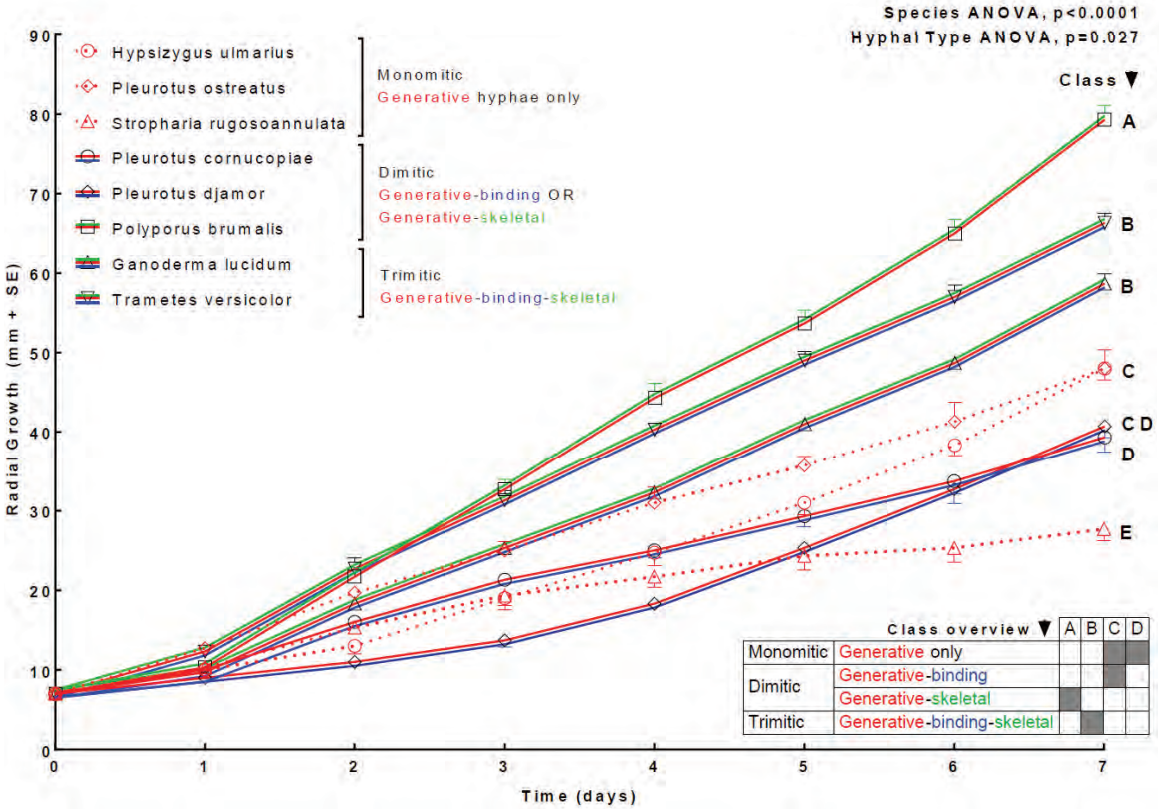

Fig. 2. Hyphal extension rate measured as radial growth (mm + SE) over 7 days for monomitic (generative only, red dotted), dimitic generative-binding (red-blue), dimitic generative-skeletal (red-green) and trimitic (generative-binding-skeletal hyphae, red-blue-green) fungi. Error bars indicate standard error within triplicate sets. Class categories were letters of comparison based on Tukey's family error rate at p≤0.05 for species dependent ANOVA.

Results

Basidiomycete hyphal types

Significant differences in 7-day hyphal extension measured as radial growth were observed for different hyphal types (ANOVA, p=0.027), although species-specific variation was more significant (ANOVA, p<0.0001). Growth performance varied within, and between the monomitic, dimitic and trimitic groups, however, monomitic species consistently had a slower hyphal extension rate than trimitic species. Dimitic species hyphal extension rate varied greatly depending on whether skeletal or binding hyphae were present in addition to the generative hyphae. Species with generative-skeletal hyphae exhibited a much higher hyphal extension rate than those with generative-binding hyphae **(Fig. 2)** which supported evidence of an inverse relationship between hyphal branching and extension rate.

Monomitic species containing only generative hyphae consistently exhibited low radial growth overall (25-52 mm over 7 days, ȳ=41 mm) falling exclusively into growth classes C and E. Lowest performing monomitic species, *S. rugosoannulata* (28 mm), (79 mm, highest radial growth) and even *P. ostreatus* and *H. ulmarius* (48 mm) underperformed by almost 40%. Dimitic species exhibited significant variation in radial growth. *P. brumalis* (generative-skeletal hyphae) achieved the highest radial growth overall (class A, 79 mm) while in contrast *P. cornucopiae* and *P. djamor* (generative-binding hyphae) achieved approximately half this value (class C, 39 mm and 41 mm respectively) and performed worse than most monomitic species. Trimitic species consistently achieved high radial growth (57-68 mm, ȳ=63 mm), falling into growth class B. Highest performing trimitic species *T. versicolor* had the second highest radial growth overall, lagging 16% behind *P. brumalis* **(Fig. 2).**

However, significant differences in growth density measured as 14-day dry weight were not observed for different hyphal types (ANOVA, p=0.198), with significant species-specific variation instead (ANOVA, p<0.0001). There was significant growth performance variation within and between the monomitic, dimitic and trimitic groups with only trimitic species consistently achieving similar results. Monomitic species comprising only of generative hyphae varied significantly, achieving both very low and high dry weights. Dimitic species, while generally high-performing, also varied significantly. The presence of generative, binding or skeletal hyphae appeared not to influence growth density **(Fig. 3).**

Monomitic species had the lowest average dry weight (109 mg), although performance did vary significantly, spanning dry weight classes A-D. Values recorded ranged from 34 mg (*H. ulmarius*) to 238 mg (*P. ostreatus*) (7 times difference) which were respectively the lowest and second highest values recorded overall. Dimitic species had the highest average dry weight (159 mg), but performance varied from 105 mg (*P. brumalis*,

generative-skeletal hyphae) to 251 mg (*P. djamor*, generative-binding hyphae) which was the highest dry weight recorded overall (over 2 times difference, classes A, B, C). Trimitic species had medium average dry weight (138 mg), falling in classes B and C, and ranging from 119 mg (*G. lucidum*) to 165 mg (*T. versicolor*) **(Fig. 3).**

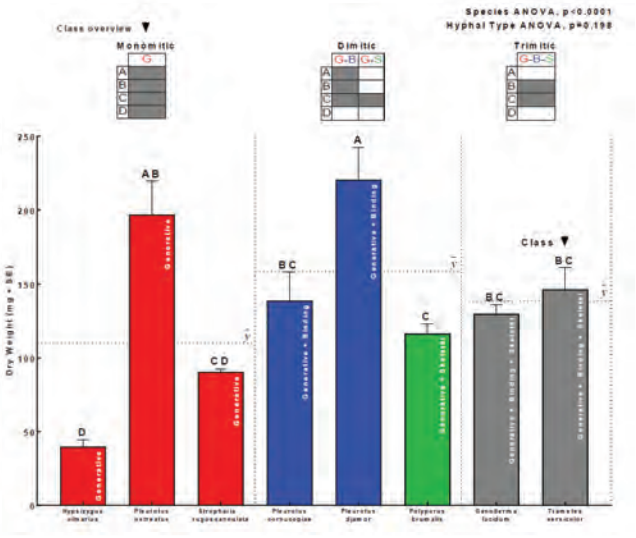

Fig. 3. Growth density measured as 14-day dry weight (mg + SE) for generative monomitic (G, red), dimitic generative-binding (G-B, blue), dimitic generative-skeletal (G-S) (green) and trimitic (G-B-S, grey) fungi. Class categories were letters of comparison based on Tukey's family error rate at p≤0.05 for species dependent ANOVA.

Pathogenicity

The 7-day hyphal extension measured as radial growth was not significantly different between pathogenic and non-pathogenic species (ANOVA, p=0.079). Instead, species-specific variation was significant (ANOVA, p<0.0001). Growth performance varied significantly within the pathogenic and non-pathogenic groups, with group representatives in most classes which ranged from slow (10-50 mm) to fast (50-90 mm). The outlier, *S. rugosoannulata*, underperformed and was the slowest species **(Fig. 4).**

Pathogenic species (47-84 mm, ȳ=67 mm, classes A-E) experienced higher average radial growth performance than non-pathogenic species (25-83 mm, ȳ=55 mm, classes A-F). However, the highest performing pathogenic species *S. vasiformis* (82 mm) was only marginally faster than the highest performing non-pathogenic species *P. brumalis* (79 mm), and the performance of most non-pathogenic species investigated was better than or comparable to at least one pathogenic species. The slowest pathogenic species, *P. cinnamomi* (50 mm) experienced radial growth only marginally faster than the non-pathogenic *P. ostreatus* and *H. ulmarius* (both 48 mm) and was outperformed by two-thirds of all species. The slowest non-pathogenic species, *S. rugosoannulata,* was almost twice as slow as the slowest pathogenic species (28 mm) **(Fig. 4).**

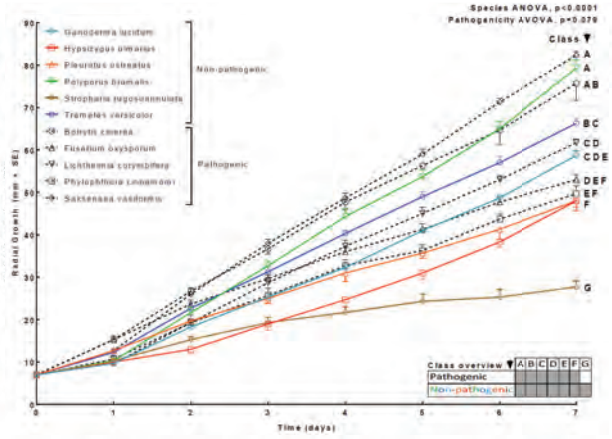

Fig. 4. Hyphal extension rate measured as radial growth (mm + SE) over 7 days for pathogenic (black dotted) and non-pathogenic fungi (coloured) fungi. Class categories were letters of comparison based on Tukey's family error rate at p≤0.05 for species dependent ANOVA.

Differences in growth density data measured as 14-day dry weight had borderline significance between pathogenic and non-pathogenic species (Non-parametric, Kruskal-Wallis, p=0.051), but variation determined by individual species was far more significant (Kruskal-Wallis, p=0.001). Growth performance was comparable within and between pathogenic and non-pathogenic groups except species-specific outliers like *H. ulmarius* and *F. oxysporum*.

Pathogenic species had higher overall average dry weight (89-316 mg, ȳ=172 mg) than non-pathogenic species (34-160 mg, ȳ=120 mg). Pathogenic *F. oxysporum*, as an outlier, experienced the highest dry weight (316 mg), however, this was not characteristic of pathogenic species with most performing approximately 50-70% worse than *F. oxysporum*. Non-pathogenic outliers included *P. ostreatus* (197 mg) and *H. ulmarius* (40 mg), but dry weight of pathogenic and non-pathogenic fungi was comparable for all other species with very similar performance noted between several fungi of opposing pathogenicity status. Non-pathogenic *H. ulmarius* had the lowest dry weight (40 mg), but this was also not characteristic of non-pathogenic species with most performing approximately 2-5 times better **(Fig. 5).**

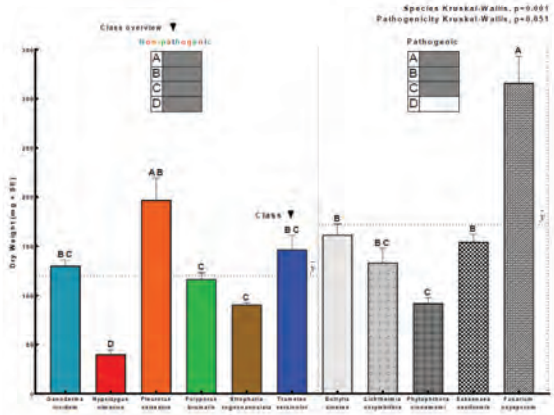

Fig. 5. Growth density measured as 14-day dry weight (mg + SE) for non-pathogenic (coloured) and pathogenic (greyscale) fungi. Class categories were letters of comparison based on cluster membership at p≤0.05 for species dependent k-means clustering.

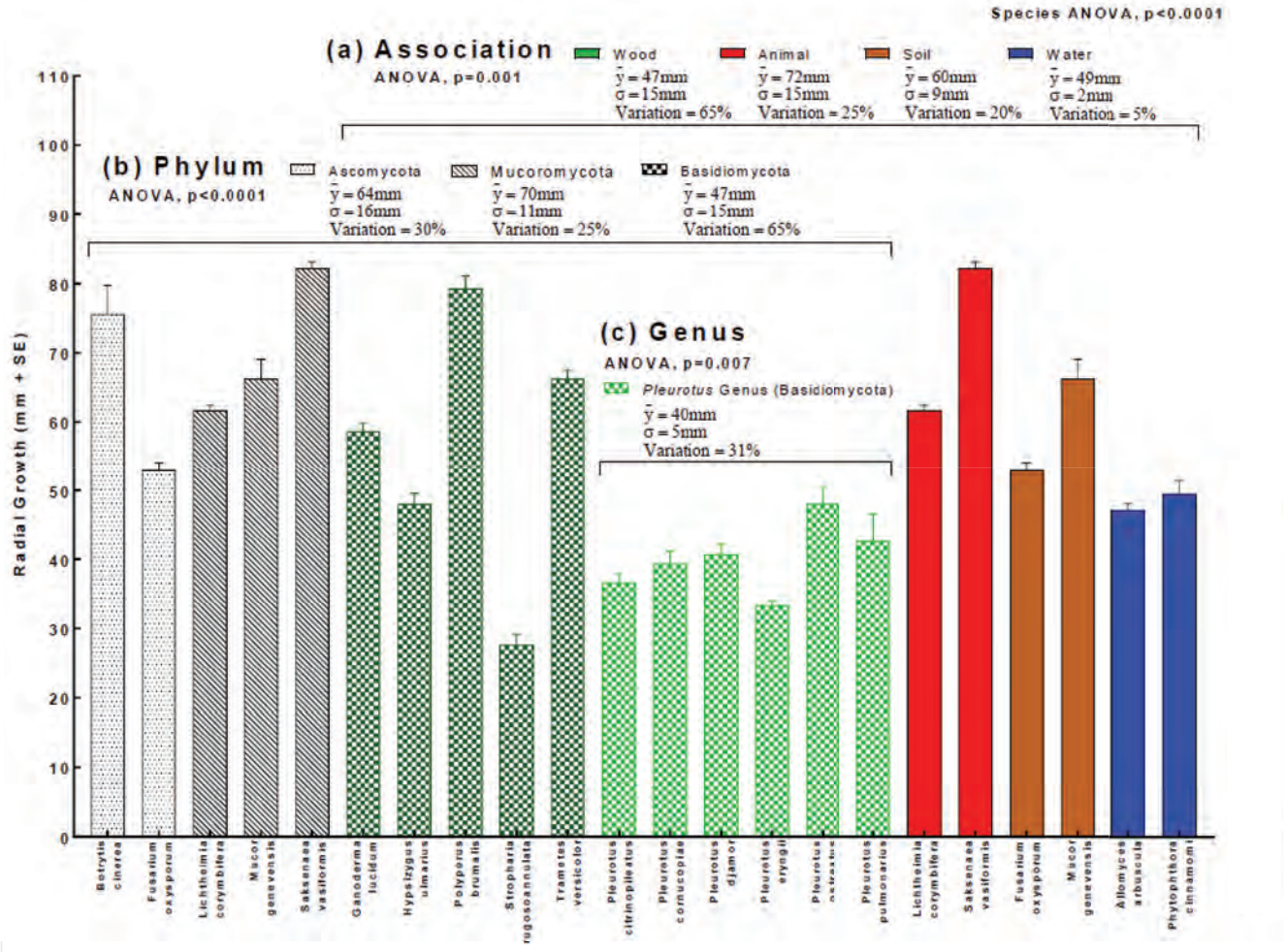

Fig. 6. Hyphal extension measured as 7-day radial growth (mm + SE) for (a) fungal association, (b) phylum and (c) species-specific variation within the same genus (*Pleurotus*). Colouring: animal (red), soil (brown), water (blue) and wood (green) fungal associations. Shading: Ascomycota (dotted), Basidiomycota (checkered) and Mucoromycota (diagonally striped) phyla. *Pleurotus* genus highlighted (light green checkered). The mean ($\bar{y}$) and standard deviation (σ) of each data set is included in addition to the percentage variation between minimum and maximum values.

Taxonomic and fungal association based classifications

Most significant differences in 7-day hyphal extension measured as radial growth were observed between different species and phyla (ANOVA, $p<0.0001$). Significant differences were also observed between the radial growth of fungi of different associations (ANOVA, $p=0.001$) with least significant differences observed between fungi within the *Pleurotus* genus (ANOVA, $p=0.007$) **(Fig. 6).**

Wood-associated fungi experienced the highest variation in radial growth based on association (σ=15 mm, variation=65%) **(Fig. 6a)** with a sample size (n=11) sufficiently large to demonstrate the heterogenous nature of fungi and preclude a correlation between the fungal association and hyphal extension rate. Although a micro trial (n = 2) of water-associated fungi displayed low variation (σ = 2 mm, variation = 5%), other micro trials conducted on the animal- (σ = 15 mm, variation = 25%) and soil- (σ = 9 mm, variation=20%) associated fungi demonstrated significant variation and demonstrated the futility of further trials.

Comparison by phylum **(Fig. 6b)** yielded similar results with Basidiomycota synonymous with wood associated fungi in this case. The significant variation (σ = 15 mm, variation=65%) present within the large sample of Basidiomycota (n = 11) coupled with micro trials of Ascomycota (n = 2) (σ = 16 mm, variation = 30%) and Mucoromycota (n = 3) (σ = 11 mm, variation = 25%) demonstrated significant variation present within and between phyla and the insignificance of phylum on radial growth.

A final investigation of genetically similar fungi within the *Pleurotus* genus (n = 6) (σ = 5 mm, variation = 31%) **(Fig. 6c)** confirmed the significant species-specific variation in radial growth between fungi and the absence of a relationship between traditional taxonomic and fungal association based classifications and hyphal extension growth performance.

Most significant differences in 14-day growth density measured as dry weight were observed between different species (including genetically similar species within the *Pleurotus* genus) (ANOVA, $p<0.0001$). Significant

differences were also observed in the dry weight of fungi between phyla (ANOVA, p=0.001) with least significant differences observed between fungi of different associations (ANOVA, p=0.005) **(Fig. 7).**

Wood-associated fungi experienced the highest association based variation in growth density (σ=55 mg, variation=82%) **(Fig. 7a)** with a sample size (n=11) sufficient to suggest that fungal association had little impact on growth density. Although a micro trial (n = 2) of animal-associated fungi did show lower variation (σ = 15 mg, variation=14%), most micro trials including those of water- (σ = 230 mg, variation = 77%) and soil- (σ = 118 mg, variation = 53%) associated fungi also demonstrated significant variation, making further trials redundant.

Comparison by phylum **(Fig. 7b)** yielded similar results with Basidiomycota synonymous with wood associated fungi for the species examined. Although, a micro trial of Mucoromycota (n=3) indicated some uniformity (σ = 11 mg, variation = 14%), significant variation (σ = 55 mg, variation = 82%) within the extensively sampled Basidiomycota (n = 11) coupled with a micro trial of Ascomycota (n = 2) (σ = 109 mg, variation = 49%) demonstrated significant variation present within and between phyla and the lack of influence of this factor on growth performance.

A final investigation of genetically similar fungi within the *Pleurotus* genus (n = 6) (σ = 65 mg, variation = 72%) **(Fig. 7c)** verified the significant species-specific variation in dry weight between fungi and the lack of correlation between traditional taxonomic and fungal association based classifications and growth density.

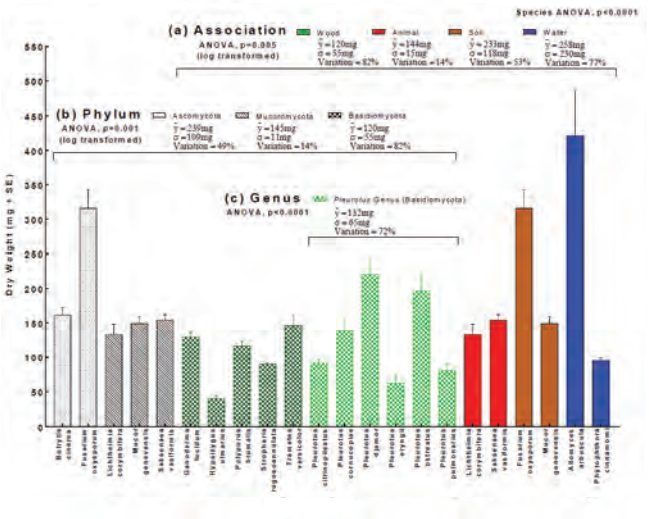

Fig. 7. Growth density measured as 14-day dry weight (mg + SE) for (a) fungal association, (b) phylum and (c) species-specific variation within the same genus (*Pleurotus*). Colouring: animal (red), soil (brown), water (blue) and wood (green) fungal associations. Shading: Ascomycota (dotted), Basidiomycota (checkered) and Mucoromycota (diagonally striped) phyla. *Pleurotus* genus highlighted (light green checkered). The mean ($\bar{y}$) and standard deviation (σ) of each data set is included in addition to the percentage variation between minimum and maximum values.

Performance overview

Significant variation in hyphal extension (28-82 mm) and growth density (40-421 mg) was present in the fungi assessed. *S. vasiformis* experienced the fastest hyphal extension over 7 days measured as radial growth (82 mm) overall, and *A. arbuscula* achieved the highest growth density over 14 days measured as dry weight (421 mg) **(Fig. 8).**

Other fungi (*B. cinerea*, *M. genevensis*, *P. brumalis* and *T. versicolor*) experienced radial growth or dry weight within 20% of values attained by the highest performing species. A further 25% of fungi (*F. oxysporum*, *G. lucidum*, *L. corymbifera* and *P. cinnamomi*) attained radial growth or dry weight within 20-40% of the highest performing species while the remaining 50% of fungi (*H. ulmarius*, *P. citrinopileatus*, *P. cornucopiae*, *P. djamor*, *P. eryngii*, *P. ostreatus*, *P. pulmonarius*, *S. rugosoannulata*) performed at least 40% worse than the highest performing species **(Fig. 8).**

The poorest growth performance was exhibited by *S. rugosoannulata* (28 mm hyphal extension) and *H. ulmarius* (40 mg dry weight). Medium (60-80% performance) and high-performing (top 20% performance) fungi attained either high radial growth or high dry weight but never both. Some low-performing fungi (0-60% performance) had more proportional radial growth and dry weight (*P. cornucopiae* and *P. ostreatus*) but overall high radial growth and high dry weight were mutually exclusive in species assessed **(Fig. 8).**

Discussion

Daily radial growth measurement on solid media was an economical, efficient and effective method for assessing the hyphal extension rate of fungi. Trends in individual hyphal extension acceleration and deceleration were visible over as little as 7 days, and growth performance comparison between fungi was also possible for this period. This two-dimensional metric, combined with a three-dimensional growth density assessment derived from fungi grown in liquid media, comprehensively described fungal growth potential and could be used to delimit low-performing species at an early stage in development and help identify high-performing species.

Growth performance varied significantly and arbitrarily meaning that for optimal species selection, fungi need to be assessed on an individual basis to establish their suitability for composite manufacturing applications. Hyphal growth involves cell wall extension and biosynthesis of wall components utilising chitin synthase isozymes with different kinetic parameters (K_M values) that vary in type and number by species (Carlile *et al.* 2001). Hyphal extension rate is also related to hyphal extension zone and colony peripheral growth zone dimensions which vary not only by species but by strain (Carlile *et al.* 2001).

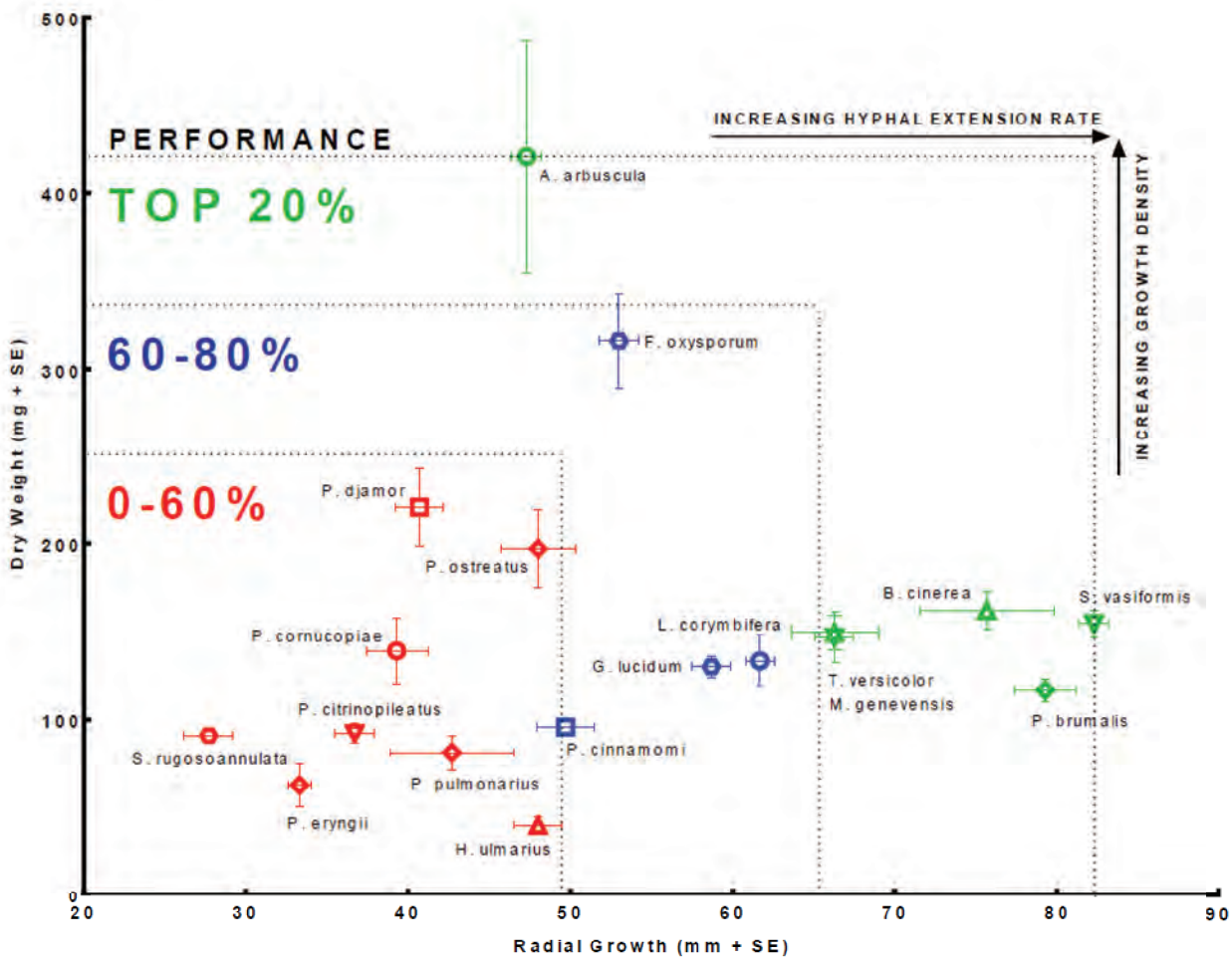

Fig. 8. Hyphal extension (7-day radial growth in mm) versus growth density (14-day dry weight in mg) for all fungi assessed grouped by percentage performance (low 0-60% - red, moderate 60-80% - blue, top 20% - green).

Hyphal extension rate and branching were inversely related for basidiomycetes in this study, with dimitic species containing sparsely branched or unbranched skeletal hyphae exhibiting much higher hyphal extension rates than dimitic species containing highly branched binding hyphae. However, an inverse relationship between growth density and branching of basidiomycetes was absent. Hyphal extension rate and branching are also inversely related for ascomycetes (Robinson & Park 1966) and the hyphal growth unit (G), which is a property of the mycelium that is mathematically linked to other hyphal and colony growth parameters (Kotov & Reshetnikov 1990), increases as branching becomes sparser (Prosser 1995). The effect of branching on dry weight (growth density) for basidiomycetes has not been extensively studied, but branching is known to increase the surface area of colonies and mediate hyphal fusion events which aid nutrient assimilation and exchange between hyphae of the same colony (Harris 2008).

Pathogenic fungi, which rapidly invade and colonise host material utilising a combination of specialised toxins, cellular process subversion and mechanical force (Kavanagh 2005; Sexton & Howlett 2006) absent in the less aggressive saprotrophic species, were expected to exhibit improved growth performance. However, no consistent or significant growth performance improvement was present in these fungi. Pathogenic species are inherently more dangerous than non-pathogenic species with their infectious nature making them more difficult to render inert and hence safe materials harder to produce. Significant risks are also present during their manufacture for either plants or animals (including humans) exposed to the manufacturing process. As such, with no significant growth performance improvement present among these species, their use should be excluded from mycelium composite manufacturing.

It is not known how variation in the substrate and environmental conditions would affect the growth performance of the fungi investigated as this varies on a species-specific basis. However, water-, animal- and soil-associated fungi provided no notable growth performance improvement and since most composite substrates are starch, cellulose or lignin-based (Haneef *et al.* 2017; Holt *et al*. 2012; López Nava *et al*. 2016; Pelletier *et al.* 2013; Travaglini *et al.* 2013), wood-associated fungi would be enzymatically best suited to digest these complex carbon

sources. *T. versicolour* and *P. brumalis* were the highest performing wood-associated fungi, achieving growth performance within the top 20% of species overall. This supports their use in mycelium composites applications over other traditionally popular species including *G. lucidum* (Haneef *et al.* 2017; Holt *et al.* 2012; Travaglini *et al.* 2013) and *P. ostreatus* (Haneef *et al.* 2017; He *et al.* 2014; López Nava *et al.* 2016).

Growth performance assessment is of critical importance due to the inherently slow nature of mycelium composite manufacturing which take days to grow (Haneef *et al.* 2017; Holt *et al.* 2012; López Nava *et al.* 2016; Pelletier *et al.* 2013; Travaglini *et al.* 2013) when compared to traditional plastic manufacturing processes which require only minutes or hours to produce (Allen 2012). However, mycelium composite manufacture is much less energy intensive than plastic manufacturing and utilises agricultural waste materials to produce low density, biodegradable products rather than petroleum derivatives (Holt *et al.* 2012; López Nava *et al.* 2016; Pelletier *et al.* 2013; Travaglini *et al.* 2013). This makes mycelium composites cost competitive with plastics but significantly more environmentally sustainable (Holt *et al.* 2012; Pelletier *et al.* 2013; Travaglini *et al.* 2013) with production time able to be reduced though informed species selection governed by growth performance screening methodologies such as those proposed in this study.

Conclusion

This study found the assessment of hyphal extension rate measured as radial growth and growth density measured as dry weight to be a simple, effective and resource conservative method for evaluating the viability of fungi for mycelium composite manufacturing. Hyphal types present, pathogenicity and traditional classification structures were not influential growth factors, with fungal growth performance highly variable both in terms of hyphal extension rate and growth density. However, growth performance can be rapidly and inexpensively determined through methods such as those outlined in this study. This makes initial growth performance screening prior to more expensive and complicated testing possible. *T. versicolour* and *P. brumalis* were the most suitable species assessed in this study based on growth performance and enzymatic compatibility with typical mycelium composite substrates. This supports their use over other traditionally used species such as *P. ostreatus* and *G. lucidum*.

Acknowledgments

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References

1. Allen, KA 2012, 'Processing of Thermoplastic Composites', *Polymer and Composites Processing*, pp. 1-10.
2. Blackwell, M 2011, 'The Fungi: 1, 2, 3 … 5.1 million species?', *American Journal of Botany*, vol. 98, no. 3, pp. 426-38.
3. Breitenbach, J & Kränzlin, F 1986, *Fungi of Switzerland: Non-gilled fungi: Heterobasidiomycetes, Aphyllophorales, Gasteromycetes*, Verlag Mykologia.
4. *Fungi of Switzerland: Boletes and agarics. Strobilomycetaceae and Boletaceae. Paxillaceae. Gomphidiaceae. Hygrophoraceae. Tricholomataceae. Polyporaceae (lamallete)*, Mykologia, 1991.
5. *Fungi of Switzerland: Agarics. Entolomataceae, Pluteaceae, Amanitaceae, Agaricaceae, Coprinaceae, Bolbitaceae, Strophariaceae*, Mykologia, 1995.
6. Carlile, MJ, Watkinson, SC & Gooday, GW 2001, 'Fungal Cells and Vegetative Growth', in *The Fungi*, Academic Press, London, pp. 85-184.
7. Corner, EJH 1953, 'The construction of polypores', in *Phytomorphology*, vol. 1. Introduction to Polyporus sulphureus, P. squamosus, P. betulinus and Polystictus microcyclus, pp. 152 - 69.
8. Haneef, M, Ceseracciu, L, Canale, C, Bayer, IS, Heredia-Guerrero, JA & Athanassiou, A 2017, 'Advanced Materials From Fungal Mycelium: Fabrication and Tuning of Physical Properties', *Scientific Reports*, vol. 7, p. 41292.
9. Harris, SD 2008, 'Branching of fungal hyphae: regulation, mechanisms and comparison with other branching systems', *Mycologia*, vol. 100, no. 6, pp. 823-32.
10. Hawksworth, DL 2001, 'The magnitude of fungal diversity: the 1.5 million species estimate revisited*', *Mycological Research*, vol. 105, no. 12, pp. 1422-32.
11. He, J, Cheng, CM, Su, DG & Zhong, MF 2014, 'Study on the Mechanical Properties of the Latex-Mycelium Composite', paper presented to Applied Mechanics and Materials.
12. Holt, G, Mcintyre, G, Flagg, D, Bayer, E, Wanjura, J & Pelletier, M 2012, 'Fungal mycelium and cotton plant materials in the manufacture of biodegradable molded packaging material: Evaluation study of select blends of cotton byproducts', *Journal of Biobased Materials and Bioenergy*, vol. 6, no. 4, pp. 431-9.
13. Howard, RJ & Valent, B 1996, 'Breaking and entering: host penetration by the fungal rice blast pathogen Magnaporthe grisea', *Annual Reviews in Microbiology*, vol. 50, no. 1, pp. 491-512.
14. Jiang, L, Walczyk, D & McIntyre, G 2014, 'A New Process for Manufacturing Biocomposite Laminate and Sandwich Parts using Mycelium as a Binder', paper presented to ASC 2014, Charlotte, NC, August 10-15.
15. Jiang, L, Walczyk, D, McIntyre, G & Bucinell, R 2016a, 'A new approach to manufacturing biocomposite sandwich structures: mycelium-based cores', paper presented to ASME 2016 11th International Manufacturing Science and Engineering Conference.
16. Jiang, L, Walczyk, D, McIntyre, G & Chan, WK 2016b, 'Cost Modeling and Optimization of a Manufacturing System for Mycelium-based Biocomposite Parts', *Journal of Manufacturing Systems*, vol. 41, pp. 8-20.
17. Jones, M, Huynh, T, Dekiwadia, C, Daver, F & John, S 2017, 'Mycelium Composites: A Review of Engineering Characteristics and Growth Kinetics', *Journal of Bionanoscience*, vol. 11, no. 4, pp. 241-57.
18. Kavanagh, K 2005, *Fungi: Biology and Applications*, Wiley.
19. Kotov, V & Reshetnikov, SV 1990, 'A stochastic model for early mycelial growth', *Mycological Research*, vol. 94, no. 5, pp. 577-86.
20. López Nava, JA, Méndez González, J, Ruelas Chacón, X & Nájera Luna, JA 2016, 'Assessment of Edible Fungi and Films Bio-Based Material Simulating Expanded Polystyrene', *Materials and Manufacturing Processes*, vol. 31, no. 8, pp. 1085-90.
21. Mendgen, K, Hahn, M & Deising, H 1996, 'Morphogenesis and mechanisms of penetration by plant pathogenic fungi', *Annual review of phytopathology*, vol. 34, no. 1, pp. 367-86.
22. Pelletier, MG, Holt, GA, Wanjura, JD, Bayer, E & McIntyre, G 2013, 'An evaluation study of mycelium based acoustic absorbers grown on agricultural by-product substrates', *Industrial Crops and Products*, vol. 51, pp. 480-5.

23. Prosser, J 1995, 'Kinetics of filamentous growth and branching', in *The growing fungus*, Springer, pp. 301-18.
24. Prosser, JI 1993, 'Growth kinetics of mycelial colonies and aggregates of ascomycetes', *Mycological Research*, vol. 97, no. 5, pp. 513-28.
25. Robinson, P & Park, D 1966, 'Volatile inhibitors of spore germination produced by fungi', *Transactions of the British Mycological Society*, vol. 49, no. 4, pp. 639-49.
26. Sexton, AC & Howlett, BJ 2006, 'Parallels in fungal pathogenesis on plant and animal hosts', *Eukaryotic Cell*, vol. 5, no. 12, pp. 1941-9.
27. Stalpers, JA, Cock, AWAMd, Robert, V, Stegehuis, G, International Mycological, A, Koninklijke Nederlandse Akademie van, W & Centraalbureau voor, S 2004, 'MycoBank : fungal databases, nomenclature and species banks'.
28. Stamets, P 2005, *Mycelium Running: How Mushrooms Can Help Save the World*, Ten Speed Press.
29. Travaglini, S, Noble, J, Ross, PG & Dharan, CKH 2013, 'Mycology Matrix Composites', paper presented to ASC - 28th Technical Conference, 9-11 September.
30. Webster, J & Weber, R 2007, *Introduction to Fungi*, Cambridge University Press.
31. Wu, L, Sun, Q, Sugawara, H, Yang, S, Zhou, Y, McCluskey, K, Vasilenko, A, Suzuki, K-I, Ohkuma, M & Lee, Y 2013, 'Global catalogue of microorganisms (gcm): a comprehensive database and information retrieval, analysis, and visualization system for microbial resources', *BMC genomics*, vol. 14, no. 1, p. 933.

Publication D

Addresses Research Question 2:

Which abundant Australian agricultural by-product (organic substrate filler phase) yields the highest mycelial (fibrous matrix phase) growth (associated with shortest manufacturing time and maximum interfacial bonding)?

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Agricultural by-product suitability for the production of chitinous composites and nanofibers utilising *Trametes versicolor* and *Polyporus brumalis* mycelial growth

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Fungal chitin

ABSTRACT

Agricultural by-products can be upcycled into environmentally-sustainable, inexpensive chitinous materials and nanofibers derived from fungal mycelium for composites, cosmetics, pharmaceuticals and water treatment applications. This study determined the suitability of common agricultural by-products as medium for fungal growth. Growth was measured by quantifying ergosterol, a unique fungal product, in solid and liquid media. The results reveal that fungi grew less on rice hull, sugarcane bagasse and wheat straw agricultural by-products than on commercial wheat grains. However, the liquid agricultural by-product blackstrap molasses facilitated very high biomass production, outperforming the commonly used laboratory nutrient malt extract. Hyphal fusion, sheet formation and hyphal diameter metrics of fungi growing on each substrate were evaluated by SEM to assess suitability for chitin nanofiber production. Utilising these materials offers a cheap, renewable, easily-isolated, and abundant alternative to problematic crustacean chitin that when implemented on a large scale could rapidly upcycle low-value agricultural by-products into high-value chitinous materials.

1. Introduction

Chitinous micro-fibers (hyphae), known collectively as mycelium, form the vegetative growth of filamentous fungi, which digest and bond organic matter to produce economical and environmentally sustainable materials for packaging and construction applications [1–6]. Mycelium acts as a continuous fibrous phase called the matrix [7,8] that interfaces with a dispersed phase of partially digested organic matter that increase material volume (filler) [7]. Using this natural biological growth as a low energy manufacturing process enables the production of environmentally friendly alternatives to synthetic planar materials (e.g. polymer films and sheets) [9] and larger low-density objects (e.g. synthetic foams and plastics) [1,2,5,6,8,10].

Agricultural by-products derived from cotton, flax, hemp, rice, sorghum and wheat are often only used as fillers in composites [1,2,6,8,11], with only a few exceptions [8–10]. Growing fungi on such digestible materials can enhance the structural properties of such fillers, thus resulting in mycelial composites. The economical nature of these materials makes mycelium composites cost-competitive with polymer materials [6,9,10] and reduces issues associated with competition for land use with food production [12,13].

However, agricultural by-products typically lack optimal fungal nutrients, such as easily utilisable simple sugars, such as fructose, glucose, sucrose, and instead contain more complex carbon sources, such as cellulose and lignin [14]. Less easily utilised substrates reduce fungal growth and interfacial bonding between hyphae and organic matter [10,11]. This adversely affects the mechanical properties of the resulting mycelial composites [10], decreases growth rates and results in an undesirably protracted manufacturing period. Determining the biocompatibility of substrates for fungal growth is vital for the production of mycelium composites and mycelium-derived chitins - but has not been previously assessed.

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The biocompatibility of agricultural by-products for fungal growth is also relevant to the production of fungal-derived chitin. Waste from the marine food industry, such as crustacean shells (shrimp, crab and krill), is currently the major source of industrial chitin [15–18]. However, crustacean-derived chitin is limited in supply by seasonal and regional variation and requires environmentally unfriendly aggressive acid and alkaline treatment for purification and demineralisation to remove calcium carbonate, proteins, lipids and pigments. It also contains the allergenic protein 'tropomyosin' [17,19]. Fungi offer a renewable, easily isolated and abundant alternative to crustacean chitin that can be rapidly produced on a large scale utilising heterotrophic growth on inexpensive agricultural by-products [16,17,19–21]. Fungal chitin also does not require demineralisation during extraction, however, the rigid chitin structure is associated with more pliable branched β-glucan, yielding a native nanocomposite architecture that can provide both strong and tough fiber networks when extracted [17,22]. The proportions of chitin and glucan can also be controlled by regulating nutritional and environmental parameters of fungal growth [22] and so fiber network properties can be optimised to suit specific applications. Rapid, large-scale production of mycelium-derived chitin from agricultural by-products is possible utilising heterotrophic growth by fungi for applications ranging from chitin nanofiber production and composites to cosmetics, pharmaceuticals and water treatment [19,23–26].

