Discover Materials

Review

A review of recent advances in fungal mycelium based composites

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Abstract
The increasing global population and rapid urbanization have led to high consumer demand for construction and other raw materials. Manufacturing of synthetic material usually generate a large amount of waste, resulting in significant environmental impact. Fungi are one of the key biological resources that can be used to develop a wide range of sustainable products including biodegradable materials with promising applications, with zero waste generation during the production process. Mycelium, the vegetative part of a fungus can be shaped either into pure mycelium materials or composites. Mycelium can grow its network in lignocellulosic material, combining separate pieces into a solid material which results in Mycelium-Based Composites (MBCs). The attributes of MBCs are influenced by the fungal species, the growth substrate, and the processing conditions. Both pure mycelium materials and MBCs have remarkable advantages as versatile materials because they are porous, elastic, low-density, low-cost and eco-friendly materials with potential applications in various industries. In this review, we provide an overview of the latest developments MBCs considering the possibility of using mycelium for the material-driven design (MDD) approach, and the potential of genetic and biochemical modifications to enhance mycelium properties. We therefore encourage researchers in material science and fungal biotechnology to strengthen their collaborative efforts and address the current challenges in this innovative field.

Keywords Fungal bioresources · Genetic modification · Material driven design · Mycelium based composites · Waste management

1 Introduction

The global population growth has resulted in an extra burden on the natural environment due to excessive waste generation and the rapid depletion of natural resources [1]. According to the 2019 World Bank report, the amount of solid waste generated worldwide is projected to increase by 70% between 2016 and 2050, from 2.01 billion tons to 3.40 billion tons per year [2]. The improper disposal of waste generated from several sources such as the construction sector, commercial centers, agriculture, domestic, and other industries has resulted in the pollution of fertile soil, water bodies,

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and air [1]. However, well-planned waste recycling procedures provide sustainable solutions to minimize detrimental effects on the environment [3, 4].

The requirement for construction and other raw materials has also increased with the increasing population globally. The manufacturing procedures for conventional building materials such as steel, concrete, and synthetic materials consume substantial energy, potentially resulting in significant environmental pollution [3, 5]. Additionally, the rapid increase in the global human population leads to increased utilization of agricultural products, which generates agricultural waste or by-products such as rice husks, rice/wheat/corn straw, cotton stalks, and coconut husks [6, 7]. The majority of these residual materials are commonly discarded or incinerated, releasing carbon dioxide and other toxic gases, along with particulate matter, into the atmosphere. They are partly used as material for composting, animal feed, animal bedding, insulation materials, and filling for road construction [4, 8–10].

In recent years, fungal mycelium has gained more attention in both fundamental and applied research as well as in innovative commercial platforms, due to its broad application potential, zero waste, and relatively less energy consumption for sterilization during the production process [11, 12, 33, 34]. Mycelium is comprised of a complex network of small white fibers with a diameter range of approximately 1–30 µm (Fig. 1). The filaments of mycelium are composed of multiple layers, which exhibit variations in protein, glucan, mannan, and chitin content [6, 11, 35].

In a mycelium-based composite, a substrate of organic matter provides nutrients that facilitate the growth of the mycelial network. These organic materials in the substrate are available as the remains of dead matter of plants and animals, as well as their waste products. They are composed of cellulose, hemicellulose, tannin, and lignin, as well as proteins, lipids, and other carbohydrates [36–38]. Mycelium can act as a natural binder, adhering to many organic substrates in its vicinity such as coffee husk, sawdust, straw, wheat bran, and bagasse forming a dense network of threads [13–15, 39, 40]. Current research has emphasized the significant potential of utilizing mycelium-based composites as a substitute for traditional materials across diverse applications [16–18]. Since these biocomposites are grown from living material rather than synthesized via chemical reactions, these materials are generally considered

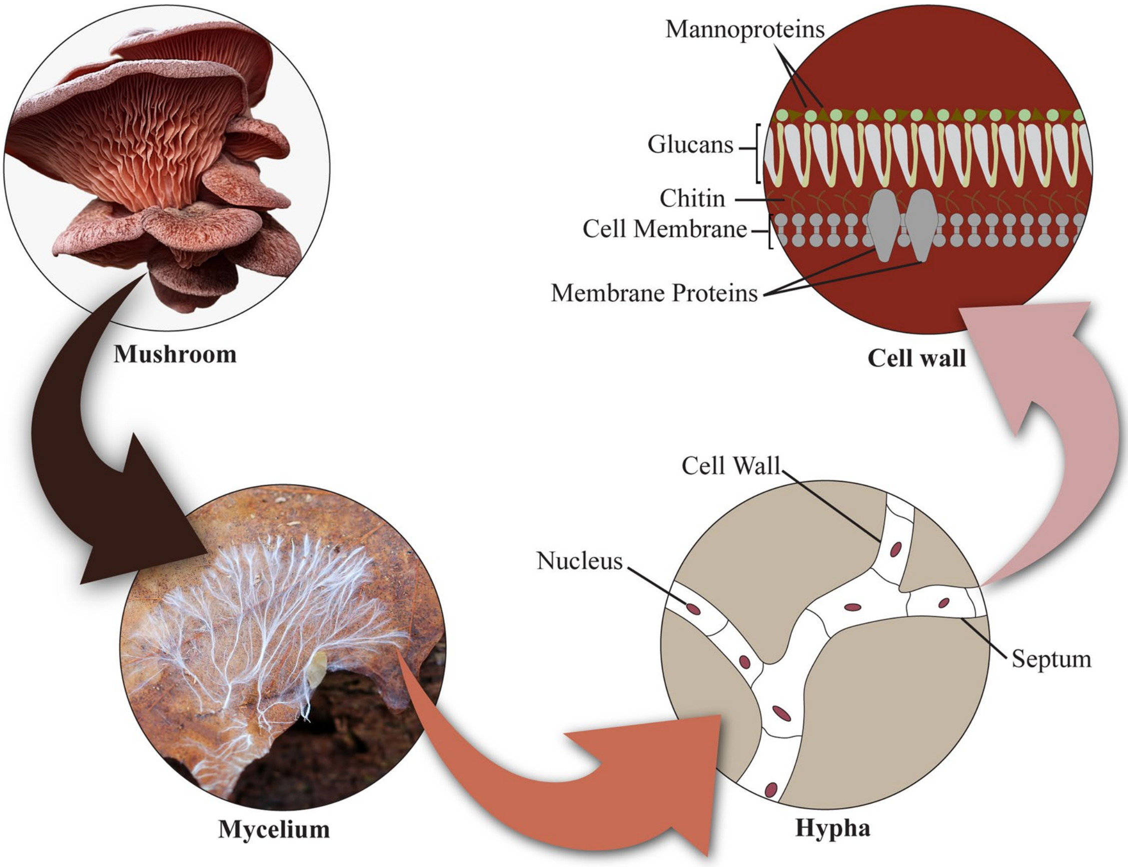

Fig. 1 Representation of mycelium, hyphae and fungal cell wall—Filamentous mycelium which makes the mushroom forming fungi are made up of multicellular structures of hyphae. The hyphae primarily made up of chitin cell wall and membrane proteins

low-cost and environmentally friendly [4]. Furthermore, the ability to use many fungal strains expands the spectrum of properties covered by mycelium-based materials, from packaging applications, structural to mechanical under the specified conditions [19, 41, 42].

