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TITOLO TESI

MushToBiom
From Edible Mushrooms to Biomaterials
A Dual-Production System to produce Food sources and Biomaterials

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PROLOGO

La motivazione principale che guida questa ricerca è un profondo impegno nel mitigare l'impatto dell'umanità sul pianeta. Questo impegno comprende diverse dimensioni: trasformare le dinamiche che conducono al degrado ambientale, come lo sfruttamento eccessivo delle risorse naturali, e ridefinire le pratiche produttive per ridurne gli effetti dannosi sull'ambiente e sulla società. L'obiettivo è promuovere sistemi sostenibili e virtuosi che mettano al centro la responsabilità ecologica, creando al contempo soluzioni accessibili e significative, capaci di incoraggiare un'adozione diffusa di comportamenti e pratiche positive.

Questi obiettivi sono fondamentali per affrontare le pressanti sfide ambientali del nostro tempo, considerando che la crisi attuale affonda le sue radici nelle attività antropiche. Creare un mondo migliore e più sostenibile richiede soluzioni concrete che permettano all'umanità di migliorare le pratiche quotidiane. Questi cambiamenti devono concentrarsi sulla trasformazione dei comportamenti dannosi e sulla comprensione dell'essenza stessa dell'essere umano come parte di un ecosistema più ampio: il mondo naturale, indispensabile per la nostra sopravvivenza e oggi devastato dall'Antropocene. Questo equilibrio è essenziale: la sostenibilità non deve sacrificare l'essenza umana, ma guidarla verso un'armonia con la natura. È verso questo mondo "rinaturalizzato" che l'umanità deve orientare i propri sforzi.

La rinaturalizzazione non consiste nel limitare il progresso umano, bensì nell'ampliare il potenziale dell'uomo. Significa riconnettere le persone alla loro vera natura in qualità di elementi integranti di un sistema complesso e interdipendente, in cui equilibri delicati governano la vita. Questa interconnessione ci ricorda che ogni essere vivente svolge un ruolo fondamentale nel mantenimento della stabilità e della salute del pianeta. Abbracciando questa prospettiva, possiamo vedere l'umanità non come una forza opposta alla natura, ma come una parte integrante della sua intricata rete vitale.

Per realizzare questa visione, ho sempre creduto che studiare la natura e i suoi fenomeni sia l'unico modo per riscoprire il ruolo dell'umanità all'interno di un sistema più ampio. La natura offre lezioni profonde su resilienza, adattabilità ed equilibrio, qualità spesso trascurate nelle attività umane moderne. Tra i tanti elementi della natura, i funghi hanno catturato particolarmente la mia attenzione. La loro intelligenza unica, la complessità e il contributo alla vita sulla Terra mi hanno spinto a esplorarli più a fondo. Sebbene abbia ancora molto da imparare, la mia ricerca sui funghi ha già rivelato intuizioni che mettono in discussione i modi convenzionali di pensare alla sostenibilità e all'innovazione.

I funghi possiedono una straordinaria capacità di creare equilibrio negli ecosistemi. Decompongono la materia organica, riciclano nutrienti e favoriscono la crescita di nuova vita. Questo ruolo di riciclatori e architetti della rigenerazione sottolinea la loro importanza nel mantenimento della salute ecologica. Ciò che è ancora più straordinario è il loro potenziale nell'insegnarci come progettare sistemi umani non distruttivi, ma rigenerativi. Attraverso l'osservazione attenta e lo studio, possiamo replicare i meccanismi usati dai funghi per regolare gli ecosistemi, applicando questi principi per creare soluzioni sostenibili per le necessità umane.

Si consideri, ad esempio, come i funghi abbiano già rivoluzionato la vita umana nel corso della storia. Dalla scoperta della penicillina, che ha salvato milioni di vite, allo sviluppo del pane lievitato e dei prodotti fermentati, i funghi hanno profondamente influenzato le civiltà. Oggi il loro potenziale va oltre. Scienziati e innovatori stanno esplorando come i funghi possano produrre materiali sostenibili in grado di sostituire quelli inquinanti, costruire strutture bio-based e persino definire infrastrutture urbane per le città del futuro. Questi progressi sono allo stesso tempo tecnologici e filosofici, poiché ci spingono a ripensare il nostro rapporto con la natura.

I funghi, regolatori e riciclatori del mondo naturale, offrono un modello per il cammino dell'umanità. Dimostrano che è possibile soddisfare i bisogni umani senza esaurire le risorse. Invece di sfruttare la natura, possiamo collaborare con essa, replicando i processi sostenibili che i funghi hanno perfezionato in milioni di anni. Questo approccio simbiotico non è solo un'opportunità, ma una necessità per costruire un futuro in cui l'umanità possa prosperare senza compromettere la salute del pianeta.

Attraverso questa ricerca, spero di contribuire a un movimento più ampio che mira a riconnettere l'umanità ai sistemi naturali da cui dipendiamo e a creare un mondo in cui la sostenibilità non sia un'aspirazione, ma una realtà. È un percorso di scoperta, guidato dall'intelligenza dei funghi e dalle lezioni che essi offrono per vivere in armonia con la Terra.

PROLOGUE

The primary motivation driving this research is a profound commitment to mitigating humanity's impact on the planet. This endeavor encompasses several dimensions: transforming the dynamics that lead to environmental degradation, such as the overexploitation of natural resources, and reshaping manufacturing practices to reduce their harmful effects on the environment and society. The aim is to foster sustainable and virtuous systems that prioritize ecological responsibility while creating accessible, meaningful solutions that encourage the widespread adoption of positive practices and behaviors.

These goals are essential for addressing the pressing environmental challenges of our time, considering that this crisis is rooted in human-centered activities. Creating a better and more sustainable world requires practical solutions that empower humanity to improve daily practices. These changes must focus on transforming harmful behaviors and understanding the real essence of humankind as part of a broader ecosystem: the natural world, which is essential for our survival and is now being devastated by the Anthropocene itself. This balance is crucial: sustainability cannot mean sacrificing humanity's essence but guiding it toward harmony with nature. It is toward this "renaturalized" world that humankind must direct its efforts.

Re-naturalization is not about limiting human progress but about expanding human potential. It means reconnecting people with their true nature as integral parts of a complex, interdependent system, where delicate balances govern life. This interconnectedness reminds us that every living being plays a critical role in maintaining the stability and health of the planet. By embracing this perspective, we can perceive humanity not as a force opposite to nature but as a participant within its intricate life network.

To achieve this vision, I have always believed that studying nature and its phenomena is the only way to rediscover humanity's role within the larger system. Nature offers profound lessons on resilience, adaptability, and balance, qualities often overlooked in modern human activities. Among the countless elements of nature, fungi have particularly captured my attention. Their unique intelligence, complexity, and contributions to life on Earth have inspired me to dive deeply into understanding them. While I still have much to learn, my research into fungi has already revealed insights that challenge conventional ways of thinking about sustainability and innovation.

Fungi possess an extraordinary ability to create balance within ecosystems. They decompose dead matter, recycle nutrients, and facilitate the growth of new life. This role as nature's recyclers and architects of renewal underscores their importance in maintaining ecological health. What's even more remarkable is their potential to teach us how to design human systems that are not exploitative but regenerative. Through careful observation and study, we can replicate the mechanisms fungi use to regulate ecosystems, applying these principles to create sustainable solutions for human needs.

Consider how fungi have already revolutionized human life throughout history. From the discovery of penicillin, which saved millions of lives, to the development of leavened bread and fermented products, fungi have profoundly shaped civilizations. Today, their potential goes even further. Scientists and innovators are exploring how fungi can produce sustainable materials to replace harmful materials, build bio-based structures, and even develop urban infrastructures for the cities of the future. These advances are technological and philosophical, as they challenge us to rethink how we interact with nature.

Fungi, the regulators and recyclers of the natural world, provide a blueprint for how humanity can move forward. They demonstrate that it is possible to meet human needs without depleting

resources. Instead of exploiting nature, we can work alongside it, replicating the sustainable processes fungi have perfected over millions of years. This symbiotic approach is not just an opportunity but a necessity for building a future where humanity thrives without compromising the planet's health.

Through this research, I hope to contribute to a broader movement that seeks to reconnect humanity with the natural systems we depend on and to create a world where sustainability is not an aspiration but a reality. It is a journey of discovery, guided by the intelligence of fungi and the lessons they offer for living in harmony with the Earth.

DEDICATION

To all the people who have walked beside me throughout this journey.

To my loved ones, who have supported me unconditionally and believed in me even when I struggled to believe in myself. Thank you for always encouraging me to give my best and for pushing me to pursue what I truly care about.

To my friends, both the old ones and those I met along the way: you have been a constant source of strength, joy, and inspiration. Each of you has contributed to this work more than you may realize.

To my professors and mentors, for your guidance, trust, and the opportunities that allowed me to grow as a researcher and as a person.

To all of you, my deepest gratitude.

This thesis is yours as much as mine.

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Thesis introduction

1. Research motivations

The construction industry, accounting for approximately 37% of global CO_2 emissions, plays a significant role in environmental degradation. This sector is the largest carbon emitter, accounting for more than one third of annual global emissions. These emissions are typically divided into operational carbon, linked to building use, and embodied carbon, associated with the production and disposal of construction materials. To achieve carbon neutral buildings, both operational and embodied carbon should be minimized throughout a building's life cycle [1]. One of the key challenges lies in the reliance on traditional, resource-intensive, and polluting building materials. Their production often involves extractive processes, high energy consumption, and substantial waste generation, leading to resource depletion and increased environmental pollution.

The pollution generated, coupled with the high demand for raw materials, highlights the need to develop low-impact solutions, such as utilizing renewable materials capable of absorbing CO_2 for the production of bio-composites, to reduce greenhouse gas emissions [2]. Effectively addressing these issues is critical to advancing global sustainability efforts and aligns with the objectives of the United Nations Sustainable Development Goals (SDGs), particularly Goal 11, which promotes the development of inclusive, safe, resilient, sustainable cities and human settlements.

For these reasons, there is an urgent need to identify and develop alternative materials with a lower environmental footprint. In this context, bio-based materials have emerged as promising candidates due to their potential to contribute to sectoral decarbonization and support the transition toward a circular economy. Derived from renewable sources and often biodegradable, these materials enable a shift from extractive, linear production models to regenerative, resource-efficient systems.

2. Research focus

This research aims to develop sustainable alternatives to conventional building materials. The construction sector relies heavily on resource-intensive, non-renewable, and often toxic materials, particularly for insulation, which contribute to resource depletion and significant waste generation. Existing insulation materials can be divided into three main categories: synthetic, mineral, and natural, all of which involve extractive production systems. A promising alternative is represented by biomaterials or bio-composites. These materials offer a viable pathway to reducing the environmental footprint of construction by replacing traditional components and mitigating the impacts associated with their production and life cycle.

This research focuses on developing a specific category of biomaterials based on mycelium known as Mycelium-based composites (MBC). Derived from the vegetative part of fungi, mycelium acts as a natural binder that can transform agricultural and organic residues into solid, lightweight, and biodegradable materials. MBCs have emerged among the biomaterials as promising candidates for sustainable applications in various fields, including packaging, design, textiles, and, more recently, construction. Leveraging mycelium means to form natural composites by transforming lignocellulosic sources into valuable solutions creating high-value products with customizable mechanical properties. One of the most significant contributions lies in the possibility of growing instead of extracting the resources from the natural environment and the ability of mycelium to recycle agricultural waste as a substrate for growing, obtaining remarkable properties

that contribute to the development of eco-friendly composites [3]. The characteristic of recycling organic waste into new bio-based products makes the MBC very advantageous, constituting a new form of a cost-effective and low-energy bio-fabrication [4] that has a real potential to be implemented in sustainable design solutions, especially for the construction field [5]. In this regard, MBCs derived from agro-industrial residues provides an alternative for sustainable building materials that are biodegradable, recyclable, and with a low carbon footprint that aligns harmoniously with the principles of the circular economy [6]. In the construction sector, MBCs are emerging for their thermal and acoustic insulation properties, lightweight nature, and complete biodegradability [7], [8]. Nevertheless, their large-scale adoption remains constrained by several significant barriers, primarily related to production complexity, biological variability, high costs related to the current state of the market, and restrictive patent protections where only a small niche of specialized producers manufacture MBCs. The lack of economies of scale, combined with biologically complex and time-intensive processes, drives up prices and hinders broader accessibility. To overcome these challenges, this thesis explores the development of an integrated dual-production system that simultaneously produces food source and biomaterials by yielding edible mushrooms and generating mycelium-based composites within a single biological process. Unlike the current paradigm, where the mushroom cultivation sector and the biomaterials sector operate independently, this thesis proposes a unified model that leverages the shared biological foundation of fungal growth. By cultivating mushrooms for both edible and structural purposes within the same process, we reduce waste, optimize energy and resource use, and significantly lower production costs by dividing them between two outputs and two markets. This method not only enhances economic feasibility but also advances a regenerative model of production that avoids the exploitation of natural resources, instead mimicking and amplifying naturally occurring systems [3]. This integrated system represents a significant advancement beyond the current state of the art, where such cross-sectorial synergy is virtually nonexistent. The research demonstrates, for the first time, how food production and material fabrication from mycelium can be industrially merged, offering a scalable, sustainable, and economically viable pathway for future applications in both agriculture and construction.

3. Research Objectives and Questions

The main objectives of this thesis are:

- To develop a scalable, integrated dual-production system capable of producing both edible mushrooms and MBCs using renewable substrates.
- To quantitatively evaluate mushroom yield and characterize the mechanical and acoustic properties of MBCs produced using the newly implemented dual-production system.
- To propose a framework for integrating fungal biomaterials into construction, addressing scalability, performance standards, and market viability.

Correspondingly, the research is guided by the following questions:

- Is it technically and biologically feasible to integrate edible mushroom cultivation and MBCs production into a single, standardized process and can a dual-output production system significantly reduce costs and improve the scalability of MBCs for industrial use?
- How can MBCs, produced through an integrated dual-output system, be optimized and standardized to serve as a sustainable alternative to conventional construction materials, thereby reducing environmental impact and CO_2 emissions in the building industry?

- What are the main advantages and limitations of implementing a dual-production system in terms of edible mushroom yield and the performance of MBCs particularly regarding mechanical and acoustic properties and how can these materials be further optimized for real-world applications in sustainable construction?
- How can circular economy principles and biomimicry, particularly inspired by fungal ecosystems, be leveraged to create a regenerative approach that minimizes resource exploitation and waste both in the agricultural and construction industry?

To address these research questions, this study adopts a multidisciplinary framework that bridges microbiology, agriculture, material science, environmental engineering, and industrial design. Microbiology and agriculture are applied to investigate and optimize the yield of edible mushroom production; materials science is employed to analyze and tailor the mechanical and physical properties of MBCs; while environmental engineering and design methodologies support the development of an efficient, scalable, and sustainable production system.

This integrated approach aims to address key limitations in current MBC research and practice. Existing literature highlights the promise of MBCs as low-impact alternatives to conventional insulation materials, yet it also reveals a fragmented market where production is confined to a few companies. Most of these focus on low-performance applications such as packaging, and only one or two entities explore the use of MBCs in interior design or acoustic panels. This research responds to these limitations by proposing a novel model that not only bypasses existing intellectual property barriers but also demonstrates how food and biomaterial production can be unified into a single, standardized process. By doing so, it seeks to distribute production costs across two value streams, agriculture and construction, thus improving cost-efficiency and increasing economic and ecological viability. The MBCs developed through this dual system are evaluated not only for their mechanical properties and biodegradability, but also for their scalability and potential integration into real-world construction practices. Ultimately, the objective is to contribute to the development of sustainable, locally adaptable building materials while simultaneously enhancing food production capacity, positioning fungal cultivation as a key component in circular and regenerative bio-based economies.

4. Thesis Structure

This thesis is structured into seven main chapters, each contributing to the progressive development and validation of a dual-production system that integrates edible mushroom cultivation with the fabrication of mycelium-based composites (MBCs) within a single, sustainable process. The research begins with a review of the state of the art on mycelium-based biomaterials, followed by the replication of existing methodologies using a novel substrate for acoustic characterization. It then moves on to the optimization of production processes aimed at improving the mechanical performance of MBCs. Building on these results, the study introduces an initial investigation into the use of spent mushroom substrate (SMS), a byproduct based on the cultivation substrate of edible mushroom cultivation, as a potential sustainable input for MBC production. Once the compatibility of this material is assessed, the research defines and experimentally validates a closed-loop dual-production model. The final chapters are dedicated to evaluating the system's feasibility in real-world applications through the development of a full-scale prototype, which serves as a proof of concept. A detailed overview of each chapter is provided in the next section.

5. Research Methods

- **Chapter 1 – Mycelium-based Biomaterials: State of the Art**

To establish a foundation for this study, an analysis of state-of-the-art methods was conducted, identifying the key steps required to produce insulation biomaterials, as illustrated in Fig. 1. This chapter explores the current production methods and application. The analysis establishes the conceptual and technical foundations upon which this research is built, while identifying critical gaps and opportunities for innovation.

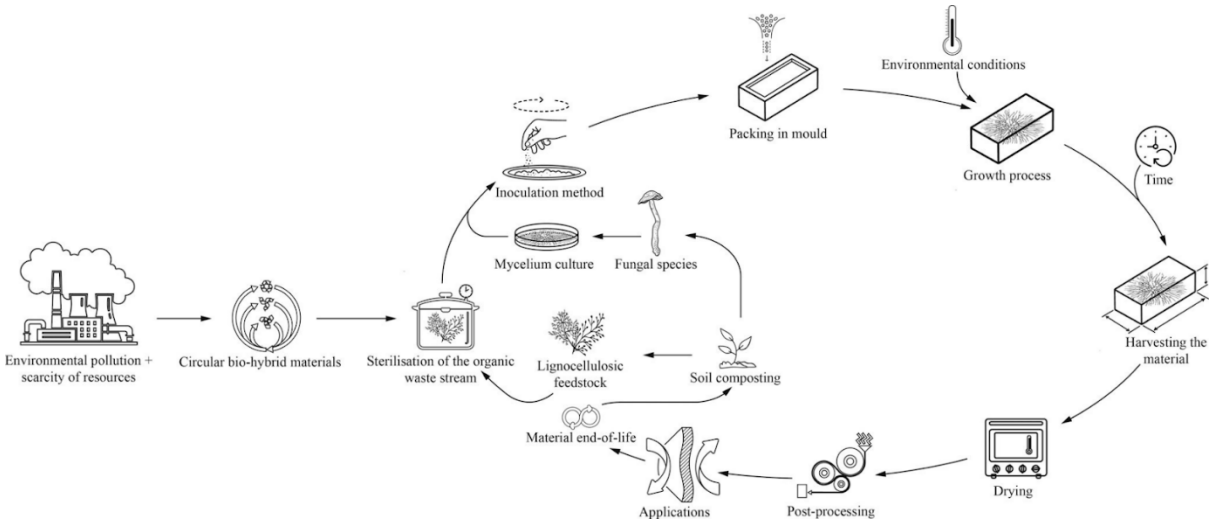

Fig. 1 Production process of MBCs reported in the literature [3]

- **Chapter 2 – MBC Production Replication with a Novel Substrate and Acoustic Characterization**

This chapter details the initial phase of the research, which involved replicating established protocols for MBC production as reported in existing literature. The goal was to evaluate the reproducibility, reliability, and scalability of these methods under controlled conditions using a new substrate never reported previously. This involved selecting a substrate among widely available lignocellulosic byproducts within the European region, such as Green Pruning Waste (GPW). Furthermore, a compatible fungal strain, *Pleurotus ostreatus* (PO), was selected for cultivation on the GPW substrate. This substrate is an untreated organic agricultural byproduct composed primarily of fibrous plant residues. Additionally, an acoustic test was performed and integrated into the cultivation phase: the growth of the mycelium was monitored over time, and its sound absorption capacity was characterized at different growing stages [9]. This approach allowed for the identification of optimal harvest windows based on desired acoustic performance, offering a practical strategy for tuning material properties through temporal control of the biological process. By systematically applying and testing these reference methodologies, the study aimed to identify critical practical constraints and inconsistencies in material performance. This replication phase served as a benchmark for comparison, providing a foundational understanding of current limitations and directly informing the experimental adjustments and innovations explored in the subsequent phases of the research.

- **Chapter 3 – Optimization of MBC Production and Mechanical Characterization**

The third phase of this research focused on developing a more sustainable and scalable production process for MBCs. As a starting point, existing methodologies from the literature were replicated to establish a comparative process, while introducing a novel lignocellulosic substrate—previously unreported in the state of the art and described in Chapter 2—as a growth medium. In this phase, a different substrate composed of woody debris from Robinia pseudoacacia (RP) was tested in combination with the fungal strain Trametes versicolor (TV), alongside an optimization of the previously explored production protocol. This pairing enabled the development of an alternative cultivation method that addressed several key inefficiencies found in conventional and patented approaches, thereby proposing a redefined process suitable for industrial-scale implementation. Experimental trials confirmed that T. versicolor successfully colonized and grew on RP, leading to the fabrication of panels with customizable properties. Furthermore, mechanical compression tests were conducted to evaluate the structural performance of the resulting composites, providing quantitative validation of their material properties. These results also demonstrated the adaptability of the new protocol to different substrate–fungus combinations. Notably, the revised methodology reduced the number of processing steps, resulting in lower energy consumption and shorter production times.

- **Chapter 4 – Methodological Advancement of SMS-based MBCs**

Building upon the methodologies and findings presented in the previous phases, the subsequent stage of this research investigated the feasibility of employing Spent Mushroom Substrate (SMS) as an alternative lignocellulosic substrate for MBCs production. SMS is the residual substrate used in edible mushroom cultivation, typically discarded after three harvesting cycles. It consists of partially degraded lignocellulosic material enriched with fungal biomass, including residual mycelium. Given its abundance and composition, SMS presents a valuable opportunity for circular reuse within a dual-production framework. According to data from innovation and agricultural research programs, approximately five kilograms of SMS are generated for every kilogram of edible mushrooms produced, underscoring the urgency of valorizing this high-volume waste stream. The main goal of this experimental phase was to evaluate the compatibility of SMS with MBC production. Specifically, the research aimed to assess whether the substrate, already biologically processed during mushroom cultivation, could effectively support further fungal regrowth and serve as a structural base for composite fabrication. To this end, the optimized production protocols previously developed for GPW and RP substrates were adapted to SMS. Different pre-treatment strategies were applied to improve the substrate's performance, and MBC panels were produced and tested to assess their mechanical properties. In addition, the same specimens were analyzed for their acoustic properties. This experiment confirmed that SMS is not only a viable byproduct for reuse but also a compatible and effective substrate for MBCs fabrication. The findings support its integration into a closed-loop system that combines food production with the creation of sustainable construction materials.

- **Chapter 5 – Definition of a dual production system for food and MBCs with mushroom yields analysis and mechanical characterization**

This phase of the research focused on optimizing the use of SMS for MBC production, marking a significant advancement in the development of a dual-production system. Unlike previous approaches that required new fungal inoculation and substrate colonization, this study demonstrated that post-harvest SMS, already structurally bound by residual mycelial networks, can be directly processed into MBC insulation panels without additional biological growth phases. This innovation simplifies the production process, eliminates key steps such as incubation, and significantly reduces time, energy consumption, and resource inputs. Moreover, since SMS

originates from food production, its reuse supports a net-zero manufacturing model by repurposing organic waste into biodegradable, high-performance materials for construction. To validate this approach, the study was divided into two key experimental components. First, edible mushroom cultivation was carried out under varying conditions to analyze yield performance and identify optimal protocols that simultaneously ensured the production of a structurally suitable SMS byproduct. The harvested SMS was then processed directly into MBC panels. These were subjected to mechanical characterization to evaluate their structural integrity. Results confirmed that SMS, even without further fungal colonization, retains sufficient cohesion and mechanical performance for potential use in sustainable building applications. Through this work, the dual-production system was formally defined and experimentally validated, showing how a single biological cycle can yield both food and functional biomaterials. This model demonstrates technical feasibility and economic scalability while aligning with circular economy and regenerative design principles.

- **Chapter 6 – Acoustic Characterization of SMS-Based MBCs**

Following the mechanical evaluation of MBC panels derived from SMS, the same specimens were subjected to acoustic testing to assess their sound absorption capabilities. This second phase of characterization aimed to explore the multifunctionality of the material and its potential for use as an insulating component in sustainable construction systems. By using the exact samples previously analyzed for mechanical properties, the research ensured consistency in composition, processing, and biological origin, allowing for a comprehensive understanding of the material's overall performance. Acoustic tests were conducted under standardized conditions to measure absorption coefficients across various frequencies and determine the material's effectiveness in mitigating airborne sound. The results demonstrated that SMS-based MBCs offer favorable acoustic performance, in addition to mechanical stability. These findings further strengthen the case for integrating MBCs into real-world construction contexts, reinforcing the value of a dual-production model that simultaneously addresses food production, material fabrication, and waste reduction.

- **Chapter 7 – Mush2Biom Prototype**

In this final chapter, the first prototype made from SMS is presented, created by directly collecting the material from a mushroom farm following the third harvesting cycle of edible mushrooms. By repurposing SMS into MBCs as a functional biomaterial for the construction sector, we not only offer a sustainable alternative to conventional building materials but also address the waste problem associated with SMS, providing economic value to the mushroom industry. This integrated approach yields environmental, economic, and practical benefits within a single solution. Innovative strategies are essential in advancing sustainable alternatives within the expanding field of bioeconomy. This is especially important when resource cultivation involves unsustainable methods with high opportunity costs, as is the case with mushroom farming. This final chapter also assesses the scalability and real-world feasibility of the proposed dual-production system. To demonstrate its practical application, a full-scale prototype was developed: a 3×3 meter wall built using SMS collected directly from a mushroom farm and transformed on-site into MBC panels. This first prototype was presented and exposed to the FUTURA EXPO fairs taken on the 7-8-9 of March in Brescia, Italy. This pilot installation serves as proof of concept, confirming both the structural integrity and functional performance of the material, and showcasing its potential for adoption in sustainable construction practices.

6. Roadmap

The research outlines a clear pathway from conceptualization to practical implementation. It emphasizes the need for collaboration between academia, industry, and policymakers to overcome adoption barriers and create a sustainable framework for biomaterial production.

7. Expectations and Limitations

While this research advances the development of sustainable materials, it acknowledges the complexities associated with biological systems, production scalability, and regulatory constraints. The study focuses on creating practical solutions within these limitations, recognizing that systemic changes in policy and industry practices are necessary for widespread adoption.

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Chapter 1

Mycelium-based Biomaterials: State of the Art

1. Introduction to MBC

In this chapter it is presented a critical review that explores the intricacies of MBCs by evaluating their potential for industrial scale-up and the practical challenges of integrating these biotechnologies into modern material applications. The review begins by outlining current MBC production methods, establishing a baseline for the research and highlighting the limitations that hinder broader adoption. By tracing the development of MBC technology, we aim to identify the key barriers and opportunities for advancement. This sets the stage for the innovative system proposed at the end of the thesis, an approach designed to make MBC production more accessible, cost-effective, and environmentally sustainable, thereby paving the way for its widespread implementation.

MBCs have emerged as promising biomaterials due to their impressive qualities and potential for diverse applications. By utilizing agricultural waste as an energy source for mycelium growth, MBC could potentially offer cost-effective and low-energy bio-fabrication, particularly advantageous for sustainable construction. In this chapter a review of the state of the art on MBCs examines cultivation procedures and materials, highlighting the potential of MBC to revolutionize the building sector with benefits including energy efficiency, cost-effectiveness, and environmental sustainability. Fungal biotechnology has the potential to advance the transition from a petroleum-based economy to a bio-based circular economy, offering sustainable products. Mycelium possesses a network of thread-like structures called hyphae and has an exploratory and invasive nature that allows fungi to quickly find and colonize new and diverse lignocellulosic-based substrates forming porous and robust structures making the MBC a compelling material for various uses in architecture, spatial structures, and load-bearing components, highlighting their versatility and potential in structural engineering and design [1], [2]. Research has shown that mycelium can be used to produce biocomposites with customizable mechanical properties, making MBC suitable for applications such as insulation foams, protective panels, architectural bricks, packaging, textiles, wound care [3], [4]. Mycelium has also been explored as a source of advanced functional materials such as biopharmaceutical application, tissue engineering [5], [6], fungal skin for robots [7], films [8], mycoremediation [9], sources of food, feed, chemicals, fuels, automotive and transportation industries, for furniture and beyond. MBC has low thermal conductivity, high acoustic absorption and fire safety properties that surpass traditional building materials such as synthetic foam. MBC can enhance the air quality within indoor environments having natural air-purifying capabilities, particularly in terms of particulate matter (PM) filtration. Additionally, mycelium has the potential to regulate indoor humidity. The hygroscopic nature of the mycelium allows it to absorb and release moisture, helping to maintain optimal moisture levels and can sequester over 16 metric tons of carbon from the atmosphere within a month [10] and up to 298 kg CO_2/m^3, meaning that the mycelium insulation material is carbon negative [11]. However, there are limitations, such as mechanical resistance, water absorption and other properties, which must be assessed when the composite is used in building materials. MBC does not have sufficient structural strength for load-bearing applications, and as such, they have been applied in non-load-bearing applications such as partitions, insulations, and acoustic damping materials [12]. The potential of MBCs is further emphasized by their capacity to revolutionize the natural products market, which is projected to exceed $200 billion by 2026 [13]. However, one of the main obstacles to market expansion is the difficulty of scaling production from small-scale applications to industrial-level manufacturing. Key challenges include long production cycles,

susceptibility to contamination, complex and multi-step fabrication processes, limited standardization in both production and material characterization, poor reproducibility, and insufficient automation [14]. Overcoming these barriers is essential to enable broader adoption of MBCs across sectors and to keep pace with the exponential growth of this emerging market, ultimately unlocking the full potential of these sustainable biomaterials [13]. The transformative potential of MBCs in shaping a sustainable future is evident, and further research and development in this field are crucial for realizing the full potential of mycelium as a versatile and sustainable resource. In this critical review are being analyzed all the factors that can change the parameters of MBC, scrutinizing the fungal strains, the substrates, the growing factors, the post-processing procedures, and the mechanical parameters reported in the state-of-the-art with the aim of tracing the guidelines for an industrial application able to widespread MBC among the construction industry. MBCs have emerged from the vanguard of biomaterial innovation as a compelling solution, promising to redefine the landscape of material science through their eco-friendly profile and symbiotic growth processes [15], [16]. This natural technology delves into the intricate network of hyphae that construct the mycelium, a self-assembling architect that engineers robust structures from lignocellulosic-based substrates, and the multitude of applications that span from architecture to healthcare [4], [17]. The versatility and adaptability of mycelium position as a cornerstone for future development across various sectors [18]. However, the path to industrial adoption is marred by challenges—the rigors of scalability, the quest for standardization, and the intricacies of a zero-waste production mandate a rigorous assessment. As we critically analyze the path forward, the ecological and economic benefits of MBCs are clear: their potential for reducing greenhouse gas emissions, their role in air quality enhancement and carbon sequestration [10], [11], and their harmonization with the circular economy [12]. Yet, it is incumbent upon us to confront the limitations head on assessing the mechanical properties, addressing water absorption concerns, and overcoming the production cycle's temporal constraints. By integrating the holistic view of MBCs as a critical component of sustainable development with the technical specifics that define their production and application, this review seeks to illuminate the scale-up opportunities that MBCs present. Through this approach, we explore how MBCs can transition from niche ecological alternatives to mainstream industrial materials, reinforcing a global shift towards materials that are not only produced sustainably but also empower a circular, bio-based economy. It is an exploration of mycelium's role in this transition, as a biological catalyst for change and an emblem of ecological stewardship. In conclusion, mycelium-based composites play a vital role in paving the way for a more sustainable and environmentally conscious approach to material design and production. In particular, the activation of the microbial commons by the uptake of microbial technologies and materials through daily acts of living, ‘democratizes’ resource harvesting (materials and energy) in ways that sustain life and return important decisions about how to run a home back to citizens. This disruptive move re-centered the domestic-commons economic axis to the heart of society, setting the stage for new vernacular architectures that support increasingly robust and resilient communities [19].

