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Characterization of mycelium-based composites as foam-like wall insulation material

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Highlights

The main objective of this research is to study the feasibility of mycelium-based composites when applying as foam-like wall insulation material, and outlined below:

- To find the most optimal and suitable substrates ratio as foam-like wall insulation material in regard to thermal conductivity and compressive strength. The selected mixture is used in the next experiment.

- To investigate a prolonged growth period for a thicker and denser outer layer of mycelium which can result in better water resistance.

- Apply drying and wetting cycles to study the influences of accelerated aging on mycelium-based composites.

- To study mycelium-based composites in hygrothermal behavior regarding moisture buffer performance.

Abstract

Nowadays circular economy and sustainability aspects of materials are taking huge roles in consumer decisions. Wall insulation materials are usually synthetic or petroleum-derived materials, which are less environmentally friendly in the overall material life cycle. Mycelium -based composites (MBC), on the other hand, utilize fungal mycelium, an interwoven network of hyphae to bind with lignocellulosic substrates and produce composites with high porosity. The main components of mycelium are natural polymers; thus, it is a biocomposite and completely biodegradable at the end-of-life cycle. Furthermore, MBC can also upcycle agricultural by-products. White-rot fungi have superior traits to decay and obtain nutrients from any lignocellulosic materials, including low-nutrients agricultural by-products. In addition, mycelium composites can be alternative sustainable materials to replace petroleum-derived foams in the current conventional insulation market. Utilizing agriculture residues to create sustainable biocomposites in the building industry that meets the ultimate goal of mitigating natural resources exploitation and reducing energy and water usage in material production.

This research aims to study the feasibility of MBC as foam-like wall insulation material by conducting experiments related to material characterizations and applying an accelerated aging test on MBC. The results showed that a prolonged growing period arose a denser mycelium outer layer in MBC, which rendered better water resistance due to the hydrophobicity of mycelium. Thermal conductivity and mechanical properties are highly dependent on substrate choices than other parameters of MBC, which coincided with literature. Additionally, influences of accelerated aging test and moisture buffer capacity of MBC were first studied in this research. The results indicated that MBC not only maintained good functional performance after the accelerated aging test (i.e. drying and wetting cycles) but also constituted good moisture buffer capacity. This means that MBC has key material essences to apply as internal wall insulation material and become one of the layers in vapor-permeable building envelope systems to passively regulate indoor relative humidity and thermal comfort.

Nomenclature

Abbreviations

G. *lucidum*	Ganoderma *lucidum*
RPS	Rapeseed Straw
MBC	Mycelium-Based Composites
EPS	Expanded Polystyrene
XPS	Extruded Polystyrene
MBV	Moisture Buffer Value
SEM	Scanning Electron Microscopy
FT-IR	Fourier Transform Infrared Spectroscopy
DWC	Drying and Wetting Cycle
RH	Relative Humidity (%)
PG	Prolong Growth
NG	Normal Growth

Table of Contents

Introduction 1
  1.1. Knowledge background of fungi 2
  1.2. Weakness of MBC 5
Literature Review 7
  2.1. Mycelium-based composites as insulation materials 7
    2.1.1. Thermal insulation materials 7
    2.1.2. Overview of MBC 10
    2.1.3. Production process of foam MBC 12
    2.1.4. MBC physical and mechanical characterizations 16
  2.2. Moisture buffer capacity of building materials 24
  2.3. Challenges with MBC apply as thermal insulation 30
Materials and methods 32
  3.1. Materials 32
  3.2. Sample fabrication and drying 33
  3.3. Methods 36
Results and Discussion 41
  4.1. Substrate mixtures refinement 41
  4.2. Physical properties of MBC 46
  4.3. Hygrothermal behavior: $MBV_{practical}$ 52
Conclusion 55
References: I
Appendices: i
  Appendix A- Compressive Strength Test Raw Data i
  Appendix B- Flexural Strength Test Raw Data (NG vs PG) iv
  Appendix C- MBV Raw Data Sheet Sample v

1. Introduction

Building envelope systems in temperate and cold climate regions rely on insulation materials in cavities and are ubiquitous and required in building codes for better indoor environment control to save energy loads. Especially in heating demand regions (latitude >40°), insulation materials play a critical role as one of the passive strategies to reduce heating loads and possibly achieve net-zero energy buildings. However, arbitrary standards and inconsistency between manufactures product sheets, laboratory testing conditions, field installing and actual performances imposed tangible difficulties for designers and commercial users to select suitable insulation materials [1][2]. In building physics and science field, it is well-known that external continuous insulation (no thermal bridge) is the most optimal insulation method in climate regions that require higher heating demand [3]. Yet, this construction method only applicable to new design projects. Most existed buildings rely on renovations with internal insulation within structural cavities to improve thermal performance. Especially, in historical preserved monuments, where altering external appearance is not allowed. Internal insulation materials indeed have a higher produced volume than insulation for exterior applications. Thus, this research is inspired by utilizing sustainable biocomposites to replace petroleum-derived synthetic foams as internal insulation.

As greenhouse gases emission, plastic wastes, a large amount of human-induced toxicity, and raw material exploitation are detrimental to the environment and human health. The building industry is one of the major sectors that accounts for the above environmental issues, along with transportation and manufacturing industry [4]. Fortunately, the building sector is aware of reducing its carbon footprint and eliminating harmful substances to be used in building design and construction. Nowadays circular economy and sustainability aspects of materials are thriving in consumer decisions and scientific research. These aspects are also encouraged in sustainable building design tools (i.e. LEED by U.S. Green Building Council or BREEM by Building Research Establishment) [4]. A circular economy is when a production process circular within a complete cycle and can be infinitely repeated. This approach and strategy not only creates zero waste in a production process, but also considers reuse and recycle at the materials' end-of-life cycle, which reduces raw material consumptions and exploitation.

Wall insulation materials are usually synthetic or petroleum-derived materials, which are less environmentally friendly in the overall material life cycle and do not align with a circular economy approach. Furthermore, petroleum-derived materials that contain toxic compounds when used in building interiors, are usually carcinogenic and imposed health risks to inhabitants [5]. On the other hand, biocomposites use less extracted raw materials at the production process and are 100% bio-degradable at the end-of-life cycle, which will not release toxic substances into the earth and soil. For instance, working with natural fibrous materials based on agricultural residues or forest residues to produce insulation particleboards, foam composites, rolls, and batts. Another advantage of using biocomposites and natural plant-based materials for internal insulation is the ability to regulate indoor environment (i.e. relative humidity (RH) and temperature) and potential reduction in energy operational cost due to their high hygroscopicity (ability to absorb and release moisture from the environment) [6], [7]. Despite a large amount of literature related to natural fiber insulation materials exists, associated production processes with natural binders were monotonous. One intriguing, self-growing, and living material, mycelium, has brought growing interests in material science and in the building material sector since a recent decade [8].

Mycelium-based composites (MBC) utilizes fungal mycelium, an interwoven network of hyphae to bind with lignocellulosic substrates and produce composites with high porosity. The main components of mycelium are natural polymers, such as polysaccharides, proteins, and lipids, that are 100% bio-degradable. Substrates (usually lignocellulosic feedstocks) in MBC not only provide nutrients for fungi to grow on but also have a major impact on the final properties of composites. Review papers summarized commercial products made by MBC across from various building materials, such as insulation materials, acoustic absorption panels, structural materials to packaging foams (i.e. furniture, particleboards, etc.) [9]–[11]. Studies have shown mycelium composites can be alternative sustainable materials to replace petroleum-derived foams used in the current conventional insulation market [9], [12]–[14]. Besides its low thermal conductivity, one study has shown it outperformed synthetic insulation materials in the fire safety aspect [12]. It released less hazardous gases and had less heat release rate; furthermore, it’s a potentially cheaper solution (per volume) due to low-cost ingredients [12]. Before introducing the production process of MBC, it is crucial to amplify details regarding background information of fungal biological and mycological mechanisms.

1.1. Knowledge background of fungi

Most of the studies in MBC chose white-rot fungi because of their well-known ability to decay lignin most efficiently, which can be grown on any lignocellulosic material in a cost-efficient perspective [15]. This type of fungi produces secret decaying enzymes to convert essential nutrients for growth. This research also used a white-rot fungi species, Ganoderma *lucidum (*G. *lucidum)* as known as wood-decay fungi as its name indicated (Figure 1a). The life cycle of fungi is a loop of spores germinate to hyphae (the root of fungi), a large surface of hyphae connects with each other to become a complicated network called mycelium. Mycelium matures into fruiting bodies, which generate spores and complete the growing cycle (Figure 2b). The sole purpose of this research only focuses on growing biomass of mycelium and not fruiting bodies of fungi. The whole growing cycle of fungi mentioned above has complex biological and chemical metabolisms, which belongs to the field of mycology.

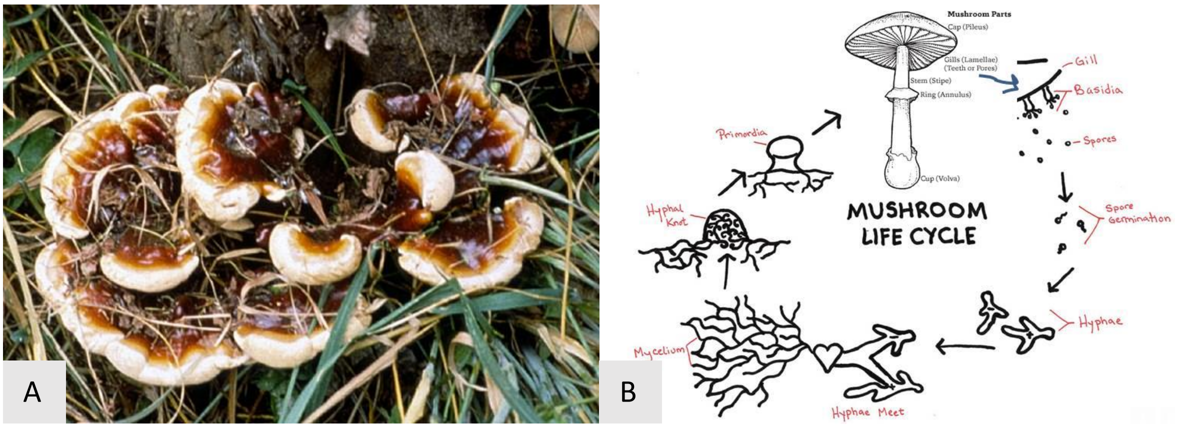

Figure 1 a) Ganoderma *lucidum* fruiting body [16] and b) a life cycle of fungi [17].

Fungi cell walls and structures are made of chitin, glucans, and glycoproteins, which are different from cell walls of plants. Chitin is the second most abundant biopolymer after cellulose and only exists in fungi and arthropods. It is a long-chain polymer of N-acetylglucosamine, a derivative of glucose when fixed with nitrogen, as Figure 2 shown. This long-chain polymer forms into anti-parallel chains and reinforces by crossed-linked to β (1,3)-glucan with covalent bonds, which represents the backbone of hyphae and mycelium [18]. The biosynthesis of chitin is known by secrete enzymes produced in fungi and detailed in [18]. As Figure 2 shown, the difference between cellulose and chitin (or chitosan) is the replacement of hydroxide to the amino group. Fungi utilize different forms of nitrogen from nutrient resources to biosynthesized chitin, i.e., nitrate nitrogen, nitrates, ammonium (NH_4^+), glutamine, etc. [19], [20].

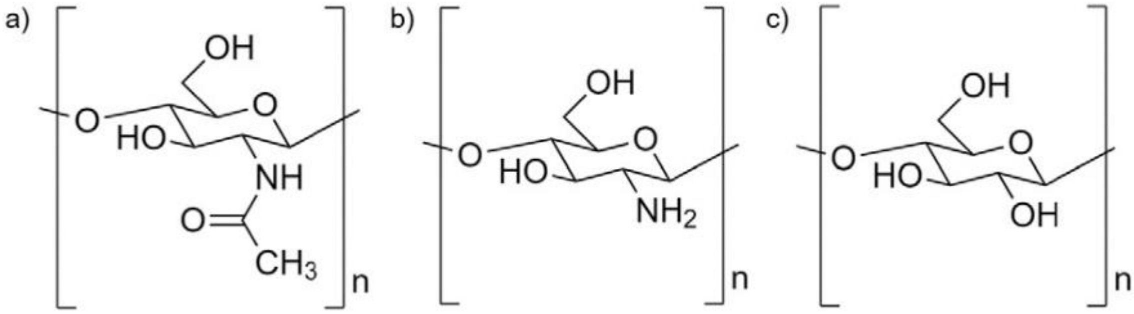

Figure 2 Molecular structures of a) chitin, b) chitosan and c) cellulose. *Note*. Reprinted from [21].

The taxonomic of G. *lucidum* belongs to Basidiomycota, *Polyporales* order, and family of *Ganodermataceae* (or *Polyporaceae*). Taxonomic classification is one of the most adapted classifications in biology, which classify fungi into hierarchal groups by recognizable features and characteristics and show as phylogenetic trees, similar to the Animalia or the Plantae Kingdom in biology [22]. Elsacker et al. [15] compiled information about commonly studied fungi in mycelium-based materials in phylogenetic trees, as Figure 3 shown. The fungi order, *Polyporales*, is among the most populist fungi order to be studied in scientific articles, and G. *lucidum* belongs to this order.

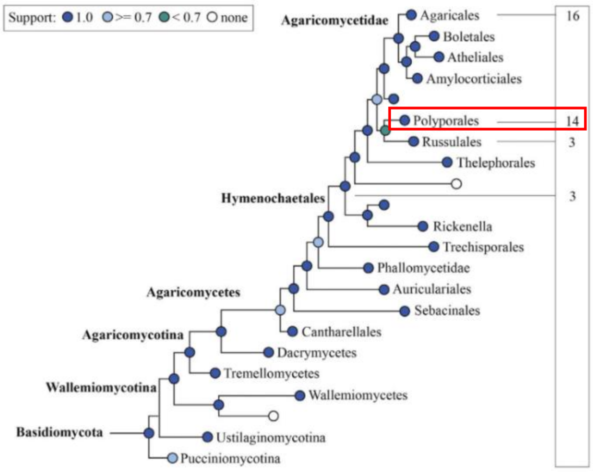

Figure 3 Phylogenetic representation at the order level of fungi species used in published papers related to mycelium-based materials. *Note*. Reprinted from [15].

Besides taxonomic classification, G. *lucidum* belongs to the saprophytic fungi group (this category is decay types of fungi), which is known by converting plant wastes into biomass for material science or medical usages (the extracts or fruiting bodies for consumption). White-rot fungi are the only type that has the ability to decay lignin completely [23].

When researched G. *lucidum* regarding growing on different lignocellulosic wastes, one study [24] showed that G. *lucidum* mycelial growth rate has a positive correlation with substrates consisted a high level of nitrogen content but a low level of cellulose (both had significant correlation at the level of 0.01). However, in-depth discussion is absent in this study and there is counter-evidence showed in another study [20]. As fungi have complicated regulatory mechanisms regarding nitrogen regulation and subjective to fungi phylogenetic. Optimizing the growing conditions of mycelium in specific fungus requires experts in microbiology, which is out of the scope of this study.

The complex biosynthesis mechanisms of fungi decaying lignocellulosic materials in molecular, biochemical, chemical aspects still require large scientific researches and lack sufficient details [23], [25]. The fundamental process of delignification known by microbiologists is the extracellular enzymes produced by fungi degraded lignin by oxidative and not hydrolytic because lignin polymers are hydrophobic and difficult to degrade due to its unspecific linkages and heterogeneity [25]. White-rot fungi produce various polysaccharide-degrading enzymes and delignification enzymes when decaying wood and plants, such as laccase, lignin peroxidase (Lip), manganese peroxidase (MnP), etc., has shown in various studies [23], [26]. The proposed delignification mechanism initiated by MnP is shown in Figure 4, fungi obtain nutrients (i.e. sugars, carbon, nitrogen, etc.) from lignocellulosic materials and produced secret enzymes to degraded lignin. In woods, white-rot fungi attach to cell lumina, jeopardize the cell structure, and colonize it with mycelium.

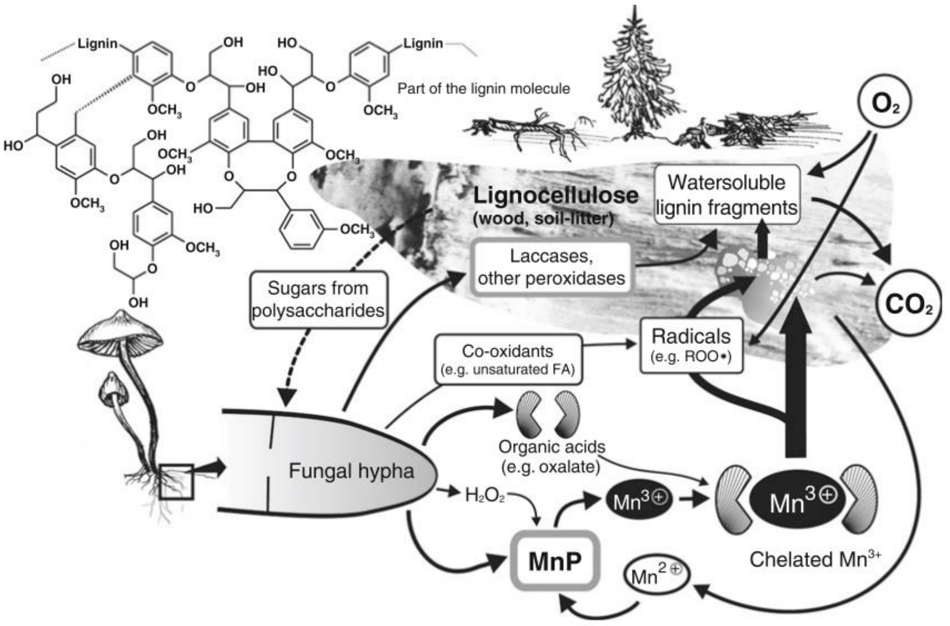

Figure 4 Proposed MnP enzymes lignin degradation mechanism. *Note*. Reprinted from [25].

1.2. Weakness of MBC

One of the important design essences when considering internal insulation is the inevitable condensation at the interfaces of insulation materials within envelope systems. It happens when a non-isothermal condition occurs (interior temperature is greater than exterior temperature; interior relative humidity (RH) is lower than exterior RH). The condensation is accumulated and absorbed by the insulation materials. The thermal conductivity of the material increases due to water is a medium with high conductivity, which jeopardizes the functional performance as an insulation layer to minimize energy transfer. Therefore, thermal conductivity, water vapor transmission characteristics, water absorption coefficient are important parameters to consider when selecting thermal insulation materials.

Similar to other natural biocomposites, there is one intrinsic weakness of MBC to be used as building materials due to a large portion of the material being fibrous particles. Both the natural microstructure of the composites and the hydrophilicity impose difficulties to apply as building materials, especially for exterior applications. Although water intake and absorption tests have been done in studies, results were troublesome to compare due to different standards and testing methods used [27]–[32]. Solutions for high water intake were hardly provided nor studied in the research field. One study had developed treatments to improve its water resistance from applying mycelium on surfaces in nanoscale [33]. Nevertheless, tests and issues related to durability of MBC have not been presented in detail or overview in any study yet.

MBC is still a fairly new material both in the commercial market and the research. A few critical review papers pointed out the research focuses and approaches were vastly different among studies, making it difficult to compare and resonate results [9], [11], [13], [22]. Therefore, this study first provided an in-depth literature review on mycelium related materials to bridge the knowledge gaps. The literature review was separated into 3 sections. Firstly, an overview of insulation materials regarding important characteristics and material comparison (i.e. wool, extruded (XPS) and expanded polystyrene (EPS)), followed by the introduction of foam MBC and valuable results from studies for comparison and discussion. Secondly, provided detailed information about materials hygrothermal behavior and related studies. Lastly, discussed the challenges for MBC when applied as insulation materials and in what perspective it can outperform other insulation materials. The literature review stood as fundamental knowledge of this research and this review also inspired many experiment set-ups in the process.

This study intends to investigate the feasibility of MBC as wall foam-liked insulation material by studying its material characterizations through systemic literature review and practical experiments, which included produced non-pressed foam MBC and experimented with several material characterizations and accelerated aging tests. The first part of the research focused on producing the most optimal substrates mixing ratio between rapeseed straw (RPS) and cellulose in thermal conductivity and compressive strength as two main selection criteria. Assumed these two substantial different substrates (in particle sizes, compositions, etc) can compromise each other and produce MBC with better performance in physical properties. The selected mixture was further studied in the next experiment to investigate the influences of a prolonged growing time on water intake and other material property aspects. This research especially investigated from water and moisture penetration perspectives while compared the performances with petroleum-derived foam insulation materials, (i.e. EPS) and conventional interior building materials (i.e. gypsum board) when applied as internal insulation materials.

Research questions, objectives, and hypotheses of this study are defined as below:

- To compare various substrates mixing ratios of RPS and cellulose in thermal conductivity and compressive strength performance. Substrate mixtures with the most optimal and suitable mixing ratio to be applied as foam-like wall insulation material in both criteria.

- To investigate a prolonged growth period for a thicker and denser outer layer of mycelium which can result in better water resistance. A thicker and denser mycelium resulted in a higher yield in chitin or chitosan, which are the main biopolymer of fungi cell wall and structure. Chitin and chitosan are hydrophobic; therefore, able to increase the water resistance of the end products.

- To study accelerated aging influences on MBC. The thermal conductivity and compressive strength were conducted again to study consequences with and without drying and wetting cycles (DWC).

- To study MBC in hygrothermal behavior in regard to moisture buffer performance. As aforementioned, the high hygroscopicity of MBC is inherent from filler substrates (i.e. agricultural residues and wood products) and the open-cell air voids in materials. Porous materials perform better when air is permittable between construction layers, which allows vapor and moisture transmission in between layers to prevent liquid water accumulation. MBC has shown compatible results in thermal conductivity when compared with synthetic foams; water absorption issues can be compensated with correct construction methods and vapor permittable building envelope systems.

2. Literature Review

The literature review was conducted systematically by researching findings from published journals with keywords in titles and selecting by abstracts in the early stage. In the later stage, literature was mostly found by the "backward snowball" effect, i.e., select resources from the reference list of interesting papers. At beginning of the first section provided an overview of insulation materials on the current market, including the key essences to be able to apply as insulation materials, materials comparisons, related durability characteristics, etc. Followed with MBC as innovative insulation material and an in-depth literature review of basic information one requires to know about mycelium and MBC, (i.e. the fundamental growth kinetics, production process, MBC material characteristics, etc.). Data extraction was performed for each paper for analysis regarding proposed research questions (section 2.1).