Specific structures and material functions of MBCs can be reached by controlling the types of substrates, fungal species, and processing methods [4, 15, 20, 43, 44]. For example, incorporating glass fines and rice husks into the substrate can substantially enhance the fireproofing of the mycelial-based composites. This is due to the ability of rice husk and glass fines to tolerate high temperatures in fire releasing a lot of silica ash and char [21]. The MBCs have been effectively used in the manufacture of products of both low value, such as packaging and gap filling, as well as high-value products intended for industrial use [15, 22, 23]. Mycelium-based foams (MBFs) and mycelium-based sandwich composites (MBSCs) represent the primary categories of composite materials derived from mycelium (Fig. 2) [9]. The MBFs are produced by cultivating fungi on small portions of agricultural waste, while MBSCs are manufactured by incorporating natural fiber fabrics (e.g. hemp, cellulose, wood) as outer layers surrounding a central core [4, 24].

The most challenging task in the fungal biocomposite production process is the selection of species or strains [25, 26]. Several criteria must be considered when selecting a suitable species of fungi, carefully considering the growth rate, mycelium density, ease of cultivation, noxiousness level, cost of growth media, and mycelium structure [27, 45–47]. Most researchers have chosen Basidiomycetous fungi for biocomposites production due to their ability to degrade lignocellulose and their natural adhesive properties. The presence of septa and anastomosis is the other important feature to select this phylum for biocomposite production [8, 48, 49]. *Ganoderma lucidum* and *Pleurotus ostreatus* are the most prevalent and commonly used fungal species within the aforementioned phylum for the production of MBCs [27–29, 50]. The microfungi belong in Ascomycota are have been used rarely to produce MBCs and found to be less successful due poor binding properties [28, 50].

Fungal mycelium has recently gained attention as a sustainable alternative to traditional materials due to its unique properties and ability to grow into complex, biodegradable structures. This article offers a comprehensive reappraisal of the most recent progress in the production of mycelium-based materials and their applications in various fields. The use of mycelium for material-driven design (MDD) is also discussed, providing how the unique properties of mycelium can be used to create innovative materials with specific properties. Furthermore, we discuss the possibility of genetic alterations to enhance the characteristics of mycelium leading to more innovations in both biotechnology and materials engineering. Overall, this review offers a thorough analysis of the most recent developments in the production of beneficial sustainable materials using fungal mycelium, leading to innovative applications of bioresources in the future.

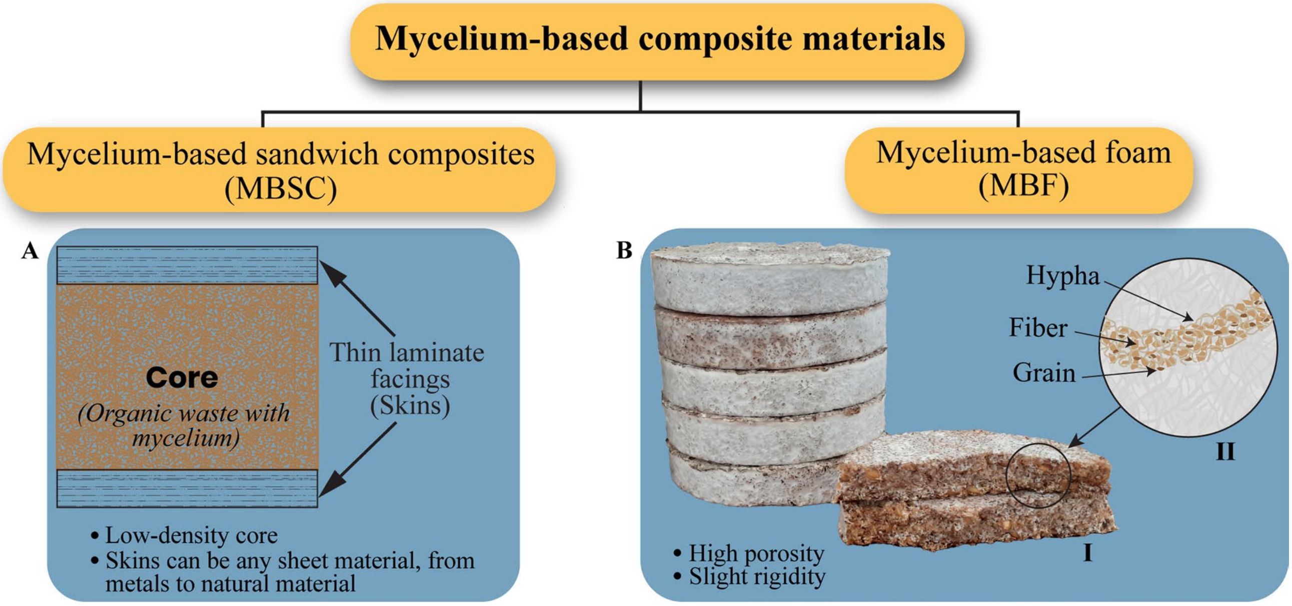

Fig. 2 **A.** Schematic diagram of a cross-section of biocomposites sandwich structures, **B**. Mycelium biocomposites: (I) Cross-sectional view of biocomposites, (II) Schematic diagram of a cross-section of biocomposites

2 Mycelium for Material Driven Design (MDD) approach

For decades, the term "material" has been at the forefront of product design research and practice agendas [4, 51]. A majority of important studies centered on how to aid designers to select appropriate materials within the constraints of the manufacturing process and/or the required shapes [27, 51]. Researchers have recently become more interested in a newly established research path that examines materials' active role in molding desired shapes [51–53]. Biomaterial studies are continually evolving to provide better alternatives to conventional materials, such as bio-based, recycled, recyclable, or smart materials.

The goal of the MDD approach is to aid in the design process when the desired subject material serves as the starting point [51]. Therefore, the experience of designing materials has been made easier with the MDD technique. The MDD technique outlines four primary action phases in sequential order (Fig. 3): "(1) understanding the material, (2) creating materials experience vision, (3) manifesting materials experience patterns, and (4) designing material/product concepts. The procedure begins with a material (or a material proposal) and concludes with a finished product or further developed material. The technique emphasizes a designer's journey from the tangible to the abstract, and then back to the tangible" [13]. The MDD process has been used to design with several raw-materials, including coffee waste, 3D printed textiles, plastic waste, electroluminescent materials, recycled textiles, and mycelium-based materials [13, 52].

Mycelium-based products can be generated in two ways: (1) by utilizing mycelium's ability to interlock with the different substrates within its network to produce a bulk material (MBCs), or (2) by collecting a liquid culture of mycelium (pure mycelium material) [37]. Therefore, the MDD process can be used to fabricate many different innovative mycelium based products as composites or pure mycelium as the starting point for the fabrication.

2.1 Understanding mycelium-based materials

Material science and engineering have traditionally focused on the links between material structures and properties, as well as the influence of processing conditions on material performance [13]. The way in which mycelium is cultivated has a significant effect on the properties of mycelium-based materials, both in terms of technical performance and experimentation outcomes. Moisture and temperature have a significant impact on the final product [24]. The material tests have been primarily concerned with the various substrates used to grow mycelium, such as agricultural and landscape waste, byproducts of food processing, and industrial waste generated from paper and fabric manufacturing [6, 13, 54]. Studies indicate that certain fungal species and substrates are not conducive to mycelium growth, and that the rate and density of growth may vary [46, 100] (Fig. 3A).