This study aimed to determine the biocompatibility of common (Australian) agricultural by-products, such as wheat straw, sugarcane bagasse, rice hulls and blackstrap molasses, to produce mycelium composites and mycelium-derived chitin; evaluate how the by-products compare with traditionally used nutrient-rich substrates, such as wheat grain and malt extract; and establish a methodology for assessing substrate suitability. This methodology can be used in future studies to select optimal substrates to ensure maximum fungal growth, bonding and growth rate. It also aimed to evaluate hyphal fusion, sheet formation and hyphal diameter metrics of the fungi growing on each substrate to assess their suitability for chitin nanofiber production where fiber geometry and sheet size, dictating the necessity and degree of nanofibrillation, are of interest.

2. Materials and methods

2.1. Materials

Assessed agricultural by-products, including wheat straw (E&A Salce, Melbourne, Australia), rice hulls (CopRice, Leeton, Australia), sugarcane bagasse and blackstrap molasses (MSF Sugar, Gordonvale, Australia), were kindly provided by local suppliers. Highly nutritious solid agricultural products, such as wheat grains (E&A Salce, Melbourne, Australia) and malt extract (Morgan's Brewing Co. Yatala, Australia), were purchased to serve as reference standards. *Polyporus brumalis* was obtained from the RMIT University fungal culture collection (Bundoora, Australia). The culture was stored under oil on a nutrient agar slope, which was subcultured onto fresh sterile malt extract agar (Neogen, Michigan) plates and incubated inverted at 25 °C in darkness for 7 d. *Trametes versicolor* was purchased from New Generation Mushroom Supplies (Melbourne, Australia). The sample was supplied as mycelium on wheat grain sealed in a plastic bag with a filter patch. This isolate was subcultured onto malt extract agar plates and incubated as above. *Agaricus bisporus* (white button) mushrooms were purchased from a local convenience store (origin: B. Fungi Kft, Ocsa, Hungary). High-performance liquid chromatography (HPLC) grade chemicals (≥ 99.0%) $CaCl_2$, ethylenediaminetetraacetic acid, ferric-sodium salt (FeNa-EDTA), KH_2PO_4, KOH, $MgSO_4{\cdot}7H_2O$, NaCl, NaOH, $(NH_4)_2HPO_4$, $ZnSO_4{\cdot}7H_2O$ and thiamine hydrochloride were purchased from Sigma-Aldrich. Ergosterol analytical standard (Sigma, ≥ 95.0%, 10 mg/mL in chloroform, molecular weight 396.65), dichloromethane (Sigma Chromasolv® Plus for HPLC, ≥ 99.9%), ethanol, methanol, isopropanol and heptane (all Sigma, ≥ 99.0%) were also purchased. Type 1 Milli-Q® ultrapure water was used for all experiments.

2.2. Fungal growth

Liquid and finely ground solid agricultural by-products were suspended (1 g/100 mL) in half-strength low nitrogen Modified Melin-Norkrans media without carbon (CNMMN) [27] in 250 mL glass jars and autoclaved at 121 °C for 20 min. CNMMN without carbon (glucose) was used to ensure that fungal mineral nutrition was not a limiting factor for growth and so that only fungal utilisation of the carbon source (agricultural by-product or reference nutrient standard) tested was limiting. Cultures were cut into inoculum disks (7 mm diameter) using the blunt base of a sterilised 1.0 mL pipette tip. A new pipette tip was used for each species. A single inoculum disk was suspended in each glass jar. Each experiment was conducted using four biological replicates in jars containing a single inoculum and incubated at 25 °C in the dark for 7 d on a Paton Scientific OP3422 orbital shaker at 100 orbits per minute (OPM). The contents of the glass jars were vacuum filtered using grade 1 chromatography paper and a filtration device (EMD Millipore XX104710) and then freeze dried in preparation for growth quantification. Freeze-dried samples were weighed using an analytical balance (OHAUS Explorer) and used in whole or in part as aliquots for analysis.

2.3. Quantification of fungal growth

Sterols were extracted using the methodology proposed by Mehra, Morrison, Coates and Lawrie [28]. Samples were kept in darkness where possible to prevent degradation of ergosterol, which is photolabile. Fungi and carbon source residue were extracted using 3 mL of 25% alcoholic potassium hydroxide (25 g KOH + 35 mL of sterile distilled water augmented to 100 mL with 100% ethanol). Samples were then vortexed vigorously for 1 min and incubated at 85 °C for 1 h before cooling to room temperature overnight. Sterile water (1 mL) and n-heptane (3 mL) were added and the samples vigorously vortexed again for 3 min. Suspensions were then allowed to partition overnight before the organic phase (n-heptane) was transferred to small glass vials and evaporated to dryness under nitrogen. The extracted sterols were finally redissolved in 1 mL of dichloromethane: isopropanol (100: 1 v/v) and filtered through 0.22 µm Teflon (PTFE) syringe filters (13 mm) (Labquip) into 2 mL amber screw-top glass vials for quantification using UHPLC.

Ergosterol was analysed through UHPLC (Agilent 1290 two dimensional liquid chromatography (2DLC) system) using a reverse-phase UPLC column (Waters Acquity C18, 1.7 µm particle size, 2.1 mm × 50 mm). Samples of 1.4 µL were injected via an auto-sampler into a methanol mobile phase with a flow rate of 0.65 mL/min. Ergosterol was identified by absorbance at 282 nm and a retention time of 1.2 min. Ergosterol standards were run before each batch of samples and a calibration curve produced. A 500 mg/L stock solution of the ergosterol standard was prepared in dichloromethane:isopropanol (100:1 v/v) and diluted as necessary. Three replicate 1.4 µL injections of five standards (0–20 mg/L) were used for each run to determine the linear relationship between peak area (y) and ergosterol concentration (x). Samples from both fungal species grown on each by-product, in addition to the nutrient standards and the negative control treatment were diluted when necessary to match the calibrated standard range and injected. Ergosterol concentrations were assessed by integrating the peak area for the standards and samples. The ergosterol contents of the negative control treatments, were subtracted from all sample results prior to analysis. Growth on malt extract was analysed by both dry weight biomass (d.wt) assessed using an analytical balance (OHAUS Explorer) and ergosterol content quantified using UHPLC to obtain a calibration of ergosterol:dry weight biomass of fungi [29].

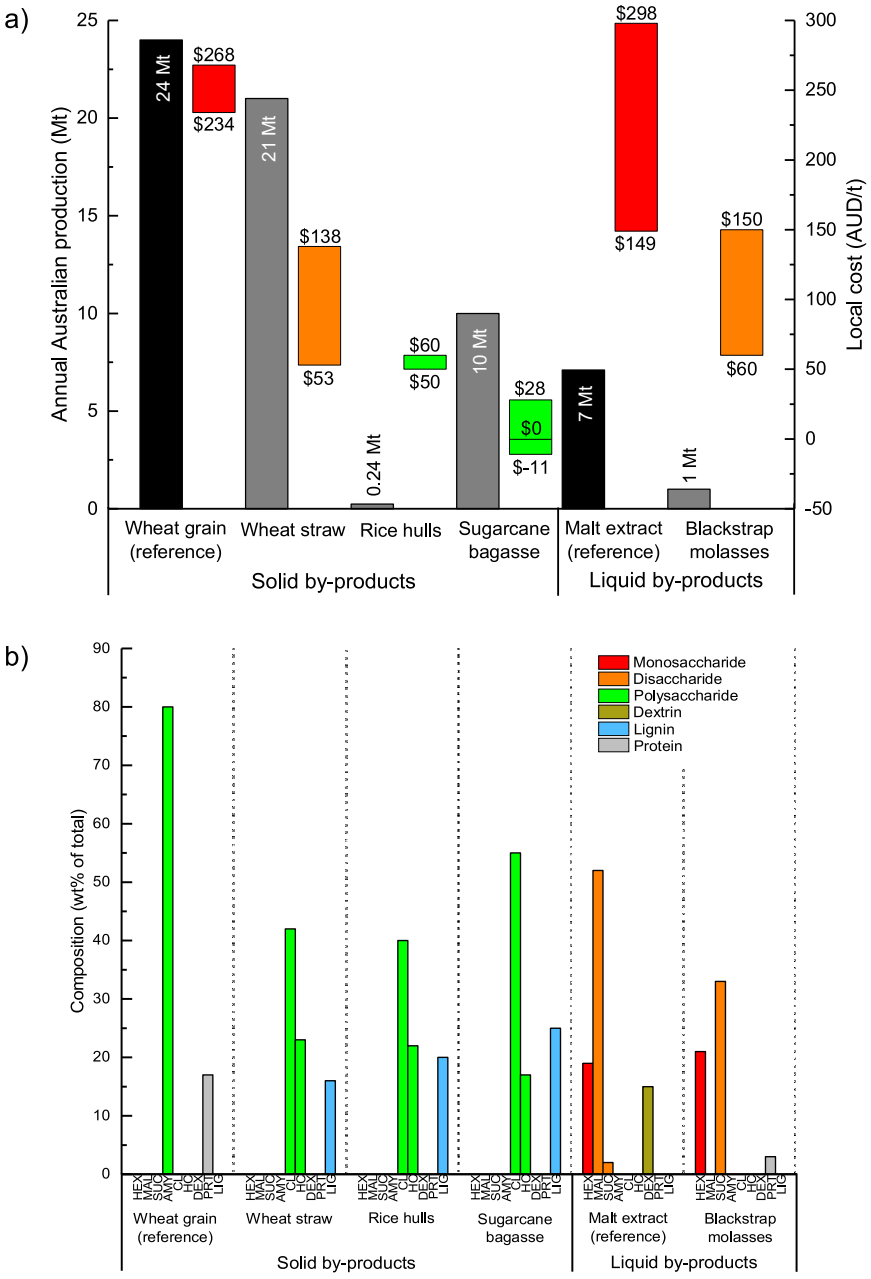

Fig. 1. a) Annual Australian production (Mt) and local cost (AUD/t) of solid and liquid agricultural by-products. Colouring: nutrient standard for reference (black), agricultural by-product (grey), $0-50/t (green), $50-150/t (orange), > $150/t (red). *Annual Australian malt extract production calculated based on annual production of malting barley using a conversion factor of 1.267 [47]. Compiled from literature [32,47–54]. Commercial values are the most recent available literature values and were adjusted for inflation to the 2018 AUD value. b) Principal organic composition of solid and liquid agricultural by-products. Colouring: monosaccharide (red), disaccharide (orange), polysaccharide (green), dextrin (brown), lignin (blue) and protein (grey). Abbreviations: HEX (hexoses: glucose and fructose), MAL (maltose), SUC (sucrose), AMY (amylum/starch), CL (cellulose), HC (hemicellulose), DEX (dextrin), PRT (protein) and LIG (lignin). Compiled from literature [14,33,55–57] (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

2.4. Assessment of hyphal morphology

Hyphal fusion (anastomosis) was examined using an environmental scanning electron microscope (FEI Quanta 200) under high vacuum. Samples were sourced from the freeze-dried material prior to quantification of ergosterol, sputter-coated with gold and subsequently imaged using an accelerating voltage of 30 kV.

Hyphal network metrics including minimum, maximum and average hyphal diameter were investigated using SEM. Micrographs were taken at four separate sites for each sample. Hyphal diameter was measured by superimposing a 10 × 10 grid of squares over each micrograph and adding an XY point on either side of each hypha for triplicate hyphae in each square using the Fiji distribution of ImageJ software (version 1.51a). This was conducted until 200 hyphal diameters had been recorded for each micrograph across four replicate micrographs (800 hyphal diameters in total for each sample).

2.5. Analysis of hyphal extension

The hyphal extension of each species was assessed for liquid by-products to supplement growth quantification via ergosterol. Blackstrap molasses and malt extract were combined with agar and half-strength CNMMN (1 g/100 mL) and autoclaved at 121 °C for 20 min. Molten agar was poured aseptically into 90 mm Petri dishes and allowed to solidify. Cultures were cut as before. A single inoculum disk was placed on the edge of each Petri dish in contact with the dish wall. Each experiment was conducted with four replicate dishes containing a single inoculum. Plates were sealed individually with Parafilm™ in groups in zip-lock bags and incubated at 25 °C in darkness.

Radial growth (mm) was measured daily from the centre of the inoculum disk to the tip of the of the longest hypha at the same time each day for 9 d. This growth period was selected based on preliminary trials that showed the fastest species-substrate combination filling a 90 mm Petri dish in this time.

2.6. Analysis of chitin-glucan polymer yield

T. versicolor mycelial biomass and *A. bisporus* mushrooms were washed with water and vacuum filtered (VWR 125 mm qualitative filter paper 413, particle retention 5–13 μm) to remove any excess moisture. Total wet biomass quantity was assessed using a laboratory balance. Biomass aliquots were also assessed using a Sartorius Cubis® micro balance pre- and post-drying at 105 °C to determine biomass dry mass/water content, which was used in conjunction with the total wet biomass quantity to evaluate the total dry biomass.

Mild extraction was then used to isolate chitin-glucan structural polymers. Biomasses were initially washed thrice with water and submerged for 5 min to remove any remaining contaminant residues. The masses were then blended for 5 min in 500 mL of water and the resulting suspension heated to 85 °C for 30 min. The suspension was then cooled to 25 °C and centrifuged at 9000 rpm for 15 min at 18 °C. The resultant residue was resuspended in a 1 M NaOH solution for 3 h at 65 °C. The suspension was again cooled and then neutralised (pH 7) by repeated centrifugation and redispersion of the residue in water.

Total polymer extract quantity and dry mass/water content were assessed as before, and these values compared with the values previously determined for mycelial and mushroom biomass. This allowed evaluation of the percentage conversion from biomass to polymer extract (conversion yield) for each species based on total dry biomass and total dry polymer extract.

2.7. Data processing and statistical analysis

Ergosterol concentration, hyphal diameter and end-point radial growth were statistically analysed using Microsoft Excel and plotted using OriginLab Pro 2017 (b9.4.2.380). Data was checked for normality using the Kolmogorov-Smirnov test on Minitab (version 18.1). For normally distributed data, ANOVA (Analysis of Variance) was performed. Class categories for normal data were generated based on Tukey's family error rate. Data not normally distributed was log-transformed to normality and analysed by ANOVA. For non-parametric data, the Kruskal-Wallis test was conducted. Class categories for non-parametric data were generated based on k-means clustering. Differences were considered significant at $p \leq 0.05$.

3. Results and discussion

3.1. Agricultural by-products

Agricultural by-products were selected based on their local abundance, cost (in Australia) **(**Fig. 1**)** and application-specific merit (e.g. silica content to enhance thermal stability). Solid by-product biocompatibility was assessed for use as main filler in as-grown mycelium composites. Liquid by-product growth yield was assessed for production of mycelial biomass to facilitate chitin nanofiber production.

Such agricultural by-products include wheat straw and sugarcane bagasse, which are both abundant and economical solid filler materials **(**Fig. 1a**)** that comprise predominantly cellulose, hemicellulose and lignin **(**Fig. 1b**)**. Sugarcane bagasse is the cheapest material overall, varying seasonally in value between $-11/t (disposal cost, AUD) and $28/t **(**Fig. 1a**)**. Although rice hulls are not as abundant as the other solid agricultural by-products in Australia, they are still economical **(**Fig. 1a**)** and can be utilised as filler in mycelium composites to improve thermal stability [4,30] since significant quantities of lignin (25–30 wt.%) and silica (15–20 wt.%) are present in addition to cellulose and hemicellulose **(**Fig. 1**b)** [31]. Rice hulls are widely available in Asia at lower cost (< $20/t) [32].

Liquid products include blackstrap molasses ($60-150/t), which is a viscous liquid by-product of sugar production comprising predominantly hexose, sucrose and proteins **(**Fig. 1b**)**. It could provide an economic alternative to malt extract ($149-298/t) **(**Fig. 1a**)** for growing fungal biomass to produce chitin nanofibers.

Highly nutritious solid (wheat grains) and liquid (malt extract) agricultural products were used as high-nutrient reference standards and accordingly had the highest costs ($149 to $298/t) **(**Fig. 1a**)**. Wheat grains are a complete source of nutrition, comprising primarily starch (~80%), proteins (~10%) and cell wall polysaccharides in addition to lipids, terpenoids, phenolics, minerals, vitamins and essential amino acids [33] **(**Fig. 1b**)**. Malt extract is also an excellent substrate, comprising about 90–92% carbohydrates including hexoses (glucose, fructose), disaccharides (maltose, sucrose) and dextrins. Nitrogenous substances also present in malt extract include proteins, peptides, amino acid, purines, pyrimidines and vitamins [34] **(**Fig. 1b**)**.

3.2. Ergosterol-biomass correlation

The common white-rot fungi *P. brumalis* and *T. versicolor* were selected for assessment based on 1) their availability, 2) growth rate [35] since growth time must be minimised, and 3) activity of their phenol-oxidizing enzymes, e.g. laccases, peroxidases, tyrosinases [36], due to the significant quantities of lignin in most agricultural by-products [14].

Ergosterol was quantified to assess fungal growth. Ergosterol is an essential sterol unique to fungi and some microscopic algae and protozoa [37]. Following extraction it is identified by its single UV absorbance peak at 282 nm and quantified using ultra-performance liquid chromatography [28]. Other sterols exhibit little or no absorbance at this wavelength [38]. Ergosterol correlates with mycelial dry weight better than alternatives, such as chitin [38,39]. Ergosterol quantification is also faster than chitin assays [38,40].

Calibration curves of the peak area against the concentration of the ergosterol standards at 282 nm provided good linear responses with correlation coefficients (R^2) > 0.96. A positive linear relationship was established between ergosterol and dry weight (biomass) for fungal growth on malt extract (detailed in the Supplementary material), characterised by the following equation:

$$\text{Ergosterol } (C_{28}H_{44}O) \text{ concentration (ppm)} = 19.75 \bullet \text{dwt (mg)} - 99.69$$

$$(R^2 = 0.80)\ (\text{ANOVA}, F = 7.63, p < 0.001)$$

This demonstrated that fungal biomass can be accurately assessed via its correlation with ergosterol in cases where the substrate and fungal growth cannot be separated and weighed individually, such as growth on solid agricultural by-products. The relationship parameters recorded were similar to those of other white-rot basidiomycetes [41] but different to those of slower-growing mycorrhizal fungi with smaller equation coefficients [28]. The parameters vary among types of fungi in general but are constant for each fungus when grown on a variety of substrates [29,41].

3.3. Solid by-products for use as filler in mycelium composites

The ergosterol content of the fungal growth on highly nutritious wheat grains was 6–12 times greater than that of the agricultural by-products, suggesting better fungal growth **(**Fig. 2**)**. The ergosterol content for each fungus did not differ among solid by-products or between fungi grown on the same substrates. Both fungi yielded 4–7 times more growth on the solid by-products than on the negative control (CNMMN).

Hyphal diameter was significantly (20–40%) greater on wheat grains than on all solid by-products **(**Fig. 2**)**. Hyphal diameter for both fungi was greater on wheat straw than on sugarcane bagasse, with diameter on rice hulls being intermediate.

The similar ergosterol contents of colonised wheat straw, rice hulls and sugarcane bagasse were supported by the similarity in their hyphal density **(**Fig. 2**)**. On wheat grains, hyphal fusion (anastomosis) resulted in pseudo-laminar sheets having an average width from 17 μm (*T.*

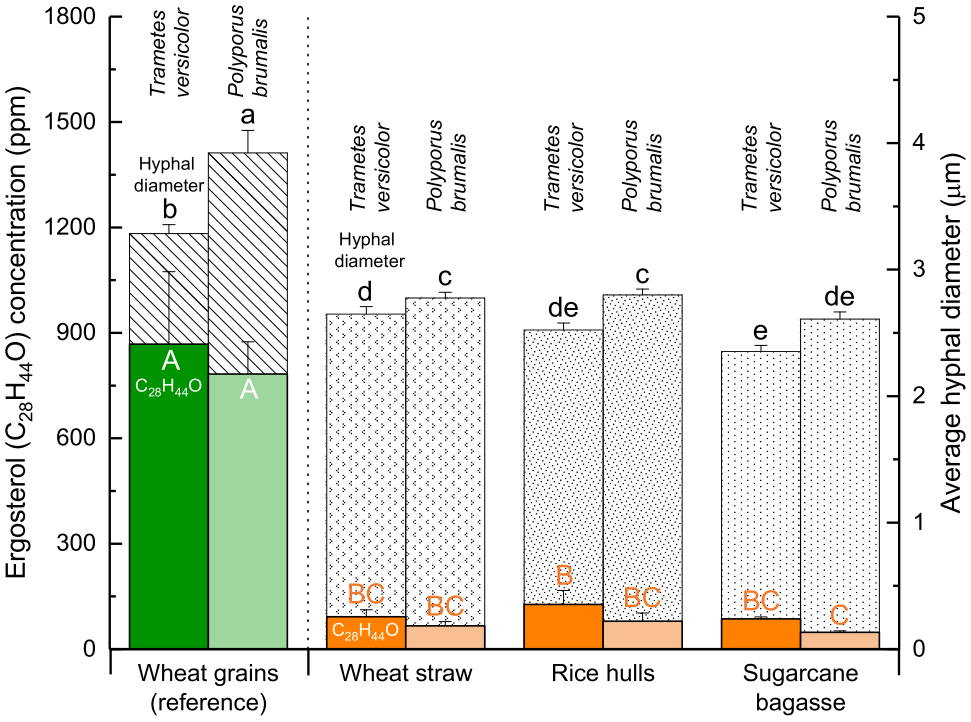

Fig. 2. Ergosterol concentration (coloured, ppm) and average hyphal diameter (black-white shaded, µm) for *T. versicolor* and *P. brumalis* grown on wheat grain reference medium (green), wheat straw, rice hulls and sugarcane bagasse solid agricultural by-products (orange). Error bars indicate standard error within sets of four replicates. Non-normal data was log transformed prior to class categorisation. Class categories were letters of comparison based on Tukey's family error rate at $p \leq 0.05$ for sample-specific ANOVA (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

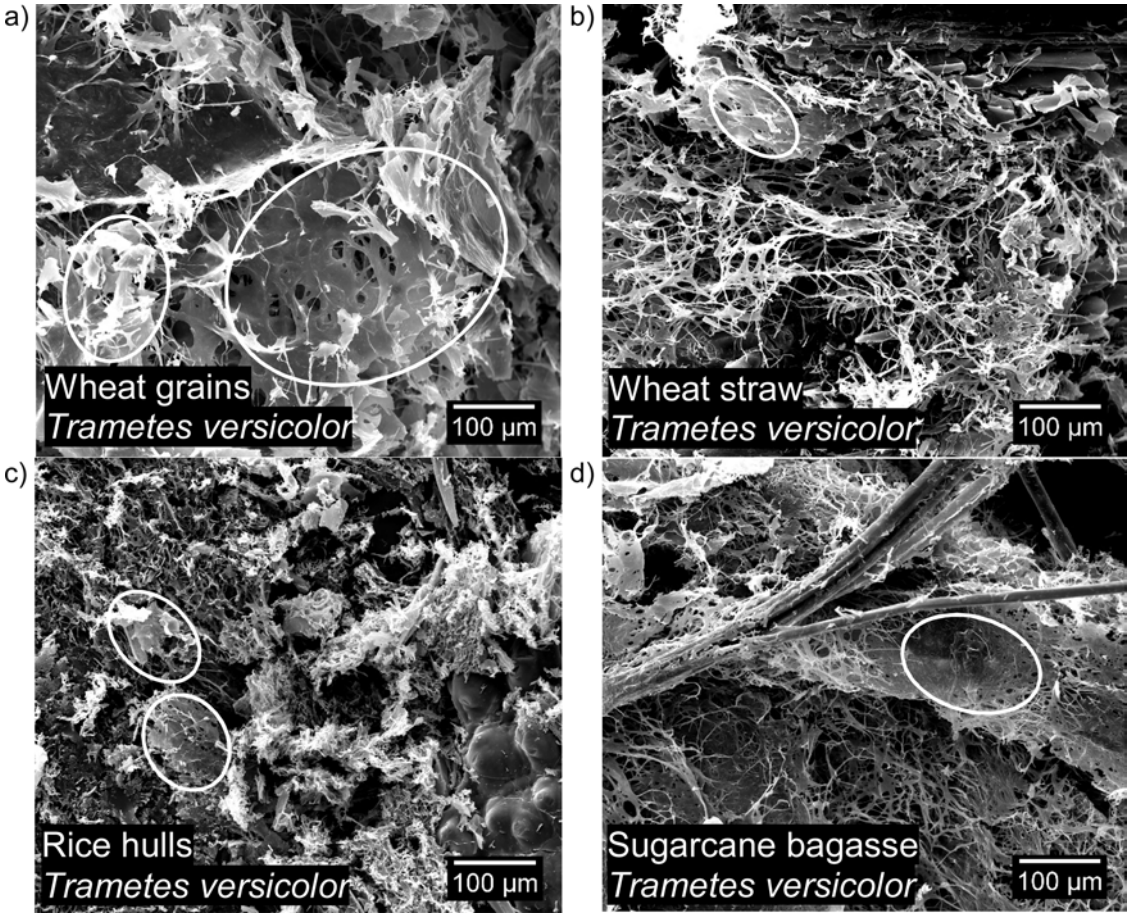

Fig. 3. SE micrographs of freeze-dried biomass prior to ergosterol quantification (UHPLC) for *T. versicolor* growing on wheat grains (reference) compared to wheat straw, rice hulls and sugarcane bagasse agricultural by-products. *P. brumalis* had a similar morphology for the same by-products. SEM are shown in the Supplementary material. Hyphal fusion and pseudo-laminar sheet formation is prominent for fungi grown on wheat grains and is highlighted using white ovals.

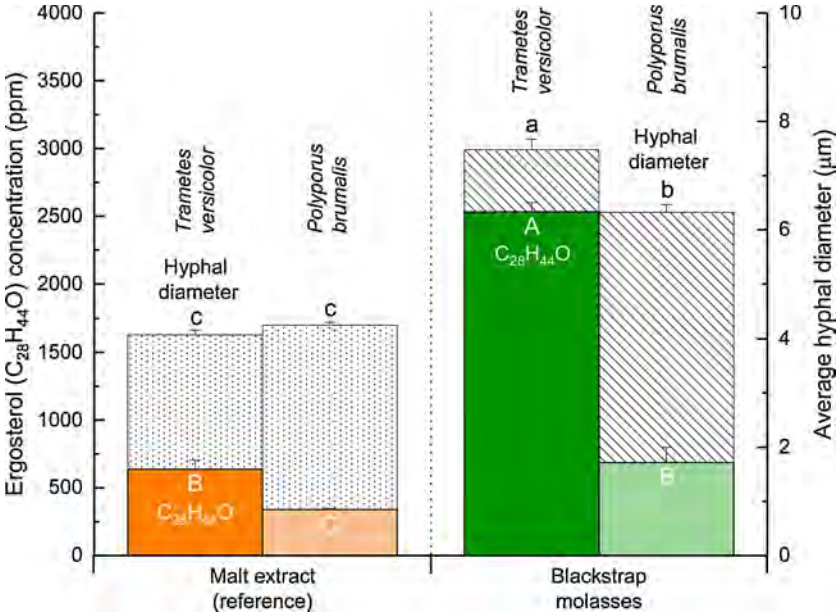

Fig. 4. Ergosterol concentration (coloured, ppm) and average hyphal diameter (black-white shaded, μm) for *T. versicolor* and *P. brumalis* growing on malt extract reference medium (orange) and blackstrap molasses liquid by-product (green). Error bars indicate standard error within sets of four replicates. Non-normal data was log transformed prior to class categorisation. Class categories were letters of comparison based on Tukey's family error rate at $p \leq 0.05$ for sample-specific ANOVA (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

versicolor) to 53 μm (*P. brumalis*) **(**Fig. 3**)**. This is common when grown in highly nutritious substrates due to the correlation between increased hyphal fusion and mature colony growth [42] and did not occur on the less nutritious solid by-products, such as wheat straw, rice hulls and sugarcane bagasse. These solid agricultural by-products alone therefore lacked the nutrients and hyphal growth required to fully bond the fibrous fillers into mycelium composites. They would have to be supplemented with a more nutrient-rich substrate, such as wheat grains prior to use [4].

3.4. Assessment of liquid by-products for production of chitin nanofibers

Liquid substrates resulted in up to 25 times greater ergosterol production than the reference solid (wheat grain) and liquid (malt extract) products (Fig. 2, Fig. 4). Both fungi also produced significantly more ergosterol on blackstrap molasses than on malt extract, the reference standard **(**Fig. 4**)**. Ergosterol production by *T. versicolor* was almost four times greater on blackstrap molasses than on malt extract, whereas that of *P. brumalis* only doubled.

The differences in ergosterol content of the fungi grown on different liquid substrates were also shown by differences in their rates of hyphal extension. *T. versicolor* had a greater hyphal extension rate on blackstrap molasses than on malt extract, whereas there was no significant difference between substrates for *P. brumalis* **(**Fig. 5**)**.

The hyphal diameter of both fungi on blackstrap molasses was 1.5–1.8 times that on malt extract **(**Fig. 4**)**. On malt extract, hyphal diameter was equal for both fungi but was 1.2 times greater for *T. versicolor* than for *P. brumalis* on blackstrap molasses.

Of more importance for use in fabrication, hyphal fusion and pseudo-laminar sheet formation varied with substrate, which was particularly apparent in *T. versicolor* hyphal networks on blackstrap molasses, which yielded sheets of hyphae up to 50 μm wide **(**Fig. 6b**)**. This hyphal fusion was probably a main factor for the much larger ergosterol content of *T. versicolor* on blackstrap molasses than on malt extract. *T. versicolor* has a trimitic (three hyphal types) generative-binding-skeletal hyphal system that contains highly branched binding hyphae leading to sheet formation (Fig. 6a, b).

For *P. brumalis,* hyphal diameter doubled on blackstrap molasses relative to malt extract **(**Fig. 6b**)** and occasionally fused into sections with widths up to 12 μm (Fig. 6c, d). The presence of straighter, less branched skeletal hyphae in the dimitic (two hyphal types) generative-skeletal hyphal system of *P. brumalis* enables directed growth of highly

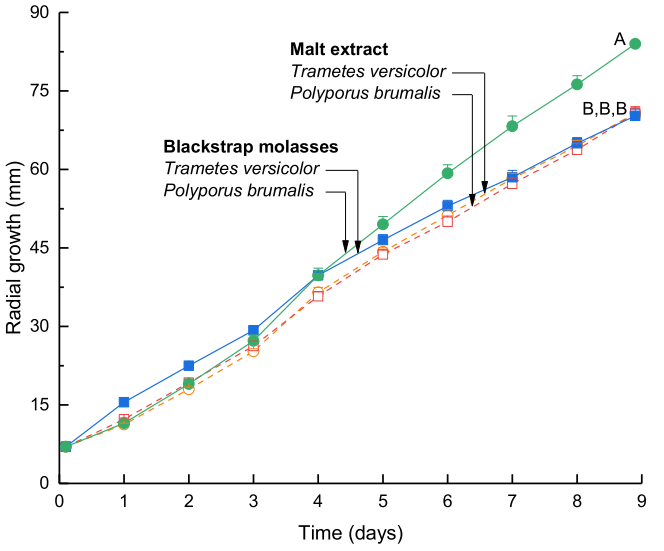

Fig. 5. Hyphal extension measured as radial growth (mm + SE) for a period of 9 d for *T. versicolor* and *P. brumalis* grown on malt extract reference medium and blackstrap molasses liquid by-product. Error bars indicate standard error within sets of four replicates. Class categories were letters of comparison based on Tukey's family error rate at $p \leq 0.05$ for sample-specific ANOVA.

orientated anisotropic (macro)fibers containing chitin-glucan nanofibers. This aids fabrication of aligned natural fiber reinforced composites where fiber geometry (e.g. aspect ratio L/D) and orientation are especially important [22,43].

Larger sheets provide significantly more biomass, which can be reduced to smaller fiber through mechanical fibrillation processes, which use shearing and impact forces to break weakly bonded nanofibers in the chitin fiber bundle for instance by high-pressure homogenisation, wet shear grinding, water jet atomisation, micro fluidisation and high-speed blending [22]. Alternatively, ultrasonication, which induces localised high-pressure regions through high frequency oscillation to loosen fibers through cavitation [44] or chemical methods (e.g. TEMPO-mediated oxidation) [45] can also be used to reduce fiber size. Smaller fibers provide a greater surface area, which increases hydrogen bonding and produces entanglements, resulting in stronger materials [22].

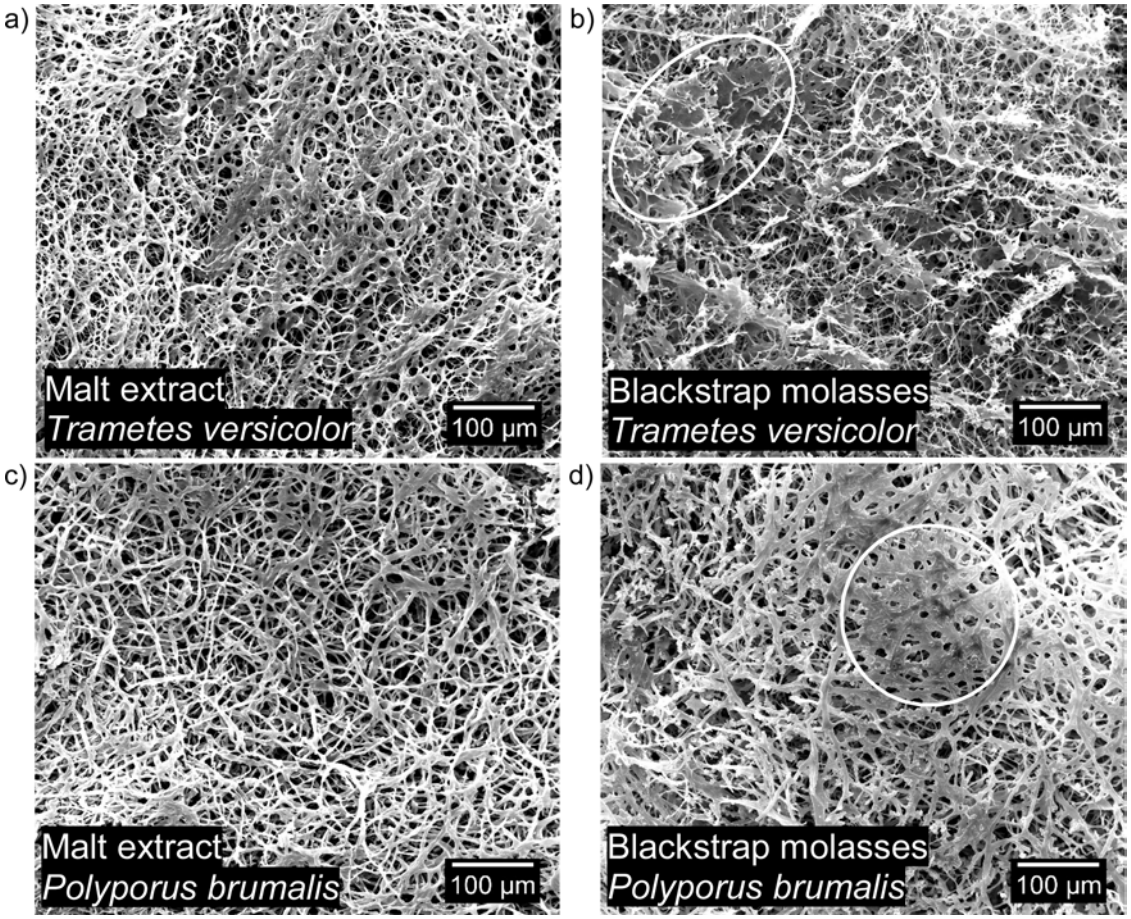

Fig. 6. SE micrographs of freeze-dried biomass prior to ergosterol quantification (UHPLC) for *T. versicolor* and *P. brumalis* grown on malt extract and blackstrap molasses. Hyphal fusion and pseudo-laminar sheet formation were prominent in blackstrap molasses samples and are highlighted using white ovals.

Table 1
Biomass and polymer extract dry mass yields (and associated water content) and overall dry polymer conversion yield (biomass to chitin-glucan) for *A. bisporus* mushrooms and *T. versicolor* mycelium.

Species	Dry mass / water content		Conversion yield (wt%)
	d.wt biomass (wt%)	d.wt extract (wt%)	
A. bisporus	5.1% (94.9% H_2O)	3.4% (96.6% H_2O)	19.3% (of mushroom)
T. versicolor	25.7% (74.3% H_2O)	9.5% (90.5% H_2O)	12.0% (of mycelium)

The large growth yield of *T. versicolor* on blackstrap molasses illustrates the viability of mycelial biomass as a source of chitin nanofibers. Common mushroom (*A. bisporus*) constituents (whole fruiting body, stipe, pileus) have been utilised as a source of fungal chitin for nanofiber production [22]. However, mushrooms take 14–21 d to grow and their use in materials science directly competes with food supply. *T. versicolor* mycelial biomass production on blackstrap molasses in this study is much more rapid (7 d) than mushrooms, also has a significant conversion yield (biomass to chitin-glucan) (Table 1) and is industrially scalable using bioreactors and continuous culture techniques [46] making it a viable source of chitin nanofibers for materials science applications.

4. Conclusion

Solid agricultural by-products such as rice hulls, sugarcane bagasse and wheat straw resulted in insufficient growth to be considered as sole substrate constituents for mycelium composites. They should instead be partly supplemented with more nutrient-rich substrates. However, the liquid agricultural by-product blackstrap molasses resulted in very high biomass, outperforming even the commonly used laboratory nutrient malt extract. Such large biomass yields for fungi grown on inexpensive agricultural by-products offer a cheap, renewable, easily isolated and abundant alternative to crustacean chitin. Rapid, large-scale production is possible utilising fungal growth for applications from chitin nanofiber production to cosmetics, pharmaceuticals and water treatment.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.procbio.2019.01.018.

References

[1] G. Holt, G. Mcintyre, D. Flagg, E. Bayer, J. Wanjura, M. Pelletier, Fungal mycelium and cotton plant materials in the manufacture of biodegradable molded packaging material: evaluation study of select blends of cotton byproducts, J. Biobased Mater. Bioenergy 6 (4) (2012) 431–439.

[2] J.A. López Nava, J. Méndez González, X. Ruelas Chacón, J.A. Nájera Luna, Assessment of edible Fungi and films bio-based material simulating expanded polystyrene, Mater. Manuf. Process. 31 (8) (2016) 1085–1090.

[3] M. Jones, T. Huynh, C. Dekiwadia, F. Daver, S. John, Mycelium composites: a review of engineering characteristics and growth kinetics, J. Bionanosci. 11 (4) (2017) 241–257.

[4] M. Jones, T. Bhat, T. Huynh, E. Kandare, R. Yuen, C.H. Wang, S. John, Waste-derived low-cost mycelium composite construction materials with improved fire safety, Fire Mater. 42 (2018) 816–825.

[5] E. Karana, D. Blauwhoff, E. Hultink, S. Camere, When the material grows: a case study on designing (with) mycelium-based materials, Int. J. Des. 12 (2) (2018) 119–136.

[6] S. Camere, E. Karana, Fabricating materials from living organisms: an emerging design practice, J. Clean. Prod. 186 (2018) 570–584.

[7] V.K. Thakur, A.S. Siṅgha, Biomass-based Biocomposites, Smithers Rapra Technology Ltd., Shropshire, U.K, 2013.

[8] M.G. Pelletier, G.A. Holt, J.D. Wanjura, E. Bayer, G. McIntyre, An evaluation study of mycelium based acoustic absorbers grown on agricultural by-product substrates, Ind. Crops Prod. 51 (1) (2013) 480–485.

[9] M. Haneef, L. Ceseracciu, C. Canale, I.S. Bayer, J.A. Heredia-Guerrero, A. Athanassiou, Advanced materials from fungal mycelium: fabrication and tuning of physical properties, Sci. Rep. 7 (2017) 41292.

[10] S. Travaglini, J. Noble, P.G. Ross, C.K.H. Dharan, State College, PAMycology Matrix Composites, 28th ASC Technical Conference2013, Mycology Matrix Composites, 28th ASC Technical Conference (2013).

[11] J. He, C.M. Cheng, D.G. Su, M.F. Zhong, Study on the mechanical properties of the latex-mycelium composite, Appl. Mech. Mater. 507 (1) (2014) 415–420.

[12] M. Harvey, S. Pilgrim, The new competition for land: food, energy, and climate change, Food Policy 36 (2011) S40–S51.

[13] R. Rathmann, A. Szklo, R. Schaeffer, Land use competition for production of food and liquid biofuels: an analysis of the arguments in the current debate, Renew. Energy 35 (1) (2010) 14–22.

[14] O. Faruk, A.K. Bledzki, H.-P. Fink, M. Sain, Biocomposites reinforced with natural fibers: 2000–2010, Prog. Polym. Sci. 37 (11) (2012) 1552–1596.

[15] W. Arbia, L. Arbia, L. Adour, A. Amrane, Chitin extraction from crustacean shells using biological methods–a review, Food Technol, Biotechnol. 51 (1) (2013) 12–25.

[16] M.A. Razak, A.B. Pinjari, P.S. Begum, B. Viswanath, Biotechnological production of fungal biopolymers chitin and chitosan: their potential biomedical and industrial applications, Curr. Biotechnol. 7 (3) (2018) 214–230.

[17] A. Hassainia, H. Satha, S. Boufi, Chitin from Agaricus bisporus: Extraction and characterization, Int. J. Biol. Macromol. 117 (2018) 1334–1342.

[18] S. Ifuku, Chitin and chitosan nanofibers: preparation and chemical modifications, Molecules 19 (11) (2014) 18367–18380.

[19] F. Di Mario, P. Rapana, U. Tomati, E. Galli, Chitin and chitosan from Basidiomycetes, Int. J. Biol. Macromol. 43 (1) (2008) 8–12.

[20] W. Liao, Y. Liu, C. Frear, S. Chen, Co-production of fumaric acid and chitin from a nitrogen-rich lignocellulosic material–dairy manure–using a pelletized filamentous fungus Rhizopus oryzae ATCC 20344, Bioresour. Technol. 99 (13) (2008) 5859–5866.

[21] C.R. Koza, G.A. Norton, J.H. van Leeuwen, Dewatering investigations on fungal biomass grown in thin stillage from a dry-mill corn ethanol plant, Biomass Bioener. 97 (2017) 65–69.