The second section is the review of porous building materials in hygrothermal behavior, specified in the NORDTEST moisture buffer values (MBV) method (section 2.22.2). Lastly, a summary section of current MBC challenges to apply as thermal insulation material (section 2.3).

2.1. Mycelium-based composites as insulation materials

2.1.1. Thermal insulation materials

Several studies developed comparative, multi-objective tools and in-depth analysis for conventional insulation materials currently in the commercial market, unconventional materials (biobased and sustainable), and innovative materials (i.e. Vacuum insulation panels, Aerogel, etc.) [1], [34], [35]. Although not a single study mentions mycelium-based material, its intrinsic characteristics belong to sustainable and plant-based materials when comparing with other insulation materials because substrates used in MBC are natural plants and fibrous materials.

As Figure 5 shown, parameters with 2 knots connected to indicators and criteria were chosen to be listed in Table 1 to compare plant-based, inorganic foams, and synthetic foams insulation materials currently on market. It should be noted that innovative materials are still under research and development stage; therefore, properties summary excluded innovative materials for comparison.

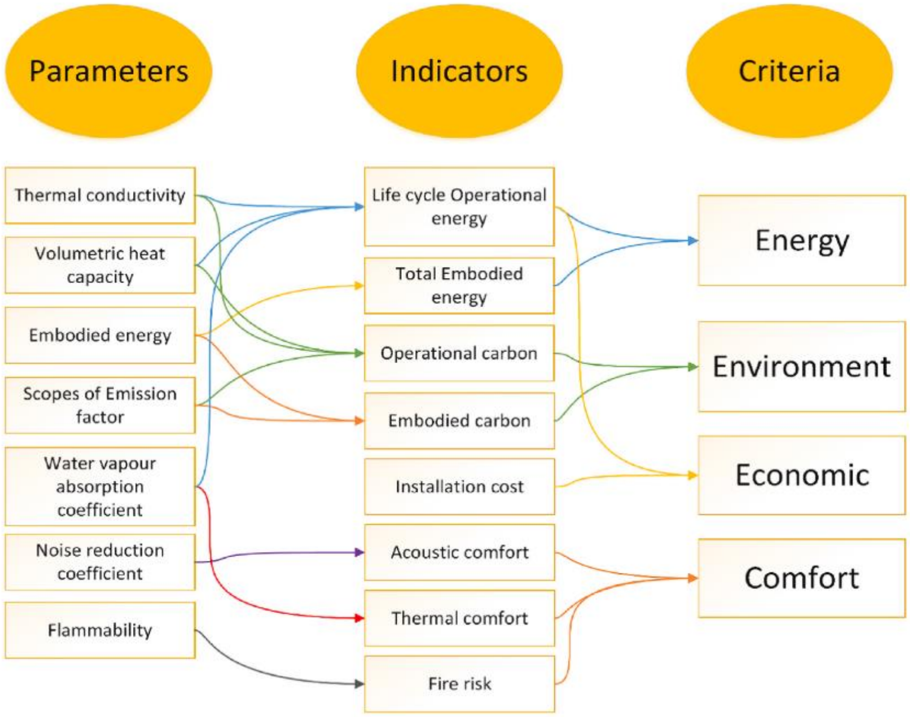

Figure 5 Kumar et al., proposed the framework to select optimum building insulation materials. *Note.* Reprinted from [35].

As Table 1 shown, plant-based insulation materials (i.e. hemp, flax, rice husk, wood fiber) have shown competitive thermal properties and potentially lower cost when compared with inorganic and synthetic insulation materials, subjective to plant selection and production methods [34], [35]. It is more versatile regarding material applications and shapes. For instance, plants fibrous can be produced as foam-like, rolls, batts, etc., to accommodate the targeted concealed spaces in building cavities. From the environmental perspective, plant-based materials have significantly lower embodied energy and carbon emission compare with others. In contrast, plant-based materials have way lower water vapor diffusion resistance factor (μ) due to high porosity and open-cell air voids, which cause durable problems when applying as insulation materials.

On the other hand, the high hygroscopicity and water wicking properties of plant-based materials provide a solution for interstitial condensation when applying as vapor-permeable internal insulation materials. The vapor-permeable characteristic provides a pathway for vapor and water to be absorbed and desorbed to reduce possibility of continuous wetting. As Figure 6 shown, the third scheme (vapor-permeable internal layer) allows liquid water to transfer between porous materials and an interior environment. Water wicking properties of these porous materials not only allow interstitial condensation caused by vapor diffusion to properly dry but also regulate indoor relative humidity when is needed and save energy and ventilation loads [36], [37].

Table 1 Conventional insulation materials properties. Data from Kumar et al. [32].

Material	Density	Thermal conductivity	Specific heat capacity	Water vapor diffusion resistance factor	Cost	Embodied Energy	Embodied Carbon
Unit	[kg/m^3]	[W.m^{-1}.K^{-1}]	[J/g*°C]	[-]	[USD/m^3]	[MJ/kg]	[kg CO$_{2\text{-eq}}$/kg]
Plant-based							
hemp	25-100	0.039-0.123	1.7-1.8	1-10	15-19.4	18.71	0.14
flax	20-100	0.033-0.09	1.6	1-5.28	15.18	39.5	20
Rice husk	130-170	0.048-0.08	1.2-2.7	2	5	1.36	0.6
Wood fiber	50-270	0.038-0.05	1.9-2.1	1-5	26.6-37.8	20.3	0.124
Inorganic (fibrous & foams)							
Glass wool	10-100	0.03-0.05	0.8-1	1-1.3	9.3-14.7	14-30.8	1.24
Rock wool	40-200	0.033-0.04	0.8-1	1-1.3	12-20	16.8	1.05
Synthetic foams							
EPS	18-50	0.029-0.041	1.25	20-100	8.6-17	80.8-127	6.3-7.3
XPS	32-40	0.032-0.037	1.45-1.7	80-170	18-23	72.8-105	7.55
Polyurethane	30-160	0.022-0.035	1.3-1.45	50-100	24.91	74-140.4	5.9
Phenolic foam	40-160	0.018-0.024	1.3-1.4	35	23	13-159	4.15-7.21

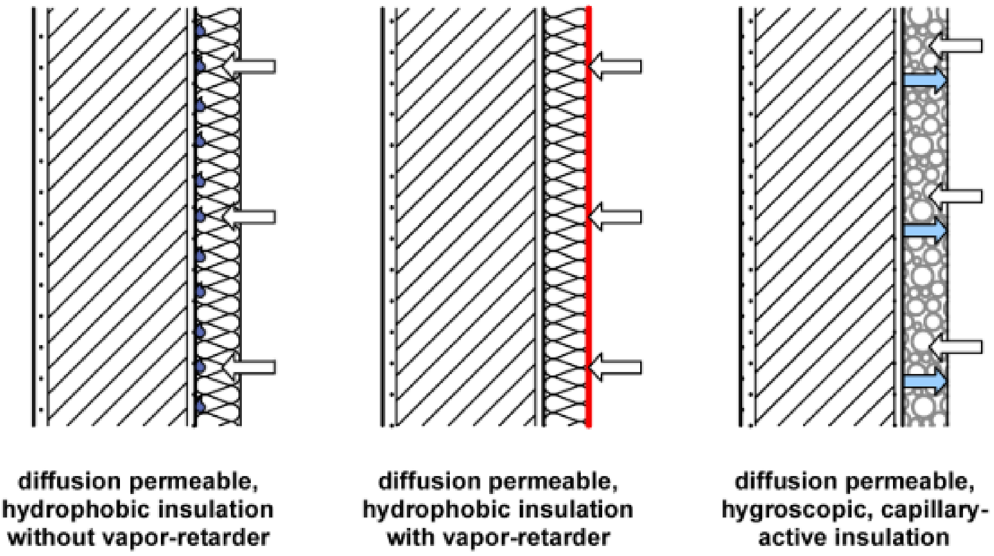

Figure 6 Three schemes of moisture transfer at the interface with hydrophobic or hygroscopic insulation materials. *Note.* Reprinted from [36].

As thermal insulation materials have a major role in saving building operational energy in a long run, more rigorous and holistic approaches in material selections and design schemes are required. Kumar et al [35], is the first study incorporated different climate regions into the selection framework of insulation materials regarding energy, environment, economic, and comfort performance criteria, as Figure 5 shown. Furthermore, the authors stated a more holistic perspective for building envelop design is urgently needed in traditional heating-demanded regions due to more extreme and intense heatwaves in past decades [35]. Multi-story dwellings suffered from severe thermal discomfort during intense heatwaves and increased peak cooling-demand due to higher thermal resistances and air-tightness of building envelope [35]. In addition, when designing building envelope systems, insulation materials with higher volume specific heat capacity (kJ/m^3*K), such as plant-based insulation materials can storage the peak solar radiation absorbing from building façades and reduce peak cooling loads by delaying indoor peak temperature caused by solar radiation in the summertime.

A few summary points to conclude the advantages of using sustainable porous and water vapor permeable materials as internal insulations:

- Sustainable plant-based materials have higher volumetric heat capacity to store heat and delay peak temperature rises inside buildings during the summertime, especially when heatwave occurs.

- Porous materials have high hygroscopic properties to regulate indoor RH and maintain indoor thermal comfort passively.

- Water wicking properties to solve interstitial condensation in material interfaces of insulation materials and other assembly layers.

2.1.2. Overview of MBC

The process of producing MBC is very similar to the edible fungal growing industry. Three major components of the material are substrates (the medium which provides nutrients for fungal growth and is the main structure of composites), fungal spawns (the growing process of hyphae creates a 3D interwoven network acts like binders), and water, as Figure 7 shown. The composition is rather simple and trivial; however, the growing conditions and processes are the important factors to determine the quality and quantity of the end materials.

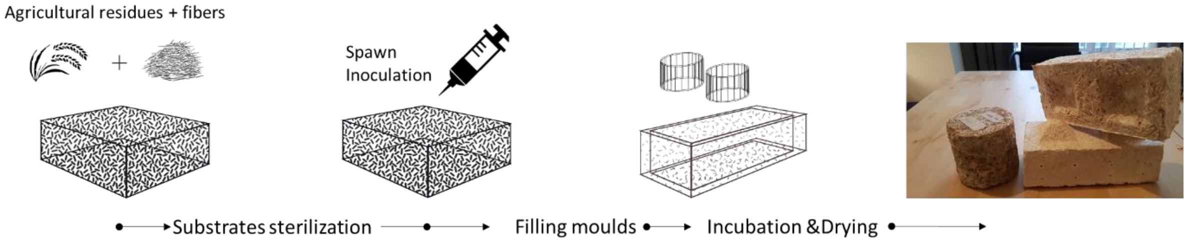

Figure 7 Production process of mycelium-based composites.

Foam composites' mycology and surface topography are highly dependent on fungal species and optimal growth conditions. In mycology, finding the optimal growing conditions for a specific genus or species of fungal will ensure the most efficient time to harvest in the most desired stage. As Figure 8 depicted, mycelium growing phases could be classified into 3 phases described below [13]:

a) Lag phase – when there is zero or low growth because fungal is focusing on acclimatization to their new physical environment. This can be better explained metaphorically as the settle-in phase when newcomers move to a new environment. Most of the energy and attention will be focusing on adaption.

b) Exponential phase – when biomass (total quantity of organisms in a given area or volume) of fungal population increases, such as cell numbers, nucleic acid, and protein contents. This phase happens when reaches optimal growing conditions, meaning most of the energy will be focusing on growing and increasing population.

c) Stationary phase – when there is no specific growth of population and biomass remain stable because nutrients are exploited or the environment is contaminated (prevent further growing for fungal).

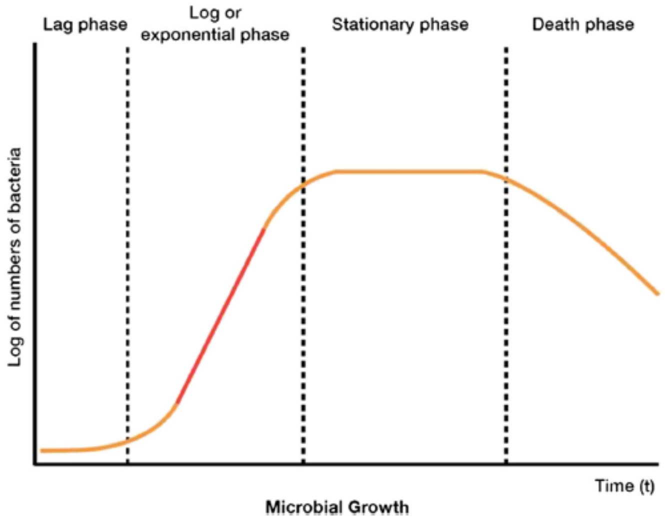

Figure 8 Fungal growth curve. *Note.* Reprinted from. [38]

An optimal growing condition by definition is to minimize the lag phase and ensure the exponential phase is reached as soon as possible to maximize growth yield and biomass [22]. The review from Jones et al. [22], compiled a very clear relationship chart between environmental growing conditions and growth kinetics of mycelium as Table 2 shown. In general, higher inoculated density (by volume), temperature, and water activity will result in better growth rate and higher maximum yield. It should be also noted that optimum values depend on taxonomic groups of fungal (genus).

Table 2 Relationship between mycelium growth kinetics and environmental growing conditions. Note. *Reprinted from* [22].

Environmental parameters	**Lag phase**	**Exponential phase**
Inoculum density ↑	↓	↑ Growth rate; ↑ Maximum yield
Temperature ↑	↓	↑ Growth rate
Water activity ↑	↓	↑ Growth rate
Extreme pH	↑	↓ Growth rate

MBC received attention in recent scientific research, 70% of papers were published between 2018 and 2019. However, literature is still scarce and most of them focused on material characterizations, which has shown that this material is still in early development. Scientific papers focused on material characterizations of MBC usually involved comparable standard tests and results in order to categorize it. Some other literature types focused on design aspects of the material, which most of them included aesthetics, design process, molds shapes, etc. [10], [39]–[42]. Others targeted in productive process such as production optimization, optimal growth, and yields of MBC and even upscaling possibilities for targeting products [32], [33], [43]–[45]. Bioengineering studies researched gene modification and deletion impact on MBC mechanical properties [46]–[49]. Review papers, patents review and smart materials overview were also published to have a broader view for mycelium-based materials [8], [9], [11], [15], [22], [50]–[53]. One study by Bucinell et al. [52] investigated the optimal way of testing tensile strength of MBC.

2.1.3. Production process of foam MBC

From a material sciences point of view, substrate choices, types and particle sizes for fungi to bind and grow on, has a tremendous impact on material mechanical and physical characteristics [11], [13], [22], [29], [31], [54]. For example, finer fibers will result in higher composites density; fibrous agricultural by-products and larger particle sizes will increase porosity and lower densities of composites, (i.e. straw). Fungi require nutrients to increase their biomass; therefore, substrates have great influences on a growth period (i.e. more nutrients less growth period vice versa). In addition to fundamental growth conditions of mycelium, substrates and water proportions also render growth conditions of mycelium (i.e. insufficient water within substrates reduce growth rate). Besides nutrients and water, fungi require a low amount of light and oxygen to grow; therefore, amount of light, airflow, temperature, and humidity all have influences on the growth rate and biomass of mycelium.

Post-processing methods such as heat-press generally will result in higher mechanical strength due to fewer air voids in-between material microstructure [29], [55]. Other parameters and their impacts on composites' characteristics were briefly listed in Table 3 and further explained in detail.

Table 3 Parameters affect material characterizations of MBC.

Parameters	**Impacts on composites' characteristics**
Fungal Strains	Surface morphology, growth rate, structural integrity, lignin degradation, mycelium thickness
Substrates	Bulk density, porosity, mechanical and physical properties
Substrate mixing proportions	Growth rate, biomass density, structural integrity
Growing conditions	Bulk density, porosity, water absorption, mechanical and physical properties
Post-processing	Mechanical and physical properties, water absorption

Fungal strains and substrates:

Fungal strains have influences on the thickness of mycelium, branching trend, and surface topography of composites. As Figure 9 shown, no particular fungal species is most common in mycelium material characterization studies (28% of published studies did not provide fungal species in reports; 32% were others). The next 3 common phylogenetic fungi mentioned in studies are all in Basidiomycota division, under Agaricomycetes class/order (Pleurotus *ostreatus* (12%), Trametes *versicolor* (12%) and G. *lucidum* (8%)) and all belong to saprotrophic group. This group of fungi specifically convert nutrients from plants and waste into mycelium biomass can be used in material science [22]. Their scientific classifications are summarized in Table 4.

Studies indicated that fungal species with trimitic hyphal systems (i.e. *Agaricales* order) resulted in higher compressive strength than species with simple hyphal systems (i.e. *Polyporales* order) [15], [22], [56]. Therefore, in order to achieve high compressive strength, P. *ostreatus* is preferable to produce MBC. However, the research laboratory supported this research only had G. *lucidum*, and information related to G. *lucidum* in literature is sufficient to compare in discussion and the results at the end. In addition, this fungal strain was chosen for this study to produce MBC.

Table 4 Scientific classifications of P. ostreatus, T. versicolor, and G. lucidum.

	Pleurotus *ostreatus*	**Trametes *versicolor***	**Ganoderma *lucidum***
Division	Basidiomycota	Basidiomycota	Basidiomycota
Class	Agaricomycetes	Agaricomycetes	Agaricomycetes
Order	Agaricales	Polyporales	Polyporales
Family	Pleurotaceae	Polyporacaea	Polyporacaea
Genus	Pleurotus	Trametes	Ganoderma

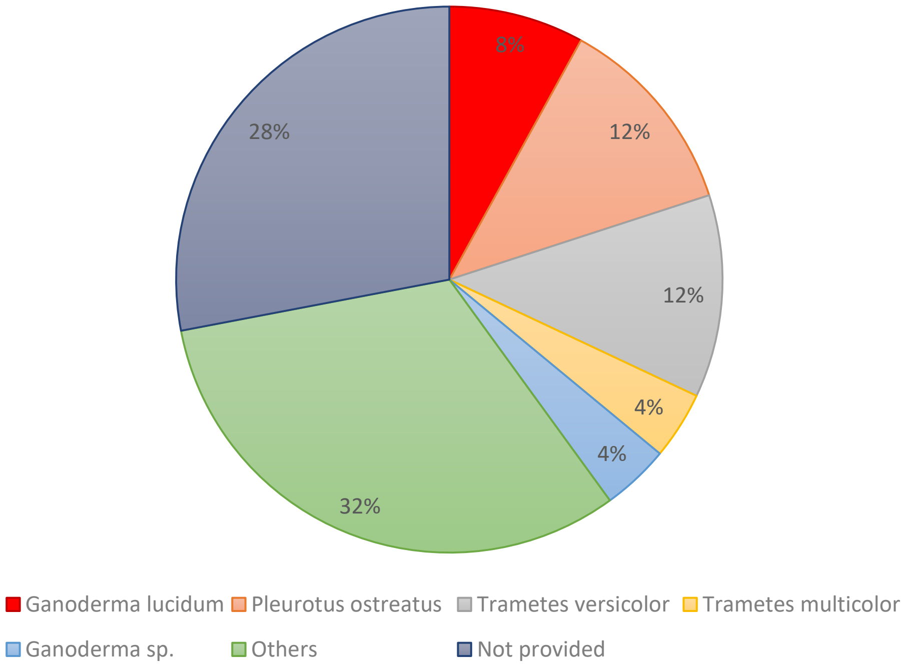

Figure 9 The main fungal species used for material characterization studies according to this literature review.

Compiled substrates choices in literature related to MBC, 70% of sample types were foam (Figure 10a) and 34% of the substrates were agriculture residues, such as rice husk, corn stover, rice hulls, etc.; 20% were cash crops (i.e. cotton, cellulose, straw, etc.); the rest were either food crops, wood by-products or not provided in the studies (Figure 10b).

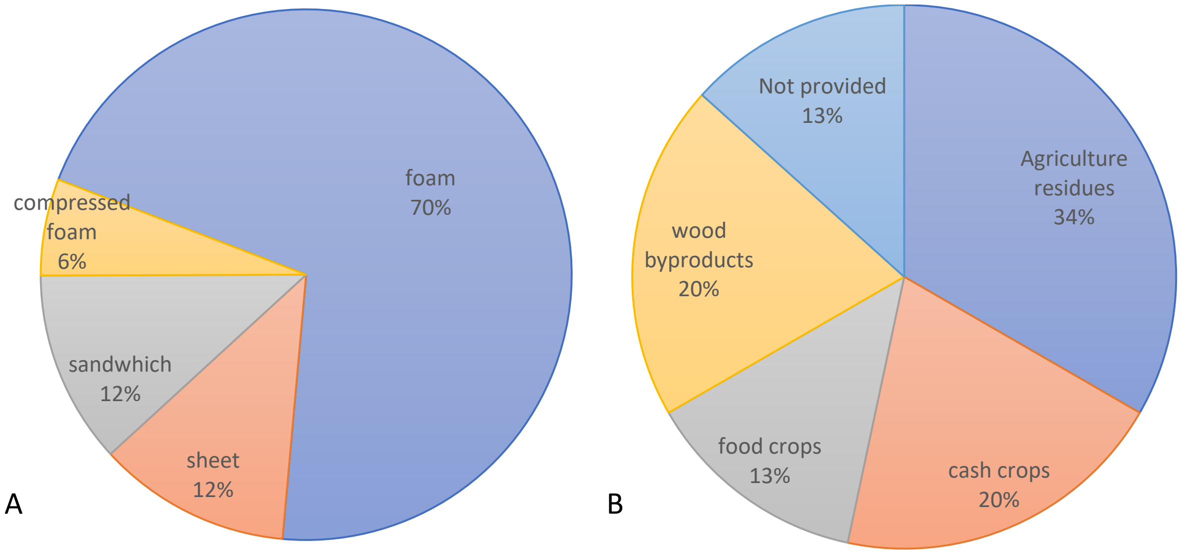

Figure 10 a) The main sample types and b) main substrates used for foam samples according to this literature review.

The most common sample types were foam because the fabrication method at the end only requires to be oven and air-dried to produce foam-like MBC. Other post-fabrication methods, including cold or heat-press, will increase the mechanical properties of MBC [29], but damage the mycelium layer on the outer surface and result in a higher water intake [31]. Moreover, produce foam-like composites requires less energy in the life cycle of production by eliminating extra processing. Therefore, in this study foam-like composite was chosen over cold or heat-press methods.