2.2 Creating material experience vision by characterization

The material experience can be visualized by technical characterization, processing method, and based on the comparison with the reference materials. Different tests can be performed to technically characterize the mycelium-based materials, including thermal conductivity, water resistance, flammability, mechanical strength, density, and the capability of the material for laser cutting. In order to enable comprehensive material characterization studies, researchers have identified two variables, strain structure (e.g. grain versus fiber) and compression of the grown material (e.g. hot and cold compressed versus uncompressed), that can be manipulated to produce diverse samples with distinct experiential qualities and technical properties [4]. The produced material can be categorized as mycelium foams and sheets. Even when the mycelium-based composites are manufactured from the same ingredients, the processing method (e.g. cold pressing or heat pressing) results in differences that allowed them to be classified into separate material groups [6]. Foam-like samples are more difficult to categorize than sheet materials since the visual qualities of the material were regarded as natural, while the way it behaved when gripped and pressed was linked with synthetic foam materials [13]. Thus, reference materials that can be compared include medium-density fiberboard (MDF), sawdust, wood, and synthetic polymer materials like Styrofoam (Fig. 3B).

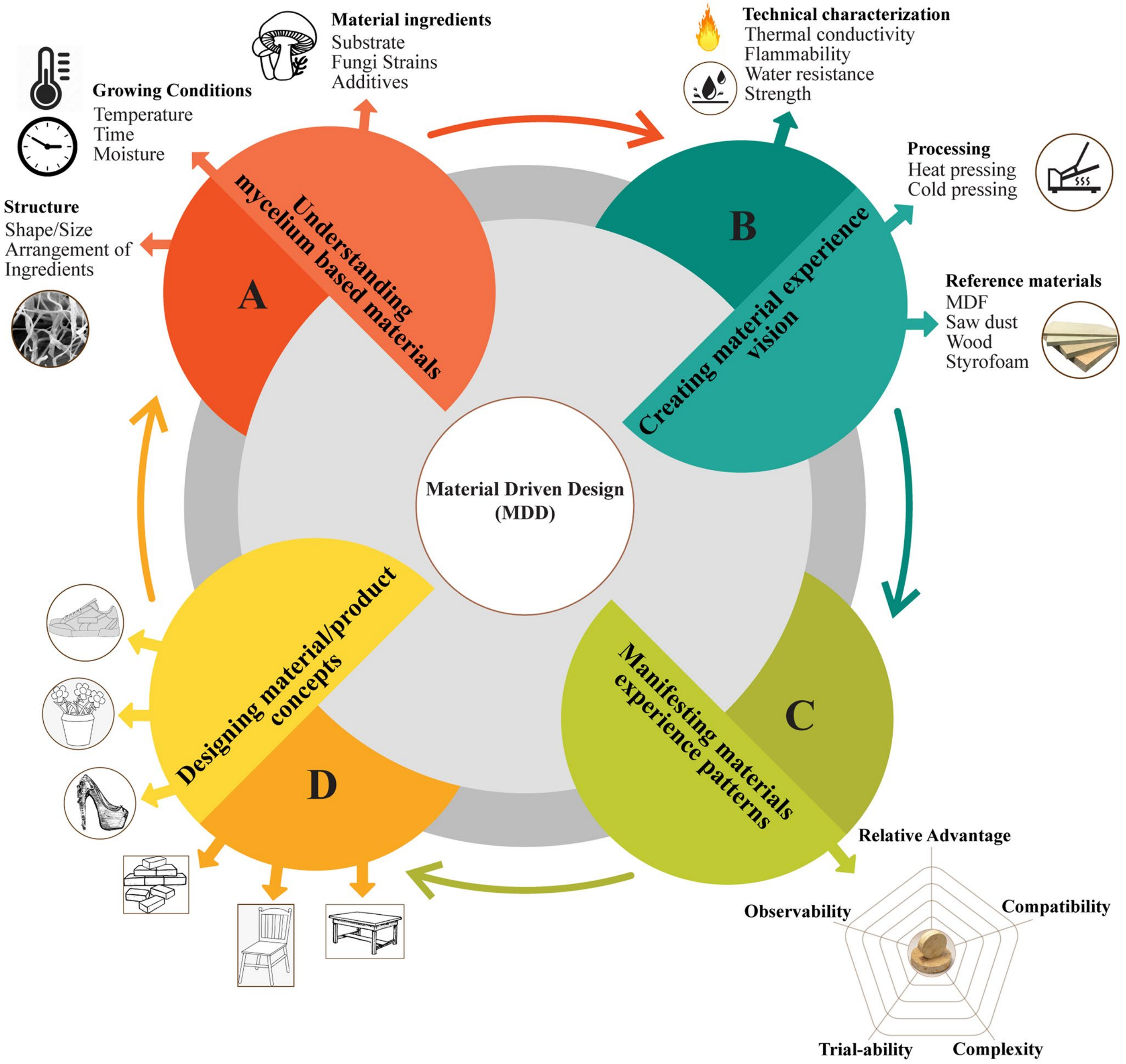

Fig. 3 Material driven design method as applied to MBCs. The MDD method consists of four primary action phases in an order of (**A**) understanding the material, (**B**) creating materials experience vision, (**C**) manifesting materials experience patterns, and (**D**) designing material/product concepts

2.3 Manifesting material experience patterns

At this point in the design process, it is necessary for the designer to improve the sensory attributes of the product, consider the visual properties of the materials used, and use common material experience patterns among individuals in the intended setting to convey the features of the product and the materials, such as their organic shape or level of transparency [13]. The theory of diffusion of innovations described by Rogers et al. [55] which elucidates the process through which new concepts, products, or technologies are embraced and disseminated among society, can be utilized to develop a tentative model for the acceptance of a material, as it [6]. An acceptance prediction spider chart is used to analyze the concepts. The acceptance prediction spider chart, took into account five perceived characteristics, namely relative advantage, compatibility, complexity, trial-ability, and observability [13]. The information gathered in this step can be organized and visualized through charts or images and also can be summarized based on a few categories, such as users (age, gender, fields, nationality), products (shape, manufacturing process, function), meanings, and materials to be considered when producing mycelium-based composite materials [56]. The materials fully covered with mycelium have an attractive appearance, since the color is revealed to be a critical factor in the perception of the material. The surface treatment must be accounted for because the mycelium-based product's texture plays a vital role when commercializing [13] (Fig. 3C).

2.4 Designing material/product concepts

The identified material experience patterns are applied to the materials in order to make them consistent with the material vision [56]. For example, an experiment conducted by Karana et al. [13] five distinct concepts, namely a plant pot, root, packaging for a coffin, second skin, furniture, and footwear, were developed using mycelium. They used the acceptance prediction spider chart described above to analyze the concepts. Upon evaluating the five concepts, the second skin was chosen as the innovative packaging for wine bottles. This design involves growing a mycelium-based substance around the bottle, conforming precisely to its shape and providing protection. Once the skin is removed, the bottle is revealed and can be used. The product concepts of mycelium-based products can range from packaging, textiles, and furniture to building materials. Therefore, it is possible to create a variety of products that are difficult to forecast using traditional methods with the careful use of the MDD approach. In general, mycelium materials have the potential to replace a range of conventional materials in a number of industries, providing long-term, ecologically advantageous solutions to the pressing environmental issues we currently face (Fig. 3D).

3 Pure mycelium materials

Although the mycelium-based composite materials are produced by colonizing lignocellulosic waste with saprotrophic fungi inside a suitable mold, pure mycelium materials (PMMs) are made entirely of mycelial biomass [25, 30–32]. These PMMs have versatile characters and appear to promise a replacement for myco-leather or present petrochemically manufactured polymeric, or animal-based leather [57].