2. Growing methods and production processes for MBC

The ascendance of mycology has uncovered the extensive capabilities of fungi, organisms that have sculpted our planet's biosphere for eons. Their profound evolutionary journey, intricately intertwined with life's fabric, is now being harnessed in the development of sustainable materials such as MBC. As elucidated in the foundational "21st-century guidebook to fungi," this kingdom, officially distinguished as a primary branch of eukaryotes based on unique cellular characteristics, unveils a trove of applications through its varied species and feeding processes [20]. Fungal strains within Ascomycota and Basidiomycota, particularly those akin to the latter, have demonstrated remarkable proficiency in deconstructing lignocellulosic matter, laying the

groundwork for MBC production. These filamentous fungi possess a toolkit of specialized enzymes enabling the transmutation of fibrous substrates into digestible sugars, an essential step in the cultivation of MBCs. The diversity among fungal species imparts distinct pathways of degradation, categorized into soft-rot, brown-rot, and white-rot fungi, each engaging in a unique interplay with their environment [20], [21]. In the domain of MBCs, the anatomy and structure of the mycelium vary dramatically among different species, impacting the mechanical properties and potential applications of the composites produced. For instance, biomaterials cultivated from trimitic species such as Trametes versicolor exhibit enhanced compressive and tensile strengths compared to monomitic species like Pleurotus ostreatus [22]. These differences highlight the need for meticulous selection and understanding of fungal species when tailoring MBCs for specific uses. The growth kinetics of these organisms is equally complex and pivotal. The hyphal extension rates, influenced by the presence of skeletal or binding hyphae, dictate the efficiency and rate of colonization, a critical consideration for scaling up production processes. The intricacies of mycelium growth, from its initiation to the eventual consolidation into a composite board or intricate shape, are influenced by a multitude of factors, including the substrate choice and the specific fungal strains employed [23], [24]. Research underscores the prominence of white-rot fungi from the Basidiomycota phylum in MBC production, given their prolific capabilities. Notable species such as Pleurotus, Ganoderma, and Trametes, among others, have been identified as prime candidates for bio-composite production due to their efficient substrate colonization and mycelium growth rates [9], [25], [26], [27], [28], [29], [30], [31]. Furthermore, the potential of brown-rot and soft-rot fungi, including species like Fomitopsis pinicola and Acremonium sp., has also been explored, broadening the spectrum of possibilities for MBC production. These species, with their varying decomposition pathways, offer additional avenues for creating composites with diverse properties and applications [32], [33]. The production of MBC necessitates a holistic comprehension of fungal biology, the interrelations between growth factors, and the mechanical and physical attributes of the composites. As we refine the growing methods and production processes, our grasp on the nuances of these fungal species will not only bolster the efficiency of MBC production but will also ensure the adaptation of these composites to the rigorous demands of industrial applications. This understanding is fundamental to unlocking the full potential of mycelium-based materials and charting a course for their sustainable industrialization.

3. Substrate selection

The foundation of MBC production lies in the substrate: a pivotal element that not only fuels the growth of mycelium but also fundamentally shapes the mechanical and structural characteristics of the resultant biomaterials. The meticulous selection of substrates is a scientific endeavor that delves deep into the intersection of biology and material science, as the substrates serve both as a nutrient source and a scaffold that guides the mycelium's development into a robust biomaterial. Substrates, spanning from organic residuals to agricultural by-products, provide a complex milieu of cellulose, lignin, and hemicellulose, each contributing distinct mechanical properties to the MBC. Cellulose microfibrils, encased within a matrix of lignin and hemicellulose, lend rigidity and structural integrity, while the interplay of these components influences everything from strength to biodegradability [20], [34]. This biochemical synergy underpins the transformative potential of lignocellulose composites, enabling the valorization of diverse waste streams such as rice hulls, sawdust, and soybean hulls into sustainable construction materials [14]. The versatility of mycelium to thrive across various substrates underlines its adaptability and aligns with the principles of a circular economy, where waste becomes the cornerstone of innovation [35]. The substrate composition's influence on MBC's mechanical and chemical properties necessitates a strategic selection based on the end-use, whether it be for construction, packaging, or any number of industrial applications [36], [37].

For instance, cellulose-rich environments discourage the formation of fruiting bodies, channeling the mycelium's energy towards creating a dense, mechanically optimized network. The presence of lignin in the substrate bolsters the composite's resilience, serving as a natural barrier to decay by regulating the bioavailability of decomposition agents and influencing enzymatic activity [38].

Given the broad array of available organic materials, substrates have been grouped into four primary categories, each with its unique contributions to the fabrication of MBCs:

Cellulose-based: These substrates are primarily composed of cellulose, offering a framework that supports the growth of mycelium while ensuring a high degree of structural integrity.

Lignocellulose-based: Incorporating both lignin and cellulose, these substrates are characteristic for their durability and resistance to decay, making them suitable for applications where longevity is critical.

Natural Fiber Textiles: Natural fibers provide a unique texture and flexibility to MBCs, facilitating the creation of materials with specific tactile properties.

Mixed lignocellulose, cellulose, and composites: A hybrid approach, these substrates merge the benefits of both cellulose and lignocellulose, along with additional components to tailor the properties of the final product to precise specifications.

Each substrate requires careful preparation to optimize the conditions for mycelial growth. Factors such as particle size, moisture content, pH, and the method of sterilization play significant roles in influencing the growth kinetics and the properties of the MBC. Industrial-scale production may demand innovative approaches to substrate preparation, often relying on local waste streams, which underscores the scalability and sustainability of MBC manufacturing [39]. In this intricate dance of biological growth and material science, the preparation and choice of substrates are not merely a matter of nutrient provision but a deliberate engineering process. This process is designed to guide the mycelium in forming self-assembling, self-repairing composites that respond dynamically to environmental conditions, characteristics that position MBCs as a frontier material for industrial and environmental applications.

4. Supplemental Additives in MBC Cultivation

The cultivation of MBC extends beyond substrate selection, venturing into the strategic deployment of supplements to create an optimized microenvironment for mycelial proliferation. These additives, rich in carbon or nitrogen, serve as catalysts that can significantly expedite hyphal growth, enhancing the mycelium's structural and mechanical properties.

Carbon and nitrogen are quintessential for the energetic and structural demands of growing mycelium. While substrates often provide a foundational array of nutrients, the judicious addition of supplements can sharpen the growth profile of the mycelium, leading to denser and stronger MBCs. Common carbon-based supplements like sawdust and flour offer a burst of energy, whereas nitrogenous additives such as rice bran and ammonium supply the building blocks for protein synthesis, which is crucial for mycelial development [40], [41].

The addition of inorganic salts like KH_2PO_4, K_2HPO_4, $MgSO_4$, and $CaCO_3$ can balance the pH and provide essential minerals that serve as cofactors in enzymatic reactions, further bolstering mycelium growth [42]. Organic compounds such as glucose directly fuel metabolic pathways, while chitosan, a biopolymer, can act as both a nutrient source and a structural enhancer to the MBC matrix [43], [44]. Other supplements, like oats and bran, can diversify the nutrient profile of the substrate, with the former being a rich source of both carbon and nitrogen [45], [46], [47].

Meanwhile, molasses, whether derived from cane or rice, provides a complex array of sugars that can be readily assimilated by the mycelium [48].

Physical additives like pumice, red laterite particles, or even sand can be incorporated to modify the texture and porosity of the MBC, potentially influencing characteristics such as compressive strength and thermal insulation [33], [49]. These particulates not only contribute to the physical matrix but also create microenvironments that can affect water retention and distribution, which are crucial for mycelial growth.

While supplementation is a powerful tool in MBC cultivation, it is not a ubiquitous requirement. The decision to supplement hinges on the existing Carbon/Nitrogen (C/N) ratio within the substrate. An optimal C/N ratio can be the deciding factor between prolific mycelial growth and stagnation or contamination. This delicate balance must be managed carefully, as excessive supplementation in non-sterile conditions can attract contaminants that compete with or hinder mycelial growth [50], [51].

In practice, the introduction of supplements must be meticulously controlled. The goal is to establish an ideal cultivation environment that accelerates mycelium growth and minimizes the risk of contamination. This approach requires a profound understanding of mycological nutrition and the interplay between substrate composition, additive selection, and the sterilization of materials. By mastering these elements, MBC production can achieve new heights in efficiency, yield, and quality, paving the way for broader industrial adoption.

5. Fiber Processing for MBC Fabrication

The journey of MBC from raw materials to functional materials is significantly influenced by the initial processing of the fibers used as the substrate. These lignocellulosic materials undergo a series of manipulations to acquire the appropriate particle size and form that align with the intended structural design of the MBC.

A critical factor in this process is the determination of the fiber dimensions, which typically range from 0.2 mm to 10 mm. The chosen size affects not just the mycelium's growth rate but also the physical attributes of the final product [42]. The processing methods like chopping, dust creation, pre-compression, or arranging into tows, determine the fiber's final orientation and distribution within the composite [52].

Innovative approaches such as sandwich panel fabrication require fibers to be cut and assembled meticulously, often in textile or mat forms, conforming to the desired shapes and thicknesses [53]. The alignment of these fibers can be random, promoting airflow and space for mycelium to flourish, enhancing the material's elasticity. Conversely, aligning the fibers in a specific direction, akin to the use of rattan or jute sheets, provides integral structural reinforcement, significantly bolstering the material's stiffness as the mycelium merges with the fibrous network [54].

The porosity of the substrate is another pivotal factor; an increase in porosity is hypothesized to amplify oxygen diffusion throughout the material, which is essential for cultivating a dense mycelium network. The orientation and configuration of the fibers thus play a dual role, they facilitate air circulation crucial for mycelial development and contribute to the mechanical resilience of the composite [45].

Additionally, the thickness and density of the fibers are paramount in maintaining appropriate moisture levels during growth. A thinner substrate may not retain sufficient moisture, leading to premature drying and suboptimal mycelial development. In contrast, a substrate with a higher density of fibers can hold moisture more effectively, creating an ideal microenvironment for mycelium cultivation.

The strategic processing of fibers for MBC production is a nuanced balance of material science and biological engineering. It requires a deep understanding of how the substrate's physical structure can influence the biological growth processes and the resulting properties of the composite. Mastering this balance is crucial for advancing the field of sustainable biomaterials and expanding the capabilities and applications of MBCs.

6. Moisture Control in MBC Growing

In the delicate art of cultivating MBC, moisture management is a pivotal aspect that can dictate the success or failure of fungal growth. Following the preparation of fibers and the addition of supplements, achieving the ideal moisture level becomes the next critical step. This phase ensures that the mycelium has the necessary water content to sustain and proliferate.

Hydration of the substrate is universally essential, regardless of the type of fiber or its size. Soaking the substrate ensures that every particle is sufficiently saturated, with a typical soaking period ranging from 24 to 48 hours, though the exact time may vary according to the substrate's absorbency [55]. Precision in hydration is key, with the moisture content customarily calculated as a percentage of the substrate's dry weight, commonly aimed between 55% to 70%, as per mycological research findings [56].

The role of moisture is fundamental: it catalyzes the elongation of hyphal cells and enables the mycelium to colonize the entirety of the substrate [35]. An alternative to soaking is the integration of hot water into the substrate to reach a homogenized state at the desired relative humidity, often targeted at around 60% [47]. This method not only adjusts the moisture but can also aid in pasteurization, reducing the risk of contamination.

Striking the right balance in moisture content is crucial; for instance, a fiber moisture content at 60% has been associated with the most vigorous mycelial growth and the maximal yield of fungal biomass. Conversely, a higher moisture content of 80% can inhibit mycelial development, highlighting the narrow window in which optimal growth conditions exist [57].

Maintaining this optimum moisture content is not merely about maximizing growth; it's about fostering an environment conducive to efficient inoculation and subsequent mycelium development. This involves not just the initial adjustment of moisture levels but also their maintenance throughout the cultivation process, as water serves as a fundamental pillar in the mycelium's metabolic activities and structural development.

The moisture level in MBC production is, therefore, not just a variable but a controlled condition that must be managed with precision and care. Understanding and maintaining the delicate moisture requirements of the mycelium can significantly impact the quality, strength, and viability of the developed biomaterials, marking it as a crucial step in the production of MBCs.

7. pH Adjustment in MBC Preparation

The successful cultivation of MBC is a multifaceted endeavor, where the pH of the growth medium plays an essential role. After ensuring the substrate is adequately hydrated and supplemented, the next vital step is to fine-tune the pH to create an optimal environment for mycelium proliferation.

Mycelium favors slightly acidic conditions, with a pH range of 5 to 6 being ideal for most fungal growth [58], [59]. To achieve this target pH, substances like calcium sulfate ($CaSO4$) or calcium carbonate ($CaCO3$) are incorporated into the substrate mixture. These compounds, when added, serve a dual purpose: they not only adjust the pH but also contribute calcium and sulfur, which are micronutrients that mycelium may require.

The choice between calcium sulfate and calcium carbonate depends on their respective effects on the substrate. Calcium carbonate tends to have a stronger neutralizing effect and can increase the pH more significantly. It's often used when the substrate is overly acidic. Calcium sulfate, while also adjusting pH, provides sulfate ions that can be beneficial for certain enzymatic activities within the fungal metabolism.

Adjusting the pH is not merely a matter of reaching a number on the pH scale; it's about creating a biochemical environment that supports mycelial health and productivity. An incorrect pH can lead to slowed growth or even cessation of mycelial development. Moreover, it can influence the bioavailability of nutrients and the efficacy of the mycelium's enzymatic systems, impacting the overall growth kinetics and the structural integrity of the final MBC product.

Thus, pH adjustment is a critical control point in the MBC production process. It requires precision and attention to the specific needs of the fungal strain being cultivated, as different strains may have slightly different pH preferences. By maintaining the appropriate pH, the production process ensures that the mycelium has the best possible conditions for growth, leading to a higher quality and more consistent biomaterial output.

8. Sterilization Methods in MBC Production

The process of creating MBC demands not only nutrient-rich and pH-balanced substrates but also a sterile environment free from competitive microbial contamination. Sterilization is thus a pivotal stage in MBC production, ensuring the substrate is conducive exclusively to mycelium growth.

Sterilization techniques must be chosen based on the specific properties of the lignocellulosic substrates commonly used in MBC production. The most prevalent methods include autoclaving and pasteurization, both of which have been widely studied for their efficacy and impact on the physicochemical attributes of the biomaterials produced.

Autoclaving is a sterilization method that utilizes pressurized steam to eliminate contaminants. The process parameters can vary widely, with temperatures ranging from 115 °C to 121 °C and durations from 15 to 90 minutes. The selection of time and temperature depends on factors such as the substrate's composition and the volume being sterilized. During autoclaving, substrates are often placed in filter patch bags or polypropylene filter bags designed to withstand high temperatures and pressure. Larger volumes of substrate require longer sterilization times to ensure even and complete sterilization throughout the material.

Another method of sterilization involves the use of hydrogen peroxide solutions, typically at concentrations of 0.3% to 10%. This chemical sterilization method can be effective, although studies have shown that autoclaving may result in a quicker spawn run, indicating a more favorable starting condition for mycelium growth.

Pasteurization is another sterilization technique, where the substrate is immersed in boiling water at 100 °C for around 100 minutes. Unlike autoclaving, pasteurization does not aim to eliminate all microorganisms. Instead, it reduces the microbial load while potentially preserving organisms that are harmless or even beneficial to mycelium growth.

Selecting the appropriate sterilization method is vital to the overall success of MBC production. It impacts not only the prevention of mold and contamination but also influences the effectiveness of the final product. Each method has its merits and can be selected based on the requirements of the specific MBC production process at hand.

In conclusion, thorough sterilization is essential to ensure the safety, integrity, and efficacy of MBCs. By creating a substrate that is free from detrimental microorganisms, mycelium is provided

with a clean slate to grow and form the desired biomaterials, essential for applications in the construction industry and beyond. The literature serves as a guide, allowing producers to choose the most suitable sterilization technique for their unique substrate and production conditions, leading to high-quality and reliable MBC products.

9. Fungal Strain: Selection, Cultivation, and Inoculation

Inoculation is the cornerstone of MBC production, where the selection of a suitable fungal strain and its successful cultivation are paramount. The process begins with cloning, a technique that involves sectioning tissue from a mushroom fruiting body to obtain a pure culture in a controlled environment, free from contamination [60].

The initial stage of mycelium cultivation often employs media such as Potato Dextrose Agar (PDA), sometimes enhanced with silica to influence the fungi's physiological activities [28], or other nutrient-rich mediums like Potato Dextrose Broth (PDB), malt extract agar (MEA), and glucose-based complete medium (CM) [61]. These mediums are incubated in petri dishes at temperatures between 21 °C to 30 °C, optimal for most fungi, particularly the Basidiomycota group which does not tolerate temperatures above 40 °C [40], [62]. The incubation period ranges from 7 to 15 days, varying with the strain. Post-colonization, the petri dishes are preserved at 4 °C to maintain the mycelium.

Preparation of the spawn, which involves integrating the cultivated mycelium into a secondary substrate, can follow various methods such as solid-state fermentation, liquid-state surface fermentation [63], or use of a pre-grown homogenized substrate [15]. The widely adopted technique of Grain Spawn involves soaking and sterilizing grains, which, after cooling, are inoculated with mycelial plugs (1 × 1 cm) cut from the pure culture [64]. These inoculated grains are then incubated in darkness at 25-30 °C for another 10-15 days [36]

Grain spawn offers a rich, nutritious platform for mycelium growth, promoting uniform distribution and rapid expansion of the fungal network. The choice of grain, that can be based on rye, rice, wheat, sorghum, millet, or corn, is dictated by the fungal strain's preferences and material availability [29]. Additionally, substrates like cotton waste can also be utilized, further underscoring the versatility of the inoculation mediums.

Inoculation can also be achieved by simply introducing the pure culture of mycelium into the substrate under sterile conditions. The integration of PDB or other cultivation mediums with the feeding substrate can significantly affect the resultant MBC's properties, such as plasticizer production and chitin composition in the mycelium's hyphae [65]. The amount of inoculum used can vary; it ranges from as low as 1wt% to an average of 10 wt% and sometimes up to 25 wt% [58], [64]. It's worth noting that lower inoculum rates below 25 wt% have been associated with prolonged growth times and heightened risks of contamination [23].

Thus, inoculation is a delicate balance of science and art. It requires a deep understanding of mycology, precise environmental control, and careful timing to ensure that the mycelium thrives, ultimately forming a strong and viable composite material suitable for various applications. The thoroughness of this process directly influences the quality and integrity of the final MBC product.

10. Incubation and Molding in MBC Production

Following inoculation, the mycelium-infused substrate enters a critical phase of incubation, which is tailored to create the optimal conditions for mycelial colonization. The substrate is typically transferred to growing bags designed to maintain a controlled environment, with temperatures ranging from 18-25°C and relative humidity levels between 60% to 95%. This stage, which can last anywhere from 5 to 10 days, is dependent on the specific fungal strain and substrate in use.

The goal during this period is to allow the mycelium to thoroughly permeate the substrate, establishing a uniform and homogenous growth.

Once the substrate is fully colonized, it is commonly transferred into molds that dictate the final shape of the MBC. These molds can vary greatly in design, depending on the intended application of the final product. The transfer to molds marks the transition from a loose substrate to a defined biomaterial with a predetermined geometry.

Interestingly, some experimental approaches in MBC production have begun to challenge the conventional two-step process of incubation followed by molding. In these instances, the inoculated substrate is directly placed into molds immediately after inoculation, thus eliminating the separate incubation stage. This method has the potential to streamline the production process and reduce handling time and labor. However, the success of this direct-to-mold strategy largely depends on the compatibility of the mycelial strain with the new growth conditions and its ability to colonize effectively within the confines of a mold.

These approaches to incubation and molding demonstrate the evolving techniques within the field of biomaterials. By optimizing these steps, producers can enhance the efficiency of MBC production and potentially unlock new applications for these sustainable materials. Whether through traditional methods or innovative direct-to-mold strategies, the incubation and molding phases are integral to achieving a fully colonized, structurally sound, and application-ready mycelium-based composite.

The remarkable adaptability of mycelium-based composites (MBC) allows for their molding or 3D printing into an array of structural forms, such as insulating bricks or complex geometries, accommodating various architectural and design applications [66]. Once the mycelium is inoculated into the substrate, producers have several methods at their disposal to guide the mycelium through the colonization process and shape it into the final product.

The direct molding method involves placing the inoculated substrate into molds immediately post-inoculation. These molds can be fashioned from a variety of materials including plastic, aluminum, wood, 3D-printed matrices, or organic fibers. An alternative approach allows for the inoculated substrate to first undergo preliminary colonization within cultivation bags for a duration ranging from 5 to 15 days, followed by transferring the partially colonized substrate into molds.

Before introducing the mycelium-infused substrate into molds, sterilization of the equipment is crucial to minimize the risk of contamination. This is often accomplished using a solution of 70% ethanol. Although this may slightly slow the mycelium growth, it substantially reduces the potential for contamination.

Post-inoculation, the sealed molds with air-permeable covers create an ideal micro-climate to facilitate further mycelial growth. This second incubation phase, under controlled conditions like the initial phase, generally spans another 5 to 15 days and is dependent on factors such as growth conditions, substrate type, and the fungal strain [15], [42].

Novel methodologies in MBC fabrication include the use of frameworks, such as a rattan skeleton, which allows the mycelium to form self-supporting structures without the need for an external mold and is particularly effective for creating double-curved surfaces [54], Additionally, innovative growth chamber designs have been developed to harness the fungi's natural respiration to drive mycelial growth into void spaces, resulting in a pure mycelium structure devoid of growth media [67].

Despite the diverse methods available for shaping MBCs, there are inherent limitations to this material, particularly when considering the production of large, continuous blocks or structures.

Due to factors like the porosity and composition of MBCs, current applications often favor small-scale elements. These smaller components can be modularly assembled into larger structures. In architectural contexts, there is a growing inclination towards modular applications of mycelium products, which could be optimized for ease of assembly and structural performance in building systems [68].

In summary, the process of shaping and maturing MBCs is characterized by a variety of techniques that offer creativity and flexibility in design. The methodologies adopted must consider the balance between maintaining mycelium growth, preventing contamination, and achieving the desired structural and aesthetic qualities of the end product. The ongoing exploration in MBC fabrication continues to push the boundaries of sustainable material science, promising an array of applications from intricate design pieces to functional architectural components.

11. Growth Conditions for MBC: Temperature, Humidity, and Time

The cultivation of MBC is highly sensitive to environmental conditions, with temperature, humidity, and incubation time being crucial factors that must be meticulously controlled throughout the various growth phases. The parameters for these conditions are largely dictated by the characteristics of the fungal species, the nature of the substrate, and the specific method employed for molding the mycelium into its final form.

During the initial substrate colonization phase, which typically occurs in bags, a temperature range of 20° to 28°C is optimal, and a high relative humidity (RH) between 80% and 95% is maintained for a period of 5 to 10 days. This high humidity is crucial to prevent desiccation of the mycelium and to support vigorous and uniform growth across the substrate [69].

For the second phase, which involves shaping the mycelium within containers, temperatures are kept between 20° to 30°C with a similar humidity range to encourage the mycelium to form a cohesive matrix around the substrate particles. The timing for this phase can vary from 3 to 10 days.

The third phase, referred to as the binding interface phase, allows for the development of search hyphae and skeletal hyphae, vital for the structural integrity of the MBC. This phase can be conducted at temperatures ranging from 15 ◦C to 30 ◦C for 10 to 30 days, again with a high humidity of 80% to 95%.

For mycelium propagation, temperatures are generally kept within the 21 °C to 30 °C range for 7 to 14 days, providing an ideal balance for mycelium expansion without promoting premature fruiting or excessive water loss [70].

The cultivation of spawn is another critical process requiring specific conditions, notably high humidity around 98% and warm room temperatures of 24-25°C, which are conducive to mycelium growth. The temperature for spawn cultivation typically ranges from 24°C to 29°C, and the relative humidity can be between 60% to 90%. The growth time for spawn can span 7 to 20 days [66].

The conditions for substrate colonization must accommodate the unique requirements of the selected fungal strain and substrate, with temperature and humidity ranges that could fluctuate from room temperature up to 40 °C, depending on the species. For instance, P. ostreatus thrives at an average temperature of 29 °C, reflecting its natural subtropical habitat [35]. The RH during substrate colonization is similarly varied, spanning from 60% to as high as 95%, with the duration of growth extending from 11 to as much as 30 days [26], [71].

Thus, the successful cultivation of MBCs is contingent upon creating and sustaining the precise environmental conditions that foster optimal mycelium growth. By carefully managing these

parameters, the mycelium can robustly colonize the substrate, ensuring that the MBC possesses the desired physical and mechanical properties for its intended application. Whether for mushroom cultivation or bio-composite production, the precise control of temperature, humidity, and cultivation time is indispensable for achieving quality and functional biomaterials.

12. Post-Processing of MBC

The final stage in the production of MBC involves a series of post-processing treatments that are essential for enhancing the material's physical, chemical, and mechanical properties. These post-processing steps are critical for stabilizing the MBC, making it suitable for practical applications, and can include drying, pressing, and coating.

Drying:

Drying is a fundamental post-processing step aimed at reducing the moisture content within the MBC, thus denaturing the fungal tissue and preventing further biological activity. This is typically achieved using an oven or air-drying techniques at temperatures that can vary significantly depending on the specific requirements of the material [33], [42], [72]. The endpoint of drying is determined by a consistent weight, indicating that moisture has been effectively removed, or when reaching a residual moisture content of about 10% to 12% by weight [69]. However, drying must be controlled to prevent molding or deformation of the mycelial fibers.

Heat Pressing:

Heat pressing is another post-growth treatment where the material is subjected to heat for a certain duration to halt fungal growth and remove moisture. The process compacts the material, realigns the fibers, and can significantly enhance the mechanical properties, such as increasing the material's stiffness [44].

Cold Pressing:

Cold pressing serves to reduce porosity and improve the stiffness of the final product by increasing the Young's modulus. Unlike heat pressing, cold pressing applies pressure at lower temperatures to enhance material properties without the influence of heat [1].

Coating:

Coating applications, ranging from natural resins to beeswax, are explored to augment the MBC's durability, stiffness, and resistance to environmental factors while retaining biodegradability. These coatings can provide a protective layer, augmenting the MBC's performance without compromising its sustainable characteristics [46], [73].

To ascertain the effectiveness of these post-processing treatments, a comprehensive analysis of the MBC is required. Parameters such as dry density, Young’s modulus, compressive stiffness, thermal conductivity, water absorption rate, and spectral analysis through FTIR are evaluated to understand the impact of each treatment and refine the post-processing protocols [52]. These post-processing treatments are not just about drying or shaping the material; they're about unlocking the MBC's potential for use in various domains, from construction to design. By employing these methods, researchers and manufacturers can fine-tune the performance and sustainability of mycelium-based materials, ensuring their functionality and longevity in intended applications.

13. Mechanical Properties of MBC

The functionality and viability of MBCs in practical applications, especially in sectors demanding structural integrity, such as construction, are largely determined by their mechanical properties.

Research in the field has provided valuable insights into the factors that influence these properties, illuminating paths to enhance the performance of MBCs for a variety of uses.

Fabrication factors play a pivotal role in defining the mechanical characteristics of MBCs. As elucidated by Elsacker et al. (2019), processing techniques, the chemical makeup of the composite, and the conditions under which the mycelium is cultivated and processed collectively impact the strength, elasticity, and resilience of the final product. This includes an exploration into how the selection of lignocellulosic substrates contributes to the formation of a three-dimensional network within the composite, which is essential for creating lightweight yet durable materials.

Mechanical testing, such as compression and three-point bending tests, provides empirical data on how these materials will perform under load. Studies have shown that the material density has a direct relationship with the Young's modulus and elastic modulus, indicating that denser materials tend to exhibit better mechanical strength [15], [22]. These findings underscore the importance of optimizing substrate particle size, choosing the right fungal species, and determining the best post-growth treatments to tailor MBCs for specific applications, ranging from construction to food production and bioremediation.