As aforementioned, substrate choices differ from agricultural residues to cash crops and wood by-products. Substrate use can be anatomized into few reasons listed below:

- Material applications:
  Substrate type has definite influences on the mechanical properties and porosity of the final composites; therefore, a substrate choice is highly dependent on a targeting application. For instance, for thermal insulation, acoustic insulation, packaging and other non-bearing load structural materials, substrates are preferable with fibrous agricultural residues and larger particle sizes to increase porosity and produce lower composites density after drying, such as straw, flax, hemp shives, etc. When pursuing a higher mechanical strength of MBC in furniture and particle boards, wood by-products are preferable due to higher lignin structures in wood cells.

- Economic and environmental considerations
  As Figure 10 (b) shown, 34% of studies chose agricultural residues over other substrate types, which manifest in circular economy. One of the advantages of utilizing saprophytic group of fungi to produce MBC is that agricultural residues and by-products can be upcycled and reused to produce new products. Most of the agricultural residues (i.e. rice husk, wheat straw) are produced in situ and not suitable for animal feedstock thus become biomass for fuel or composite wastes to burn on fields [51]. Saprophytic group fungi are able to grow on any lignocellulosic material,

even on low-nutrients content substrates, transform into valuable and wide range of product types. From economic and environmental perspectives, choosing agricultural or wood by-products over cash crops to produce MBC reduces cost, waste, and carbon emission (when burning as biomass).

- Fungi growing medium and substrate mixing proportions
  Saprophytic group fungi (white, brown, and soft rot fungi) known in literature have their specific ways of degrading materials and obtain nutrients differently [25]. Different fungi and strains have preferences on the sources of their nutrients, such as prefer cellulose over hemicellulose or vice versa [15], [31]. In addition, when a specific fungal strain is decided to produce MBC, substrate chemical composition is required to design for the optimal growth conditions. However, information regarding this is urgently needed in the literature to provide more in-depth overviews.

Mixing proportions
Among these studies, most of them did not provide mixing proportion for samples, one of the review paper pointed out that more than half of the current literature published are afflicted with the commercial companies in mycelium-based production so that mixing proportions were not mentioned for public knowledge [54]. However, the general guideline regarding mixing proportions to achieve optimal conditions is explained in the next paragraph.

Initial moisture contents of composites depend on mixing proportions of samples before inoculation. There are two ways to inoculate samples, with spores (liquid) and with pre-inoculated nutrient-rich substrates (solid), such as grains or sawdust-based substrates [9]. The advantages of inoculated with spores include evenly distributions and lower densities than using solid inoculation method [30]. However, inoculation with spores requires nutrient-rich medium (high-grade crops) to stimulate initial growths of fungi (i.e. lag phase). Interestingly, the impact on material properties from different inoculation methods has only been studied in one literature and the study did not provide detail mixing proportions and growing conditions in methodology, which made it difficult to replicate the research [30]. Arguably, solid inoculation results in higher densities can be mitigated by substrate types and sizes. In addition, solid inoculation is preferable over liquid inoculation in this research.

From an optimal growth perspective, 10%-32% of inoculum density in an identical medium (by volume) was suggested, depended on inoculation mediums and methods [22]. According to Wimmers et al. [57], initial moisture content of 65% in substrates (before reaching saturation points) had better growth than 45%. This coincided with Jones et al. [22], which indicated that water activity levels (equilibrium relative humidity) ranged between 0.6-0.8 resulted in optimal growth. The water activity level is highly dependent on the capacity of water retention of the substrates, which has a direct impact on water availability for fungi growth [58]. On the other hand, water activity has slightly less effect in a post lag phase, which indicates that the solid inoculation method is possible to use less water in mixing proportions [22]. Therefore, 10%-32% of inoculum density (in volume) and water ratio between 60-80% (in weight) were general guidelines when designing mixing proportions in this research.

Growing conditions and drying protocols:
Several studies indicated that incubation temperature and humidity varied among fungal species, substrates, nutrients, and targeted growth periods [9], [13], [22]. Most selected fungal strains for mycelium composites belong to mesophilic, which means growing temperature between 15°C to 40°C is preferable. Consolidated from the literature, incubation temperature ranged from 21°C to 28°C; RH maintained in 50% [12], 65% [55], and 95% [32] during the growth periods. Growth periods among studies normally ranged from 14 days to 28 days.

Some studies demolded samples in the middle of growth periods to achieve surface homogeneity of mycelium growth and strengthen the mycelium network on sides which first enclosed by molds [29], [31]; others skipped this step. Damaging the mycelium network during a growing phase has shown to stimulate mycelial growth and colonization process in mycology studies [15], [59]–[61]. Therefore, the demolding process was implemented in this research.

After a certain growth period, oven and air-dried the samples to terminate the mycelium growth was the most common way in academic literature. Drying duration and temperature have no standard and reasoning to back it up in literature since sample sizes and initial moisture contents were vastly different among studies.

Above all, there is a lack of a systematic way to analyze optimal growing conditions and mixing proportions among different studies due to variance in fungal strains and research methodologies. Therefore, the valuable information extracted from MBC studies were fragmented. A better way to determine whether the MBC meets the optimal growth and can be harvested in a correct period is to study matrixes of parameters affecting growth rate, such as fungal strains, environmental conditions, and substrates proportions specifically and holistically.

2.1.4. MBC physical and mechanical characterizations

In this section, the inter-study of various properties of MBC was discussed to further understand this material. Table 5 shown the mechanical and physical properties of foam MBC in studies. These properties were selected to compare with existing thermal insulation materials.

Table 5 Physical and mechanical properties of foam composites extracted from the literature (non-press).

Substrates	Density	Compressive strength	Thermal conductivity	Water intake	FT-IR	References
Unit	[kg/m^3]	[MPa]	[W.m^{-1}.K^{-1}]	[%]		
Cotton carpel	*	*	0.13	198		[30]
Straw	100	-	-	436		[29]
Sawdust	170	-	-	43		
Cotton	130	-	-	279		
Sawdust pulp	240-280	0.65	0.05-0.07			[62]
Hemp	99	0.5-1.2[a]	0.04	24.45	Y	[31]
Straw	94	-	0.0419	26.78		
Sawdust	-	0.5~-1.3	-	-	Y	[56]
Wood shavings	-	-	0.051-0.055	-		[57]

a. Test stopped when a fixed strain was reached (between 70%-80%)
*. Normalized to XPS

Thermal Conductivity:

As Table 5 shown, thermal conductivity ranged from 0.04 to 0.13 (W.m^{-1}.K^{-1}), only [30] had the lowest thermal conductivity value in the studies; the rest of the studies showed fairly consistent values between 0.04 to 0.07 (W.m^{-1}.K^{-1}) disregard differences in fungal species and substrates. This might due to different testing standards were chosen for measuring thermal conductivity. However, which standard was used in measuring thermal conductivity was not provided in Holt et al. [30]. As Table 6 shown, [31] used the transient method (ASTM D5334) resulted in 0.04 (W.m^{-1}.K^{-1}), whereas [57] used a steady measurement obtained values from 0.051-0.055 (W.m^{-1}.K^{-1}). This research used the transient method with a needle probe to measure thermal conductivity of samples according to ASTM D5334.

Table 6 Thermal conductivity testing standards chosen in literature.

	Thermal conductivity testing standards	References
ASTM D5334	Standard Test Method for Determination of Thermal Conductivity of Soil and Soft Rock by Thermal Needle Probe Procedure	[31]
NBN EN 12667	Determination of thermal resistance by means of guarded hot plate and heat flow meter methods—Products of high and medium thermal resistance	
ASTM C518-17	Standard Test Method for Steady-State Thermal Transmission Properties by Means of the Heat Flow Meter Apparatus	[57]

Foam MBC overall has slightly higher conductivity comparable to synthetic foams (ranged between 0.03-0.04 (W.m^{-1}.K^{-1}) used in building applications as insulation materials due to its open-cells porosity. In addition to material porosity, substrates choices, such as hemp and straw are both well-documented low thermal conductivity natural materials. Another advantage of applying foam MBC as insulation materials over synthetic foams is fire safety. Current insulation materials on the market for building applications release toxic gases and matter when combust. Foam MBC, on the other hand, is 100% biodegradable and will release less or non-toxic gases when combusting [12]. Consequently, thermal conductivity is one of the most important material characteristics to explore in this research considering the potentials to replace synthetic foams in the current market.

Water intake & moisture resistance

Water absorption is an innate disadvantage of applying biocomposites as building materials because of high durability requirements in building codes. MBC foam incorporated properties from the substrates fillers as high porosity from air voids between particles. Unlike common synthetic petroleum-derived foams are closed cells; these air voids are open cells in MBC resulted in high water intake due to capillary suction. Besides capillary suction from these air voids, substrates used are usually hydrophilic materials. The high water intake of this material imposed great risks for material degradation, molds formation, and thermal conductivity increase.

Water intake results were subjective to immersion time and measured hours in studies (Table 7). When compared [29] and [31], both studies used the same substrate (i.e. straw) had a water intake variance of 94%. One explanation can be that the testing hours were different between the two. However, there is no figure nor data from the same hours to be compared between the two. Interestingly, the same substrate used (i.e. cotton) when water intake immersion time was closer between the two, resulted in a 29% variance between [29] and [30]. This indicated that when comparing water intake results from different studies, immersion time should be discussed.

Table 7 Water intake results versus immersion hours in different studies.

References	[29]		[31]	[30]
Immersion time	192 hours		24 hours	168 hours
Substrate	Straw	Cotton	Cotton carpel	Straw
Water intake (%)	436	279	198	26.78
Variation between studies (%)	**94**	**29**	**-**	**-**

Water absorption/water intake had inconsistent results among various studies due to many factors, such as different research methodologies, testing standards, and sample preparation protocols. This also made it difficult to compare between studies. Two studies indicated that water intake after partial immersion is independent of types of fungi, substrates, or pressing conditions (non-press, cold/hot-press) [29], [30]. Contradictory, review paper [9] stated that hot or cold-press can reduce water intake due to smaller air voids between particles. One study [31] showed way less water intake within 24 hours (between 24.45% to 30.28% depends on substrates) than other studies ranged from 180% to 350% (Table 5). The possible explanation given by the study was that samples were covered by well-growth and dense outer layer of mycelium, which was hydrophobic resulted in way less water intake than other studies. Furthermore, hemp samples had the densest mycelium layer, which also had the least water intake than the other 2 substrate types[31]. In other words, water intake and density of mycelium layer seemed to have an inversed relationship.

As for testing standards (Table 8), the partial immersion method was more common to be used in studies compare with total immersion. The difference between the two is that the bottom face of the test sample must be 10mm below the water surface in partial immersion; 50mm below for total immersion. Besides different testing standards chosen in studies, the water intake represented results were also hard to compare due to various immersion hours.

Table 8 Water intake testing standards chosen in literature.

Water intake testing standards		**References**
ASTM C1134	Standard Test Method for Water Retention of Rigid Thermal Insulations Following Partial Immersion	[30]
ASTM C1585-04	Standard Test Method for Measurement of Rate of Absorption of Water by Hydraulic-Cement Concretes	[31]
NBN EN ISO 15148	Hygrothermal performance of building materials and products—Determination of water absorption coefficient by partial immersion	

With further investigations of fungal composition in literature, chitin can form into a chain of stabilized chemical structures with internal hydrogen bonds, which explains the hydrophobic properties of mycelium layer [47]. Pure mycelium film also has shown high values of water contact angle (around 120°); hence has shown low water intake in the study [28]. Therefore, this research aims to investigate the relationship between a longer growth period and water resistance. Water uptake measurement set-up in this research was inspired by standard with partial immersion (i.e. ASTM C1134 and EN/ISO 15148) since internal insulation materials are rarely exposed to rainwater but more to interstitial condensation.

Density & mechanical strength

The density and mechanical strength of composites are highly dependent on substrates used for foam MBC [27], [29]–[31], [62]. As Table 5 shown, straw used in [29] and [31] showed densities variance only in 6%. In other words, substrates selection will be associated with material applications. For example, to use as lightweight insulation foam in between walls, substrate choices should be fibrous agricultural by-products and larger particle sizes to increase porosity and lower densities after drying, (i.e. straw). Furthermore, [31] showed that mycelium had better growth in substrates with larger particle sizes, namely, less fine substrates perform better growth. One possible reason is that finer substrates create less airflow and lower oxygen availability for fungi to grow.

Mechanical strength and density have a positive correlation, which indicates the inversed correlation with porosity and compressive strength. As aforementioned, high porosity results in low density, which is favorable for insulation materials. On the other hand, compressive strength is slightly less critical for non-structural materials. When compared compressive strength of XPS and EPS foams (ranged from 0.03-0.69 MPa) for building insulation applications, foam MBC with particulate substrates (i.e. wood by-products, sawdust) has shown competitive results (ranged from 0.5-1.3MPa in literature, Table 5). Interestingly, Islam et al. [63] showed that particle size has no impact on compressive strength (stress-strain curve).

Besides substrates have a critical impact on the compressive strength of foam composites, fungal strains also have some influences on them. According to [22], fungal species with trimitic hyphal systems (i.e. polyporales order) produce composites with higher compressive strength than species with simple hyphal systems (i.e. agarics order). This has shown in Bruscato et al. [56], when tested compressive strength with samples grew on the same substrates and three different fungal strains (Pycnoporus *sanguineus,* Lentinus *velutinus,* Pleurotus *albidus*), resulted in 1.3, 1.3, 0.4 MPa in compressive strength respectively. Pycnoporus *sanguineus* and Lentinus *velutinus* belong to polyporales order with complex trimitic hyphal systems, which appeared to have a woody and leathery surface morphology. In order to have foam composites with good compressive strength, one chooses particulate substrates over fibrous substrates and fungal strains with complex hyphal systems. Nevertheless, literature in existence has shown that MBC can compete with synthetic foams for non-structural materials (i.e. XPS, EPS ranged from 11-50 kg/m^3) [9], [13], [31], [54].

As Table 9 shown, most foam MBC related literature used different testing standards for measurements. Moreover, some of the chosen standards were applied for wood-based materials, plastics, or even hydraulic-cement concrete, which have very different characteristics than mycelium-based material. The reason for choosing certain standards was not narrated in studies.

Table 9 Density & strength testing standards chose in literature.

Density & strength testing standards		References
ASTM C165	Standard Test Method for Measuring Compressive Properties of Thermal Insulations	[30]
ASTM D-792	Standard Test Methods for Density and Specific Gravity (Relative Density) of Plastics by Displacement	[64]
ASTM D3501	Standard Test Methods for Wood-Based Structural Panels in Compression	[31]

Wall insulation materials require to withstand certain compressive stress and sustain its own weight when applying between space cavities; therefore, compressive strength is decided to be one of the main mechanical characteristics to be tested in this research with ISO standard bench.

Chemical characterization

MBC inherit properties from substrates and mycelium in combination, more to that, the production process involves mycology and biology phenomenon. It is intriguing to investigate the changes of components between undecayed substrates and substrates with mycelium. There are many methods to analyze and understand the compositions, such as Fourier Transform Infrared Spectroscopy (FT-IR) or X-Ray diffraction (XRD). XRD is only for crystalline structure and is not suitable for organic materials. FT-IR works better with organics materials and is a reliable method. FT-IR is using infrared vibration to determine functional groups and compositions in a material. FT-IR analysis usually has wavenumbers in wavelength (cm^{-1}) in the x-axis and absorbance or transmittance (%) in the y-axis. Each peak in a different wavenumber represents each unique functional group. For example, bands at around 3000 cm^{-1} regions correspond to functional groups of C-H stretching. In low infrared wavelength regions are usually used to identify absence bands to accurately identify materials. FT-IR can be used to dissect the decaying process of fungal on different substrates; compare components of composites with pure mycelium and undecayed substrates; and analyze different layers of composites in various sections/plans of samples. For example, use FT-IR to investigate if further growth period will result in a thicker mycelium outer layer. Currently, there were only two studies that have done FT-IR tests and analysis on foam MBC compositions [31], [56], and some important peaks were listed in Table 10.

Table 10 Important peaks and attributions in MBC from literature.

Peak (cm^{-1})	Attribution	Constituent	References
3278,3280	O-H stretching hydrogen bonds	cellulose	[31], [56]
2922,2924	C-H stretching vibrations, CH_2, CH_2OH	cellulose	[31], [56]
1551	C = C stretching of aromatic ring	lignin	[23]
1505, 1510	C =C stretching of aromatic ring	lignin	[23], [65],[66]
1370, 1375	CH bending	Cellulose, hemicellulose, chitin	[23], [67]
890, 896	Glucan β-anomer C-H bending, C-H deformation	cellulose	[31], [56]

According to Bruscato et al. [56], the infrared spectra of three different fungal species were exactly the same (Figure 11), which included polysaccharides, nucleic acids, proteins, and lipids. Elsacker et al. [31] showed a more in-depth analysis with FT-IR by comparing chemical compositions of mycelium composites with hemp and flax substrates versus pure mycelium and undecayed fiber. The study found both hemp and flax composites decreased in peak intensities of lignin to carbohydrate ratio and increased in peak intensities of chitin to polysaccharide ratio compared with undecayed fiber. Results were consistent with other studies that lignin was decomposed when substrates interacted with mycelium. The decomposition of lignin might compensate for the strength of composites but this result revealed that substrate conditions (i.e. sizes, processing methods, etc.) have a greater influence on mechanical strength than chemical compositions of the mycelium and fiber [31].

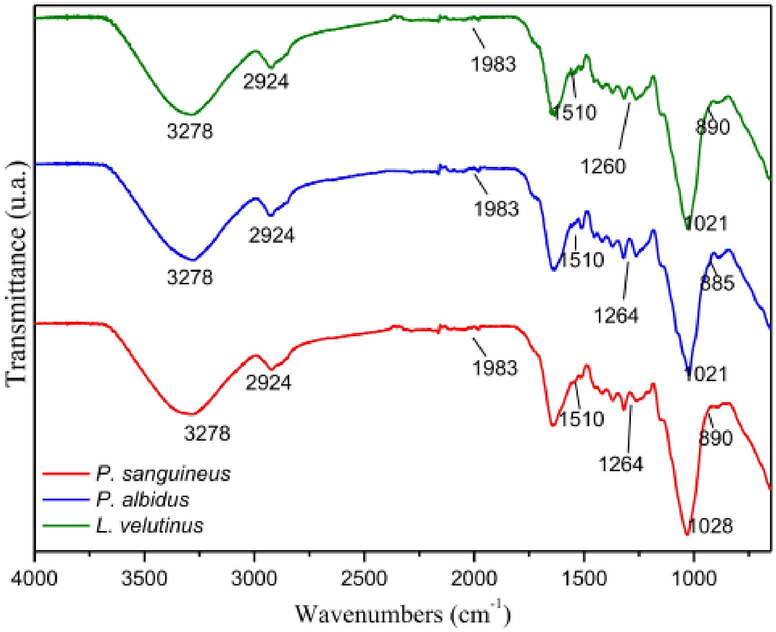

Figure 11 Infrared spectra of foam samples. *Note.* Reprinted from [56].

Surface morphology

Microscopy is a technique to study the surface of the materials, scanning electron microscopy (SEM) and cryo-SEM are commonly used in the literature related to mycelium-based materials. Cryo-SEM is commonly used when samples contain high water contents and conducted the images under freezing temperatures. Cyro-SEM is specifically designed for biological samples when traditional electron beam and vacuum tend to kill the biological samples while extracting images.

MBC after drying can be studied with traditional SEM in low voltage (ranged from 5-15kV) to understand the surface microstructure because the mycelium growth is already terminated. SEM images of substrates, mycelium, and mycelium with substrates are shown in Figure 12-Figure 13. SEM images of mycelium clearly show the tubular hyphae and the interwoven network. When combined with substrates, the interwoven hyphae network colonized the surface of substrates and had a distinct microstructure from substrates.

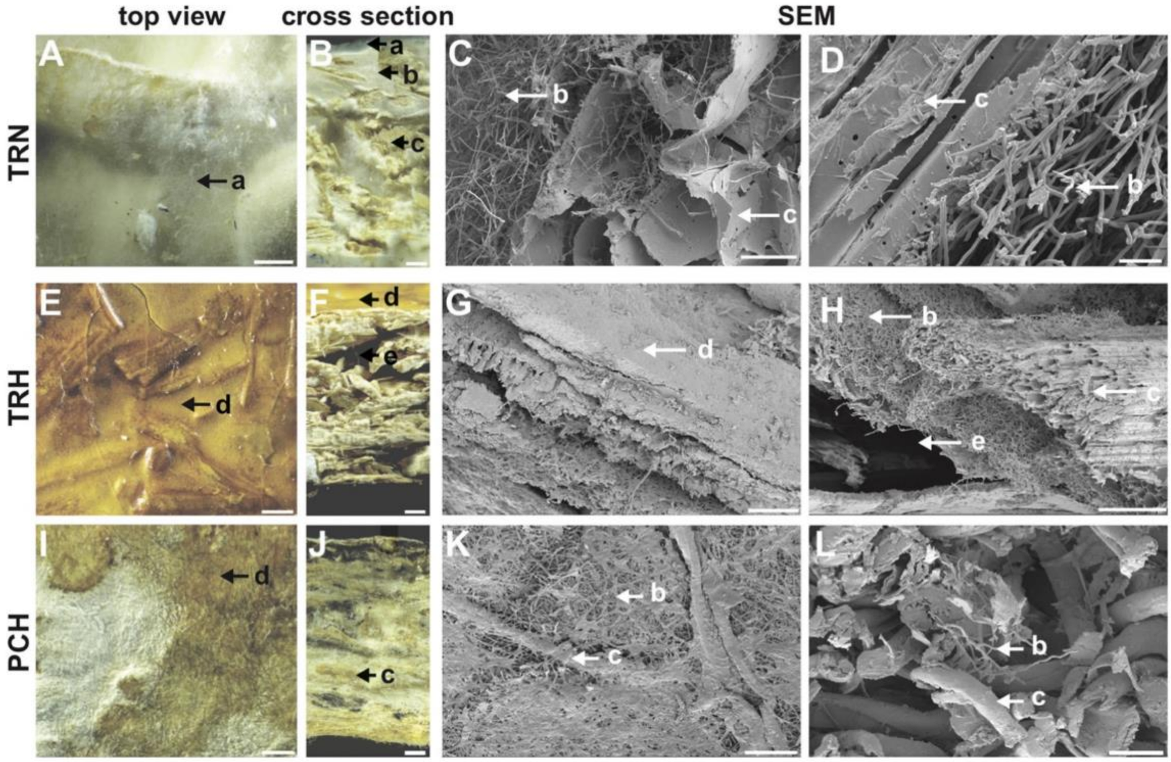

Figure 12 Stereomicroscopy and cryo-SEM images of composites with various substrates (TRN, TRH, PCH) a) aerial hyphae b) mycelium c) substrate d) fused hyphae e) air voids. *Note.* Reprinted from [29].