[22] W. Nawawi, Renewable Chitin Based Nanomaterials From Fungi, doctor of Philosophy, Imperial College London, London, U.K, 2016.

[23] X. Liang, X. Fan, R. Li, S. Li, S. Shen, D. Hu, Efficient removal of Cr (VI) from water by quaternized chitin/branched polyethylenimine biosorbent with hierarchical pore structure, Bioresour. Technol. 250 (2018) 178–184.

[24] M. Szczęsna-Antczak, K. Struszczyk-Świta, M. Rzyska, J. Szeląg, Ł. Stańczyk, T. Antczak, Oil accumulation and in situ trans/esterification by lipolytic fungal biomass, Bioresour. Technol. (2018).

[25] Y.A. Skorik, A.V. Pestov, Y.G. Yatluk, Evaluation of various chitin-glucan derivatives from Aspergillus niger as transition metal adsorbents, Bioresour. Technol. 101 (6) (2010) 1769–1775.

[26] C.G. Geromel-Costa, J.J. Corbi, G.R. Gorni, V. Colombo, R.C. Correa, A. Fiamingo, S.P. Campana-Filho, Adsorption of metals by crosslinked chitosan beads in sugarcane contaminated streams, Biomass Bioenery 119 (2018) 128–134.

[27] D.H. Marx, W.C. Bryan, Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont Pisolithus tinctorius, For. Sci. 21 (3) (1975) 245–254.

[28] S. Mehra, P. Morrison, F. Coates, A. Lawrie, Differences in carbon source utilisation by orchid mycorrhizal fungi from common and endangered species of Caladenia (Orchidaceae), Mycorrhiza 27 (2) (2017) 95–108.

[29] J.-E. Nylund, H. Wallander, Ergosterol Analysis As a Means of Quantifying Mycorrhizal Biomass, Academic Press Ltd., London, U.K, 1992.

[30] M. Jones, T. Bhat, C. Wang, K. Moinuddin, S. John, Xi'an, ChinaThermal Degradation and Fire Reaction Properties of Mycelium Composites, 21st International Conference on Composite Materials2017, Thermal Degradation and Fire Reaction Properties of Mycelium Composites, 21st International Conference on Composite Materials (2017).

[31] M.S. Ismail, A. Waliuddin, Effect of rice husk ash on high strength concrete, Constr. Build. Mater. 10 (7) (1996) 521–526.

[32] DP CleanTech, Understanding Rice Husk As a Biomass Fuel, viewed 17 May 2018 (2013) http://www.dpcleantech.com/files/Understanding-rice-husk-as-a-biomass-fuel-EN-V1-2013.9.4.pdf.

[33] P.R. Shewry, M.J. Hawkesford, V. Piironen, A.-M. Lampi, K. Gebruers, D. Boros, A.A.M. Andersson, P. Åman, M. Rakszegi, Z. Bedo, J.L. Ward, Natural variation in grain composition of wheat and related cereals, J. Agr. Food Chem. 61 (35) (2013) 8295–8303.

[34] S. Soni, Microbes: a Source of Energy for 21st Century, New India Publishing, Delhi, India, 2007.

[35] M. Jones, T. Huynh, S. John, Inherent species characteristic influence and growth performance assessment for mycelium composite applications, Adv. Mater. Lett. 9 (1) (2018) 71–80.

[36] K. Nakade, Y. Nakagawa, A. Yano, T. Sato, Y. Sakamoto, Characterization of an extracellular laccase, PbLac1, purified from Polyporus brumalis, Fungal Biol. 114 (8) (2010) 609–618.

[37] C. Mille-Lindblom, E. von Wachenfeldt, L.J. Tranvik, Ergosterol as a measure of living fungal biomass: persistence in environmental samples after fungal death, J. Microbiol. Meth. 59 (2) (2004) 253–262.

[38] J.I. Pitt, A.D. Hocking, Fungi and Food Spoilage, Springer, Berlin, Germany, 2009.

[39] S. Matcham, B. Jordan, D. Wood, Estimation of fungal biomass in a solid substrate by three independent methods, App. Microbiol. Biotechnol. 21 (1) (1985) 108–112.

[40] L. Seitz, D. Sauer, R. Burroughs, H. Mohr, J. Hubbard, Ergosterol as a measure of fungal growth, Phytopathology 69 (11) (1979) 1202–1203.

[41] O. Niemenmaa, S. Galkin, A. Hatakka, Ergosterol contents of some wood-rotting basidiomycete fungi grown in liquid and solid culture conditions, Int. Biodeter. Biodegr. 62 (2) (2008) 125–134.

[42] N.D. Read, A. Fleißner, M.G. Roca, N.L. Glass, Hyphal Fusion, Cellular and Molecular Biology of Filamentous Fungi, ASM Press, Sterling, VA, 2010, pp. 260–273.

[43] W. Steinmann, A.-K. Saelhoff, Essential Properties of Fibres for Composite Applications, Fibrous and Textile Materials for Composite Applications, Springer, Berlin, Germany, 2016, pp. 39–73.

[44] Y. Lu, Q. Sun, X. She, Y. Xia, Y. Liu, J. Li, D. Yang, Fabrication and characterisation of α-chitin nanofibers and highly transparent chitin films by pulsed ultrasonication, Carbohyd. Polym. 98 (2) (2013) 1497–1504.

[45] Y. Fan, T. Saito, A. Isogai, Chitin nanocrystals prepared by TEMPO-Mediated oxidation of α-Chitin, Biomacromolecules 9 (1) (2008) 192–198.

[46] K. Kavanagh, Fungi: Biology and Applications, Wiley, Hoboken, NJ, 2005.

[47] Food and Agriculture Organization of the United Nations, Agribusiness Handbook - Barley Malt Beer, (2009) viewed 20 May 2018 http://www.fao.org/fileadmin/user_upload/tci/docs/AH3_BarleyMaltBeer.pdf.

[48] Australian Bureau of Infrastructure Transport and Regional Economics, Freightline 3 - Australian Sugar Freight and Transport, Canberra, (2015) viewed 18 May 2018 https://bitre.gov.au/publications/2015/files/Freightline_03.pdf.

[49] Australian Bureau of Agricultural and Resource Economics and Sciences, Agricultural Commodities - March 2017, (2017) viewed 19 May 2018 http://www.agriculture.gov.au/abares/Documents/agricultural-commodities-report-march-2017.pdf.

[50] Australian Rural Industries Research and Development Corporation, Furfural Chemicals and Biofuels From Agriculture, (2006) viewed 18 May 2018 https://rirdc.infoservices.com.au/downloads/06-127.pdf.

[51] Bioenergy Australia, Bioenergy in Australia - Status and Opportunities, (2012) viewed 19th May 2018 http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.694.4104&rep=rep1&type=pdf.

[52] G. Kent, Cairns, AustraliaThe Value of Bagasse to an Australian Raw Sugar Factory, 29th Annual Conference of the Australian Society of Sugar Cane Technologists2007, The Value of Bagasse to an Australian Raw Sugar Factory, 29th Annual Conference of the Australian Society of Sugar Cane Technologists (2007) 453–459.

[53] Meat and Livestock Australia, Expanded Use of Molasses for Intensive Beef Cattle Feeding, (2008) viewed 22 May 2018 https://futurebeef.com.au/wp-content/uploads/Expanded-use-of-molasses-for-intensive-beef-cattle-feeding.pdf.

[54] RaboResearch Food and Agribusiness, Agribusiness Monthly - May 2017, (2017) viewed 22 May 2018 https://services.rabobank.com/publicationservice/download/publication/token/e0tdkv0ByCDgS7MVlRWl.

[55] E. Bridson, A. Brecker, Design and Formulation of Microbial Culture Media, Methods in Microbiology, Elsevier, New York City, NY, 1970, pp. 229–295.

[56] F. Imrie, Fermentation media: sugar and molasses, Process Biochem. 34 (1) (1969) 35.

[57] A. Rhodes, D.L. Fletcher, Principles of Industrial Microbiology, Pergamon Press, Oxford, U.K, 1966.

Electronic supplementary material for:

Agricultural By-product Suitability for the Production of Chitinous Composites and Nanofibers

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List of Figures

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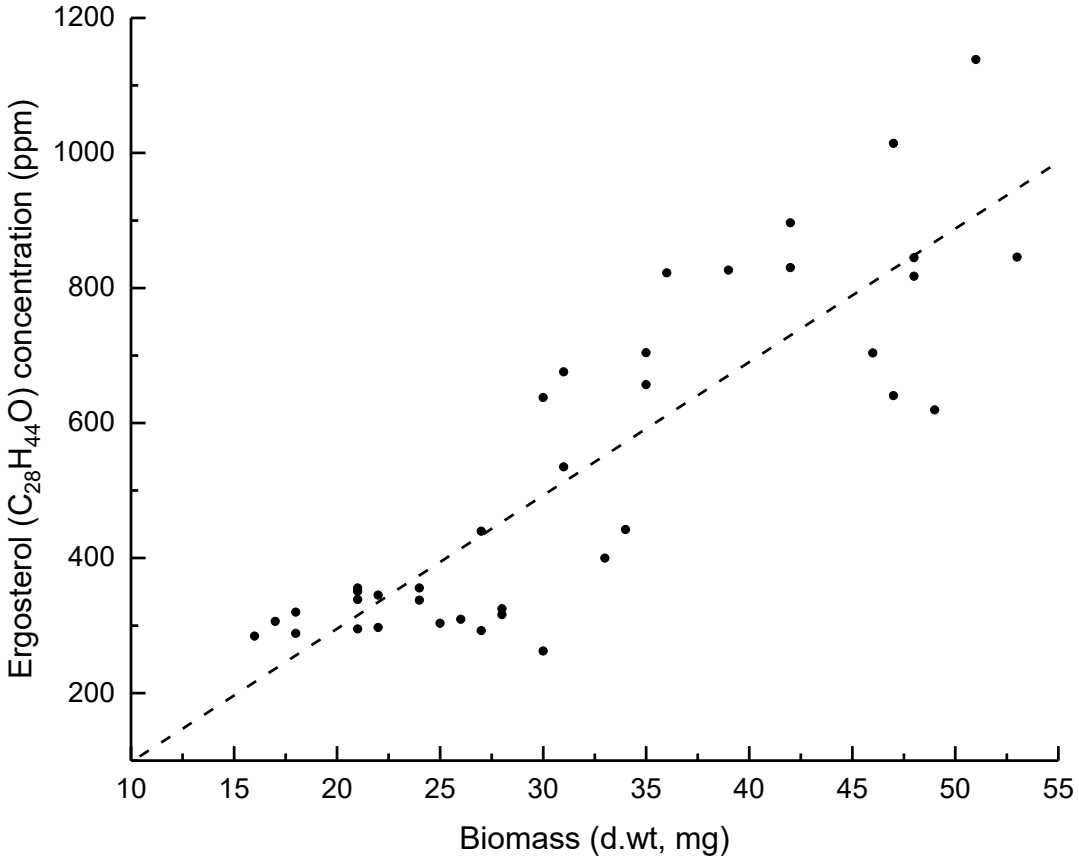


Figure S1. Positive linear correlation between ergosterol concentration (ppm) and biomass (d.wt, mg) for samples, varying in biomass, grown in malt extract in 100 mL CNMMN with concentrations of 0.2, 0.4, 0.6, 0.8 and 1 g/L.

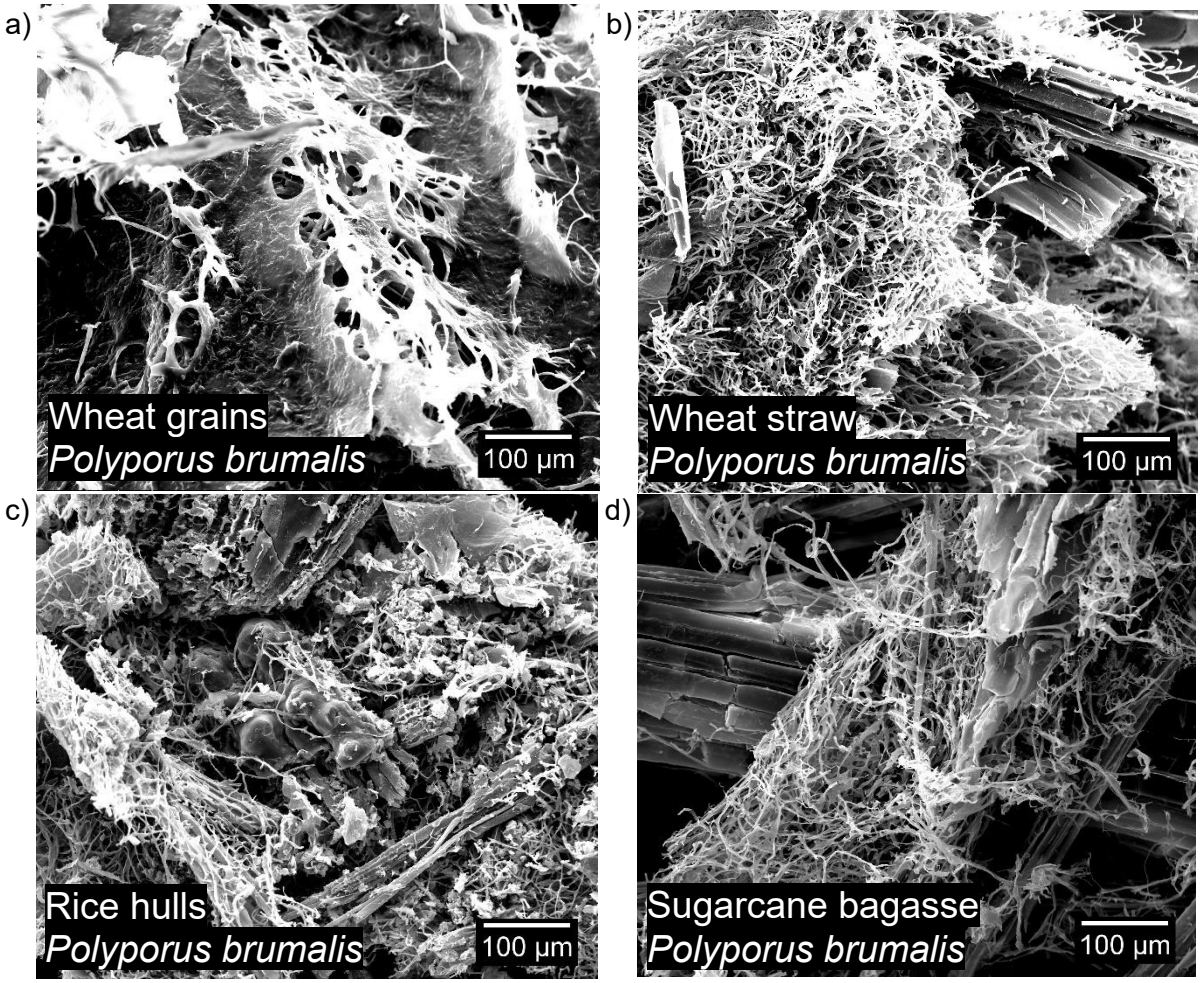


Figure S2. Micrographs of freeze-dried biomass prior to ergosterol quantification (HPLC) for *P. brumalis* growing on wheat grains (reference) compared to wheat straw, rice hulls and sugarcane bagasse agricultural by-products.

Reviewer comments:

Reviewer #1 (Remarks to the Author):
This study which aims at using agricultural waste biomass for the production high value chitin is very interesting. Although chitin production and its processing are well-known subjects even coming from the food industry as waste, fungal chitin is in demand. Such an approach of production chitin is appreciated. The work has been logically and carefully written, all the performed experiments fit into the scheme. The provided illustration are very clear.

I therefore recommend the publishing of this article.

Authors' response:
The authors thank Reviewer #1 for their very positive feedback on this article. The reviewer's appreciation for the novelty and necessity of this work in addition to their praise for the experimental and written elements of this work are graciously received and appreciated. No changes are noted by this reviewer and as such no changes have been implemented in the manuscript.

Reviewer #2 (Remarks to the Author):
The document focuses on the mycelium growth by the use common Australian of agricultural by-products to produce chitinous materials. The manuscript is well written and pretty clear. The topic is interesting related to the search of different applications for the agricultural residues and new carbon sources to the production of mycelial growth.

Authors must include in the title the names of the microorganisms.

Figure 1 is well described and presents important information. Nevertheless, why did the authors use literature information for carbon source characterization instead of performed at the lab?

Figure 5 did not present the error bars in all the treatments

The information of table 1 must be determined by the authors to corroborate the chitin content of the different treatments

Reviewer query 1: Authors must include in the title the names of the microorganisms.

Authors' response:
The authors thank the reviewer for this comment. The title has been updated to include the names of the microorganisms utilised. The article is now entitled:

Agricultural By-product Suitability for the Production of Chitinous Composites and Nanofibers Utilising *Trametes versicolor* and *Polyporus brumalis* Mycelial Growth

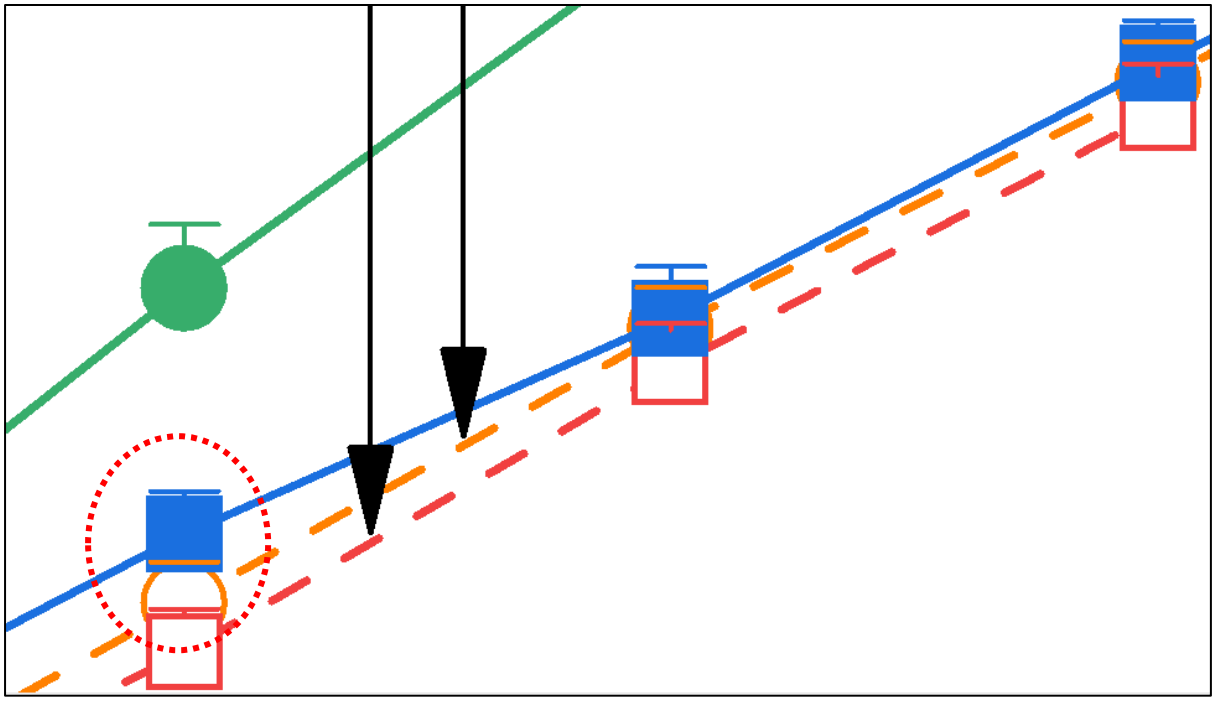
Reviewer query 2: Figure 1 is well described and presents important information. Nevertheless, why did the authors use literature information for carbon source characterization instead of performed at the lab?

Authors' response:
The authors thank the reviewer for this comment. The authors used carbon source composition sourced from literature since the by-products in question have already been comprehensively characterised, both commercially and academically, and are subject to only limited biological variation. The authors also primarily refer to this information qualitatively, comparing compositions in terms of components and not quantities, to support discussion of the nutritional merits of each by-product. Fungal growth utilises groups of nutrients, with all

nutrients essential and no nutrient consequently of sole importance. As such composition of by-products is important in a qualitative sense with highly accurate composition values for individual compounds unnecessary. Fungal growth itself is the important parameter and was assessed using very high accuracy HPLC quantification of ergosterol.

Reviewer query 3: Figure 5 did not present the error bars in all the treatments

Authors' response:
The authors thank the reviewer for this comment. Figure 5 does include error bars for all data series, however the error is so low for some of the series that it is necessary to zoom in to see the error bars, as illustrated below:



Reviewer query 4: The information of table 1 must be determined by the authors to corroborate the chitin content of the different treatments

Authors' response:
The authors addressed this query by assessing chitin-glucan structural polymer yield from *Agaricus bisporus* mushrooms and *Trametes versicolor* mycelial biomass.

The *Materials and methods* section was updated to include the following:

"*T. versicolor* mycelial biomass and *A. bisporus* mushrooms were washed with water and vacuum filtered (VWR 125 mm qualitative filter paper 413, particle retention 5-13 µm) to remove any excess moisture. Total wet biomass quantity was assessed using a laboratory balance. Biomass aliquots were also assessed using a Sartorius Cubis® micro balance pre- and post-drying at 105°C to determine biomass dry mass/water content, which was used in conjunction with the total wet biomass quantity to evaluate the total dry biomass.

Mild extraction was then used to isolate chitin-glucan structural polymers. Biomasses were initially washed thrice with water and submerged for 5 min to remove any remaining contaminant residues. The masses were then blended for 5 min in 500 mL of water and the resulting suspension heated to 85°C for 30 min. The suspension was then cooled to 25°C and

centrifuged at 9000 rpm for 15 min at 18°C. The resultant residue was resuspended in a 1 M NaOH solution for 3 h at 65°C. The suspension was again cooled and then neutralised (pH 7) by repeated centrifugation and redispersion of the residue in water.

Total polymer extract quantity and dry mass/water content were assessed as before, and these values compared with the values previously determined for mycelial and mushroom biomass. This allowed evaluation of the percentage conversion from biomass to polymer extract (conversion yield) for each species based on total dry biomass and total dry polymer extract."

The *Results and discussion* section has been amended and now reads:

"The large growth yield of *T. versicolor* on blackstrap molasses illustrates the viability of mycelial biomass as a source of chitin nanofibers. Common mushroom (*A. bisporus*) constituents (whole fruiting body, stipe, pileus) have been utilised as a source of fungal chitin for nanofiber production [22]. However, mushrooms take 14-21 d to grow and their use in materials science directly competes with food supply. *T. versicolor* mycelial biomass production on blackstrap molasses in this study is much more rapid (7 d) than mushrooms, also has a significant conversion yield (biomass to chitin-glucan) (Table 1) and is industrially scalable using bioreactors and continuous culture techniques [46] making it a viable source of chitin nanofibers for materials science applications."

Table 1 was replaced.

Reviewer #3 (Remarks to the Author):
This manuscript is well organized and written along with the evaluation of hyphal fusion, sheet formation and hyphal diameter metrics of the fungi growing on each substrate to assess their suitability for chitin nanofiber production. Overall it is quite interesting paper. Then, this manuscript will be acceptable for PRBI Journal only if the following issues are corrected and improved by the authors.

Authors' response:
The authors thank Reviewer #3 for their positive feedback on this article. The reviewer's interest in this article is appreciated. No changes are noted by this reviewer and as such no changes have been implemented in the manuscript.

Reviewer #4 (Remarks to the Author):
This manuscript is well written, and presentation is good, but authors used very simple microbiological methods to culture fungi using different substrates as source of nutrition

Authors' response:
The authors thank Reviewer #4 for their positive feedback on this article. The authors contend that the most appropriate microbiological methods for the work completed were used. Reliable and very commonly practised inoculation protocols were followed in order to minimise the risk of contamination in this study. Basic microbiological methods were only a tool to generate samples which could then be tested using more advanced scientific protocols. No changes are noted by this reviewer and as such no changes have been implemented in the manuscript

Reviewer comments:

Reviewer #2 (Remarks to the Author):
Accept

Authors' response:
The authors thank Reviewer #2 for their recommendation to accept the manuscript. The authors are glad to have been able to address the reviewer's feedback to their satisfaction.

Reviewer #5 (Remarks to the Author):
The manuscript focuses on the fungal growth on some agricultural by-products. It is clear and well written but, the comparation of the results to those obtained by other authors using other organic wastes or lignino-cellulosic materials will provide higher value to the study.

Authors' response:
The authors thank Reviewer #5 for their positive feedback on the manuscript. The reviewer's appreciation for the clarity and value of the manuscript is very gratefully received and acknowledged. The author's also wish to thank the reviewer for their suggestion pertaining to comparison of the results of the study with other literature concerning organic wastes and lignocellulosic materials. Unfortunately, a fair comparison of the results obtained in our study with other literature is not possible for the following reasons:

Biomass cannot be measured directly unless the growth medium is a liquid phase which can be readily separated from the biomass. Subsequently ergosterol concentration is a common method for quantifying fungal biomass growing in solid substrates. Ergosterol concentration is directly correlated with fungal biomass as outlined by Matcham, Jordan and Wood (1985) and Pitt and Hocking (2009), amongst others. This correlation is also characterised in our manuscript for the species in question. The relationship between fungal biomass and ergosterol is detailed in *Figure S1* of the supplementary material.

Ergosterol quantity per unit biomass, in addition to biomass quantity itself, is highly dependent on a large number of factors detailed in Webster and Weber (2007), Kavanagh (2005), Jones et al. (2017) and others. These factors include:

- Fungal species
- Growth duration
- Inoculum density
- Growth conditions (temperature, relative humidity, pH and others)
- Macronutrient concentration of substrate
- Micronutrient concentration of substrate

These parameters must remain constant for a fair comparison of fungal biomass growing on various lignocellulosic substrates when quantified using ergosterol concentration. Any change in these factors will result in altered ergosterol concentrations compromising their comparative reference value. The noted parameters were subsequently stringently controlled in our study, allowing comparison of the materials within our manuscript. Although the literature documents fungal growth on other lignocellulosic materials, no studies allowing a fair comparison were able to be identified by the authors. Examples are illustrated below:

Mehra et al. (2017) utilise slow growing mycorrhizal fungi on various complex insoluble and simple soluble carbon sources produced during litter degradation. Their study yielded low ergosterol concentrations, primarily attributable to the slow growing nature of the fungi in question. Their study therefore contributes little value as a comparative reference. Different species, inoculum density and growth duration were used which affected the ergosterol

concentrations measured. This compromises the potential for fair comparison of the lignocellulosic substrates they tested with those in our manuscript. A fair comparison of species performance also cannot be completed due to the differences in substrate type and the other previously noted parameters.

Niemenmaa, Galkin and Hatakka (2008) tested white and brown rot fungi, which were similar to the species used in our study, on various woods. They achieved similar ergosterol concentrations to those attained in our study. However, none of the species used in their study are the same as those of our study. They also utilise a different inoculum density, growth duration and growth conditions and report ergosterol concentrations in biomass specific units, while neglecting to characterise the relationship between biomass and ergosterol. A fair comparison of lignocellulosic substrates is subsequently not possible. A fair comparison of species performance is also not possible due to the differences in substrate type and the other previously noted parameters.

Eikenes et al. (2005) used one of the two species utilised in our study (*Trametes versicolor*), growing on birch wood. Their study's primary focus was substrate mass loss since their research interest was long-term wood decay resulting from fungal invasion. They utilise extended growth durations (4, 8, 12, 16, 20 weeks) in addition to different inoculation densities and growth conditions. The variation in these parameters affects the ergosterol concentrations reported in their study and makes a fair comparison of their lignocellulosic substrates with our manuscript impossible. A fair comparison of species performance is also not possible due to the differences in substrate type and the other previously listed parameters.

Since literature concerning ergosterol concentration cannot be compared without consistency in all growth parameters the authors regret that the recommended revision is not possible. Subsequently, no changes have been made to the manuscript.

References:

Eikenes, M, Hietala, AM, Alfredsen, G, Fossdal, CG & Solheim, H 2005, 'Comparison of quantitative real-time PCR, chitin and ergosterol assays for monitoring colonization of Trametes versicolor in birch wood', *Holzforschung*, vol. 59, no. 5, pp. 568-73.

Jones, M, Huynh, T, Dekiwadia, C, Daver, F & John, S 2017, 'Mycelium Composites: A Review of Engineering Characteristics and Growth Kinetics', *Journal of Bionanoscience*, vol. 11, no. 4, pp. 241-57.

Kavanagh, K 2005, *Fungi: Biology and Applications*, Wiley, Hoboken, NJ.

Matcham, S, Jordan, B & Wood, D 1985, 'Estimation of fungal biomass in a solid substrate by three independent methods', *Applied Microbiology and Biotechnology*, vol. 21, no. 1, pp. 108-12.

Mehra, S, Morrison, P, Coates, F & Lawrie, A 2017, 'Differences in carbon source utilisation by orchid mycorrhizal fungi from common and endangered species of Caladenia (Orchidaceae)', *Mycorrhiza*, vol. 27, no. 2, pp. 95-108.

Niemenmaa, O, Galkin, S & Hatakka, A 2008, 'Ergosterol contents of some wood-rotting basidiomycete fungi grown in liquid and solid culture conditions', *International Biodeterioration & Biodegradation*, vol. 62, no. 2, pp. 125-34.

Pitt, JI & Hocking, AD 2009, *Fungi and food spoilage*, Springer, Berlin, Germany.

Webster, J & Weber, R 2007, *Introduction to Fungi*, Cambridge University Press, Cambridge, U.K.

Publication E

Addresses Research Question 3:

How does mycelium thermally decompose and what are its thermal degradation properties?

SCIENTIFIC REPORTS

OPEN

Thermal Degradation and Fire Properties of Fungal Mycelium and Mycelium - Biomass Composite Materials

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Mycelium and mycelium-biomass composites are emerging as new sustainable materials with useful flame-retardant potentials. Here we report a detailed characterisation of the thermal degradation and fire properties of fungal mycelium and mycelium-biomass composites. Measurements and analyses are carried out on key parameters such as decomposition temperatures, residual char, and gases evolved during pyrolysis. Pyrolysis flow combustion calorimetry (PCFC) evaluations reveal that the corresponding combustion propensity of mycelium is significantly lower compared to poly(methyl methacrylate) (PMMA) and polylactic acid (PLA), indicating that they are noticeably less prone to ignition and flaming combustion, and therefore safer to use. The hyphal diameters of mycelium decrease following pyrolysis. Cone calorimetry testing results show that the presence of mycelium has a positive influence on the fire reaction properties of wheat grains. This improvement is attributable to the relatively higher charring tendency of mycelium compared to wheat grain, which reduces the heat release rate (HRR) by acting as a thermal insulator and by limiting the supply of combustible gases to the flame front. The mycelium growth time has been found to yield no significant improvements in the fire properties of mycelium-wheat grain composites.

A new class of composite materials can be made by growing mycelium on various types of biomass, and the resultant composites have recently received significant attention due to their low density and biodegradable properties[1–7]. Mycelium is the vegetative growth of filamentous fungi, comprising of a network of micro-filaments with diameters ranging between 1 and 30 μm, depending on the type of species and growth environment[3]. Mycelium grows on organic substrates through apical tip expansion of hyphae from a spore or inoculum, under ambient conditions[8]. The hyphal colonies interact randomly through hyphal fusion (anastomosis) to form a fibre network structure[9], which acts as a natural self-assembling glue to bind the substrates and form a composite material. The key incentives for the use of mycelium composites are their low cost, low environmental impact and carbon footprint, low density, reduced energy consumption and most importantly, their biodegradability[1,2,10]. Mycelium composites are currently being used in non-structural applications (e.g. packaging and insulation)[1,11]. Although the mechanical properties of mycelium composites based on biomass are inferior to those of conventional engineered (glass or carbon fibre) composites, advanced processing techniques have enabled their use in semi-structural applications (e.g. furniture, decking, etc.)[3–5,12]. Furthermore, the wide variety of substrates on which mycelium grows combined with advanced processing techniques provides manufacturers with new opportunities to customize the material to meet specific requirements (e.g. impact resistance, thermal and acoustic insulation, etc.)[2,7,13].

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Figure 1. Representative mycelium (*T. versicolor*) – wheat grain composite grown for 18 d and dried at 50 °C for 48 h.

Much of the potential applications of mycelium-based composites are intended for high fire prone environments (e.g. packaging and building insulation). However, very little is known about the thermal degradation and fire properties of mycelium and its composites. To meet the stringent fire safety regulations, it is imperative to characterize the flame-retardant properties of mycelium and mycelium composites. The heat, smoke and gases released by a burning composite can also make fire-fighting extremely hazardous and increase the likelihood of serious injury and death[14]. Large quantities of organic matter present in mycelium composites can act as a fuel source and may escalate the fire risk by shortening the ignition time, increasing heat release and other fire risk factors such as flame spread and smoke toxicity, although these are yet to be quantified. These issues and the effect of incubation period (growth time), which controls the relative mycelial mass, on the fire properties of mycelium composites need to be thoroughly quantified to enable wide practical applications.

Here, we investigate the thermal degradation properties and subsequent changes in the morphological and chemical structure of mycelium. A single mycelium species (*Trametes versicolor*) was selected in this study based on its growth kinetics[15] and its availability. Parameters such as the onset of decomposition, residual char, evolved gases and heat release were measured. Changes to the physical structure, reduction in hyphal diameters and chemical composition following pyrolysis were investigated to gain an in-depth understanding of the thermal degradation and decomposition mechanisms. Also investigated is the effect of incubation period (growth time) for composites incorporating an organic substrate on their respective fire properties. The results from this first study provide new understanding and quantitative data on the fire safety of mycelium composites.

Materials and Experimental Methodology

Mycelium Composite Preparation. Fungal inoculum of the commonly used species (*T. versicolor*) was purchased from New Generation Mushroom Supplies Pty. Ltd. as mycelial mass growing on wheat grains. Wheat grains (supplied by E&A Salce Pty Ltd) were selected as a substrate material for their high nutritional value[16] and to match the inoculum composition. The substrate (wheat grains) was soaked in Type 1 Milli-Q® water for 48 h and sterilised (autoclaved at 121 °C and 103.4 kPa for 90 min) before use. A fixed amount (25 wt%) of fungal inoculum was then mixed with the substrate using a sterilised blender. Inoculum content lower than 25 wt% resulted in longer growth times and increased the risk of contamination by other competing microbial species. The blended mixture was then evenly distributed into sterile plastic moulds (100 × 100 mm) and incubated under standard atmospheric conditions for 6, 12 and 18 d. Following incubation, the specimens were dried at 50 °C for 48 h to dehydrate and denature the fungus. A representative mycelium-wheat grain composite is shown in Fig. 1.

Coupled Thermogravimetric Analysis and Fourier Transform Infrared Spectroscopy (TGA-FTIR and TGA-GCMS). To assess the mass loss and nature of the species evolved during thermal decomposition, measurements were conducted using a Pyris 1 TGA interfaced with a time-resolved FTIR (Perkin Elmer Frontier) and GCMS (Clarus SQ 8 S). A mycelial mass of approximately 10 mg was peeled from the composite, placed in an alumina crucible and heated from 25 °C to 600 °C at a heating rate of 30 °C/min under nitrogen atmosphere (50 ml/ min). All samples were inspected under an optical microscope prior to TGA measurements to ensure any bonded substrate (i.e. wheat grain particles) invisible to the naked eye were removed. The residue obtained following TGA was collected for further investigation into changes to the chemical composition.

The gases evolved during heating were piped (gas flow 50 mL/min) via a pressurized heated transfer line and analysed continuously by the FTIR equipped with a thermostated conventional gas cell. The infrared spectra were acquired in the 4000–600 cm^{-1} wavenumber range. At 300 °C, the evolved gas was automatically collected (*ca.* 80 µL) and injected into the gas chromatograph equipped with a standard non-polar fused silica capillary column which was then inter-phased to a mass spectrometer. Analyses of the average mass spectra calculated at the chromatographic peak middle height were carried out with NIST-MS Search Software.

Scanning Electron Microscopy and Energy Dispersive X-Ray Spectroscopy (SEM-EDS). SEM imaging and elemental analysis of mycelium before and after pyrolysis was performed using an FEI Quanta 200 Environmental Scanning Electron Microscope with an Oxford X-Max^N 20 Energy Dispersive X-ray Spectrometer attached. An accelerating voltage of 30 kV was used with a spot size of 5. The EDS spectra were analysed using the AZtecEnergy EDS software. An average spectrum was obtained based on individual spectra from 30 different sites.

Transmission Electron Microscopy (TEM). The *T. versicolor* hypha specimens were fixed in a 100 mM cacodylate buffer (pH 7.0) solution containing 2.5% glutaraldehyde, 2% paraformaldehyde followed by washing thrice with 100 mM cacodylate buffer. The specimens were post-fixed with 1% osmium tetroxide for 1.5 h followed by washing thrice with distilled water. The samples were gradually dehydrated with increasing gradients of 50% to 100% ethanol for 15–30 min each. Following dehydration, the samples were infiltrated twice with Spurr's resin before polymerisation. The samples were post-polymerised at 60 °C for 48 h. Ultra-thin sections (~90 nm) were cut using the Leica Ultracut Ultramicrotome on carbon-formvar copper grids. The samples were post-stained with heavy metals and imaged using a JEOL-1010 transmission electron microscope (TEM) at 80 kV with the Gatan Microscopy Suite software (v 2.3) (Gatan Inc., Pleasanton, USA).

X-ray Photon Spectroscopy (XPS). The surface chemistry of the mycelium was assessed using a K-alpha X-ray Photoelectron Spectrometer (XPS) instrument (Thermo Fisher Scientific, USA) with a monochromated Aluminium Kα X-ray source. X-ray spot size was 30–400 μm in 5 μm steps. Scans spanned from 1400 to 0 eV binding energy. Peak analysis was performed by means of peak decomposition to fit a Gaussian function using the Thermo Scientific^{TM} Avantage Software (v 5.9902, b 06552). XPS spectra detail environment sensitive surface chemistry only as opposed to EDS which is a bulk analysis tool. As such XPS is considered to be less accurate than EDS for the elemental analysis of mycelial biomass and is included in the supplementary material for reference purposes only.

Fire Reaction Testing. *Pyrolysis Combustion Flow Calorimetry (PCFC).* The PCFC measurements were performed on mycelium using a Fire Testing Technology Ltd. (Gosport, UK) micro-scale combustion calorimeter according to ASTM D7309[17]. For each run, an accurately weighed (*ca*. 20 mg) sample was heated to *ca*. 900 °C at a heating rate of 1 °C/s, in a stream of nitrogen (80 cm^3/min). The volatile thermal degradation products, thus obtained, were then mixed with a stream of pure oxygen (at a flow rate of 20 cm^3/min) prior to entering a combustion chamber maintained at 900 °C. Each sample was run in duplicate. A more detailed description of the method including operating parameters is described by Cogen *et al.*[18]. The following quantities were measured: peak heat release rate (PHRR); temperature at peak heat release rate (TPHRR); total heat release (THR); heat release capacity (HRC) and percentages of the char residues[19]. These fire reaction properties of mycelium were compared against those of commonly used synthetic thermoplastic polymers PMMA and PLA for bench-marking purposes.

Cone Calorimetry. The flammability characteristics of the as-received wheat grains and mycelium-wheat grain composites grown for 6, 12 and 18 d were assessed using a three-cell cone calorimeter (Fire Testing Technology, UK) operated in the horizontal testing mode. The samples (100 mm × 100 mm) were exposed to a constant incident thermal heat flux of 50 kW/m^2 in accordance with ISO 5660[20] with the ignition event initiated by the pilot spark ignitor. The heat exposed surface was positioned 25 mm from the cone heater. The fire reaction parameters measured were time to ignition (TTI), heat release rate (HRR), mass loss and smoke density.

Results and Discussion

Thermal Degradation and Evolved Gas Analysis. Results of the mass loss and volatiles released during thermal decomposition measured by simultaneous thermogravimetric analysis and Fourier-transform infrared spectroscopy (TGA-FTIR) are shown in Fig. 2a. The TGA mass loss - temperature profiles exhibited three distinct stages with no significant differences between the thermal degradation characteristics of mycelium grown for 6, 12 and 18 d. The representative FTIR profiles of the major volatiles evolved were determined by relating the IR absorbance peaks to specific wave numbers (namely 3566 cm^{-1} for H_2O, 3016 cm^{-1} for CH_4, 2185 cm^{-1} for CO and 2359 cm^{-1} for CO_2), and are shown in Fig. 2b. Monitoring the evolution of volatiles as a function of temperature, the FTIR analysis unveiled a complex degradation pattern characterized by the release of various flammable and non-flammable gaseous products.

The first degradation stage (25–200 °C) is attributed to the evaporation of free and chemically bonded water (H_2O) leading to a mass loss of 5 wt% between 100 and 200 °C. The FTIR spectra (Fig. 2b) indicated mostly water being released up to 150 °C. A much larger second stage mass loss (*ca*. 70 wt%) occurs between 200–375 °C, presumably due to the decomposition of the organic constituents (e.g. amino acids, polysaccharides, chitin, etc.). FTIR analysis of the gases evolved showed that the mass loss in the second stage is accompanied by the release of a multi-component volatile mixture mainly composed of CO_2 and H_2O. The release of non-flammable gases such as CO_2 and H_2O is a critical finding since these gases may hinder flaming combustion by diluting the concentration of flammable gases evolved, thereby improving the fire performance. The FTIR profiles of CO_2 and H_2O reached their peak values at approximately 350 °C. Absorbance bands in the region of 2850 and 3030 cm^{-1} (Fig. 2c) revealed C-H stretching vibration due to the presence of volatile hydrocarbon moieties which are likely composed primarily of CH_4. Moreover, absorbance peaks between 2000 and 2250 cm^{-1} show the presence of CO in the gaseous products, although the peak intensities are relatively low compared to those of CO_2.