Comparative analyses of MBCs utilizing different substrates have revealed significant variations in mechanical behavior. For instance, MBCs produced from sawdust typically have higher densities, which can translate to greater strength, whereas those derived from rice straw might demonstrate higher water absorption and average shrinkage capacities [25]. This suggests that the choice of substrate is not merely a question of availability or cost but is integral to the engineering of the composite's properties.

The interplay of fabrication processes, substrate selection, and post-growth treatments is complex and demands a thorough understanding to optimize MBCs for construction-related applications. By carefully managing these factors, it is possible to develop mycelium composites that not only meet but exceed the necessary standards for building materials, offering sustainable, lightweight, and strong alternatives to traditional construction materials.

14. Overview of Traditional Materials Compared with Biomaterials

As the main focus of this research centers on designing a dual-production system for Food and MBCs where the first attempt of this research were focused on optimize MBC production with specific properties to potentially start substituting the class of synthetic, mineral, and natural insulations traditionally used in construction it is reported a brief introduction of the traditional insulative materials used nowadays such as:

- Mineral-Based Insulating Materials that required an High Energy Consumption in terms of production of materials like fiberglass, rock wool, and aerogels requires high temperatures, leading to significant energy use and CO_2 emissions. furthermore this materials neet the extraction of raw materials (silica sand, basalt rocks, volcanic minerals) that can lead to soil degradation and biodiversity loss and the Disposal of this mineral-based materials are non-biodegradable and contribute to non-recyclable landfill waste
- Synthetic-Based Insulating Materials creating Environmental Concerns as they are Fossil Fuel Dependence as the Materials like EPS, XPS, polyurethane, and PVC are derived from petroleum and natural gas, depleting non-renewable resources. Furthermore the Manufacturing and disposal of this materials generate greenhouse gases and may release toxic substances into the environment and they are Low Recyclability as Most synthetic insulators are not easily recyclable, resulting in significant waste accumulation.

Furthermore these materials release Microplastic that can break down into small particles, which enter ecosystems and the food chain.

- Natural-Based Insulating Materials, create Environmental Concerns in terms of Land Use: Intensive farming (e.g., hemp, flax, or cotton) requires large land areas, potentially competing with food production or causing deforestation. Chemical Treatments: Many natural materials require treatments for fire resistance or pest control, reducing their overall sustainability. Limited Availability: Harvesting natural resources (e.g., cork, wool) can be seasonal and localized, leading to transportation emissions and supply limitations.
- Hybrid or Innovative Insulating Materials creating Environmental Concerns as they are Difficult Recycling: Hybrid insulators often combine synthetic and natural materials, complicating end-of-life recycling processes. Requires High Energy Production: Some innovative materials (like thermoceramic coatings or bio-based foams) require advanced manufacturing processes with high energy demands. Derived from Fossil Fuel Components: Despite improvements, many innovative insulators still rely on synthetic components derived from petroleum or natural gas.
- Mixed-Origin Insulating Materials creating Environmental Concerns for the High Carbon Footprint: Producing cellular concrete blocks or gypsum-fiber panels involves significant CO_2 emissions. Separation Challenges: Combining different materials (e.g., cellular glass and gypsum) makes recycling at the end of their life cycle difficult. Limited Durability: Some mixed materials degrade faster, requiring replacements and generating additional waste over time.

Sustainable biomaterials are essential for reducing reliance on fossil-based resources, addressing waste management challenges, and fostering circular economic systems. In the construction sector, sustainable biomaterials can play a pivotal role in mitigating climate change by reducing carbon emissions and promoting resource efficiency. This study positions MBCs as a viable solution for achieving these goals, emphasizing their potential to revolutionize material production and usage in the construction industry.

15. Industrial Scaling-Up Opportunities for MBC

The transition from laboratory-scale experiments to industrial-scale production of MBCs for construction applications is a domain ripe with potential, thanks to the pioneering research that underscores their feasibility and sustainability.

Elsacker et al. (2020) have laid down a comprehensive framework that may serve as a blueprint for large-scale MBC production, highlighting how mycelium can be harnessed to create lignocellulosic composites suitable for extensive construction applications. Jones et al. (2018) take this further by focusing on the application of waste-derived mycelium composites as construction materials, examining their fire safety and mechanical integrity, which are critical parameters for building materials.

Waste-derived materials are also the focus of Jones et al. (2019), who investigate the creation of mycelium nanopapers. Their research points to the adaptability of mycelium composites, capable of having tunable mechanical and surface properties essential for diverse construction needs.

Carcassi et al. (2022) address another vital aspect of industrial scaling: the environmental impact. Their work on assessing the carbon footprint of bio-based composites for building insulation is a step towards quantifying and reducing the ecological footprint of construction materials.

Stelzer et al. (2021) extend the conversation on environmental impact by performing a life cycle assessment of fungal-based composite bricks. Their work provides comparative data between lab-scale and potential industrial-scale production, an essential consideration for gauging scalability and sustainability.

Finally, Bitting et al. (2022) acknowledge the challenges of scaling up but also spotlight the opportunities mycelium-based materials offer for architectural applications. Their insights are valuable for directing future research and development efforts aimed at overcoming the barriers to industrial adoption. Collectively, these studies present a compelling case for the industrial-scale application of MBCs in the construction sector. They underscore the sustainable, cost-effective nature of MBCs and affirm their mechanical competencies, all of which are integral to making them viable alternatives to traditional building materials. For industrial scaling-up to become a reality, an analysis of the life cycle assessment (LCA), cost, production process, and energy requirements is crucial. It is through this lens that MBCs can be evaluated not just for their environmental benefits but also for their economic and practical viability in large-scale construction scenarios. The research paves the way for a new era in construction, one that leverages the unique properties of mycelium for creating eco-friendly, cost-effective, and structurally competent materials that can revolutionize the way we build.

16. Conclusions and Future Directions

The exploration of MBCs in this review reveals a confluence of sustainability, innovation, and potential within the construction sector. The biotechnological advancements in cultivating mycelium and transforming it into a viable building material underscore a significant leap towards more sustainable practices. With their intrinsic benefits such as low thermal conductivity, natural air-purifying capabilities, and carbon sequestration, MBCs present a promising alternative to conventional building materials. However, the road to widespread industrial application is laden with challenges that must be surmounted. These include enhancing the mechanical properties to suit load-bearing applications, reducing water absorption rates to improve durability, and developing standardized, scalable production methods that can meet the demands of the construction industry while maintaining the ecological integrity of the biomaterials. Looking to the future, the path is clear: further research is imperative to optimize the cultivation and processing of MBCs, to better understand their long-term behavior in various environmental conditions, and to develop new techniques that could streamline their production. Moreover, the industry must embrace a paradigm shift towards biofabrication and circular economy principles, integrating MBCs into the mainstream of construction material options. The potential market growth for natural products based on mycelium technology is robust. Yet, to capitalize on this growth, innovations in automation, production speed, and contamination control are necessary. In parallel, a regulatory framework that supports the adoption of biobased materials will be instrumental in propelling the market forward. In conclusion, MBCs are at the cusp of revolutionizing material science and construction. As this field matures, it is poised to redefine not just how materials are produced, but also how societies conceptualize and implement the ethos of sustainable living. Through continued interdisciplinary collaboration and innovation, MBCs can transition from a promising concept to an integral component of a resilient and environmentally conscious built environment.

Table 1. Analysis of the state of the art comparing the literature about MBC production

CITATION	RATIO	SUBSTRATES	SUPPLEMENTS	FUNGAL STRAINS	STERILIZATION METHODS	MOLDING	GROWING CONDITIONS	POST-PROCESSING	FINAL RESULT
[74]	The agricultural by-products were tested as both sole constituents and in 50–50% mixtures ratios	switch-grass, rice straw, sorghum stalks, flax shive, kenaf and hemp.		basidiomycetes		16 cm × 16 cm molds at a depth sufficient to generate a finished nominal thickness of 2.5 cm	environment chamber under dark warm humid conditions for 4–6 days		acoustic absorption panels
[53]	kenaf/hemp mix and later a corn stover/hemp mix both 50/50% by weight	Natural fiber textile as jute, hemp, and cellulose were used as skin, while agricultural waste as core, and bioresin as matrix				Thermoformed trays	The incubation process lasts for 5 days and takes place on growth racksat room temperature (24 ◦C).	Drying the composite at high temperature (>80◦C) in a convection oven for almost an entire day	sandwich structures
[75]	25 wt% of fungal inoculum mixed with the substrate	wheat grain		Trametes versicolor	autoclaved at 121 °C and 103.4 kPa for 90 min	10 × 10 cm sterile plastic molds	incubated under standard atmospheric conditions for 6, 12 and 18 days	dried at 50 °C for 48 h	mycelium-biomass composites
[56]		Rapeseed straw, beech sawdust, and non-woven low-quality cotton fibers	bran	Pleurotus ostreatus, and Trametes multicolor	sterilized in autoclavable bags	34 × 34 × 4 cm, PET-G of Plastic thermo-formed molds	25 °C for 14 days in the dark and once demolded kept at the same conditions for 10 more days	Heat (150 °C) or cold (20 °C) pressing was performed with a mechanical multi-plate press for 20 min at F b 30 kN. And then dried at environmental conditions for 24-48 h	product design and manufacturing objects
[76]	3% inoculum added to the substrate mixture	Pruning residues of apple and vinecrops	1% flour and 3% wheat straw	Colorius sp.; Trametes sp.; Ganoderma sp.	autoclaved at 100°C for 1 hour.	incubation vessels, or filtered bags and then growing vessels.	23 °C for overall 14 days at 95% humidity	After incubation, the samples were oven-dried for 48 hours at 60 °C.	mycelium-based composites
[44]	20% wt of fibres, 70% wt of sterile demineralised H2O and 10% wt of mycelium spawn	hemp, flax, flaxwaste, softwood, and straw		T. versicolor	autoclave for 20 minutes at 121℃.	PVC molds	28℃ for 8 days and after demolded in the laminar flow and incubated in a micro box for another minimum of 8 days without molds	convection oven at a temperature of 70 ℃ for 5 to 10 hours	MBC
[77]		sawdust, straw and a mixture of sawdust and straw	wheat bran	Pleurotus Ostreatus	autoclave for 45 minutes in 121℃.	Formworks are cubes of 5x5x5 cm3 made of plastic covered MDF and plexiglass	Growth chambers that have 99% relative humidity and a temperature of 24±1 °C for cultivation for a total of 14 days + 3 days into molds in a dark environment that has a temperature of 24±1 °C	Drying rack for 6 hours using a fan, which led the samples to lose 1/3 of their weight. Consequently, they are placed in the oven for 90 minutes in 90 °C	MBC
[67]	56.3% ground corn stover, 27% grain spawn, 2.4% maltodextrin, 0.8% calcium sulfate and 13.5% of a complex of nutrients and mineral mixture	ground corn stover	2.4% maltodextrin, 0.8% calcium sulfate and 13.5% of a complex of nutrients and mineral mixture	Ganoderma sp.	sterilization	grown in a specially designed growth chamber. The chamber is designed such that as the fungi grows and respires, thereby producing carbon dioxide (CO2) at elevated temperatures between 30° to 35 °C		the biopolymer was cut away from the substrate and dried at 43 °C	pure mycelium culture for acoustic absorption panels.
[78]	38% of ground woodchips; 1% flour and 3% wheat straw; mixed with 62% distilled water and 3% inoculum	pruning residues of apple and vine crops	1% flour and 3% wheat straw	Trametes versicolor, Trametes ochracea and Ganoderma sessile	autoclaved at 100 °C for 1 h.	Polypropylene Microbox incubation vessels (118ODþ00118/80 model, Vol. 565 mL)	23 C and 50% relative humidity for a total of 12 days	oven-dried for 48 h at 60 °C.	MBC
[48]	5:2:1 by mass for C:RB:CH with 350 mL water added to the mix. The SD mix contains a ratio of 5:3:1 by mass for C:SD:CH with	Rice Bran bricks (RB); Rice Bran (RBM); Sawdust bricks (SD); Sawdust (SDM); Clay Bricks (C); Clay (CM)	rice and sugarcane molasses (SCM)			20 cm length × 9 cm width × 6 cm height steel brick mold	33 days of incubation	oven at 110 °C to 115 °C for not less than 24 h	mycelium bricks

	450 mL of water added to this mix.								
[43]		Bamboo culms of Dendrocalamus asper	Chitosan with mycelium-enriched bamboo	Ganoderma lucidum	autoclaved at 121 °C for 1 h	Cultivation bags	Incubated at 25 °C to 28 °C, relative humidity of 65% to 80% and at pH of 5 to 6 for 1 to 4 weeks in a filtered air environment. Then the mycelium was then left to grow on the extruded struts for 20 days, in air at 23 ± 0.5 °C and 65–70% humidity.		extrudable paste of mycelium-bound composites with complex 3D shapes
[70]	The final water to substrate ratio (w/w) with oat husk 1:1, oat and birch sawdust 1:2, oat straw 1:2, rapeseed cake 4:3.	Oat husk and rapeseed cake, oat husk without milling, pine sawdust, oat straw, birch sawdust		Trichoderma asperellum, Agaricus bisphorus. Lentinula edodes, Pleurotus ostreatus, Ganoderma lucidum, Pleurotus ostreatus sajor caju, Pleurotus ostreatus florida, Kuehneromyces mutabilis, Flammulina velutipes.	autoclaved at 120 °C for 20 min		21 ± 1 °C in the laboratory cabinet. After 14 days, the cultures were visually inspected for quantitative and qualitative growth properties.		MBC
[79]	The mycelium pellets 10% w/w were mixed with the substrates	hexane extracted rose flowers (Rosa damascena Mill.) and the steam distilled lavender straw (Lavandula angustifolia Mill.)	MgSO4, KH2PO4, K2HPO4, peptone, yeast extract, and CO3	Ganoderma resinaceum	autoclavable mushroom growing bags (SacO2, Belgium) (100 × 350 mm) and sterilized at 121 ◦C for 45 min	3D-printed molds (55 × 55 × 55 mm), made of poly-lactic acid (PLA)	grow in the mushroom growing bags in darkness at 25 ◦C for 7 days + 7 days incubated at 25 ◦C, 95% humidity in the moulds + 6 days de-molded in the incubation chamber	drying oven SLW 32 (POL-EKO-APARATURA, Poland) at 60 °C for 8 h	MBC
[80]	inoculated straw with 10% spawn	Barley straw		Lentinus crinitus	moistened for 12 h and then pasteurized at 60 °C for 1 h		incubated for seven days at 25 °C and then for 8 days were filled with the colonized straw, cover with a plastic mat and incubated for 7 days more.	pressed with two different loads, group G1 (n=6) was loaded with 3922.66 Pa for two hours and group G2 (n=6) was pressed with 17651.97 Pa for two hours + dried at 45 °C for 72 h	MBC
[81]	sawdust, wheat bran, and CaCO3 were dry mixed with W/W percentages of 88%, 10% and 2% Water and dry EFB were mixed at a ratio of 5:4 by weigh; DMC:Organic wheat grains, wheat bran, and CaCO3 were dry mixed with weight percentages (wt%) of 88%, 10%, and 2% + 20 wt% water	Sawdust from Albizia chinensis, and empty fruit bunch (EFB) fibers	wheat bran and calcium carbonate CaCO3	Ganoderma lucidum	2 L pp bags, hot sealed, sterilized at 120 °C for 60 min	180 cm in length x 30 cm in width moulds designed according to the size of the pressing machine	26–28 °C with 70–80% humidity for 2 weeks	kept in an oven at a temperature of 70 °C for 2 days	mycelium-bound composites (DMCs) resembling commercial particle boards
[44]	200 g hemp fibres + the fibre mixture was supplemented with 10 wt% of mycelium spawn	particle boards from hemp chips, Bacterial Cellulose mixed with hemp fibres	bacterial cellulose and other organic additive	Bacterial cellulose K. xylinus, Trametes versicolor	autoclave at 121 °C for 15 min. MBC: autoclave at 121 °C for 20 min	18,5 × 18,5 × 7,8 cm microbox containers (SacO2, Deinze, Belgium) with a depthfiltration system on top	Incubated at 26 °C during 10 days and then samples were removed from the containers and incubated for 2 days more	heat-press compression, either at 70 °C or at 200 °C. The BC-mycelium samples were compressed with an Instron 5900R with an oven built around by applying a maximum force of 30 kN at 2 kN/min.	bio-fabricated and biodegradable composite materials
[33]	3% (w/w) of the mycelium inoculum was inoculated on top of the substrate.	Cotton stalk, wheat bran	natural reinforcement particles and carbonate sand	Pleurotus ostreatus, Oudemansiella radicata, and Acremonium sp.	Sterilized for 2 h under 0.12 MPa at 120 ◦C	cylinder polypropylene bag with a diameter of 8 cm and a height of 9,5 cm	temperature (24 ± 1 ◦C) and relative humidity (50%) in the dark. The colonization periods varied with the different fungi. O. radicata (28 days on average) P. ostreatus (31 days) and	dryer at 24 ◦C for 72 h	MBC

							Acremonium sp (37 days).		
[82]	rice bran volume ratio to the substrate is 9:1	hemp, rice straw, lacquer tree wood chips, and oak wood chips	rice bran	Pleurotus ostreatus	sterilized for 90 min at 121 ◦C.	10 cm × 10 cm x 1 cm (w x d x h) stainless steel mold	22 ◦C (±2 ◦C), relative humidity of 65% (±5%), with ventilation and no light for 7 days	Dryer (L'EQUIP FilterPro 6, Korea) at 60 ◦C for 24 h	Sound absorbent, insulation, and fire-resistant materials.
[83]		wood chips and two wood panels as skin based on spruce solid wood, chipboard, MDF, and poplar plywood		Ganoderma Lucidum	autoclaving or irradiation with gamma rays	formwork for the mycelium panel core	temperature about 25°C and humidity of 80–90%	denaturation with heat ca. 80°C	MBC sandwich panels
[47]	Substrate 95% and fungal strain 5%	Bran/cotton and bran/hemp	Bran	Mycelium inoculum is grown by Mogu	polypropylene thermal resistant and microfiltered SacO2 bags (PP50/SEU4/V4051) sterilized for 90 min at 123 ◦C at 1500 bar	Plastic thermo-formed mould covered with perforated cellophane foil (0.35 µm)	growing room with 75 % of humidity and at 25 °C for 14 days in the dark		MBC
[84]	5% rice bran, 1% calcium carbonate, 2% calcium sulfate, and 0.2% sodium sulfate on a dry mass basis. The ratio of fungal inoculum to substrate mass was 1:100 (w/w)	Lignocellulosic residues (sawdust, corn husk, rice straw)	rice bran, calcium carbonate, calcium sulfate, and sodium sulfate on a dry mass basis	Ganoderma fornicatum, Ganoderma williamsianum, Lentinus sajorcaju, and Schizophyllum commune	(3.50 inches wide and 12.5 inches long) polypropylene bags sealed with cotton-plugged polyvinyl chloride pipe rings, covered with pieces of paper, and autoclaved at 121 ◦C for 60 min.	cylindrical plastic boxes with a ratio diameter and height of 2:1 (8,6 cm in diameter and 4,3 cm in height). Molds of 8,5 cm in diameter and 1,3 cm in height. Molds designed from acrylic clear sheets shaped 16,5 × 1,9 cm, neck 5,7 × 1,3 cm, a rectangular shape (12,7 × 1,2 × 0,3 cm), and a rectangular shape (6,3 × 1,2 × 1,2 mc)	incubated at 30 ◦C in darkness for 14–21 days substrate that was colonized with each fungal mycelium was put into the mold, pressed using a unidirectional cold press machine (Shop press ZX0901E-1, New Taipei, Taiwan) set at 0.5 MPa for 10 min, and incubated at 30 ◦C. After three days of incubation, MBCs were removed from the mold and incubated in a plastic box for another three days until the mycelia covered the sides	dried in an oven at 70 °C for 24 to 72 h	MBC
[25]	Each type of agricultural waste was mixed with 5.0% rice bran, 1.0% calcium carbonate, 2.0% calcium sulfate and 0.2% sodium sulfate (Kupradi et al., 2017). Then, the mixtures were adjusted to a relative humidity of 60% by adding water.	Two different agricultural wastes namely the sawdust of rubber tree and rice straw	rice bran, calcium carbonate, calcium sulfate and sodium sulfate	Earliella sp., Hexagonia sp., Lentinus sp., Pleurotus sp., Pycnoporus sp.	substrate mixture was placed onto a glass plate (9 cm in diameter). The plates were autoclaved at 121 °C for 60 min.	glass plates	The plates were incubated at 30 °C for 14 d in darkness + myco-composite samples were removed from the glass plates, placed in separate plastic boxes and incubated at 30 °C. After 3 d incubation,	dried at 70 °C for 24 h	MBC
[37]	10% Teff bran (on a dry weight basis) was added to each prepared substrate + 3% of calcium sulfate (on a dry weight basis) + Each substrate (1000 g) with 60 to 70% moisture was inoculated with 10% spawn (100 g)	sawdust, bagasse, and coffee husk	Teff bran and calcium sulfate	Pleurotus ostreatus	sterilized in an autoclave at 121°C for 60 min	11 cm × 8 cm x 4 cm size plastic mold	dark room at (22 + 1°C) + after molding three incubation periods (7, 14, 21 days) [26, 31], under aseptic conditions and incubation temperature was adjusted to 22 + 1°C	heat of about 50°C for 48 h was applied	Mycoblock

[46]	This substrate was composed of 30 g of bamboo fibers, 15 g of oats and 45 ml of deionized water + 30 g of the mycelium spawn was added to the organic substrate at a mass ratio spawn: substrate of 1:1	Dendrocalamus asper were obtained from bamboo sheets for Bamboo microfibers substrate	Oat	Pleurotus ostreatus	This substrate mixture was mixed thoroughly before being sterilized in an autoclave (Hiclave HG 80, Hirayama, Japan) at 121 °C for an hour.	ice cube tray 3 × 3 × 1cm3	The samples were then kept in a cupboard in the dark for 28 days at a room temperature of 22 °C and a relative humidity of 80% for a maximum of 28 days		MBC for in-door application
[71]	mixtures ratios: straw and sawdust ratios of 1 to 1, 2, 3, 7, and one with sawdust only + Sterilized substrates were inoculated with the spawns by 7% of the dry weight in a sterilized environment.	Oakwood pellets and wheat straw	whole wheat flour	Pleurotus ostreatus	Bags were sterilized in the autoclave machine at 121 ◦C temperature for 40 min	the formworks for this experiment were made of cardboard covered with plastic tapes to avoid the hyphae feeding on the cardboard and decrease the cardboard's humidity level by capillary action. All formworks were cubes of 5 cm in length	temperature was set to 21 ◦C, and the relative humidity to 95%. The room was kept dark at three different durations of growth for 5, 6, and 7 weeks	oven for 48 h at 92 ◦C.	MBC
[22]	10 wt% Pleurotus ostreatus or Coprinus comatus grain spawn, 22.5 wt% dry substrate (chipped and sieved straw), and 67.5 wt% water for both batches.	straw		Pleurotus ostreatus and Coprinus comatus	the first batch of substrate was sterilized for 30 min at 121 ◦C and 16 psi and the second was pasteurized	molds built based on the ASTM testing requirements	6.5 weeks (i.e., 46 days) in a dark opaque tent. The temperature and relative humidity inside the tent were 25.7 ± 0.2 ◦C and 50.5 ± 5.6%. Batches were grown at a different time of the year: from 21 March to 6 May 2020 (first batch) and from 29 June to 14 August 2020 (second batch).	dried, baked, compacted then dried, or compacted then baked. Myceliated materials were dried in an oven for a total of 12 h at 40 ◦C. Oven for 6 h at 100 ◦C to bake. Hydraulic press 20 TON shop press	MBC
[85]	MYCELIUM SPAWN: 15 g of rapeseed straw and 30 g of recycled cellulose with 35 or 70 g of water + with 3% spawn based on the dry weight MYCOBLOCK:96 .5kgofrecycled cellulose and96.5kgofrape seed strawbyhandinbat ches of2.1kg, followed bymixing 450.33 Lofwater(4.9Lper batch) + 3% of spawn (63 g per bag)	Rapeseed straw and recycled cellulose		Trametes betulina	sterilization 121° for 30 min	poly (methylmethacrylat e) (PMMA) molds (20cm × 20cm × 40cm; about 9.44 kg of the substrate into each mold) and ripening box (polypropylene crates with small slits,where four blocks can be placed without touching each other)	5 °C for 8 days,after which the colonized substrate was cast into molds. Growth was prolonged for 4 days at 25 °C in the incubator, after which the mycelium biocomposite was transferred to a ripening box for another 2 days at 25 °C in the incubator.	heat treatment at 60 °C for 4 hours	lifecycle assessme nt of MBC as 20 × 20 × 40 cm mycoblock
[86]		Mycelium and bamboo and Wood chips, sawdust and fibrous waste products from the regional food industry such as sugarcane and cassava root were used as substrates		Ganoderma Lucidum			grown under varying conditions		MycoTree is a spatial branching structure made out of load-bearing mycelium component s.

[35]	rice husk, 2% (w/v)CaCO 3, and 10% (w/w) rice bran + moisture content was set to 50% (w/w), and 20% (w/w) commercial spawn of P. ostreatus was added	Sugarcane bagasse, sawdust, rice husks	2% (w/v) calcium carbonate (CaCO3) and 10% (w/w) rice bran	Pleurotus ostreatus	autoclaved at 121 °C for 15 min to sterilise.	rectangular plastic container (10 cm × 10 cm × 5 cm)	incubated at different temperatures (20, 25, 30, 35, and 40 °C) f and the process was stopped on the 11th day of the growing period	oven at 100 °C for 24 h	biofoam
[54]		Natural rattan framework as a supportive structure for mycelium substrate + Short, chopped fibers such as wood chips and long continuous fibers such as hemp are compared as a hosting environment		Pleurotus ostreatus	sterilized via pasteurization		20-25 °C over 3 weeks		Mycelium-based composites products
[58]	The mixture was then blended with 60 wt% (weight percentage) of water, and mixed with 1 wt% of colonized mycelium spawn.	Wood-veneer made of maple (Acer pseudoplatanus) choosen among Maple “beech (Fagus sylvatica), oak (Quercus robur), and spruce (Picea abies) veneers and willow branches of the genus Salix americana. Substrate made of Hemp hurds	mixed with wheat bran to enhance the growth of mycelium, while calcium sulfate (CaSO4) was added in a dry condition to adjust the pH of the mixture to the desirable threshold of 5 to 6, suitable for mycelium growth	Ganoderma lucidum	sterilized at 121 ◦C for 60 min.	molds	The colonized substrate was eventually left in the incubation room at 26–28 ◦C with 70–80% humidity for two weeks to develop the full mycelium network and then was placed into molds with similar conditions to the previous phase for an additional 3–6 days until the mycelium network was observed to have covered the substrate surface. Then, the mold was removed, and the samples were left in the incubation for another 3-5 days	drying oven and kept there at a temperature range of 60–70 ◦C for 2–3 days.	structural performance of mycelium composites for interior use integrating continuous wood fibers into the mycelial matrix as reinforcement
[61]	hemp shives and rapeseeds straw were hydrated with 150 wt% of water in separate cultivation bags and 5 wt% overgrown millet spawn was added to the wet substrate.	hemp shives (Hemparade) and rapeseed straw (Optistraw)		Fomes Fomentarius	The mixture was sterilized by autoclaving	plastic tube of 7 cm diameter and 6–7 cm	Bags were incubated for 14 days at 25 °C in the dark after inoculation and then placed into samples that were incubated for 7 days in the mould followed by another 7 days after removing the mould. To reduce the risk of contamination and ensure a high relative humidity of 80–100%, incubation was done in a disinfected closed plastic box (IKEA, Sweden) with two sterile sponges soaked in sterile distilled water.	Finally, growth of F. fomentarius was stopped by drying the samples in an oven (B5090E, Heraeus, Germany) at 60 °C for 2 days	Mycelium composite
[21]	fibres and hessian were prepared at 40% Moisture Content and then principal substrates were mixed with 16 wt% G. lucidum spawn	European beech wood (Fagus sylvatica) + Longitudinal fibres were introduced in a specimen series by using common reed fibres (Phragmites australis) + Fibres perpendicular to compressive stress were studied with use of rattan fibres (Calamus manan) + hemp-based hessian jacket (Cannabis sativa)		Ganoderma lucidum	sterilised at 121 ◦ C for 15 min	the principal substrates were massaged to break them down and formed with the fibres and hessian into aerated PETG moulds.	and incubated in PP filtered bags for 7 days at 25 °C in the dark. Once colonised. The principal substrates were massaged to break them down and formed with the fibres and hessian into moulds. the formed specimens were incubated for 21 days at 25 °C in the dark	oven dried for 48 h at 60 °C.	MBC