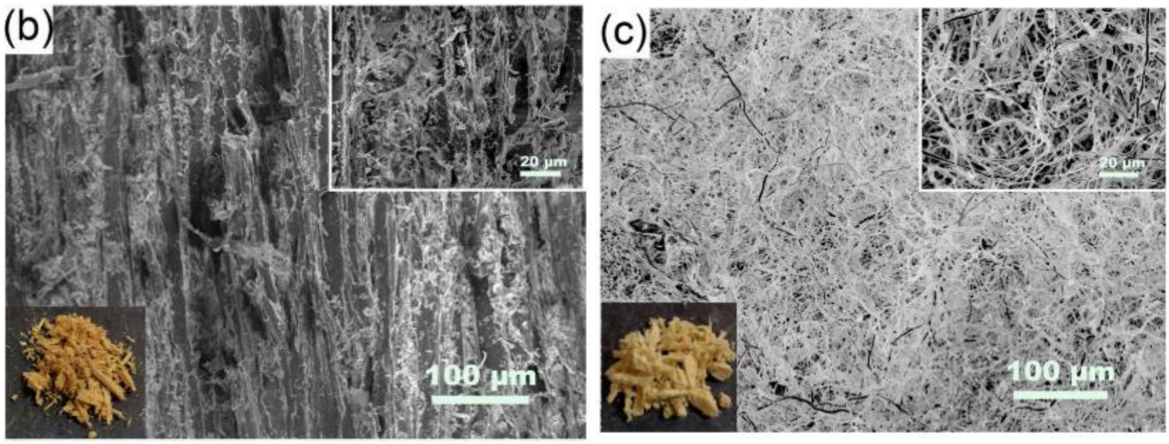

Figure 13 Morphology of b) wood-mycelium particles c) pure mycelium. *Note.* Reprinted from [33].

2.2. Moisture buffer capacity of building materials

From building physics perspective, heat transfer modes are combined with convection, conduction, and radiation in dynamic states. In order to simplify the physics phenomenon into equations and study building thermal dynamic, initial and boundary conditions are necessary to be set. Considering the scope of this study is about thermal insulation and is mainly on the material level. A few equations explain heat and moisture transfer in materials and its hygrothermal behavior are listed and explained below for clarification.

Fourier's law of heat transfer:
Fourier's law states the rate of heat transfer through a material is proportional to the negative gradient in the temperature, as equation (1) shown.

$$q_x = -\lambda \frac{\mathrm{d}T}{dx}, q_y = -\lambda \frac{\mathrm{d}T}{dy}, q_z = -\lambda \frac{\mathrm{d}T}{dz} \tag{1}$$

Where $q_{x,y,z}$ = heat flux [W/m^2], λ = thermal conductivity [W.m^{-1}.K^{-1}],
T = thermodynamic temperature [K]

The gradient sign is negative because temperature is a vector from high to low. When a material is anisotropic, the thermal conductivity can be different in different directions (i.e. wood or any material made of natural fiber) and does not have same direction as the heat flow.

This equation is the fundamental theory to solve heat conduction problems in an empirical relationship. For instance, when a material is isotropic and thermal conductivity is known, the heat flux can be solved with temperature differences in the cross area. Thermal conductivity (W.m^{-1}.K^{-1}) is an essential material property when considering thermal insulation. A higher conductivity value means the heat conduction (heat flux quantity) is larger than materials with less thermal conductivity values when other variables stay consistent.

Moisture transfer and Fick's law
Moisture transfer is more complicated than heat and energy transfer from conduction because the gradients (driving forces) of the regime combine with various parameters and potentials. The moisture transfer induced by pressure diffusion (vapor pressure) is caused by temperature and RH differences between two spaces and interfaces of material from building physics perspective, which is stated by Fick's law of diffusion, shown as equation (2).

$$q_x = -\mu \frac{dP_v}{dx}, q_y = -\mu \frac{dP_v}{dy}, q_z = -\mu \frac{dP_v}{dz} \tag{2}$$

Where $q_{x,y,z}$ = moisture flux [kg/m^2,s], P_v = vapor pressure, μ = the moisture permeability of the material

The gradient sign is negative because vapor pressure is a vector from high to low. The moisture permeability (μ) of material is, how much the material will allow moisture to pass through; therefore, for a vapor retarder is very small and for a porous material is vice versa.

The pressure gradient mentioned in above equation (P_v) subjective to temperature and absolute humidity. According to ideal gas law and Daltons law, partial pressure is proportional to temperature, and total pressure exerted by a mixture of gases is the sum of partial pressures of the individual gases. Partial pressure of air and partial pressure of water vapor is different due to the differences in molecule weights; therefore, the humidity ratio in a given temperature (K) and 1 atmosphere (≈10^5 Pa) can be derived as equation (3).

$$humidity\ ratio\ (x) \approx \frac{P_v/R_v\,T}{P_a/R_aT} \tag{3}$$

Where x = [kg vapor/ kg dry air], P_v = water vapor partial pressure, P_a = air partial pressure, T = temperature [K], R_v = gas constant of water vapor 462 [J/kgK], R_a = gas constant of air 287.1 [J/kgK]

The humidity ratio mentioned above is the concentration of water vapor present in the air, which is also called absolute humidity (g/ kg) and is subjective to change as temperature or pressure change. In building physics, ambient pressure is always assumed to be at 1 atmosphere (≈10^5 Pa) when determines saturation pressure, i.e. dew point or evaporation temperature. For every temperature, there is a maximum water vapor pressure and when this pressure exceeds, saturation happens and is called saturation pressure (P_{sat}). The relationship between saturation pressure (P_{sat}) and RH in each temperature (K) can be derived as equation (4). This means at 100% RH in a given temperature, the air is saturated with water.

$$RH\ (\%) = \frac{P_v}{P_{sat}} \tag{4}$$

Above equations stood as fundamental background knowledge for practical experiment set-ups and facilitated the testing methods design in this research. As aforementioned, there is no one common testing standard that can be applied to foam MBC. Most of the studies performed tests according to ASTM, NBN EN, and ISO standards, as Table 6~Table 9 shown. Standards were chosen according to similar material characteristics with MBC or according to its targeted applications. For example, if the research focused on replacing XPS as packing material, the chosen testing standards should also apply for XPS and packing material required properties. However, there is a lack of a systemic way of testing standards justification in literature. Thus, in this research, some physical properties tests (i.e. compressive strength, thermal conductivity) conducted according to ISO/ASTM standards, some experiment set-ups were designed in situ followed the intrinsic characteristics of the material (i.e. water absorption test and DWC test).

Moisture buffer values

In building physics, moisture transfer via water vapor and liquid water between assembly layers. This phenomenon is critical when designing and constructing building envelope systems, i.e. exterior insulation, interior insulation, closed-cell or open-cell porous materials, etc. Studies have shown that building industries are interested in materials that can passively regulate RH and off-set the extreme peak loads for active ventilation in daily or seasonal variation [68]–[71].

Most building materials are porous, and moisture can be stored inside building materials There are 3 transfer potentials when one needs to understand the moisture transfer regime in porous materials, RH of surrounding conditions, capillary suction inside the material pores, and gradient of partial pressure [36]. Capillary suction of material is normally determined by the moisture retention curve, which is a plot of the moisture content versus the capillary suction (Pa) in static laboratory measurement. Studies have shown the large discrepancy between moisture retention curves of high-porous and hygroscopic materials obtained from static measurements and dynamic measurements due to the effects of desaturation rates involved [6], [72]. In addition, the dynamic measurement (including adsorption and desorption) measurement is preferable when determining the moisture transfer regime in building materials. Moisture buffer value (MBV) measurement is one of the dynamic measurements in the literature, and the most well-known moisture buffer capacity measurement is defined by the NORDTEST method [37], [68], [69], as Figure 14 shown.

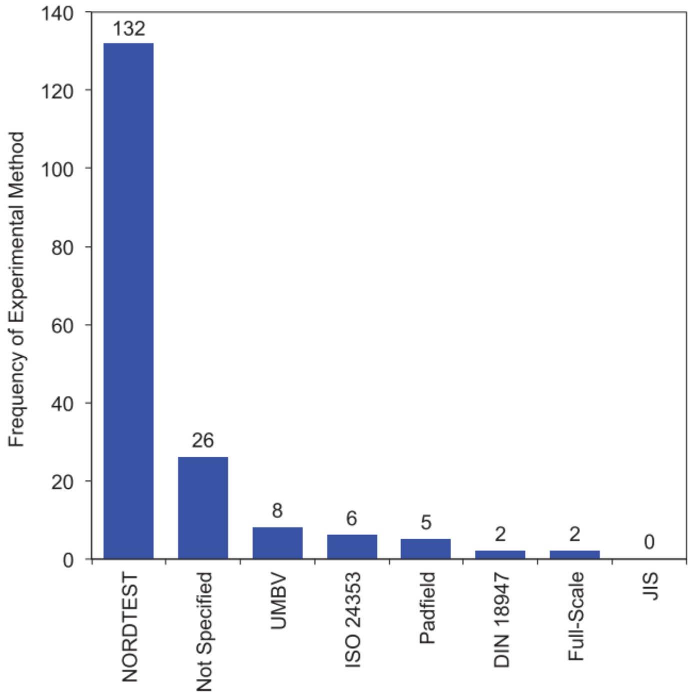

Figure 14 Frequency of methods used to determine MBV in experiments. *Note.* Reprinted from [37].

In order to define and properly categorize materials ability or capacity to regulate moisture in the material level, ideal moisture buffer values (MBV_{ideal}) is defined below:

> “The moisture exchange (moisture uptake/release) within periodic variation normalized with the change in surface relative humidity. [69]“

In theory, the MBV_{ideal} is derived from a thermal effusivity ($W.\sqrt{s}.m^{-2.}K^{-1}$) of a material, which is a heat transfer over a surface of a material when a surface temperature changes over time. The MBV_{ideal} replaces heat effusivity with moisture effusivity as equation (5) shown.

$$b_m = \sqrt{\frac{\delta_p \cdot \rho_0 \cdot \frac{d\mu}{d\varphi}}{p_{sat}}} \quad [kg/(m^2 Pa\sqrt{s})] \tag{5}$$

Where δ_p = water vapor permeability[kg/(m*s*Pa)], ρ_0 = dry density of a material [kg/m^3], μ = moisture content [kg/kg], φ = relative humidity [-], p_{sat} = saturation vapor pressure [Pa].

In this ideal case, saturation vapor pressure (p_{sat}) is given by the test conditions, other variables all related to standard material properties [68]. However, moisture effusivity in this ideal case is only treated when surface humidity is discontinuous change (step-change). This is rarely the case in building physics. In addition, the MBV_{ideal} is required to be shown as moisture change over exposure time under periodic variation of RH by Fourier sinusoidal functions ($T_p = T_1 + T_2$). T_1 is 8 hours in high 75% RH; T_2 is 16 hours in low 33% RH; and in total T_p resulted in 24 hours as the boundary condition, as Figure 15 shown [68]. The derived MBV_{ideal} approximation proposed by the NORDTEST method is shown as equation (6).

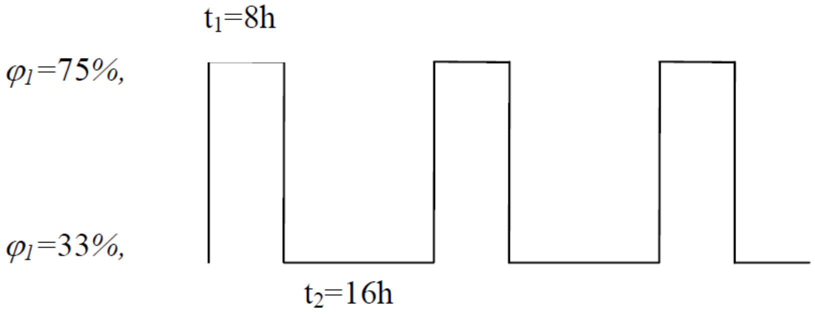

Figure 15 Periodic variation of the boundary condition to determine ideal MBV. *Note.* Reprinted from [68].

$$MBV_{ideal} \approx \frac{G(t)}{\Delta RH} = 0.00568 \cdot p_{sat} \cdot b_m \cdot \sqrt{t_p} \quad [g/(m^2\%RH)] \tag{6}$$

At the material level, MBV_{ideal} is independent of a surrounding environment and neglects the air boundary layer. It means that it has no resistance to the vapor exchange between a material and a surrounding environment. Another important assumption, when used MBV_{ideal}, is that a thickness of a material exceeds its penetration depth since the moisture transfer and retention inside porous material is highly non-linear.

A moisture penetration depth of a material defined by the NORDTEST method is a thickness point (x_p) of a material where the RH is equal to 1% of the amplitude of surface RH variation [68], as Figure 16 illustrated.

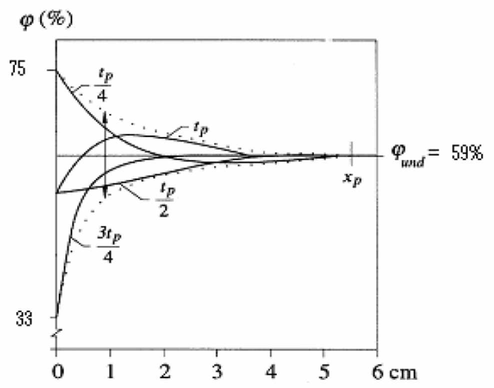

Figure 16 The moisture distribution at different times during the period time (tp) due to a periodic boundary condition at the boundary. The amplitude of the variation (dotted lines) decreases with the depth (x-axis). *Note.* Reprinted from [68].

The calculation method followed Kirchhoff's potential in the one-dimensional case, the flow potential and RH reference can be arbitrary for any material [68].

Practical moisture buffer values

MBV_{ideal} is the upper limit when compare with $MBV_{practical}$, the detailed comparison is explained and illustrated in Abadie & Mendonca [73]. According to [73], when the Biot number as a factor between [0.1; 10], the $MBV_{practical}$ is highly sensitive toward testing conditions and imposes the precision of the experimental results [73].

The Biot number defined in Rode et al. is shown as equation (7) [68]:

$$B_i = \frac{L}{\delta_v \cdot Z_{s,v}} \tag{7}$$

Where L = thickness of a one-sided sample or half the thickness of a two-sided sample, δ_v = water vapor permeability of the sample [m^2/s], $Z_{s,v}$ = the moisture surface resistance included both the material and surface film resistance due to boundary air layer.

When MBV_{ideal} is incorporated into building performance simulation models, the relationship between ideal and practical is important but this is not within the scope of study. Considering, the tested sample (MBC) material properties, such as thermal effusivity and penetration depth are unknown. Another difficulty is that MBC is highly heterogeneous material, which creates a large discrepancy when compared MBV_{ideal} with $MBV_{practical}$. Hence, it is preferable to choose $MBV_{practical}$ to compare with a reference sample done in Rode et al. [68] for the integrity of this research.

The ultimate purpose of using $MBV_{practical}$ is to align comparable results with constrained and actual conditions and to represent building envelope assembly as a whole. Furthermore, to provide a realistic overview of material and assembly performance for real application in the building industry.

As defined in Rode et al. [68]: “The $MBV_{practical}$ indicates the amount of water that is transported in or out of a material per open surface area, during a certain period of time, when it is subjected to variations in relative humidity of the surrounding air.” The unit of $MBV_{practical}$ is [g/(m^2 %RH)].

$MBV_{practical}$ experiment is required to meet certain requirements and testing conditions to render buffer capacity at the system level, instead of at the material level. The robustness of the designed protocol was tested throughout various materials and research institutes, and the results showed good alignment between the institutes with the same tested materials (mean $MBV_{practical}$ of 1 [g/(m^2 %RH)] deviation in between) [68]. Most of the building internal furnishing materials showed $MBV_{practical}$ values between 0.5-1.2; therefore, a classification was developed to show and compare the moisture buffer capacity of these building materials, as Figure 17 shown. The testing protocol and sample preparation are further explained in the Materials and methods section.

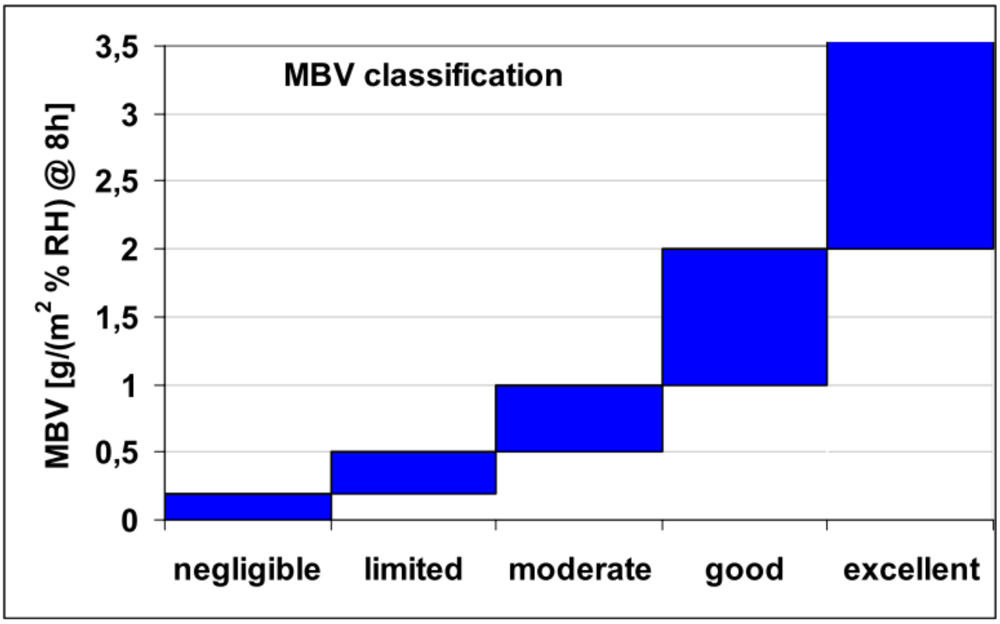

Figure 17 $MBV_{practical}$ classification of internal furnishing building materials. *Note.* Reprinted from [69].

2.3. Challenges with MBC apply as thermal insulation

When applied as building materials, MBC has shown promising results for thermal insulation and fire safety to replace synthetic and petroleum-derived insulation materials. Many parameters affect the material properties of mycelium composites, such as filler substrates, fungal species, growing conditions, and pressing methods. Mechanical and physical characteristics strongly depend on substrate types, (i.e. density and strength). Fungal strains with a more complex hyphae network can strengthen the mechanical properties of final products. As aforementioned, this material is still in early development and most information regarding material characterizations of MBC is fragmented. Lacking consistency and transparency in research methodology impose difficulties in comparing and analyzing results between studies. Testing standards also vary among studies and depend on targeting applications in research. Therefore, the valuable information extracted from studies is limited. Besides a lack of systemic and holistic overview to understand MBC, production, upscaling, and durable issues are required to be addressed in scientific research.

Production and upscaling:

MBC is still under early development phase, disregarding material applications and fields, and are required cooperation between cross-disciplines to optimize the production process and compete with petroleum-derived synthetic materials. Most fungi used in current MBC belong to saprotrophic group (especially, white-rot fungi) because of its easy accessibility and its ability to grow on wide varieties of lignocellulosic materials (including low nutrients agricultural residues). One of the superior traits of this group of fungi has indefinite hyphae growth, which is only limited to a surface area of substrates to grow on [15]. However, the actual biology mechanisms between fungi and substrates are complicated and still required more in-depth inputs from mycologists. Only by understanding the actual mechanisms of obtaining nutrients and growth, one is able to optimize the growth conditions and production process.

Genetically modified fungal strains are also under discussion in the mycelium-developing field. Although it might unlock more potentials and opportunities to outperform wild-strains and possibly increase mechanical and physical properties of MBC. Arguably, this path deviates from sustainable circular economy globally and locally by allowing certain organizations and companies to uphold secret “recipes”. This situation is also observed in the literature review, where more than 80% of studies did not specify mixing proportions.

Overall, the great potential of applying MBC as insulation materials are foreseeable but only by the cooperation between open source knowledge base and local circular economy driving force. In short, more transparency in the scientific research field when disclosing research background and methodologies is recommended to enable MBC compatible with conventional insulation materials in the market.

Durability issues:

As materials are applied as building materials, durability and longevities requirements are stringent, from environmental and economic perspectives. Durability can have different definitions listed below [74]:

- The ability to perform a function until a limit state is reached.
- Decreasing its functional performance after aging or the evolution of the properties regarding positive and negative changes.
- A material effective life span between the building commissioning until its performance level drop below its failure threshold.

Different building material applications can suffer from various durability issues. For instance, the main concern for internal insulation materials is the moisture transfer at the interfaces of insulation and structural materials due to the largest temperature gradient occurred at the interfaces. As moisture transfer (liquid water when condensate or vapor content in the air) can cause swelling and shrinkage of insulation materials. Worst case scenario, when liquid water is absorbed by insulation materials, the functional performance as thermal insulation will dramatically decrease.

From the literature review on MBC and thermal insulation materials, the intrinsic disadvantage of this 100% biodegradable material is the high water intake similar to other natural biocomposites. Although water absorption tests have been published in studies, the information provided is fragmented due to inconsistency testing methods and production processes of MBC. Most of the solutions for water intake reduction rely on extra resin surface coating at the end of final products. Solutions such as extra coating added another uncertainty in coating durability and cohesion bonding between coating and composites. Another solution made possibly of studying the natural characteristics of MBC in detail and improve water resistance without extra additives.

Elsacker et al. [31] showed way less water intake within 24hours than other studies and provided the possible explanation that samples were covered by well-growth and dense outer mycelium layer. This explanation coincides with literature about mycelium and chitin polymer extracted from fungi [22], [28], [47]. Molding, packing methods and total growth time have shown impacts on water absorption performance in mycelium composites [15]. For instance, molding and packing methods that have better air exchange during growing periods result in denser outer layers of mycelium. A longer growth period (under optimal growing condition) will also result in a more intense mycelium outer layer. Engineered fungi strains in gene modification (i.e. deletion of hydrophobin gene) can stimulate exponential growth phase in an early stage when compared with wild-strains, which means increase mycelium biomass in a short growth period [47]. However, the gene modification technique is not within the discussion scope of this research.