The biological parameters of the organism and the growing environment completely define the basic qualities of PMMs [34, 58, 59]. By cultivating the fungi separately from its substrate, pure mycelium biomass can be created. This method generates sustainable materials that possess adjustable characteristics, such as foam, paper, leather, and polymer-like properties [59]. The PMMs are usually derived from a liquid mycelium culture [57]. Fungi can ferment in liquid in either static or machine-shaken containers. When grown in a static liquid culture, fungi form a layer of hyphae on the surface of the liquid. When dried, the resulting material has qualities that are similar to leather, paper, or plastic. The final properties of the mycelium-based products, including translucency, color, and stiffness, may differ based on the additives introduced during their growth period (e.g. ethanol or glycerol) [13]. Furthermore, newly introduced fermentation processes are capable of functionalizing these unique materials when integrated with appropriate post-growth treatment [11]. As the range of features of mycelium-based materials continues to expand, a growing number of consumer products from various companies are being introduced. These products include high-performance foams used in garments or skincare products (MycofexTM), leather-like fabrics (MyloTM, ReishiTM, MyleaTM, ForagerTM), and alternative products (AtlastTM) [59, 60]. Due to the adaptability of novel material applications and possible feedstock diversification, PMMs represent an emerging technology.

Fungal materials can be produced through fermentation techniques that employ low-value agricultural by-products as a nutritious medium for promoting mycelium growth. Additionally, co-cultivation with one or more species is possible [60, 61]. The PMMs can be produced utilizing a variety of fermentation processes. Due to the requirement of direct contact between fungi and their food source, the fungi grow closely attached to their substrate. For this reason, liquid fermentation has traditionally been the preferred method for extracting metabolites, enzymes, or mycelial biomass [4, 59, 62].

In addition to liquid fermentation, pure mycelial biomass can also be produced on solid substrates. Recent developments in solid-state fermentation (SSF) have revealed particular cultivation conditions that promote extensive growth of aerial hyphae, as evidenced by research on *Ganoderma* sp. [24]. This hypha is distinguished by its outward development, away from the feeding material and into the air. To obtain optimal growth behavior, temperature (± 30 °C), humidity (40–99%), and CO_2 (50–70 k ppm) must be controlled inside incubation chambers [63]. Commercial mushroom growers prefer using lower concentrations of CO_2 and lower temperatures for the best fruiting yields. These conditions are considered ideal to avoid the mycelium from differentiating into fruiting bodies [4]. In PMMs utilized for textiles and flexible foams, flexibility is preferred over rigidity. Different chemical treatments are used to offer flexibility, durability, and a protective coating to the fungal tissue [34]. PMMs are commonly coated to extend durability and prevent abrasion. Polylactic acid (PLA) can function as an efficient coating material that is easily applicable by dissolving it in water. Subsequently, mycelium absorbs the solution while the water evaporates [59]. A study conducted by [64] found that *Fomitopsis iberica* was the most suitable strain for the development of leather-like materials. They used twenty-one wood decay fungi (WDF) strains based on the homogeneity, color, and consistency of the mycelia to evaluate the major components and texture of the produced materials, as well as the equivalent pure mycelia developed in liquid culture. In addition, the structural composition of mycelium can serve as a viable option for wound care materials and as a biomedical scaffold for cultivating biopsies. The examples given in this section indicate that, over the past 10 years, there has been an increasing tendency toward creating consumer products based on PMMs, opening up new research opportunities.

4 Mycelium-based composites

Many studies have concentrated on biocomposites rather than pure mycelium materials. The rationale behind this is that the MBCs frequently incorporate natural lignocellulosic waste streams. This approach allows for simpler attainment of a feasible material in terms of shape-ability, durability, and cost, as opposed to pure mycelium materials [27, 37, 59]. As alternatives to conventional materials, architects and designers started to use mycelium-based products such as packaging items, kitchen utensils, furniture, biocement, blocks, masonry units, and synthetic leather [18]. A few innovative enterprises in this area include Evocative Design (www.ecovative.com/), MOGU (www.mogu.bio/), MycoTex (www.mycotex.nl/), and MycoWorks (www.mycoworks.com/) started to design mycelium-based composites products in the world [4].

4.1 Mycelium-based composite manufacturing process

The generalized process to produce mycelium-based materials consists of several steps (Fig. 4) [4, 8, 12, 36]. To initiate the development, the substrate must first be sterilized and inoculated with fungal spawn (mycelium). Any cellulose and lignin-rich material (e.g. straw, wood, and hemp) can be used as a substrate.

The substrate should have high cellulose content for two reasons: (1) fungi can grow rapidly in a cellulose-rich environment since it acts as its carbon and energy source, and (2) high cellulose content provides high tensile strength to the protective material [37, 65]. The substrate should provide the necessary nutrients for growth of mycelium,

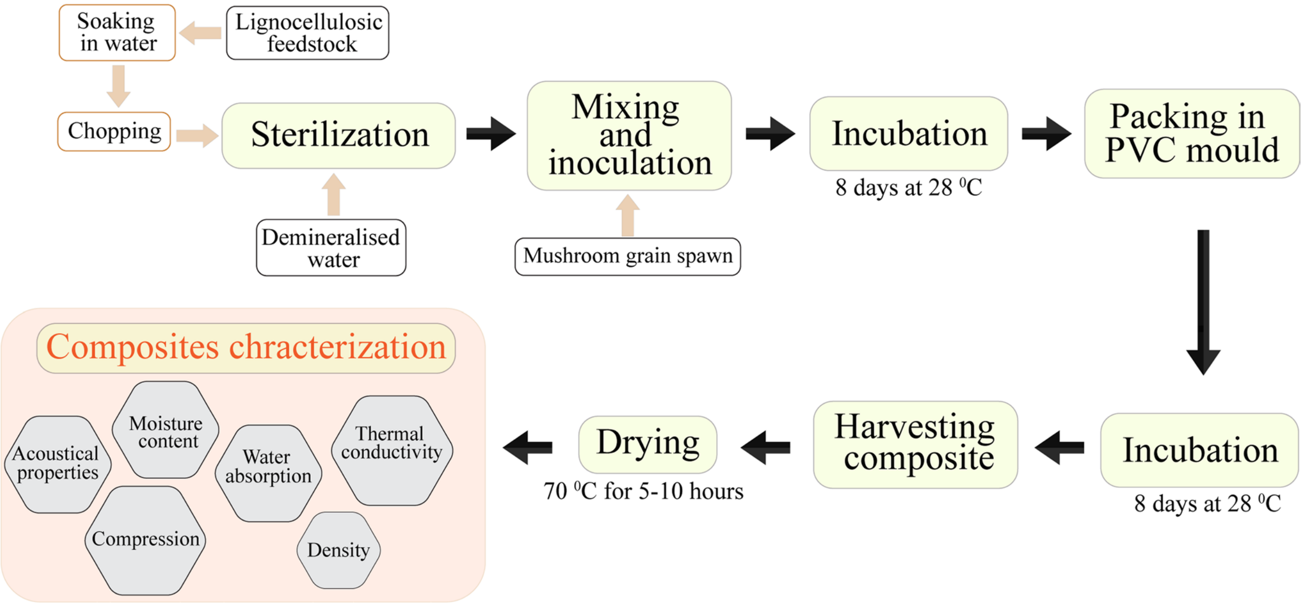

Fig. 4 A generalized process for biocomposites production. The process include main steps of feed sterilization, mixing, inoculation of fungi, incubation, packing in PVC mold, harvesting, drying and characterization for desired properties

including carbon, nitrogen, vitamins, and minerals, along with a suitable amount of water. Mycelium should be grown in the dark to prevent the production of fruiting bodies [8]. The optimum temperature during growth should between 25 and 30 ℃. This temperature can be maintained without high cost for energy especially in tropical countries where the temperature ranges between 25 and 30 ℃ throughout the year. The moisture content usually needs to be high (60–90%). Relatively, the CO_2 content should be kept high to prevent the growth of mushrooms and to stimulate mycelium growth [1]. The composite is shaped into the appropriate appearance after its initial growth. Before drying, the composite should be grown for an extended period of time. Drying, heat pressing, or cold pressing can be used at last to achieve the desired properties [4].