[87]	The principal substrates, fibres and hessian were prepared at 40% moisture content with mineralized water. The principal substrates were then mixed with 16 wt% spawn	European beech wood (Fagus sylvatica) + Longitudinal reinforcement was introduced in the specimens group by using 5 mm diameter rattan fibres (Calamus manan), and hessian was used for other groups (Cannabis sativa)		Ganoderma lucidum	sterilised at 121 ºC for 15 min.	aerated PETG moulds	incubated in polypropylene filtered bags for 7 days at 27 ºC in the dark. Once colonised, the principal substrates were broken down and formed with the sterile fibre and hessian into alcohol cleaned moulds. The formed specimens were incubated for 21 days at 27 ºC in the dark	then oven-dried for 48 h at 60 ºC	MBC
[69]		Beechwood		Ganoderma lucidum, Pycnoporus sanguineus		molds	Incubation for 5 and 7 days, at a temperature ranging from 20 °C to 28 °C, and at humidity ranging from 80% to 95% + shaping containers defining the shape of the composite material, and incubating the mixture in a second incubation phase for a time between 3 and 10 days, at a temperature ranging from 20 °C to 30 °C, and at humidity ranging from 80% to 95% + Joining at least two basic units through the binding interface and incubating for a time between 10 and 30 days, at a temperature in the range of 15 °C to 30 °C, and at a humidity in the range of 80% to 95%	Denaturizing the specimen at a temperature range of 65 °C to 90 °C and obtaining a mycelium-based lignocellulosic composite with a residual moisture content of 10% to 12% by weight based on the total weight of the composite	mycelium and chipped wood composite for building components
[88]	Ratio of Mycelium and Substrates 1:2	hay, rice shell, beech shavings, walnut shell	rice shell powder	Pleurotus Ostreatus	sterilization	Then the mixture is sterilized and allowed to grow in cling film-coated molds with dimensions of 4x4x16cm.After the molding process, the top of the mold is covered with stretch film and holes are drilled on it for the sample to breathe.	grow in bags for 7 days to create a more comfortable growth environment before molding. The incubation medium created for mycelium composite samples; is completely dark, with a relative humidity of ~75-80% and set at 24°C. At the end of the first period of 7 days, the mixture in the bag was poured into the sterile container, crushed and mixed until the whiteness dissipated and molded. The samples left to grow in the mold were removed from the molds at the end of 7 days to ensure equal growth on each surface. The specimens, which were planned to have a growth period of 7 days, were dried. The remaining samples were taken from the growth medium according to the planned periods of 14, 21, and 28 days.	The specimens, which were planned to have a growth period of 7 days, were dried at 100°C for 45 minutes to stop the growth of mycelium.	MBC

[26]		Yellow birch (Betula alleghaniensis Britt.) wood veneers		Trametes versicolor	wood particles was poured into a filter patch bag and steam-sterilized at 121 °C for 60 min.	square Petri dishes (80 mm × 80 mm × 14 mm) 50 g (dry weight: 19.8 (±1.3) g) per dish.	The filter bags were incubated at 28 ◦C, 80% RH for 8 days, then the mixture was transferred to a stand mixer with a paddle mixing blade and was mixed at speed 2 for 2 min.The mixture was then packed in square Petri dishes and incubated at 28 ◦C, 80% RH for up to 30 days.	After specific incubation periods, half of the sampleswere oven-dried for 48 h at 50 ◦C to produce as-grown foams. The other half of the samples were hot-pressed using a laboratory press to produce high-density panels. The hot-pressing process was conducted at 180 ◦C for 8 min with a thickness control of 4 mm using metal stops (pressure was not controlled during the process).	grown foams and hot-pressed (densified) panels
[89]	the amount of mycelium used for inoculation was 10% of the weight of sterilised susbtrate.	beech sawdust (FS), oak sawdust (Q), bleached cellulose pulp (CS1), shredded cardboard (SC), shredded newspaper (SN), cotton fibres (CO), soy silk fibres (SF), wheat bran (WB), straw (ST), burlap (B), clay (C) and sand (S) + burlap fabric made of woven from jute		Pleurotus ostreatus and Ganoderma lucidum	the substrate was put in a polypropylene microfilter bag and sterilised in a pressure coocker at 121 "C for 45 min.	moulds 10x10x2 cm	22 to 24"C in an environment protected from light sources for 14 days	dried over a heating source	MBC
[90]	65% moisture content by adding water. Each prepared substrate mixture contained 100 g of dry weight material, 185 g of water, and 18 g of wheat bran.	Paper-based waste products (sorted office paper, cardboard, and newsprint)	10% (w/w) wheat bran	Pleurotus ostreatus	polypropylene autoclavable bags autoclaved for 45 min at 121 ◦C.	rectangular acrylic formworks	The mycelium was left to grow in the bags for 12 days with 99% relative humidity and a temperature of 24 ± 1 degree Celsius. After 12 days of growth in bags, the cultivated mycelium mixtures were transferred to the frameworks for 16 days in the same environmentally controlled growth room	oven at 90 ◦C for 24 h	mycelium-based acoustic panel prototypes.
[91]		Silica source substrate and polypropylene fabric		F. oxysporum (ATCC MYA-1198),	sterilized at 120 °C for 15 min				fire-resistant behaviors of novel fungal fibers
[28]		rye berries feedstocks		Pleurotus ostreatus	A filter patch bag with 454 g of rye berries was sterilized at 121 ◦C for 2.5 h by using saturated steam at 15 psi p	silicone gel molds	which was observed clearly by naked eyes after 3 days. After a 7-day incubation the mixture of fungi spawn and feedstocks were removed from bag and manually ground homogenized. 40 g, 42 g, and 45 g of myceliated feedstocks were transferred to molds. The substrate was allowed to grow in the mold for four days, and it was flipped over for additional four days to minimize the errors generated due to sampling. The incubation environment was controlled as 65% RH and 25 ◦C.	oven for 24 h at 65 ◦C	Mycelium-composite insulation brick

[60]	ratio of spawn to substrate is calculated by weighing the damp substrate and taking 5–10% of this weight for the grain spawn	European beech (Fagus sylvatica (S1)) was taken in the form of sawdust (S2)		Pleurotus ostreatus	into a polypropylene microfilter bag (S5). The sterilisation process lasts 20 min once the autoclave has reached a temperature of 121 °C	mould fabrication involves a process of laser cutting a PVC/PE foil	dark environment with temperatures ranging from 20 to 24 °C (growth process). After 14 days	left to dry. In order to make sure that the fungal growth is terminated, the sample should be exposed to a minimal temperature of 40 °C for 30 min (passive solar heating is being used for this step)	MBC Blocks
[92]	inoculation of the fungus (three different concentrations: 15%, M1; 20%, M2; and 25%, M3)	plastic and sugarcane bagasse		Pleorotus ostreatus	121°C for 15 min at 1 atm of pressure	20 × 15 × 3 cm wooden molds	The substrate was added to mycelium and placed in sterile 1 L beakers and incubated at 26 ◦C for 10 days and then placed into the moulds and incubated at 26°C for 14 days under aseptic conditions + After 14 days, the molds were removed, and the biomaterial-based blocks were incubated for 5 days at 26°C.	oven at 60°C for 24 h.	biomaterial -based blocks
[64]	The amount of fungi spawn in the mixture was set to a fixed 10%	Bamboo or wood shavings		Trametes versicolor, Fomitopsis pinicola, Agaricus bisporus	sterilized	mixed in polypropylene bags for 5 days and then transferred to ylindrical and cuboid molds while applying subtle pressure to obtain a more compact assortment	5 days + 28◦C for 10 days,	oven at 70 ◦C for 10 h	mycelium-based bio-composites (MCBs) for construction applications
[27]	and mixtures of wheat bran + wheat straws, wheat bran + sawdust and wheat bran + coconut husk fibers at a ratio of 1:3.	wheat bran, minced wheat straws; coconut husk fibers; broadleaves sawdust		A. biennis RECOSOL73, B. adusta RECOSOL20, D. tricolor RECOSOL10, F. fomentarius RECOSOL11, I. lacteus RECOSOL61, P. ostreatus RECOSOL32, L. arcularius RECOSOL40, T. versicolor RECOSOL94	sterilized through autoclaving at 121°C	molded in a “baking tin” shape between two metal sheets 15 mm thickness (10 mm at wrinkles), 45 mm height, 80 mm diameter at bottom and 120 mm diameter at top	pre-screening 25°C for 12–32 days; final results 25°C for 3 days after that period, the cover the metal sheet was removed, and cultures incubated further for 27 days, assuring humidity.	Resistance to temperature was tested by placing fragments in an oven at 150 ◦C for 1 h and subsequently at 200 ◦C for 2 h	MBC
[93]	the mass ratio of chopped straw to wheat bran was set as 83:17	rice straw, wheat straw and corn straw,	wheat bran	Ganoderma lucidum	autoclave at 121°C for 2 h	mold and then packed with a sterilization bag	28 ◦C for 14 days.	oven at a temperature of 60°C for 36 h	MBC
[29]	For pine sawdust a solid:liquid ratio of 1:2.5 (w/w) and then 10% (w/w) of the fungal spawn was mixed with each substrate	(pine sawdust, oak shavings, tree of heaven wood chips, wheat straw and shredded beech wood). 31. Wheat straw and four different wood by-products (pine (Pinus spp.) sawdust, oak (Quercus spp.) shavings, tree of heaven (Ailanthus altissima) (Mill.) Swingle (1916) wood chips and shredded beech (Fagus sylvatica L., 1753) wood	10% w/w oat bran	Trametes versicolor, Pleurotus ostreatus, P. eryngii, Ganoderma carnosum, Fomitopsis pinicola	121 ◦C for 45 min.	9 × 12 × 4 cm molds	The bags were incubated at 25 ± 2 ◦C in the dark until the mycelium had colonized the material completely, then the substrate was kneaded and introduced molds for 4 to 5 days’ incubation in the same conditions.	oven dried at 60 ± 2 ◦C for 48 h	insulation panels

[49]		Hemp hurds, hemp fiber, red laterite particles	calcium and carbohydrate – starch	Pleurotus ostreatus		packed into molds (30 × 10 × 5 cm)	mycelium mixed with hemp hurds were grown in filter patch bag for 5 days by supplying nutrients (calcium and carbohydrate – starch) and water + 5 days in moulds growing conditions (temperature of 25 ◦C and relative humidity of 60%)	air-dried for 48 hours before desiccation in an oven at 93 ◦C for 30 minute	mycelium blocks
[94]	the ash chips were seeded at a 1:10 ratio with colonized rye grain, enriched with 3 tablespoons of un bleached flour	Insecticide- and fungicide-free ash chips	bleached flour and unbleached flour	Pleurotus ostreatus	sterilized for 40 min at 121 ◦C	The shavings were placed densely in 12 cm x 7.6 cm x 9 cm	incubated for 14 days at 25◦C or until the chipswere fully colonized. When the wood shavings were fully colonized, they were mixed with 3 tablespoons of unbleached flour and then it was placed in the molds and incubated for 14 days at 25 ◦C,	24-hour period in an oven at 80 ◦C	bio-based insulation boards
[62]	VISIT -> Investigation of Mycelium-Miscanthus composites as building insulation material" (Dias et al., 2021, p. 0)	Miscanthus and mycelium	VISIT -> Investigation of Mycelium-Miscanthus composites as building insulation material" (Dias et al., 2021, p. 0)	Ganoderma resinaceum and Pleurotus ostreatus	VISIT -> Investigation of Mycelium-Miscanthus composites as building insulation material" (Dias et al., 2021, p. 0)	VISIT -> Investigation of Mycelium-Miscanthus composites as building insulation material" (Dias et al., 2021, p. 0)	VISIT -> Investigation of Mycelium-Miscanthus composites as building insulation material" (Dias et al., 2021, p. 0)	VISIT -> Investigation of Mycelium-Miscanthus composites as building insulation material" (Dias et al., 2021, p. 0)	eco-friendly building insulation material
[57]		Rubber wood sawdust (RBS)		Pleurotus ostreatus	sterilization process using an autoclave at a temperature of 121 °C for 20 min	.sterile plastic case with dimensions of 210 mm × 150 mm × 30 mm	dark room with a temperature of 29 °C for 21 days.	composite to heat pressing using a hot press machine. The pressure applied was 5 MPa, and the pressing duration was set at 20 min. The heat pressing stage was conducted at two different temperatures, 130 °C and 180 °C	MBC
[40]	Following the sterilisation process, the ratio of fungal spawn (P. ostreatus) and substrate mass (SCGs) was 1:100 (w/w)	Spent coffee grounds (SCGs), natural pineapple fibers (NPFs), sawdust	16% rice bran as a nitrogen source, 4% flour as a carbon source, and 2% pumice as a mineral source, based on their dry mass	Pleurotus ostreatus	substrates were autoclaved at 121 °C for 20 minutes	aluminium-formed moulds,	room temperature with 70%–80% relative humidity in a growth chamber and placed in a semi-permeable polypropylene bag for 28 days	oven at a temperature of 80 °C for 3 days	MBC
[95]	The mixing ratio for rice husk into each substratewas 1:10, i. e., 10% rice husk addedto each substrateas. Also, 10 g of calcium carbonate was added to bagasse substrate + 10 to 15% of mycelium spawn was added into each mould of substrate	three different substrate types namely, bagasse (B + RH), coconut husk (CH + RH) and a mixture of coconut husk plus bagasse (CH + B + RH)..	Rice husk (RH) was added to all substrates for mycelium nutrients.	Pleurotus ostreatus	autoclavable plastics and autoclaved for approximately 2 h at 121 ◦C and 1.5 bar	moulds	A dark room was setup with an air-conditioning unit at 25 ◦C and 28 ◦C.	oven for 2 h at 150 ◦C	constructio n and packaging materials
[96]	Substrates, rice bran, and calcium carbonate powder were mixed together in a ratio of 100:10:1,	rice husk, sawdust, sugarcane bagasse, and teak leaves	rice bran and calcium carbonate	Pleurotus florida and Pleurotus citrinopileatus	autoclave at 121 ◦C for 30 min.		Room temperature for 30 days	oven at 60 ◦C for 10 h to inactivate	fungal foam.

[72]		Development of Rice Husk and Sawdust Mycelium Based Bio composites		Pleurotus ostreatus	autoclaving at 121° for 90 min	detachable pieces of 350 GSM-laminated waste paper. The assembly of moulds was aided by manual paper staplers. The size 55 × 55 × 60 mm and 60 × 20 × 75 mm	After 15 days, the composite materials grown into bags were transferred to moulds. The final incubation period lasted between 15 and 25 days as per the research design.	Upon harvesting, all composites were oven-dried at 80 °C for 15 h to denature the fungus	insulation foam materials
[97]	150 g of beech sawdust, 15 g potato extract glucose broth, 6 g gypsum, 15 g corn cob powder, 3 g calcium carbonate and 300 ml double distilled water each.	beech wood sawdust	15 g potato extract glucose broth, 6 g gypsum, 15 g corn cob powder, 3 g calcium carbonate	Trametes versicolor and Trametes pubescens	autoclaved at 121 ◦C for 20 min	Petri dishes and laminated cardboard molds (21 × 10 × 3.5 cm) were filled and clasped.	After 9 days under room temperature and dark, humid conditions. The molds were incubated in a sterile, humid environment at room temperature in the dark. Molds were incubated for a total of 21 days for T. pubescens and 25 days for T. versicolor. The composites were dried in an oven (EB53, Jouan GmbH, Unterhaching, Germany) at 65 ◦C for at least 24 h and 46 h for Petri dishes and cardboard molds, respectively.	The composites were dried in an oven at 65 ◦C for at least 24 h and 46 h for Petri dishes and cardboard molds, respectively.	MBC
[98]	each mixed substrate was consisted of one selected substrate and fixed substrate (FS) with a mass ratio of 1:1. The fixed substrate was mainly composed of cottonseed husk and nutrients.	five selected substrates (rice straw, bagasse, coir-pith, sawdust, and corn straw) and accessory substrates (cottonseed husk, corn cob, and wheat brain)	Perlite, calcium carbonate, Magnesium sulfate, Lignin	Pleurotus ostreatus	sterilized in autoclave at 115° for 15 mins	moulds	incubate at 25°C with 90% RH for 25 days	oven at 70°C for 12 hours	mycelium bio-composite s
[59]		Hemp fiber (HF) and peanut shell (PS)		Pleurotus ostreatus	sterilized	petri dishes.	microbiological incubator at 24 °C in a humid environment (70%–78% RH) for 21 days.	dried for 20 min at 170 °C and 24 h at 60 °C	Mycelium-Based Foams (MBFs)
[41]	inoculated using freshly grown P. ostreatus (10% w/v)	Mycelium growth on four types of textile residues (white and colored cotton and polyester mixtures)	The moisture content was maintained at 70% using a solution containing 5 g/L of glucose as an additional carbon source and 0.5 g/L ammonium sulphate as a nitrogen source." (Saini et al., 2023, p. 2)	Pleurotus ostreatus	sterilised using an autoclave at 121 °C, 15 psi for 20 minutes	moulds	27 °C for 4 weeks and 75% humidity	The fungal-grown textile was kept at 105 ± 1 °C for 24 h to deactivate the fungus.	Myceliu-based composite s from textiles residues
[99]	VISIT-> Pohl et al., 2022 - Establishment of the basidiomycete Fomes fomentarius for the production of composite materials	VISIT-> Pohl et al., 2022 - Establishment of the basidiomycete Fomes fomentarius for the production of composite materials	VISIT-> Pohl et al., 2022 - Establishment of the basidiomycete Fomes fomentarius for the production of composite materials	VISIT-> Pohl et al., 2022 - Establishment of the basidiomycete Fomes fomentarius for the production of composite materials	VISIT-> Pohl et al., 2022 - Establishment of the basidiomycete Fomes fomentarius for the production of composite materials	VISIT-> Pohl et al., 2022 - Establishment of the basidiomycete Fomes fomentarius for the production of composite materials	VISIT-> Pohl et al., 2022 - Establishment of the basidiomycete Fomes fomentarius for the production of composite materials	VISIT-> Pohl et al., 2022 - Establishment of the basidiomycete Fomes fomentarius for the production of composite materials	VISIT-> Pohl et al., 2022 - Establishm ent of the basidiomy cete Fomes fomentariu s for the production of composite materials

[45]	25 wt% bamboo, 12.5 wt% oats, 37.5 wt% water, and 25 wt% inoculum containing the fungus. The weights mentioned are the dry weight of the ingredients.	Dendrocalamus asper Bamboo	instant oatmeal	Pleurotus ostreatus	sterilized in the autoclave at 121 °C for an hour.	After the desired shapes of the inoculated substrates were formed, they were placed between two plastic cups to allow air circulation and avoid light.	The samples were also moisturized to maintain a minimum relative humidity of 80 % and each growth experiment was conducted up to 28 days at a temperature of 21 ± 0.5 °C.	without further treatment.	mycelium is grown onto porous woodpile struts structures to increase the final mechanica l properties.
[100]	VISIT ->Walter and Gürsoy, 2022 - A Study on the Sound Absorption Properties of Mycelium-Based Composites Cultivated on Waste Paper-Based Substrates	VISIT ->Walter and Gürsoy, 2022 - A Study on the Sound Absorption Properties of Mycelium-Based Composites Cultivated on Waste Paper-Based Substrates	VISIT ->Walter and Gürsoy, 2022 - A Study on the Sound Absorption Properties of Mycelium-Based Composites Cultivated on Waste Paper-Based Substrates	VISIT ->Walter and Gürsoy, 2022 - A Study on the Sound Absorption Properties of Mycelium-Based Composites Cultivated on Waste Paper-Based Substrates	VISIT ->Walter and Gürsoy, 2022 - A Study on the Sound Absorption Properties of Mycelium-Based Composites Cultivated on Waste Paper-Based Substrates	VISIT ->Walter and Gürsoy, 2022 - A Study on the Sound Absorption Properties of Mycelium-Based Composites Cultivated on Waste Paper-Based Substrates	VISIT ->Walter and Gürsoy, 2022 - A Study on the Sound Absorption Properties of Mycelium-Based Composites Cultivated on Waste Paper-Based Substrates	VISIT ->Walter and Gürsoy, 2022 - A Study on the Sound Absorption Properties of Mycelium-Based Composites Cultivated on Waste Paper-Based Substrates	VISIT - >Walter and Gürsoy, 2022 - A Study on the Sound Absorption Properties of Mycelium-Based Composite s Cultivated on Waste Paper-Based Substrates
[101]	grains with the mycelium to the culture bag filled with the S substrate (weight ratio 1:5)	1. (S) stalk fibers in comparison with 2. the commercial mushroom grow kit, a mixture of wheat bran, hardwood saw dust (fruit wood), spent coffee grounds, and other agriculture wastes for growing substrate and mycelium 3. (HCM) hardwood mixed with coffee grounds and mycelium		Pleurotus eryngii		design a two-part mold to make the mycelium-based biocomposite sample of a type IV dog bone-shape according to the ASTM D638 standard	room temperature (around 25°C) and use the ultrasonic humidifier at high humidity (relatively 98%) for 14 days (SM) and for 7 days (HCM).	three different temperatures for each material (i.e., 80°C, 90°C, and 100°C) and three different applied press forces, which are 1, 2, and 3 metric tons.	Mycelium-based wood composite s
[42]		Poplar and birch sawdust	glucose, KH2PO4, K2HPO4, MgSO4, and CaCO3	Trametes versicolor	sterilized in an autoclave at 121 ◦C for 30 min	100 mm × 100 mm × 15 mm polypropylene molds	Bags with inoculated substrate were incubated in a dark room with a temperature of 27°C and 67% relative humidity (RH) for 2 weeks + The substrate mixture containing mycelium was removed from the culture bags and placed in a blender again for 15 min to ensure a more uniform distribution of mycelium and molded under controlled conditions of temperature (27°C) and relative humidity (67%)	oven at 60°C for 12 h	mycelium composite s with multiscale hierarchica l porous structure,

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Chapter 2

MBC Production Replication with a Novel Substrate and Acoustic Characterization

1. Objective

This chapter presents the first experimental phase of the research, which involved replicating and adapting established protocols for the cultivation of MBCs based on state-of-the-art literature, with the aim of evaluating their reproducibility, reliability, and potential for industrial scalability. Given the growing relevance of MBCs as sustainable biomaterials, this phase was crucial for identifying current methodological limitations and laying the groundwork for subsequent innovations.
In this initial phase, a novel substrate, never reported in literature, was used to test the reproducibility of the methodology. The resulting material was evaluated throughout the mycelium's growth process on the substrate and subsequently subjected to acoustic characterization to assess its performance as a potential sound-absorbing biomaterial.

2. Introduction

In this initial phase, a novel substrate based on Green Pruning Waste (GPW) never previously reported in the literature, was used to test whether the established production methodology could be successfully applied to a new and unconventional growing medium. This approach aimed to assess not only the flexibility and robustness of the protocol, but also the potential for diversifying the raw materials involved in MBC production, with an eye toward circular economy strategies and the reuse of organic waste.

The resulting material was monitored during the entire growth cycle of the mycelium to verify the biological compatibility of the new substrate with the fungal strain of Pleurotus spp. and to observe its development over time. Subsequently, the material underwent acoustic characterization in order to evaluate its performance as a sound-absorbing biomaterial and to understand how the evolution of mycelium growth influences its acoustic behavior. Furthermore, the development of innovative biomaterials with acoustic functionalities is currently a highly active area of research. Due to its biological nature, mycelium exhibits properties that evolve over time. For real-world applications, mycelium growth is typically halted once the desired characteristics are achieved. Specifically, when focusing on acoustic performance, determining the optimal point at which to stop growth is crucial to maximize either sound absorption or sound insulation.

Given the small size of the samples, a four-microphone impedance tube proves to be a practical measurement tool. This method also offers the advantage of enabling the evaluation of both the sound absorption coefficient and transmission loss, along with other relevant parameters useful during the research and development phase.

This study presents measurements performed on MBCs samples, beginning with the characterization during the growth of mycelium in the substrate and continuing through to the inerted biological form. A custom-built setup was used to allow the mycelium to grow directly within the measurement holders, thereby avoiding any external interference with the growth process. Tests were conducted at regular time intervals to monitor how the acoustic behavior evolved over time. The findings indicate that as the mycelium matures, its acoustic performance improves.

Another motivation for conducting this type of experiment and subsequent acoustic characterization was to align with recent European directives promoting the development of sustainable alternatives to conventional, polluting insulation materials. This research also aims to address the growing concern highlighted in the latest edition of the EEA Report No 22/2019, which identifies environmental noise, produced by road traffic, railways, aircraft, and industrial activities, as one of the most significant environmental threats to human health. According to the report,

such noise pollution is responsible for the loss of approximately one million healthy life years annually, due to effects including annoyance, sleep disturbance, and ischemic heart disease.

More recently, the European Union has highlighted noise pollution as not only a health hazard but also a pressing ecological issue. This is especially urgent in the context of growing urban populations, resource scarcity, and climate change, all of which will necessitate a fundamental shift in how materials are used and how construction is approached [2], [3].

Biomaterials could offer viable solutions to some of these challenges. In line with this, a composite mycelium-based material made from urban green waste, such as pruning residues, has been investigated. This approach aligns with the circular economy model, transforming waste into a valuable resource, while also ensuring the material is entirely biodegradable and composed of natural substances. Another benefit of this biomaterial is its cost-effective production compared to conventional petroleum-based foams, combined with its capacity for natural degradation at end-of-life, making it suitable for composting [4], [5].

MBCs have garnered significant attention in recent years, with numerous biomass cultivation methods now standardized. Their properties have been examined extensively in relation to both sound absorption and insulation. The overarching goal is to develop a material that can be used as an acoustic panel. Potential applications include noise-reducing panels for traffic and building sites, created through sustainable production processes. This research compares two different cultivation techniques to determine which method yields superior acoustic insulation performance.

3. Materials and Methods

The following section outlines the methodology used for mycelium cultivation. Two strains of *Pleurotus* species were selected for solid-state fermentation on substrates composed of GPW collected from both public and private urban areas. These fungal strains are stored in the culture collection of the Environmental and Agro-food Microbiology Platform at the University of Brescia. The strains were initially propagated on Potato Dextrose Agar (PDA) in Petri dishes, incubated in the dark at 26.5°C. For the purpose of acoustic characterization, two types of samples, labelled “a” and “b”, were prepared. Each type included three replicates and one negative control based on substrate only, resulting in a total of seven samples. All samples were composed of fungal biomass and GPW with particle sizes between 2.5 and 5 cm, used as the growth substrate. Each sample were based on 200 gr of GPW that was hydrated with distilled water, placed in plastic containers, and sterilized in an autoclave at 121°C for 20 minutes. The sterilized GPW were then inoculated with *Pleurotus* sp. and incubated at 25°C for 14 days in sealed plastic containers until nearly full colonization by the hyphal network was observed as shown in Fig. 1. Sample group “a” was exposed to alternating with 12-hour light and dark cycles to simulate a circadian cycle, while group “b” was kept in constant darkness.

GPW	GPW with Pleurotus spp.
	

Fig. 1 Growing Substrate: GPW before inoculation (left) and GPW with Pleurotus spp. 14 days after inoculation (right)

After colonization, the substrates were manually remixed and transferred into sterilized aluminium tubes with an inner diameter of 46 mm and a length of 75 mm, which served as sample holders for the acoustic tests by using the impedance tube. Following 21 days of incubation, the tubes were heat-treated above a critical temperature to terminate fungal activity and render the material inert. Acoustic tests were conducted weekly over the three-week growth period both on samples “a” and “b” as shown in Fig. 2-3, and an additional test was performed on the inert samples, resulting in a total of four acoustic measurements per sample.

Day 2	Day 9	Day 16
		

Fig.2 Samples “a” Time Lapse: 2nd, 9th and 16th day of growing after preparation of samples in aluminium tube.

Day 2	Day 9	Day 16
		

Fig.3 Samples type “b” Time Lapse: 2nd, 9th and 16th day of growing after preparation of samples in the aluminium tube

The acoustic tests were conducted following the ASTM E2611 Standard Test Method for Normal Incidence Determination of Porous Material Acoustical Properties Based on the Transfer Matrix Method. This method utilizes a four-microphone impedance tube as show in the graphical representation in Fig. 4, also referred to as a dual standing-wave tube, which is fitted with four microphones. The setup enables the evaluation of normal-incidence acoustic parameters and offers the advantage of requiring only a small-sized specimen, typically a cylindrical sample with a diameter of just a few millimeters.

A loudspeaker is installed at one end of the tube and generates a broadband noise signal, either white or pink noise. The sound travels through the tube as the plane waves up to the cut-on frequency. Beyond this frequency, wave propagation no longer adheres to the plane wave assumption, rendering the method’s theoretical foundations invalid.

The sample is placed in a sample holder located in the central section of the tube, between two pairs of microphones. The second endpoint of the tube is usually equipped with a low-reflection termination.

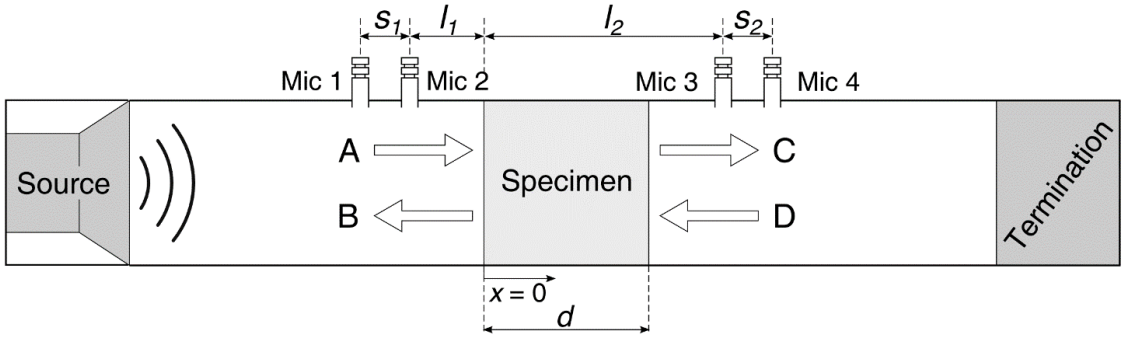

Fig. 4 Graphical representation of a four-microphone impedance tube.

As illustrated in Fig. 4, the acoustic wave inside the impedance tube can be divided into its components by processing the signals from the four microphones. This enables the differentiation of the sound field into forward and backward propagating waves on both sides of the test

specimen. The incident and reflected wave components, labeled A, B, C, and D in Fig. 4, are then used to calculate the elements of the transfer matrix, following the procedures outlined in the ASTM E2611 standard.