Besides improving water resistance stands alone as insulation material for MBC. Tests and issues related to durability of MBC have not been presented in detail or as an overview in any study yet, such as comparing performances with and without accelerated aging tests. An aging-related test is commonly carried out in a climate chamber which can alternate extreme temperature and RH level or used drying and wetting cycle (DWC) to stimulate aging process. In addition, this research aims to explore the accelerated aging and the consequences of MBC with and without DWC. To summarize above findings, the main objective of this research is to study the feasibility of MBC to apply as foam-like wall insulation materials.

3. Materials and methods

3.1. Materials

All materials were provided by the AVANS CoEBBE laboratory in Den Bosch. Fungus species, G. *lucidum* was pre-inoculated on wheat grains and preserved in a freezer prior to the mixing with substrates. Chosen substrates were cellulose fiber (provided by Recell® 90.90) and rapeseed straw (RPS), 2 substrates which have very different characteristics in various aspects. The compositions of the 2 substrates are shown in Table 11.

Table 11 Composition of RPS and cellulose fiber.

	Cellulose (%)	**Hemicellulose (%)**	**Lignin (%)**	**Ash (%)**	**Protein (%)**	**References**
RPS	49	15	21	2	2	[75]
Cellulose fiber	65-80	< 15	<10	<10		Manufacture product sheet

RPS is an agricultural by-product from rapeseed oil manufacturers, which has high contents in cellulose (49%), hemicellulose (15%), and lignin (21%) [75]. Similar to other straw-based biomass, RPS has a higher content of hydrophobic waxy cuticle than wood, which indicated the reasons it has less water intake than dry cellulose fiber [76]. The added water remained in the autoclave bag for sterilization and for fungal to utilize. RPS substrate mixing proportion was according to [31] because this study showed great water resistance of the RPS composites. The cellulose fiber mixing proportion ratio was not found in literature, thus was determined in situ.

The dry cellulose fiber contained less lignin (<10%) and without the waxy outer layer, the added water was absorbed immediately by the fiber, while RPS fiber had more water remained without absorbing. Notably, the mixing proportion (in weight percentage) of cellulose fiber was 31.25% fiber, 62.5% water, and 6.25% inoculation fungal (Table 12), the added water percentage was within optimal growth water percentage in weight ratio. Although the inoculated fungal weight (100g) remained the same for both substrates, when considered in ratio proportion it was lower than RPS mixing proportion (6.25% and 10% respectively, Table 12).

Table 12 Sample mixing proportion for the initial growth.

	Substrate (g)	**Water (g)**	***G. lucidum* (g)**	**Ratio in weight**
RPS	200	700	100	20% : 70% : 10%
Cellulose	500	1000	100	31.25% : 62.5% : 6.25%

Substrate mixing proportion (Table 12) was only used for initial growth inside autoclaves bags for 7 days, each autoclave bag contained one substrate type (Figure 18). It should be noted here that all produced samples in all experiments kept the same substrate-water-inoculation mixing proportion (in weight ratio) for initial growth (Table 12). The mixtures of two substrates occurred at the molding stage (sample fabrication phase), and procedures were elaborated further in the next section.

3.2. Sample fabrication and drying

Initial growth period in autoclave bags:
Substrates with added water in autoclave bags were first sterilized in the autoclave for 40 minutes at 121°C and left to cool down until reached 40°C. The solid inoculation method was chosen for this experiment instead of the liquid method with spawns. The pre-inoculated wheat grains were broke into pieces, hand-mixed with substrates, and left to grow in autoclave bags in the growing chamber at 30°C and 58% RH for 7 days, as Figure 18 shown.

Figure 18 Cellulose (left) and RPS (right) with pre-inoculated wheat grains before incubation.

Incubation period and molding for substrate mixtures refinement experiment:
After 7 days of growth in autoclave bags (Figure 19), the substrate mixtures with grown mycelium were broken into small pieces and hand packed tightly into 4x4x16cm EPS molds according to the ratios (in weight, Table 13). The molding process occurred in the laminar flow environment to prevent potential contamination and covered with porous polyurethane film afterward (Figure 20). Since the bulk densities of two substrates inoculated with mycelium were unknown, the determination of weight for each substrate for the mixtures could not be decided prior to molding.

Table 13 Samples with various mixing ratios for incubation.

Sample ID	RPS mixture (g)	Cellulose mixture(g)	Ratio in mixture weight
100RPS	108	-	100% RPS : 0% Cellulose
75RPS25C	126	42	75% RPS : 25% Cellulose
50RPS50C	80	80	50% RPS : 50% Cellulose
25RPS75C	50	150	25% RPS : 75% Cellulose
100C	-	160	0% RPS : 100% Cellulose

Note: each sample group had 3 specimens for tests and analysis

During the molding process, the 100RPS mixture was first to mold to justify the weight needed when completely fill up the EPS molds for 3 specimens and followed by the 100C mixture. After these 2 mixtures, the total weight needed to fill the molds was determined, the rest 3 mixtures (75RPS25C, 50RPS50C and 25RPS75C) were decided by adding and subtracting gradually to fill up the molds but kept the weight according to design ratios. Notably, in constant volume (3*4x4x16 cm), the 100C mixture was heavier than the 100RPS mixture due to the particle sizes of cellulose fiber was substantially smaller than RPS fiber; therefore, more compact and condense.

Supposedly, samples moved to the growing chamber again for another 7 days growth period with molds and another 7 days without molds to stimulate mycelium growth in better homogenous on surfaces and denser mycelium network, which resulted in a total growth period of 21 days, as Figure 20 shown. Due to a certain circumstance, the samples were left in molds to grow for 6 weeks (42 days) instead of 21 days before drying. However, this did not affect the experiment goal, which was comparing substrate mixtures in thermal conductivity and compressive strength because all samples still had the same incubation period.

Figure 19 Ganoderma lucidum grew on substrates for 7 days in autoclave bags (white color); RPS (left); cellulose (right).

Figure 20 Samples hand-packed in the laminar airflow environment (left) into molds with various substrate ratios and incubated in the growing chamber (right).

Incubation periods for physical properties experiment:

The substrate used for material properties measurement was 100% RPS. After 7 days of growth in autoclave bags, the mixture was broken into pieces, hand-packed tightly into the EPS molds, and moved to the growing chamber for 7 days; molds were removed afterward for another 7 days to stimulate mycelium growth in better homogenous on surfaces and denser mycelium networks, which resulted in a total growth period of 21 days, as Figure 21 shown. The difference between prolonged growth (PG) samples and normal growth (NG) samples was the amount of incubation period. PG samples had an extra 7 days incubation period, which resulting in 28 days of a total growth period.

Drying process:

After certain incubation periods, samples were oven-dried for 24 hours at 65°C until weights were stable. Samples were weighed before and after drying. When samples were demolded from the EPS molds, all dimensions were assumed to be 4x4x16 (256 cm^3) and dimensions were measured after drying to calculate shrinkage percentages.

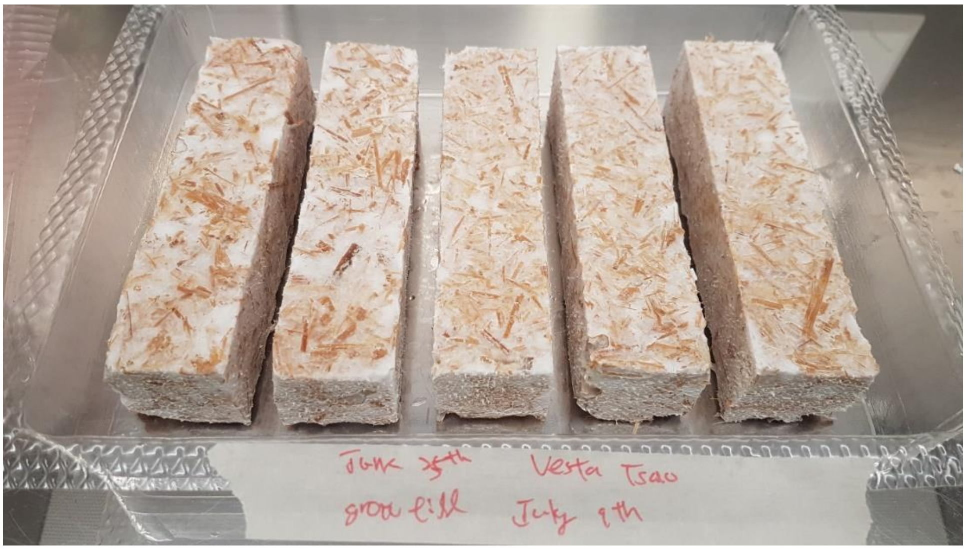

Figure 21 Normal growth samples demolded after 7 days of incubation period and left in the sterilized box for a longer growth.

3.3. Methods

Properties of samples

Initial moisture content, shrinkage percentage, and dry composites bulk density were calculated for each sample as equation (8)~(10) shown, where: W_w = wet weight; W_d = dry weight; V_w = wet volume; V_d = dry volume. Results are shown as Table 14. It should be noted that initial moisture content was determined after the growth period of samples, with mycelium influences.

$$\text{Initial moisture content} = \frac{\mathrm{W_w} - \mathrm{W_d}}{\mathrm{W_d}} \times 100\ [\%] \tag{8}$$

$$\text{Shrinkage} = \frac{\mathrm{V_w} - \mathrm{V_d}}{\mathrm{V_w}} \times 100\ [\%] \tag{9}$$

$$\text{Dry Bulk Density} = \frac{\mathrm{W_d}}{\mathrm{V_d}}\ \ [\mathrm{kg}/m^3] \tag{10}$$

Table 14 Samples properties and associated tests in this research.

Label	Tests	Weight before drying [g]	Weight after drying [g]	Dry bulk density [kg/m³]	Initial Moisture content [%]	Dry Volume [cm³]	Average shrinkage [%]
Samples used in substrate mixtures refinement experiment							
100RPS	Th, C	95 ± 3	29 ± 2	156 ± 9	228 ± 37	188 ± 10	27 ± 4
75RPS25C	Th, C	151 ± 7	40 ± 3	236 ± 14	274 ± 9	171 ± 2	33 ± 6
50RPS50C	Th, C	142 ± 5	45 ± 1	237 ± 6	216 ± 14	193 ± 7	25 ± 3
25RPS75C	Th, C	188 ± 8	62 ± 2	385 ± 14	205 ± 14	160 ± 2	37 ± 6
100C	Th, C	146 ± 7	53 ± 4	373 ± 29	178 ± 8	141 ± 0	45 ± 11
Samples used in physical properties & hygrothermal behavior experiment							
Normal	Th, C, F	86 ± 3	19 ± 1	102 ± 5	331± 15	196 ± 14	16 ± 5
Prolong	Th, C, F	74 ± 2	17 ± 0	103 ± 3	327 ± 7	167 ± 2	22 ± 1
Normal	DWC, Th, C	89 ± 2	20 ± 0	118 ± 7	343 ± 4	172 ± 5	21 ± 3
Prolong	DWC, Th, C	84 ± 3	17 ± 1	101 ± 9	371 ± 10	101 ± 9	19 ± 7
Normal	W, FL, MBV	78 ± 3	17 ± 1	110 ± 3	362 ± 9	154 ± 3	14 ± 3
Prolong	W, FL	74 ± 5	17 ± 0	98 ± 3	346 ± 26	171 ± 10	20 ± 7

Note:
Th: thermal conductivity test; C: compressive test; FL: Flexural test; F: FT-IR; DWC: drying and wetting cycle; W: water absorption test; MBV: moisture buffer value
Each sample group had 3 specimens for tests and analysis, shown as mean ± standard deviation

Thermal conductivity

The transient method (non-steady method) was used by following ASTM D5334-08 with thermal needle probe (measured ranged of 0.035-0.2 ($W.m^{-1}.K^{-1}$) with an accuracy of 5% reading +0.001 ($W.m^{-1}.K^{-1}$) from ISOMET model 2104 to measure thermal conductivity of samples. No predrilling hole was needed for samples and measurement was performed under stable thermal condition (steady ambient temperature). Considering thermal conductivity is one of the most critical parameters for insulation materials, it was conducted in substrate mixtures refinement and material properties experiments. In the latter case, it was conducted between NG and PG samples, and with and without DWC to understand consequences of accelerated aging tests.

Compressive strength & flexural strength

Material is required to sustain its own weight to be applied as non-structural-bearing wall thermal insulation material. Therefore, compressive strength tests were conducted in both experiments, same reasons as thermal conductivity tests.

Compressive strength of samples was determined by using an ISO standard Instron load bench with a 30kN load cell at ambient conditions. Sample sizes were not full sizes (4 x4 x16cm) but sliced to cubicles (3.5 x 3.5cm) in the substrate mixtures experiment and (3.0 x 3.0 cm) in the material properties experiment. It should be noted that the initial contact surfaces of the load cell and samples were not completely flat because the natural characteristic of the MBC contained rough surfaces. In substrate mixtures experiment, the test was stopped when a fixed strain of 43% was reached in the testing specimens, while the material properties experiment stopped when a fixed strain reached 67%. In substrate mixtures experiment, samples were mixtures of various ratios of two kinds of substrates; therefore, the maximum loads were inconsistent. As the result, the maximum load at fixed strain (43%) was extracted from each sample raw data for comparison. In contrast, the latter experiment samples consisted of same materials; therefore, the maximum load at fixed strain (67%) was determined without the necessity of raw data extraction. The compressive strength at the fixed strain was averaged with standard deviation between 3 specimens with each sample batch and compared between different substrates mixing ratios, NG versus PG and with and without DWC.

Flexural strength was tested in material properties experiment only because the purpose was to compare with conventional insulation materials. PG versus NG samples (each had 3 specimens) were tested by using the Instron load bench (the same standard machine used in compressive strength test) with a 30kN load cell at ambient conditions in a three-point bending setup. The elongation rate was 3 mm/min and a span distance was 100mm. Load versus elongation rate data were recorded until failure and results were shown as flexural stress at tensile strength (MPa). It should be noted that the initial contact surfaces of the load cell and samples were not flat due to the rough surfaces of MBC.

Water Absorption

Water intake over time was measured for both NG and PG samples. For each specimen (2 groups x 3 specimens, resulted in 6 total) was partially submerged (10mm below water surface) for water intake measurement over 48 hours. The specimen was hanged by an especially designed hook and connected to a scale for continuous weighing, as Figure 22 shown. The initial weight and wet weight were measured for consistency reasons. However, due to the unknown initial weight of samples with the device and the initial water buoyant force since the samples were floated on the water surface. The data were analyzed with the equation (11) for analysis and graphing purposes.

$$\text{Relative Absorption } \% = \frac{\mathrm{W_{48h}} - \mathrm{W_{ini}}}{\mathrm{W_{ini}}} \times 100\ [\%] \tag{11}$$

Figure 22 Water absorption test and thermal conductivity test set-up.

Drying and wetting cycles (DWC)

Specimens of NG and PG were completely immersed in water for wetting cycles (25°C), and drying cycles were performed with a forced airflow chamber (40°C). In order to define the appropriate duration for cycles, other samples (100RPS, 50RPS50C, and 100C) were wetted and dried for a few cycles before the actual specimens were tested. The initial weight and volume of dried samples were measured before and after wetting and drying to determine mass variation over time. Considering that after 24 hours of complete water immersion and 24 hours of drying, the sample was able to absorb and release 90% of its water uptake/release. In total, 3 DWC were conducted: 24 hours immersed in water followed by 24 hours of drying in the airflow chamber. Initial weight and volume of samples were recorded to monitor changes after DWC.

Chemical and surface characterizations

The FT-IR and SEM were used to analyse surface differences (between NG and PG) in chemical compositions and microstructure of samples. The FT-IR was acquired on PerkinElmer FT-IR spectrometer and equipped with PIKE technologies GladiATR to record in single bounce Attenuated Total Refractance (ATR). The background automatic atmospheric reference was obtained before the measurement of samples. All samples were measured with 4 scans per sample from 400 to 4000 cm^{-1}. One sample from each group (PG and NG) and undecayed RPS were used for measurement. Small specimens were extracted from various locations by small tweezer from top and side of the samples to ensure the reproducibility of the data (Figure 23).

The surface morphology (intensities of mycelium growth on surfaces) of the NG and PG were studied by Phenom ProX and backscattered electron detector (BSD) with a voltage of 15kV, which was within the range showed in the literature. First, the small samples (4 pieces from different locations) were gently extracted with a tweezer and mounted with double-sided carbon tape. Samples were coated with a 15nm gold layer to ensure good electrical conductivity.

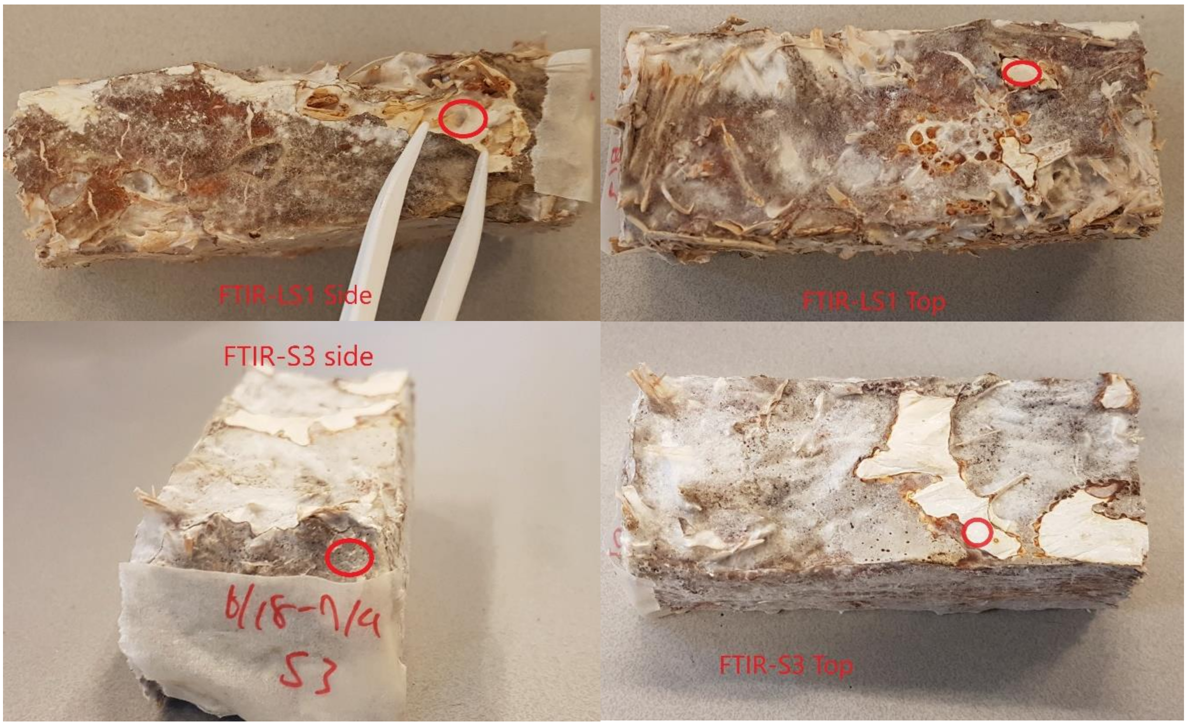

Figure 23 FT-IR Samples extraction configures for PG sample (LS1) and NG sample (S3) top and side.

Practical Moisture Buffer Values

The NORDTEST method was chosen to evaluate the $MBV_{practical}$ of MBC with EPS and gypsum board ($CaSO_4$) as reference materials. Before measurement, samples were stored in 23°C ± 2°C and 50%RH ± 0.5 %RH condition, aluminium tape was used to cover either 5/6 or 4/6 sides of sample surfaces, with minimum 0.01 m^2 exposed surface areas (single or doubled sided). MBC was double-sided to ensure minimum surface exposure of 0.01 m^2, and other samples had met minimum surface exposure with single-sided. Samples were placed in the climate chamber (± 0.2°C and ± 0.5%RH) with air velocity of 0.28 m/s (10% of maximum air velocity of the climate chamber) and exposed to cyclic step-change in RH between 75%RH and 33%RH for 8 and 16 hours respectively for minimum 3 stable cycles (quasi-steady), stable cycles defined as [68]:

- The change in mass (Δm) is less than 5% between the last three cycles (days).
- The differences between weight gain and weight loss within each cycle should be less than 5% of Δm.

Sample weighing was performed outside the climate chamber without disturbance of air velocity and every measurement for each cycle happened less than 30 seconds. The change in mass (Δm) is determined as the average between the weight gain during the absorption period and weight loss during the desorption period. Sample mass was weighted every turn of the cycles (8/16 hrs), with the last absorption cycle (8hrs and 75%RH) acquired at least 5 times of measurement.

MBC and gypsum board, in total 6 cycles (8/16 hours is one cycle) were performed, whereas EPS only required 4 cycles. Every group of samples contained 3 specimens. $MBV_{practical}$ was the average between 3 samples with standard deviation, shown as equation (12), where A is exposed surface area (m^2).

$$\mathrm{MBV}_{\mathrm{practical}} = \frac{\overline{\mathrm{m}_{\mathrm{absorbed\,,desorbed}}}}{\mathrm{A} \times \Delta\mathrm{RH}} = \frac{\Delta\mathrm{m}}{\mathrm{A} \times \Delta\mathrm{RH}} \quad [\mathrm{g/m^2} \times \%\mathrm{RH}] \tag{12}$$

It should be noted here that 3 specimens were placed in the climate chamber at once, which deviated from the NORDTEST method (one specimen each measurement). The sample dimensions and information are shown in Table 15 and Figure 24.

Table 15 MBV samples dimensions and information.

Sample Type	Sample ID	Averaged Initial Weight [g]	Averaged thickness [mm]	Averaged exposed surface area [m^2]	Single or double-sided exposure
Mycelium	S7,S8,S9	19.17 ± 1.01	26.3 ± 0.47	0.012	Double
EPS	ES1,ES2,ES3	6.90 ± 0.42	19	0.013	Single
Gypsum	GS1,GS2,GS3	372.36 ± 0.97	12.5	0.04	Single

Figure 24 MBV samples a) mycelium b) gypsum board c) EPS d) samples in the climate chamber.

4. Results and Discussion

4.1. Substrate mixtures refinement

The research objective of this experiment was to find the most optimal and suitable substrate mixtures ratio to be applied as foam-like wall insulation materials regarding thermal conductivity and compressive strength. The two chosen substrates, RPS and cellulose had distinctly different natural characteristics regarding particle sizes, initial moisture intake, bulk density, and compositions which rendered the possibility the mixtures of two enable to produce MBC in better functional performance.