When the growing process has ceased, the sample can be remolded if necessary. By applying a suitable coating, the quality and durability of the material can be improved [8]. The production process of MBCs has several advantages over the manufacturing of other synthetic composites, including lower production costs, lower energy consumption, and lower density. In addition, MBCs have been shown to have a smaller impact on the environment and produce fewer CO_2 emissions compared to wood composites or bioplastics [13, 66]. Therefore, the establishment of industrial scale production plants for MBCs is an eco-friendly approach for prospective investors. Once the setup of a production plant is established, the cost of continuous production is very low due to low or no energy consumption.

Table 1 Comparison of the physical, mechanical, fire resistance, acoustic absorption, moisture uptake, manufacturing and end of life properties of mycelium composites, wood products (hardwood (HW), softwood (SW), (plywood (PW)) and typical synthetic foams (polystyrene (PS), polyurethane (PU) and phenolic formaldehyde resin (PF)) used in construction

Material property	Mycelium composites	Wood products	Synthetic foams	References
Compressive strength (MPa)	0.17–1.1	SW: ∥ 35–43, ⊥ 3–9 HW: ∥ 68–83, ⊥ 12.7–15.6 PW: 8–25	PS: 0.03–0.69 PU: 0.002–48 PF: 0.2–0.55	[27, 46]
Tensile strength (MPa)	0.03–0.18	SW: ∥ 60–100, ⊥ 3.2–3.9 HW: ∥ 132–162, ⊥ 7.1–8.7 PW: 10–44	PS: 0.15–0.7 PU: 0.08–103 PF: 0.19–0.46	[100, 102]
Density (kg/m^3)	59–552	SW: 440–600 HW: 850–1030 PW: 460–680	PS: 11–50 PU: 30–100 PF: 35–120	[100]
Flexural strength (MPa)	0.05–0.29	SW: 9.9–11.5 HW: 10.3–11.5 PW: 35–78	PS: 0.07–0.70 PU: 0.21–57 PF: 0.38–0.78	[27, 100]
Fire resistance	No silica: low 50 wt% silica: high	SW: low HW: low PW: low	PS: very low PU: very low PF: very high	[46]
Acoustic absorption (NRC)	> 70–75%[b]	SW/HW: 0.05–0.15 PW: 0.1–0.23	PS: 0.2–0.6 PU: 0.2–0.8	[10, 46]
Moisture uptake (wt%)	40–580	SW/HW: 5–190 PW: 5–49	PS: 0.03–9 PU: 0.01–72 PF: 1–15	[27, 100]
Manufacturing process	Fungal growth	SW/HW: milling PW: lathing, pressing, resin infusion	Polymerization and expansion	[5, 100]
Material cost ($US/kg)	0.07–0.17[a]	HW: 3–11 SW: 0.7–1.4 PW: 0.5–1.1	PS: 2.1–2.3 PU: 8.2–10.4 PF: 1.7–1.9	[46, 102]
Biodegradability	All constituents	Wood constituents	None	[46]
Degradation time	Weeks-months	Years-decades	Decades-centuries	[46]
End of life	Garden composting	Recycling, incineration, landfill	Recycling, incineration, landfill	[46, 102]

NRC = noise reduction coefficient, with 0 indicating total reflection and 1 indication total absorption of sound. No noise reduction coefficient is available for mycelium composites. The mechanical properties of wood vary parallel (∥) or perpendicular (⊥) to the wood grain. (a) Cost of raw materials only. (b) Acoustic absorption at 1000 Hz

4.2 Properties of mycelium-based composites

When producing MBCs, several material properties must be addressed including mechanical properties, thermal conductivity, thermal degradation, acoustic absorption, water absorption, fire safety, and termite resistance [46, 101]. The Table 1 presents a critical assessment of MBCs in comparison to synthetic materials such as polystyrene, polyurethane, and phenolic formaldehyde resin, as well as wood products such as plywood, softwood, and hardwood.

MBCs demonstrate thermal conductivities comparable to or lower than those of commonly used thermal insulation materials. Furthermore, MBCs exhibit 70–75% greater acoustic absorption than traditional ceiling tiles, plywood, and polyurethane foams [34]. High moisture absorption and the presence of many open areas inside the material limit their applications and further research is required to address these limitations. However, the increasing popularity of MBCs' material research and commercialization, combined with their beneficial material properties, suggest that they are a cost-effective, efficient, and environmentally sustainable emerging technology that could make a significant contribution to the future of green construction.

5 Mycelium-based nanomaterials

Studies of MBCs focus on utilizing coarse material compositions, including sawdust, rice husks, leaves, bio-waste, and other similar materials. There are very few but remarkable articles available that discuss mycelium-reinforced composites using nanomaterials [67–69]. Nanotechnology has revolutionized the material industry over the last two decades. Recently, several microorganisms have been introduced in the field of nanomaterials for their capacity to produce biopolymers. However, these advance biopolymers are functionally different from regular structural MBCs and usually originates from some Ascomycetes (moulds) or their components.

In the recent past, Bacterial cellulose-based materials have found extensive use in various nanomaterial applications, including drug delivery, wound healing, and bio-sensing [70]. Now, the selection the fungi are more frequent to produce bio-based materials [69, 71]. Trabelsi et al. [69] revealed *Pleurotus ostreatus* grown on polyacrylonitrile (PAN) nanofiber mats producing mycelium/PAN nanocomposites which can be used for many applications such as biotechnology and medicine, water purification and filtration, architecture, and vertical farming.

A study by [72] developed carbon nanotube (CNT) composites using polycyclic aromatic hydrocarbons (PAHs) degrading fungal mycelium (*Penicillium oxalic*) for pyrene removal. PAHs have been known as one of the major environmental pollutants from industrial effluents with a severe threat to human health [73]. While various physical, chemical, and biological methods are available to eliminate PAHs from the environment, the limited bioavailability and slow removal rate of these compounds often hinder the widespread use of biodegradation techniques. According to the study conducted by [72] the CNT composite was able to entirely remove pyrene, present at 20 mg L^{-1}, within a duration of 48 h.

A new bioceramic hybrid was developed by [74] to enhance water treatment. The researchers achieved this by functionalizing fungal mycelia of *Aspergillus fumigatus* with halloysite nanotubes to regulate hyphal dispersal and sorption behavior. Their findings show that environmentally friendly ceramic materials (halloysites) could be employed as a functional surface doping agent to improve filamentous fungi's water treatment capability.

A recent study suggests that proposed a solution for producing coated bean seeds using nanofibers of polyethylene oxide (PEO) as a transport system for arbuscular mycorrhizal fungi (AMF) [75]. The researchers determined that PEO nanofibers do not negatively impact the infectivity of AMF. Since this method is an easy-to-apply and economically viable technology, it can be used as a possible option for the seed coating material, for the application of AMF inoculants in agriculture, ecological remediation, forestry, horticulture, and other related fields. Jones et al. [67] carried out a study to manufacture nano-papers from mycelium-derived chitin-chitosan or chitin-glucan that demonstrated superior mechanical properties to existing mycelium materials. Due to the existence of lipid residues in the nano-papers, the mycelium-derived nano-papers had a higher hydrophobicity level than pure chitin and other natural polysaccharides, such as starch and cellulose. To eliminate these contaminants, HCl (hydrochloric acid) or H_2O_2 (hydrogen peroxide) treatments were applied, allowing the properties related to mechanics, thermal, and surface of the nano-papers to be fine-tuned. The variations in surface morphology, wettability, and mechanical performance highlight the versatility of these cost-effective and environmentally friendly materials, indicating their potential suitability for various applications, including paper, packaging, coatings, and membranes.