Through this approach, impedance tube measurements based on the transfer matrix method make it possible to establish the relationship between the acoustic state variables (such as pressure and velocity) at the two sides of the sample:

$$\begin{bmatrix} p_0 \\ u_0 \end{bmatrix} = \begin{bmatrix} T_{11} & T_{12} \\ T_{21} & T_{22} \end{bmatrix} \begin{bmatrix} p_d \\ u_d \end{bmatrix} \qquad (1)$$

where *p* is the sound pressure and *u* is the particle velocity, and 0 and *d* subscripts indicate the front (source side) and back (low reflection coefficient side) surfaces of the sample, respectively. The terms T_{ij} are the elements of the transfer matrix T. This method is particularly convenient for the acoustic characterization and optimization of complex layered structures, whose total transfer matrix, and, consequently, acoustic properties, can be obtained as the product of the individual transfer matrices multiplied in the correct sequence [6]:

$$T_{total} = T_1 T_2 \dots T_n \qquad (2)$$

The absorption coefficient α (hard backed) is obtained as:

$$\alpha = 1 - \frac{T_{11} - \rho c T_{21}}{T_{11} + \rho c T_{21}} \qquad (3)$$

Another quantity that can be derived by this measurement is the sound insulation of the specimen, expressed by the sound transmission loss (TL). The TL is related to the sound transmission coefficient *τ* through the formula TL = 10 $\log_{10}(1/\tau)$. Impedance tube measurements can provide further valuable information, such as the propagation wavenumber, the characteristic impedance, and the speed of sound in the tested specimen. When using an impedance tube to estimate the transmission loss, one must consider that the resulting TL can be assumed to be independent of boundary conditions only for materials that are considered as an equivalent fluid, that is, whose shear modulus is negligible [6].

The custom-made impedance tube used for the tests consists of two segments of 1200 mm length and a nominal internal diameter of 46 mm. With this cross-section, the plane-wave assumption necessary to estimate the particle velocity from the microphone couples is valid up to 3800 Hz. The source endpoint holds a 100 mm loudspeaker enclosed in a sealed and isolated volume, while the second endpoint can be limited by an anechoic termination. For high-frequency measurements, in the range of 100-3500 Hz, the microphone ports are spaced 45 mm apart. The sample is in a separate tube segment of appropriate length, installed between the two measurement sections described above. A picture of the impedance tube is shown in Fig. 5.

The microphones are housed in O-ring-equipped ports and connected to an OROS OR36 multichannel analyzer, also generating the test signal. A pistonphone has been used to calibrate microphones. The measured transfer functions have been saved and post-processed using a self-built code based on the ASTM E2611 standard procedure. The script provides the acoustic parameters of the samples, such as the sound absorption coefficient, the sound transmission loss, the propagation wavenumber, the speed of sound in the material, and the characteristic acoustic impedance.

Fig. 5 Picture of the impedance tube including the anechoic termination (left), the sample holder (centre), and the loudspeaker (right).

4. Results

This section reports the experimental results obtained using the four-microphone impedance tube. As described in the previous section, a loudspeaker generates plane waves that propagate along the tube. When these waves encounter the test sample, part of the acoustic energy is reflected, while the remaining portion is either transmitted through or absorbed by the material. The transmitted wave then reaches the downstream end of the tube, where most of its energy is dissipated by a near-anechoic termination. Assuming the use of a symmetrical sample, only a single load, thus a single termination, is required for accurate measurements. To account for any discrepancies in microphone phase and amplitude responses, their positions are interchanged during the measurement process. Based on the resulting transfer functions, a wave decomposition technique is applied to determine the pressure and particle velocity amplitudes on both the upstream and downstream sides of the specimen.

Once these quantities are known, the transfer matrix can be calculated according to the methodology defined in ASTM E2611 Standard using the following expression:

$$[T] = \begin{bmatrix} \frac{p_d u_d + p_0 u_0}{p_0 u_d + p_d u_0} & \frac{p_0^2 - p_d^2}{p_0 u_d + p_d u_0} \\ \frac{u_0^2 - u_d^2}{p_0 u_d + p_d u_0} & \frac{p_d u_d + p_0 u_0}{p_0 u_d + p_d u_0} \end{bmatrix} \quad (4)$$

where the subscripts 0 and *d* denote upstream and downstream respectively. The characteristics of the matrix when using symmetric samples in the test are as follows,

$$T_{11} = T_{22} \text{ and } T_{11}T_{22} - T_{12}T_{21} = 1 \quad (5)$$

Once the transfer matrix components are known, the following parameters can be easily found:

Transmission coefficient :

$$\tau = \frac{2e^{ikd}}{T_{11} + \frac{T_{12}}{\rho c} + \rho c\, T_{21} + T_{22}} \quad (6)$$

Transmission loss:

$$TL = 20log(1/\tau) \quad (7)$$

Reflection coefficient:

$$R = \frac{T_{11}-\rho cT_{21}}{T_{11}+\rho cT_{21}} \quad (8)$$

Absorption coefficient:

$$\alpha = 1 - |R|^2 \quad (9)$$

where *d* is the length of the sample along the axis, ρ = density of air in the tube, and *c* = speed of sound in the tube.

This procedure has been executed for all the mycelium samples described in the previous section, and their transmission losses and absorption coefficients have been computed and represented together in the following graphs in fig. 6 and 7.

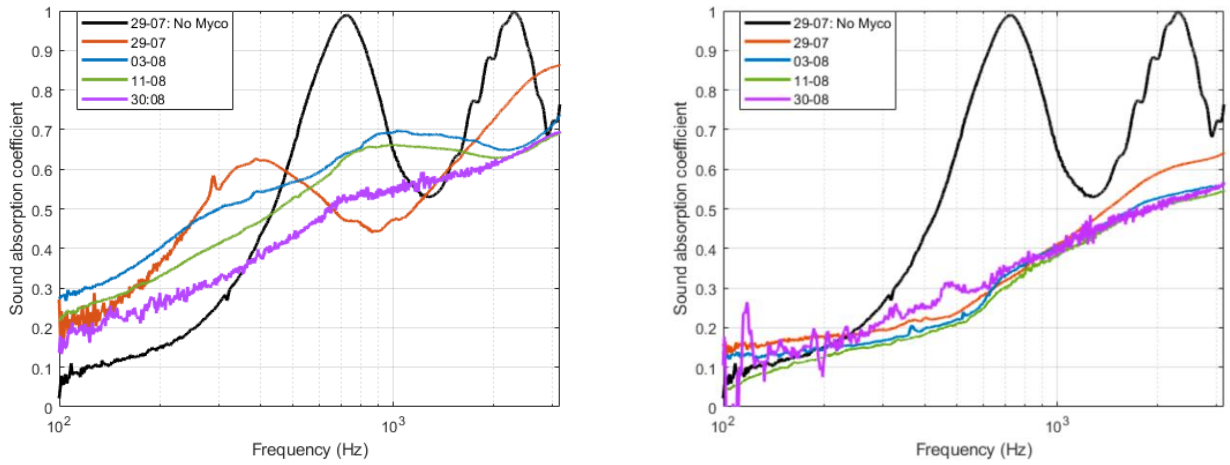

Fig.6 Absorption coefficient of the sample “a” (left) and “b” (right) during their growing phases

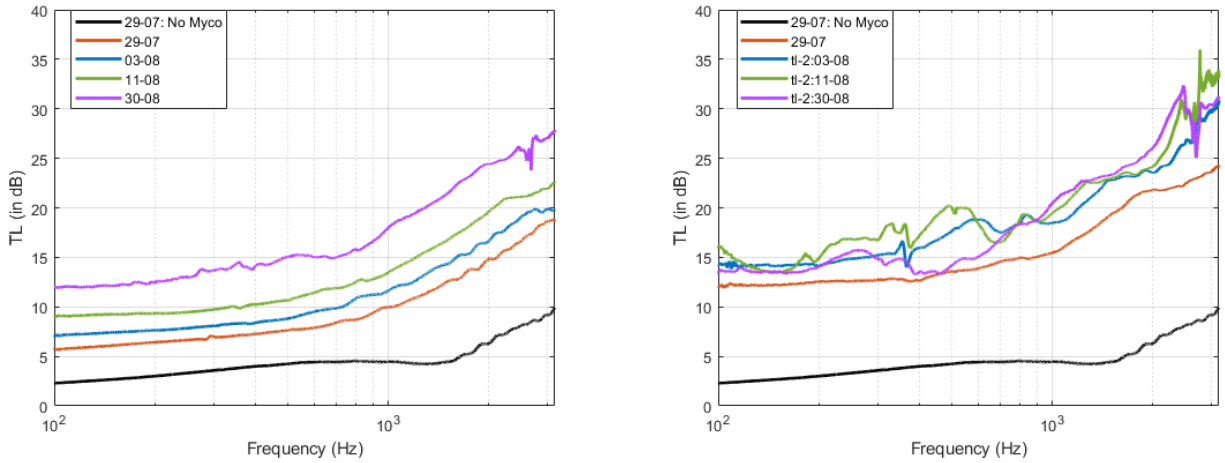

Fig.7 Transmission loss of sample “a” (left) and “b” (right) during their growing phases

5. Discussion

From Figure 6 and Figure 7, it can be seen that the absorption coefficients of samples “a” and “b” have a similar trend and the sample grown without mycelium (black line) show a quarter wavelength resonator behavior different from the other graphs. This is mainly due to the highly porous nature of the substrate. This leads to peaks and troughs in the absorption coefficient, the frequencies of which are related to the length of the sample. This implies the presence of substrate alone is not very effective in obtaining a broadband sound absorption. However, as the mycelium grows, the absorption coefficient approaches a flatter spectrum. Additionally, the curves become bumpy with the growth of mycelium, for both samples. In the case of the sample “a”, the absorption coefficient decreases with time, whereas for the sample “b”, it stays nearly the same, meaning that the different growing conditions, as explained in the materials and methods paragraph, affect the acoustic properties. The absorption coefficient of the sample “a” is better than that of the sample “b” for almost all the frequencies investigated.

The transmission losses (TL) of samples “a” and “b” show a much clearer trend. The sample without mycelium has a much lower TL than the other samples. Additionally, the TL improves as mycelium grows, which is evident in Figure 8. In Figure 9, this trend is seen for higher frequencies (>700 Hz) but for lower frequencies, this trend cannot be established clearly. Another point of observation is that the TL stays at around 15 dB for all the stages of growth for sample “b” at frequencies lower than about 700 Hz. Sample “b” shows a better TL than sample “a” by about 2 dB for all investigated frequencies. When the samples “a” and “b” are rendered inert, their properties stop varying with time.

The absorption coefficient improves compared to the one measured on the 30th of August, implying that the absorption properties are improved by the growth of the mycelium. The absorption coefficient and the TL curves measured after two weeks are comparable. The best improvement was obtained in the middle-frequency range (400 Hz-1200 Hz). As concerns the TL, the sound insulation performances increased with time for sample “a”, while for sample “b” after the first improvement the TL does not show a clear enhancement with time.

6. Conclusions

The acoustic properties of mycelium samples during their growth phase have been analyzed in terms of absorption coefficient and transmission loss (TL) for two different samples. The absorption coefficients showed a clear improvement with the presence of mycelium. Moreover, as the mycelium develops, the absorption coefficient tends to approach a maximum value. Since living mycelium cannot be used in practical applications, its properties are relevant only once it is made inert. Therefore, it is crucial to maximize the absorption coefficient before the inactivation process. Further analysis should be carried out to closely examine the precise behavior of the absorption coefficient during the final stages of mycelium growth. On the other hand, transmission loss demonstrates a more consistent and clearly defined behavior during the growing phase. The presence of mycelium enhances TL across the entire frequency range investigated. As with the absorption coefficient, a more detailed examination of TL in the later growth stages may yield valuable insights. Mycelium offers a broad range of potential applications. The absorption coefficient, as observed in this study, does not exhibit distinct peaks or abrupt variations. Instead, it increases steadily with frequency, indicating effective broadband noise absorption. Similarly, transmission loss remains stable without sudden spikes or dips. In summary, mycelium can be effectively used for broadband noise absorption and attenuation. Additionally, the methodology for producing MBCs has been successfully replicated using a new type of substrate, demonstrating the versatility and reliability of the approach. This replicability forms the foundation for the subsequent experiments presented in the following chapters, which aim to refine the production process of MBCs and control over key variables such as the substrate to mycelium ratio and to explore the properties of this biomaterial.

NOTE: Parts of this chapter are based on the Proceedings of the International Congress on Sound and Vibration *"VARIATION OF THE ACOUSTIC CHARACTERISTICS OF MYCELIUM-BASED BIOMATERIALS WITH TIME"*, authored by Chiara Dognini, Somayan Basu, Edoardo Piana, and Emanuela Gobbi. The content has been adapted, expanded, and reorganized to fit the context and structure of this dissertation. The author wishes to express sincere gratitude to Professor Edoardo Piana for their support and collaboration throughout the development of this research.

7. References

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Chapter 3

Optimization of MBC Production and Mechanical Characterization

1. Objective

This chapter presents the experimental phase in which the production methodology for MBCs, tested and discussed in Chapter 2, was reinterpreted with the aim of eliminating some of the most critical and resource-intensive steps commonly cited in literature. To achieve this, an untested industrial byproduct was introduced as an alternative growth substrate, and a new cultivation protocol was developed. This revised approach was designed to enhance sustainability, streamline the production process, reduce environmental impact, and increase the feasibility of MBCs for scalable, real-world applications. The resulting material was then mechanically characterized, and its properties were compared to those reported in the current state of the art.

2. Introduction

The use of MBCs as sustainable biomaterials has garnered considerable attention due to their versatile applications across various industries. Derived from the vegetative part of fungi, these composites present a broad spectrum of uses, ranging from construction materials and packaging to textiles and medical applications. Demonstrating superior technical characteristics, such as sound absorption, thermal insulation, and low embodied energy, MBCs stand as environmentally friendly alternatives to traditional building materials. Several factors related to the production methods influence the mechanical behavior and quality of MBC. Customizable properties can be achieved based on the manufacturing process employed as also reported and shown in Chapter 2. Despite their significant potential, the widespread adoption of MBC has been hindered by the absence of efficient manufacturing processes at the industrial level. Tackling this challenge and advancing sustainable practices in the construction industry are imperative for fostering the ecological transition and shaping the future of a green building market. MBCs have emerged as revolutionary biomaterials with the potential to transform traditional materials form various industrial sectors into sustainable alternatives [1] offering a wide range of applications, including furniture, insulation panels, and eco-friendly leather [2]. These composites, derived from the vegetative part of fungi, are grown on substrates made from agricultural or renewable waste materials, aligning with the principles of the circular economy [1]. Research has shown that MBC possess customizable technical characteristics and can be molded into different shapes and sizes, opening new design possibilities [3]. Furthermore, they have been found to be safer and more environmentally friendly than traditional materials, contributing to a reduction in the construction industry's carbon footprint [4]. As reported in the Chapter 1, the mechanical behavior of MBC is influenced by factors such as fungal strains, substrates, cultivation conditions, and post-processing treatments highlighting the need for efficient manufacturing processes at an industrial level to facilitate widespread adoption [5]. While the potential of MBC to create sustainable and environmentally friendly materials is evident, their adoption has been limited by the lack of standardized manufacturing processes for industrial scale-up and the expensive costs at which they are commercially sold today. In addition, this new generation of biomaterials needs further study to meet the mechanical demand from governance, as MBCs are still mechanically weak. However, recent studies have explored the use of additives like chitosan and bacterial cellulose to reinforce MBC [6], indicating potential avenues for enhancing their mechanical characteristics [7]. The application of MBC extends beyond construction, with research demonstrating their

versatility in various contexts, including 3D printing, packaging, household appliances, and textiles [8]. Moreover, the development of MBC using waste materials showcases the potential for utilizing agricultural by-products for sustainable biomaterials [9]. The integration of MBC into different industries aligns with the principles of sustainable manufacturing and the circular economy, emphasizing the importance of addressing pollution associated with building materials through sustainable practices [10]. While challenges related to manufacturing processes and standardization exist, ongoing research and innovations in biomaterials offer potential solutions to enhance the mechanical properties of MBC. In our study, we analyzed and compared protocols to standardize and adapting an efficient manufacturing process to a new lignocellulosic substrate derived from the woody debris from understory for potential industrial scale-up. Specifically, we reported the first attempt to improve and enhance the cultivation procedures for a biomaterial based on mycelium and the small branches, twigs, and pieces of wood that have fallen naturally or been left behind after vegetation management (such as thinning or pruning) in the understory layer of a forest. Reusing woody debris from the understory is important because it allows for the recovery of a valuable biomass resource that would otherwise go to waste. Instead of leaving this material to decompose or burning it on site, which can release unnecessary CO_2 and pollutants, it can be processed and used as a renewable source of energy, such as biofuel or heat, helping to reduce reliance on fossil fuels. This not only supports more sustainable energy systems but also contributes to rural economies by creating jobs in biomass collection and processing. Moreover, it aligns with the principles of circular forestry, where even non-commercial residues are given value and function within the forest ecosystem. On the other hand, removing understory woody debris is sometimes necessary to reduce the risk of wildfire, especially in dry or fire-prone areas where this material acts as highly flammable fuel. Its presence in large quantities can also promote the spread of pests or diseases, as decaying wood provides a habitat for insects and fungi that may harm healthy trees. Furthermore, an overabundance of debris can hinder forest regeneration by limiting light and space for young plants, while also affecting the accessibility and visual quality of recreational forest areas. For these reasons, managing and sometimes removing this material plays a critical role in both ecological conservation and forest safety. Additionally, the research endeavors to evaluate the mechanical properties of this biomaterial and compare them to those reported in the literature [11], [12], thereby contributing valuable insights into growing protocols. The research emphasis lies in formulating a protocol aligned with the principles of the circular economy, integrating a sustainable life cycle assessment (LCA), minimizing energy consumption, and achieving near-zero costs for raw materials recovered from waste.

3. Materials and methods

3.1 Fungal Strain

The study outlines cultivating procedure for MBC using the strain of Trametes *versicolor* (TV) on a substrate derived from repurposed organic waste derived from woody debris. For the following experiment, we used a substrate made of Robinia pseudoacacia (RP), known as black locust. RP was selected as a substrate due to its challenging proliferation in the United States and Europe especially in the green areas, posing issues associated with its invasive nature and pest attributes. Additionally, the choice was influenced by the robust physical properties of its highly durable wood, which has already been used in building applications in the past. To prepare for the experiment, the mycelium of TV was harvested from a pruned strain in northern Italy and subsequently isolated. The collected fungal sample underwent a sequential process: initial washing with distilled water, immersion in 70% alcohol for 30 minutes, and placement under a biological fume hood followed by exposure to UV light for 30 minutes to ensure thorough sterilization. Following sterilization, the fungus was sectioned, and a sample from the inner part was positioned at the center of a Petri dish containing Potato Dextrose Agar (PDA). The Petri dish

with TV was kept in a dark environment at room temperature for 2 days, when the first hyphae emerged. A portion of the outermost hyphae was then isolated and placed into a new Petri dish with 30 mm diameter containing PDA. This crucial step facilitated the isolation of TV from any contamination. Subsequently, the new Petri dish was incubated in darkness at room temperature for 7 days until the hyphae covered the full diameter. Fungal DNA analysis was conducted at the Università Cattolica del Sacro Cuore di Piacenza (Dipartimento di Scienze animali, della nutrizione e degli alimenti - DIANA) confirming the strain of TV.

3.2 Substrate

Two sets of experiments were developed, characterized by RP substrate with different particle sizes: 1-3 cm (substrate A) and 3-5 cm (substrate B). These sizes were achieved through the trimming and sewing of the RP substrate. The diverse particle sizes were intentionally chosen to assess the mycelium's growth rate and examine the impact of fibers on mechanical properties. Both A and B substrates underwent a 12-hour immersion in water with 175 grams of dried substrate. Following this period, excess water was carefully removed. The final weight of substrate A reached 425 grams, indicating an impressive water absorption that elevated the weight nearly 2.4 times. In contrast, substrate B concluded with a final weight of 330 grams, corresponding to a water absorption that increased the weight by approximately 1.9 times. These variations in water absorption quantities are contingent on the particle size and the final composition of the fibers.

3.3 Procedure

Four samples, each weighing 100 grams, were prepared using substrate A, while an additional four samples, each weighing 80 grams, were crafted using substrate B. These substrates were carefully inserted into autoclavable polypropylene micro-boxes, measuring 125 mm (length) x 65 mm (width) x 80 mm (depth), equipped with airtight lids and breathable strips. The discrepancy in weight is attributed to the volume of the substrate and the necessity to achieve samples with a thickness of 30 mm. This thickness allows sufficient space for air circulation within the micro-boxes. Subsequently, the substrates underwent sterilization through one cycle of autoclaving at 121°C for 20 minutes. Following sterilization, three out of the four samples from each series (A and B) were inoculated with 1/6 of a 30 mm diameter petri dish containing TV. Non-inoculated samples were used as negative controls for each experiment (A and B). The samples were then incubated in a dark environment at room temperature for 8 weeks. During the incubation period, samples were manually shaken after the first week to redistribute the mycelium throughout the substrates. Additionally, each sample was flipped horizontally and then vertically once a week, promoting uniform exposure of all sides and facilitating water movement through capillarity aided by gravity. After 8 weeks, the samples were ready to be rendered inert using a hot press at 90°C, without applying any load, and heated until a stable weight was reached. The dried samples exhibited an average weight loss of 60% from their initial weight. Prior to testing, the samples were left at room temperature overnight. This protocol, designed with a focus on resource and energy efficiency, differs from traditional production methods [3], that involve growing mycelium initially on petri dishes and then in grains at 24-25° Celsius temperature; and once the grains are fully colonized, they are seeded into the substrate. However, in this experiment, this step was omitted, and the mycelium grown directly on the petri dishes was used to inoculate the substrate that was incubated at room temperature, thereby reducing costs associated with material and energy recovery during incubation.

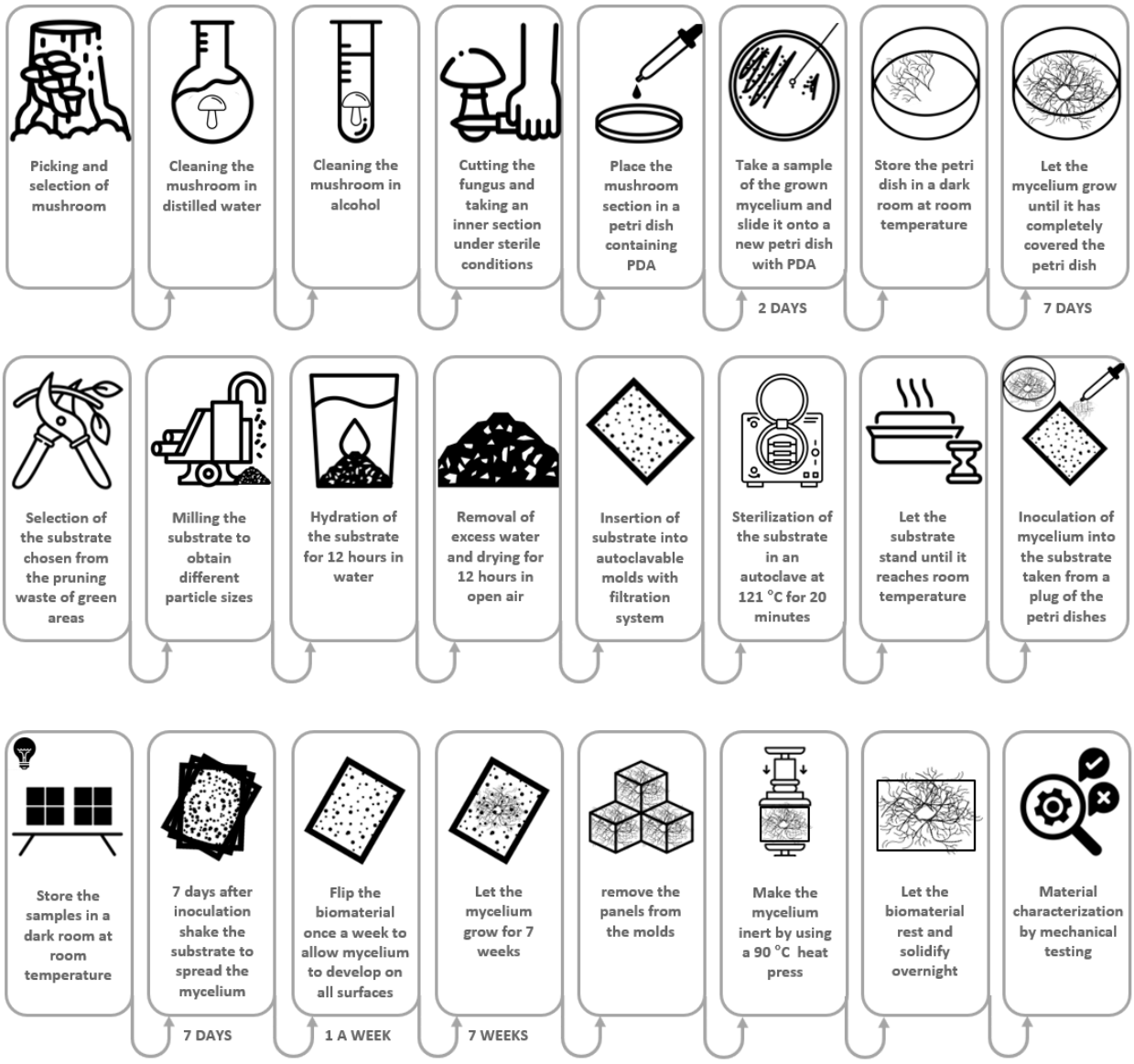

Fig. 1 The Scheme represents the procedure for the cultivation of MBC from the fungal strain isolation to the post-procedure for mechanical characterization.

3.4 Mechanical samples

The obtained samples from groups A and B had different compositions. Samples A, made from finer fibers, were more homogeneous and foam-like material, and at equal volume they were heavier than samples from group B. The inner section had been completely colonized presenting a more homogeneous fiber arrangement and fewer interstitial spaces compared to group B. Those in group B had greater strength and weren't foam-like material to the touch. From the section it was visible how internally the mycelium had completely colonized the substrate and was more compactly resistant than group A, given the inhomogeneous fiber arrangement and greater interstitial spaces. Both groups of specimens had been equally colonized on the surface.

Fig. 2 Images of the MBC samples obtained from substrates A and B and the fungal strain TV.

3.1 Mechanical characterization

Mechanical properties of the prepared systems were carried out under both uniaxial tensile and compression tests by means of an electromechanical dynamometer (Instron, Mod. 3366).
Tensile tests were carried out only on systems featuring larger cells, on three bar-shaped specimens (cross-section: 30x10 mm; gauge length: 60 m). For the compression tests thick rectangular specimens (cross-section: 55 x 55 mm; height: 30 mm) were used, preparing three samples for both A and B groups of samples. Both types of specimens were prepared by carefully cutting the panel with a sharp cutter. The tests were carried out at room temperature and at a crosshead speed equal to 5 mm/min until failure for the tensile tests, and up to the large strains (about 80%) under compression.The results are displayed as stress vs. strain curves, evaluating the apparent stress, s σ, as

$$\sigma = \frac{F}{A} \quad (1)$$

where F is the force measured along the test and A the overall specimen cross-section measured before the test, and the apparent strain as

$$\varepsilon = \frac{\Delta l}{l0} \quad (2)$$

where Δl represents the measured crosshead displacement and $l0$ the specimen length before testing (gauge length for tensile tests; initial height for compression tests); stress and strain are termed apparent at the light of to the non-homogeneous nature of the specimens, which incorporate larges voids and wood fragments.

4. Results

Tensile test results were carried out only on systems featuring larger cells, are reported in Fig. 3a as apparent stress *vs.* strain curves. A brittle behavior is displayed, with an important failure at strains lower than 5%, preceded by local micro-cracking and followed by a diffuse propagation regime that involves further cracking of the specimen before eventual rupture. The brittle behavior,

consisting of a large number of local failures throughout the specimens, has to be clearly ascribed to the non-homogeneous structure, including large voids and wood macro-particles whose adhesion with the mycelium is probably weak. An attempt at evaluating the material stiffness, as initial linear trend of the stress *vs.* strain curve, show that the material average apparent Young's modulus is equal to 7 MPa (± 4MPa), as typical of flexible polymer foams, while the material strength, evaluated as maximum apparent strain before failure, has an average value equal to 60 MPa (±30 MPa). The remarkable value of the error allows to quantify the scattering, as evident from the stress vs. strain curves, likely influenced by the inherent material structure.

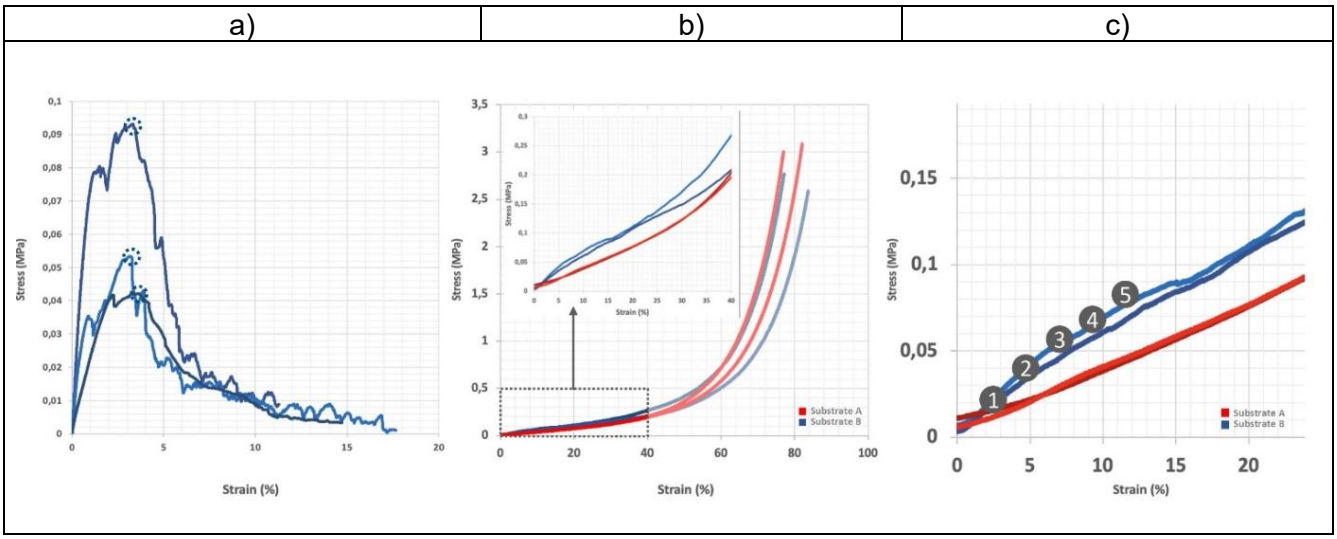

Fig. 3 Apparent stress vs. strain curves measured in a) tensile tests in larger cells substrates and b) compression tests on both substrate A (blue line) and substrate B (red line). Inset of graph b): magnification of the initial part of the compression stress vs. strain curves. The graph c) is a zoom in of the graph b).