Average initial moisture content and shrinkage of MBC with various substrate mixtures (by weight in percentage) are shown in Table 14 and Figure 25. The result aligned with a visual inspection that substrates contained more RPS had less average shrinkage than mixtures with more cellulose. The causes of volume shrinkage can be influenced by two factors: water evaporated after drying and fiber particle sizes. Assumed the volume shrinkage was mainly caused by water evaporation after drying, mixtures with the high initial moisture contents (i.e. mixtures with RPS) would result in higher volume shrinkage. In contrast, the mixture of 100% cellulose with the lower moisture content (178%) resulted in the highest volume shrinkage (45%). Therefore, the results indicated that initial fiber particle sizes had more impact than initial moisture contents of composites in regard to volume shrinkage, as RPS had substantially larger sizes than cellulose fiber. When hand-packed into molds, larger particle sizes created more air voids in between fiber, which allowed better growth for fungi. As light and air exchange are required to stimulate mycelium growth and bind with substrates surrounding it.

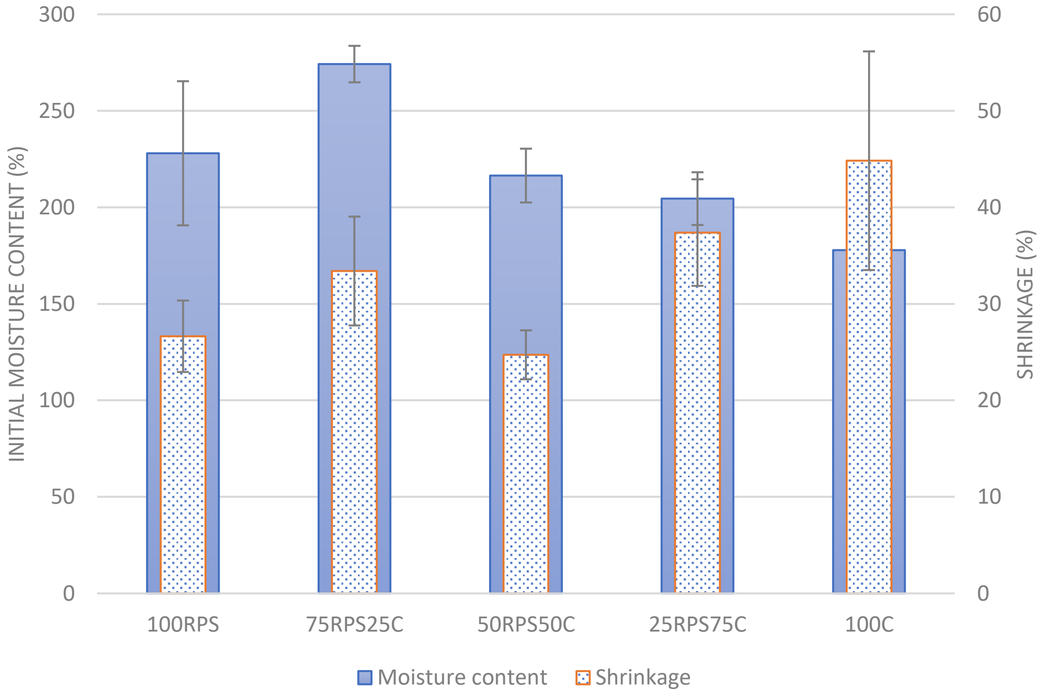

Figure 25 Various substrate mixture ratios in average initial moisture content and shrinkage of composites after drying.

Arguably, one study showed no significant volume shrinkage between the same substrate in different fiber sizes (loose, chopped, tow) [31]. In contrast, one study showed substrates with relatively smaller particle sizes had smaller shrinkage than substrates with larger particle sizes [30]. In short, the more variables in inoculated substrates mixing proportions are, the more unpredictable and uncertainties are shown in dimension stability [15], [30].

As for thermal conductivity, despite RPS as a natural fiber itself is a low thermal conductivity medium, the mycelium composites after drying also showed good potential in thermal conductivity, which outperformed other mixtures. The results showed that adding cellulose fiber into mixtures increased density and thermal conductivity (Table 16), which imposed negative effects to apply as insulation materials. A possible explanation was the cellulose fiber with substantially smaller particle sizes than RPS fiber created more condense and compact composites, which induced fewer air voids and spaces for the mycelium to grow and fuse. This matched with the study showed that when particles pre-processed to dust as substrates had the worst growth of mycelium compared with non-processed or chopped [31].

Table 16 Various substrate mixture ratios in dry bulk density, thermal conductivity and compress strength of composites after drying.

Label	**Dry bulk density [kg/m^3]**	**Thermal conductivity [W.m^{-1}.K^{-1}]**	**Compressive Strength @ fixed strain 43% [MPa]**
100RPS	156 ± 9	0.057 ± 0.0	0.452 ± 0.048
75RPS25C	236 ± 14	0.075 ± 0.005	0.608 ± 0.133
50RPS50C	237 ± 6	0.072 ± 0.003	0.690 ± 0.046
25RPS75C	385 ± 14	0.084 ± 0.003	0.845 ± 0.090*
100C	373 ± 29	0.085 ± 0.004	0.145 ± 0.037

Note:
Each sample group had 3 specimens for tests and analysis, shown as mean ± standard deviation
*One of the samples stopped before reach to fixed strain; mean with 3 specimens instead of 4

In contrast to thermal conductivity, compressive strength at fixed strain 43% showed that mixtures ratio of two substrates outperformed the other two pure mixtures (only RPS or only cellulose), shown in Table 16 (raw data refer to Appendix A). As substrate has a major impact on density and machinal properties; moreover, density has a positive correlation with compressive strength, as Figure 26 shown. The mixture of 25RPS75C had the highest density (385 kg/m^3), which resulted in the highest compressive strength. Notably, 100% cellulose substrate performed the worst in compressive strength, despite its density was the second highest (373 kg/m^3). This indicated that cellulose fiber performed the worst as a substrate itself. Possible reasons for the failure of cellulose as a substrate might due to the mixing proportion was not optimal for the mycelium to grow, both in autoclave bag and in mold, i.e. lower inoculation percentage (<10% in weight) or a lack of air exchange during growing. Another anticipation is that the substantial differences in particle sizes of two substrates had caused the variations in compressive strength. Arguably, [30] conducted compressive strength versus different particle sizes (other factors stayed the same) showed no correlation between substrate particle sizes and compressive strength. Nevertheless, G. *lucidum*-based materials induced a wider range regarding compressive strength in studies, as Figure 27 shown [15]. According to [15], G. *lucidum*-based materials act more like plasticizers because its mycelium constituted more lipid and protein when compared with fungi from a different order.

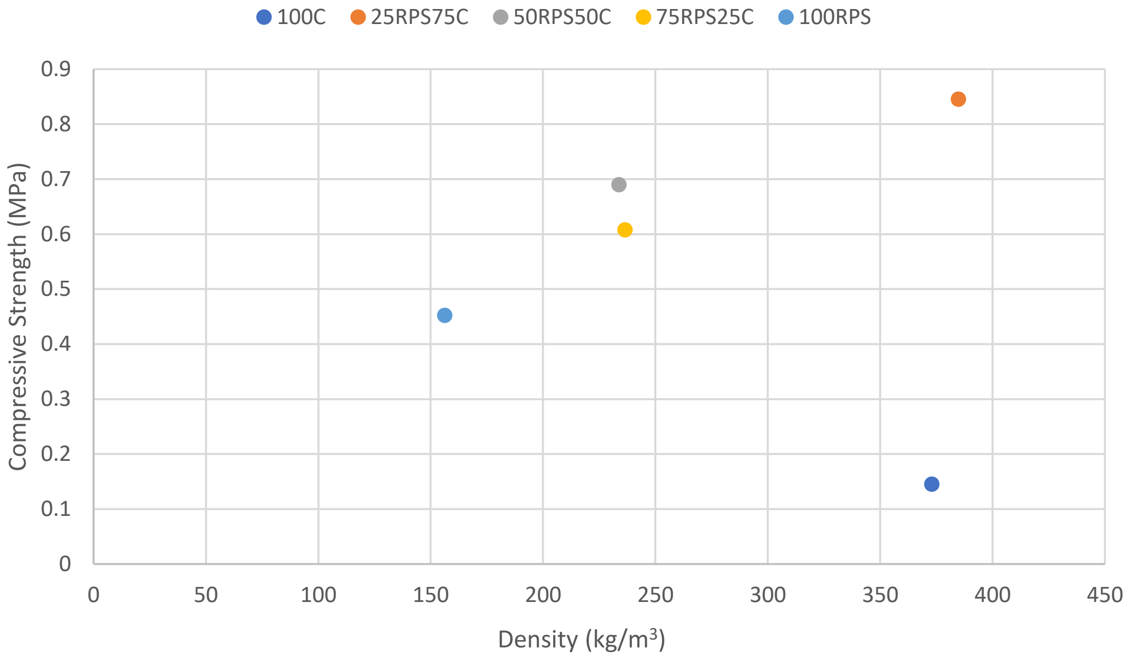

Figure 26 Compressive strength at fixed strain (43%) versus density of mycelium composites.

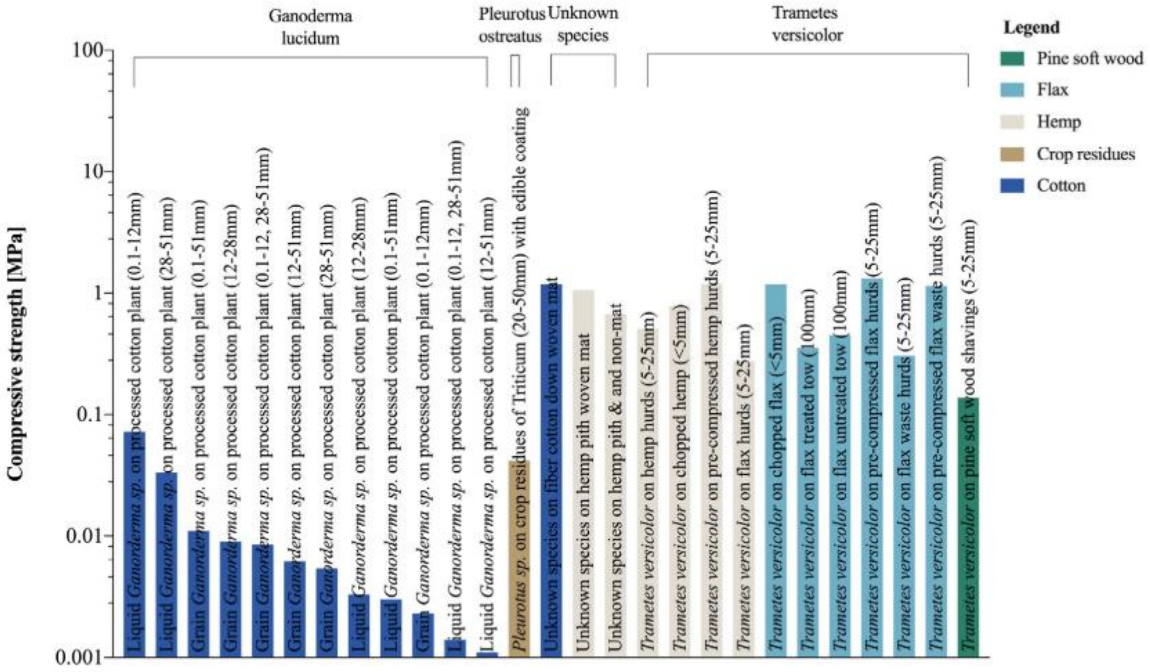

Figure 27 Compressive strength (MPa) of mycelium-based materials from various studies. *Note*. Reprinted from [15].

Besides considering mycelium influences in compressive strength performance, natural plant fiber with higher lignin content had intrinsic characteristics to reinforce compressive strength in various fiber composites studies. Also, it was expected that RPS fiber had higher compressive strength than cellulose fiber, disregard the influences of mycelium growth.

This research was limited to understand the optimal mixing proportions for both fibers at the initial growing phase. However, as the growing period of mycelium commonly ranged between 14-28 days, and the molding process occurred at 7 days after initial growth in autoclave bags. The exponential growing phase of mycelium continued when samples grew in molds. Furthermore, the literature stated that damaging the mycelium network during the growth phase has been shown to stimulate mycelial growth and colonization of substrates [15], [59]–[61]. This occurred in the molding process in this research when two substrates with grown mycelium were hand-packed into molds with various ratios (in weight) to create the mixtures of substrates. The initial optimal growing proportion was not critical when determining mycelium biomass grew on the two substrates in the end.

Another unexpected result of this research was that the original growing period of this experiment aimed at 14 days, but due to a certain circumstance, the samples were left to grow for 48 days (6 weeks). Although this had no influence on deciding optimal and suitable substrates mixtures, extended prolonged growth jeopardized the growth of mycelium biomass and network because the nutrients obtained were used in the growing of fruiting bodies. The extended growth period also made the delignification (decaying process) occurred in a longer time, which had impacts on lignocellulose contents in substrates, thus affected material properties, such as volume shrinkage and initial moisture contents. As Figure 28 shown, the brown watery woody part indicated that the growing phase of fruiting bodies was initiated, when the hydrophobic tissues formed on aerial hyphae [58].

Figure 28 Fruiting body growing state was initiated.

To summarize, the substrate with 100% RPS performed the best in thermal conductivity with the lowest value (0.057 $W.m^{-1}.K^{-1}$), which was 24%-33% lower than others. The density of 100RPS was the lowest with acceptable compressive strength and volume shrinkage compare with others. In future work, it is recommended to have statistical analysis in significance level due to high heterogenicity in samples and different parameters involved.

To compete with synthetic foam insulation materials (i.e. EPS or XPS), density is also critical which correlates with compressive strength [5]. As results indicated, density and compressive strength of MBC had a linear relationship, similar to EPS/XPS products in the current market [5], [77]. Conventional EPS and XPS in the market currently have densities ranging from 11-50 (kg/m^3); therefore, it is difficult to directly extrapolate compressive strength by densities of two materials. The compressive strength of EPS/XPS conducted in the current testing standard only required the stress [MPa] at 10% strained, which also imposed difficulties to extrapolate the direct comparison with MBC. Furthermore, EPS in the study showed more resistance to strain but different deformation performance from the mycelium-based samples [54].

Due to the above reasons, referred to literature that had conducted compressive strength tests of MBC versus EPS was used to determine compatible compressive strength [54]. When the samples with the lowest density (185.6 kg/m^3) at 20% fixed strain to compare with EPS (21 kg/m^3) at 20% fixed strained, the compressive stress was approximately the same between the two (approximately 0.12MPa) in the study [54]. As 100% RPS composite produced in this research had lower density (156 kg/m^3) compare with the literature [54], the compressive strength of this mixture at 43% fixed strain (2-fold) was 0.45 MPa (more than 3-fold of 0.12MPa). Therefore, 100RPS produced in this research was compatible with synthetic foams and showed good potential to be applied as interior insulation material. In addition, 100RPS was selected to further study the material’s physical properties and hygrothermal behavior.

4.2. Physical properties of MBC

Water absorption test:

One main hypothesis of this research is to study a prolonged growing period of MBC results in water absorption reduction due to denser mycelium at the outer surface. As Figure 29 shown, the NG samples resulted in roughly 80% of average relative water absorption (blue solid line), whereas the average of PG samples was (green solid line) 55% after 48 hours of partial immersion underwater. PG samples had 25% less average relative water absorption than NG samples. This coincided with literature and proved the hypothesis with evidence, that chitin compositions in fungi cell walls created a natural hydrophobic surface layer in composites to prevent the entry of water [15], [31], [58]. While compared with one study [33] that improved composites water resistance by hybridized with cellulose nanofibrils, the improvement of this research was relatively less, 40 % and 31% respectively. Nevertheless, this research investigated the intrinsic material compositions instead of binding with extra additives. Further tests related to material compositions and surface morphology were used to identify the differences between NG and PG samples.

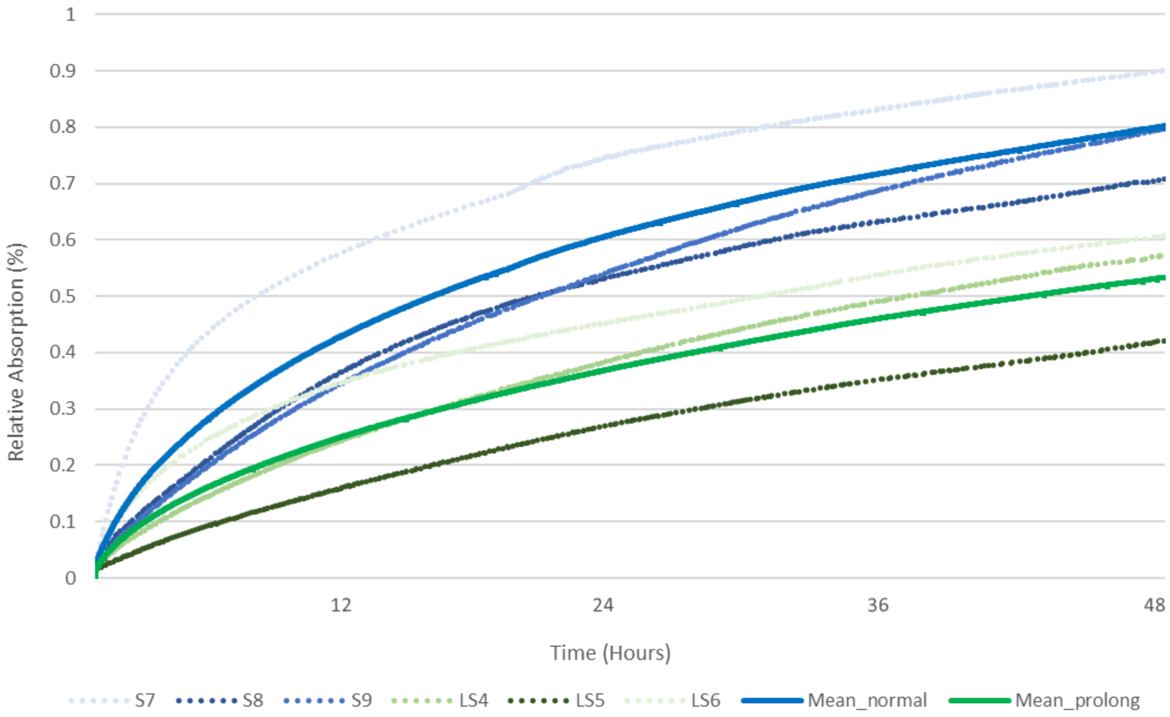

Figure 29 Relative water absorption test NG (blue lines) versus PG (green lines) samples and their mean values (two solid lines).

A longer growth period also resulted in the formation of a denser hydrophobic surface layer. The hydrophobic layer is induced by emergent hydrophobin protein, the unique gene only exits in fungi [78]. This protein gene has a phenomenal trait, which is not known from other proteins, can assemble into an amphipathic membrane from hydrophobic and hydrophilic environments [58]. One study showed that the deletion of hydrophobin in the SC3 gene resulted in a more hydrophilic mycelium layer when compared with wild-strain, which indicated that hydrophobin genes play a critical role in forming the hydrophobic coating on the substrates[47]. An additional test which can quantify the hydrophobin genes is recommended to strengthen the hypothesis above in a future study.

Chemical characterization:

FT-IR can be used to analyze the chemical composition of materials to understand the changes between undecayed substrates (RPS pure) versus MBC (decayed substrates). Most of the changes occurred in the fingerprint region (1800-500 cm^{-1}), as Figure 30 shown. All the samples from mycelium composites had very similar patterns and peaks. Undecayed substrate showed the same peaks when referred to the literature [75]. Thus, the peak differences between undecayed substrate and mycelium composites were the focus and other peaks were not further discussed.

According to the literature, bands at 1370-1375 cm^{-1} indicated strong signals of chitin [31], [67]; however, the peak was also observed in the undecayed substrate. Pure RPS in literature also has bands at 1370 cm^{-1} and 1170 cm^{-1}, assigned to the bending of C-H groups and the stretching vibrations of the C-O-C groups of hemicellulose and cellulose [75]. The peaks of chitin and chitosan (1370, 1375 cm^{-1}) were difficult to identify when compared spectra between undecayed fiber and mycelium-composites due to the peaks were mostly similar between the two [7], [54]. Moreover, when analyzed FT-IR bands from extracted pure mycelium film (1371-1375 cm^{-1}) than foam-like composites, the band signals of chitin were also weak, which showed the autogenous difficulties utilizing FT-IR to identify chitin peaks [20]. Nonetheless, the delignification process was shown in FT-IR result. The undecayed substrate had a prominent peak at 1595 cm^{-1}, which was the typical stretching of aromatic C=C groups of lignin, and the peak was absent in mycelium-composites [31], [75]. When subtracted mycelium composites from the undecayed substrate (dot lines) had clearly shown the absence peak.

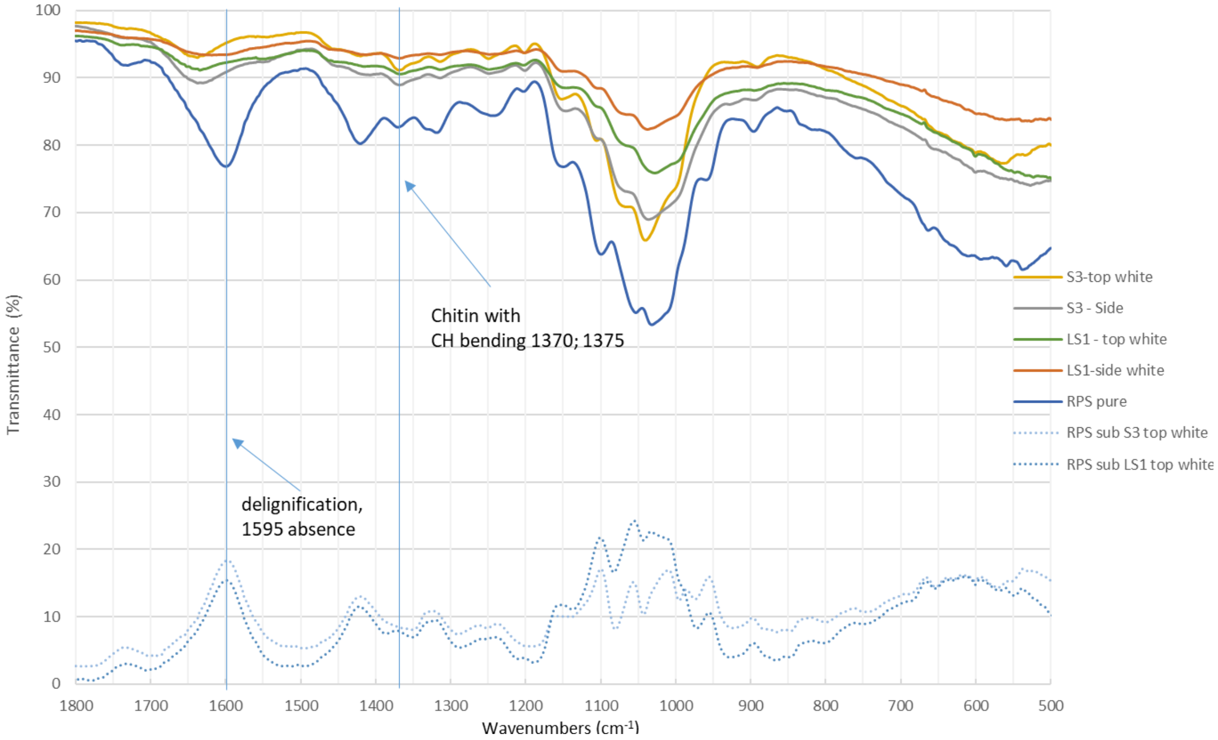

Figure 30 FT-IR of mycelium composites and undecayed RPS.