To further investigate secondary products during the thermal decomposition of mycelium, the evolved gases were also analysed using GCMS sampling of the volatiles evolved from the TGA at 300 °C. The gas chromatogram as shown in Fig. 2d, revealed the presence of several other organic species, many of which were aromatic

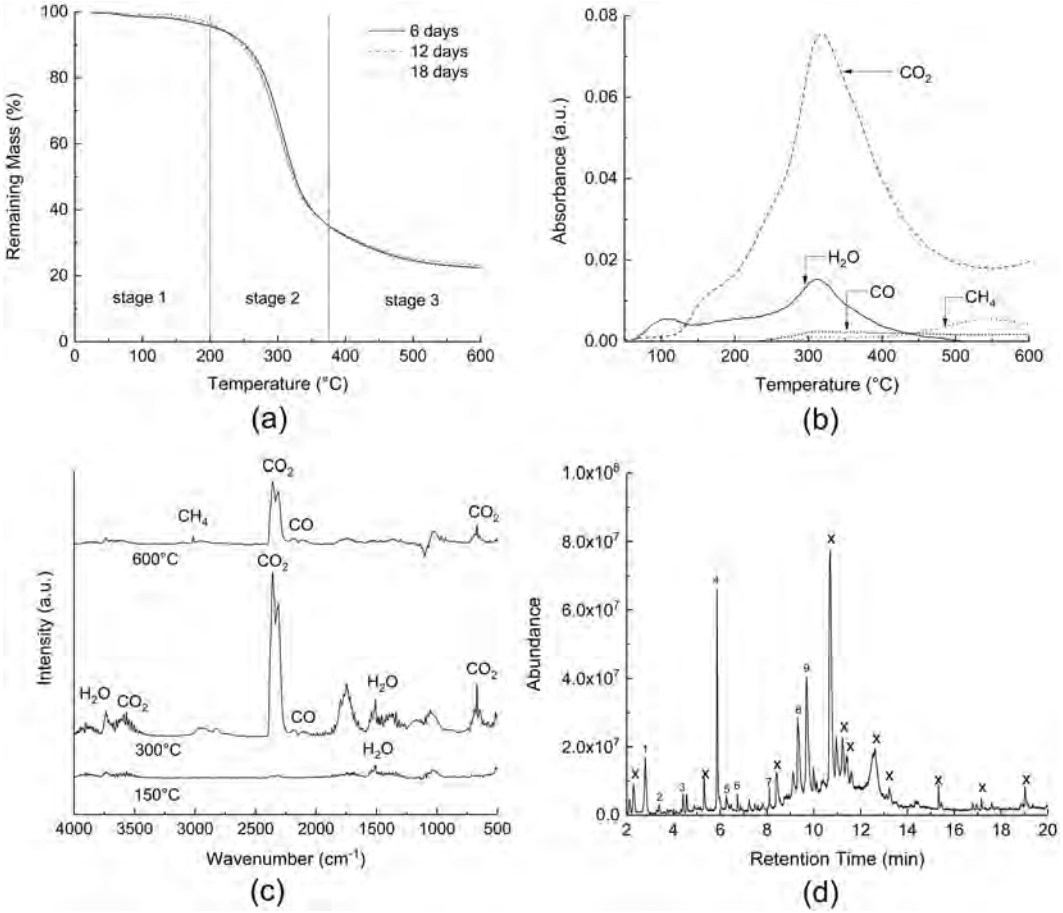

Figure 2. (**a**) TGA-mass loss temperature curve of mycelium grown for 6, 12 and 18 d between 25–600 °C under nitrogen (**b**) FTIR profiles of volatile products released expressed as a function of temperature (**c**) Offset Infrared spectra of the gases released at 150, 300 and 600 °C and (**d**) GC chromatogram of volatiles evolved at 300 °C respectively. Peaks marked with an 'x' symbol represent unidentified species.

Peak Number	Compounds	Retention Time (min)
1	Furan, 3-methyl	2.79
2	Urea, methyl-	3.39
3	Disulphide, dimethyl	4.40
4	Furfural	5.86
5	2-Furanmethonal	6.25
6	4-Cyclopentene-1,3-dione	6.73
7	2-Furancarboxaldehyde, 5-methyl-	8.09
8	Diisooctyl phthalate	9.69
9	Heptasiloxane, hexadecamethyl-	9.97

Table 1. Anticipated volatiles released at 300 °C obtained from GCMS.

in nature. The GCMS system was calibrated to identify gases between 45–450 atomic mass units (AMU) and hence could not identify low molecular weight gases such as CO_2 and H_2O. The wide variety and complex nature of compounds in mycelium made it difficult to identify all the gaseous products with high accuracy and hence, only products with a database match probability of greater than 60% are reported and listed in Table 1. Evidence for the presence of disulphide, dimethyl and heptasiloxane, hexadecamethyl- as volatile compounds released during decomposition of mycelium is supported through the detection of sulphur and silicon in the residual char via EDS measurements (Fig. 3a). No silicon was detected in the residue following decomposition, thereby suggesting its evolution in the gaseous phase. Likewise, a reduction to the carbon/sulphur peak intensity ratio in the residual char also suggests that sulphur derivatives may have been released as a gaseous product. The third and final decomposition stage (450–600 °C) involves further degradation of the primary residual char during which

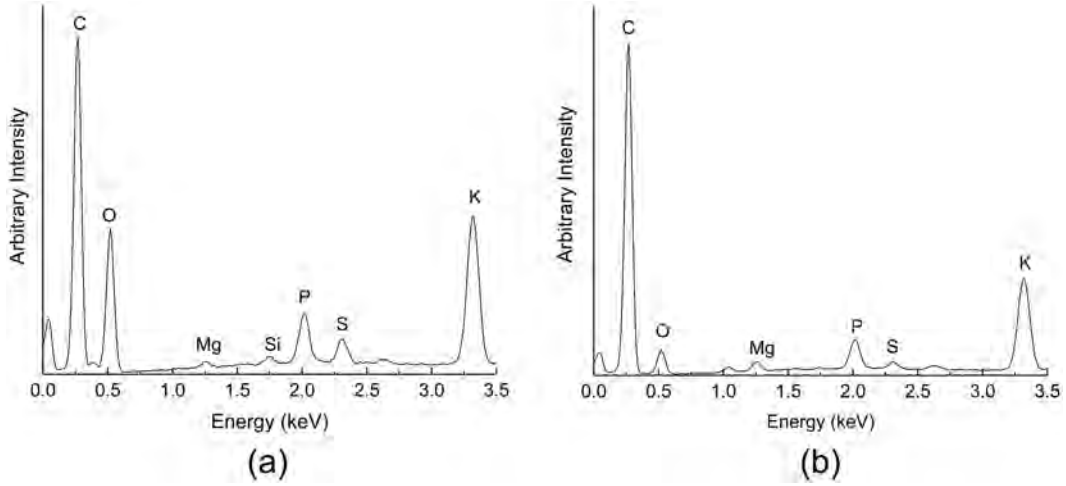

Figure 3. EDS spectra of mycelium (**a**) before and (**b**) after pyrolysis, respectively.

the chemical constituents have been completely reduced, resulting in the production of methane (CH_4) and the consequent formation of a carbonaceous char residue. The carbon residue is expected to be fully amorphous due to the relatively low heating temperatures (<1000 °C without catalyst) in comparison to graphitizing temperature greater than 1400 °C[21]. The residual mass was found to be approximately 23 ± 0.6 wt% at 600 °C with a negligible drop at higher temperatures and is consistent with other studies performed on similar species of mycelium[2]. The relatively higher residue yield for mycelium compared to most thermoplastic[22–24] and thermoset[25–28] polymers implies a potentially lower tendency to form smoke and toxic volatiles during thermal decomposition/combustion, thereby suggesting improved fire safety for the former[29].

The EDS measurements detected the presence of potassium and phosphorus in the residue, although their respective roles in the thermal decomposition of mycelium are still unclear. Phosphorus is widely used as a flame retardant in polymers and virtually any phosphorus compound can provide some degree of fire retardancy[14]. Since no phosphorus-based volatiles were detected, it is presumed that phosphorus was active within the condensed phase by promoting char formation through the production of phosphoric and polyphosphoric acids[14,25,30].

Morphological characterisation. Changes to the physical structure of mycelium after thermal decomposition were also investigated using the SEM and TEM. Micrographs were taken at various separate sites before and after pyrolysis and representative micrographs provided in Fig. 4. The hyphal diameters were measured using the Fiji distribution of ImageJ (v 1.51a) software.

As seen in Fig. 4, the fibrous network structure is retained after pyrolysis albeit with a substantial (10–30%) reduction in the hyphal diameter based on average hyphal diameter for 600 SEM hyphal diameter measurements and TEM sectional analysis after pyrolysis at 600 °C for 1 h. A 66% reduction in cell wall thickness was also identified based on TEM following pyrolysis. The retention of this fibrous structure is likely due to the presence of chitin in the cell walls of mycelium[31]. Chitin is a linear polymer of the acetylated amino sugar N-acetylglucosamine that forms microfibrillar arrangements in living organisms[32]. Chitin has a tensile strength greater than those of carbon fibres and steel[33] and possesses excellent thermal stability and flame-retardant properties[34,35].

This is an important finding which shows that the hyphal diameter can be tuned using thermal treatment. Species with a hyphal diameter of 1–3 μm can be pyrolysed to produce micro/nano sized carbon fibres that can then be used to toughen polymer-matrix composites for structural and non-structural engineering applications.

Combustion Properties of Mycelium Composites

Pyrolysis Flow Combustion Calorimetry. In investigating the fire reaction properties of pure mycelium, PCFC was chosen over cone calorimetry since it provides a rapid screening technique that requires only a few milligrams of a solid material and provides a direct comparison on the impact of replacing a synthetic polymer with mycelium as a binder in a composite material. In addition, the influence of sample morphology, thickness, heat losses through the sample-holder, etc. of conventional cone measurements are eliminated. A summary of properties measured using PCFC is shown in Table 2.

In addition to mass loss and evolved gases, the thermal degradation of mycelium is also accompanied by energy release in the form of heat. The heat release rate of a burning material is commonly accepted as the most important fire reaction property since it strongly influences factors such as flame spread, secondary ignitions, smoke generation, etc.[36].

The heat release rate profile of mycelium fluctuates considerably with increasing temperature due to various chemical and thermal events that occur simultaneously during thermal degradation. Figure 5 shows an initial induction period (up to 100 °C) during which the material does not release any heat (indicative of a non-combustion phase). The mass loss (Fig. 2a) during this stage is independent of oxygen consumption (Fig. 5), and thus can be attributed fully to the loss of physically adsorbed water. The increase in heat release between 100 and 200 °C can

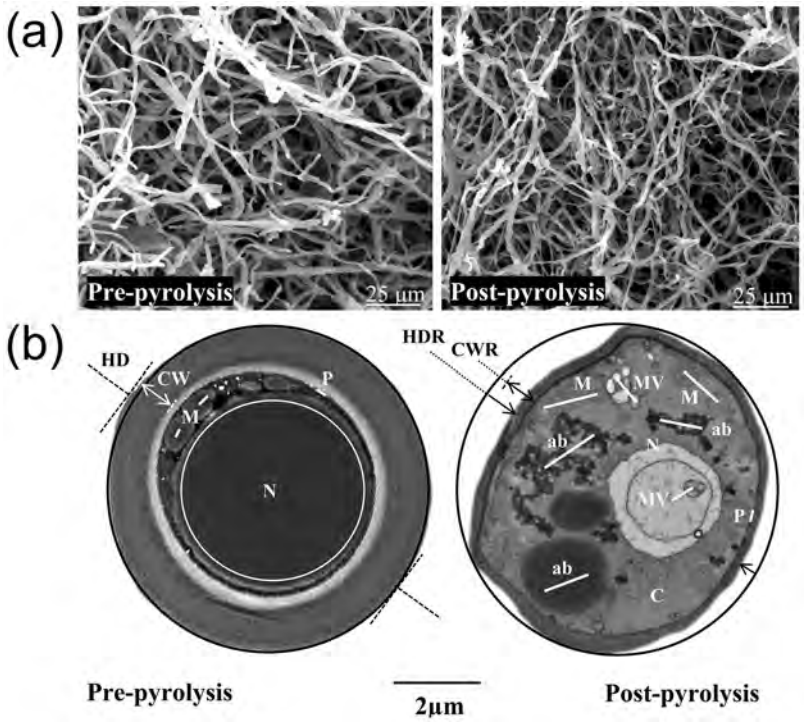

Figure 4. (**a**) Topographic SEM micrographs and (**b**) TEM transverse section micrographs detailing the ultrastructural, hyphal diameter and cell wall thickness changes in *T. versicolor* hypha pre- and post-pyrolysis (up to 600 °C in nitrogen). Abbreviations: ab, accumulation body; C, cytoplasm; CW, cell wall; CWR, cell wall reduction; HD, hyphal diameter; HDR, hyphal diameter reduction; M, mitochondria; MV, multivesicular; N, nucleus; P, plasmalemma.

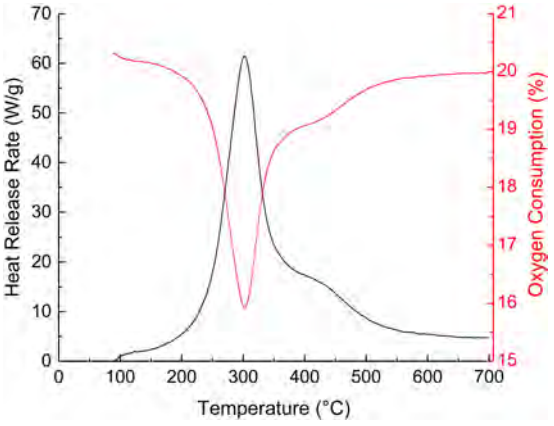

Figure 5. Effect of increasing temperature on the heat release rates and oxygen consumption of mycelium.

Sample	Temp to PHRR (°C)	pHRR (W/g)	THR (kJ/g)	Heat Release Capacity (J/gk)	Char yield (wt %)
Mycelium	300 ± 1	67 ± 2	6.8 ± 0.1	70 ± 1	23 ± 1
PMMA	399 ± 2	446 ± 6	24.6 ± 0.2	439 ± 6	0
PLA[23,37]	385	375	17.8	489	0.6

Table 2. Fire reaction properties of mycelium and other thermoplastic polymers measured using PCFC.

be attributed to the release of flammable low molecular weight volatiles. Mycelium starts to decompose at approximately 225 °C. This event is accompanied by a rapid rise in the HRR spectrum reaching a peak HRR value at 300 °C, after which the heat release rate gradually decreases with temperature. The oxygen consumption reached a

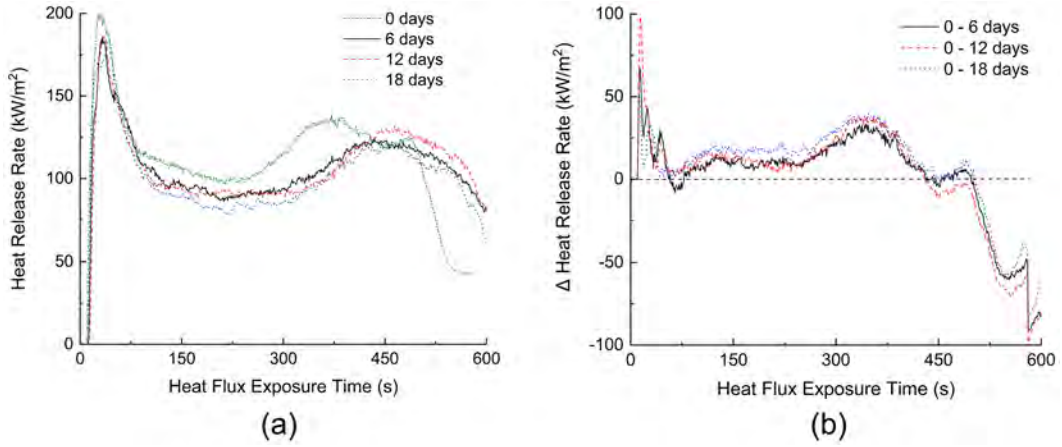

Figure 6. (**a**) Heat release rate data for composites grown for 0, 6, 12 and 18 d at an incident heat flux of 50 kW/m^2 and (**b**) calculated Δ heat release rates, respectively.

maximum value (*ca*. from 21 to 16 vol %) at the same time/temperature as the peak HRR (*ca*. 300 °C). The char yield obtained from PCFC tests was consistent with that from the TGA at approximately 23 wt%.

The fire reaction properties of mycelium are compared against commercially available polymers such as PMMA and PLA. The values for mycelium (PHHR = 67 W/g; THR = 6.8 kJ/g) are significantly lower when compared to both PMMA (PHHR = 446 W/g; THR = 24.6 kJ/g) and PLA (PHHR = 375 W/g; THR = 21.5 kJ/g)[23,37], indicating that this material is less combustible and has improved fire safety over PMMA and PLA. The average heat release capacity of mycelium (69.5 J/gK) is also significantly lower than that of PMMA (439 J/gK) and PLA (489 J/gK)[23,37], indicating superior resistance to flaming combustion. The improved flaming combustion resistance may be attributed to the higher residual char produced of mycelium (23 wt %) in comparison to PMMA (0 wt %) and PLA (0.6 wt %)[23,37]. The presence of char inhibits oxygen migration at the solid/gas phase interface thereby limiting the flaming combustion process.

Effect of Incubation Period on Fire Reaction Properties of Composites. In addition to pure mycelium, the fire reaction properties of composites made from mycelium and wheat grain were measured experimentally using a cone calorimeter at an incident heat flux of 50 kW/m^2, with temperatures ranging from 600 to 700 °C[38–40]. The composites were grown for 6, 12 and 18 d to ascertain the differences, if any, in their fire reaction properties associated with growth time. The fire reaction properties were compared against those of the as-received wheat grains without mycelium (i.e. growth time of 0 d). As shown in Fig. 6a, the HRR profiles for all four samples are similar, with a rapid increase following ignition to reach an initial peak value. However, the peak heat release rate, which is considered a critical property controlling the maximum temperature and flame spread rate,[14,36] was marginally higher (*ca*. 10%) for the 0-d sample (200 kW/m^2) relative to that of composites grown for 6, 12 and 18 d (180 kW/m^2). This observation suggests that the addition of mycelium results in marginal improvements to the fire reaction properties of the wheat grain substrate. The dissimilarity in flaming-combustion intensities of the 0, 6, 12 and 18-d composites is clearly depicted in Fig. 6b, wherein the ΔHRR-time profile is shown. The ΔHRR values were obtained by subtracting the HRR data measured for 0-d samples from those measured for 6, 12 and 18 d (i.e. $HRR_{0\ d}$ minus $HRR_{6,\ 12,\ 18\ d}$). The presence of the mycelium in the composite reduced the flaming intensity of the 6, 12 and 18-d samples during the initial thermal exposure (e.g. first 350 s) as revealed by the positive ΔHRR values over this period.

The lower PHRR values for the 6, 12 and 18-d samples can be attributed to the formation and growth of a mycelium rich surface layer between 0.9 to 1.5 mm thick depending on growth time (Fig. 7). The hyphal density in the core was significantly lower than at the surface, with negligible differences observed as a function of growth periods. The lower hyphal densities in the core can be attributed to the reduced oxygen diffusion into the bulk of the composite, an essential ingredient for mycelial growth. TGA performed on mycelium and wheat grain (Fig. 8a) showed that mycelium starts to decompose at relatively lower temperatures compared to the wheat grain. However, mycelium is a relatively higher charring material with approximately 23 wt% remaining at 600 °C compared to 19 wt% for wheat grains at the same temperature. The relatively higher mass retention for the 6, 12 and 18-d samples was also confirmed by the cone calorimetry results and is shown in Fig. 8b. The presence of a surface char layer reduces the heat release by acting as a thermal insulator and by limiting the supply of combustible gases and oxygen to the flame front. Following the initial peak, the HRR drops rapidly due to the emergence of a temporary thermally-protective carbonaceous char layer resulting from the thermal degradation of mycelium. However, with continued thermal exposure, the temporary char may degrade thereby exposing the underlying materials and resulting in a second HRR peak event. The absence of mycelium in the 0-d sample meant that the flaming combustion process was uninhibited resulting in a relatively higher second peak HRR value in comparison to the mycelium-coated samples.

The fire reaction properties of mycelium composites grown for 6, 12 and 18 d were similar. To elucidate why these composites would achieve the same fire retardation effect despite different growth times, the surface morphologies of the samples were investigated using SEM. As shown in Fig. 9, mycelium composites show a

Figure 7. Cross section of the mycelium-wheat composite showing the differences in hyphal density at the surface and the core.

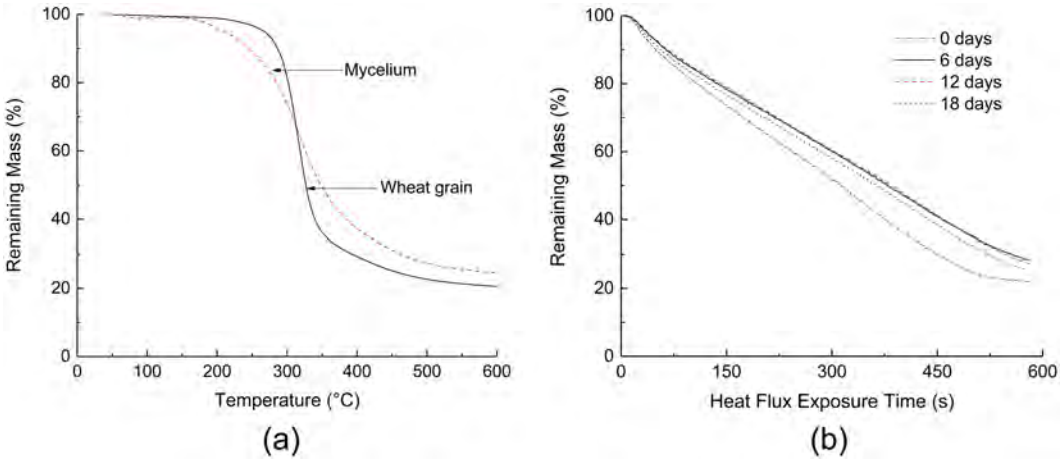

Figure 8. (**a**) TGA – mass loss curves for mycelium and wheat grain tested at 30 °C/min under nitrogen and (**b**) mass loss data for composites grown for 0, 6, 12 and 18 d obtained from cone calorimetry.

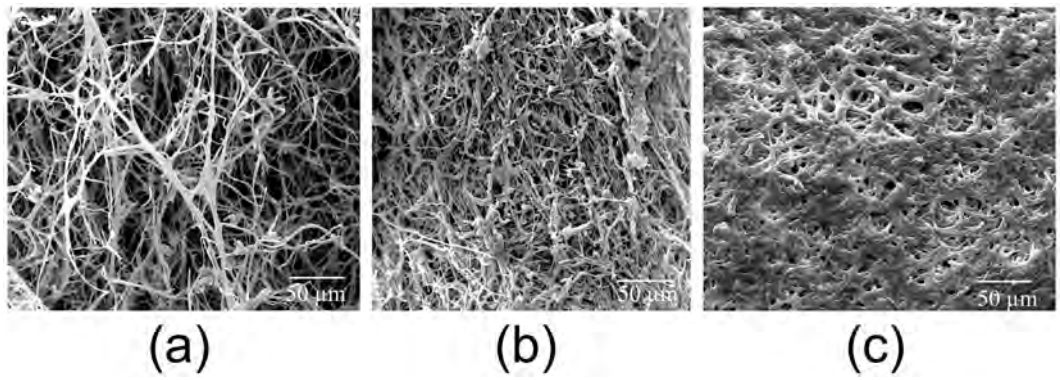

Figure 9. SEM images of mycelium surface layer on composites grown for (**a**) 6 d (**b**) 12 d and (**c**) 18 d.

significant increase in the surface hyphal density with increasing growth time. In addition, an increase in the thickness of the surface layer was also observed. The average thickness was measured using an optical microscope and found to be 0.9 ± 0.1, 1.2 ± 0.2 and 1.5 ± 0.2 mm for the 6, 12 and 18-d samples, respectively. However, the increase in thickness of the mycelium coating was not sufficient to cause significant variation in the fire reaction properties of the composite. This observation might point to the existence of a low threshold mycelial density required to enable effective fire retardation in mycelial composites.

Conclusion

The thermal degradation and fire safety of mycelium and mycelium-wheat grain composites have been characterised using various experimental techniques. Thermogravimetric analysis revealed that the growth time has no discernible effect on the thermal degradation characteristics of mycelium. FTIR and GCMS analysis have identified the complex thermal degradation patterns accompanied by the release of multiple flammable and non-flammable

gaseous products. The fibrous structure of mycelium is retained following pyrolysis, albeit with a reduction in its diameter. The fire reaction properties of mycelium have found to be superior to other competing thermoplastic polymers (PMMA and PLA) due to its tendency to form relatively higher char yields. The presence of mycelium is responsible for an improvement in the fire reaction properties of wheat grains. However, beyond 6 d, the growth time has been found to have no significant effect on the fire reaction properties of mycelium-wheat grain composites. Mycelium has been found to possess certain flame-retardant properties (e.g. high char residue and release of water vapour) and could be used as an economical, sustainable and fire-safer alternative to synthetic polymers for binding matrices.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Abhijith, R., Ashok, A. & Rejeesh, C. R. Sustainable packaging applications from mycelium to substitute polystyrene: a review. *Materials Today: Proceedings.* **5**, 2139–2145 (2018).
2. Haneef, M. *et al.* Advanced Materials From Fungal Mycelium: Fabrication and Tuning of Physical Properties. *Scientific Reports.* **7**, 41292 (2017).
3. Islam, M. R., Tudryn, G., Bucinell, R., Schadler, L. & Picu, R. C. Morphology and mechanics of fungal mycelium. *Scientific Reports.* **7**, 13070 (2017).
4. Jiang, L., Walczyk, D., McIntyre, G., Bucinell, R. & Tudryn, G. Manufacturing of biocomposite sandwich structures using mycelium-bound cores and preforms. *Journal of Manufacturing Processes.* **28**, 50–59 (2017).
5. Jiang, L., Walczyk, D., McIntyre, G. & Chan, W. K. Cost modeling and optimization of a manufacturing system for mycelium-based biocomposite parts. *Journal of Manufacturing Systems.* **41**, 8–20 (2016).
6. Jones, M., Huynh, T., Dekiwadia, C., Daver, F. & John, S. Mycelium Composites: A Review of Engineering Characteristics and Growth Kinetics. *Journal of Bionanoscience* **11**, 241–257 (2017).
7. Pelletier, M. G., Holt, G. A., Wanjura, J. D., Bayer, E. & McIntyre, G. An evaluation study of mycelium based acoustic absorbers grown on agricultural by-product substrates. *Industrial Crops and Products.* **51**, 480–485 (2013).
8. Fricker, M., Boddy, L. & Bebber, D. *Biology of the Fungal Cell*. 309–330 (Springer, 2007).
9. Glass, N. L., Rasmussen, C., Roca, M. G. & Read, N. D. Hyphal homing, fusion and mycelial interconnectedness. *Trends in Microbiology.* **12**, 135–141 (2004).
10. Arifin, Y. H. & Yusuf, Y. Mycelium Fibers as New Resource for EnvironmentalSustainability. *Procedia Engineering.* **53**, 504–508 (2013).
11. Holt, G. A. *et al.* Fungal Mycelium and Cotton Plant Materials in the Manufacture of Biodegradable Molded Packaging Material: Evaluation Study of Select Blends of Cotton Byproducts. *Journal of Biobased Materials and Bioenergy.* **6**, 431–439 (2012).
12. Abrams, M. *Construction Materials Made from 'Shrooms*, http://www.asme.org/engineering-topics/articles/construction-and-building/construction-materials-made-from-shrooms (2014).
13. Gonzalez, E. M. Growing architecture through mycelium and agricultural waste. Columbia University. http://aar.gsapp.org/ie/files/MAYORAL_0910_GROWING-ARCHITECTURE-THROUGH-MYCELIUM-AND-AGRICULTURAL-WASTE.pdf (2010).
14. Mouritz, A. P. & Gibson, A. G. *Fire Properties of Polymer Composite Materials*. Vol. 143 (Springer, 2006).
15. Jones, M., Huynh, T. & John, S. Inherent Species Characteristic Influence and Growth Performance Assessment for Mycelium Composite Applications. *Advanced Materials Letters.* **9**, 71–80 (2018).
16. Shewry, P. R. *et al.* Natural Variation in Grain Composition of Wheat and Related Cereals. *Journal of Agricultural and Food Chemistry* **61**, 8295–8303 (2013).
17. ASTM D7309 - 13, Standard Test Method for Determining Flammability Characteristics of Plastics and Other Solid Materials Using Microscale Combustion Calorimetry (ASTM International, West Conshohocken, PA, 2013).
18. Cogen, J. M., Lin, T. S. & Lyon, R. E. Correlations between pyrolysis combustion flow calorimetry and conventional flammability tests with halogen-free flame retardant polyolefin compounds. *Fire and Materials.* **33**, 33–50 (2009).
19. Tretsiakova-McNally, S. & Joseph, P. Pyrolysis Combustion Flow Calorimetry Studies on Some Reactively Modified Polymers. *Polymers.* **7**, 453 (2015).
20. ISO 5660-1, Reaction-to-fire tests–Heat release, smoke production and mass loss rate–Part 1: Heat release rate (cone calorimeter method) and smoke production rate (dynamic measurement) (International Organization for Standardization, 2015).
21. Tang, J., Etacheri, V. & Pol, V. G. Wild Fungus Derived Carbon Fibers and Hybrids as Anodes for Lithium-Ion Batteries. *ACS Sustainable Chemistry & Engineering.* **4**, 2624–2631 (2016).
22. Cho, J. H. *et al.* Bioinspired Catecholic Flame Retardant Nanocoating for Flexible Polyurethane Foams. *Chemistry of Materials.* **27**, 6784–6790 (2015).
23. Fox, D. M., Lee, J., Citro, C. J. & Novy, M. Flame retarded poly(lactic acid) using POSS-modified cellulose. 1. Thermal and combustion properties of intumescing composites. *Polymer Degradation and Stability.* **98**, 590–596 (2013).
24. Renneckar, S., Zink-Sharp, A. G., Ward, T. C. & Glasser, W. G. Compositional analysis of thermoplastic wood composites by TGA. *Journal of Applied Polymer Science.* **93**, 1484–1492 (2004).
25. Braun, U. *et al.* Influence of the oxidation state of phosphorus on the decomposition and fire behaviour of flame-retarded epoxy resin composites. *Polymer.* **47**, 8495–8508 (2006).
26. Cardona, F., Rogers, D. & Erp, G. V. Investigation by TGA—FTIR of the Effect of Styrene Content and Thickness on the UV-curing of Vinylester Resins. *Journal of Thermoplastic Composite Materials.* **20**, 601–615 (2007).
27. Guo, Z., Liang, X., Pereira, T., Scaffaro, R. & Thomas Hahn, H. CuO nanoparticle filled vinyl-ester resin nanocomposites: Fabrication, characterization and property analysis. *Composites Science and Technology.* **67**, 2036–2044 (2007).
28. Yee, R. Y. & Stephens, T. S. A TGA technique for determining graphite fiber content in epoxy composites. *Thermochimica Acta.* **272**, 191–199 (1996).
29. Joseph, P. & Ebdon, J. R. In *Fire Retardant Materials*. 220–263 (Woodhead Publishing, 2001).
30. Granzow, A. Flame retardation by phosphorus compounds. *Accounts of Chemical Research.* **11**, 177–183 (1978).
31. Bartnicki-Garcia, S. Cell Wall Chemistry, Morphogenesis, and Taxonomy of Fungi. *Annual Review of Microbiology.* **22**, 87–108 (1968).
32. Ravi Kumar, M. N. V. Chitin and chitosan fibres: A review. *Bulletin of Materials Science.* **22**, 905 (1999).
33. Webster, J. & Weber, R. *Introduction to Fungi*. 3 edn, (Cambridge University Press, 2007).
34. Moussout, H., Ahlafi, H., Aazza, M. & Bourakhouadar, M. Kinetics and mechanism of the thermal degradation of biopolymers chitin and chitosan using thermogravimetric analysis. *Polymer Degradation and Stability.* **130**, 1–9 (2016).
35. Pan, H. *et al.* Formation of self-extinguishing flame retardant biobased coating on cotton fabrics via Layer-by-Layer assembly of chitin derivatives. *Carbohydrate Polymers.* **115**, 516–524 (2015).

36. Mouritz, A. P., Mathys, Z. & Gibson, A. G. Heat release of polymer composites in fire. *Composites Part A: Applied Science and Manufacturing.* **37**, 1040–1054 (2006).
37. Bhat, T., Chevali, V., Liu, X., Feih, S. & Mouritz, A. P. Fire structural resistance of basalt fibre composite. *Composites Part A: Applied Science and Manufacturing.* **71**, 107–115 (2015).
38. Bhat, T., Kandare, E., Gibson, A. G., Di Modica, P. & Mouritz, A. P. Compressive softening and failure of basalt fibre composites in fire: Modelling and experimentation. *Composite Structures.* **165**, 15–24 (2017).
39. Kandare, E., Luangtriratana, P. & Kandola, B. K. Fire reaction properties of flax/epoxy laminates and their balsa-core sandwich composites with or without fire protection. *Composites Part B: Engineering.* **56**, 602–610 (2014).
40. Li, S., Deng, L., Xu, C., Wu, Q. & Wang, Z. Making a Supertough Flame-Retardant Polylactide Composite through Reactive Blending with Ethylene-Acrylic Ester-Glycidyl Methacrylate Terpolymer and Addition of Aluminum Hypophosphite. *ACS Omega.* **2**, 1886–1895 (2017).

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Author Contributions

M.J. prepared all samples and assisted in most experimental testing, analysis and manuscript preparation. T.B. wrote the manuscript text and performed most of the experimental testing and analysis. A.T. and P.J. performed the PCFC testing. R.Y. and T.B. completed the cone calorimetry testing. C.D. performed the TEM imaging. E.K., J.M., S.J. and C.W. supervised the work and provided technical expertise. All authors reviewed the manuscript.

Additional Information

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Electronic supplementary material for:

Thermal Degradation and Fire Properties of Fungal Mycelium and Mycelium - Biomass Composite Materials

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List of Figures

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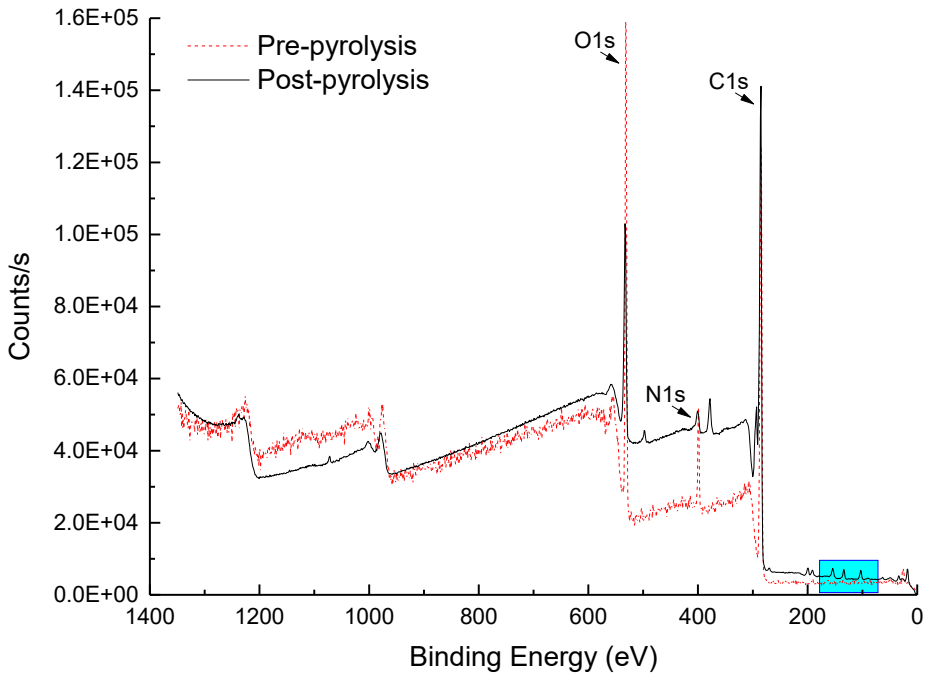
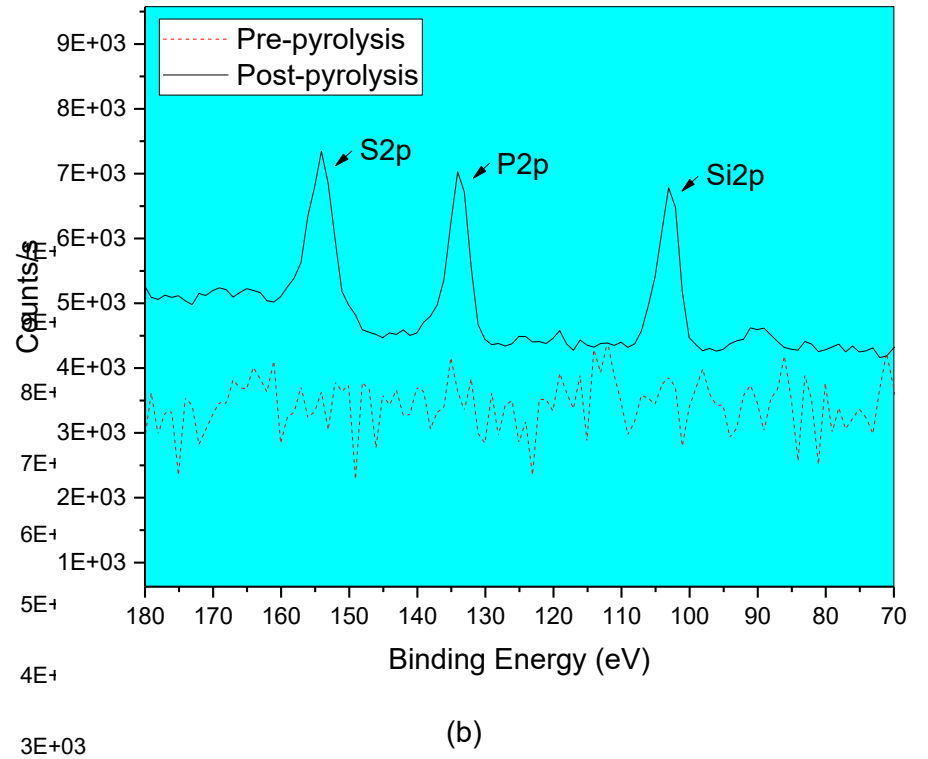


(a)



(b)

Figure S1. X-ray photoelectron spectroscopy (XPS) spectra for a) C, N, O and b) P, S, Si for *T. versicolor* mycelial biomass pre- (red) and post-pyrolysis (black). Note: XPS provides environment sensitive, surface based elemental analysis only. It characterises surface chemistry that may be affected by the atmosphere, pyrolysis based chemical reactions and other factors. The energy-dispersive x-ray spectroscopy (EDS) bulk analysis spectra included in the main article are suggested to provide more reliable elemental analysis information for whole mycelial biomass. This information is provided for reference only.

Reviewer comments:

Reviewer #2 (Remarks to the Author):
The manuscript titled “Thermal Degradation and Fire Properties of Fungal Mycelium and Mycelium - Biomass Composite Materials” reports the investigation of the thermal degradation properties and subsequent changes to the morphological and chemical structure of mycelium is novel. It is a well-documented manuscript with solid data. It can be published in Scientific Reports after minor revision.

The authors should provide TEM or high resolution TEM to confirm the change of structure indicated by the authors.

The author should provide more characterizations to confirm the chemical structure change such as XPS.

Reviewer query 1: The authors should provide TEM or high resolution TEM to confirm the change of structure indicated by the authors.

Authors’ response:
The authors addressed this query by acquiring TEM images of the *T. versicolor* hypha. The following amendments were made to the manuscript:

The *Materials and Experimental Methodology* section was updated to include the following:

“Transmission Electron Microscopy (TEM)
The T. versicolor hypha specimens were fixed in a 100 mM cacodylate buffer (pH 7.0) solution containing 2.5% glutaraldehyde, 2% paraformaldehyde followed by washing thrice with 100 mM cacodylate buffer. The specimens were post-fixed with 1% osmium tetroxide for 1.5 h followed by washing thrice with distilled water. The samples were gradually dehydrated with increasing gradients of 50% to 100% ethanol for 15-30 min each. Following dehydration, the samples were infiltrated twice with Spurr's resin before polymerisation. The samples were post-polymerised at 60°C for 48 h. Ultra-thin sections (~90 nm) were cut using the Leica Ultracut Ultramicrotome on carbon-formvar copper grids. The samples were post-stained with heavy metals and imaged using a JEOL-1010 transmission electron microscope (TEM) at 80 kV with the Gatan Microscopy Suite software (v 2.3) (Gatan Inc., Pleasanton, USA).”

Figure 4. Was updated to include TEM micrographs of mycelium pre- and post-pyrolysis. This supplemented the already provided SEM micrographs of mycelium pre- and post-pyrolysis.

The *Morphological characterisation* section was also updated and now reads:

“Changes to the physical structure of mycelium after thermal decomposition were also investigated using the SEM and TEM. Micrographs were taken at various separate sites before and after pyrolysis and representative micrographs provided in Figure 4. The hyphal diameters were measured using the Fiji distribution of ImageJ (version 1.51a) software. As seen in Figure 4, the fibrous network structure is retained after pyrolysis albeit with a substantial (10-30%) reduction in the hyphal diameter based on average hyphal diameter for 600 SEM hyphal diameter measurements and TEM sectional analysis after pyrolysis at 600°C for 1 h. A 66% reduction in cell wall thickness was also identified based on TEM following pyrolysis. The retention of this fibrous structure is likely due to the presence of chitin in the cell walls of mycelium[31]. Chitin is a linear polymer of the acetylated amino sugar N-acetylglucosamine that forms microfibrillar arrangements in living organisms[32]. Chitin has a tensile strength greater than those of carbon fibres and steel[33] and possesses excellent thermal stability and flame-retardant properties[34-35].”

Reviewer query 2: The author should provide more characterizations to confirm the chemical structure change such as XPS.

Authors' response:
XPS was completed at the reviewer's request and the acquired data is now included in the supplementary material. The authors have retained EDS in the manuscript and included the XPS in the supplementary material to avoid duplication in discussion of the results. EDS was preferentially retained because XPS spectra detail environment sensitive surface chemistry only as opposed to EDS which is a bulk analysis tool. As such XPS is considered to be less accurate than EDS for the elemental analysis of mycelial biomass and is included in the supplementary material for reference purposes only.