The results of compression tests are shown, for both A and B substrates, in Fig. 3 b. The stress vs. strain curves displays the typical three regime trend of cellular materials and foams: a first highly compliant, linear elastic response (strain up to 5%); a subsequent deformation ibn which the cells deform by collapsing (strain up to about 50%); a final, increase in stress as the cells become highly compacted under compression. The overall behavior of the substrates is very similar and seems not to depend on the cell size distribution, so that, due to some scattering, the curves groups of the two materials practically overlap. Nonetheless, distinctions emerge in the first two regimes between the two systems, as revealed by a closer examination of this region (inset of Fig. 3 graph b). Specifically, the material with larger cells, substrate B, demonstrates higher stiffness, evaluated as 0.7 ± 0.1 MPa, almost twice that of finer cell, substrate A, (0.28 ± 0.06 MPa). Further, substrate B undergoes a change in slope upon entering the cell collapse region, indicating an inherent strength in the cellular structure itself. By contrast, the material with substrate A have a distribution exhibits slightly lower stiffness and a smoother transition towards the cell collapse region.

5. Conclusions

Following the conducted tests, a comparison was made between the mechanical results obtained and those documented where the initial trend of the curve is not regular, and irregularity points are labeled by numbers 1-5 in Fig. 3c. Similar irregularities were witnessed in other works in literature, see for comparison [11]. These peculiar points are a consequence of the non-homogeneous and local deformation occurring in the beginning part of compression. The Fig.3 graph c) illustrates that the compression curve from the experiments with substrate B (featuring fiber particles with size 3-5 cm) aligns with literature-reported results despite employing a more sustainable protocol with low cost and energy consumption. The compression curve of the substrate B specimens has a similar trend as that obtained from the literature [11] with an elastic modulus between 7 ± 4 MPa and a peak stress 60 ± 30 kPa that is very comparable to the one in the literature with elastic modulus 0.5 MPa and a peak stress at 69 kPa. These findings highlight the potential to reduce production steps for biomaterial panels by almost 50%, while maintaining comparable mechanical performance. In a broader context, the mechanical properties of Mycelium-Based Composites (MBC), as observed across various studies, are comparable to synthetic polymer-based foams, indicating room for improvement. MBC is composed of low-weight substrate mixtures exhibit compressive strength like polystyrene foams but are weaker than polyurethane and phenolic formaldehyde resins. Notably, MBC stands out as sustainable and biodegradable biomaterials that can be cultivated using locally available resources. Research indicates that, compared to conventional materials from the cement and petrochemical industry, MBC significantly reduces environmental pollution. Moreover, the versatility of cultivating MBC on various substrates, including waste or recalcitrant materials, offers a potential solution for reintegrating building waste into the circular economy instead of ending up in landfills. In conclusion, our ongoing efforts are focused on pioneering a novel biomaterial production method designed for seamless scalability at an industrial level. This innovation signifies more than just a leap forward in efficiency; it represents a conscientious step towards a more sustainable future. Envisioning a production model that aligns with circular economic principles, undergoes rigorous scrutiny through Life Cycle Assessment, and adheres to emerging regulations driving ecological transition, we aim to redefine the landscape of biomaterial production, setting a new standard for industry-wide sustainability.

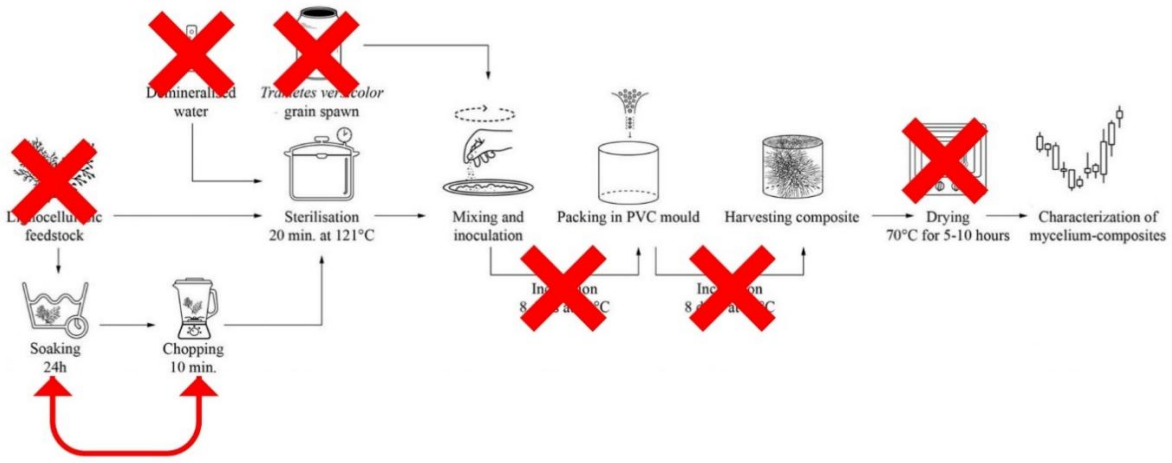

Fig. 4 comparison with cultivation procedures reported in the literature and the possibility of decreasing the steps required for MBC production by about 50%.

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Chapter 4

Methodological Advancement of MBC based on Spent Mushroom Substrate (SMS)

1. Objective

The objective of this chapter is to evaluate the feasibility of reusing Spent Mushroom Substrate (SMS) as a viable raw material to produce MBCs. This phase of the research was crucial for assessing both the material compatibility and the functional performance of SMS when repurposed for construction applications. SMS, a byproduct of edible mushroom cultivation composed of exhausted lignocellulosic material and residual mycelium, is often underutilized, contributing to environmental and economic challenges. This study investigates the potential of transforming this agricultural waste into high-performance, biodegradable building materials. By integrating SMS into the broader dual-production framework, this chapter not only establishes a sustainable process based on the methodology reported in Chapters 2 and 3 but also reinforces the vision of a regenerative bioeconomy. In this model, the byproducts generated by mushroom cultivation are transformed into low-cost, energy-efficient MBCs. Moreover, the results lay the foundation for the development of a dual-production system capable of generating both edible mushrooms and MBCs within a single, circular production cycle.

2. Introduction

The cultivation of edible mushrooms results in the production of considerable agricultural residues, with approximately 5 kg of SMS generated for every 1 kg of fresh mushrooms harvested [1], [2]. This byproduct is often underutilized, leading to notable environmental and economic challenges. After the third fruiting cycle, SMS is generally regarded as spent, particularly for species like *Pleurotus ostreatus* (P. ostreatus), whose yield declines significantly after the second flush due to ongoing nutrient depletion in the substrate [3], [4], [5]. Since continued cultivation becomes economically impractical, the substrate is typically discarded. Traditional SMS disposal methods—such as incineration, land spreading, burial, composting, or landfilling—result in the inefficient use of lignocellulosic materials, require substantial land, and pose environmental hazards including mosquito breeding, unpleasant gas emissions, and contamination of surface and groundwater systems [6], [7]. Additionally, the bulk nature of SMS, along with its high salt and moisture content, contributes to elevated transportation costs [8], which in turn significantly increase the overall expenses associated with SMS storage and disposal [9]. To address these issues, it is crucial to develop efficient treatment technologies that enable more sustainable management of the large volumes of SMS waste [10]. Recent research has explored sustainable strategies to valorize SMS for various applications, including animal feed, heavy metal adsorption, biofertilizers, soil amendments, and the production of biofuels, bioethanol, and bio-oil [11], [12], [13], [14], [15]. These approaches are in line with green biotechnology and sustainability objectives, as SMS, a byproduct generated in large quantities, remains rich in bioactive compounds such as enzymes, antibiotics, and carbohydrates formed during the development of the fruiting body [16]. Despite

nutrient depletion, SMS retains its value as a lignocellulosic resource [17], typically composed of residues like straw or cotton along with remaining mycelium. The valorization of SMS contributes to waste reduction and improved resource efficiency in the mushroom industry [18]. This is especially relevant for SMS derived from *P. ostreatus*, which accounts for 27% of global mushroom production due to its cost-effective and environmentally friendly conversion of a variety of byproducts into edible biomass [19], [20]. Owing to its widespread cultivation and demonstrated effectiveness in mycelium-based composite (MBC) production, SMS from *P. ostreatus* was selected for this study. This choice is supported by previous findings that highlight the superior performance of *P. ostreatus* in comparison to other mushroom species in MBC manufacturing [21], [22]. Common fungal species used for MBCs include both trimitic and monomitic varieties, which are capable of producing key enzymes that effectively break down lignin, a major component of the cultivation substrate [23], [24]. *P. ostreatus* is particularly recognized for its strong lignin-degrading ability. MBCs offer versatile and sustainable alternatives to traditional materials, with properties that can be tailored through changes in composition, structure, and treatment methods, enabling use across industries such as packaging, cushioning, and padding [25]. These materials also deliver environmental advantages, including reduced carbon emissions, decreased fossil fuel dependency, and enhanced circularity [26].

This research explores the feasibility of transforming SMS into MBCs by evaluating a range of processing techniques. Twelve experimental treatments were designed to assess SMS as a novel cultivation medium, with the setup managed using Design Expert software. The treatments were divided into two main categories: sterilized and unsterilized SMS. The unsterilized version was assessed for its potential in direct application, while the sterilized SMS aimed to serve as a neutral substrate by eliminating microbial contaminants. Within both categories, several modifications were introduced: nitrogen supplementation to support fungal growth and improve substrate quality [27], addition of fresh mycelium to increase colonization efficiency, and supplementation with fresh substrate. The cultivation phase lasted 10 days under controlled environmental conditions. Mycelial growth was evaluated through measurements of biomass yield and growth rate. A minimum colonization threshold of 50% surface coverage was set to ensure sufficient substrate cohesion for subsequent processing steps [28]. Treatments exceeding this threshold were selected for further transformation into samples for mechanical and acoustic testing. Mechanical analysis showed that SMS-based MBCs can achieve compressive strength values nearing 10 MPa and a Young's Modulus of 45 N/mm². Acoustic testing indicated that the best-performing formulations achieved sound absorption coefficients above 0.9 at 1600 Hz and maintained values above 0.75 across the high-frequency range (2400–6400 Hz), highlighting their potential for use in acoustic insulation. These findings illustrate how agricultural waste can be repurposed into innovative materials, promoting resource efficiency, minimizing waste, and advancing sustainable development. This study provides a pioneering example of industrial symbiosis by establishing a connection between the agricultural and construction sectors in support of a more sustainable future.

3. Materials and methods

This study aimed to optimize MBC production by assessing different cultivation treatments to identify optimal conditions for mycelium colonization on SMS. The most effective treatments were selected for post-processing techniques, including heat-pressing, to prepare samples for mechanical and acoustic characterization. This section outlines the methodology, encompassing SMS preparation, mycelium cultivation, and post-treatment procedures for MBC evaluation. An overview of the experimental design and methodological workflow is illustrated in Fig. 1, providing a visual representation of the procedures adopted in this study.

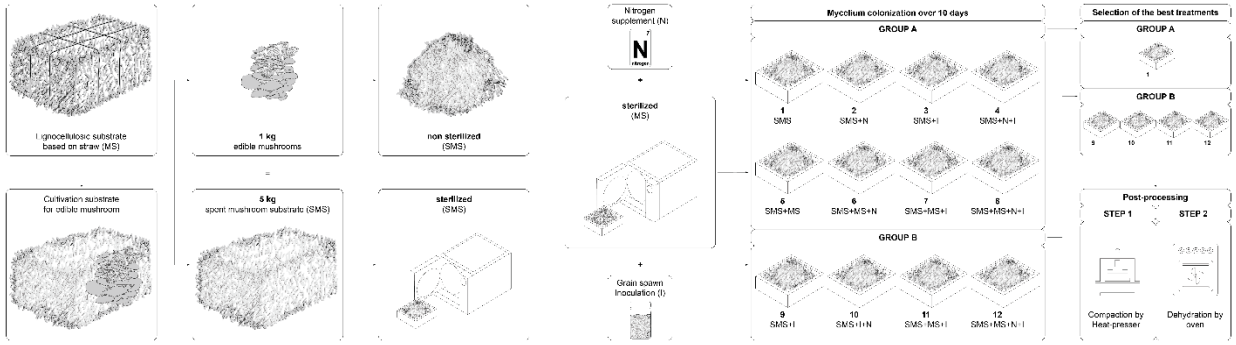

Fig. 1 Schematic representation of the experimental workflow for SMS-based MBCs production

3.1. Materials

3.1.1. Spent Mushroom Substrate

The SMS from *P. ostreatus* was repurposed as a new cultivation substrate for mycelium colonization for the fabrication of MBCs. The SMS was collected after the third fruiting cycle based on a mixture consisting of 50% cottonseed hull pellets and 50% chopped wheat straw by volume, equivalent to approximately 80% hull pellets and 20% straw by weight. Additionally, 0.18 kg of All Season Organic supplement and 0.36 kg of millet spawn were added to each bag, and then water was added to achieve a humidity content of 65%. When the SMS was collected after cultivation, the water content was measured, as the initial value of 65% decreases over time due to evaporation. This parameter is one of the main factors influencing fungal growth on a specific substrate [29].

3.1.2. *Pleurotus ostreatus*

The mycelial strain of *P. ostreatus* (Strain 123 *Pleurotus ostreatus*) was supplied in the form of commercial spawn and purchased from Lambert Spawn in a pre-spawn bag as the one reported in the literature [30]. The same *P. ostreatus* strain used in edible mushroom cultivation, where the SMS was collected, was selected for inoculation to ensure compatibility between the SMS and the new inoculum. This strain is widely

recognized for its suitability in biomaterial production, alongside *Ganoderma lucidum*, as one of the most commonly used fungal species for MBCs [31].

3.2. Methods

3.2.1. Preparation of the SMS as a new cultivation substrate for MBCs

The collected SMS was manually grinded and to ensure consistency, the moisture content of the SMS was measured, and the necessary amount of water was added to restore the target level achieving a humidity level of 65%, as reported in literature [32].

To evaluate the potential of reusing SMS, 12 treatments were designed by using Design Expert software, dividing the experiments into two groups: non-sterilized (Group A) and sterilized (Group B). In the latter, the mycelium presented in the SMS was deactivated due to sterilization, necessitating a new inoculation with fresh mycelium. The substrate was distributed into twelve autoclavable, microperforated polypropylene bags: Group A required 8 bags and Group B required 4 bags, each containing 3 kg of SMS to ensure uniformity in treatment conditions and consistent heat penetration during sterilization. Group B substrates were sterilized in an autoclave at 121 °C for 45 minutes, following the protocol reported by Ghazvinian et al. (2019) [33], to eliminate microbial contaminants without compromising the substrate's structural or nutritional properties. This process requires precise control of temperature, pressure, and duration, as incomplete sterilization can result in contamination, jeopardizing the production process.

Both groups incorporated additional treatments, including nitrogen supplementation (N), new inoculation (I), and fresh substrate addition (MS). N supplementation was added to adjust the carbon-to-nitrogen (C/N) ratio of the SMS, which becomes nutrient-depleted after the third flush. Studies have shown that the C/N ratio significantly influences mycelial growth rates and biomass yield [34]. MS was added to replenish nutrients depleted during previous mushroom cultivation cycles and to enhance mycelial colonization. Both N and MS, before being used, were sterilized following the same procedure reported before and, after cooling to room temperature, were mixed into their respective bags. Once the bags were mixed with their respective mix of SMS, MS, and N, the *P. ostreatus* in the form of grain spawn was added for inoculation and manually mixed under a safety cabinet to ensure uniform distribution within the bags. Tables 1 and 2 detail the experimental design for SMS-based MBCs production of Group A and Group B treatments, respectively. For each treatment, six replicates weighing 500 grams each were prepared and placed into sterilized aluminum trays (110x170x75 mm), totaling 72 trays. The aluminum trays have been autoclaved and cooled beforehand. The filled trays were wrapped in plastic film and incubated under controlled conditions in an incubator at 23.5°C and 75% humidity for 10 days.

Table 1 Experimental Design for Group A treatments based on Spent Mushroom Substrate (SMS), Fresh Substrate Addition (MS), Inoculation (I), and Nitrogen (N)

Treatment #	Spent Mushroom Substrate (SMS)	Fresh Substrate (MS)	Inoculation (I)	Nitrogen (N)
1	100%	0%	0%	0%
2	100%	0%	0%	3%
3	100%	0%	6%	0%
4	100%	0%	6%	3%
5	50%	50%	0%	0%
6	50%	50%	0%	3%
7	50%	50%	6%	0%
8	50%	50%	6%	3%

Table 2 Experimental Design for Group B treatments based on Spent Mushroom Substrate (SMS), Fresh Substrate Addition (MS), Inoculation (I), and Nitrogen (N)

Treatment #	Spent Mushroom Substrate (SMS)	Fresh Substrate (MS)	Inoculation (I)	Nitrogen (N)
9	100%	0%	6%	0%
10	100%	0%	6%	3%
11	50%	50%	6%	0%
12	50%	50%	6%	3%

3.2.2. Analysis and quantification of fungal biomass

Daily photographs were taken over 10 days to monitor mycelium colonization, with Fig. 2 presenting one representative replicate for each of the 12 treatments (the complete collection of images of all replicates is available upon request). This monitoring process enabled the identification and evaluation of treatments exhibiting the highest levels of biomass development on the sample surfaces. Effective biomass colonization on the surface of MBCs is critical for achieving uniform mycelium distribution throughout the composite material. Such uniformity is essential for enhancing the composite's mechanical strength, as it forms a continuous fungal hyphae network that securely binds the substrate. Additionally, thorough colonization minimizes the risk of contamination by other microorganisms, ensuring consistent mechanical and structural properties across the entire composite. Fungal biomass analysis was carried out using RStudio's *ImageJ* software [35], which facilitated the quantification of biomass by assessing the same area on each of the six replicates across Treatments 1 through 12. The software was configured to detect the white regions corresponding to mycelial biomass. For acoustic and mechanical testing, only treatments achieving at least 50% surface biomass colonization, calculated as the average and the median among the six replicates of each of the twelve treatments, were selected, as reported in Table 3. This threshold was

established as the minimum requirement to produce a compact and dense biomaterial suitable for transformation into various specimens for further mechanical and acoustic characterization. The Treatments surpassing this threshold were 1, 9, 10, 11, and 12.

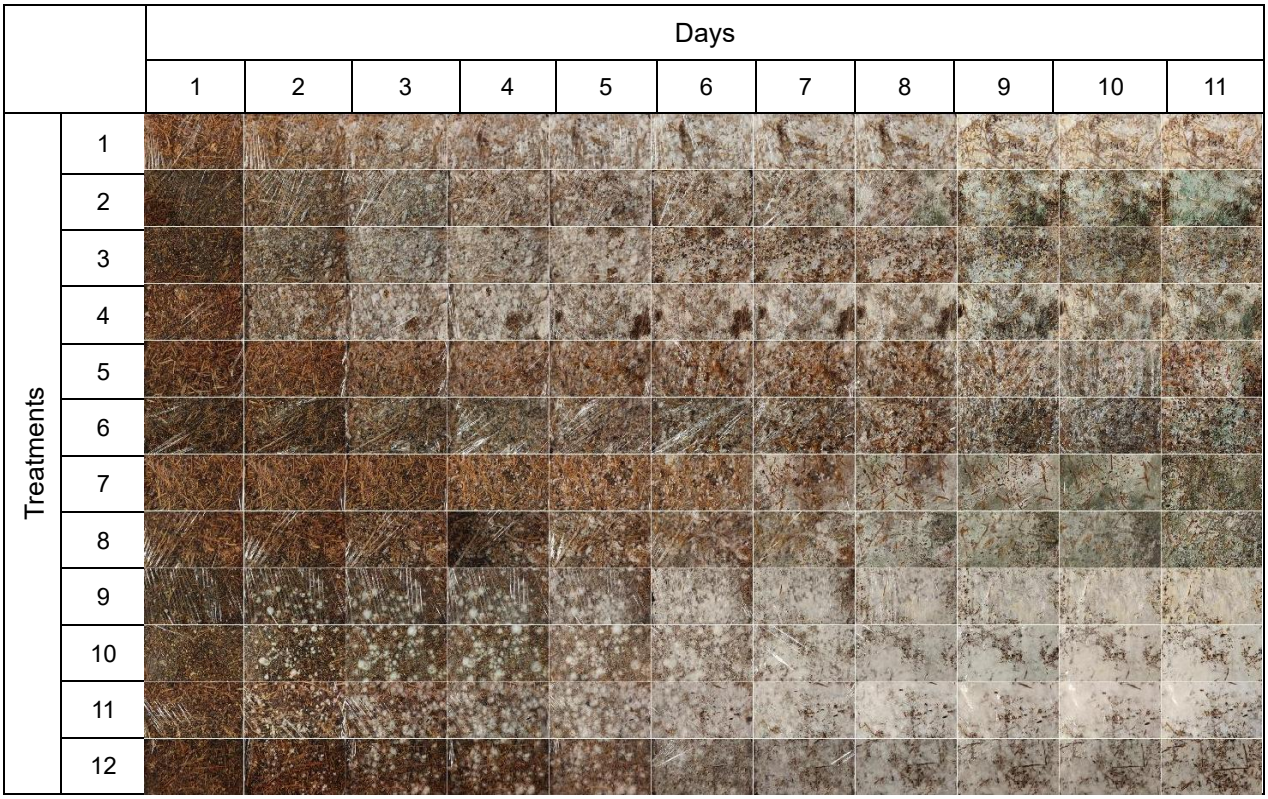

Fig. 2 Representative pictures of one for each of the 12 MBC treatments during the 10 days of growth

Table 3 Biomass colonization detected with RStudio's ImageJ software, and the average and median of each treatment found after 10 days of growth

Treatment #	Replicates	Biomass Colonization	Biomass Colonization Average (%)	Biomass Colonization Median (%)
1	**1**	**71.48**	**54.82**	**56.50**
	2	**58.43**		
	3	**40.62**		
	4	**69.18**		
	5	**34.65**		
	6	**54.58**		
2	1	58.71	40.37	36.16
	2	45.10		

	3	38.11		
	4	32.66		
	5	33.42		
	6	34.22		
3	1	36.75	38.49	37.56
	2	34.53		
	3	38.38		
	4	50.61		
	5	39.03		
	6	31.67		
4	1	40.11	43.46	40.56
	2	60.22		
	3	43.95		
	4	38.64		
	5	36.88		
	6	41.02		
5	1	26.64	29.94	28.70
	2	34.50		
	3	26.67		
	4	30.75		
	5	37.76		
	6	23.36		
6	1	29.95	32.77	32.53
	2	30.70		
	3	34.22		
	4	35.36		
	5	35.54		
	6	30.85		
7	1	38.48	45.13	43.45
	2	50.52		
	3	36.92		
	4	57.98		

	5	43.06		
	6	43.85		
8	1	50.61	47.68	49.68
	2	39.58		
	3	37.38		
	4	48.77		
	5	58.06		
	6	51.70		
9	**1**	**86.26**	**81.29**	**82.46**
	2	**85.40**		
	3	**74.90**		
	4	**79.78**		
	5	**85.15**		
	6	**76.27**		
10	**1**	**73.17**	**78.81**	**77.65**
	2	**85.21**		
	3	**76.97**		
	4	**78.34**		
	5	**86.39**		
	6	**72.81**		
11	**1**	**73.98**	**78.88**	**79.68**
	2	**80.65**		
	3	**81.38**		
	4	**78.71**		
	5	**83.63**		
	6	**74.94**		
12	**1**	**73.76**	**70.35**	**70.07**
	2	**63.51**		
	3	**56.18**		
	4	**66.40**		
	5	**82.94**		
	6	**79.32**		

In Group A (non-sterilized treatments), only Treatment 1 reached 50% colonization, demonstrating the potential for mycelium to continue growing on SMS without additional interventions. However, Treatments 2, 3, and 4 exhibited rapid colonization during the first three days, followed by a plateau, with no further increase in biomass growth (Fig. 2). Treatments 5, 6, 7, and 8 showed visible signs of contamination starting from day six, suggesting that the addition of fresh substrate (MS) mixed with SMS may have negatively impacted growth.

In contrast, all treatments in Group B (sterilized treatments) achieved more than 70% biomass colonization, highlighting the positive impact of sterilization on mycelium growth and development. These results underscore the effectiveness of sterilization in enhancing the colonization process and reducing contamination risks.

3.2.3. Preparation of the specimens for mechanical and acoustic characterization

After 10 days of growth, treatments with more than 50% surface biomass colonization were subjected to post-treatment using a Carver press. This procedure is one of the most promising ways for improving the mechanical properties of MBCs. These techniques have shown great promise in enhancing the structural integrity of MBCs and improving the bonding between the mycelium fibers and the reinforcement materials [36]. The process involved applying heat at 121°C and 1.5 metric tons of pressure for 10 minutes. Heat-pressing plays a vital role in fabricating MBCs, enhancing their mechanical properties by compacting the fibers into a denser and stronger material, thereby broadening potential applications across various industries [37], [38], [39], [40]. The MBCs were then fully inactivated in an oven at 60°C to remove residual moisture, stabilizing the samples at approximately 35% of their initial weight. Heat-pressing and oven drying are favored by industry as they are the fastest dehydration processes [41]. For mechanical testing, the treated MBCs were cut into cubes measuring 50×50×50 mm, following ASTM C109 standards, as outlined by Ghazvinian and Gürsoy (2022) [33], as shown in Fig. 3. For each of the Treatments 1, 9, 10, 11, and 12, three replicates were prepared for mechanical testing.

For acoustic absorption testing, four replicates of the Treatments 1, 9, 10, 11, and 12, were prepared by cutting circular samples of two different diameters: large (100 mm) and small (29 mm), both having a thickness around 30 mm, as shown in Fig. 4. The samples were precisely cut and sanded to fit the circular cross-section of the impedance tube, ensuring an airtight seal for accurate measurements.

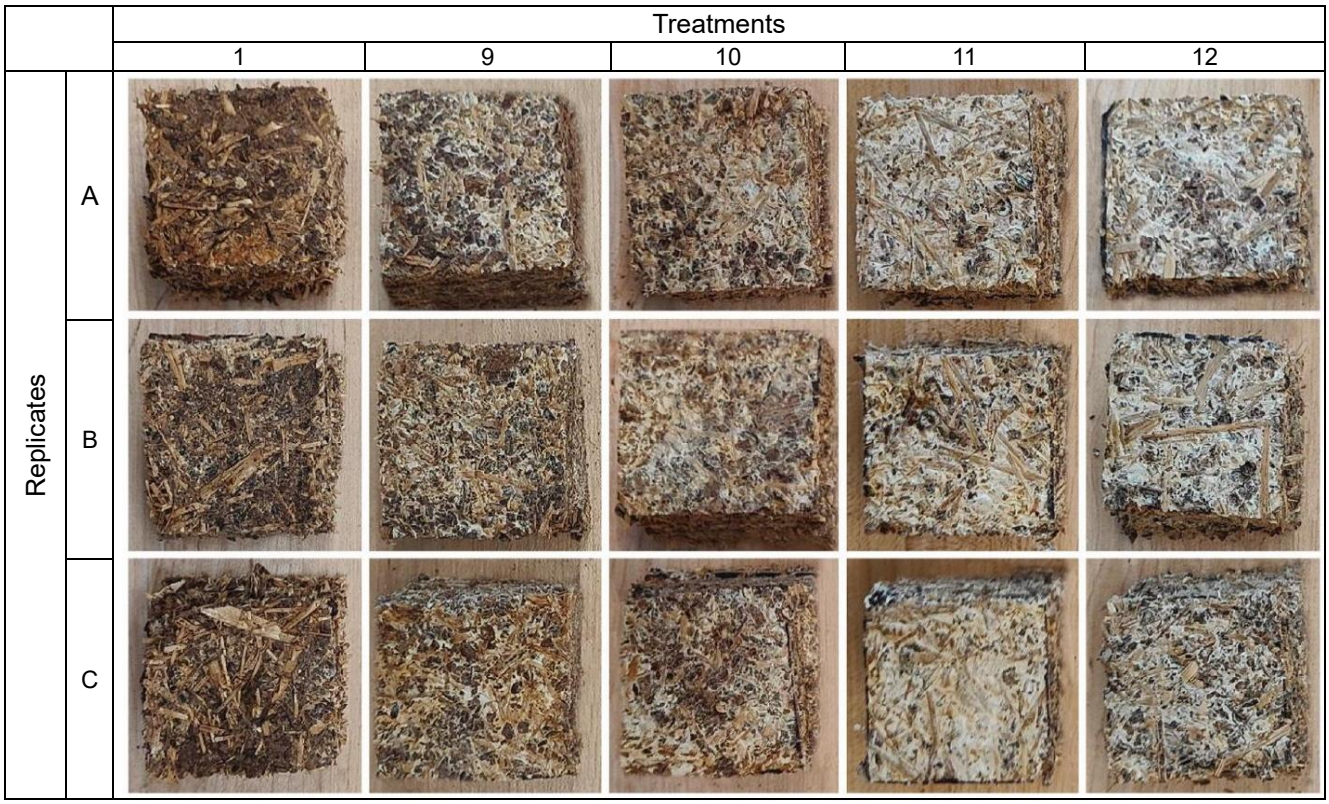

Fig. 3 Top view of the samples 50×50×50 mm with three replicates (A, B, C) from the selected Treatments (1, 9, 10, 11 and 12) prior to compressive strength testing.