Surface morphology:

SEM was performed to show the surface morphology differences between PG and NG. As expected, the white mycelium (tubular hyphae) without fiber in PG samples (Figure 32c) had less porosity than NG samples (Figure 31a), which indicated more biomass of mycelium in PG samples [28]. When considered the interlocking colonization of mycelium on RPS, the NG samples images showed less binding with mycelium (Figure 31b) than PG samples (Figure 32 d). Tubular hyphae are clearly identifiable due to their distinct interwoven structures when fused with fiber, and the images matched with the study produced RPS mycelium composites [29]. Above all, the hypothesis of a prolonged growth induced more intense mycelium layer on the composites is shown when compared with the SEM images of two. Moreover, this result coincided with the water absorption result that PG samples had relatively 25% less water absorption than NG samples due to the denser hydrophobic mycelium layer on the outer surface. However, the covered areas of the hydrophobic surfaces on both samples were highly heterogenous, which imposed difficulties to connect the definite influences of hydrophobic surface intensities and water absorption results. Therefore, physical property tests were performed to further investigate the differences between NG and PG.

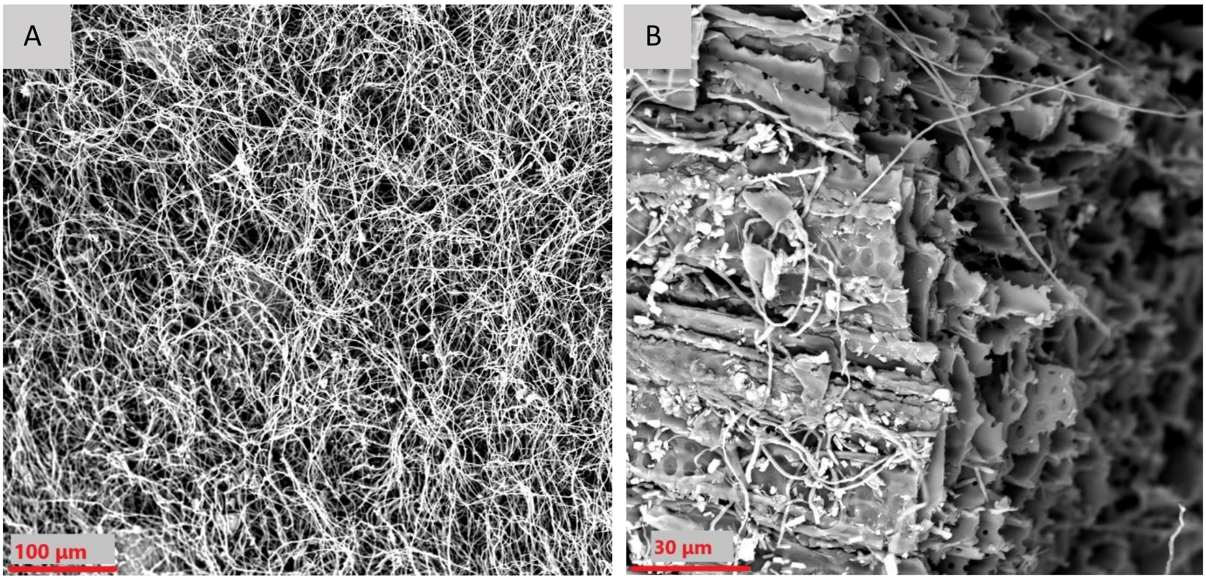

Figure 31 Surface morphology of NG samples A) top surface B) side surface with fiber.

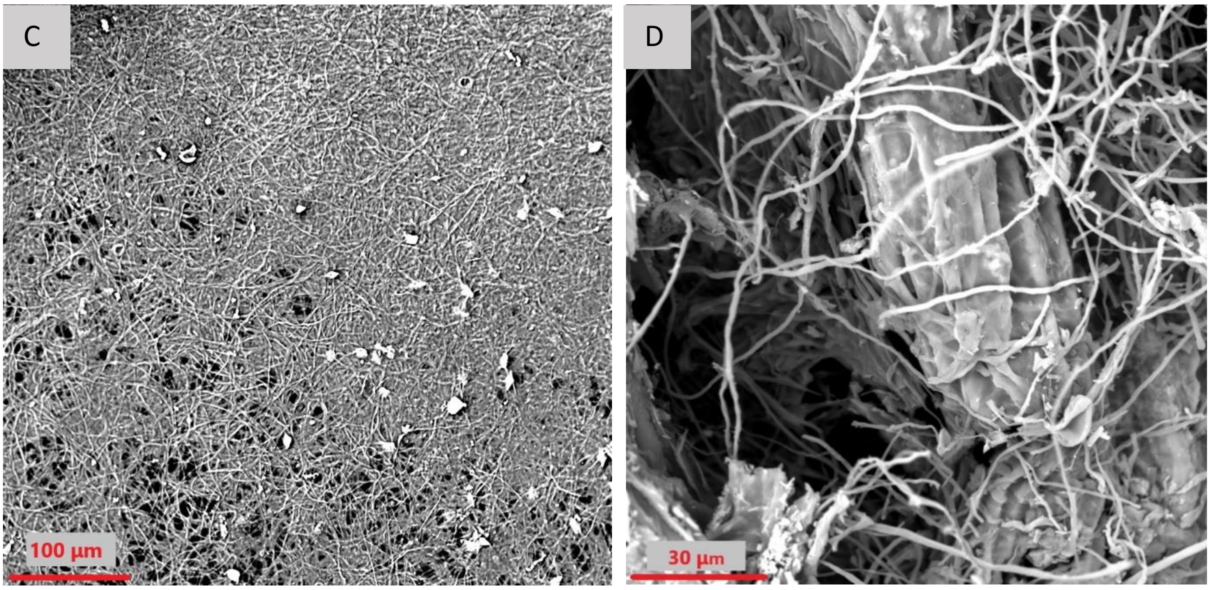

Figure 32 Surface morphology of PG samples C) top surface D) side surface with fiber.

Physical properties:

Table 17 depicted average flexural stress at the tensile strength of both samples (raw data refer to Appendix B). Flexural stress of NG samples showed 6% higher flexure stress than PG. NG samples were 11% higher in density than PG samples, which showed the variations in flexural stress between the two samples were insignificant.

Table 17 Flexure stress of PG and NG, and comparison with literature and EPS.

	Density [kg/m^3]	Flexural stress [MPa]	References
PG	98 ± 3	0.15 ± 0.01	This research
NG	110 ± 3	0.16 ± 0.01	This research
Variation	**11%**	**6%**	
T. *multicolour* with RPS	100	0.22	[29]
P. *ostreatus* with RPS	130	0.06	[29]
EPS	20	0.22	[77]

As results illustrated, mechanical properties had negligible influence by growing time but more by substrate choices and densities (Figure 35 & Table 17). This indicated that the differences between NG and PG only existed on the mycelium covered area intensity on the surfaces but not the interlocking degree with substrates. Intriguingly, the produced samples in this research have shown good performance in flexural stress when compared with literature used the same substrate but with different fungal strains [29], 0.15-0.16 MPa and 0.06 MPa, respectively. These results agreed with the literature that G. *lucidum* presents higher tensile strength and more plasticity than P. *ostreatus* due to its complex hyphal system [15][28]. When compared with synthetic insulation material (i.e. EPS), the flexural stress of MBC is slightly lower, subjective to the fungal strains used.

Thermal conductivity had insignificant differences between the two, showed that thermal conductivity is highly dependent on substrate choices than growing time (Figure 33).

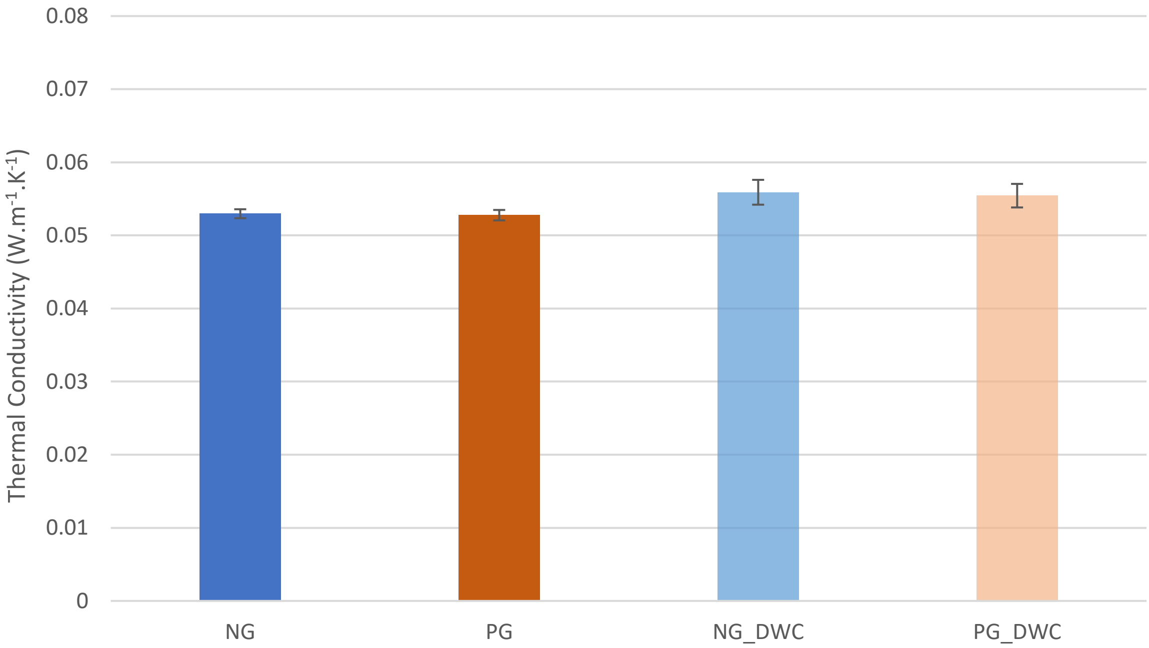

Figure 33 Thermal conductivity NG versus PG and with and without DWC.

DWC assessment:

When samples with DWC were visually inspected, the mycelium surface had discoloration and appeared more veins on the samples, as Figure 34 shown. The discoloration can be explained by the leaching solution from RPS extractives during the immersion period [75]. NG samples before and after DWC had insignificant density variation (0.9%), whereas PG samples before and after DWC had a density reduction of 11%. Significant weight loss and density reduction occurred in PG samples after DWC, as Table 18 shown. The dry bulk density changes of DWC samples were determined by the dry weight and volume before and after DWC.

Table 18 PG and NG samples density changes before and after DWC.

	PG	**NG**
Weight loss after DWC (%)	12 ± 1	8 ± 1
Density before DWC [kg/m^3]	101 ± 2	119 ± 5
Density after DWC [kg/m^3]	91 ± 6	118 ± 3
Variation (%)	**11**	**0.9**

Thermal conductivity of PG versus NG, and with and without DWC test had insignificant differences as Figure 33 shown. The result indicated that after 3 DWC cycles, the MBC could maintain good performance in thermal conductivity. Anticipation is that thermal conductivity is highly related to material porosity (i.e. open or close air voids in materials). Thus, liquid water-filled voids evaporated after drying and thermal conductivity maintained its original level.

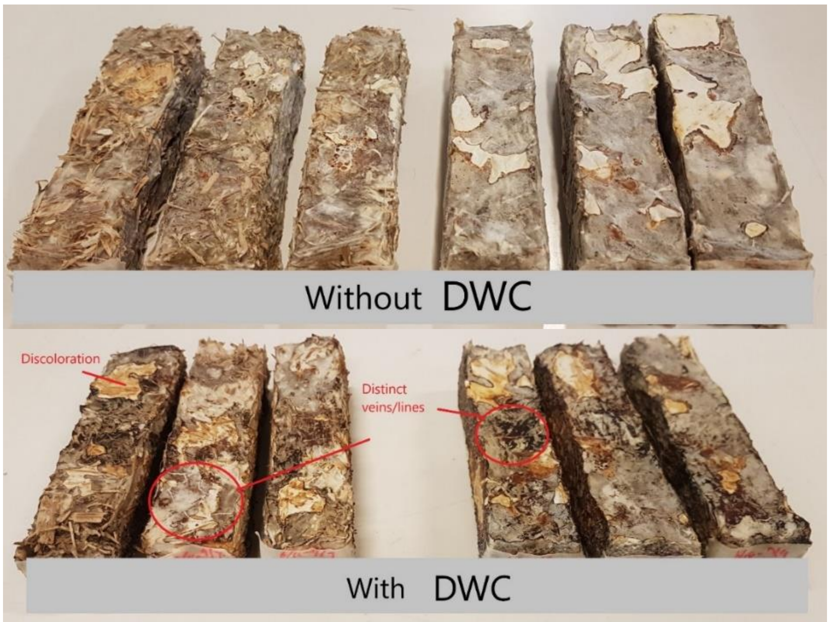

Figure 34 PG (left) and NG (right) samples with and without DWC test.

Compressive strength of PG versus NG without DWC had insignificant differences because the density differences of the two groups were small (103, 102 kg/m^3, respectively). In contrast to the expected lower compressive strength of both PG and NG samples with DWC, the results of NG samples with DWC showed insignificant differences, as Figure 35 shown (normalized with sample densities). As aforementioned, MBC density and compressive strength have a positive relationship; therefore, two explanations can be derived from this result. First, density was the major cause of differences in compressive strength. Second, DWC had an insignificant impact on compressive strength of MBC.

The average density of NG samples with DWC had significantly higher density compare with groups without DWC (118 kg/m^3, 102 kg/m^3, respectively), whereas PG samples with DWC had the lowest density of all (91 kg/m^3). The lower density might be correlated with the compressive strength reduction of prolonged samples with DWC.

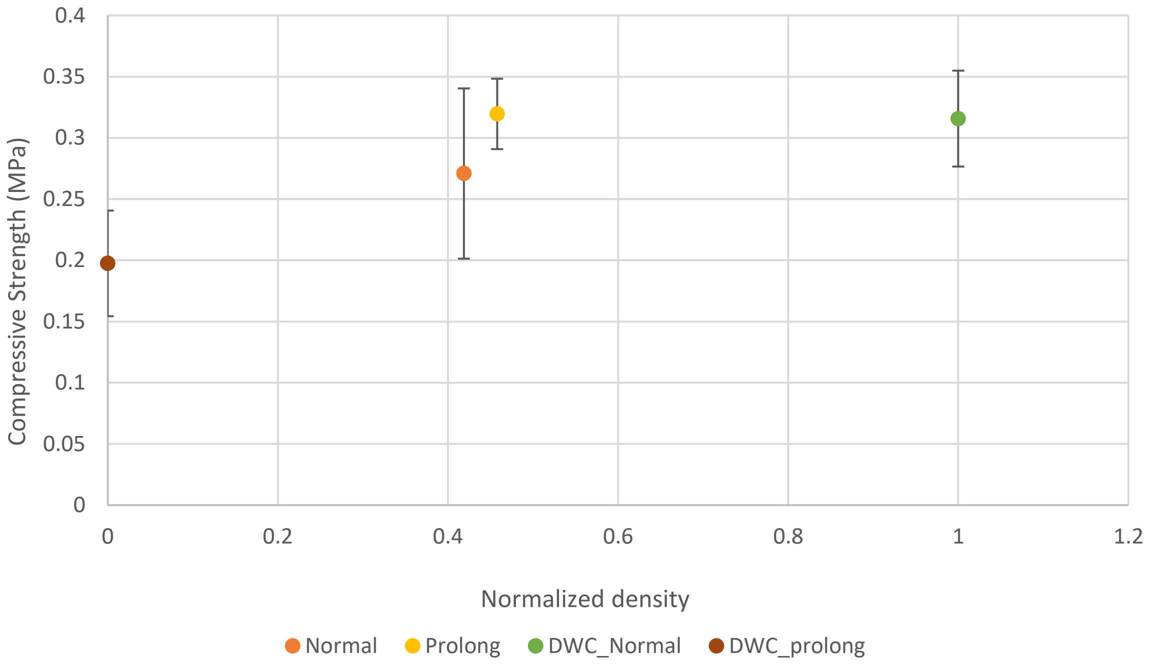

Figure 35 Compressive strength PG versus NG, with and without DWC normalized with density.

Arguably, if density was linearly correlated with compressive strength in MBC, then NG samples with DWC (118 kg/m^3) should have resulted in higher compressive strength than samples were not conducted with DWC test (102 kg/m^3), but this was not seen. Therefore, DWC cannot be ruled out from inducing performance reduction in mechanical characteristics of MBC. Possible explanations were the exposing composites with the complete immersed method for 24 hours had caused the swelling in fiber and leaching extractives. Drying afterward caused damage to fiber structure and thus reduced compressive strength [79].

Above all, the extended growing period resulted in better water resistances in MBC because of the denser hydrophobic mycelium outer layer, other physical properties had no significant differences between NG and PG indicated that the interlocking level of mycelium into the substrates maintained the same. Intriguingly, influences of accelerated aging tests on MBC had shown insignificant differences in thermal conductivity but a slight decrease in compressive strength. Therefore, this research showed that MBC can maintain its functional performance after accelerated aging tests.

4.3. Hygrothermal behavior: $MBV_{practical}$

The raw data of mass changes over the adsorption and the desorption period (33%RH and 75%RH) of mycelium composites, gypsum, and EPS samples are shown in Figure 36. The measurement discontinued over the weekend (between 96 hours – 144 hours) and continued afterward, during the non-measured period the samples stayed in the climate chamber. The first absorption mass changes after the non-measured period were not counted in the results. In the absorption period of the last cycle (8hrs/75%RH), samples mass changes were measured at least 5 times; thus, data scattered after 217 hours for mycelium and gypsum and 168 hours for EPS (Figure 36).

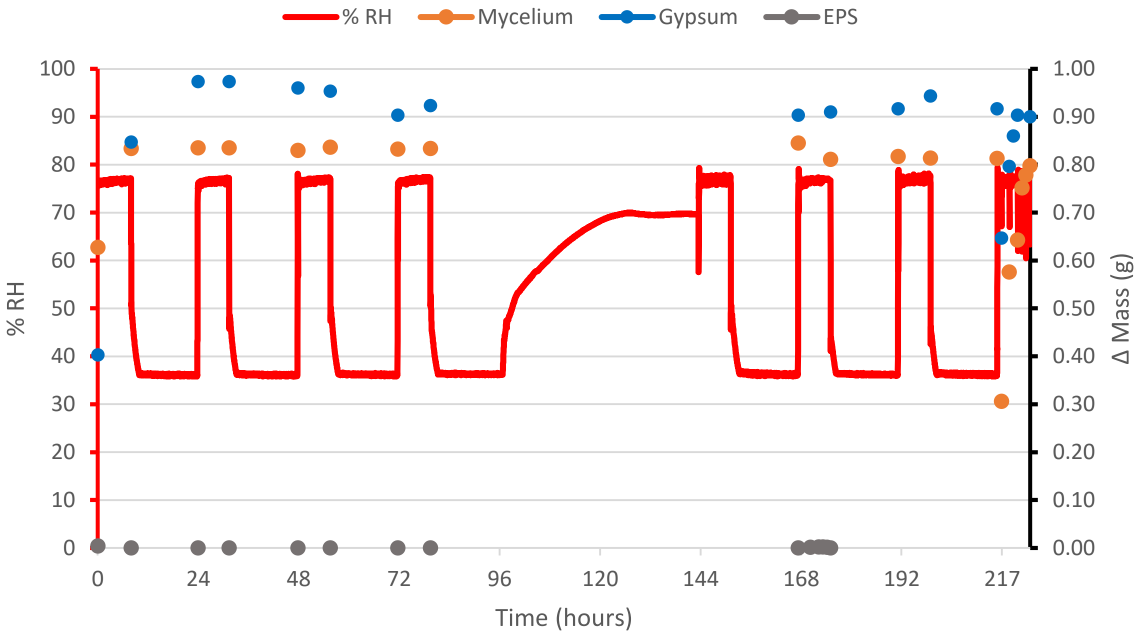

Figure 36 Measured different samples of mass changes during the adsorption and desorption period (raw data).

To determine the quasi-stable cycles of samples, the calculated MBVs basis of changes of mass in relation to a given cycle is shown in Figure 37, which indicated that after cycle 4 all samples reached to quasi-stable cycles, where daily changes of mass and MBV were less than 5% between the last 3 cycles and between each absorption and desorption periods. Therefore, MBVs of mycelium and gypsum boards were the average of daily absorption and desorption mass changes in cycle 4-6, whereas MBV of EPS was the average of 2-4 cycles since EPS mass changes were insignificant between cycles, as shown in Table 19 & Figure 37.