Several fungi (e.g. *Phanerochaeete chrysosporium* to synthesize Cds quantum dots) are also used as a base material in the production of nanomaterials for a variety of applications such as packaging, biomedical, cosmetics, construction, and designing [76]. Fungi-mediated nanoparticles are generated by introducing metal precursors into the fermentation culture, which are subsequently assimilated by the mycelium biomass and transformed into the intended nanoparticles [34]. Despite the potential of nanomaterials in the production of mycelium composites, research in this area remains relatively constrained. There is a huge potential to incorporate nano-based MBCs and pure mycelium materials to improve the properties of mycelium-based composites.

6 Diverse applications of fungal biomaterials and trends in innovative business startups.

The bio-based economy is a sustainable approach that replaces traditional fossil-based resources and production methods with renewable biomass to produce the materials necessary for our daily consumption [71, 77, 78]. The recent trend in fungal biotechnology will facilitate the development of biomaterials derived from fungi [71]. Fungal enzymes, as well as fungal bioactive compounds, are widely employed in the biofuels, food, and detergent sectors, and fungal bioactive compounds are used in human and veterinary medicine [79–81]. The metabolic products of fungal species are of interest to researchers and industry, and the mycelium structure of fungi is also attracting new attention [82]. Considering applied or granted patents on fungal biomaterials, most patents are held by companies based in the United States, Europe, and China [12]. Ecovative Design LLC currently holds the largest percentage, 45%, of available patents related to the field (Table 2). Other companies with notable patent shares include Ford Global Tech (19%), Shenzhen Zeqingyuan Tech Dev Service Co Ltd (17%), and MycoWorks Inc (6%) [71]. The recent applications in protective packaging, automotive supplementary material, electrical circuit boards, and construction material are considered the most recent trend of applications of fungal biomaterials [15].

6.1 Protective packaging applications

Green biocomposites, which are made with 100% bio-based materials, could be a sustainable replacement for petroleum-based synthetic packaging in a variety of applications. Mycelium has the ability to grow in the shape of a mold, which makes it a suitable option for creating diverse protective packaging materials. Moreover, by controlling growing conditions and selecting the substrate, it is possible to produce mycelium materials with specific structural properties such as strength and density [34].

In 2017, the company Shenzhen Teq Development (Patent no CN106633990) produced a protective packaging material based on mycelium using corn stalks as the main substrate [71]. The product has many benefits including being low in weight and biodegradable. In 2015, the Ecovative company designed a packaging material with an orange-red colour by utilizing a strain of *Pycnoporus cinnabarinus*. The coloration was achieved without the use of artificial pigments. In addition to its bright appearance, this naturally occurring pigment has high buoyancy, making it a potential candidate for the production of sea buoys [71].

6.2 Automotive industry

Several patents have been granted for the fabrication of MBC materials in the automobile industry to use as an alternative material [54]. Ford Automotive Components Holdings LLC developed a specialized method for an injection molding process in 2012 (US Patent US8313939B2). In this method, a liquid mushroom mixture is injected into a mold and then heated. The finished casting is used for creating tubular structures or vehicle interiors. The fungal biomaterial provides parts fit for use in vehicles both aesthetically and structurally, cutting down costs by eliminating manufacturing steps and removing the need for adhesive [71]. Fungal-based biomaterials possess sound-absorbing and insulating properties, owing to their low heat conductivity. Consequently, biocomposites derived from mycelium can serve as a substitute for synthetic foams commonly utilized in various parts of automobiles such as roofs, doors, engines, bumpers, cavities, seats, and dashboards. The mycelium-based materials exhibit similar or superior characteristics in terms of absorbing sound, mitigating impacts, insulating, and offering lightweight construction as compared to traditional synthetic foams. Additionally, these biocomposites provide better fire resistance as an added advantage to the automotive industry [18].

Table 2 Examples of granted patents published between 2016 - 2022 on usage of fungal material

Applicant	Year	Publication number and country	Details
Ecovative LLC.	2018	US10144149-USA	The production method for stiff mycelium bound parts for furniture and fixations
	2018	US9914906-USA	Production method for dehydrated mycelium elements
	2018	US9879219-USA	Production method for dehydrated mycelium elements
	2018	US10154627-USA	Production method for dehydrated mycelium elements
	2017	US9714180-USA	The production method for an absorbing and remediating composite material for contaminants
	2017	US9803171-USA	Production method for dehydrated mycelium elements
Earthform inc	2022	KR102463058-South Korea	The manufacturing method of biodegradable eco-friendly packaging material using mushroom mycelium and beer hop residue and biodegradable eco-friendly packaging material manufactured thereby
LANG LASER—System	2022	DE 102021107059-Germany	Process for the production of compostable packaging and/or insulating material
Mycoworks	2018	US9951307-USA	The production method for dehydrated mycelium elements for building or construction
	2016	US9410116-USA	Production method for dehydrated mycelium elements
University of Alaska Anchorage	2020	US10604734-USA	Thermal insulation material from mycelium and forestry byproducts
	2018	CN108699507-China	Heat-barrier material from mycelium and forestry byproducts
Shenzhen tech	2017	CN105292758-China	Production method for organic packaging material

6.3 Electrical circuit boards

In 2016, the Ecovative Company produced a mycelium sheet by introducing a mycelium culture to a substrate consisting of potato dextrose agar or broth. The solution was infused with metal salts, including Al_2O_2, $CuSO_4$, or $CuCl_2$. Through this process, the thin mycelium sheets took the shape of the wiring layout, thereby sequestering the metal salts [71]. Researchers have taken an interest in cultivating mycelium on agricultural byproducts and waste as a potential strategy for developing low-energy construction materials and a waste recycling approach. Utilizing mycelium as a material offers several advantages compared to traditional options, including lower density, biodegradability, reduced costs, and minimal environmental impact. Since the mycelium-based bio-foams offer low density, they have a lower thermal conductivity. Because of this quality, they offer a lot of potential as alternative insulation materials in the construction and development of infrastructures [34]. Bharath et al. [14] developed interior surfaces for printed circuit boards (PCBs) using mycelium composites. The composite laminate was developed using natural rise husk and epoxy resin. They assessed some essential properties such as tensile, bending properties, thermal properties, dielectric properties, micro-drilling, flammability, and moisture absorption, and those mechanical properties were sufficient for justifying its use in PCBs.

Mycelium demonstrates noteworthy natural low-frequency absorption capabilities (< 1500 Hz), making it a superior acoustic absorber in comparison to cork. Thus, mycelium has the potential to replace conventional ceiling tiles as a means of mitigating noise pollution.

Fungal biomaterials are more fire-resistant than conventional building materials such as polystyrene insulation and particleboard, and they also resist termites [46]. Fungal-based products are a safer alternative to highly flammable petroleum-based materials as they emit fewer amounts of CO_2 and smoke during combustion. Additionally, they take a longer time to reach the flashover point [34]. Based on the aforementioned uses, there is great potential to create materials derived from mycelium that can be utilized for protective packaging applications, automobile parts, and circuit items. Still, polystyrene remains the most widely utilized packaging material globally [14]. Therefore, the development of commercially viable biocomposite materials will be a great alternative to replace synthetic materials.