The *Materials and Experimental Methodology* section was also updated to include the following:

"The surface chemistry of the mycelium was assessed using a K-alpha X-ray Photoelectron Spectrometer (XPS) instrument (Thermo Fisher Scientific, USA) with a monochromated Aluminium Kα X-ray source. X-ray spot size was 30-400 μm in 5μm steps. Scans spanned from 1400 to 0 eV binding energy. Peak analysis was performed by means of peak decomposition to fit a Gaussian function using the Thermo Scientific™ Avantage Software (v5.9902, b. 06552). XPS spectra detail environment sensitive surface chemistry only as opposed to EDS which is a bulk analysis tool. As such XPS is considered to be less accurate than EDS for the elemental analysis of mycelial biomass and is included in the supplementary material for reference purposes only."

Publication F

Addresses Research Question 4:

How do the fire reaction and fire safety properties of mycelium composites compare with synthetic polymer foam, such as extruded polystyrene, and engineered wood, such as particleboard, and can their properties be improved through incorporation of industrial by-products, such as glass fines?

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RESEARCH ARTICLE

WILEY

Waste-derived low-cost mycelium composite construction materials with improved fire safety

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Summary

Mycelial growth attracts academic and commercial interest because of its ability to upcycle agricultural and industrial wastes into economical and environmentally sustainable composite materials using a natural, low-energy manufacturing process able to sequester carbon. This study aims to characterise the effect of varying ratios of high silica agricultural and industrial wastes on the flammability of mycelium composites, relative to typical synthetic construction materials. The results reveal that mycelium composites are safer than the traditional construction materials considered, producing much lower average and peak heat release rates and longer time to flashover. They also release significantly less smoke and CO_2, although CO production fluctuated. Rice hulls yielded significant char and silica ash which improved fire performance, but composites containing glass fines exhibited the best fire performance because of their significantly higher silica concentrations and low combustible material content. Higher concentrations of glass fines increased volume-specific cost but reduced mass-specific and density-specific costs. The findings of this study show that mycelium composites are a very economical alternative to highly flammable petroleum-derived and natural gas-derived synthetic polymers and engineered woods for applications including insulation, furniture, and panelling.

KEYWORDS

composite, cost, fire safety, mycelium, waste utilisation

1 | INTRODUCTION

Mycelium composites are a new type of novel, economical, and environmentally sustainable materials that have attracted increasing academic and commercial interests over the past decade.[1] Mycelium is the vegetative growth of filamentous fungi that bonds organic matter through a network of hyphal microfilaments in a natural biological process that can be exploited to produce composite materials.[1-5] This process is cost-effective because mycelium can grow on and bind agricultural and industrial waste materials (eg, rice hulls, sugarcane bagasse, wheat, barley straw, and glass fines) that have little or no commercial value[6,7] and convert them into high-value composite materials for multiple applications. Furthermore, the wide variety of utilisable substrates coupled with technological improvements in processing means that manufacturers can customise mycelium composites to meet specific structural and functional requirements including impact resistance, fire resistance, and thermal and acoustic insulation.[1-4]

The diverse range of useful properties of mycelium composites makes them suitable as low-cost and sustainable alternatives to widely used, highly flammable petroleum-derived and natural gas-derived synthetic polymers (eg, plastics including insulation foams) and resin-based engineered woods (eg, particleboard). These traditional construction materials have been identified as the main cause of severe and fatal fire incidents worldwide[8-11] because of significant heat release and the toxic fumes generated during combustion.[8,9] Modern

houses constructed from unprotected engineered woods collapse over 3 times faster than older wood-based constructions.[12] Plastic foams are often major contributors to fires involving rapid flame spread which generate high volumes of smoke and toxic gases[13] including carbon monoxide and hydrogen cyanide.[14]

This study aims to investigate the effect of varying ratios of different substrates comprising of agricultural (rice hulls) and industrial (glass fines) wastes on the fire safety and cost of mycelium composites and compare these parameters to synthetic construction materials. Rice hulls are the hard coatings that protect rice seeds or grains during the growing season.[15] They account for 20% to 22% of rice paddy (rice grain, bran, and husk)[16] and are readily available in large quantities worldwide (167 MT/annum).[17] Rice hulls are considered a low-grade agricultural by-product. They have limited use in animal bedding, boiler fuel, and as a filler for building materials, glass production, and road construction but are largely discarded as waste.[15] As a result, rice hulls are a suitable primary substrate for mycelium composites, and their utilisation reduces waste and associated disposal costs.

Glass fines are very small pieces of glass that are too fine to be efficiently recycled because of the presence of similar-sized organic and inorganic contaminants which cannot be easily separated.[6,18] They account for 35% to 50% of recovered glass in Australia (~616 kT/annum), or 20% to 29% of annual Australian glass waste (~1.1 MT/annum).[6,19,20] Glass fines are typically used in asphalt, abrasive blasting, road aggregates, brick making, water filtration, and insulation batts[6] but are considered a waste material and mostly discarded.[21] This makes glass fines a low-cost additive that can be used to impact thermal stability in mycelium composites.

This is the first study into the growth process of mycelium composites using industrial (glass fines) and agricultural (rice hulls) wastes, the thermal degradation properties of mycelium grown on different substrates, and the fire safety of the resultant composites.

2 | MATERIALS AND EXPERIMENTAL METHODOLOGY

2.1 | Composite constituents

Rice hulls (CopRice, Leeton, Australia) and glass fines (The Alex Fraser Group, Melbourne, Australia) were selected as economic waste materials for this study based on their local availability, cost, and silica content (Table 1). Wheat grains (E&A Salce, Melbourne, Australia) were also selected as a highly nutritious high-growth comparison substrate and inoculation medium.

The common white-rot fungus *Trametes versicolor* was selected to bond these waste materials based on its growth performance[24] and possession of phenol-oxidising enzyme (eg, laccase, peroxidase, tyrosinase) activity[25,26] which is required for the digestion of the lignin in rice hulls.[27] The fungal inoculum was purchased from New Generation Mushroom Supplies (Melbourne, Australia) as a mycelial mass on digested wheat grain sealed in plastic bags including filter patches.

2.2 | Preparation of mycelium composites

Rice hull and wheat grain substrates (primary function to support fungal growth) were soaked for 48 hours in type 1 Milli-Q® ultrapure water and sterilised at 121°C and 103.4 kPa for 40 minutes before use. Glass fine additives (primary function to modify material properties) were sterilised as above. The substrates and additives were then combined in varying ratios with *Trametes versicolor* wheat grain inoculum using a sterilised blender. The inoculated substrate was deposited

TABLE 1 Availability, cost, and silica content of rice hulls and glass fines locally sourced in Australia

Waste	Waste Type	Availability (kT/annum)	Cost (AUD$/tonne)	Silica Content (wt%)
Rice hulls	Agricultural	240	50-60	15-20
Glass fines	Industrial	385-580	<50	70-90

Data extracted from literature.[6,7,19,20,22,23]

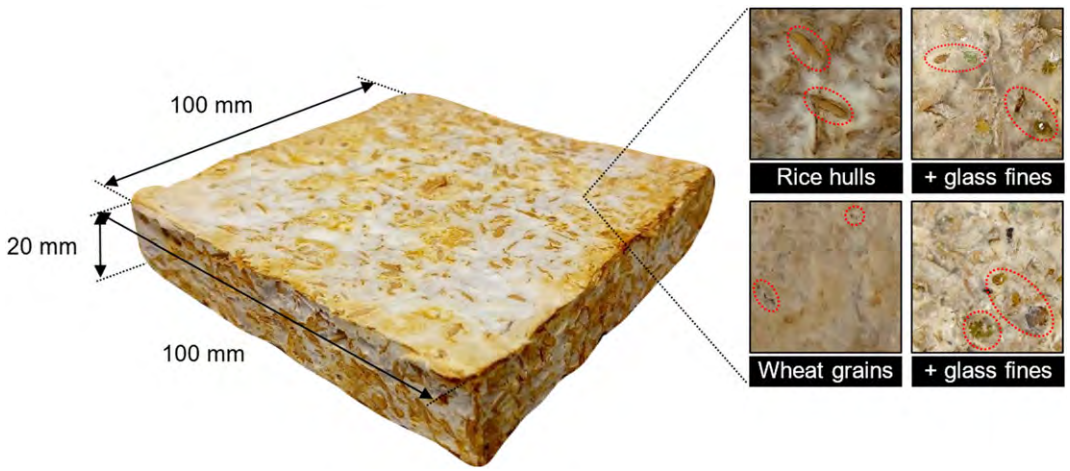

FIGURE 1 Representative mycelium composite as a dehydrated square tile with surface profiles for each composite sample produced, ie, rice hulls, rice hulls + glass fines, wheat grains, and wheat grains + glass fines [Colour figure can be viewed at wileyonlinelibrary.com]

into sterile plastic moulds which were sealed and incubated under standard atmospheric conditions (25°C, 50% RH) for 12 days allowing the hyphal growth to bind the substrates and additives. Following the incubation period, specimens were dried at 50°C for 48 hours to completely remove adsorbed moisture and denature the fungal material (Figure 1).

Commercially available ClimaFoam® extruded polystyrene (XPS) foam (Knauf Insulation, Brisbane, Australia) and STRUCTAflor® YELLOWtongue® particleboard (CarterHoltHarvey Woodproducts, Melbourne, Australia) were cut to the same dimensions as the mycelium composites and used for comparison of fire reaction and fire safety properties.

2.3 | Fire reaction testing

The fire reaction properties of the mycelium composites, XPS foam, and particleboard were assessed using a 3-cell cone calorimeter (Fire Testing Technology, UK) operated in the horizontal testing mode. All samples (100 mm long × 100 mm wide × 20 mm thick) were exposed to a constant incident thermal heat flux of 50 kW/m^2 simulating a well-developed room fire per ISO 5660.[28] The heat-exposed specimen surface was positioned 25 mm from the cone heater. The fire reaction parameters including the time to ignition (t_{ig}), heat release rate (HRR), mass loss rate, and smoke (CO, CO_2, and soot) release were measured.

2.4 | Scanning electron microscopy and elemental analysis

Hyphal growth was examined using an FEI Quanta 200 environmental scanning electron microscope under high vacuum. Samples were removed from the surfaces of the mycelium composite dehydrated tiles, gold coated, and subsequently imaged using an accelerating voltage of 30 kV. Multiple sites were imaged to ensure consistency.

Elemental analysis of as-received uncoated glass fines was also completed using the attached Oxford X-Max^N 20 energy-dispersive X-ray spectrometer and subsequent spectra analysis performed using AZtecEnergy EDS software.

2.5 | Thermogravimetric analysis

Thermogravimetric analysis (TGA) was performed on mycelial masses sourced from the surfaces and centre of each composite type in

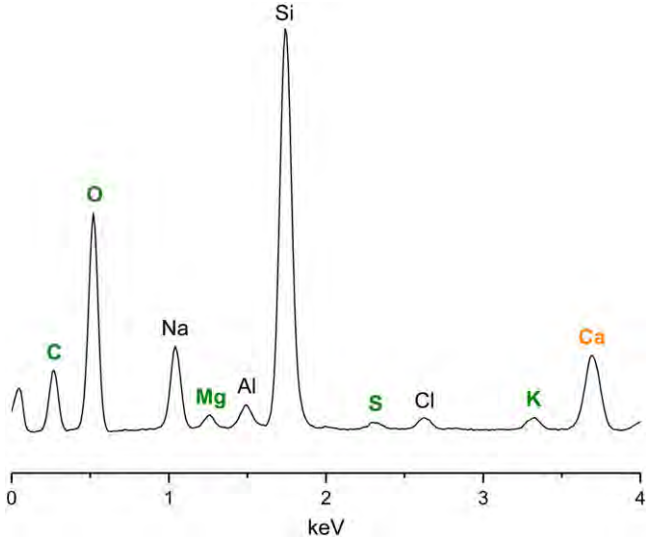

FIGURE 2 EDS analysis of glass fines as received including fungal macronutrients (C, K, Mg, O, S) and micronutrients (Ca) [Colour figure can be viewed at wileyonlinelibrary.com]

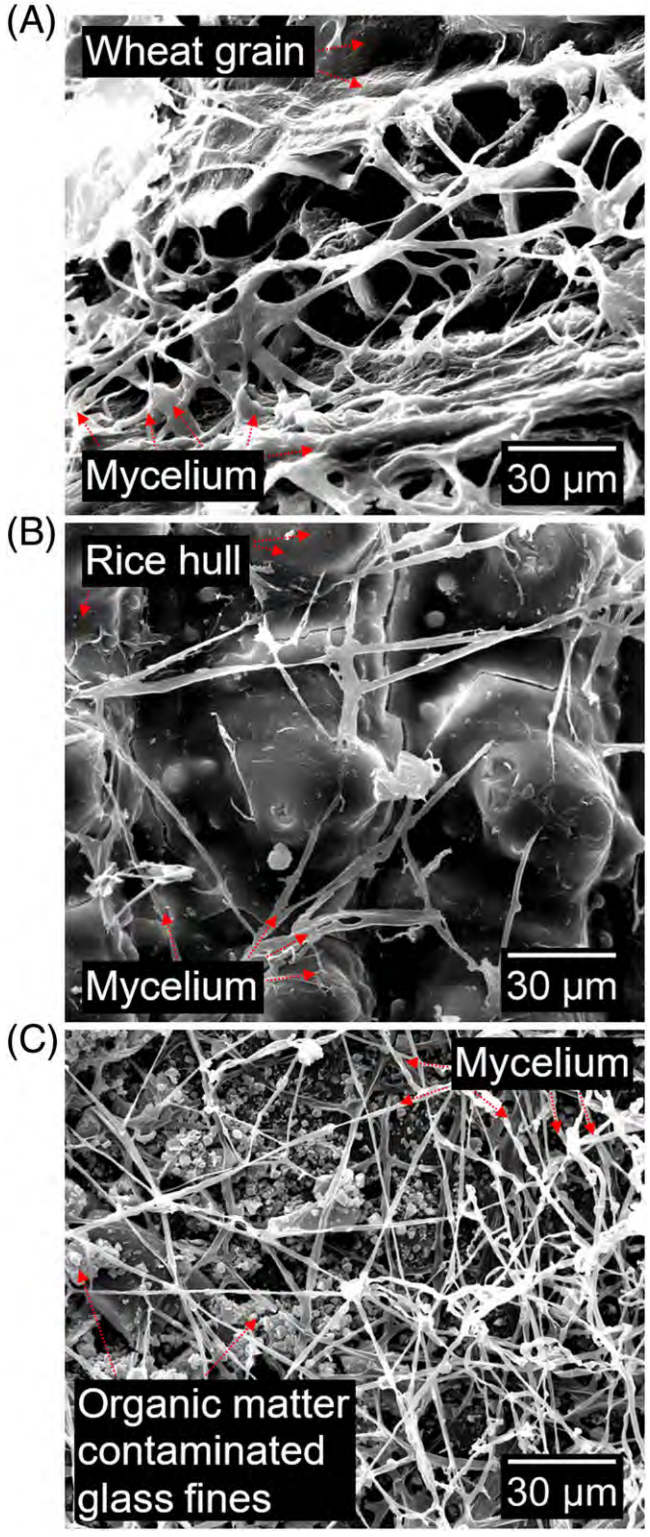

FIGURE 3 Filamentous hyphal growth (mycelium) on (A) wheat grains, (B) rice hulls, and (C) glass fines [Colour figure can be viewed at wileyonlinelibrary.com]

nitrogen at a heating rate of 20°C/minute using a PerkinElmer Pyris 1 instrument to determine the onset decomposition temperature (temperature at which the material starts disintegrating) and residual char content.

3 | RESULTS AND DISCUSSION

3.1 | Substrate and additive biocompatibility

Mycelium composites were first produced using wheat grain, a highly nutritious substrate that fuelled maximum fungal growth and provided a mycelium-rich standard for comparison with rice hull-derived and glass waste-derived mycelium composites. Wheat grains are a complete source of nutrition comprising primarily of starch (~80%), proteins (~10%), and cell wall polysaccharides in addition to lipids, terpenoids, phenolics, minerals, vitamins, and essential amino acids.[29] This makes them an ideal substrate for mycelium composites. However, wheat grains are also a commercial food source valued at $AUD251/tonne,[30] and as such, whole wheat grain-derived mycelium composites are not economically viable.

Rice hulls and organic matter-contaminated glass fines are not as nutrient rich as wheat grains but still contain nutrients capable of supporting fungal extracellular digestion and growth. Rice hulls primarily contain cellulose (~50 wt%), hemicellulose, and lignin (25-30 wt%) (carbon and nitrogen-based feedstocks).[22] Glass fines comprise primarily of silica (SiO_2) and contain up to 30 wt% organic surface matters including well-documented fungal macronutrients (C, O, Mg, S, K) and micronutrients (Ca)[31] (Figure 2). Industrial wastes have not been used so far in the manufacture of mycelium composites,[1-5] although mycelium has previously been noted to grow anaerobically from soil particles on nutrient-free silica gel.[32]

Rice hulls could be used for up to 75 wt% of the mycelium composite, supplemented by 25 wt% inoculated wheat grains, without significantly compromising mycelial growth or interfacial bonding. Filamentous fungal (hyphal) growth spread adequately upon all substrates and additives,[33] undergoing dichotomous or lateral branching (Figure 3) and binding them together[34] to form the composite materials. However, the hyphal density and mycelium content of the composites varied based on the nutritional properties of the substrates with significantly faster hyphal extension and denser growth occurring on the highly nutritious wheat grains than the less nutritious rice hulls (Figure 3A, B). Hyphae also readily spread on glass fines, although their high inorganic content limited growth to a weak surface spread (Figure 3C) that had to be supplemented by at least 50 wt% organic material (ie, wheat grains or rice hulls) to facilitate sufficient growth to properly bond the constituents and hold the composites together.

3.2 | Flammability and fire safety

Time to ignition (t_{ig}) defines the start of flaming combustion and is important because composites often yield high-temperature flames following ignition, which contribute to the rapid spread of fire.[10] The times to ignition for mycelium composites grown on wheat grains (12 seconds) and rice hulls (7 seconds) were similar to that of the XPS foam (9 seconds) but significantly shorter than the particleboard (26 seconds) (Table 2).

This indicated that the constituents of the mycelium composites reached their endothermic decomposition temperatures earlier than those in the particleboard.[10] This was not unexpected because mycelium primarily consists of organic polysaccharides, proteins, lipids, organelles, and cytoplasm in generative hyphae[33] and exhibits mediocre thermal degradation properties typical of organic materials (eg, plant matter and natural fibres).[2,36] Mycelium exhibited no flame-retardant properties and functioned purely as a binding matrix phase in the composite. Thermogravimetric analysis of mycelium grown on different substrates showed that the mycelium began to decompose at approximately 250°C (Figure 4) with decomposition occurring through a series of reactions that broke the organic constituents down into low molecular weight volatiles.[36] Stable char consisting of primarily amorphous carbon started to form at 500°C (~25 wt%) with a negligible drop in mass at higher temperatures. There was negligible difference in the thermal degradation characteristics of mycelium grown on different substrates or sourced from different locations within the composite.

However, there were significant differences in fire performance between mycelium composites utilising different substrates. Heat release rate (Figure 5) is commonly accepted as the most important fire reaction property because of its role in fire growth and spread.[37,38] Heat released from burning material provides additional

TABLE 2 Summary of cone calorimetry performance and fire safety parameters

Type	Sample	ρ (kg/m^3)	t_{ig} (second)	RHR_{180} (kW/m^2)	PHRR (kW/m^2)	t_{fo} (second)	TSR (m^2/m^2)	COP_{180} (g)	CO_2P_{180} (g)
Synthetic	ClimaFoam® extruded polystyrene (XPS) foam	33	9	114	503	61	1184	0.48	15.2
	STRUCTAflor® particleboard	689	26	134	200	173	64	0.47	30.0
Mycelium composite[a]	75 wt% wheat grains	359	12	107	185	94	70	0.33	23.8
	75 wt% rice hulls	193	7	85	133	75	40	0.02	14.6
	25 wt% wheat grains +50 wt% glass fines	589	12	42	79	370	5	0.39	10.2
	25 wt% rice hulls +50 wt% glass fines	450	7	33	85	311	0.9	0.91	6.3

ρ, density calculated from the volume and mass; t_{ig}, time to ignition; RHR_{180}, average heat release rate from ignition to 180 seconds after ignition; PHRR, peak heat release rate; t_{fo}, estimated time to flashover in room fire test[35]; TSR, total smoke release; COP_{180}, carbon monoxide produced from ignition to 180 seconds after ignition; CO_2P_{180}, carbon dioxide produced from ignition to 180 seconds after ignition.

[a]Inoculated using 25 wt% wheat grain inoculum.

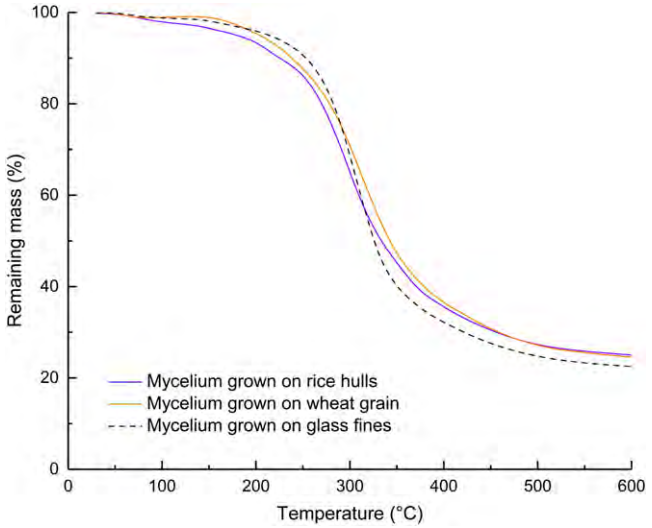

FIGURE 4 TGA mass loss curves for mycelial mass sourced from the surface and centre of wheat grain-based, rice hull-based, and glass fines-based mycelium composites [Colour figure can be viewed at wileyonlinelibrary.com]

thermal energy to fires and strongly influences their behaviour[10] and reaction properties including surface flame spread, smoke generation, and carbon monoxide emission.[39,40] The average heat release rate (RHR_{180}), which is considered the most appropriate variable for predicting full-scale fire properties,[41] indicated that mycelium composites comprising of wheat grains released marginally less heat (107 kW/m^2) than the XPS foam (114 kW/m^2) but significantly less heat than the particleboard (134 kW/m^2). Even greater reductions were achieved by substituting the wheat grain substrate for rice hulls (85 kW/m^2) (Figure 5A). The peak heat release rate (PHRR), which is considered a critical property controlling maximum temperature and flame spread rate,[10] was also much lower for the rice hull-based mycelium composites (133 kW/m^2) relative to the wheat grain-based mycelium composites (185 kW/m^2), synthetic XPS foam (200 kW/m^2), and particleboard (503 kW/m^2) (Table 2).

The lower heat release of rice hull-based mycelium composites is attributable to significantly higher residue following decomposition of the rice hulls comprising of amorphous carbon-based char (approx. 20 wt%) and embedded silica (approx. 20 wt%) during combustion.[36,42] Char is derived from organic rice hull constituents, especially lignin which is an aromatic compound containing cyclic rings of very stable bonds which do not easily break apart or react with other constituents.[43] These rings decompose into aromatic fragments which are the principal constituents from which char is formed. The formation of char is known to increase flame retardancy by acting as a thermal insulation barrier because of its low thermal conductivity[10] and to reduce smoke because of the ability of char to impede the release of ultrasmall fragments of fibre into the smoke plume.[44,45] In the presence of oxygen (on air-exposed surfaces), char will oxidise leaving inert silica behind as the main constituent of the surface residue.[36] Progressive accumulation of these silica layers results in the formation of a silica-ash layer which acts as a thermal barrier, preventing oxygen flow to the composite core. This lack of oxygen flow prevents further oxidation which insulates the virgin materials in a shielding effect similar

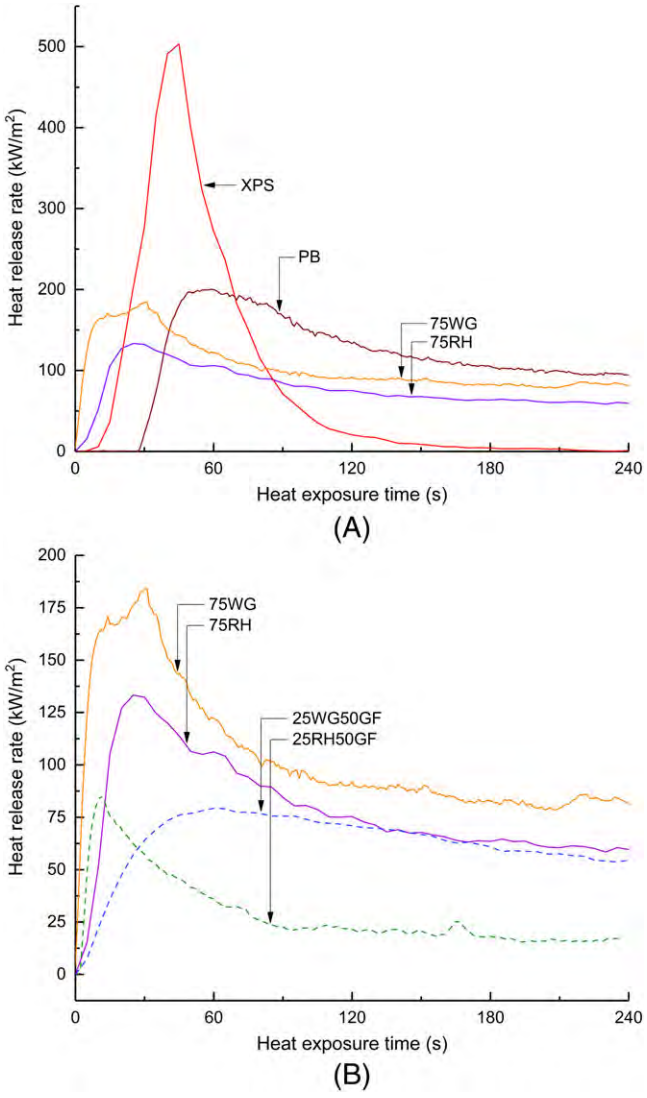

FIGURE 5 Heat release rate (kW/m^2) of the wheat grain-based and rice hull-based mycelium composites with increasing exposure time at a thermal heat flux of 50 kW/m^2 compared to (A) synthetic extruded polystyrene (XPS) foam and STRUCTAflor® particleboard and (B) mycelium composites incorporating 50 wt% glass fines. Abbreviations: 75RH, 75 wt% rice hulls; 75WG, 75 wt% wheat grains; 25RH50GF, 25 wt% rice hulls + 50 wt% glass fines; 25WG50GF, 25 wt% wheat grains + 50 wt% glass fines; XPS, ClimaFoam® extruded polystyrene (XPS) foam; PB, STRUCTAflor® particleboard [Colour figure can be viewed at wileyonlinelibrary.com]

to fire retardancy improvements noted in organic polymers because of combustion of silicones.[46]

Inclusion of glass fines resulted in even greater reductions in average and PHRR in both wheat grain-based (RHR_{180} from 107 to 42 kW/m^2 and PHRR from 185 to 79 kW/m^2) and rice hull-based (RHR_{180} from 85 to 33 kW/m^2 and PHRR from 133 to 85 kW/m^2) mycelium composites (Figure 5B). The presence of glass fines significantly increased the silica content of the composites which would otherwise be almost negligible for wheat grains[47] and a modest 15 to 20 wt% silica in rice hulls.[22] Silica in rice hulls is biosynthesised through the polymerisation of silica acid by living organisms and distributed as hydrated grains.[48] The greater mass retention of mycelium composites containing glass fines, characterised by a very low mass

loss rate (dm/dt) and low overall mass loss (Figure 6), reflected a lower flammable constituent content than composites comprising only of rice hulls or wheat grains (Figure 6) which contain cellulose, starch, and other flammable organics[10,22] that contribute to greater mass loss and heat release (Figures 5, 6, and 7 and Table 2).

The reduced heat release rates of the mycelium composites containing glass fines resulted in longer estimated times to flashover (t_{fo}). Estimated time to flashover was calculated in accordance with ASTM E1354-17 and is based on time to ignition (t_{ig}), material density

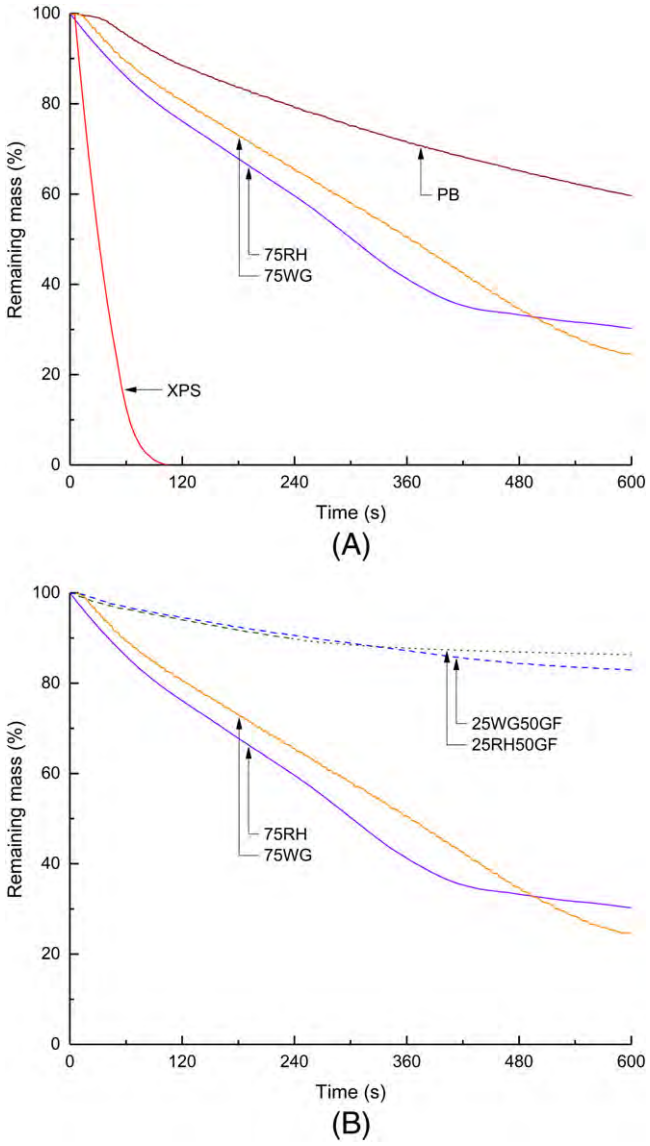

FIGURE 6 Remaining mass (%) of the wheat grain-based and rice hull-based mycelium composites with increasing exposure time at a thermal heat flux of 50 kW/m^2 compared to (A) synthetic extruded polystyrene (XPS) foam and STRUCTAflor® particleboard and (B) mycelium composites incorporating 50 wt% glass fines. Data are extrapolated from 240 to 600 seconds for 25 wt% rice hulls + 50 wt% glass fines as the sample ceased to burn, reaching a negligible heat release rate within 240 seconds. Abbreviations: 75RH, 75 wt% rice hulls; 75WG, 75 wt% wheat grains; 25RH50GF, 25 wt% rice hulls + 50 wt% glass fines; 25WG50GF, 25 wt% wheat grains + 50 wt% glass fines; XPS, ClimaFoam® extruded polystyrene (XPS) foam; PB, STRUCTAflor® particleboard [Colour figure can be viewed at wileyonlinelibrary.com]

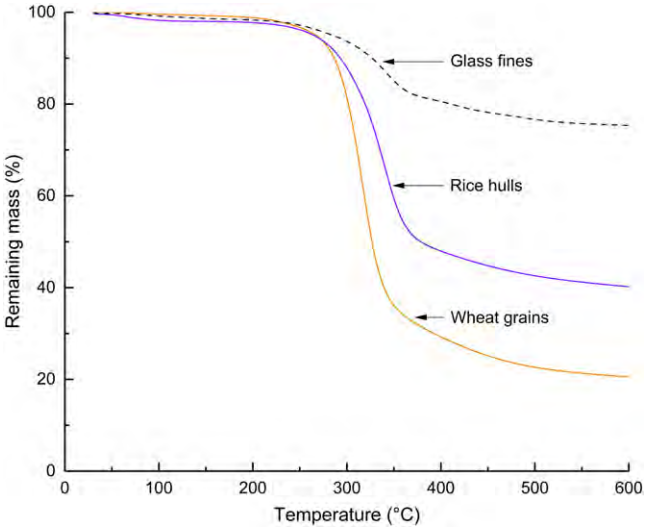

FIGURE 7 TGA mass loss curves for the wheat grain, rice hull, and glass fine constituents of the mycelium composites [Colour figure can be viewed at wileyonlinelibrary.com]

(ρ) and heat release rate (through total heat release over 300 seconds, THR_{300}) through the equation $t_{fo} = 0.07\frac{t_{ig}^{0.25}\rho^{1.7}}{THR_{300}^{1.3}} + 60$.[35] Flashover is a common and very dangerous occurrence in residential and building fires which often involve abundant combustible material.[49] Flashover is the near-simultaneous ignition of all exposed materials in an enclosed area. It occurs if the ignition temperatures of the materials are reached before the initial fuel source is exhausted or the fire extinguished.[50] Fires that reach flashover are approximately 10 times more dangerous than fires that do not, and as such, steps should be taken to prevent flashover.[49,50] The presence of glass fines significantly increased the estimated time to flashover in both wheat grain-based (94 to 370 seconds) and rice hull-based (75 to 311 seconds) mycelium composites and resulted in the mycelium composites far outperforming both XPS foam (61 seconds) and particleboard (173 seconds) (Table 2).

While heat release and flash over are important fire safety parameters, the majority of fire-related fatalities occur because of exposure to toxic gases rather than burns, generalised trauma, or other causes.[10,51] Short-term exposure to smoke consisting of small fragments of fibre and ultrafine carbon particles is not considered a serious health hazard to humans but is an important safety concern because dense smoke can reduce visibility, cause disorientation, and hinder firefighting efforts.[10] Carbon monoxide (CO) is generally considered the greatest individual hazard, and even very low levels can cause incapacitation and death (eg, 1500 ppm will cause death within an hour).[52] In contrast, carbon dioxide (CO_2) concentration must be more than 60 times higher (100 000 ppm) to cause death over the same period.[10]

Mycelium composites released less smoke (0.9-70 m^2/m^2) and CO_2 (6.3-23.8 g) than synthetic construction materials (64-1184 m^2/m^2 smoke and 15.2-29.9 g CO_2) over 180 seconds from ignition. Mycelium composites comprising of rice hulls also produced significantly less CO (0.02 g) than the synthetic samples (0.47 g) over 180 seconds from ignition; however, the inclusion of glass fines in

the mycelium composites resulted in a significant increase in CO produced (0.91 g) (Table 2). Similar behaviour was observed in the samples comprising of wheat grains and glass fines (0.39 g) compared with the wheat grain only samples (0.33 g), although the increase was minimal. Despite the total CO production being higher, glass fines reduced CO production during the first 30 seconds of combustion in wheat grain-based samples; no initial reduction was observed in rice hull-based samples. This was most likely attributable to incomplete combustion resulting from reduced oxygen supply (Figure 8).

Despite igniting quickly, mycelium composites had much lower average (up to 4 times) and peak (up to 6 times) heat release rates and longer estimated time to flashover (up to 6 times) than the synthetic construction materials tested. They also released significantly less smoke (up to 1315 times) and CO_2 (up to 4 times), although CO production fluctuated. Lower cost rice hull waste-based composites yielded superior fire performance to composites comprising of the more expensive wheat grains. Glass fine waste also improved all fire reaction and safety properties excluding CO production. Mycelium composites exhibited far better fire safety parameters than the traditional construction materials tested overall, and with rice hull-optimised and glass fine-optimised compositions, their widespread use would provide a cheap, sustainable means of significantly reducing the flammability and fire danger of modern houses and buildings.

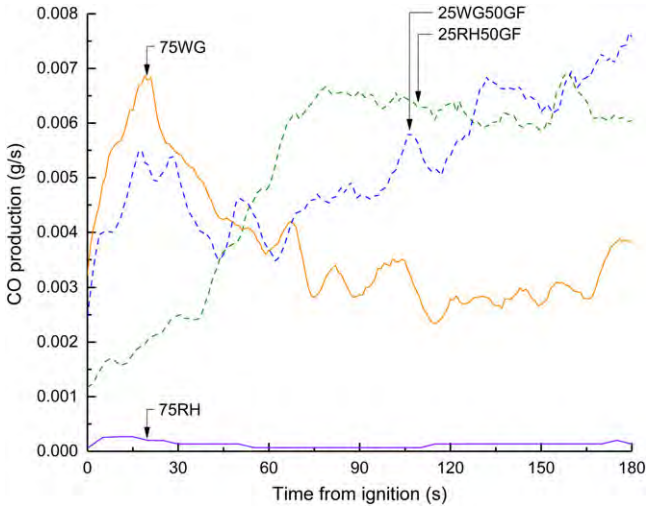

FIGURE 8 Carbon monoxide (CO) production (g/seconds) of wheat grain and rice hull-based mycelium composites with and without glass fines. Time from ignition (seconds) is the time from the point of ignition (ie, t = 0 is the time of ignition). Total COP_{180} values were determined by calculating the area under the curves. Abbreviations: 75RH, 75 wt% rice hulls; 75WG, 75 wt% wheat grains; 25RH50GF, 25 wt% rice hulls + 50 wt% glass fines; 25WG50GF, 25 wt% wheat grains + 50 wt% glass fines [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | Composition and cost

Raw material cost and subsequently the ratio of substrate to additive used are the most significant factors in the market price of mycelium composites because their natural biological manufacturing process has almost negligible associated cost. Mass-specific cost ($AU/kg) theoretically decreased with increased glass fine content because glass fines have a lower economic value per kilogram than rice hulls (Figure 9). However, glass fines are much heavier than rice hulls which caused a significant increase in material density (kg/m^3) and theoretical volume-specific cost ($AU/m^3) with increased glass fine content. While the increased density and volume-specific costs lessen the economic benefits of glass fines, overall the theoretical density-specific cost was reduced with increased glass fine content (Figure 9). The volume-specific cost is most appropriate for construction applications because materials are typically purchased and installed based on volume-specific or area-specific requirements. Subsequently, for construction applications, higher concentrations of glass fines resulted in increased costs.

3.4 | Fire safety and cost overview

The overall fire performance of all samples relative to volume-specific cost was assessed to establish how compositional variation affected the viability of mycelium composites as alternatives to typical synthetic construction materials. Cost-specific time to ignition (t_{ig}) and time to

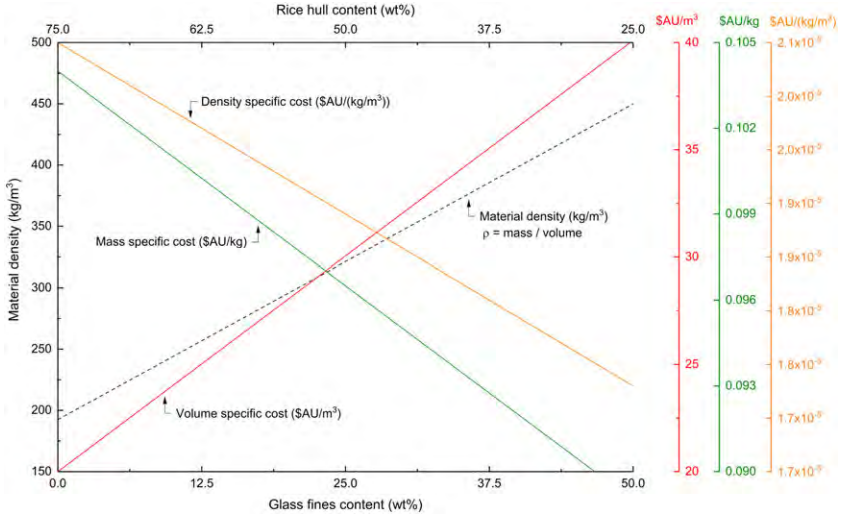

FIGURE 9 Effect of rice hull and glass fine content on the material cost ($AU) and density (kg/m^3) of mycelium composites. Commercial values reflect the most recent available literature and are adjusted for inflation to the 2018 $AU value. All samples contained 25 wt% wheat grains valued at $AUD251/tonne,[30] 25 to 75 wt% rice hulls valued at $AUD55/tonne,[7] and 0 to 50% glass fines valued at $AUD24.5/tonne.[6] Economic values are mean values for commodities procured within Australia. Rice hulls can be procured in Asia for <$AUD20/tonne[30] [Colour figure can be viewed at wileyonlinelibrary.com]

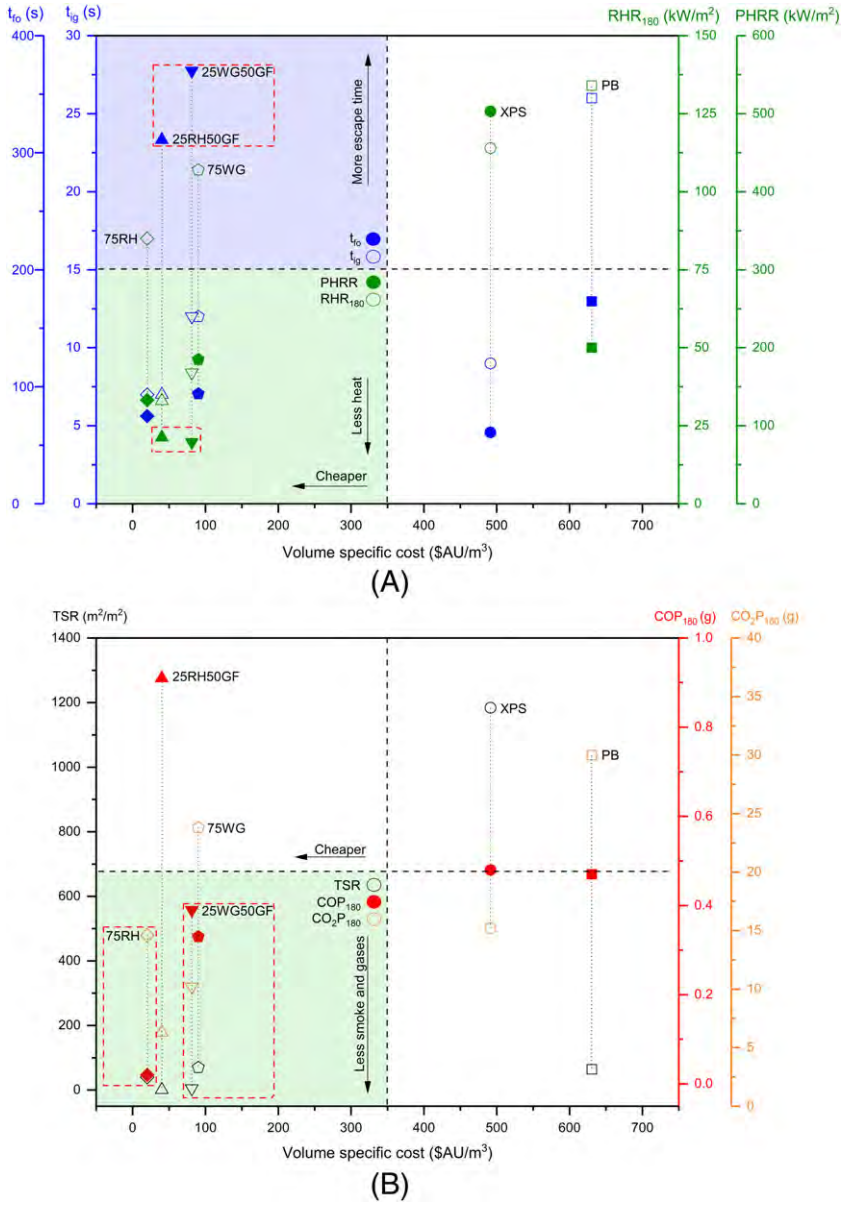

FIGURE 10 Overview of the volume-specific cost of mycelium composites and synthetic construction materials with respect to (A) fire performance and (B) smoke and gas release. For fire performance, the safest and cheapest materials were identified in the left-hand quadrants. Materials associated with more escape time have longer times to ignition (t_{ig}, seconds) and flashover (t_{fo}, seconds) (upper quadrants). Materials associated with less heat generation have lower average (RHR_{180}) and peak (PHRR) heat release rates (kW/m^2) (lower quadrants). For smoke and gas release, safer and more cost-effective materials were identified in the green quadrant. Safer materials have lower total smoke release (TSR), CO (COP_{180}), and CO_2 (CO_2P_{180}) production. Costs of mycelium composites are derived from Figure 9. Cost of extruded polystyrene (XPS) is $AU15.06/m^2 (30-mm thickness).[53] Cost of particleboard is AU$11.35/m^2 (18-mm thickness).[54] Commercial values reflect the most recent available literature and are adjusted for inflation to the 2018 $AU value. Abbreviations: 75RH, 75 wt% rice hulls; 75WG, 75 wt% wheat grains; 25RH50GF, 25 wt% rice hulls + 50 wt% glass fines; 25WG50GF, 25 wt% wheat grains + 50 wt% glass fines; XPS, ClimaFoam® extruded polystyrene (XPS) foam; PB, STRUCTAflor® particleboard [Colour figure can be viewed at wileyonlinelibrary.com]

flashover (t_{fo}) (blue) were plotted with average (RHR_{180}) and PHRR (green) to identify the safest and cheapest materials (left-hand quadrants). All mycelium composites were significantly cheaper and safer than the synthetic construction materials considered. Rice hull-based and wheat grain-based mycelium composites incorporating 50 wt% glass fines (25RH50GF and 25WG50GF respectively) had the longest times to flashover overall. This is the most critical property to human escape in fires. These compositions also had the lowest average and PHRR and were 6 to 12 times cheaper (AU$40/m^3 and AU$81/m^3 respectively) than extruded polystyrene (XPS, AU$491/m^3) and particleboard (PB, AU$630/m^3) (Figure 10A).