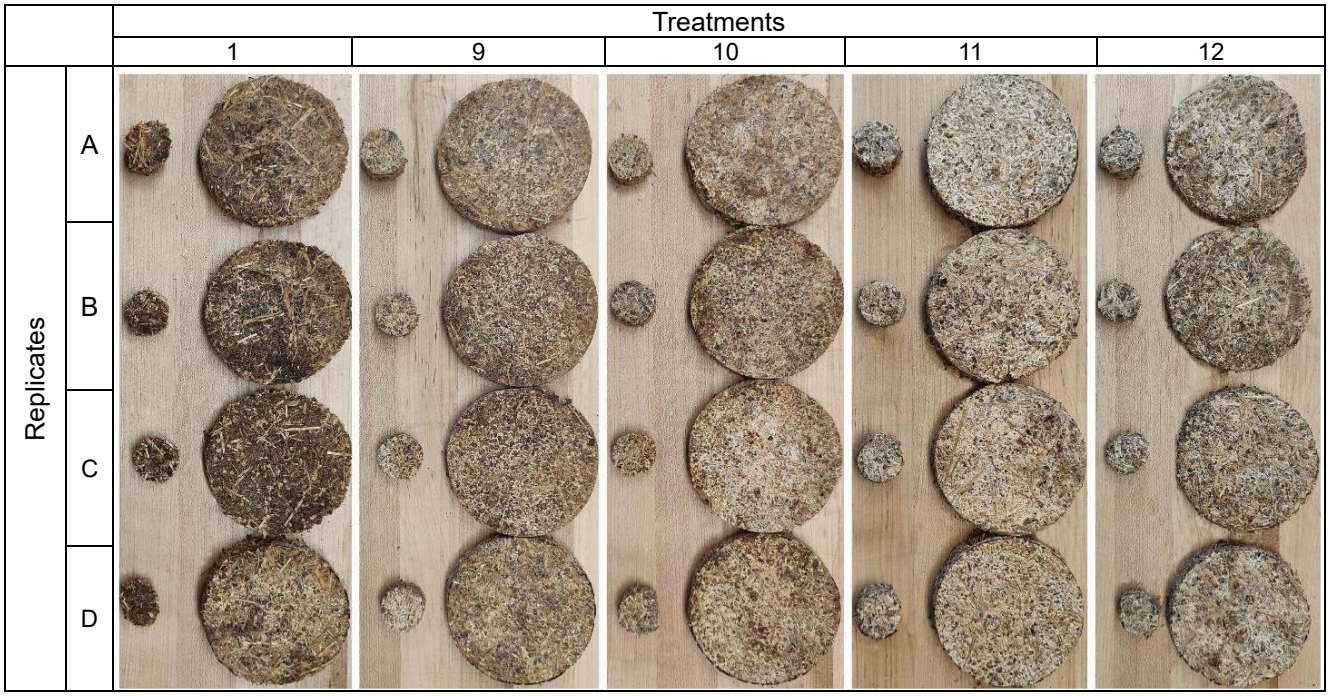

Fig. 4 Top view of the small (29mm) and the large (100mm) samples with 4 replicates (A, B, C, D) from the selected Treatments (1, 9, 10, 11 and 12) prepared for acoustic absorption tests

3.2.4. Testing and analysis of the samples for compressive strength

Compression testing was conducted using an MTS machine at a velocity of 0.05 mm/s, compressing the cubic samples to 80% strain following the same procedure reported by Ghazvinian and Gürsoy (2022) [33]. The stress-strain behavior exhibited two distinct regions: the first region (up to 20% strain) corresponded to the deformation and compaction of the porous structure without collapse; the second region (above 40% strain) reflected structural collapse and subsequent gradual compaction. Material strength was evaluated based on the maximum stress observed during testing. Two types of maximum stress were identified: the first is the local peak stress for specimens that experienced failure, typically due to transverse splitting, and the second is the maximum stress applied by the dynamometer for specimens that exhibited continuous compaction without structural failure. In the latter case, the peak stress was defined as the highest stress value within the range of 0 to 0.70 strain, following a methodology similar to that reported by Ghazvinian and Gürsoy (2022) [33]. Additionally, material stiffness was determined by analyzing the slope of the stress-strain curve in its steepest region. Young's modulus was calculated in the second region (approximately 40% strain), and the range of values across all samples was determined. This analysis follows the framework outlined by Dognini and Gürsoy (2024) [42], providing a robust methodology for assessing the compressive properties of MBCs. Furthermore, the statistical analysis was carried out. Stress-strain data were collected, and for each material, the mean and standard deviation of stress values were analyzed at corresponding strain levels. One-way ANOVA was applied at selected strain points to evaluate differences among materials. As the tests were conducted in triplicate, the values were expressed as mean ± SD. Statistical significance was detected by analysis of variance (ANOVA, value of $p < 0.05$ indicated statistical difference).

3.2.5. Testing and analysis of the samples for acoustic absorption

Acoustic absorption tests were performed in four replicates using materials derived from Treatments 1, 9-12. A Brüel & Kjær Type 4206 Impedance Tube, as shown in Fig. 5, was employed for the measurements, with large samples being characterized for low frequencies and small samples for high frequencies.

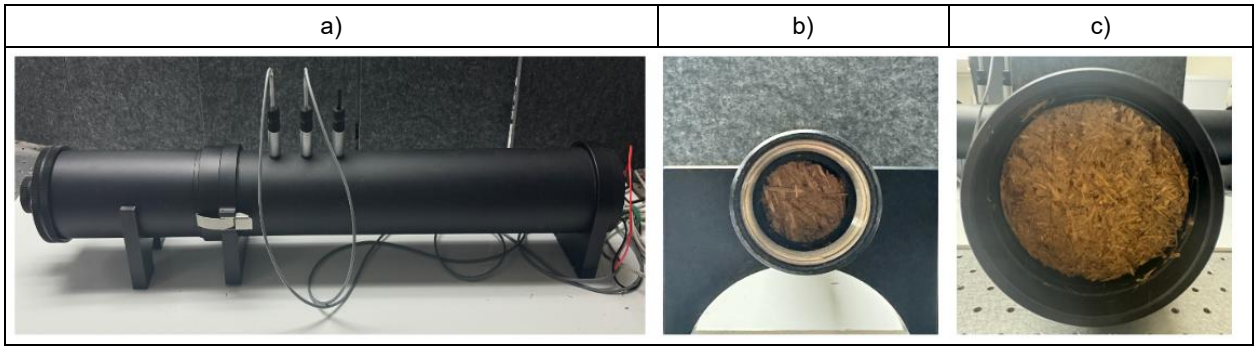

Fig. 5 Image of a) Brüel & Kjær Type 4206 Impedance Tube used for the acoustical tests, samples in b) the small impedance tube, and c) the large impedance tube

For each SMS treatment, a total of eight samples (four large and four small) were tested to determine the sound absorption coefficient (α) across specific frequency ranges. The large impedance tube (100mm) measured absorption coefficients in the frequency range of 50 Hz to 1600 Hz, while the small impedance tube (29mm) covered the range from 500 Hz to 6400 Hz. The tests were conducted following ASTM E1050-19 standards [43] for sound absorption coefficient measurements, ensuring consistency and reliability of the results across a range of frequencies. Mean values and standard deviations were computed for selected frequencies in both the low (0–1600 Hz) and high (1600–6400 Hz) frequency ranges. One-way ANOVA was performed only at selected frequency values to assess statistically significant differences among treatments. The analysis was conducted in quadruplicate, and the values were expressed as mean ± SD. Statistical significance was detected by analysis of variance (ANOVA, value of $p < 0.05$ indicated statistical difference).

4. Results and Discussion

4.1. Mechanical Tests: Compressive Strength

The compressive strength analysis of 50×50×50 mm specimens revealed distinct mechanical behaviors based on the composition and treatment applied. The stress–strain curves of the three replicates from Treatments 1, 9-12 are presented in Fig. 6, highlighting differences in material performance and showing the median curve for each treatment. This figure shows the qualitative assessment of mechanical behavior and intra-group variability across the entire deformation process. Key mechanical parameters, including maximum stress and material stiffness, were evaluated up to 70% strain to ensure consistent and comparable measurements across samples as reported in Table 4. This threshold was selected to capture the most structurally relevant phases of deformation while avoiding the influence of post-peak instability and densification. Additionally, 70% strain represents the last strain level reached by all three replicates in one treatment group, providing a cutoff for consistent data inclusion. All specimens exhibited a non-linear behavior, starting at zero stress, increasing to a peak stress point, and then declining after surpassing it. This behavior reflects an initial elastic phase, where the strain gradually increases up to approximately 20%, followed by a secondary phase characterized by pore collapse and progressive densification of the cellular structure. During this phase, the Treatments show a transition from elastic to plastic deformation, and in some cases, strain softening. Although densification contributes to the overall mechanical response, the quantitative analysis was intentionally limited to the pre-densification range (up to 70% strain), where the response remains most consistent and representative of functional performance under compressive loads.

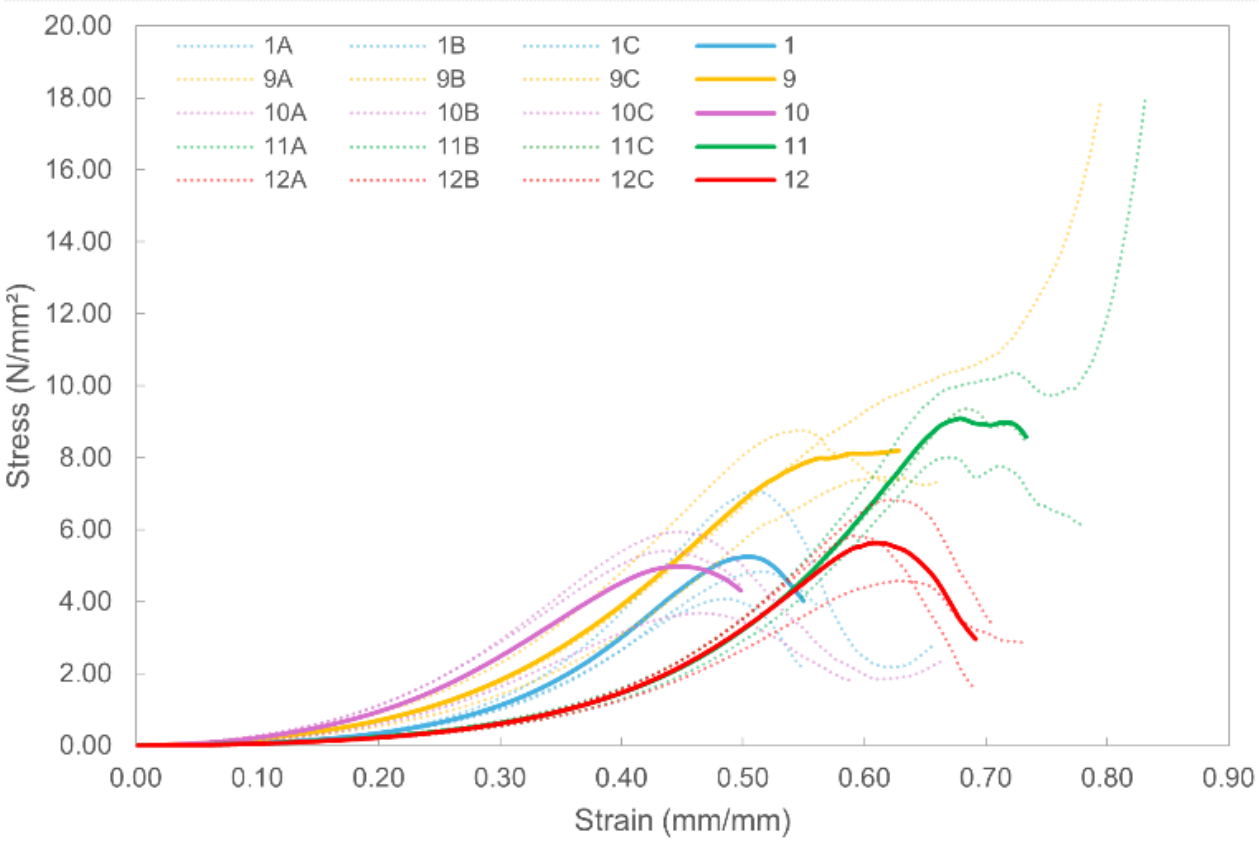

Fig. 6 Mechanical compression test of the Treatments 1, 9-12, tested in triplicate using 50×50×50 mm specimens. Dashed lines represent the individual replicates, while solid lines indicate the median curve for each treatment.

Table 4 Peak stress (MPa), Strain where the Peak Stress occurred (mm/mm) and Young's modulus (N/mm^2) of the best Treatments occurred into the range of 70% Strain.

Treatment #	Replicates	Peak Stress (MPa)	Strain (mm/mm)	Young's Modulus (N/mm^2)
1	1A	4.07	0.48	21.96
	1B	7.07	0.50	37.11
	1C	4.84	0.51	23.71
9	9A	7.47	0.61	24.09
	9B	8.76	0.54	33.55
	9C	10.74	0.70	28.47
10	10A	3.68	0.46	15.40
	10B	5.95	0.44	24.44
	10C	5.40	0.43	21.56

11	11A	10.16	0.70	45.77
	11B	8.01	0.67	38.48
	11C	9.36	0.68	37.01
12	12A	5.84	0.59	28.48
	12B	4.58	0.62	19.14
	12C	6.83	0.61	32.83

In addition to the stress–strain curves, Fig. 7 provides a summary of the two key mechanical parameters extracted within the 70% strain range, analyzing the peak compressive stress (reported in Fig. 7a) and Young's modulus (reported in Fig. 7b). Treatments 11 exhibited both the greatest peak stress and the highest stiffness considering the mean value reported; Treatments 1 and 10 showed lower values both in term of peak stress and stiffness, in line with their earlier structural failure.

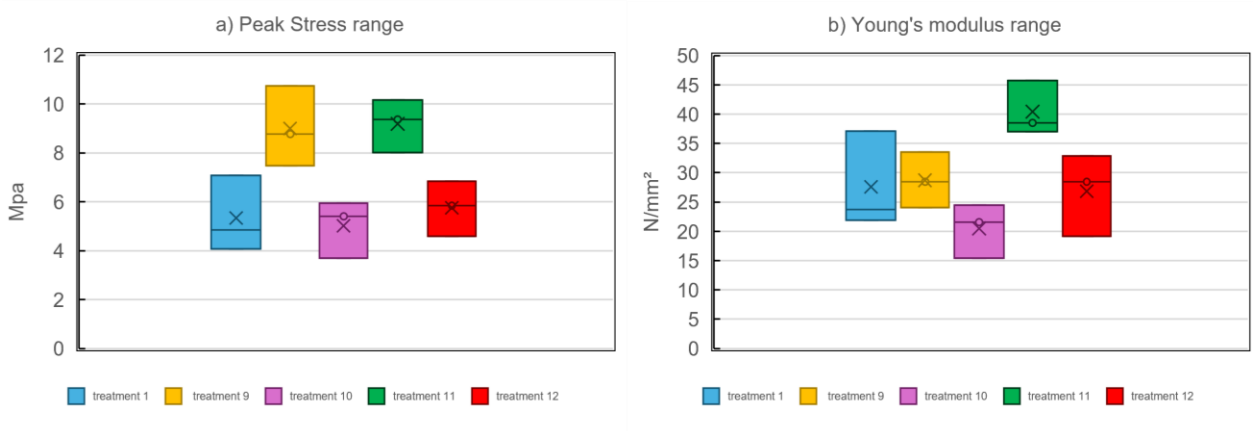

Fig. 7 Summary of mechanical properties calculated from uniaxial compression tests on mycelium-based composite (MBC) specimens (Treatments 1, 9-12), evaluated within the 70% strain range. (a) Peak compressive stress (MPa), defined as the highest stress value reached by each specimen within the defined range. (b) Young's modulus (N/mm²), calculated as the slope of the initial linear region of the stress–strain curve, corresponding to the elastic phase. For each treatment, the box indicates the range between minimum and maximum values across replicates, and the central cross marker (×) represents the mean.

For statistical evaluation, the compressive mechanical properties were analyzed by measuring stress (N/mm²) at increasing strain levels, and the results are reported as mean ± standard deviation in Table 5. A one-way ANOVA was performed at each strain level to assess statistical differences among treatments. Fig. 8 presents the mean stress values and corresponding standard deviations at selected strain intervals up to 70% strain. However, at 70% strain, Treatments 1, 9, 10, and 12 had already experienced structural failure, resulting in stress values of 0±0. Only treatment 11 retained measurable stress at this level. Therefore, the variance across groups was insufficient, and the ANOVA yielded a non-significant result (p = 0.39), as reported in Table 5. This final strain

level should therefore be interpreted as a structural limit for most samples, rather than a statistically robust point of comparison.

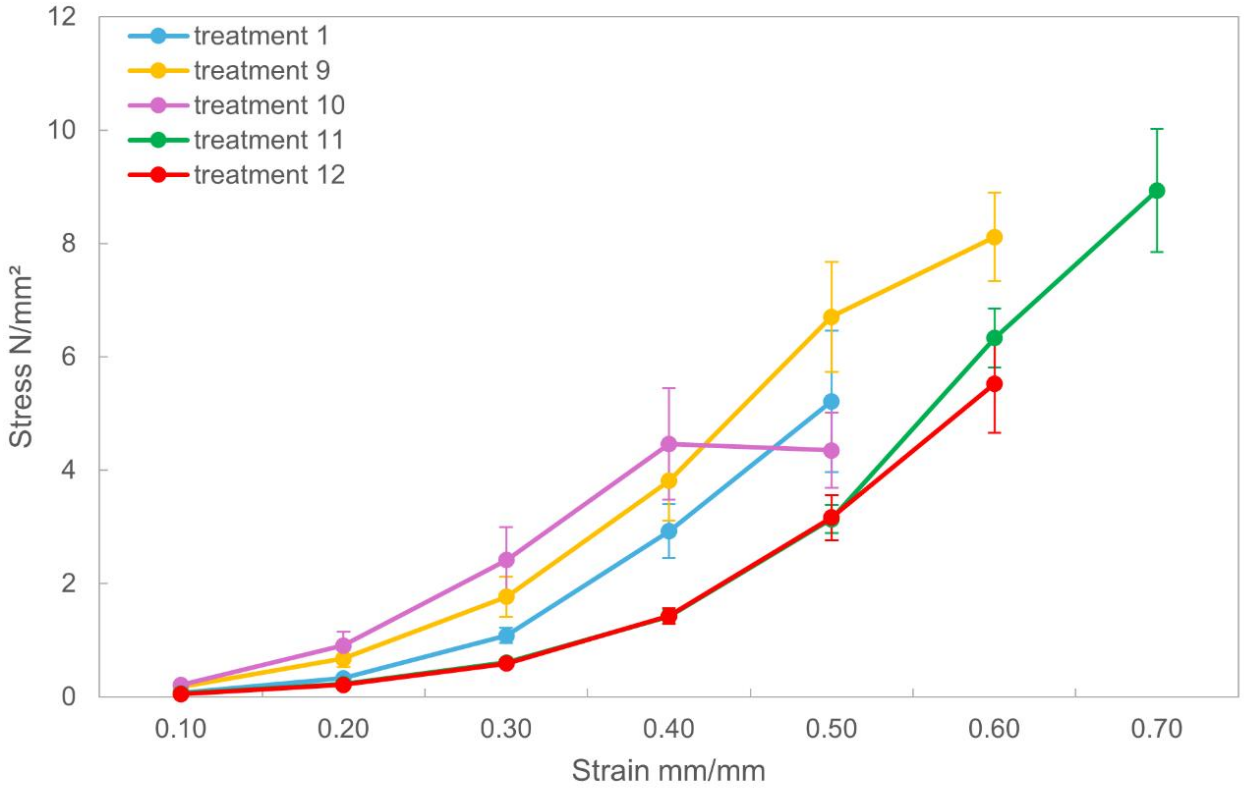

Fig. 8 Mean compressive stress (N/mm²) as a function of strain (mm/mm) for each Treatment, based on three replicates. Error bars represent the standard deviation (±SD) at each strain level, indicating the variability among specimens within each Treatment group.

Table 5 Mean stress values (N/mm²) ± standard deviation for the best treatments at increasing strain levels, with ANOVA statistical analysis

Strain mm/mm	**Treatment 1 (mean±SD)**	**Treatment 9 (mean±SD)**	**Treatment 10 (mean±SD)**	**Treatment 11 (mean±SD)**	**Treatment 12 (mean±SD)**	***F*-statistic**	***p*-value**
0.10	0.070 ± 0.005	0.177 ± 0.051	0.209 ± 0.060	0.058 ± 0.005	0.050 ± 0.002	8.88	0.00
0.20	0.333 ± 0.018	0.677 ± 0.150	0.911 ± 0.238	0.230 ± 0.023	0.215 ± 0.016	11.78	0.00
0.30	1.081 ± 0.132	1.769 ± 0.353	2.415 ± 0.586	0.608 ± 0.058	0.590 ± 0.048	12.67	0.00
0.40	2.926 ± 0.475	3.814 ± 0.697	4.468 ± 0.982	1.425 ± 0.122	1.429 ± 0.141	11.10	0.00
0.50	5.219 ± 1.249	6.708 ± 0.973	4.354 ± 0.668	3.141 ± 0.245	3.165 ± 0.398	7.13	0.01
0.60	0.000 ± 0.000	8.117 ± 0.779	0.000 ± 0.000	6.331 ± 0.519	5.532 ± 0.876	12.74	0.00
0.70	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	8.936 ± 1.087	0.000 ± 0.000	3.23	0.39

Among the tested formulations, Treatment 1, based on 100% non-sterilized SMS without reinoculation (I) or nitrogen (N) supplementation (Group A), generally exhibited the lower and less consistent mechanical performance compared to the other treatments. While compressive stress values for Treatment 1 were not always the lowest at early strain levels (0.10–0.30 mm/mm), significant differences became more evident from 0.40 mm/mm onward, as confirmed by ANOVA analysis ($p < 0.01$). The stress–strain curves showed relatively early failure (0.50 strain) and modest peak stress values ranging from 4.07 to 7.07 MPa. The Young's modulus also varied widely 21.96–37.11 N/mm², reflecting poor internal cohesion. As reported in Table 5 and Fig. 8, Treatment 1 showed significantly lower mean stress values at intermediate strain levels (0.40–0.50 strain), confirming its weaker structural integrity relative to the other groups.

In contrast, Group B treatments, which employed sterilized and reinoculated substrates, displayed markedly better mechanical behavior. Treatment 9, composed of 100% sterilized SMS inoculated with *P. ostreatus*, showed strong but slightly variable performance. Peak stress values ranged from 7.47 to 10.74 MPa, with failure occurring between 0.54 and 0.70 strain. Although it achieved a high early stiffness ranking among the best performers up to 0.50 mm/mm strain, it failed structurally before 0.70 mm/mm strain. The variability observed across replicates was also evident in the broad peak stress range and standard deviation, suggesting some degree of non-uniformity. Treatment 10, which shared the same base formulation as Treatment 9 but included nitrogen (N) supplementation, displayed reduced performance. It exhibited the lowest mechanical properties within Group B, with peak stress values between 3.68 and 5.95 MPa and the lowest Young's modulus range (15.40–24.44 N/mm²). Nevertheless, it showed the highest mean stress at early strain levels (0.10–0.30 mm/mm), indicating a stiffer response in the initial loading phase. Failure occurred at 0.60 strain, and ANOVA results confirmed a decline in statistical relevance beyond this point. Treatment 11, consisting of a 50% sterilized SMS and 50% MS, inoculated without nitrogen (N) supplementation, exhibited the most robust and consistent mechanical profile. It recorded the highest peak stress (8.01–10.16 MPa) and Young's modulus values (37.01–45.77 N/mm²), with strain at failure extending to 0.70 mm/mm across all replicates. Statistically, Treatment 11 achieved the highest mean stress values from 0.50 strain onward with the only formulation to retain structural integrity at 0.70 strain, resulting in a non-significant ANOVA due to lack of variance ($p = 0.39$). Its performance demonstrates the beneficial synergy of sterilization, reinoculation (I), and fresh substrate addition (MS). Treatment 12, based on the same substrate mix of Treatment 11 but with nitrogen (N) supplementation, showed intermediate results. Peak stress values ranged from 4.58 to 6.83 MPa, and the Young's modulus varied between 19.14 and 32.83 N/mm². Although slightly improved over Treatment 11, it failed before reaching 0.70 strain and showed only moderate stress values during statistical comparison, suggesting that nitrogen (N) supplementation did not contribute positively to mechanical reinforcement.

Taken together, the results reveal a clear pattern: sterilization and reinoculation are essential to obtain structurally cohesive and mechanically performant SMS-based MBCs. The addition of fresh substrate (MS) (as in Treatments 11 and 12) further enhances these properties, particularly in terms of stiffness and strain tolerance. In contrast, nitrogen (N)

supplementation alone (Treatments 10 and 12) does not consistently improve mechanical behavior and may compromise network formation. Treatment 11 emerges as the optimal formulation, offering the best combination of stiffness, strength, and ductility across the examined strain range.

In addition, a comparison was carried out between the compressive strength of Treatments 1, 9, 10, 11, and 12 and values reported in the literature for MBCs, as summarized in Table 6.

Table 6 Comparison of compression strength (MPa) of Treatments 1, 9, 10, 11 and 12, alongside with results from studies of mycelium-based biomaterials, the results are reported as mean ± standard deviation.

	Sample's dimensions (mm)	**ASTM**	**Compression Strength (MPa)**
Sawdust with Lentinus sajor-caju [44]	86 ⌀ x 43 h	N/D	1.87 ± 0.03
Corn husk with Ganoderma williamsianum [44]	86 ⌀ x 43 h	N/D	0.62 ± 0.01
Rice straw with Ganoderma williamsianum [44]	86 ⌀ x 43 h	N/D	0.36 ± 0.02
Hemp hurds and paper with Fomes fomentarius [45]	N/D	ASTM D1621-16	0.14 ± 0.00
Hemp hurds and paper with Trametes pubescens [45]	N/D	ASTM D1621-16	0.14 ± 0.01
Hemp hurds and paper with Ganoderma lucidum [45]	N/D	ASTM D1621-16	0.32 ± 0.03
Treatment 1	50 x 50 x 50	ASTM C109	5.22 ± 1.25
Treatment 9	50 x 50 x 50	ASTM C109	8.12 ± 0.78
Treatment 10	50 x 50 x 50	ASTM C109	4.47 ± 0.98
Treatment 11	50 x 50 x 50	ASTM C109	8.94 ± 1.09
Treatment 12	50 x 50 x 50	ASTM C109	5.53 ± 0.88

While compressive strength alone does not determine structural applicability, it remains a critical indicator of material stiffness and load-bearing capacity.
Among the literature the compressive strength values remain relatively low, typically below 2 MPa, with rice straw with Ganoderma williamsianum showing only 0.36 ± 0.02 MPa, and corn husk composites reporting 0.62 ± 0.01 MPa. Even more technically processed composites, such as hemp hurds and paper with various fungal strains tested according to ASTM D1621-16, report compressive strength values ranging between 0.14 and 0.32 MPa. In contrast, the experimental samples presented in this study exhibit significantly higher compressive performance as shown by Treatment 11 (8.94 ± 1.09 MPa). Generally, according to the literature, compressive strength in MBCs consistently ranges between 29 kPa and 567 kPa [46]. The materials developed in this study exceed that upper range by more than an order of magnitude, thus placing them well beyond the mechanical limits of traditional mycelium composites and suggesting potential for use in semi-structural or protective applications, where stiffness and compressive resistance are required.

4.2. Acoustic tests

Acoustic absorption test was carried out for Treatments 1, 9-12, using impedance tube across two frequency ranges: low frequencies (100–1600 Hz) by testing large samples (100 mm Ø), and high frequencies (500–6400 Hz) by testing smaller samples (29 mm Ø). Four replicates were tested per treatment. The absorption coefficients (α) of each treatment are presented in Fig. 9a that reported the low frequencies and Fig. 9b that reported the high frequencies.