7 Fine-tuning and growth dynamics of mycelium-based composites

Materials derived from mycelium are biodegradable and are developed based on the principles of the circular economy. Because mycelium can grow on a broad range of substrates, including waste or difficult-to-process materials, low-energy processing can be utilized to halt its biological activity [83]. In addition, mycelia from white-rot fungi have the ability to efficiently decompose lignin through the secretion of multifunctional enzymes, which makes them ideal candidates for transforming waste substrates into new and readily accessible resources [19].

The physical and chemical properties of materials derived from mycelium depend on various factors, including the strain type, growth medium, and growing conditions. Hydrophobicity and high-temperature tolerance are two of mycelia's most remarkable properties as biomaterials [34]. Tuning up these properties is essential as the fungal biomaterials usually contain the inherent qualities of the feed and the fungus used. Various studies have been conducted to evaluate how small changes in the feeding substrate can affect and adjust the shape, mechanical properties, chemical content, and hydrodynamics of the mycelium [37]. Antinori et al. [19] conducted a study in which they utilized *G. lucidum* in a standard fungal medium (potato dextrose broth (PDB)) supplemented with either alkali lignin or D-glucose to increase the hydrophobicity or hydrophilicity of the final mycelia, respectively. Alkali lignin, which is a partially hydrolyzed version of the complex molecule found in wood, was used as it is functionally identical to the original lignin and is digested by the same enzymes at similar rates. D-glucose, on the other hand, is a monosaccharide that is easily digested by mycelia and is already present in PDB among more complex polysaccharides [84].

Antinori et al. [19] revealed that adding glucose to mycelia causes them to become more porous, making them more susceptible to adsorb water moisture. In contrast, a reduced concentration of lignin in the growth medium led to faster and more concentric mycelial growth from the inoculum than in conventional PDB, while minimizing the formation of numerous sparse colonies. Furthermore, samples grown with lignin were thinner and denser, whereas mycelia grown on D-glucose-rich media are thicker and more flexible [19]. In comparison to the samples grown on the other two substrates, the samples grown on the lignin substrate had a less entangled and smoother hyphal network. Tube-like structures became more prominent in D-glucose-grown samples, whereas elongated thread-like features were seen primarily in lignin-grown samples. The rate of expansion in the area occupied by the mycelium varied according to the feeding media, with the highest rate (5.4 ± 2.6) cm^2 /day for the lignin medium and the lowest rate (2.5 ± 0.8) cm^2 /day for PDB medium. They observed the highest final weight of *G. lucidum* when grown in the glucose medium with a value of 506 ± 28 mg. It is noteworthy that, following three weeks of growth, the weight of all three samples in each medium appeared to be consistent. Antinori et al. [19] also observed that mycelia grown in a D-glucose-rich medium exhibited greater thickness and porosity (approximately 85% porosity) with intermediate density, while mycelia grown in lignin-rich medium exhibited less thickness and porosity (around 68%) with higher density.

Fine-tuning the properties of fungal mycelium composites is an essential part to obtain desirable characteristics. While modifying the growth medium, fungal strain, substrate, and growing conditions, the mechanical, chemical, and physical properties can be varied.

8 Tools in modern biotechnology to improve mycelium properties

Various tools in biotechnology including industrial fermentation, strain improvement, recombinant DNA technology, gene editing, and silencing have been experimentally used in mycelium-based material design and development recently. For instance, several fungal species belonging to either Basidiomycota or Ascomycota have been successfully used to cultivate mycelium in bulk for material applications according to the desired applications and fermentation process [59]. Various species of white-rot Basidiomycota that belong to the orders Agaricales and Polyporales have been recognized as promising candidates for effectively cultivating mycelial materials on lignocellulosic substrates. Such species include the genera *Ganoderma, Pleurotus, Trametes, Schizophyllum*, and *Fomes* [25]. The frequently encountered Ascomycetes such as *Aspergillus*, *Penicillium*, and *Trichoderma* are used for biotechnological applications when grown in bioreactor setups. When selecting a strain, it is essential to consider the specific composition of the cell wall. The ratio of chitin/protein content in fungal cell walls can differ across various species [41]. The selection of the appropriate species can significantly impact the efficiency of post-processing, such as the number of available chemical crosslinking sites, as well as the properties of the resulting material. As a result, it is crucial to identify and select the most suitable species based on the fermentation setup, substrate, and the intended application of the material before strain development. Genetic alterations can be used to improve strains, which have already been used in many biotechnological applications [85].

8.1 Genetic modifications of fungi to improve mycelium properties

Genetic modification of living organisms has revolutionized the use of living systems in various applications in medicine, agriculture, food, and environmental technology [61, 86]. While recombinant DNA technology is widely known as the primary tool for the genetic modification of living organisms, other technologies such as various gene editing tools and RNA interference technology (RNAi) have also widely been applied in the biotechnology of microorganisms.

The emergence of gene editing tools such as CRISPR-Cas9 has enabled a recent advancement in targeted genetic modification [87]. CRISPR-Cas9 is considered a versatile genetic modification with a wide range of applications. Therefore, it is more accurate, cheaper, faster, and more efficient than other genome editing approaches [88]. CRISPR-Cas9-based systems have been built for a variety of filamentous fungi, although there are challenges known to the methods [89–92]. This approach enables the avoidance of host-specific transcription systems for non-model organisms. It involves the utilization of Cas9 ribonucleoprotein complexes (RNPs) assembled in vitro, which can be used across various species without the need for species-specific adaptations. The application of polyethylene glycol (PEG)-mediated protoplast transformation eliminates the requirement for species-specific adaptations, such as promoter validation, plasmids, and genetic elements, as is the case with in vivo expression systems. The CRISPR-Cas9 system has been used to disrupt the secondary metabolism of filamentous fungi [93] and to control the synthesis of ligninolytic enzymes in white-rot fungi [94]. This could be further used to fine-tune the fungal properties and grow patterns in mycelium-based materials.

8.2 Fungal hydrophobins

The extracellular active proteins called hydrophobins are low molecular weight (7–15 kDa) and small cysteine-rich proteins produced by filamentous fungi [95]. These proteins are involved in the development of hydrophobic aerial structures,

Table 3 Classification of fungal hydrophobins and examples for each class

Class	Characteristics of hydrophibin	Hydrophobin type	Fungal species	Reference(s)
Class I	■ Compact, globular structure ■ Exhibit strong hydrophobicity ■ Involved in the formation of aerial structures	SC3, SC4, SC16	*Schizophyllum commune*	[45, 95, 99, 103]
		ABH1, ABH3	*Agaricus bisporus*	[95, 99, 103]
Class II	■ Extended, rod-like structure ■ Exhibit hydrophobicity ■ Associated with surface coatings and adhesion	CRP	*Cryphonectria parasitica*	[95, 96]
		CFTH1	*Claviceps fusiformis*	[95, 96]
		NC2	*Neurospora crassa*	[98]
		MHP1	*Magnaporthe grisea*	[95, 96]
		HFB1	*Trichoderma reesei*	[96]

including spores, fruiting bodies (such as mushrooms), aerial hyphae, and the adhesion of hyphae to hydrophobic surfaces, as well as in signaling [96]. It is most remarkable that they can self-assemble into an amphipathic membrane at any hydrophilic-hydrophobic interface. Based on differences in hydropath patterns, solubility, and the type of layers' form, they can be divided into two categories: class I and class II (Table 3) [95]. Both ascomycetes and basidiomycetes present Class I hydrophobins. Until now, class II hydrophobins have been found in ascomycetes only [97]. While class I hydrophobins can be made to adhere very strongly to various surfaces, this is not seen for class II hydrophobins which disconnect more easily.