Cost-specific total smoke release (TSR, black), CO (COP_{180}, red), and CO_2 (CO_2P_{180}, orange) production were also plotted (safest and cheapest materials associated with green quadrant). All mycelium composites produced less smoke than the synthetic construction materials. Compositions of 75 wt% rice hulls (75RH) had the lowest CO production overall and a low CO_2 production and were 24 to 31 times cheaper ($AU20/m^3) than extruded polystyrene and particleboard. Compositions of 25 wt% wheat grains + 50 wt% glass fines (25WG50GF) also had very low CO and CO_2 production (Figure 10B).

4 | CONCLUSION

This study has found mycelium composites to be an economical, sustainable, and thermally safer alternative to petroleum-derived and natural gas-derived synthetic construction materials. In particular, mycelium composites had much lower average and PHRR and longer estimated time to flash over than the synthetic construction materials considered. They also released significantly less smoke and CO_2. Rice hulls yielded significant char and silica ash which improved fire performance, but composites containing glass fines exhibited the best fire performance because of their significantly higher silica concentrations and low combustible material content. Increased concentrations of glass fines increased volume-specific cost but reduced mass-specific

and density-specific costs. Overall, mycelium composites were very economical and exhibited far better fire safety parameters than the traditional construction materials tested. Their widespread use in civil construction would enable better fire safety in buildings.

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CONFLICT OF INTEREST

None.

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REFERENCES

1. Jones M, Huynh T, Dekiwadia C, Daver F, John S. Mycelium composites: A review of engineering characteristics and growth kinetics. *J Bionanosci*. 2017;11(4):241-257.
2. Haneef M, Ceseracciu L, Canale C, Bayer IS, Heredia-Guerrero JA, Athanassiou A. Advanced materials from fungal mycelium: Fabrication and tuning of physical properties. *Sci Rep*. 2017;7:41292.
3. Holt G, Mcintyre G, Flagg D, Bayer E, Wanjura J, Pelletier M. Fungal mycelium and cotton plant materials in the manufacture of biodegradable molded packaging material: Evaluation study of select blends of cotton byproducts. *J Biobased Mater Bio*. 2012;6(4):431-439.
4. Pelletier MG, Holt GA, Wanjura JD, Bayer E, McIntyre G. An evaluation study of mycelium based acoustic absorbers grown on agricultural by-product substrates. *Ind Crop Prod*. 2013;51:480-485.
5. Islam M, Tudryn G, Bucinell R, Schadler L, Picu R. Morphology and mechanics of fungal mycelium. *Sci Rep*. 2017;7(1):13070.
6. Sustainability Victoria. Market summary—recycled glass. 2014. http://www.sustainability.vic.gov.au/-/media/SV/Publications/Government/Waste-and-resource-recovery/Recycled-materials-in-pavement/Market-Analysis-Glass-Sept-2014-PDF.pdf?la=en Accessed December 14, 2017.
7. DP CleanTech. Understanding rice husk as a biomass. *Fuel*. 2013. http://www.dpcleantech.com/files/Understanding-rice-husk-as-a-biomass-fuel-EN-V1-2013.9.4.pdf Accessed December 14, 2017
8. Cho JH, Vasagar V, Shanmuganathan K, Jones AR, Nazarenko S, Ellison CJ. Bioinspired catecholic flame retardant nanocoating for flexible polyurethane foams. *Chem Mater*. 2015;27(19):6784-6790.
9. Miao Z, Xingna L, Zhen C, Ji W, Wenhua S. Experimental study of the heat flux effect on combustion characteristics of commonly exterior thermal insulation materials. *Procedia Engineer*. 2014;84:578-585.
10. Mouritz AP, Gibson AG. *Fire properties of polymer composite materials*. New York: Springer Science & Business Media; 2007.
11. Xu Q, Jin C, Jiang Y. Compare the flammability of two extruded polystyrene foams with micro-scale combustion calorimeter and cone calorimeter tests. *J Therm Anal Calorim*. 2017;1-8.
12. Izydorek MS, Samuels MD, Zeeveld PA, Smyser JP. Report on structural stability of engineered lumber in fire conditions. Underwriters Laboratories; 2008. https://www.ul.com/global/documents/corporate/aboutul/publications/newsletters/fire/fsa_issue_3_2009.pdf Accessed December 11, 2017.
13. Melville H. *Fire and cellular polymers*. New York: Springer; 1986:1-4.
14. Stec AA, Hull TR. Assessment of the fire toxicity of building insulation materials. *Energ Buildings*. 2011;43(2):498-506.
15. Defonseka C. *Introduction to polymeric composites with rice hulls*. Smithers Information Limited: Surrey; 2014.
16. Prasad CS, Maiti KN, Venugopal R. Effect of rice husk ash in whiteware compositions. *Ceram Int*. 2001;27(6):629-635.
17. Food and Agriculture Organization of the United Nations. Rice Market Monitor. 2017. http://www.fao.org/fileadmin/templates/est/COMM_MARKETS_MONITORING/Rice/Images/RMM/RMM-Jul17.pdf Accessed December 18, 2017.
18. APC Environmental Management. Market development study used glass for Western Australia Waste Management Board. 2006. https://www.wasteauthority.wa.gov.au/media/files/documents/used_glass_market_study.pdf Accessed December 15, 2017.
19. Department of the Environment and Energy. Australian National Waste Report. 2016. https://www.environment.gov.au/system/files/resources/d075c9bc-45b3-4ac0-a8f2-6494c7d1fa0d/files/national-waste-report-2016.pdf Accessed December 14, 2017.
20. West D. NSW State of Environment Report Section 7 (Waste and Recycling) Fact or Fiction? 2016. http://www.smh.com.au/cqstatic/gxut1y/BAwaste.pdf Accessed December 18, 2017.
21. Alex Fraser Group. Leading the way in glass recycling. 2014. http://www.alexfraser.com.au/article/News/Leading_the_way_in_glass_recycling Accessed December 18, 2017.
22. Ismail MS, Waliuddin A. Effect of rice husk ash on high strength concrete. *Construct Build Mater*. 1996;10(7):521-526.
23. Australian Rural Industries Research and Development Corporation. Furfural chemicals and biofuels from agriculture. 2006. https://rirdc.infoservices.com.au/downloads/06-127.pdf Accessed December 15, 2017.
24. Jones M, Huynh T, John S. Inherent species characteristic influence and growth performance assessment for mycelium composite applications. *Adv Mater Lett*. 2018;9(1):71-80.
25. Bourbonnais R, Paice M, Reid I, Lanthier P, Yaguchi M. Lignin oxidation by laccase isozymes from Trametes versicolor and role of the mediator 2, 2′-azinobis (3-ethylbenzthiazoline-6-sulfonate) in Kraft lignin depolymerization. *App Environ Microb*. 1995;61(5):1876-1880.
26. Nakade K, Nakagawa Y, Yano A, Sato T, Sakamoto Y. Characterization of an extracellular laccase, PbLac1, purified from Polyporus brumalis. *Fungal Biol*. 2010;114(8):609-618.
27. Faruk O, Bledzki AK, Fink H-P, Sain M. Biocomposites reinforced with natural fibers: 2000–2010. *Prog Polym Sci*. 2012;37(11):1552-1596.
28. The International Organization for Standardization. ISO 5660-1: 2002: heat release, smoke production and mass loss rate–Part 1: Heat release rate (Cone calorimeter method). 2002.
29. Shewry PR, Hawkesford MJ, Piironen V, et al. Natural variation in grain composition of wheat and related cereals. *J Agr Food Chem*. 2013;61(35):8295-8303.
30. RaboResearch Food and Agribusiness. Agribusiness monthly. 2017. https://www.rabobank.com.au/-/media/rabobank-au/files/pdf/agribusiness%20monthly/2017/pdf_agri-monthly-au-may-2017.pdf Accessed December 17, 2017.
31. Kavanagh K. *Fungi: Biology and applications*. New Jersey: Wiley; 2005.
32. Wainwright M, Ali TA, Killham K. Anaerobic growth of fungal mycelium from soil particles onto nutrient-free silica gel. *Mycol Res*. 1994;98(7):761-762.
33. Webster J, Weber R. *Introduction to fungi*. Cambridge: Cambridge University Press; 2007.
34. Prosser JI. Growth kinetics of mycelial colonies and aggregates of ascomycetes. *Mycol Res*. 1993;97(5):513-528.
35. ASTM International. Standard test method for heat and visible smoke release rates for materials and products using an oxygen consumption calorimeter. 2017.

36. Jones M, Bhat T, Wang C, Moinuddin K, John S. Thermal degradation and fire reaction properties of mycelium composites. In 21st International Conference on Composite Materials. Xi'an, China. 2017.
37. Babrauskas V, Peacock RD. Heat release rate: The single most important variable in fire hazard. *Fire Safety J*. 1992;18(3):255-272.
38. Babrauskas V, Fires Q. Why was the fire so big. HHR: The role of heat release rate in described fires. *Fire Arson Investig*. 1997; 47:54-57.
39. Sorathia U, Divisjón C, Lyon R. A review of fire test methods and criteria for composites. *SAMPE J*. 1997;33(4):23-31.
40. Mouritz A, Mathys Z, Gibson A. Heat release of polymer composites in fire. *Compos Part a Appl S*. 2006;37(7):1040-1054.
41. Brown J, Fawell P, Mathys Z. Fire-hazard assessment of extended-chain polyethylene and aramid composites by cone calorimetry. *Fire Mater*. 1994;18(3):167-172.
42. Zhao Q, Zhang B, Quan H, Yam RCM, Yuen RKK, Li RKY. Flame retardancy of rice husk-filled high-density polyethylene ecocomposites. *Compos Sci Technol*. 2009;69(15):2675-2681.
43. Gosselink RJA. Lignin as a renewable aromatic resource for the chemical industry. 2011. http://edepot.wur.nl/186285 Accessed December 14, 2017.
44. Gilwee W. Fire retardant materials in aviation. In: *4th Annual Plastics Symposium*; 1975.
45. Parker J, Kourtides D. New fireworthy composites for use in transportation vehicles. *J Fire Sci*. 1983;1(6):432-458.
46. Hshieh FY. Shielding effects of silica-ash layer on the combustion of silicones and their possible applications on the fire retardancy of organic polymers. *Fire Mater*. 1998;22(2):69-76.
47. Lanning FC, Eleuterius LN. Silica and ash in seeds of cultivated grains and native plants. *Ann Bot*. 1992;69(2):151-160.
48. Bansal V, Ahmad A, Sastry M. Fungus-mediated biotransformation of amorphous silica in rice husk to nanocrystalline silica. *J Am Chem Soc*. 2006;128(43):14059-14066.
49. Liu J, Chow W. Study on flashover in small residential units with an open kitchen by. *Nonlinear Dyn*. 2014. https://repository.up.ac.za/handle/2263/44744 Accessed December 16, 2017.
50. Clarke FB. Physiological effects of smoke: Managing escape. *ASHRAE J*. 1997;39(3):47.
51. Babrauskas V, Levin BC, Gann RG, et al. Toxic potency measurement for fire hazard analysis. *Fire Technol*. 1992;28(2):163-167.
52. Hirschler MM. Fire hazard and toxic potency of the smoke from burning materials. *J. Fire Sci*. 1987;5(5):289-307.
53. Knauf Insulation. Product Price List. 2015. http://www.knaufinsulation.com.au/sites/knaufinsulation.com.au/files/KIAU1115282BR%20Product%20Guide%20Australia%20LR.pdf Accessed January 15, 2018.
54. Bord. Pressed Timber Veneers & Specialty Panel Products. 2016. https://www.bord.com.au/assets/bord-products-price-list-april-2016.pdf Accessed January 2, 2018.

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Reviewer comments:

Reviewer #2 (Remarks to the Author):
My overall review of this article is that it contains significant findings that are relevant to advancing mycelium-based materials to becoming commercially accepted alternatives to traditional building materials. I strongly encourage the authors to make the minor corrections suggested below and suggested modifications so the paper can be published.

Page 2, Line 19: change "customise" to "customize."

Page 3, Line 18: The clarity of the sentence may be enhanced with the inclusion of a "," after "...grown on different substrates..."

Page 3, Line 49: change "sterilised" to "sterilized."

Page 6, Line 26: change "utilising" to "utilizing."

Page 7, Line 11: change "oxidise" to "oxidize."

Figure 6: I believe these two figures should be separated so they can be enlarged with separate headings. Combining them makes it difficult to read the details of the figures.

Reviewer query 1: Page 2, Line 19: change "customise" to "customize."

Authors' response:
Completed as requested. Now reads "customize."

Reviewer query 2: Page 3, Line 18: The clarity of the sentence may be enhanced with the inclusion of a "," after "...grown on different substrates..."

Authors' response:
Completed as requested. Now reads "This is the first study into the growth process of mycelium composites using industrial (glass fines) and agricultural (rice hulls) wastes, the thermal degradation properties of mycelium grown on different substrates, and the fire safety of the resultant composites."

Reviewer query 3: Page 3, Line 49: change "sterilised" to "sterilized."

Authors' response:
Completed as requested. Now reads "sterilized."

Reviewer query 4: Page 6, Line 26: change "utilising" to "utilizing."

Authors' response:
Completed as requested. Now reads "utilizing"

Reviewer query 5: Page 7, Line 11: change "oxidise" to "oxidize."

Authors' response:
Completed as requested. Now reads "oxidize"

Reviewer query 6: Figure 6: I believe these two figures should be separated so they can be enlarged with separate headings. Combining them makes it difficult to read the details of the figures.

Authors' response:
Completed as requested. Figure has been divided into Figures 6 (a) and (b). Curve division is consistent with Figures 5 (a) and (b)

The authors thank reviewer 2 for their support and feedback. All suggested corrections have been addressed.

Reviewer #4 (Remarks to the Author):
This paper contains significant and interesting results regarding flammability of mycelium composite materials and their economic assessment, which merits publication on "Fire and Materials" a lot. However, I would like the authors to clarify the following doubtful items and to correct the descriptions or data if necessary.

1) On the first line of page 3, there is a statement "Glass fines are very small (< 60 mm diameter) glass pieces", but I feel that a glass piece with the diameter of 60 mm appears to be rather coarse than fine. Is the definition on "< 60 mm" in diameter certainly correct?

2) In the Section 2.4 regarding "SEM and elemental analysis" on page 4, there is a description as "a spot size of 5". I would like to ask the authors to explain the definition of the "spot size" in brief and to show us the unit of "5".

3) In the sentence "Similar behaviour was observed in the samples comprising of wheat grains and glass fines (0.33 g) compared with the wheat grain only samples (0.39 g), although the increase was minimal." of the third paragraph of page 8, I suppose the two figures, 0.33 and 0.39, must be opposite and should be changed if Table 2 is completely correct.

4) Time to peak HRR of "75WG" in Figure 5 (b) (i.e., ca. 150 s) seems to be about 5 times as long as that in Figure 5 (a) (i.e., ca. 30 s) on page 19 although the data must be similar. I suppose the scale of heat exposure time of "75WG" and "25WG50GF" in Figure 5 (b) may possibly be incorrect and the graphs should desirably be revised after the authors' careful reconsideration.

5) I suppose that the availability and cost of the waste (e.g., rice hulls, glass fines, etc.) and the material products (e.g., extruded polystyrene, particleboard, etc.) may possibly vary and fluctuate with time or period. So I think it desirable for the authors to clarify the time or period of the adopted data, for example, by describing "as of 2017", "as of 2015 to 2017" or something like that, somewhere in this paper.

Reviewer query 1: On the first line of page 3, there is a statement "Glass fines are very small (< 60 mm diameter) glass pieces", but I feel that a glass piece with the diameter of 60 mm appears to be rather coarse than fine. Is the definition on "< 60 mm" in diameter certainly correct?

Authors' response:
Although glass fines are technically defined as contaminated glass waste of < 60 mm diameter (as referenced in manuscript), the authors appreciate that the higher end of this range may not reflect material most commonly defined as "glass fines". As such the sizing description has been removed and the definition rephrased to "Glass fines are very small pieces of glass that are too fine to be efficiently recycled due to the presence of similar-sized organic and inorganic contaminants which cannot be easily separated".

Reviewer query 2: In the Section 2.4 regarding "SEM and elemental analysis" on page 4, there is a description as "a spot size of 5". I would like to ask the authors to explain the definition of the "spot size" in brief and to show us the unit of "5".

Authors' response:
The spot size is the cross-sectional diameter that the cone of the beam makes on the surface of the sample and affects 1) the resolution of the image and 2) the number of electrons generated (therefore the graininess of the image). It is analogous to the volume control of an amplifier. In consultation with the electron microscopists at The Royal Melbourne Institute of Technology University Microscopy and Microanalysis Facility it has been decided to omit spot size data from the research article as it is not a necessarily inclusion and may confuse readers.

Reviewer query 3: In the sentence "Similar behaviour was observed in the samples comprising of wheat grains and glass fines (0.33 g) compared with the wheat grain only samples (0.39 g), although the increase was minimal." of the third paragraph of page 8, I suppose the two figures, 0.33 and 0.39, must be opposite and should be changed if Table 2 is completely correct.

Authors' response:
The authors are especially grateful for this feedback. There was indeed an error with the in-text values identified by reviewer 4 which has now been rectified.

Reviewer query 4: Time to peak HRR of "75WG" in Figure 5 (b) (i.e., ca. 150 s) seems to be about 5 times as long as that in Figure 5 (a) (i.e., ca. 30 s) on page 19 although the data must be similar. I suppose the scale of heat exposure time of "75WG" and "25WG50GF" in Figure 5 (b) may possibly be incorrect and the graphs should desirably be revised after the authors' careful reconsideration.

Authors' response:
The authors are especially grateful for this feedback. There was indeed a scaling error for the "75WG" function in Figure 5(b) which has now been rectified.

Reviewer query 5: I suppose that the availability and cost of the waste (e.g., rice hulls, glass fines, etc.) and the material products (e.g., extruded polystyrene, particleboard, etc.) may possibly vary and fluctuate with time or period. So I think it desirable for the authors to clarify the time or period of the adopted data, for example, by describing "as of 2017", "as of 2015 to 2017" or something like that, somewhere in this paper.

Authors' response:
The most recent values for material costs are utilised in this study. However, the year of publication for these values varies. The labelling of every value with the year it was published would be akin to referencing the values. References have already been provided for all values which detail the exact time of validity. However, to further clarify the time-dependent validity of the data used, a sentence reading "Commercial values reflect the most recent available literature and are adjusted for inflation to the 2018 $AU value" has been included in the captions of Figure 9 and 10.

The authors thank reviewer 4 for their support and feedback. All suggested corrections have been addressed.

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Publication G

Addresses Research Question 5:

Can the mechanical properties of mycelium be improved by removing non-structural hyphal elements using chemical treatment?

Article

Cite This: *Biomacromolecules* XXXX, XXX, XXX−XXX

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Waste-Derived Low-Cost Mycelium Nanopapers with Tunable Mechanical and Surface Properties

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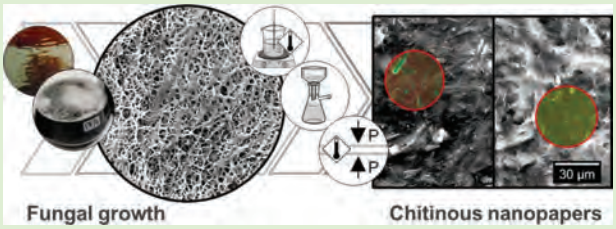
Supporting Information

ABSTRACT: Mycelium, the vegetative growth of filamentous fungi, has attracted increasing commercial and academic interest in recent years because of its ability to upcycle agricultural and industrial wastes into low-cost, sustainable composite materials. However, mycelium composites typically exhibit foam-like mechanical properties, primarily originating from their weak organic filler constituents. Fungal growth can be alternatively utilized as a low-cost method for on-demand generation of natural nanofibrils, such as chitin and chitosan, which can be grown and isolated from liquid wastes and byproducts in the form of fungal microfilaments. This study characterized polymer extracts and nanopapers produced from a common mushroom reference and various species of fungal mycelium grown on sugarcane byproduct molasses. Polymer yields of ∼10−26% were achieved, which are comparable to those of crustacean-derived chitin, and the nanopapers produced exhibited much higher tensile strengths than the existing mycelium materials, with values of up to ∼25 MPa (mycelium) and ∼98 MPa (mushroom), in addition to useful hydrophobic surface properties resulting from the presence of organic lipid residues in the nanopapers. HCl or H_2O_2 treatments were used to remove these impurities facilitating tuning of mechanical, thermal, and surface properties of the nanopapers produced. This potentially enables their use in a wide range of applications including coatings, membranes, packaging, and paper.



1. INTRODUCTION

The vegetative growth of filamentous fungi (mycelium) has attracted increasing academic and commercial interest over the past decade as a natural binder for packaging, acoustic and thermal insulation, and textile materials.[1−5] Mycelium binds organic matter through a network of hyphal microfilaments in a natural biological process which allows them to be exploited to produce useful composite materials.[1,2,6−8] Mycelium-derived materials have several key advantages over traditional synthetic materials including their low cost, low environmental impact and carbon footprint, reduced energy consumption, and biodegradability.[6,9,10] Unfortunately, mycelium-derived materials are typically limited to mechanical properties resembling foams and natural materials. Mycelium composites comprise a combination of fungal mycelium and undigested lignocellulosic material and have foam-like mechanical properties with ultimate tensile strengths of up to 1.1 MPa.[6] Conversely, the mycelial biomass comprises only fungal mycelium and exhibits material properties typical of natural materials, such as wood and cork, with tensile strengths of up to 9.6 MPa for *Schizophyllum commune*.[11] Limitations in the strength of mycelium composites result from the often low-strength agricultural waste or byproducts utilized in these composites as fillers, which are weakly bonded by a hyphal filament matrix,[3] while the strength of the mycelium itself is limited by the presence of non-structural elements, such as cytoplasm, proteins, and lipids present in the fungal biomass.[12]

The mechanical performance of mycelium-derived materials can be improved by eliminating the use of these low-strength wastes and byproducts as composite fillers, and instead utilizing them solely as nutrient sources for fungal growth and removing non-structural elements from the isolated mycelium. This process constitutes the conversion of agricultural biomass into natural polymers within the fungal

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Table 1. Fungal Species Used in This Study by Biomass Component Utilized, Polymers Present in the Cell Wall, Biosafety Level, and Hyphal Branching Type[a]

species	biomass	fibrillar polymers	hyphal branching	biosafety
A. bisporus	fruiting body	chitin−glucan	monomitic	1
A. arbuscula	mycelium	chitin−glucan	sympodially/dichotomously	1
M. genevensis	mycelium	chitin−chitosan	sympodially/monopodially	1
T. versicolor	mycelium	chitin−glucan	trimitic	1

[a]Compiled from Kavanagh,[12] Webster and Weber,[15] American Type Culture Collection,[24] Bioresource Collection and Research Center,[25] and the U.S. Department of Health and Human Services.[26]

biomass, such as nitrogenous polysaccharide-based nanofibers, that can be extracted from the cell walls of the hyphae within the mycelial biomass. Chitin is a linear macromolecule composed of acetylated *N*-acetylglucosamine that is also the main component of the exoskeleton of most insects and other arthropods.[13] It is strong with a nanofibril tensile strength of ∼1.6−3.0 GPa[14] due to hydrogen bonding along the chains which gives them rigidity.[15]

Mycelium-derived chitin offers a cheap, renewable, easily isolated, and abundant alternative to more expensive, seasonally and regionally limited, allergenic crustacean chitin.[16−18] The fungal chitin structure is also associated with more pliable branched β-glucan or chitosan, providing a native nanocomposite architecture that is both strong and tough.[19] Chitin derived from mycelium is also more viable than fungal chitin derived from mushrooms, which takes much longer to grow and if derived from edible mushrooms is more expensive, directly competing with food supply.

This study aimed to produce nanopapers produced from mycelium-derived chitin−glucan or chitin−chitosan exhibiting better mechanical properties than existing mycelium materials. Emphasis was on cost and environmental impact with only cheap agricultural byproducts and natural fungal growth used to obtain chitinous fungal biomass. Structural polymers, such as chitin and chitosan, were then extracted from this fungal biomass using simple alkaline treatment, followed by vacuum filtration and hot-pressing to produce homogenous nanopapers. The morphology, composition, and molecular structure of the nanopapers were then analyzed in addition to their physical, mechanical, and surface properties.

2. EXPERIMENTAL SECTION

2.1. Materials. *Allomyces arbuscula* and *Mucor genevensis* were obtained from the RMIT University Fungal Culture Collection (Bundoora, Australia). The cultures were stored under oil on a nutrient agar slope, which was subcultured onto fresh sterile malt extract agar (Neogen, Michigan) plates and incubated inverted at 25 °C in darkness for 7 d. *Trametes versicolor* was purchased from New Generation Mushroom Supplies (Melbourne, Australia). The sample was supplied as a mycelium on wheat grains sealed in a plastic bag with a filter patch. This isolate was subcultured onto malt extract agar plates and incubated as above. *Agaricus bisporus* (white button) mushrooms were purchased from a local convenience store (origin: B. Fungi Kft, Ocsa, Hungary). Blackstrap molasses was purchased from Nortem Biotechnology (El Puerto de Santa Maria, Spain). NaOH (≥97.0%), H_2O_2 (34.5−36.5%), and HCl (37% ACS reagent) were purchased from Sigma-Aldrich. Deionized water was used for all the experiments.

2.2. Species and Medium Selection. Fungal species were selected for use in this study based on their fibrillar structure polymers, biosafety level, hyphal branching, and growth performance (Table 1). Fibrillar cell wall polymers, such as chitin, chitosan, and glucan, were of primary interest in this study, but it should be noted that cell walls also contain polysaccharides (e.g., galactose, mannose, and fucose), phosphate, proteins, lipids, and mineral salts.[12] *T. versicolor* is a widely available trimitic white-rot fungus containing chitin and glucan fibrillar cell wall polymers. It is commonly used in mycelium-based materials science applications[3,6,20] and is known to have a high growth rate and biomass yield.[21] *A. arbuscula* also comprises a chitin−glucan cell wall structure but has been noted to have much higher cell wall chitin content than other fungi.[22] It is also sympodially/dichotomously branched giving it a higher growth rate than the sympodially/monopodially branched *M. genevensis*, which was selected for its chitin−chitosan cell wall structure rather than its growth rate. Only safe species (biosafety level 1) were used in this study. The mycelium (vegetative rootlike structure) of these species was grown on the sugarcane byproduct blackstrap molasses, an exceptional fungal nutrient with biomass yields comparable to or better than those of the commonly used laboratory nutrient malt extract.[23] Production of mycelial biomass from blackstrap molasses constituted a sustainable and rapid upcycling of low-cost liquid waste into chitin−glucan or chitin−chitosan fibrillar polymers, which could directly compete with more expensive crustacean- and fruiting body (mushroom)-derived chitin. The common white button mushroom (*A. bisporus* fruiting body) was also included in this study for reference (Table 1).

2.3. Fungal Growth. Molasses liquid media were prepared by diluting blackstrap molasses using water (1 g/10 mL) and autoclaving at 121 °C for 20 min. Shallow liquid bodies (approximately 100 mL) were aseptically dispensed into 1 L glass vessels. Isolate cultures of each fungal species were cut into inoculum disks with a diameter of 7 mm and suspended in the liquid media, which was left at room temperature (25 °C) for 14 d on an IKA KS260 Basic orbital shaker at 50 rpm. The resulting mycelia (fungal biomass) were then washed with water and vacuum filtered (VWR 125 mm qualitative filter paper 413, particle retention 5−13 μm) to remove any excess water. The total wet biomass quantity ($WM_{\text{fungal biomass}}$) was assessed using a laboratory balance. Triplicate 50 mg samples were then removed from the wet biomass and weighed pre- (wet mass, WM_{sample}) and post-drying (dry mass, DM_{sample}) at 105 °C for 12 h using a Sartorius Cubis microbalance. The water content of each sample was calculated, and the average water content ($\overline{\text{water content}}$) of the wet fungal biomass was obtained from the average of the triplicate samples. The total dry fungal biomass ($DM_{\text{fungal biomass}}$) produced was then ascertained by subtracting the water content from the total wet biomass quantity.

$$\text{Water content (\%)} = \left(1 - \frac{DM_{\text{samples 1,2,3}}\,(\text{g})}{WM_{\text{samples 1,2,3}}\,(\text{g})}\right) \times 100 \quad (1)$$

$$DM_{\text{fungal biomass}}\,(\text{g}) = WM_{\text{fungal biomass}}\,(\text{g}) \times (100 - \overline{\text{water content}}) \quad (2)$$

2.4. Extraction and Treatment of Natural Polymers. Mycelial biomass was initially washed thrice with water and submerged for 5 min to remove any remaining molasses residue. The biomass was then blended for 5 min in 500 mL of water and the resulting suspension was heated to 85 °C for 30 min. The suspension was then cooled to 25 °C and centrifuged at 9000 rpm for 15 min at 18 °C. The resultant residue was resuspended in a 1 M NaOH solution for 3 h at 65 °C.

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Biomacromolecules XXXX, XXX, XXX−XXX

The suspension was cooled to 25 °C and then neutralized (pH 7) by repeated centrifugation and redispersion of the residue in water.

The total dry polymer extract quantity ($DM_{polymer\ extract}$) was assessed using the total wet polymer extract quantity and triplicate wet polymer extract samples dried and assessed as before for fungal biomass. These values were compared with the values previously determined for the dry mass of the fungal biomass grown, which allowed evaluation of the percentage conversion from fungal biomass to polymer extract (yield) for each species based on the total dry biomass ($DM_{fungal\ biomass}$) and total dry polymer extract ($DM_{polymer\ extract}$). The polymer extract hydrogel was stored at 4 °C until further use.

$$\text{Yield}\ (\%) = \frac{DM_{polymer\ extract}\ (g)}{DM_{fungal\ biomass}\ (g)} \times 100 \quad (3)$$

2.5. Preparation of Nanopapers. Chitin−glucan or chitin−chitosan polymer extracts were suspended in 500 mL of water to allow for the production of nanopapers with a grammage of 50 g/m^2. The suspensions were vacuum filtered (VWR 125 mm qualitative filter paper 413, particle retention 5−13 μm), peeled, and cold-pressed for 5 min between blotting papers between metal plates under a 5 kg mass to remove excess moisture. The extracts were then hot-pressed at 120 °C for 15 min under 500 kg to achieve the final nanopapers. The entire nanopaper production process is summarized in Figure 1.

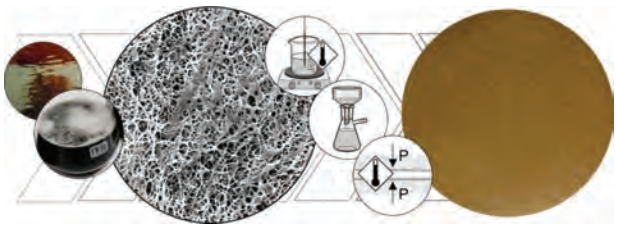

Figure 1. Nanopaper production process. A molasses medium is initially inoculated with the species of interest which grows to form a thick hyphal network. The biomass can then be treated using NaOH, the residue collected, vacuum-filtered and hot-pressed to produce the final nanopaper.

2.6. Morphological and Elemental Analysis of the Nanopapers. Scanning electron microscopy (SEM) imaging and energy dispersive spectroscopy (EDS) elemental analysis of each nanopaper was performed using a Zeiss Supra 55 VP scanning electron microscope with an Oxford X-Max^N 20 energy dispersive X-ray spectrometer attached. An accelerating voltage of 30 kV was used. The EDS spectra were analyzed using AZtecEnergy EDS software. An average spectrum was obtained based on individual spectra from 12 different sites.

C, H, N, S, and O elemental analysis was completed for triplicate 2 mg samples using a EuroVector EA 3000 CHNS−O elemental analyzer. Complete digestion of phosphorus and (earth) alkaline elements in the samples was then completed by digestion in sulphuric acid (phosphorus), followed by mineralization of the samples through combustion at 280 °C (phosphorus) or 590 °C (earth alkaline elements), with the residue dissolved in diluted nitric acid (pH ≈ 3.0). P was determined through detection of *ortho*-phosphate using an Agilent Cary 8454 ultraviolet−visible diode array spectrophotometer and the K, Ca, Mg, and Na content analyzed using a PrinCE Crystal 310 Capillary Electrophoresis instrument, with detection registered using a TraceDec conductivity detector.

Carbohydrate analysis was carried out by high performance anion exchange chromatography (HPEAC). A 300 mg freeze-dried sample was mixed with 3 mL of 72% sulfuric acid at 30 °C for 60 min. The acid was then diluted with water to a 4% concentration and the mixture was placed in an autoclave at 121 °C for 60 min. HPEAC was performed on the previously diluted acid hydrolase with a Dionex ICS3000 chromatograph equipped with a CarboPac PA20 column. Sugar recovery standards were prepared and pretreated under identical hydrolysis conditions prior to HPAEC analysis in order to analyse their recovery throughout the procedure.

2.7. Analysis of the Molecular Structure of the Nanopapers. IR spectra were recorded using an Agilent Cary 630 Fourier transform infrared (FT-IR) instrument with a single reflection diamond attenuated total reflection (ATR)-module and KBr optics (beam splitter). Three spectra were recorded from different portions of the material to verify homogeneity. Spectra were recorded across the full accessible range from 4000 to 400 cm^{-1}.

Solid state nuclear magnetic resonance spectroscopy (ssNMR) was performed on a Bruker AVANCE NEO 500 MHz wide bore system using a 2.5 mm magic angle spinning probe. Nanopapers were first frozen using liquid nitrogen, ground using a pestle and mortar, and passed through a 75 μm sieve to produce a fine homogenous powdered sample for analysis. The resonance frequency for ^{13}C NMR was 125.78 MHz, the MAS rotor spinning was set to 14 kHz. Cross-polarization was achieved by a ramped contact pulse with a contact time of 1 ms. During acquisition ^{1}H was high power decoupled using SPINAL with 64 phase permutations. The chemical shifts for ^{13}C are reported in ppm and are referenced external to adamantane by setting the low field signal to 38.48 ppm.

2.8. Physical and Mechanical Analysis of the Nanopapers. The nanopapers were cut into dog bone shaped specimens (shape according to type 1BB, BS EN ISO 527-2, 2012) using a Zwick ZCP 020 manual cutting press. Specimens had a parallel width of 2 mm and an overall length of 30 mm. The thickness of each specimen was determined using an Anyi Measuring digital outside micrometer. Tensile tests were performed using a model 5969 Instron dual column universal testing system, equipped with a 1 kN load cell and a Gig ProE iMETRIUM noncontact video extensometer. The specimens were fixed between metal clamps using blotting papers to avoid perforation of the samples. The testing velocity was 1 mm/min with the gauge length set to 12 mm. The elastic modulus (E) was analyzed in the linear elastic region as a secant between strength values separated by 0.2% strain. The tensile strength (σ) was calculated from the maximum load and specimen cross-sectional area.

Nanopaper skeletal density was analyzed for 10 replicate measurements using a Micromeritics AccuPyc II 1340 helium gas displacement pycnometry system with a 1 cm^3 chamber.

2.9. Surface Energy Analysis of the Nanopapers. Advancing contact angles of polar (water) and nonpolar (diiodomethane) droplets of test liquids on the nanopapers were determined using a Krüss DSA30 drop shape analyzer. An initial contact angle measurement was recorded 0.5 s after dosing with double sessile drops (3 μL drop of each test liquid), followed by 10 drops (0.2 μL each) dosed and measured with 0.5 s delay between each drop. A total of 100 measurements at 10 individual sites were recorded and screened individually for each nanopaper to ensure that the droplet profiles were well-formed and level. The contact angle and surface free energy were calculated using the Owens, Wendt, Rabel, and Kaelble model[27] using Krüss Advance software (v 1.5.1).

Surface free energy values were verified, and the surface area was assessed using a Surface Measurement Systems inverse gas chromatography (iGC) Surface Energy Analyzer. The surface energy of mycelium-derived nanopapers was determined at 30 °C and 0% relative humidity. Nanopaper samples were cut and inserted into a measurement column (inner diameter 4 mm and outer diameter 6 mm). The specific surface area of the samples was initially determined by octane retention at various coverages, with specific surface area computed using the Brunauer−Emmett−Teller (BET) model from the peak maxima. A series of alkanes (hexane, heptane, octane, nonane, and decane) were used for the determination of the surface energy in addition to the polar probes, dichloromethane, acetone, acetonitrile, and ethyl acetate. The vapors were passed over the nanopaper samples and the retention times and volumes recorded. The total surface energy was computed using the Dorris and Gray method[28] based on the retention times and coverages of the various organic solvents from the peak maximums.

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Table 2. Elemental Analysis for Fungal Polymer Extracts

	elemental composition (wt % of total mass)									
species	C	H	N	O	S	P	K	Ca	Mg	Na
A. bisporus (fruiting body)	41.1	6.2	3.4	47.2	0.02	0.05	0.1	0.4	0.1	0.1
A. arbuscula (mycelium)	47.1	7.1	1.9	38.6	0.2	0.1	0.1	4.8	0.1	0.1
post H_2O_2 treatment	47.4	6.8	1.5	37.6	0.06	0.2	0.1	0.1	0.1	0.1
post HCl treatment	48.3	7.1	1.7	37.8	0.09	0.1	0.1	0.1	0.1	0.1
M. genevensis (mycelium)	47.8	7.5	1.7	38.7	0.08	0.1	0.9	4.8	0.1	0.2
T. versicolor (mycelium)	28.5	4.6	0.3	47.1	0.02	0.05	0.1	20.1	0.1	0.1

Table 3. Sugar Composition Based on Fraction of Total Sugars Present

	sugar composition (wt % of total sugars)					
species	arabinose	galactose	glucosamine	glucose	mannose	other
A. bisporus (fruiting body)	0.1	0.3	42.3	52.0	5.1	0.2
A. arbuscula (mycelium)	4.3	33.1	13.7	42.2	5.9	0.8
post H_2O_2 treatment	3.5	25.4	18.8	45.0	6.7	0.6
post HCl treatment	4.1	27.0	17.9	43.6	6.9	0.7
M. genevensis (mycelium)	2.8	25.0	22.8	43.3	5.7	0.4
T. versicolor (mycelium)	0.0	0.0	12.3	86.3	0.5	0.9

The ζ-potential of the mycelium-derived nanopapers was determined as a function of pH using an Anton Paar SurPASS electro kinetic analyzer. The ζ-potential was measured in an adjustable gap cell (100 μm), with electrolyte solution (1 mM KCl) being pumped through the cell at pressures steadily increased to 300 mbar. The pH was controlled by titrating 0.05 mol/L KOH and 0.05 mol/L HCl into the electrolyte solution and the ζ-potential was determined from the streaming potential.

2.10. Thermal Degradation Analysis. Nanopaper thermal degradation properties were assessed using TA Instruments Discovery thermogravimetric analysis (TGA). Paper fragment samples of approximately 10 mg were placed in a platinum high temperature crucible and heated from 30 to 1000 °C at a heating rate of 20 °C/min. Samples were tested in air and nitrogen atmospheres (both 25 mL/min).