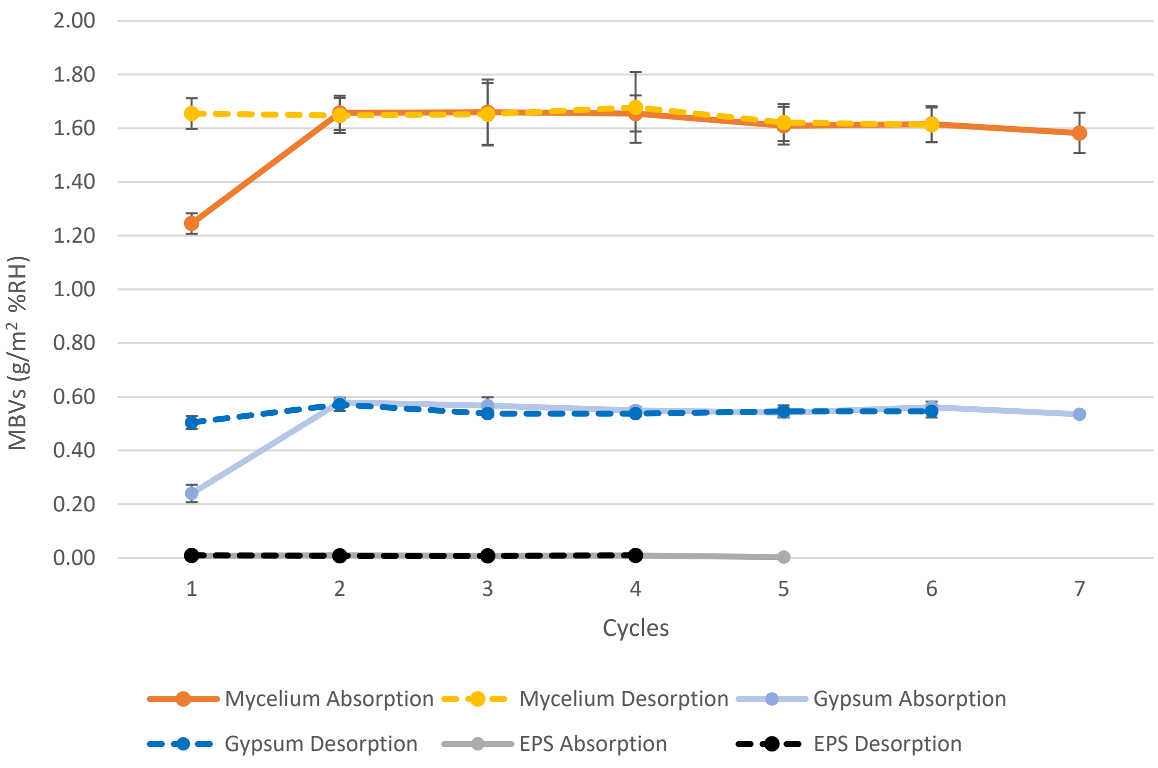

Figure 37 Calculated MBV_s of various samples versus cycles.

The final MBV of gypsum conducted and calculated in this research coincided with Rode et al. [68], which is 0.57 (g/m^2 %RH) with the same sample thickness of 12.5 mm and dimensions (200 x 200 mm). The result deviated approximately 0.02 (g/m^2 %RH) from the reference can be explained by the measurement errors and different experiment set-ups (i.e. air velocity differences, amount of samples in a climate chamber, etc.), and the discrepancy is lower than average differences of 1 (g/m^2 %RH) in [69] between various institutes measured results. Therefore, the MBV measured and calculated in this research is comparable with other conventional building materials on the market. The results have shown that mycelium composite is a good material to regulate indoor air humidity, as [68], [69] stated that most materials studied were ranged in between 0.5-1.2 (g/m^2 %RH), and mycelium composite obtained $MBV_{practical}$ of 1.6 (g/m^2 %RH) (Table 19).

Table 19 Mycelium composites, gypsum and EPS MBVs were compared with other building materials measured with the NORDTEST method.

Sample Type	**Mean MBV$_{practical}$**	**S.D**	**References**
Mycelium composites	1.632	0.024	This research
Gypsum	0.547	0.005	This research
EPS	0.002	0.000	This research
Gypsum	0.57	0.01	[68] (LTH)
Concrete	0.37	0.04	[68] (LTH)
Cellular concrete	0.96	0.06	[68] (LTH)
LW aggregate concrete with stucco	0.72	0.08	[68] (LTH)
Brick	0.35	0.02	[68] (LTH)
Birch panels	0.61	0.05	[68] (LTH)
Fiberboard	2.4	0.47	[37]
Cellulose	3.1	-	[80]
Gypsum	1.1	-	[80]

Note:

[68] LTH = Lund University, Sweden. Gypsum studied in this research had the same dimension as in LTH for reference; thus, materials MBVs were chosen to compare with LTH measured values instead of other institutes.

[80] This study had a significantly higher MBV of gypsum (2-fold) than other studies.

The measured and calculated MBV of MBC showed the good potential of this material to be applied as an internal wall assembly layer, which can regulate indoor RH to maintain thermal comfort and reduce ventilation loads. It was anticipated that MBC had good MBV than other common porous building materials due to its high hygroscopic and porosity. Porosity, which is the only physical material intrinsic properties (i.e. thickness, density, porosity), has been confirmed by statistical analysis with a positive correlation with MBV [37].

Furthermore, similar to other plant-based insulation materials, the water wicking properties of MBC prevent the chances of accumulated condensation at material interfaces. This means the high hygroscopicity of MBC can be overlooked when considering to be applied as an insulation layer in a complete envelope system since building envelope design constitutes various materials that serve specific functional layers within assemblies. In addition, it is recommended to study MBC in combination with other assembly layers to understand hygrothermal performance as a whole system.

5. Conclusions

This research studied the feasibility of MBC material to apply as foam-like wall insulation material. The related results regarding each research question were summarized below:

- 100% RPS substrate performed the best in thermal conductivity with the lowest density and good dimension stability. The compressive strength of 100% RPS was compatible with EPS.

- A prolonged growing period resulted in a denser mycelium outer layer in MBC, which rendered 25% less relatively water absorption than NG samples, due to the hydrophobicity of mycelium. Other physical and mechanical properties (i.e. thermal conductivity, flexural and compressive strength) of MBC are highly dependent on substrate choices than growing time, which agreed with the literature.

- MBC with DWC test showed insignificant differences in thermal conductivity and a slight decrease in compressive strength when compared with MBC without DWC. This indicated that MBC can maintain good functional performance after the accelerated aging test.

- The high MBV of MBC (1.6 g/m^2 %RH) suggested a good potential to apply as internal building material to regulate RH and maintain adequate thermal comfort.

Above all, the results and outcomes of this research depicted that MBC performed well as internal wall insulation material and was compatible with petroleum-derived synthetic foams (i.e. XPS and EPS). Remarkably, this research was the first study to investigate the influences of the accelerated aging test on MBC and moisture buffer capacity of MBC. This finding reinforced the possibility of applying MBC as an internal wall insulation layer, with additional properties to regulate indoor air RH and the potential to reduce operational energy cost.

Future work & recommendations

A scientific approach to stimulate more mycelium growth in the exponential growth phase in a shorter period is needed in future research. The intrinsic complexity biomechanism between substrates and fungi induced difficulties to purify the influences of a single parameter on MBC. Therefore, further investigation is needed to confirm optimal growing conditions and mixing proportions for mycelium to grow on both substrates prior to the mixtures and molding process. Furthermore, future research should consider a reference group of each substrate without fungi when conducting thermal conductivity and compressive strength tests to clearly distinguish the influence of substrate or fungi. In future work, it is recommended to have statistical analysis in significance level due to high heterogenicity in samples and different parameters involved.

Notably, applying a DWC test on MBC had never been done in other studies. In future research, it will be more convenient to compare before and after DWC on the same batches of samples, so that the density and compressive strength performance changes can be easily monitored and discussed. For instance, produce a sample with a larger size and cut into smaller pieces to compare in an accelerated aging test (i.e. DWC) instead of testing on different sample batches. Moreover, FT-IR and SEM should be used to analyze chemical compositions and surface morphology differences between DWC and without DWC samples, which was absent in this research. An additional test that can quantify the hydrophobin genes is also recommended to strengthen the hypothesis of a thicker and denser mycelium in an outer layer results in better water resistance. Other durability tests in future studies can also provide more insights about MBC, such as long duration in a climate chamber and weathering tests.

As for the NORDTEST MBV measurement, NG versus PG should be conducted in the future to examine a prolonged growing period in the moisture buffer capacity of MBC. Moreover, $MBV_{practical}$ can be listed as one of the material characterizations to be studied in future research (e.g. influences of substrates, growing period, fabrication process, etc.). Additionally, more insights into MBC regarding its hygrothermal behavior can be studied in combination with other construction layers (i.e. timber frame, lightweight concrete, etc.) for understanding the intra-and inter-relations between different layers.

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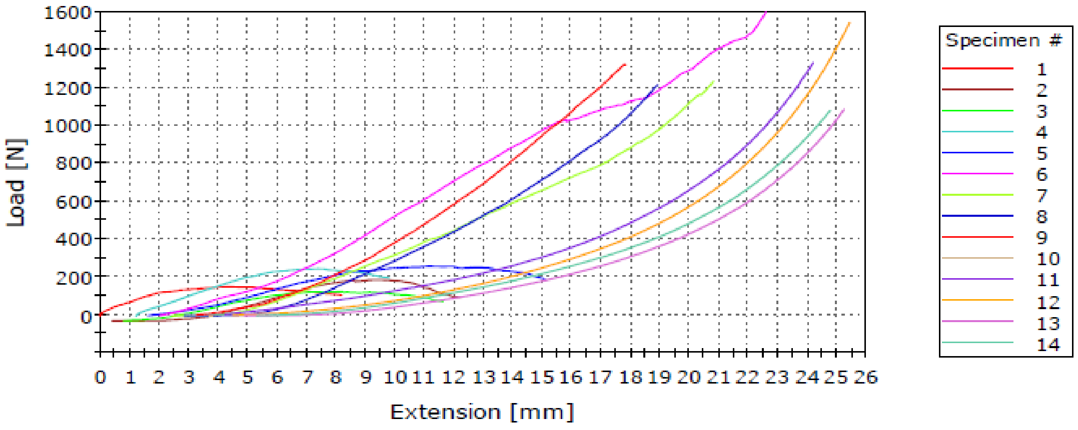
Appendices:

Appendix A- Compressive Strength Test Raw Data

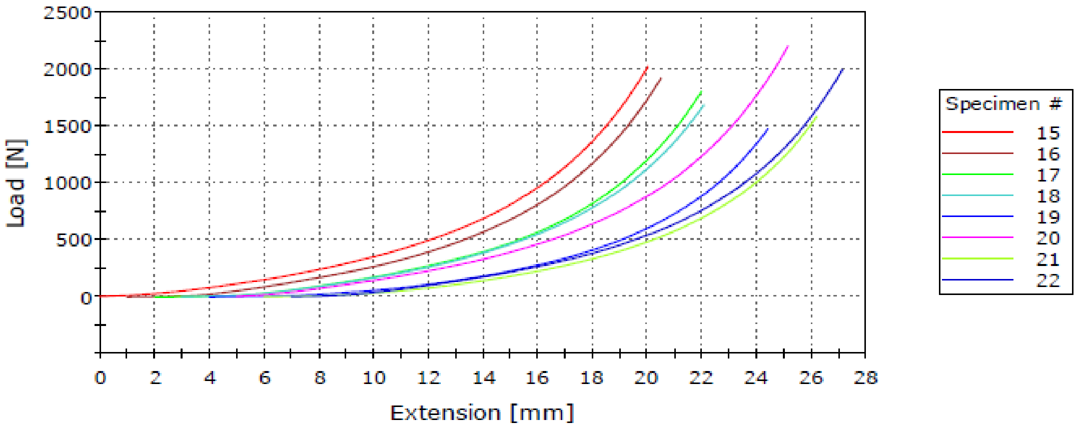
Substrate Mixtures Refinement:

	Compressive stress at Maximum Load [MPa]	Specimen label
1	0.12362	100% cel
2	0.15389	100% cel
3	0.10226	100% cel
4	0.20084	100% cel
5	0.21121	100% cel
6	1.30168	75 cel/25 rps
7	1.00411	75 cel/25 rps
8	0.98910	75 cel/25 rps
9	1.07884	75 cel/25 rps
10	-0.00045	
11	1.08527	100% RPS
12	1.25550	100% RPS
13	0.88330	100% RPS
14	0.87904	100% RPS
15	1.64659	50/50
16	1.56267	50/50
17	1.46727	50/50
18	1.37002	50/50
19	1.20137	25 cel/75 rps
20	1.79230	25 cel/75 rps
21	1.28868	25 cel/75 rps
22	1.62318	25 cel/75 rps

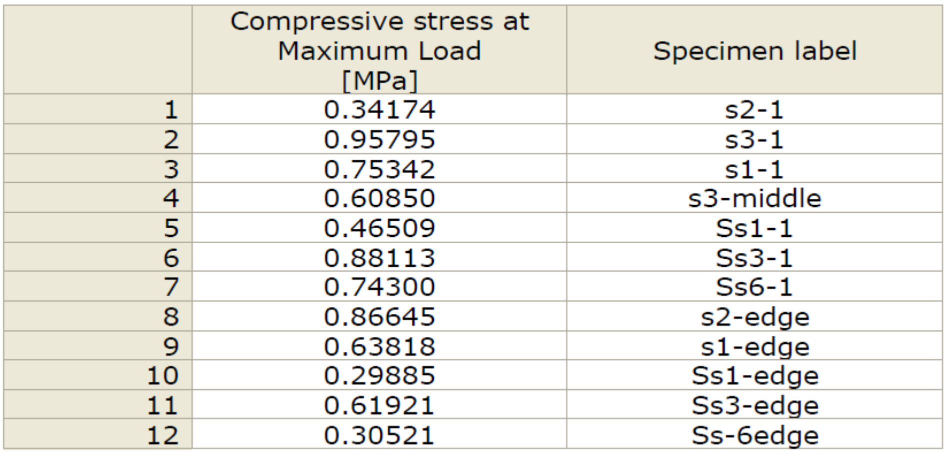
Specimen 1 to 14



Specimen 15 to 22

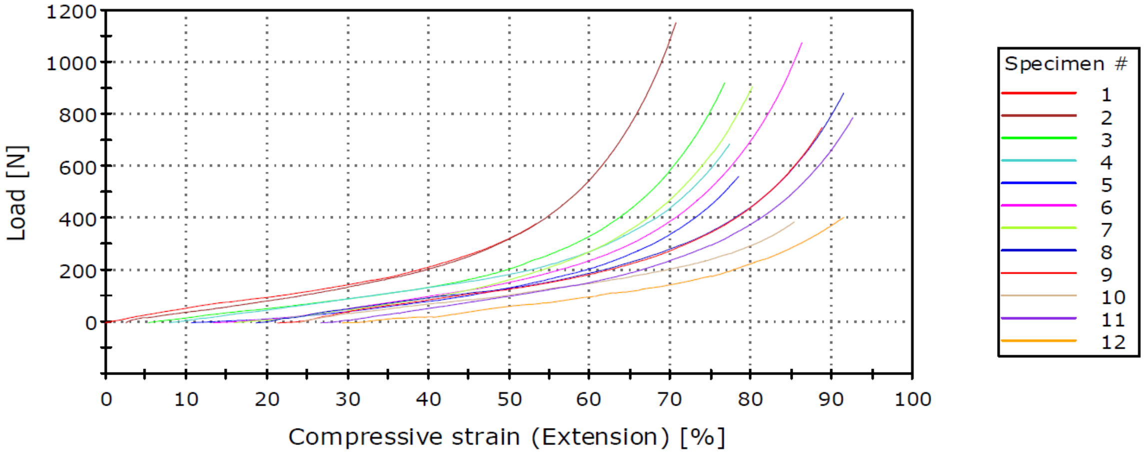


100% RPS Without Drying and Wetting Cycles:



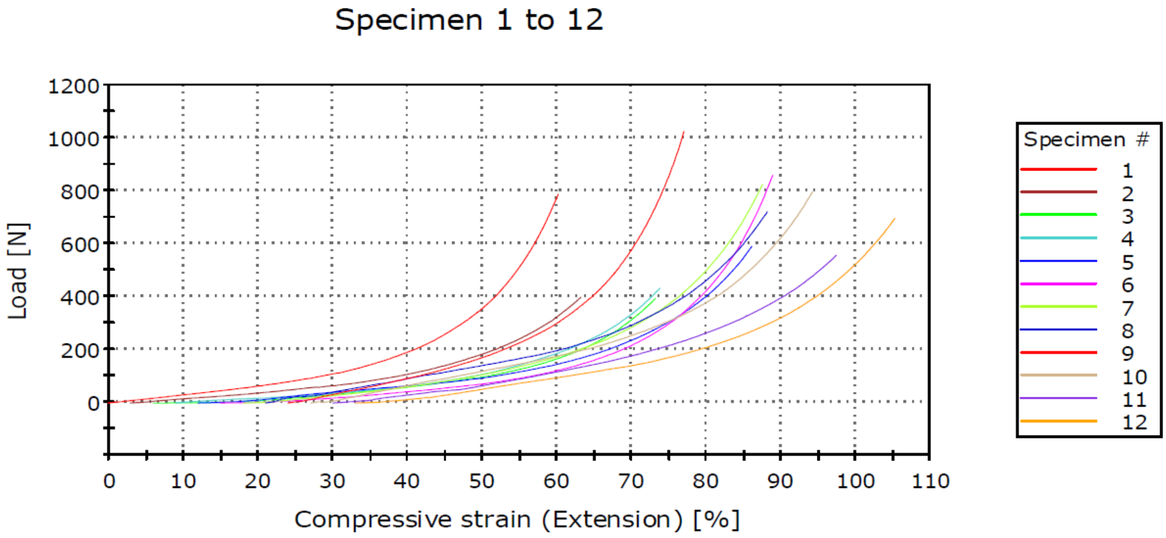
	Compressive stress at Maximum Load [MPa]	Specimen label
1	0.34174	s2-1
2	0.95795	s3-1
3	0.75342	s1-1
4	0.60850	s3-middle
5	0.46509	Ss1-1
6	0.88113	Ss3-1
7	0.74300	Ss6-1
8	0.86645	s2-edge
9	0.63818	s1-edge
10	0.29885	Ss1-edge
11	0.61921	Ss3-edge
12	0.30521	Ss-6edge

Specimen 1 to 12



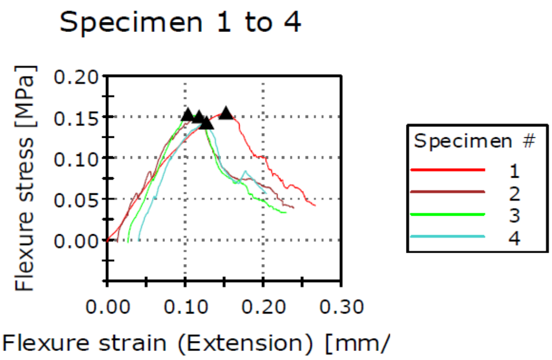
__100% RPS With Drying and Wetting Cycles:__

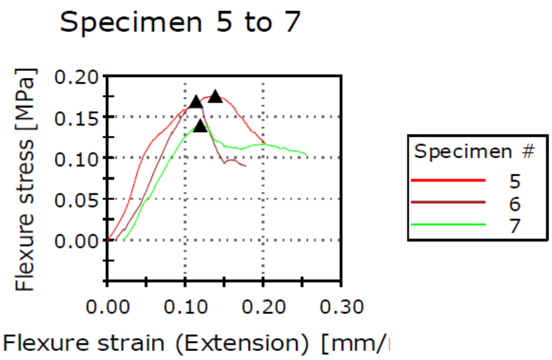
	Compressive stress at Maximum Load [MPa]	Specimen label
1	0.53550	LS9middle
2	0.29968	LS9edge
3	0.32715	LS8middle
4	0.38378	LS8edge
5	0.48913	LS7edge
6	0.73080	LS7middle
7	0.75293	S2middle
8	0.58585	s2edge
9	0.90543	S4middle
10	0.68098	s4edge
11	0.43249	s5edge
12	0.65329	s5middle



Appendix B- Flexural Strength Test Raw Data (NG vs PG)

	Load at Tensile strength [N]	Specimen label	Flexure stress at Tensile strength [MPa]
1	38.18437	testing	-----
2	24.25044	prolong s9	-----
3	28.74660	prolong s8	0.15178
4	27.07343	prolong s7	0.13870
5	40.97695	normal s4	0.17512
6	37.38408	normal s5	0.16571
7	31.84987	normal s6	0.13969





Appendix C- MBV Raw Data Sheet Sample

Cycle: Measured Date: Begin Time: Humidity: Note

Sample ID								
Initial Weight								
Weight								

Cycle: Measured Date: Begin Time: Humidity: Note

Sample ID								
Weight								

Cycle: Measured Date: Begin Time: Humidity: Note

Sample ID								
Weight								

Cycle: Measured Date: Begin Time: Humidity: Note

Sample ID								
Weight								

Cycle: Last Measured Date: Begin Time: Humidity: Note

Sample ID								
Weight 1								
2								
3								
4								
5								

Abstract

Nowadays circular economy and sustainability aspects of materials are taking huge roles in consumer decisions. Wall insulation materials are usually synthetic or petroleum-derived materials, which are less environmentally friendly in the overall material life cycle. Mycelium -based composites (MBC), on the other hand, utilize fungal mycelium, an interwoven network of hyphae to bind with lignocellulosic substrates and produce composites with high porosity. The main components of mycelium are natural polymers; thus, it is a biocomposite and completely biodegradable at the end-of-life cycle. Furthermore, MBC can also upcycle agricultural by-products. White-rot fungi have superior traits to decay and obtain nutrients from any lignocellulosic materials, including low-nutrients agricultural by-products. In addition, mycelium composites can be alternative sustainable materials to replace petroleum-derived foams in the current conventional insulation market. Utilizing agriculture residues to create sustainable biocomposites in the building industry that meets the ultimate goal of mitigating natural resources exploitation and reducing energy and water usage in material production.

This research aims to study the feasibility of MBC as foam-like wall insulation material by conducting experiments related to material characterizations and applying an accelerated aging test on MBC. The results showed that a prolonged growing period arose a denser mycelium outer layer in MBC, which rendered better water resistance due to the hydrophobicity of mycelium. Thermal conductivity and mechanical properties are highly dependent on substrate choices than other parameters of MBC, which coincided with literature. Additionally, influences of accelerated aging test and moisture buffer capacity of MBC were first studied in this research. The results indicated that MBC not only maintained good functional performance after the accelerated aging test (i.e. drying and wetting cycles) but also constituted good moisture buffer capacity. This means that MBC has key material essences to apply as internal wall insulation material and become one of the layers in vapor-permeable building envelope systems to passively regulate indoor relative humidity and thermal comfort.