The first hydrophobin genes were discovered in *Schizophyllum commune* in 1991 [45]. In *S. commune,* at least four distinct hydrophobin genes namely SC1, SC3, SC4, and SC6 genes can be found. Starting in 1991, researchers recognized a similarity in the sequence between the hydrophobins of *S. commune* and the protein RodA that forms rodlets in *Aspergillus nidulans*. They also noted that mutants of *Aspergillus nidulans* and *Neurospora crassa* that lack rodlets have a poor ability to disperse spores in the air [98]. The identification of hydrophobin structures and properties in the fungi strains mentioned above facilitated the prompt identification of comparable hydrophobins in additional fungi [95]. Several biotechnological applications of hydrophobins have been identified including surface coating, biosurfactants, cosmetics, electrodes, biosensors, emulsifiers, and pharmaceuticals [96].

8.3 Impact of hydrophobin gene deletion on properties of mycelium

The study by Appels et al. [99] successfully demonstrated the use of genetic engineering to tune up mycelium materials of *S. commune* (Fig. 5). The researchers evaluated the effects of removing the hydrophobin gene SC3 (Δsc3 mycelium) on the physical characteristics of the mycelium in the fungus *S. commune*. The study found that the deletion strain exhibited a 3–4 times higher Young's modulus (E) and maximum tensile strength compared to the wild types, namely *G. lucidum and P. ostreatus*. Furthermore, the absence of SC3 hydrophobins, a cell wall protein in *S. commune*, was found to affect the composition of the cell wall. This resulted in an increase in the number of schizophyllan, while the amount of cross-linked glucan to chitin decreased [45]. In a different instance, the introduction of a *Saccharomyces cerevisiae* CDA1 chitin deacetylase-encoding gene controlled by a glyceraldehyde-3-phosphate dehydrogenase (GPD) promoter into a strain utilized for mycelium material production led to the development of materials with a notably higher compressive modulus [34].

Furthermore, by utilizing GPD's constitutive promoter to engineer the expression of -1,3-glucan synthases (BGS1 and BGS2), the resulting transformants of *Ganoderma* sp displayed an increase of 135–165% in their -glucan content [59]. In addition to fungi, the incorporation of genetically modified bacterial strains during co-cultivation can provide significant benefits in terms of enhancing material properties and preventing contamination. For instance, co-cultivating with engineered *Bacillus* organisms that secrete polygama-glutamic acid as a biofilm led to a twofold improvement in elastic modulus. Additionally, engineering melanin synthesis conferred improved UV protection to mycelium materials [34, 99].

9 Conclusions

The field of research in mycelium composites encompasses a variety of applications that involve the incorporation of living organisms in the design process, surpassing the superficial imitation of natural systems via "bio-collaboration". The properties of materials derived from mycelium depend on the strain of fungi, growth medium, and environmental

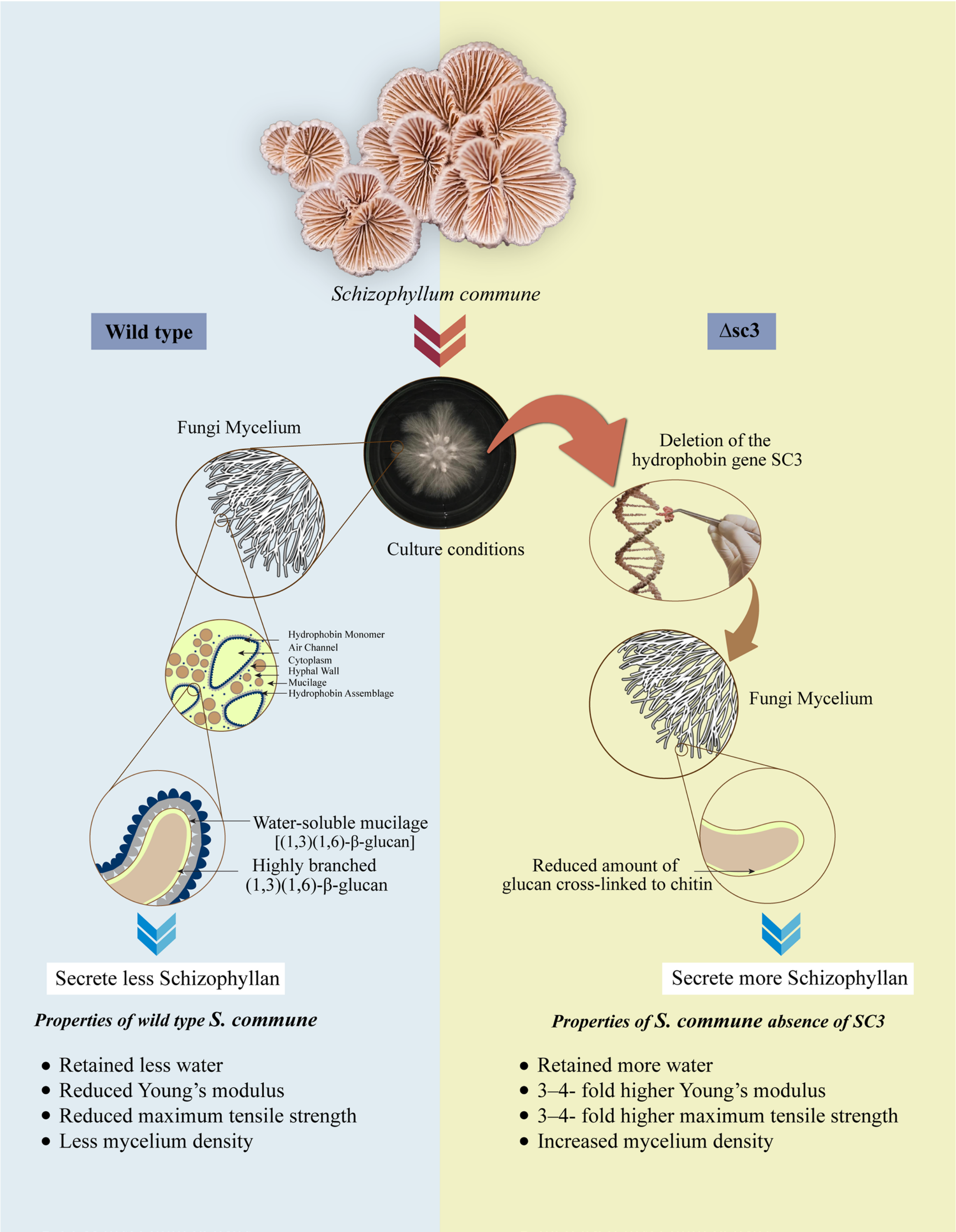

Fig. 5 Properties of *S. commune* wild type and *S. commune* in the absence of SC3 gene (ΔSc3) as described by [99]. The deletion of hydrophobin gene SC3 I increase the section of schizophyllan leading to improved favourable material characters of mycelia such as higher water retention, improved Young Modulus, relatively increased tensile strength and high mycelium density

conditions. The incorporation of nanotechnology and genetic modifications can be used to further improve the process of producing mycelium materials. Furthermore, a comprehensive cost of production analysis is highly recommended before the establishment of large scale production plants of MBCs. Many bio-based materials still face challenges such as maintaining high quality, coping with strain and substrate variations, and adapting to diverse environmental conditions. Research efforts should prioritize addressing these hurdles by fine-tuning solutions for each specific case. Therefore, to produce pure mycelium materials and composites with desirable properties, more research should be focused on discovery of suitable species fungi, improving biological characters of mycelium either by genetic modification and extensive testing of suitable feed preparations together with systematic cost–benefit analyses.

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Data and code availability The authors declare that the data supporting the findings of this study are available within the paper. Should any raw files of images be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Declarations

Ethics approval and consent to participate Not applicable.

Competing interests The authors declare no competing interests.

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