3. RESULTS AND DISCUSSION

3.1. Dry Mass and Polymer Yield of the Mycelial Biomass. The mycelial biomass typically had lower yields (biomass to chitin−glucan or chitin−chitosan polymer extract, corrected for the presence of inorganic Ca salts present in the extracts) (9.6 and 15.0% for *T. versicolor* and *M. genevensis*, respectively) than *A. bisporus* fruiting bodies (19.2%), except for *A. arbuscula*, which had a yield of 25.8% (Table S1 Supporting Information). Thus, fungal chitin sources typically had similar or slightly lower yields than processed crustacean chitin (8−33% yield).[29] This makes fungal chitin a viable, renewable, easily isolated, and abundant alternative to crustacean chitin, which can be rapidly produced on a large scale utilizing heterotrophic growth on inexpensive agricultural byproducts.[17] Nanopapers were then produced and characterized for each species. Mild alkaline extraction was utilized in this study to preserve the natural qualities of the polysaccharide-based nanofibers, with longer deproteination durations resulting in a higher degree of chitin deacetylation.[30,31] NaOH was utilized in this study, however extraction can also be achieved using other cost effective and environmentally sustainable methods utilizing biological fermentation.[32] Bacteria that produce protease, such as *Serratia marcescens*, can be utilized to remove proteins, while bacteria producing lactic acid, such as *Lactobacillus paracasei*, can be used for harsher demineralization if necessary.[33]

3.2. Chemical and Elemental Analysis of the Polymer Extract. Polymer extracts derived from *A. bisporus* fruiting bodies had significantly higher N (3.4 wt %), glucosamine (42.3 wt %), and glucose (52%) contents than those derived from the mycelium (*A. arbuscula*, *M. genevensis* and *T. versicolor*), which indicated the presence of significantly higher chitin−chitosan and glucan fractions in these extracts (Tables 2 & 3). *M. genevensis* and *A. arbuscula* mycelium-derived polymer extracts had similar elemental N contents (1.7 and 1.9 wt %, respectively), however, *M. genevensis* extracts had a significantly higher glucosamine content (22.8 wt %) than *A. arbuscula* extracts (13.7 wt %). This most likely resulted from the lower content of other carbohydrate compounds in the extracts derived from *M. genevensis*. Both *A. arbuscula* and *M. genevensis* also contained significant amounts of glucose (42.2 and 43.3%, respectively) associated with chitin-linked glucan. Polymer extracts produced from biopolymers extracted from *T. versicolor* had very low N concentrations (0.3 wt %); sugar analysis revealed that glucose was the prevalent sugar in these extracts (86.3%), most likely associated with large concentrations of glucan and a low glucosamine content. Polymers extracted from *A. arbuscula* and *M. genevensis* were also associated with significant quantities of arabinose, galactose, and mannose, which are common sugars in many fungal exopolysaccharides.[34]

ATR−FTIR spectroscopy (Figure 2a) confirmed the presence of chitin in most samples. −NH stretching was visible at 3228 cm^{-1}, in addition to an amide I band associated with C═O stretching at 1624 cm^{-1}. Amide II and III bands, resulting from −NH deformation, were also visible at 1544 and 1320 cm^{-1}, respectively, with the amide III band confirming the presence of a secondary amide. The lack of −NH deformation-based interruption in the amide I signal, and the absence of an amide II band, supported the low concentrations of chitin in *T. versicolor* extracts. Conversely, strong amide I−III band signals in *A. bisporus* fruiting body-derived polymer extracts supported the higher chitin content of these extracts. The carbohydrate backbone of the glucan and chitin polymer structure was also visible as an −OH band in all extracts at 3317 cm^{-1}, a −CH bands at 2920 and 2850 cm^{-1}, and a C−O−C band at 1026 cm^{-1}.

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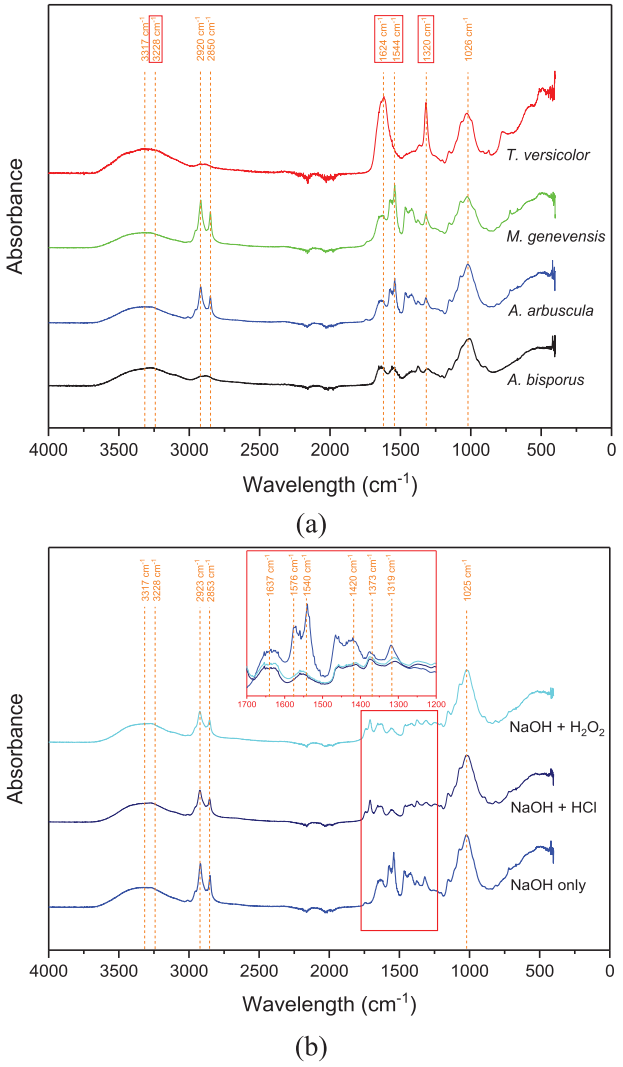

Figure 2. ATR−FTIR spectra for (a) nanopapers treated using only NaOH [mycelium-derived: *A. arbuscula* (blue), *M. genevensis* (green) and *T. versicolor* (red) and fruiting body-derived: *A. bisporus* (black) nanopapers]. Bands associated with −NH stretching and amides I−III are marked with red bounding boxes. (b) *A. arbuscula* nanopapers treated with NaOH + H_2O_2 (cyan) or NaOH + HCl (navy). Inset: magnification of bands associated with amides I−III.

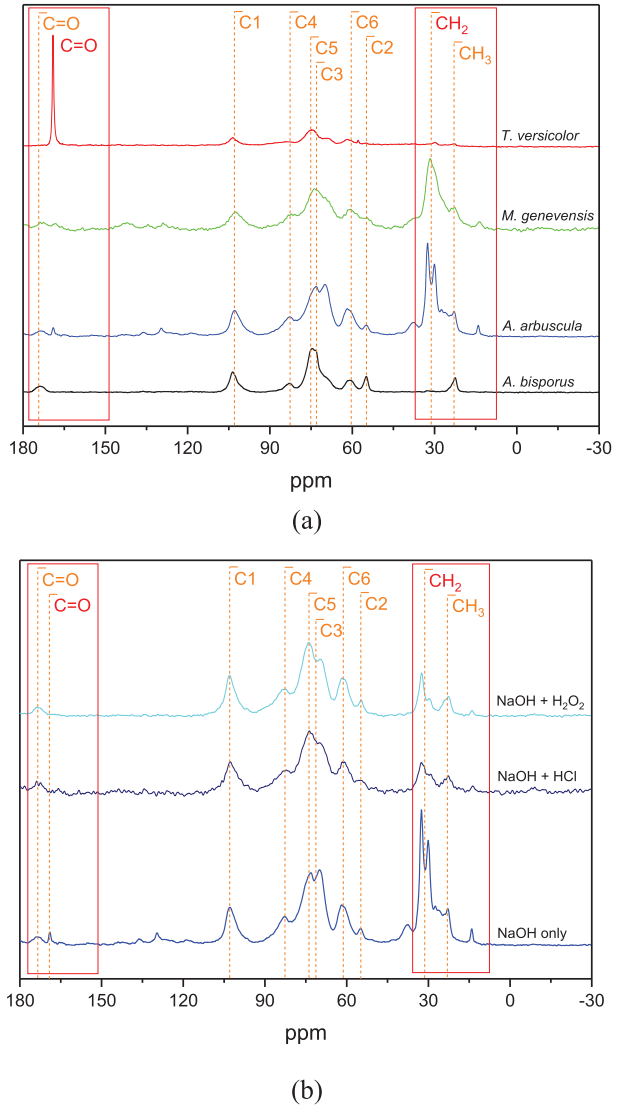

Figure 3. ^{13}C ssNMR spectra for (a) nanopapers treated using only NaOH [mycelium-derived: *A. arbuscula* (blue), *M. genevensis* (green) and *T. versicolor* (red) and fruiting body-derived: *A. bisporus* (black) nanopapers] and (b) *A. arbuscula* nanopapers treated with NaOH + H_2O_2 (cyan) or NaOH + HCl (navy). Colors: orange text (C1-6, C═O, CH_3) is associated with chitin, red text (C═O, CH_2) is associated with organic lipid and inorganic Ca carbonate and oxalate impurities. All peak integrals were referenced at C1.

Chitosan was also present in all polymer extracts, with ssNMR indicating that all extracts were at least partially deacetylated as confirmed by reduced $-CH_3$ and C═O signals at 22.5 and 173.5 ppm, respectively (Figure 3a). All samples exhibited C1-6 signals associated with the polysaccharide backbone as in chitin, chitosan, and glucan between 55 ppm and 104 ppm. *A. bisporus* showed the strongest $-CH_3$ and C═O signals and thus the highest fraction of acetylated monosaccharide units (30.8%) (Table S2 Supporting Information). This suggested that 30.8% of the sugars contained within this extract were chitin, with chitosan comprising a further 11.5% of the sugars present and other sugars representing 57.7% (Table S3 Supporting Information). *A. arbuscula* and *M. genevensis* still displayed signals indicating the presence of chitin, but the significantly reduced intensity and lower fractions of acetylated monosaccharide units suggested a lower chitin content (11.4 and 12.8% of the sugars present, respectively). This suggested the dominance of nonchitin polysaccharides (77.2−86.3% sugars other than chitin or chitosan) and a higher degree of deacetylation in these extracts, which were calculated to contain 2.3−10.0% chitosan (Table S3 Supporting Information). *T. versicolor* did not exhibit signals associated with $-CH_3$ and C═O, indicating a lack of chitin and the presence of chitosan or glucan instead. Measured ζ-potentials (Figure S2 Supporting Information) supported these results with higher isoelectric points (IEPs) associated with higher chitosan concentration papers and lower IEPs associated with papers containing more chitin. Pure chitin typically has an IEP of approximately 3.5,[35] while chitosan normally has an IEP of 7−9.[36,37] *M. genevensis* nanopapers had higher chitosan concentrations and IEPs of approximately 3.53.

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Biomacromolecules XXXX, XXX, XXX−XXX

Conversely, nanopapers with lower chitosan concentrations, such as *A. arbuscula* and papers with higher chitin concentrations, such as *A. bisporus* had lower IEPs (3.19 and 2.87, respectively). The ζ-potentials of *T. versicolor* nanopapers could not be assessed as the samples disintegrated during the measurement. This was most likely because of the large concentrations of inorganic calcium salts in these nanopapers, which significantly compromised their physical and mechanical properties.

All mycelium-derived polymer extracts only treated using NaOH contained large quantities of inorganics (Table 2). Ca salts were especially prevalent in mycelium-derived samples (~5−20%), most likely derived from the molasses growth medium which contained 0.7 wt % Ca. Fungi growing in Ca rich environments typically contain Ca biomineralized in hyphae as calcite ($CaCO_3$) and calcium oxalate.[38] Biomineralization of hyphal filaments was visible using EDS point detect analysis, elemental composition mapping (Figure S3 Supporting Information) and ssNMR results (Figure 2a), which displayed a C═O peak associated with carbonate or oxalate at 169 ppm in all mycelium-derived papers, most prevalently in *T. versicolor*. ssNMR also indicated the presence of lipid residues in *A. arbuscula* and *M. genevensis*, with a $-CH_2$ peak at 31 ppm. However, it should be noted that lipid residues are not uncommon in chitin.[39]

A. arbuscula extract was subsequently selected for additional treatments to remove the organic and inorganic impurities detected based on its high conversion yield and growth rate compared to *M. genevensis* and *T. versicolor*. 1 M concentrations of H_2O_2 and HCl were used with a solid to solvent ratio of 1:15 (0.3 g polymer extract in 4.5 mL H_2O_2 or HCl solution) and constant stirring for 1 h at ambient temperature. Both H_2O_2 and HCl treatments did not significantly affect elemental or sugar composition but were almost completely effective in removing all inorganic Ca salts from the samples, with only trace quantities of Ca remaining after treatment (Table 2). Lipid residue concentrations also decreased, which was indicated by significant reductions in the ssNMR $-CH_2$ peaks at 31 ppm (Figure 3b) and slightly more negative ζ-potentials between pH 5 and 10 resulting from greater chitin-based charge availability (Figure S2 Supporting Information). These reductions constituted ~22 wt % of the total polymer extract mass for H_2O_2 and ~23 wt % for HCl. ATR−FTIR spectra showed reductions in the amide II signals at 1576 and 1540 cm^{-1} and the amide III signals at 1373 and 1319 cm^{-1} associated with deacetylation of chitin in the treated nanopapers (Figure 2b). The IEP of HCl-treated papers also increased slightly and these papers had higher ζ-potentials at pH 2, supporting some degree of deacetylation on the paper surface (Figure S2 Supporting Information). ssNMR indicated that the fraction of acetylated monosaccharide units, and hence the chitin content, increased from 11.4 to 14.0% following HCl treatment, potentially associated with glucan cleavage, and that the chitosan content of the sugars present also increased from 2.3 to 3.9% (Tables S3 Supporting Information). H_2O_2-treated extracts showed no signs of deacetylation based on ssNMR peaks and peak integrals, which indicated that the fraction of acetylated monosaccharide units (chitin content) increased from 11.4 to 17.2%, while the chitosan content decreased from to 2.3 to 1.6% (Table S3 Supporting Information). H_2O_2-treated nanopapers also experienced a slight decrease in IEP from 3.19 to 2.99 (Figure S2 Supporting Information). This was most likely attributable to acid or carboxyl groups formed on the surface of the H_2O_2-treated papers during oxidation.

3.3. Physical and Mechanical Properties of the Nanopapers. Nanopapers produced from fibrils extracted from mycelia and fruiting bodies, exhibited dense surface morphologies lacking large pores or obvious fibrillation (Figure 4a−d). *A. bisporus* fruiting body-derived nanopapers exhibited

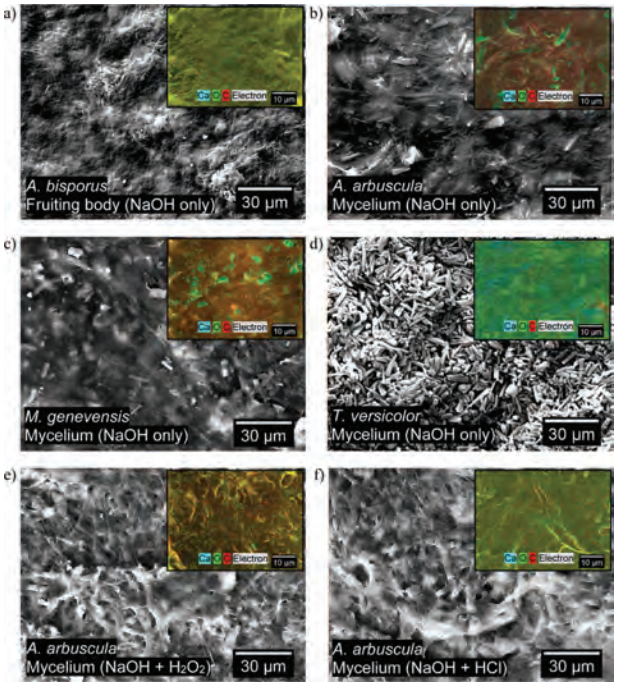

Figure 4. SEM micrographs (800× magnification) detailing the surface morphology of (a) *A. bisporus* fruiting body-derived, (b) *A. arbuscula*, (c) *M. genevensis* and (d) *T. versicolor* mycelium-derived nanopapers treated with NaOH only, and *A. arbuscula* nanopapers treated with (e) NaOH + H_2O_2 or (f) NaOH + HCl.

a very densely bonded surface morphology of microfibrils containing very few Ca impurities (Figure 4a). *A. arbuscula* had a marginally more visible fiber structure than *M. genevensis*, however both species appeared to have a hyphal filament structure biomineralized with Ca salts and interfaced with a surface layer of a lipid reside (Figure 4b,c). This biomineralization and organic interfacing was confirmed by EDS mapping (Figure S3 Supporting Information) but could be removed using H_2O_2 and HCl treatments, which exposed the hyphal filaments (Figure 4e,f). Conversely, *T. versicolor* had a very fragmented surface morphology, which contained significantly higher (~4 times) concentrations of Ca salts and no visible hyphal filaments (Figure 4d).

The nanopapers produced from fibrils extracted from *M. genevensis* had the highest ultimate tensile strength of the mycelium-derived samples (24.7 MPa), being significantly stronger than *A. arbuscula* (16.0 MPa) and in particular *T. versicolor* (0.9 MPa) (Figure 4). *M. genevensis* nanopapers were also more than twice as stiff as *T. versicolor* papers, which has an elastic modulus of 0.7 GPa, although *A. arbuscula* papers had a similar elastic modulus (1.8 GPa). The high chitin−chitosan contents of *M. genevensis* nanopapers compared to *A. arbuscula* and *T. versicolor* is potentially responsible for these differences in mechanical performance. The presence of higher

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Table 4. Density (ρ), Elastic Modulus (E), Ultimate Tensile Strength (σ_{UTS}) and Strain to Failure (ε_f) of Nanopapers Produced from Fungal Fruiting Bodies and Mycelium

species	ρ (g/cm^3)	E (GPa ± SE)	σ_{UTS} (MPa ± SE)	ε_f (% ± SE)
A. bisporus (NaOH only)	1.7 ± 0.003	6.5 ± 0.4	97.6 ± 8.3	1.8 ± 0.2
A. arbuscula (NaOH only)	1.4 ± 0.001	1.8 ± 0.4	16.0 ± 0.8	0.9 ± 0.3
NaOH + H_2O_2 treatments	1.2 ± 0.002	1.9 ± 0.1	19.2 ± 1.2	1.0 ± 0.1
NaOH + HCl treatments	1.3 ± 0.001	1.7 ± 0.1	14.3 ± 0.9	0.9 ± 0.1
M. genevensis (NaOH only)	1.3 ± 0.001	1.9 ± 0.2	24.7 ± 0.9	1.5 ± 0.1
T. versicolor (NaOH only)	2.0 ± 0.009	0.7 ± 0.01	0.9 ± 0.1	0.1 ± 0.01

chitosan contents also explains the larger strain to failure of *M. genevensis* (1.5%), which was higher than *A. arbuscula* (1.1%) and *T. versicolor* (0.1%) (Table 4).

A. bisporus fruiting body-derived nanopapers had better mechanical performance than mycelium-derived nanopapers, with much higher ultimate tensile strengths (up to 97.6 MPa) and elastic moduli (up to 6.5 GPa) (Figure 5) (Table 4). Their

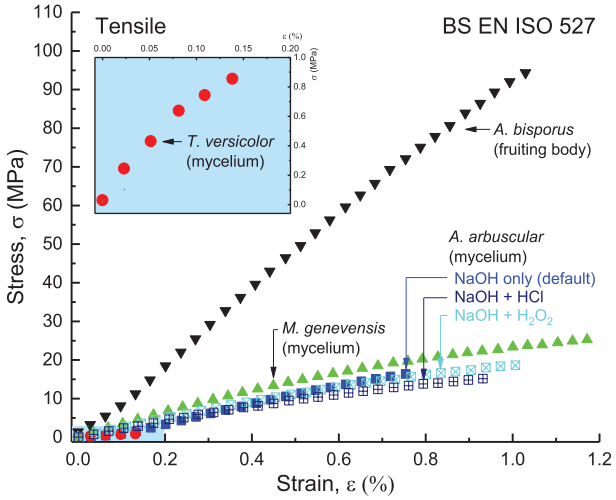

Figure 5. Tensile stress–strain curve for nanopapers treated using only NaOH [solid markers: mycelium-derived *A. arbuscula* (blue), *M. genevensis* (green) and *T. versicolor* (red) and fruiting body-derived *A. bisporus* (black) nanopapers] and *A. arbuscula* nanopapers treated with NaOH + H_2O_2 (hollow cyan markers with a diagonal cross) or NaOH + HCl (hollow navy markers with a vertical cross). Inset: magnification of *T. versicolor* mycelium-derived nanopaper stress–strain curve.

superior performance was most likely attributable to their significantly higher chitin and chitosan contents and the lack of Ca impurities in these papers. *A. arbuscula* mycelium-derived nanopapers were treated with H_2O_2 and HCl to remove Ca impurities and subsequently improve the purity of the polymer extracts used in the production of the mycelium-derived nanopapers. Despite successful removal of the Ca impurities, only a minor improvement in mechanical performance was achieved in the treated papers. The H_2O_2 treatment increased the fraction of acetylated monosaccharide units and provided some improvement in the tensile strength (16.0–19.2 MPa) but HCl treatment had no positive effect on tensile performance (Table 4). It has been noted in the literature that the acetyl group can contribute to the formation of hydrogen bonds with a higher acetylation degree providing greater resistance against fracture.[40] More significant improvements in performance may have been hindered by deacetylation of chitin in the nanopapers, glucan cleavage, a reduction in fibril–fibril bonding and increased porosity (reduced density) because of the removal of organic lipid residue that would otherwise bridge the fibrils (Figure 4e,f).

Despite variations in tensile performance, the mycelium-derived nanopapers produced in this study matched or significantly outperformed all the currently known mycelium-derived materials. Historically, mycelium composites have been characterized exclusively as foams, with low densities and elastic moduli, despite physical processing such as hot pressing (Figure 6). The mycelium-derived nanopapers exhibited higher

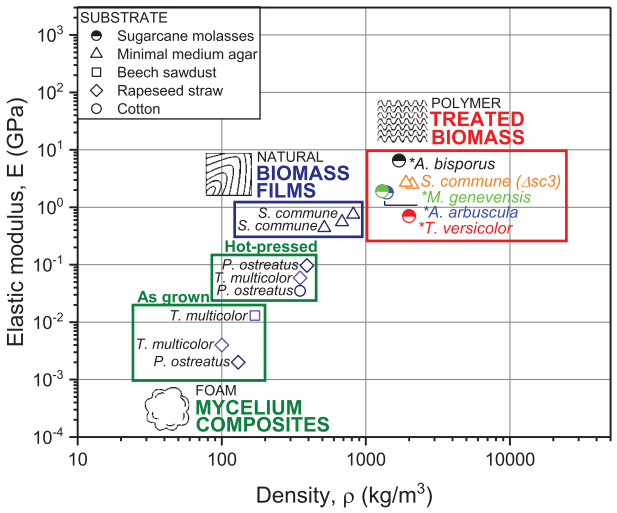

Figure 6. Comparison of the elastic modulus (GPa) and density (kg/m^3) of mycelium materials. The new chemically treated and hot-pressed nanopapers derived from the mycelium and fruiting bodies produced in this study (*) are compared to the existing, as-grown, and hot-pressed mycelium composite materials, mycelial biomass, and genetically modified Δsc3 biomass arranged by species and substrate. Data are obtained from the reports by Appels et al.[3,11] and Ashby et al.[42]

ultimate tensile strengths (up to 24.7 MPa) than mycelium composites (0.01–1.1 MPa)[3,6] and mycelial biomass grown in controlled environments (5.1–9.6 MPa).[11] They also exhibited similar tensile performance to several advanced mycelium materials utilizing genetic modification of the SC3 hydrophobin (Δsc3) gene and controlled growth environments to produce schizophyllan rather than glucan-linked chitin (15.6–40.4 MPa).[11] However, the nanopapers produced in this study have the advantage of being able to be grown in any environment and being universally applicable to all fungal biomass rather than species and strain specific genetic modification. They were characterized as polymers, based on their density (1.3–2.0 g/cm^3) and elastic moduli (up to 1.9

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Biomacromolecules XXXX, XXX, XXX–XXX

GPa), which were also similar to or higher than existing mycelium composites (0.001−0.097 GPa), mycelial biomass (0.4−0.9 GPa) and Δsc3 mycelium materials (1.2−2.5 GPa).[3,11] Overall, mycelium-derived nanopaper mechanical performance was comparable to the commercial copy paper and some plastics.[41]

3.4. Surface Properties of the Nanopapers. A further important aspect of mycelium-derived materials is their surface properties. Mycelium-derived nanopapers (*A. arbuscula* and *M. genevensis*) were hydrophobic, with high advancing water contact angles (~106° and ~101°, respectively) compared to *A. bisporus* fruiting body-derived nanopapers (~87°) and cellulose nanopapers (~19−90° depending on their lignin content)[43−45] (Figure 7). The hydrophobicity of mycelium-derived materials has previously been noted, with static water contact angles from 115 to 122° reported.[6,11] Both mycelium-derived papers also exhibited lower surface energies than *A. bisporus* fruiting body-derived nanopapers (28−31 mJ/m^2 compared to 39 mJ/m^2) and had higher BET surface areas (1.7−2.1 m^2/g compared to 0.7 m^2/g) (Figure 7). BET surface areas were assessed using iGC, which was also used to confirm the lower surface tensions. Total surface energies of 36 and 40 mJ/mm^2 were recorded, respectively, for *A. arbuscula* and *M. genevensis* mycelium-derived nanopapers, while 46 mJ/mm^2 was recorded for *A. bisporus* fruiting body-derived papers at 0.1 surface coverage (Figure S5a Supporting Information). Chitin and chitosan theoretically exhibit slightly higher surface energy than other polysaccharides, such as cellulose and starch, because of the presence of amino and amide moieties.[46] The dispersive surface energy component of chitin or chitosan is approximately 30 mJ/m^2, a value typical of macromolecules, with a similar polar component contribution owing to the dominance of −OH surface groups (total surface energy of approximately 60 mJ/m^2) and a water contact angle of ~50°.[46]

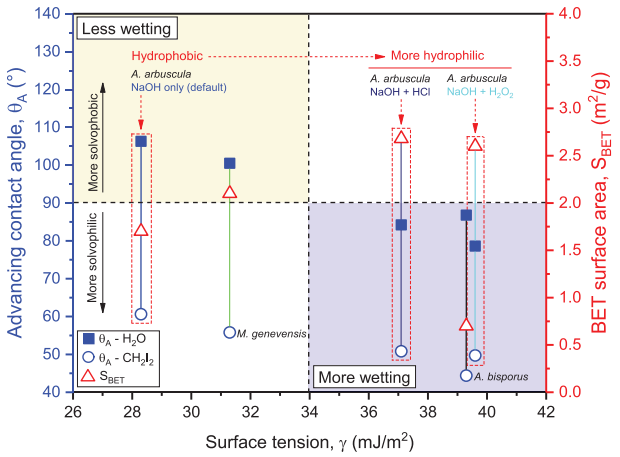

Figure 7. Advancing contact angle (blue markers, °), surface tension (mJ/m^2) and BET surface area (hollow red markers, m^2/g) measurements for *A. arbuscula* (blue connecting line) and *M. genevensis* (green connecting line) NaOH treated mycelium-derived nanopapers. *A. bisporus* fruiting body-derived nanopapers (black connecting line) and *A. arbuscula* NaOH and H_2O_2 (cyan connecting line) or HCl (navy connecting line) treated nanopapers are also displayed, with the transition between hydrophobic and hydrophilic properties following HCl or H_2O_2 treatment marked in red. *T. versicolor* has been omitted from this figure as it was unable to be tested.

However, nonpolar impurities have been noted to be responsible for lower polar surface energy components in less pure chitin.[47] Nonpolar impurities responsible for the aroma of fungi, such as alcohols and acid derivatives[48] are probably responsible for the hydrophobicity and low surface energies of mycelium-derived nanopapers. In particular, the high concentrations of lipid residues in *A. arbuscula* and *M. genevensis* nanopapers were most likely responsible for the hydrophobic properties demonstrated by these papers. Hydrophobic properties could make mycelium-derived nanopapers useful for applications including coatings. *T. versicolor* mycelium-derived nanopapers did not support stable droplets, instead absorbing them on contact, and were consequently unable to be assessed.

A. arbuscula mycelium-derived nanopapers treated with H_2O_2 and HCl displayed more hydrophilic behavior than papers only treated with NaOH. The advancing water contact angles of these papers were significantly lower than NaOH only treated papers, with reductions from ~106° to ~79° or ~84° following H_2O_2 or HCl treatment, respectively (Figure 7). The H_2O_2 and HCl-treated papers also had higher surface energies than papers only treated with NaOH (37−40 mJ/m^2 compared to 28 mJ/m^2) (Figure 7). This increase in surface energy was confirmed using iGC, which recorded a total surface energy increase following HCl or H_2O_2 treatment from 36 to 46 or 49 mJ/mm^2, respectively, at 0.1 surface coverage (Figure S5b Supporting Information). The increased hydrophilicity and surface tension of H_2O_2 and HCl-treated papers most likely resulted from the removal of lipid residues present in *A. arbuscula* nanopapers only treated using NaOH. This reduction in lipid residues is also likely responsible for the higher BET surface areas of H_2O_2 or HCl-treated samples. The more fibrous surface morphology of H_2O_2 and HCl-treated papers, stripped of Ca salts, coupled with their lower water contact angles and higher surface tensions could potentially make these papers suitable for use as membranes in filtration systems.

3.5. Thermal Degradation Properties of the Nanopapers. The mycelium itself typically exhibits a three-stage degradation process. Initially, free and chemically bonded water evaporates between 25 and 200 °C (~5 wt %). A much larger mass loss then follows between 200 and 375 °C, with onset of decomposition at ~280−290 °C, associated with the degradation of the organic constituents, such as proteins and polysaccharides (~70 wt %). Finally, from 450 to 600 °C the primary residual char further degrades to form the final carbonaceous char residue.[49] The mycelium typically yields a char residue of approximately 23 wt % in a nitrogen atmosphere[6,49] and demonstrates thermal degradation and fire reaction properties typical of organic materials.[50] All the mycelium- and fruiting body-derived nanopapers, except for *T. versicolor*, exhibited a three stage thermal degradation typical of mycelium with char residues of ~20−23 wt % in a nitrogen atmosphere (Figure 8a). *A. bisporus* fruiting body-derived papers fully thermally decomposed in an air atmosphere, however *A. arbuscula* and *M. genevensis* mycelium-derived papers had an inorganic residue of ~8−9 wt %, attributable to their Ca content (Figure 8a). *T. versicolor* mycelium-derived nanopapers exhibited a multi-stage thermal degradation process up to 800 °C and a final inorganic residue of ~34 wt % in air atmospheres, supporting the significant biomineralization of this species and the lower organic content

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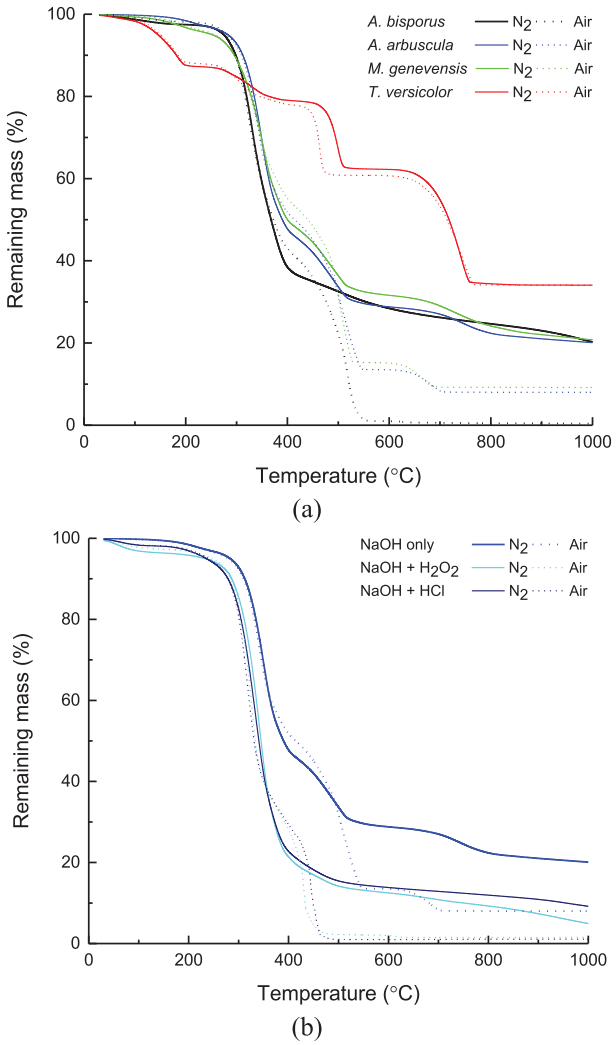

Figure 8. TGA-mass loss temperature curves for (a) nanopapers treated using only NaOH [mycelium-derived *A. arbuscula* (blue), *M. genevensis* (green) and *T. versicolor* (red) and fruiting body-derived *A. bisporus* (black) nanopapers] and (b) *A. arbuscula* nanopapers treated with NaOH + H_2O_2 (cyan) or NaOH + HCl (navy).

of these nanopapers compared to the other mycelium-derived nanopapers (Figure 8a).

A. arbuscula mycelium-derived nanopapers treated with H_2O_2 or HCl exhibited a three-stage thermal degradation process with a slight reduction in the onset decomposition temperature. A reduction in char residue under a nitrogen atmosphere was also observed, with a drop from ∼20 wt %, for papers only treated using NaOH, to ∼4 or ∼9 wt % for papers treated using NaOH + H_2O_2 or NaOH + HCl, respectively (Figure 8b). H_2O_2 and HCl-treated nanopapers fully thermally decomposed in an air atmosphere, with negligible inorganic char present above 600 °C (Figure 8b). This verified the effectiveness of the H_2O_2 and HCl treatments in removing inorganic impurities from mycelium-derived nanopapers grown on Ca-rich substrates, such as sugarcane molasses.

4. CONCLUSIONS

Fungal growth provides a low-cost method for on-demand generation of natural nanofibrils, such as chitin and chitosan, from agricultural wastes and byproducts. These nanofibrils were obtained via mild alkaline extraction of a common mushroom reference and various species of fungal mycelium grown on the sugarcane byproduct molasses and hot pressed to produce nanopapers. Mycelium-derived nanopapers were more hydrophobic than pure chitin and other natural polysaccharides, such as cellulose and starch, resulting from the presence of lipid residues within the nanopapers. Mycelium-derived polymer extract yields were competitive with crustacean chitin and nanopapers produced from the extracts exhibited much higher tensile strength than most existing mycelium materials, with comparable properties to paper and some plastics. Further hydrogen peroxide or hydrochloric acid treatments removed organic and inorganic impurities rendering the mycelium-derived nanopapers hydrophilic. Nanopapers derived from common mushrooms were hydrophilic, contained fewer lipid residues and inorganic contaminants than those derived from mycelium and had higher tensile performance. These variations in surface morphology, wettability, and mechanical performance highlight the customizable properties of these cheap and environmentally sustainable materials making them potentially suitable for a wide range of applications, including coatings, membranes, packaging, and paper.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biomac.9b00791.

Biomass and extract dry mass yields, associated water content, and overall total dry polymer yield, energy-dispersive X-ray spectroscopy (EDS) spectra and elemental composition maps, zeta potentials as a function of pH, iGC for total surface energy as a function of surface coverage, ^{13}C ssNMR peak integrals, fraction of acetylated monosaccharide units, degree of acetylation and chitin and chitosan contents as a percentage of total sugars present (PDF)

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Author Contributions

M.J. performed all the experimental design and sample preparation, yield assessment, SEM, EDS, TGA and contact angle goniometry experimental work, data analysis, and writing. K.W. contributed to sample preparation and performed the tensile tests. M.K. contributed to sample preparation and FTIR and ssNMR data analysis. J.T. performed the elemental analysis and FTIR. H.K. performed the ssNMR. E.K. performed the sugar analysis. A.M. performed the iGC and zeta potential measurements. All the authors contributed to editing and proofreading. A.M., A.B., and S.J. supervised the work. All the authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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Biomacromolecules XXXX, XXX, XXX−XXX

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■ REFERENCES

(1) Jones, M.; Huynh, T.; Dekiwadia, C.; Daver, F.; John, S. Mycelium Composites: A Review of Engineering Characteristics and Growth Kinetics. *J. Bionanoscience* **2017**, *11*, 241−257.
(2) Holt, G. A.; Mcintyre, G.; Flagg, D.; Bayer, E.; Wanjura, J. D.; Pelletier, M. G. Fungal mycelium and cotton plant materials in the manufacture of biodegradable molded packaging material: Evaluation study of select blends of cotton byproducts. *J. Biobased Mater. Bioenergy* **2012**, *6*, 431−439.
(3) Appels, F. V. W.; Camere, S.; Montalti, M.; Karana, E.; Jansen, K. M. B.; Dijksterhuis, J.; Krijgsheld, P.; Wösten, H. A. B. Fabrication factors influencing mechanical, moisture- and water-related properties of mycelium-based composites. *Mater. Des.* **2019**, *161*, 64−71.
(4) Camere, S.; Karana, E. Fabricating materials from living organisms: An emerging design practice. *J. Clean. Prod.* **2018**, *186*, 570−584.
(5) Karana, E.; Blauwhoff, D.; Hultink, E.; Camere, S. When the material grows: A case study on designing (with) mycelium-based materials. *Int. J. Des.* **2018**, *12*, 119−136.
(6) Haneef, M.; Ceseracciu, L.; Canale, C.; Bayer, I. S.; Heredia-Guerrero, J. A.; Athanassiou, A. Advanced Materials From Fungal Mycelium: Fabrication and Tuning of Physical Properties. *Sci. Rep.* **2017**, *7*, 41292.
(7) Pelletier, M. G.; Holt, G. A.; Wanjura, J. D.; Bayer, E.; McIntyre, G. An evaluation study of mycelium based acoustic absorbers grown on agricultural by-product substrates. *Ind. Crops Prod.* **2013**, *51*, 480−485.
(8) Islam, M.; Tudryn, G.; Bucinell, R.; Schadler, L.; Picu, R. Morphology and mechanics of fungal mycelium. *Sci. Rep.* **2017**, *7*, 13070.
(9) Abhijith, R.; Ashok, A.; Rejeesh, C. R. Sustainable packaging applications from mycelium to substitute polystyrene: a review. *Mater. Today: Proc.* **2018**, *5*, 2139−2145.
(10) Arifin, Y. H.; Yusuf, Y. Mycelium Fibers as New Resource for Environmental Sustainability. *Procedia Eng.* **2013**, *53*, 504−508.
(11) Appels, F. V. W.; Dijksterhuis, J.; Lukasiewicz, C. E.; Jansen, K. M. B.; Wösten, H. A. B.; Krijgsheld, P. Hydrophobin gene deletion and environmental growth conditions impact mechanical properties of mycelium by affecting the density of the material. *Sci. Rep.* **2018**, *8*, 4703.
(12) Kavanagh, K. *Fungi: Biology and Applications*; Wiley: Hoboken, NJ, 2005.
(13) Rinaudo, M. Chitin and Chitosan—General Properties and Applications. *Prog. Polym. Sci.* **2006**, *31*, 603−632.
(14) Bamba, Y.; Ogawa, Y.; Saito, T.; Berglund, L. A.; Isogai, A. Estimating the Strength of Single Chitin Nanofibrils via Sonication-Induced Fragmentation. *Biomacromolecules* **2017**, *18*, 4405−4410.
(15) Webster, J.; Weber, R. *Introduction to Fungi*; Cambridge University Press, 2007.
(16) Ifuku, S. Chitin and chitosan nanofibers: Preparation and chemical modifications. *Molecules* **2014**, *19*, 18367−18380.
(17) Di Mario, F.; Rapanà, P.; Tomati, U.; Galli, E. Chitin and chitosan from Basidiomycetes. *Int. J. Biol. Macromol.* **2008**, *43*, 8−12.
(18) Hassainia, A.; Satha, H.; Boufi, S. Chitin from Agaricus bisporus: Extraction and characterization. *Int. J. Biol. Macromol.* **2018**, *117*, 1334−1342.
(19) Nawawi, W. M. F. W.; Lee, K.-Y.; Kontturi, E.; Murphy, R.; Bismarck, A. Chitin nanopaper from mushroom extract: natural composite of nanofibres and glucan from a single bio-based source. *ACS Sustainable Chem. Eng.* **2019**, *7*, 6492−6496.
(20) Jones, M.; Bhat, T.; Huynh, T.; Kandare, E.; Yuen, R.; Wang, C. H.; John, S. Waste-derived low-cost mycelium composite construction materials with improved fire safety. *Fire Mater.* **2018**, *42*, 816−825.
(21) Jones, M.; Huynh, T.; John, S. Inherent Species Characteristic Influence and Growth Performance Assessment for Mycelium Composite Applications. *Adv. Mater. Lett.* **2018**, *9*, 71−80.
(22) Griffin, D. H. *Fungal Physiology*; Wiley: Hoboken, NJ, 1996.
(23) Jones, M. P.; Lawrie, A. C.; Huynh, T. T.; Morrison, P. D.; Mautner, A.; Bismarck, A.; John, S. Agricultural by-product suitability for the production of chitinous composites and nanofibers utilising Trametes versicolor and Polyporus brumalis mycelial growth. *Process Biochem.* **2019**, *80*, 95−102.
(24) American Type Culture Collection Fungi and Yeast Products. https://www.lgcstandards-atcc.org (accessed Aug 3, 2018).
(25) Bioresource Collection and Research Center Taiwan Fungal Flora Knowledge. http://www.bcrc.firdi.org.tw/fungi/index.jsp (accessed Aug 3, 2018).
(26) U.S. Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*, 2009.
(27) Owens, D. K.; Wendt, R. C. Estimation of the surface free energy of polymers. *J. Appl. Polym. Sci.* **1969**, *13*, 1741−1747.
(28) Dorris, G. M.; Gray, D. G. Adsorption of n-alkanes at zero surface coverage on cellulose paper and wood fibers. *J. Colloid Interface Sci.* **1980**, *77*, 353−362.
(29) Gopalan Nair, K.; Dufresne, A. Crab shell chitin whisker reinforced natural rubber nanocomposites. 1. Processing and swelling behavior. *Biomacromolecules* **2003**, *4*, 657−665.
(30) Percot, A.; Viton, C.; Domard, A. Optimization of chitin extraction from shrimp shells. *Biomacromolecules* **2003**, *4*, 12−18.
(31) Li, J.; Revol, J.-F.; Marchessault, R. H. Effect of degree of deacetylation of chitin on the properties of chitin crystallites. *J. Appl. Polym. Sci.* **1997**, *65*, 373−380.
(32) Arbia, W.; Arbia, L.; Adour, L.; Amrane, A. Chitin extraction from crustacean shells using biological methods−a review. *Food Technol. Biotechnol.* **2013**, *51*, 12−25.
(33) Jung, W. J.; Jo, G. H.; Kuk, J. H.; Kim, Y. J.; Oh, K. T.; Park, R. D. Production of chitin from red crab shell waste by successive fermentation with Lactobacillus paracasei KCTC-3074 and Serratia marcescens FS-3. *Carbohydr. Polym.* **2007**, *68*, 746−750.
(34) Mahapatra, S.; Banerjee, D. Fungal exopolysaccharide: production, composition and applications. *Microbiol. Insights* **2013**, *6*, MBI.S10957.
(35) Wysokowski, M.; Klapiszewski, Ł.; Moszyński, D.; Bartczak, P.; Szatkowski, T.; Majchrzak, I.; Siwińska-Stefańska, K.; Bazhenov, V.; Jesionowski, T. Modification of chitin with kraft lignin and development of new biosorbents for removal of cadmium(II) and nickel(II) ions. *Mar. Drugs* **2014**, *12*, 2245−2268.
(36) Gouda, M.; Elayaan, U.; Youssef, M. M. Synthesis and Biological Activity of Drug Delivery System Based on Chitosan Nanocapsules. *Adv. Nanoparticles* **2014**, *03*, 148.
(37) Swain, S. K.; Dey, R. K.; Islam, M.; Patel, R. K.; Jha, U.; Patnaik, T.; Airoldi, C. Removal of Fluoride from Aqueous Solution Using Aluminum-Impregnated Chitosan Biopolymer. *Sep. Sci. Technol.* **2009**, *44*, 2096−2116.
(38) Burford, E. P.; Hillier, S.; Gadd, G. M. Biomineralization of Fungal Hyphae with Calcite (CaCO3) and Calcium Oxalate Mono- and Dihydrate in Carboniferous Limestone Microcosms. *Geomicrobiol. J.* **2006**, *23*, 599−611.
(39) Reid, D. G.; Mason, M. J.; Chan, B. K. K.; Duer, M. J. Characterization of the phosphatic mineral of the barnacle Ibla cumingi at atomic level by solid-state nuclear magnetic resonance: comparison with other phosphatic biominerals. *J. R. Soc. Interface* **2012**, *9*, 1510−1516.
(40) Cui, J.; Yu, Z.; Lau, D. Effect of Acetyl Group on Mechanical Properties of Chitin/Chitosan Nanocrystal: A Molecular Dynamics Study. *Int. J. Mol. Sci.* **2016**, *17*, 61.
(41) Crompton, T. R. *Physical Testing of Plastics*; Smithers Rapra, 2012.

DOI: 10.1021/acs.biomac.9b00791
Biomacromolecules XXXX, XXX, XXX−XXX

(42) Ashby, M. F.; Shercliff, H.; Cebon, D. *Materials: Engineering, Science, Processing and Design*; Butterworth-Heinemann, 2018.
(43) Operamolla, A.; Casalini, S.; Console, D.; Capodieci, L.; Di Benedetto, F.; Bianco, G. V.; Babudri, F. Tailoring water stability of cellulose nanopaper by surface functionalization. *Soft Matter* **2018**, *14*, 7390−7400.
(44) Wang, Q.; Du, H.; Zhang, F.; Zhang, Y.; Wu, M.; Yu, G.; Liu, C.; Li, B.; Peng, H. Flexible cellulose nanopaper with high wet tensile strength, high toughness and tunable ultraviolet blocking ability fabricated from tobacco stalk via a sustainable method. *J. Mater. Chem. A* **2018**, *6*, 13021−13030.
(45) Lee, K.-Y.; Quero, F.; Blaker, J. J.; Hill, C. A. S.; Eichhorn, S. J.; Bismarck, A. Surface only modification of bacterial cellulose nanofibres with organic acids. *Cellulose* **2011**, *18*, 595−605.
(46) Cunha, A. G.; Gandini, A. Turning polysaccharides into hydrophobic materials: a critical review. Part 2. Hemicelluloses, chitin/chitosan, starch, pectin and alginates. *Cellulose* **2010**, *17*, 1045−1065.
(47) Cunha, A. G.; Fernandes, S. C. M.; Freire, C. S. R.; Silvestre, A. J. D.; Neto, C. P.; Gandini, A. What Is the Real Value of Chitosan's Surface Energy? *Biomacromolecules* **2008**, *9*, 610−614.
(48) Wekesa, N.; Lilechi, D.; Sigot, A.; Cheruiyot, J.; Kamau, R.; Kisiangani, P. Volatile and Non-polar Chemical Constituents of Cultivated Oyster Mushroom Pleurotus ostreatus. *Int. J. Pharmacogn. Phytochem. Res.* **2016**, *8*, 477−479.
(49) Jones, M.; Bhat, T.; Kandare, E.; Thomas, A.; Joseph, P.; Dekiwadia, C.; Yuen, R.; John, S.; Ma, J.; Wang, C. Thermal Degradation and Fire Properties of Fungal Mycelium and Mycelium-Biomass Composite Materials. *Sci. Rep.* **2018**, *8*, 17583.
(50) Jones, M.; Bhat, T.; Wang, C.; Moinuddin, K.; John, S. Thermal Degradation and Fire Reaction Properties of Mycelium Composites. *21st International Conference on Composite Materials, Xi'an, China*, Xi'an, China, 2017.

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Biomacromolecules XXXX, XXX, XXX−XXX

Electronic supplementary material for:

Waste-derived Low-cost Mycelium Nanopapers with Tunable Mechanical and Surface Properties

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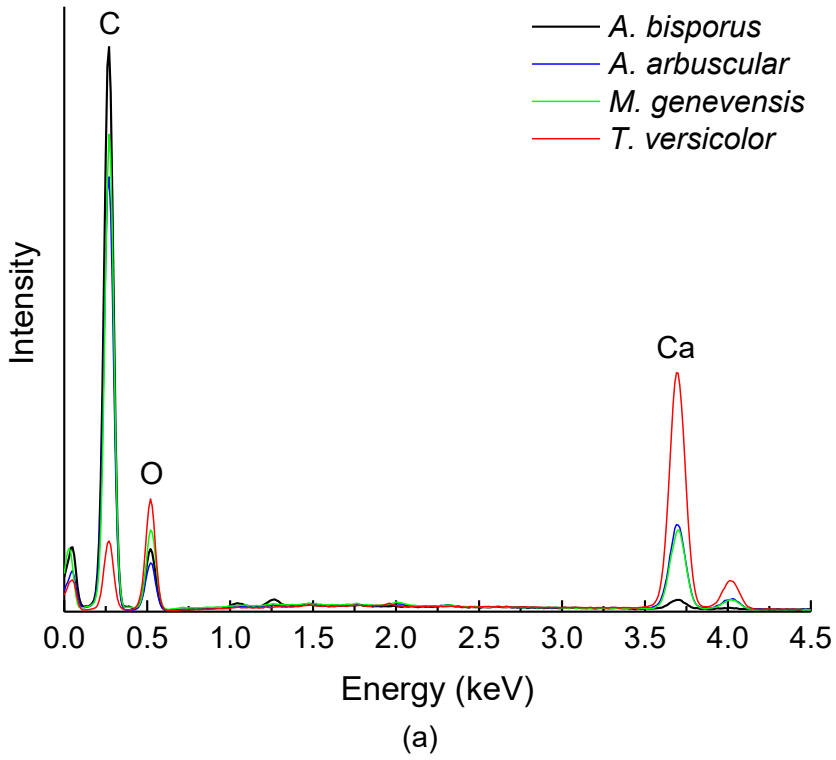
List of Figures

List of Tables

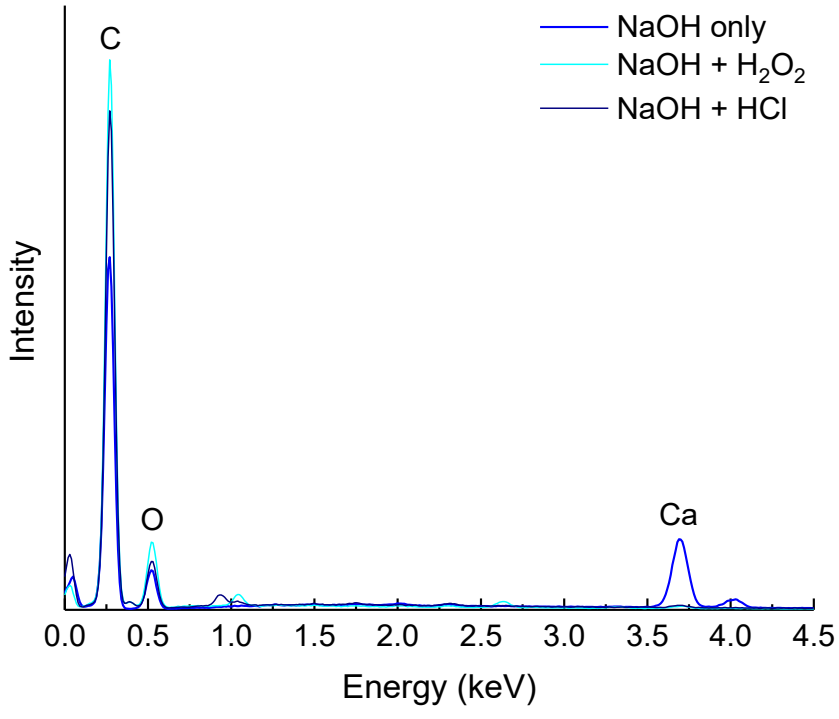
Table S1. Biomass and extract dry mass yields (and associated water content) and overall total dry polymer yield (total: biomass to chitin-glucan or chitin-chitosan + contaminants, corrected for Ca: yield corrected for inorganic Ca salt content in extracts) for selected species.

Table S2. ^{13}C ssNMR peak integrals associated with $-CH_3$ and C=O signals and fraction of acetylated monosaccharide units (X_{acetyl}) (at 22.5 ppm and 173.5 ppm respectively) for nanopapers treated using only NaOH (mycelium-derived: *A. arbuscula*, *M. genevensis* and fruiting body derived: *A. bisporus*) and *A. arbuscula* nanopapers treated with NaOH + H_2O_2 or NaOH + HCl. *T. versicolor* samples were omitted from the analysis as they did not exhibit the peaks of interest.

Table S3. Sugar content (glucosamine and other sugars), fraction of acetylated monosaccharide units (X_{acetyl}), degree of acetylation (D.A.) (X_{acetyl}/glucosamine) and chitin (D.A. × glucosamine) and chitosan ([100−D.A.] × glucosamine) contents as a percentage of total sugars present in nanopapers treated using only NaOH (mycelium-derived: *A. arbuscula*, *M. genevensis* and fruiting body derived: *A. bisporus*) and *A. arbuscula* nanopapers treated with NaOH + H_2O_2 or NaOH + HCl. *T. versicolor* samples were omitted from the analysis as they did not exhibit the peaks of interest in ssNMR. Chitin, chitosan and other sugar content (total 100% of sugars present in the nanopapers) highlighted in bold.

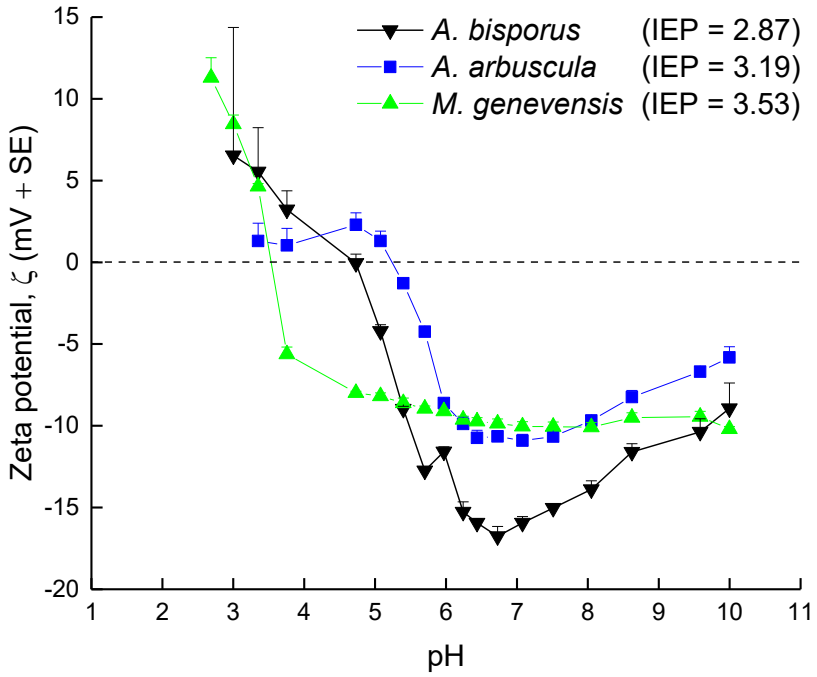


(a)

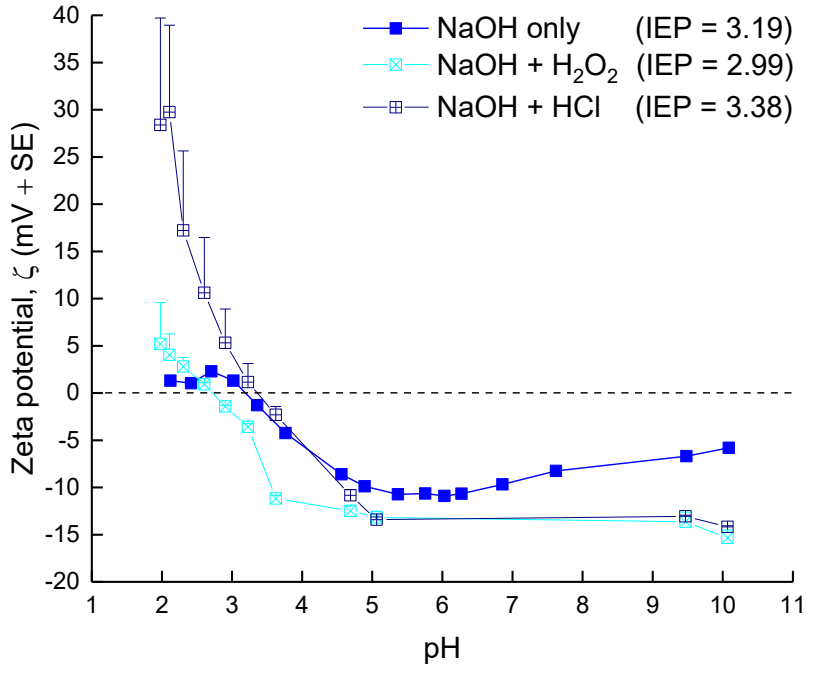


(b)

Figure S1. Energy-dispersive x-ray spectroscopy (EDS) spectra for (a) nanopapers treated using only NaOH (mycelium-derived: *A. arbuscula* (blue), *M. genevensis* (green) and *T. versicolor* (red) and fruiting body derived: *A. bisporus* (black) nanopapers) and (b) *A. arbuscula* nanopapers treated with NaOH + H_2O_2 (cyan) or NaOH + HCl (navy).



(a)



(b)

Figure S2. Zeta potential as a function of pH for (a) nanopapers treated using only NaOH (mycelium-derived: *A. arbuscula* (blue), *M. genevensis* (green) and *T. versicolor* (red) and fruiting body derived: *A. bisporus* (black) nanopapers) and (b) *A. arbuscula* nanopapers treated with NaOH + H_2O_2 (cyan) or NaOH + HCl (navy).

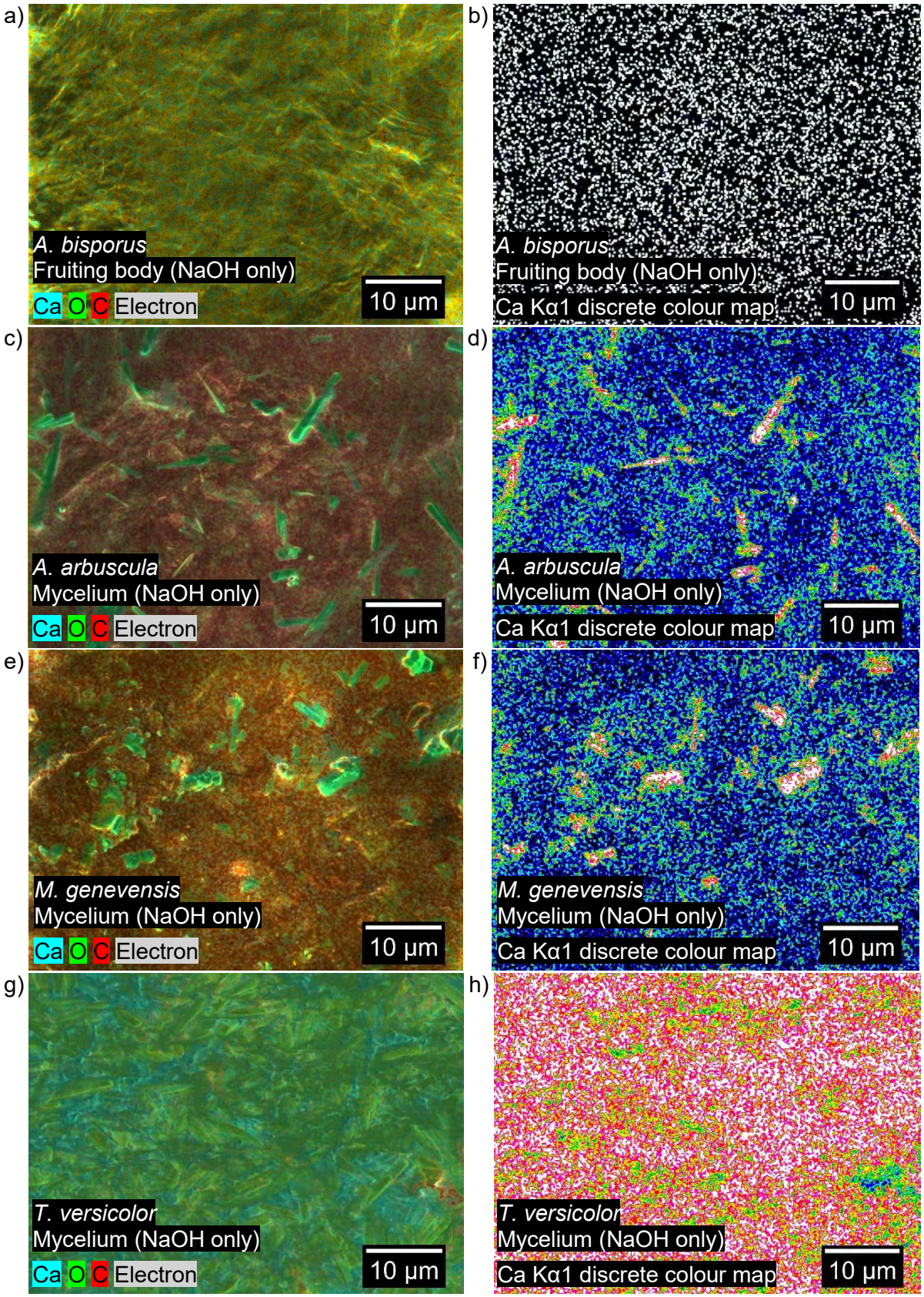


Figure S3. Energy-dispersive x-ray spectroscopy (EDS) elemental composition maps for nanopapers treated using only NaOH (mycelium-derived: *A. arbuscula*, *M. genevensis* and *T. versicolor* and fruiting body derived: *A. bisporus* nanopapers).

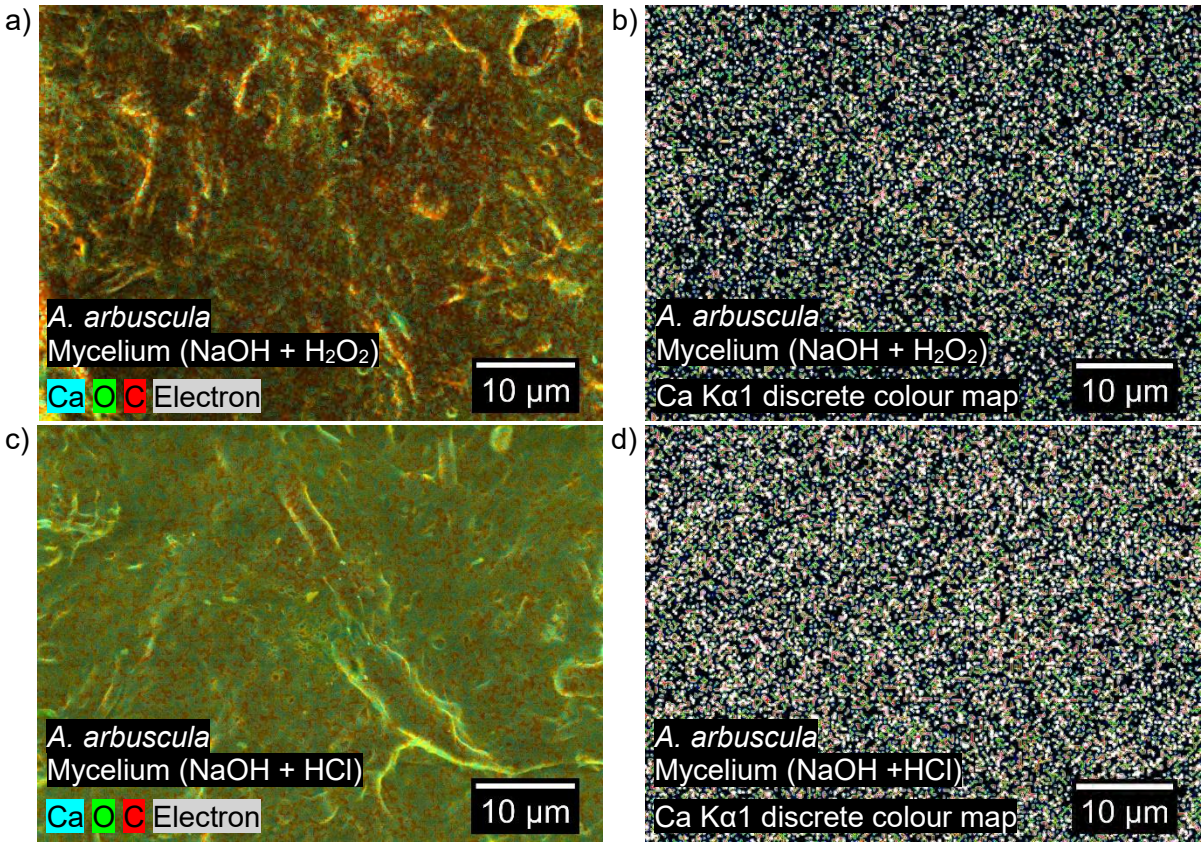
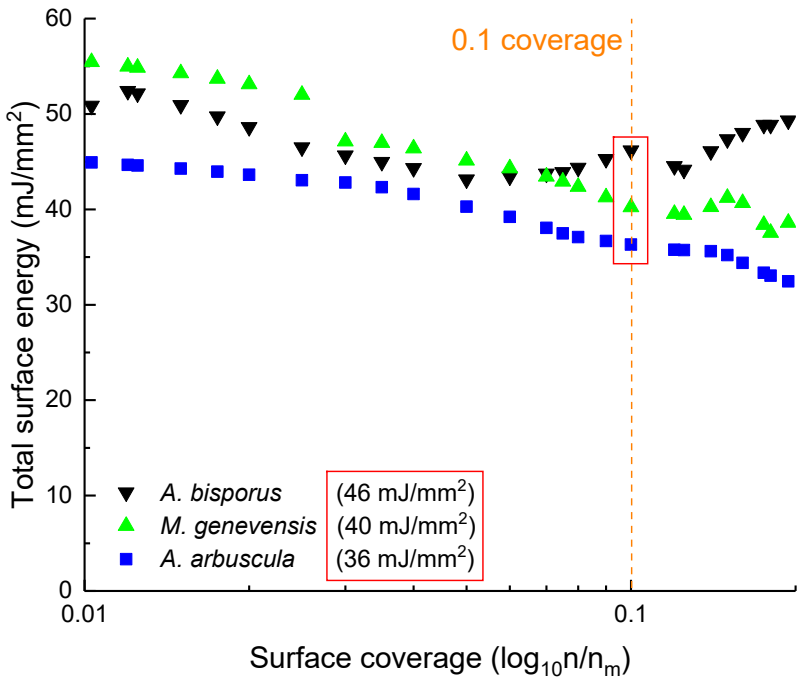
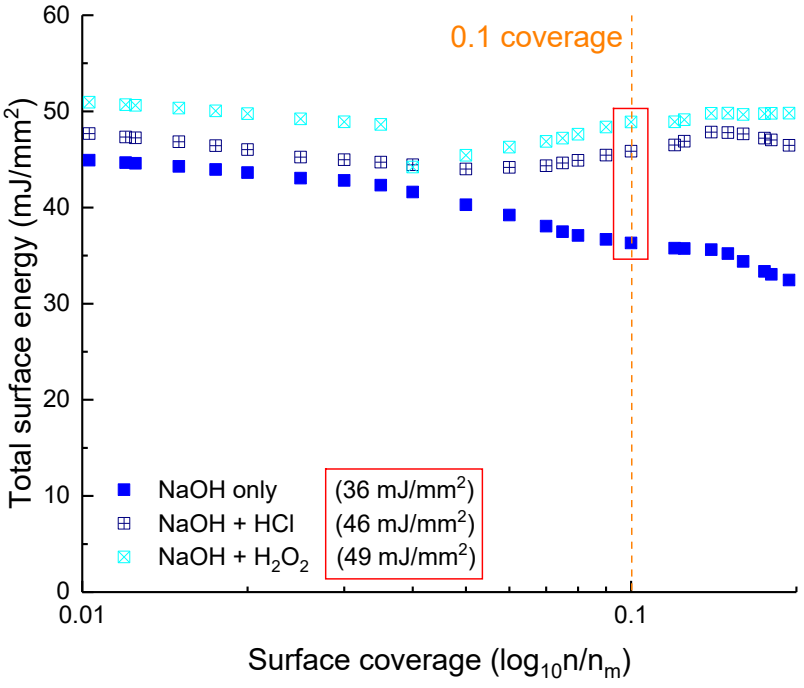


Figure S4. Energy-dispersive x-ray spectroscopy (EDS) elemental composition maps for *A. arbuscula* nanopapers treated with NaOH + H_2O_2 or NaOH + HCl.



(a)



(b)

Figure S5. Inverse gas chromatography (iGC): total surface energy (mJ/mm^2) as a function of surface coverage for (a) nanopapers treated using only NaOH (mycelium-derived: *A. arbuscula* (blue), *M. genevensis* (green) and *T. versicolor* (red) and fruiting body derived: *A. bisporus* (black) nanopapers) and (b) *A. arbuscula* nanopapers treated with NaOH + H_2O_2 (cyan) or NaOH + HCl (navy). *T. versicolor* samples were ommited from iGC analysis since contact angle goniometry was not performed on these films and hence no data validation was required.

Table S1. Biomass and extract dry mass yields (and associated water content) and overall total dry polymer yield (total: biomass to chitin-glucan or chitin-chitosan + contaminants, corrected for Ca: yield corrected for inorganic Ca salt content in extracts) for selected species.

Species	Dry mass / water content		Yield (wt%)
	d.wt biomass (wt%)	d.wt extract (wt%)	Total / Corrected for Ca
A. bisporus	5.1% (94.9% H_2O)	3.4% (96.6% H_2O)	19.3% (19.2% corrected)
A. arbuscular	13.3% (86.7% H_2O)	10.3% (89.7% H_2O)	27.1% (25.8% corrected)
M. genevensis	16.4% (83.6% H_2O)	9.7% (90.3% H_2O)	15.8% (15.0% corrected)
T. versicolor	25.7% (74.3% H_2O)	9.5% (90.5% H_2O)	12.0% (9.6% corrected)

Table S2. ^{13}C ssNMR peak integrals associated with -CH_3 and C=O signals and fraction of acetylated monosaccharide units (X_{acetyl}) (at 22.5 ppm and 173.5 ppm respectively) for nanopapers treated using only NaOH (mycelium-derived: *A. arbuscula*, *M. genevensis* and fruiting body derived: *A. bisporus*) and *A. arbuscula* nanopapers treated with NaOH + H_2O_2 or NaOH + HCl. *T. versicolor* samples were omitted from the analysis as they did not exhibit the peaks of interest.

Species	Peak integrals			X_{acetyl}
	C=O	CH_3	Average	(%)
A. bisporus (NaOH only)	0.2641	0.3526	0.3084	30.8
A. arbuscular (NaOH only)	0.1182	0.1102	0.1142	11.4
--- NaOH + H_2O_2 treatments	0.1227	0.2210	0.1719	17.2
--- NaOH + HCl treatments	0.1323	0.1484	0.1404	14.0
M. genevensis (NaOH only)	0.1103	0.1450	0.1277	12.8

Table S3. Sugar content (glucosamine and other sugars), fraction of acetylated monosaccharide units (X_{acetyl}), degree of acetylation (D.A.) (X_{acetyl}/glucosamine) and chitin (D.A. × glucosamine) and chitosan ([100−D.A.] × glucosamine) contents as a percentage of total sugars present in nanopapers treated using only NaOH (mycelium-derived: *A. arbuscula*, *M. genevensis* and fruiting body derived: *A. bisporus*) and *A. arbuscula* nanopapers treated with NaOH + H_2O_2 or NaOH + HCl. *T. versicolor* samples were omitted from the analysis as they did not exhibit the peaks of interest in ssNMR. Chitin, chitosan and other sugar content (total 100% of sugars present in the nanopapers) highlighted in bold.

Species	Glucosamine	Other	X_{acetyl}	D.A.	Chitin	Chitosan
	(%)	(%)	(%)	(%)	(%)	(%)
A. bisporus (NaOH only)	42.3	**57.7**	30.8	72.8	**30.8**	**11.5**
A. arbuscular (NaOH only)	13.7	**86.3**	11.4	83.2	**11.4**	**2.3**
--- NaOH + H_2O_2 treatments	18.8	**81.2**	17.2	91.5	**17.2**	**1.6**
--- NaOH + HCl treatments	17.9	**82.1**	14.0	78.2	**14.0**	**3.9**
M. genevensis (NaOH only)	22.8	**77.2**	12.8	56.1	**12.8**	**10.0**

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Authors' response:
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The manuscript has been formatted using the suggested template and all journal titles abbreviated using the CASSI abbreviations index provided.

Editor query 2: Reference 19 and 23 are incomplete: bibliographic information should be added if available, otherwise add doi.

Authors' response:
Thank you to the editor for bringing this missing information to the attention of the authors. Reference 19 and 23 have been updated and now read:

(19) Nawawi, W. M. F. W.; Lee, K.-Y.; Kontturi, E.; Murphy, R.; Bismarck, A., Chitin nanopaper from mushroom extract: natural composite of nanofibres and glucan from a single bio-based source. ACS Sustainable Chemistry & Engineering **2019,** 7 (7), 6492-6496.

(23) Jones, M.; Lawrie, A.; Huynh, T.; Morrison, P.; Mautner, A.; Bismarck, A.; John, S., Agricultural By-product Suitability for the Production of Chitinous Composites and Nanofibers. Process Biochemistry **2019,** 80, 95-102.

Editor query 3: Reference 37 is incomplete (journal title missing) and should be correctly formatted.

Authors' response:
The authors are grateful for the identification of this missing information and incorrect formatting. Reference 37 has been updated and now reads:

(37) Swain, S.; Dey, R.; Islam, D. M.; Patel, R.; Jha, U.; Patnaik, T.; Airoldi, C., Removal of Fluoride from Aqueous Solution Using Aluminum-Impregnated Chitosan Biopolymer. Separation Science and Technology **2009,** 44 (9), 2096-2116.

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"Supporting Information

Biomass and extract dry mass yields, associated water content and overall total dry polymer yield, energy-dispersive x-ray spectroscopy (EDS) spectra and elemental composition maps, zeta potentials as a function of pH, inverse gas chromatography (iGC) for total surface energy as a function of surface coverage, ^{13}C ssNMR peak integrals, fraction of acetylated monosaccharide units, degree of acetylation and chitin and chitosan contents as a percentage of total sugars present."

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Thank you for this comment. Figure 6 (previously Figure 5) has been completely redesigned and does not contain any copyrighted elements. It is now a completely original figure. The caption for this figure has been updated to read "Data from Appels, et al.[3, 11] and Ashby, et al.[42]". A copy of reference 42 has been provided as requested.

Reviewer comments:

Reviewer #1 (Remarks to the Authors):
None.

Reviewer query 1: Abstract, line 32: I think the use of "very poor" is not correct. It depends what the aim is of the use of the material how poor the properties are. Same holds for " better used". The strength of mycelium materials is their width of applications. The same holds for line 10, page 2

Authors' response:
The authors thank Reviewer #1 for their valuable feedback and appreciate the points that the reviewer has made. The term "very poor" has been replaced in the revised manuscript with "foam-like". The terms "better utilised" have also been changed to "alternatively utilised".

Reviewer query 2: Abstract: The authors should add some quantitative data of the materials produced. In this way the reader will immediately acknowledge the potential of the material

Authors' response:
Thank you for this excellent suggestion. The abstract has been updated and the following now appears within the abstract text.

"Polymer yields of ~10-26% were achieved, which is comparable to those of crustacean-derived chitin, and the nanopapers produced exhibited much higher tensile strengths than existing mycelium materials, with values of up to ~25 MPa (mycelium) and ~98 MPa (mushroom), in addition to useful hydrophobic surface properties resulting from the presence of organic lipid residues in the nanopapers."

Reviewer query 3: P1, Line 60: replace bonds to binds

Authors' response:
The term "bonds" has now been changed to "binds".

Reviewer query 4: First paragraph of introduction: the authors should be more clear about the two different fungal materials; the composites and the pure mycelium. The pure mycelium is stronger. Yet, it can be made more strong by extracting non structural components

Authors' response:
The authors thank Reviewer #1 for their suggestions to improve the clarity of the manuscript. The introduction has been amended and now reads as follows:

"Unfortunately, mycelium-derived materials are typically limited to mechanical properties resembling foams and natural materials. Mycelium composites comprise a combination of fungal mycelium and undigested lignocellulosic material and have foam-like mechanical properties with ultimate tensile strengths of up to 1.1 MPa.[6] Conversely, mycelial biomass comprises only fungal mycelium and exhibits material properties typical of natural materials, such as wood and cork, with tensile strengths up to 9.6 MPa for *Schizophyllum commune*.[11] Limitations in the strength of mycelium composites result from the often low-strength agricultural waste or by-products utilised in these composites as filler, which are weakly bonded by a hyphal filament matrix,[3] while the strength of mycelium itself is limited by the presence of non-structural elements, such as cytoplasm, proteins and lipids present in the fungal biomass.[12]

The mechanical performance of mycelium-derived materials can be improved by eliminating the use of these low-strength wastes and by-products as composite fillers, instead utilising them solely as nutrient sources for fungal growth and removing non-structural elements from the isolated mycelium."

Reviewer query 5: Second paragraph: pre mycelium can have a similar strength when compared to chitin (see Appels et al., 2018). So the argument that extraction of chitin is better makes no sense

Authors' response:
Thank you to Reviewer #1 for this comment. The authors are familiar with the work of Appels et al., 2018 and comment on the work later in the manuscript. Appels et al., 2018 utilise genetic modification of the SC3 hydrophobin (Δsc3) gene and controlled growth environments to produce mycelium materials with mechanical properties which would not be considered typical of either mycelium composites or mycelial biomass. Their work is specific to schizophyllan generation in the species *Schizophyllum commune* and is based on existing work completed by van Wetter et al., 1996 and 2000.

The work is indeed valuable and while it is clear that genetically modified fungal growth with improved mechanical performance is more desirable than post growth treated materials it is also worth noting that genetic modifications are much more complicated to achieve than post treatments, are species and gene specific and may not be achievable in all species of fungi. Appels et al., 2018 had the benefit of a significant body of existing work available to them when completing their genetic modification of the species *Schizophyllum commune*. It is likely that the genetic modification of any other species would be very time consuming and potentially not possible depending on the respective cell wall composition and genes present in any other given species. Our study utilised post treatments since they have the advantage of being universally applicable to all fungal biomass rather than species, strain and growth environment specific genetic modification techniques, and achieved similar results to Appels et al., 2018. There are also numerous ethical concerns associated with genetically modified organisms, making post-treatments a preferable option in a lot of cases.

Reviewer query 6: The authors should cite the articles that relate to the cell wall composition of the different fungi in Table 1. Is it really true that Mucor has no glucan ? this is surprising because normally cell walls need an elastic component

Authors' response:
Thanks for this suggestion. The cell wall compositions specified in Table 1 come from the textbook "Fungi: Biology and Applications" (Kavanagh, 2005), which is cited. Kavanagh (2005) in turn derives the table from the textbooks "Modern Mycology" (Deacon, 2000) and "The Fungi" (Carlile, 2001), which in turn are derived from numerous works over many years. The table in question in these textbooks describe the fibrillar and matrix polymers of entire phyla and is not species specific, hence draws on a quantity of sources too large to cite in a typical research paper. As such, Kavanagh (2005) is the only source cited.

Species within the *Mucor* genus typically have cell walls comprising chitin and chitosan as their primary constituents. They also contain polysaccharides (galactose, mannose and fucose), phosphate, proteins (at least 13 common amino acids), lipids (readily extracted and bound), purines and pyrimidines (RNA type), Mg^{2+} and Ca^{2+} (Bartnicki-Garcia & Nickerson, 1961).

The following sentence has been added to the manuscript to clarify this within the manuscript:

"Fibrillar cell wall polymers, such as chitin, chitosan and glucan, were of primary interest in this study, but it should be noted that cell walls also contain polysaccharides (e.g. galactose, mannose and fucose), phosphate, proteins, lipids and mineral salts."

The "Polymers" label in Table 1 has also been altered to read "Fibrillar polymers".

Reviewer query 7: P8, line 21: you mention chitin based fibers; but they are not. They are a mixture of several polysaccharides. Check the whole manuscript for this.

Authors' response:
The authors thank Reviewer #1 for their comment. The term "chitin-based fibers" has been changed to "polysaccharide-based fibers" throughout the manuscript.

Reviewer query 8: P12, line 52: I was surprised to read that the A. arbuscula mycelium was chosen for purification. This fungus showed the lowest chitin content of the selected fungi; therefore not the fungus of choice for future studies.

Authors' response:
Thanks a lot for raising this point. Species within the *Allomyces* genus are frequently noted in literature as having among the highest chitin cell wall concentrations (Griffin, 1996). As such, *A. arbuscula* seemed a logical choice for purification, being a species of particular academic interest for this reason. *A. arbuscula* was also the species with the second highest mycelium-derived chitin content, lagging only slightly behind *M. genevensis* (11.4% chitin compared to 12.8%), as outlined in Table S3 of the Supplementary Material. *A. arbuscula* also primarily contains chitin-glucan rather than chitin-chitosan (*M. genevensis*) making it a more applicable reference species for mycelium material applications, which almost exclusively use species from phylum *Basidiomycota* (chitin-glucan cell walls).

Additional Questions:

Please rate the overall importance of this manuscript to the field of biomacromolecules (10 – High Importance/ 1 – Low Importance): 8

Please rate the element of novelty in the research reported (10 - High Novelty / 1 - Low Novelty): 8

Please rate the interest to scientists working in the field of biomacromolecules (10 - High Interest / 1 - Low Interest): 8

Please rate the focus of the manuscript on the interface of polymer science and the biological sciences (10 - High Focus / 1 - Low Focus): 8

Are the conclusions adequately supported by the data presented?: Yes

Are any compounds reported adequately characterized?: Yes

Are the literature references appropriate and up to date?: Yes

Overall quality and clarity of the manuscript: Good

Would you like to bring this manuscript to the attention of the Editor as a particularly newsworthy or noteworthy manuscript?: Yes

Reviewer #2 (Remarks to the Authors):
The paper entitled "Waste-derived Low-cost Mycelium Nanopapers with Tunable Mechanical and Surface Properties" by Jones et al. is a very interesting work about the use of mycelium as "chitin-factories" for the production of "nanopapers" in a context of circular bioeconomy. The topic is attractive, the manuscript is very well written and the characterization is comprehensive and useful for other researchers. Congratulations to the authors.

Authors' response:
The authors wish to thank Reviewer #2 for their compliments on the quality, thoroughness and value of the manuscript and congratulations. The reviewer's positive feedback is very greatly appreciated.

Reviewer query 1: I only have a small demand. Please, add an initial figure with a schematic representation of the nanopaper fabrication and some photos of the final materials.

Authors' response:
The authors thank Reviewer #2 for their valuable suggestion. An additional figure, now entitled "Figure 1" has been added to the manuscript, which details the fabrication of the nanopapers and provides a representation of the final materials.

Additional Questions:

Please rate the overall importance of this manuscript to the field of biomacromolecules (10 – High Importance/ 1 – Low Importance): 9

Please rate the element of novelty in the research reported (10 - High Novelty / 1 - Low Novelty): 9

Please rate the interest to scientists working in the field of biomacromolecules (10 - High Interest / 1 - Low Interest): 9

Please rate the focus of the manuscript on the interface of polymer science and the biological sciences (10 - High Focus / 1 - Low Focus): 8

Are the conclusions adequately supported by the data presented?: Yes

Are any compounds reported adequately characterized?: Yes

Are the literature references appropriate and up to date?: Yes

Overall quality and clarity of the manuscript: Excellent

Would you like to bring this manuscript to the attention of the Editor as a particularly newsworthy or noteworthy manuscript?: Yes