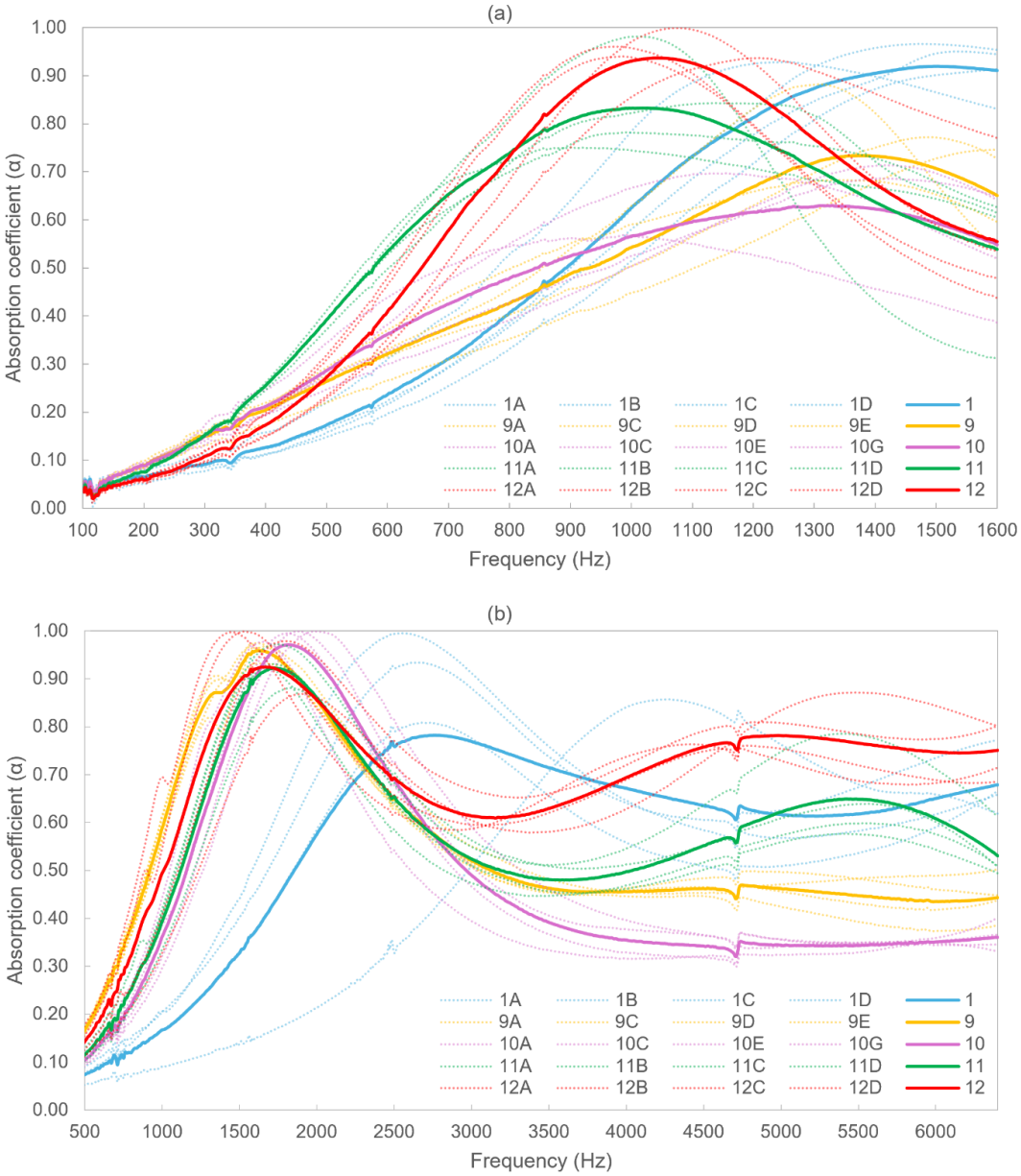

Fig. 9 Averaged sound absorption coefficient (α) measurements of low frequency (a) samples (100 mm), and high frequency (b) samples (29 mm)

The corresponding average values and standard deviations are summarized in Fig. 10a that reported the low frequencies and Fig. 10b that reported the high frequencies, with statistical outcomes reported in Table 7.

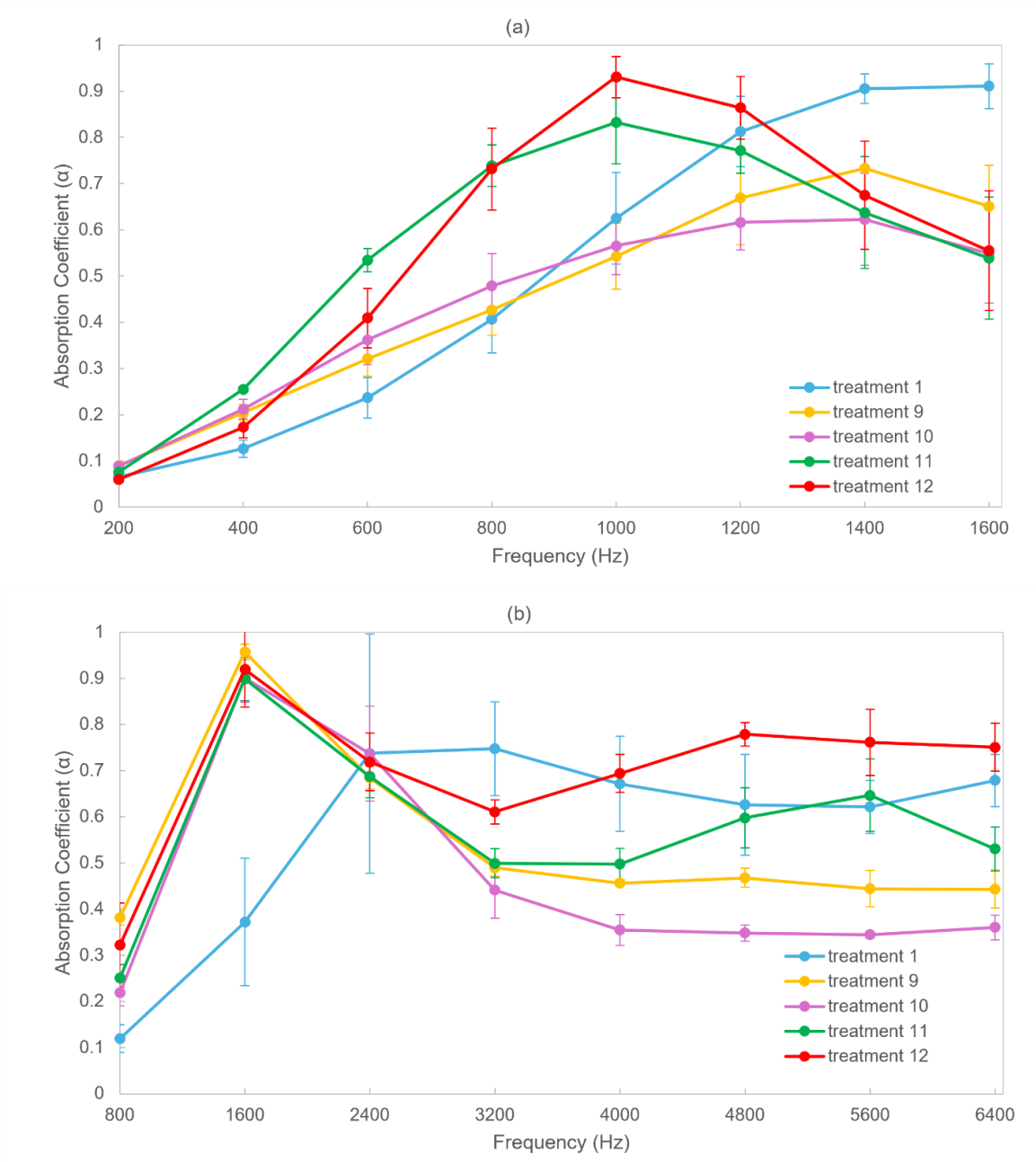

Fig. 10 Sound absorption coefficient (α) of the best Treatments across low (a) and high (b) frequency ranges with statistical comparison (ANOVA)

Table 7 Mean stress values (N/mm²) ± standard deviation for the best treatments across frequencies from 200 Hz to 1600 Hz, and from 800 Hz to 6400 Hz with ANOVA results

Low Frequency (Hz)	Treatment 1 (mean±SD)	Treatment 9 (mean±SD)	Treatment 10 (mean±SD)	Treatment 11 (mean±SD)	Treatment 12 (mean±SD)	*F*-statistic	*p*-value
200	0.066 ± 0.011	0.090 ± 0.006	0.089 ± 0.005	0.075 ± 0.001	0.060 ± 0.004	14.15	0.00
400	0.127 ± 0.019	0.205 ± 0.017	0.212 ± 0.021	0.255 ± 0.006	0.173 ± 0.023	20.73	0.00
600	0.237 ± 0.044	0.321 ± 0.038	0.362 ± 0.053	0.534 ± 0.025	0.409 ± 0.064	16.66	0.00
800	0.407 ± 0.073	0.427 ± 0.054	0.478 ± 0.070	0.738 ± 0.045	0.732 ± 0.089	17.73	0.00
1000	0.625 ± 0.099	0.543 ± 0.071	0.565 ± 0.062	0.832 ± 0.090	0.930 ± 0.045	15.42	0.00
1200	0.813 ± 0.076	0.669 ± 0.101	0.616 ± 0.059	0.771 ± 0.048	0.864 ± 0.068	5.94	0.00
1400	0.905 ± 0.032	0.733 ± 0.059	0.622 ± 0.100	0.637 ± 0.121	0.674 ± 0.117	4.61	0.01
1600	0.911 ± 0.049	0.651 ± 0.089	0.548 ± 0.107	0.539 ± 0.132	0.555 ± 0.129	6.67	0.00

High Frequency (Hz)	Treatment 1 (mean±SD)	Treatment 9 (mean±SD)	Treatment 10 (mean±SD)	Treatment 11 (mean±SD)	Treatment 12 (mean±SD)	*F*-statistic	*p*-value
800	0.119 ± 0.030	0.381 ± 0.016	0.219 ± 0.029	0.251 ± 0.029	0.323 ± 0.091	13.50	0.00
1600	0.372 ± 0.138	0.957 ± 0.016	0.900 ± 0.052	0.899 ± 0.047	0.920 ± 0.082	29.34	0.00
2400	0.737 ± 0.259	0.685 ± 0.025	0.737 ± 0.103	0.687 ± 0.045	0.719 ± 0.062	0.12	0.97
3200	0.748 ± 0.102	0.490 ± 0.018	0.441 ± 0.061	0.499 ± 0.032	0.611 ± 0.026	14.13	0.00
4000	0.671 ± 0.104	0.456 ± 0.006	0.355 ± 0.033	0.497 ± 0.034	0.694 ± 0.041	21.35	0.00
4800	0.626 ± 0.109	0.468 ± 0.021	0.348 ± 0.018	0.598 ± 0.065	0.779 ± 0.025	22.78	0.00

5600	0.621 ± 0.058	0.444 ± 0.040	0.345 ± 0.007	0.646 ± 0.078	0.761 ± 0.072	25.59	0.00
6400	0.679 ± 0.057	0.443 ± 0.042	0.360 ± 0.026	0.530 ± 0.047	0.751 ± 0.052	37.05	0.00

For the low frequency, as shown in Fig. 9a, all treatments demonstrated an increase in absorption coefficient with frequency, reaching absorption coefficients above 0.5 by 1000 Hz. Treatment 1 demonstrated a gradual increase in absorption, peaking at 0.911±0.049 at 1600 Hz, though values at lower frequencies remained significantly lower (e.g., 0.625±0.099 at 1000 Hz), presenting overall performance weaker compared to the other treatments and suggesting limited early-range effectiveness and greater variability. All samples demonstrate varying peak frequencies, generally ranging between 1000 Hz and 1600 Hz.

Treatments 9 and 10 show consistent performance, maintaining absorption coefficients above 0.6 across most of the frequency range. Treatment 9 achieved strong mid-range performance, reaching 0.733±0.059 at 1400 Hz, though its values remained slightly below those of the top-performing at 1000 and 1200 Hz such as treatments 11 and 12. Treatment 10 showed moderate performance, with absorption peaking at 0.622±0.100 at 1400 Hz, but generally underperformed compared to the others, especially below 1000 Hz.

Treatment 11 recorded high absorption values in the mid-frequency range, with 0.832±0.090 at 1000 Hz and consistent performance up to 1400 Hz with 0.637±0.121, indicating balanced absorption characteristics, even if it exhibits a slight decline at higher frequencies.

Treatment 12 outperformed all others in this frequency range, peaking at 0.930±0.064 at 1000 Hz, and maintaining an absorption coefficient above 0.85 from 1000 to 1400 Hz, whereas other samples experienced varying degrees of decline. Overall, Treatment 12 demonstrates the best absorption across the low frequency range, particularly excelling in the mid and high frequencies, making it ideal for applications requiring broad-spectrum sound absorption, while Treatments 9, 10, and 11 offer moderate and consistent performance, making them suitable for general-purpose acoustic applications. The one-way ANOVA showed statistically significant differences ($p<0.01$) at all frequencies in this range, with particularly high F-statistics at 400 Hz ($F=20.73$) and 800 Hz ($F=17.73$), confirming that the acoustic response at low frequencies is strongly influenced by material formulation.

The graph in Fig. 9b illustrates the acoustical absorption coefficients (α) of the smaller samples (1, 9-12) measured across a frequency range of 500 Hz to 6400 Hz. All Treatments showed distinct behavior in the high-frequency range, achieving maximum absorption coefficients of 0.9 or higher at specific frequency ranges, with Treatments 9, 10, 11, and 12 nearing perfect absorption coefficients around 1600 Hz, aligning with optimal mid-frequency sound absorption performance. In contrast, Treatment 1 shows a distinct peak of 0.737±0.259 at 2400 Hz, which differs from the other samples, indicating its performance is shifted toward higher frequencies and maintains relatively stable values

of absorption coefficient around 0.6–0.7 from 2400 Hz onward. However, it also showed the largest variability across replicates and frequencies.

Treatment 9 is the standout performer at mid-range frequencies, achieving near-perfect absorption, with the peak reaching 0.957±0.016 at 1600 Hz, but experienced a sharp drop to 0.490±0.018 at 3200 Hz, with further reduction at higher frequencies. Treatment 10 showed a similar trend, with a maximum of 0.900±0.052 at 1600 Hz, followed by a progressive decline of the absorption coefficient below 0.4 beyond 4000 Hz. Treatment 11 maintained good absorption values, reaching 0.899±0.047 at 1600 Hz and stabilizing between 0.50–0.65 from 3200 to 6400 Hz, indicating effective performance across a wide frequency band. Treatment 12 again demonstrated the most stable and balanced high-frequency response, maintaining an absorption coefficient at around 0.70 from 1600 Hz onward and peaking at 0.920 ± 0.082 at 1600 Hz, indicating its suitability for applications requiring effective sound absorption across a broader frequency range. Treatment 12 provides the most balanced performance across the entire frequency spectrum. Treatment 1, despite its distinct peak at 3200 Hz, exhibits weaker absorption overall, making it less effective compared to the other samples. These findings highlight the varying acoustical properties of SMS-based MBCs and their potential applications tailored to specific frequency ranges. Statistical analysis confirmed significant differences (p<0.01) at nearly all frequency points, with particularly high F-statistics at 1600 Hz (F=29.34) and 6400 Hz (F=37.05). The only exception was at 2400 Hz, where no significant difference was observed between treatments (F=0.12, p=0.97). This suggests that, at this frequency, a convergence of material responses may occur due to shared geometric or acoustic properties, such as similar resonance or wave interference effects in the test setup.

These results highlight the dual functionality of SMS-based MBCs, offering both structural strength and acoustic performance. Reinoculation with *P. ostreatus* (Group B) significantly enhances mechanical properties, while incorporating MS further improves structural robustness. N supplementation, however, exhibits limited benefits for both mechanical resistance and acoustic absorption. The acoustic properties, combined with the mechanical findings, suggest that SMS-based MBCs have significant potential for applications requiring sound-damping and structural support, particularly in temporary or modular construction settings. Importantly, while heat-pressing contributes to the improvement of mechanical strength by increasing material density and enhancing bonding within the matrix, it simultaneously reduces porosity, which may adversely affect acoustic performance. This trade-off reflects a known limitation in physical processing techniques: as highlighted in recent studies, methods such as cold and hot pressing have been shown to significantly enhance the structural integrity of MBCs by eliminating air pockets, increasing density, and strengthening the interaction between mycelium fibers and reinforcement materials [42]. However, since acoustic absorption is closely linked to porosity, a denser and less porous structure may result in reduced sound-damping capacity. Therefore, optimizing the balance between mechanical reinforcement and functional acoustic performance remains a critical design consideration for the development of SMS-based mycelium composites.

In addition, a comparative evaluation of the acoustic performance of the developed treatment with the Noise Reduction Coefficient (NRC) was calculated for each treatments and compared with other MBCs reported in the literature as shown in Table 8.

Table 8 Comparison of acoustic absorption coefficients (α) and NRC values of Treatments 1, 9, 10, 11 and 12, alongside with results from studies of various bioacoustics materials.

		Frequencies (Hz)				
	Thickness	**250**	**500**	**1000**	**2000**	**NRC**
Mogu "Plain" [47]	40 mm	0.30	0.50	0.40	0.40	0.40
Shredded Cardboard [48]	38 mm	0.06	0.01	0.46	0.96	0.40
Bamboo Biocomposites [49]	40 mm	0.11	0.39	0.98	0.75	0.55
Hemp w/ Lime, Water, Shives [50]	40 mm	0.05	0.10	0.16	0.14	0.10
Rice Husk and Resin [51]	40 mm	0.26	0.48	0.89	0.41	0.50
Treatment 1	30 mm	0.05	0.07	0.17	0.58	0.20
Treatment 9	30 mm	0.07	0.17	0.59	0.86	0.40
Treatment 10	30 mm	0.05	0.10	0.36	0.94	0.35
Treatment 11	30 mm	0.05	0.12	0.39	0.86	0.35
Treatment 12	30 mm	0.06	0.14	0.49	0.86	0.40

The NRC provides a standardized, single-value indicator of a material's sound-absorbing capacity across the mid-frequency range (250–2000 Hz), which is particularly relevant for typical human speech environments such as classrooms, offices, and auditoriums [52]
As summarized in Table 8, the experimental treatments exhibited NRC values ranging from 0.20 (Treatment 1) to 0.40 (Treatment 12). Notably, Treatment 12 matched the NRC performance of established sustainable references such as shredded cardboard and Mogu "Plain", both at 0.40. Among all materials considered, Bamboo Biocomposites achieved the highest NRC (0.55), demonstrating excellent absorption across the targeted frequency range. In contrast, the hemp-based sample showed the lowest NRC value (0.10), indicating limited mid-frequency absorption.
These findings confirm that the materials developed in this study, particularly Treatment 12, are not only viable but also competitive with several bio-based acoustic materials reported in the literature. Their performance supports their potential use in sustainable sound-absorbing applications, offering an environmentally friendly alternative with promising acoustic properties.

5. Conclusions

This study demonstrates the significant potential of repurposing SMS into MBCs, providing an innovative solution for waste management and sustainable material development. Among the tested formulations, mechanically, all samples exhibited a typical non-linear stress–strain response, with peak stress values reached between 0.43 and 0.70 mm/mm strain. The formulation based on sterilized SMS mixed with fresh substrate addition (MS) and reinoculated with *P. ostreatus* (Treatment 11) exhibited the best balance between mechanical resistance and strain tolerance and the relatively high peak stress values indicate that the material can withstand considerable force before breaking, making it suitable for applications requiring moderate load-bearing capacity,

such as lightweight structural components. It showed superior peak stress up to 10.16 N/mm² and the highest Young's modulus up to 45.77 N/mm², and the ability to sustain load up to 70% strain, making it suitable for applications requiring both stiffness and deformation tolerance, such as cushioning or structural panels. The ability to tolerate higher strain before failure demonstrates that the material is not only strong but also flexible to a certain extent. This balance between strength and flexibility is valuable in applications where deformation under load is acceptable or necessary, such as cushioning materials or impact-resistant panels. Furthermore, the high Young's modulus values signify a stiff material with minimal deformation under applied stress. This makes it particularly well-suited for applications where rigidity is critical, such as rigid boards, panels, or other structural components requiring dimensional stability. Treatment 9 showed lower strain tolerance in comparison to Treatment 11 suggesting that the material is less flexible and may be more prone to brittle failure. This limits its use in applications where significant deformation or impact absorption is needed. Additionally, the Young's modulus values show moderate stiffness and structural cohesion, offering enough rigidity for applications such as insulating panels or protective casing. The mechanical properties of Treatment 10 suggest that while N supplementation may negatively impact the material's strength and stiffness, it could still serve in applications where lightweight, biodegradable, or low-mechanical-performance materials are sufficient. For instance, it could be used in eco-friendly disposable items, or low-strength fillers. Treatment 12 reported variabilities that indicate inconsistencies in the material's stiffness and structural properties that could be a disadvantage for applications requiring uniform performance but may still work for less critical uses where variability is acceptable.

Acoustically, Treatment 12 provided the best broadband absorption across both low (100–1600 Hz) and high (500–6400 Hz) frequency ranges. It consistently achieved coefficients $\alpha>0.85$ in mid-frequencies and maintained $\alpha>0.75$ at high frequencies, confirming its effectiveness as a sound-absorbing material. Treatments 9 and 11 also performed well, especially at mid-frequencies, while Treatment 1 had limited absorption below 1000 Hz but peaked at higher frequencies (1500–2400 Hz). Treatment 10 showed high absorption at specific frequencies but declined sharply above 2400 Hz. Statistical analysis confirmed significant differences ($p<0.05$) among treatments for both mechanical and acoustic properties, especially in the mid-strain and mid-frequency ranges. Notably, all treatments showed similar acoustic absorption at 2400 Hz, possibly due to shared microstructural resonance behavior or material densification at that specific frequency band.

Overall, this research confirms that the combination of sterilization, fresh substrate addition, and reinoculation with *P. ostreatus* are key to optimizing both mechanical and acoustic properties in SMS-based MBCs. These features indicate that the material can withstand considerable force before breaking, making it suitable for applications requiring moderate load-bearing capacity, such as lightweight structural components. The ability to tolerate higher strain before failure demonstrates that the material is not only strong but also flexible to a certain extent. This balance between strength and ductility is especially valuable in applications where energy absorption or deformation underload is required, such as cushioning elements, impact-resistant panels, or interior modular systems.

Importantly, the integration of mechanical robustness and acoustic performance identified in this study enhances the multifunctional potential of these materials. According to

Alaneme et al. (2023) [53], low thermal conductivity, high acoustic absorption, and fire safety are key drivers promoting the adoption of MBCs in the construction industry, particularly for insulation, paneling, and furnishings. The findings presented here align with and expand upon these known advantages: Treatment 11 not only meets but potentially exceeds these criteria, combining excellent acoustic performance with high compressive strength and flexibility properties rarely addressed together in existing literature. This integration of multifunctionality underscores the promise of SMS-based MBCs for hybrid applications, especially in sustainable, low-impact architecture, where thermal, acoustic, and mechanical performance must coexist in a single material. Moreover, by repurposing SMS into MBCs, this study supports a circular economy model that reduces waste management costs for mushroom farms while supplying sustainable biomaterials to the construction sector. This dual-product approach increases resource efficiency, decreases dependence on non-renewable materials, and promotes sustainability in both agriculture and building industries.

Ultimately, this research not only addresses critical environmental concerns but also charts a path toward more resource-efficient and sustainable industrial practices. It highlights the potential of SMS-based MBCs as multifunctional, biodegradable materials suitable for temporary architecture, insulation, acoustic panels, and low-load structural applications. This study serves as a foundational step for the following chapters, where the development and implementation of a dual production system, capable of generating both a food source and biomaterials, is explored in depth.

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Chapter 5

Definition of a dual production system for food and MBCs with mushroom yields analysis and mechanical characterization

1. Objective

This chapter presents the definition and validation of the integrated dual-production process, in which edible mushroom cultivation and MBC fabrication are combined within a single, circular system. Building on the groundwork laid in Chapters 2 and 3, which enabled the identification and testing of a reliable MBC growth methodology, and on the findings of Chapter 4, which demonstrated the compatibility of SMS as a viable feedstock for MBCs, this chapter advances the research by developing and evaluating a unified process capable of producing both edible mushrooms and mycelium-based composites simultaneously. The chapter is divided into two main sections: the first focuses on analyzing and comparing mushroom yield with the mechanical performance of the resulting MBCs, aiming to assess the balance between food production and material quality. The second section provides an in-depth acoustic characterization of the MBCs, considering their primary application in sound insulation and noise control. Together, these analyses serve to evaluate the technical viability and functional performance of the dual-process system, confirming its potential for sustainable industrial applications.

2. Introduction

This study introduces a novel and innovative approach to produce edible mushrooms and transforming the waste generated by the cultivation of edible mushrooms into MBCs, producing products both for the agrifood sector and construction industry as an outcome. The prospect of cultivating edible mushrooms and developing MBCs presents an opportunity for a circular economy framework minimizing waste and optimizing resources. By repurposing cultivation substrates into sustainable building materials, a zero-waste solution can be achieved, promoting sustainability in manufacturing processes. In the realm of sustainable and circular bioeconomy solutions, innovative strategies are pivotal for resource utilization. One such promising approach involves the dual-purpose system that not only enhances the value proposition of mushroom farming but also contributes to sustainable material innovation, offering a scalable and eco-friendly alternative to traditional manufacturing practices for both food production and biotechnological applications in construction field and beyond. The cultivation of *Pleurotus ostreatus*, commonly known as the oyster mushroom, is recognized for its ease of cultivation, rapid growth, minimal susceptibility to diseases and pests, high nutritional value, and adaptability to various lignocellulosic substrates, making it a suitable candidate for cultivation using simple and cost-effective methods [1]. However, as previously reported, conventional mushroom cultivation results in the generation of significant amounts of waste [2], which constitute one of the most significant environmental issues for the mushroom industry [3] presenting challenges in disposal and raising environmental concerns [4]. Traditional methods of cultivating mushrooms, particularly *Pleurotus* species, typically involve a production cycle that is interrupted after three crop cycles, called flushes, due to diminishing returns and increasing costs. Prolonged cultivation beyond this point leads to decreased mushroom production and increased costs, potentially surpassing the revenue generated from production. To address this issue, transforming SMS into

valuable MBCs offers a sustainable solution to develop composites with low embodied energy and high CO_2 sequestration by capturing and storing it within the material, thereby preventing its release back into the atmosphere [5]. Integrating the production of edible mushrooms with the development of MBCs optimizes resource utilization, minimizes waste, and enhances economic viability. In conclusion, the integration of *Pleurotus ostreatus* cultivation for both food and MBC production represents a sustainable and innovative approach contributing to sustainable agriculture, and eco-friendly material innovation especially for the building and construction sector. This study aims to establish a sustainable model for mushroom cultivation and MBC production by quantifying the production of edible mushrooms and characterizing the structural properties of MBCs cultivated on SMS after being heat treated. This innovative approach streamlines the current production process of MBCs and surpasses the study reported in Chapter 4 by directly converting waste of mushroom cultivation to sustainable building materials. Typically, MBCs are produced using methods like those employed in the cultivation of edible mushrooms, but the process is halted before fruiting bodies (mushrooms) develop. This study assesses how cultivating MBCs from SMS affects their mechanical properties while analyzing the parameters that affect edible mushroom production (Figure 1).

Fig. 1 Mush2Biom production process from edible mushroom cultivation to mycelium-based composites

3. Methodology

The methodology used for this study was divided into two main parts. The first part focused on cultivating edible mushrooms, collecting data on the amount of mushrooms harvested, and analyzing total growth times. The second part focused on investigating the potential of transforming SMS into MBCs and testing their mechanical properties. Specifically, the SMS were heat-treated and tested for compressive strength after the first fruiting cycle (I flush), the second fruiting cycle (II flush), and, before fruiting occurred (pre-flush). Through this integrated methodology, the feasibility and benefits of reusing SMS for MBC production are demonstrated, paving the way for adoption of circular economy in agricultural and sustainable materials production practices.

3.1 Edible Mushroom Cultivation

The first step in the cultivation process involved preparing the substrates. Two substrates were chosen for the cultivation of *Pleurotus ostreatus*: Straw (S), and Straw-Cotton (SC) mixtures, both of which are widely used as compatible substrates for the cultivation of both *Pleurotus ostreatus* mushrooms and MBCs. These substrates were placed in cultivation bags in two different ways, one in which the bags are formed into rectangular prisms (R) and the other in which the bags are simply filled with the substrates into circular shapes (C), as shown in figure 2, to assess potential differences in mushroom yield and the ease of processing the SMS by heat-pressing and oven dehydration after harvesting the mushrooms. Circular bag shapes (C) simply follow the ways cultivation bags are processed in mushroom farming. With the rectangular prism bag shapes (R), the possibility of pre-shaping the cultivation bags prior to post-processing to ease MBC production is explored. For the S group, the mix was composed of 95% straw and 5% nitrogen based on dry weight. For the SC group, the mix consisted of 80% cotton, 15% straw, and 5% nitrogen based on dry weight. The dry weight of each mix was considered equal to 35% of the overall weight of the substrate, and 65% of water was added to the mixture to get the needed relative humidity in the bags for Pleurotus ostreatus cultivation. S and SC substrates were then placed into autoclave bags with filters to allow transpiration. For each cycle (Pre-flush, Flush I, Flush II), and each bag form (C and R), three bags were prepared, making a total of 36 bags: 18 for the S group and 18 for the SC group. The bags were sterilized by autoclaving for 45 minutes at 121°C and left to cool down overnight before inoculation with 6% dry weight of Pleurotus ostreatus spawn, which had been previously stored at 4°C. Each bag had six holes to allow fruiting. The bags were then incubated within environmentally controlled growth rooms at 23°C with a humidity of 75% for 10 days, during which the mycelium colonized the substrates. The temperature was then reduced to 18°C to initiate mushroom fruiting. The mushrooms were harvested and weighed during both the first and second flush cycles to analyze and compare the yield and cultivation time in the S and SC groups, as well as the C and R subgroups. The pre-flush phase was considered complete when the substrates were fully colonized on the surface by mycelium, and the end of each flush was determined by the last day mushrooms were harvested.

Rectangular prisms shape (R)	Circular shape (C)
	

Fig. 2 Rectangular prisms (R) and circular shapes (C) bags.

3.2 Mechanical Testing of MBC Produced Using SMS

MBCs were produced using SMS after the first fruiting cycle (Flush I), the second fruiting cycle (Flush II), and as a negative control before any fruiting occurred (Pre-flush). The rationale for examining the MBCs produced from SMS up to the second fruiting cycle aligns with industrial edible mushroom cultivation practices, where production is typically ceased after the second cycle due to considerations of cost-effectiveness. Colonized bags from each group were post-treated using a Carver Hydraulic Unit Model #3925 for 25-ton manual presses with heating plates of 200x200 mm. The heat press was set to 121°C for 30 minutes. The colonized substrates were not taken out of the autoclave bags during heat pressing. Heat pressing ensured a relatively homogeneous distribution of heat across the SMS. Following this stage, the resulting MBCs were fully dehydrated in the oven at 90°C until their weights stabilized. The MBCs from each group were cut into three cubes of (50x50x50mm) for mechanical testing, as shown in figure 3. The tests were conducted in accordance with ASTM C109 standards, using the approach previously outlined by [6], employing an MTS machine. The experiments involved subjecting the cubic samples to compression up to 80% at a velocity of 0.05 mm/s. The material stiffness was determined by analyzing the slope of the stress-strain curve in the steepest region having curves in two different regions. The first region corresponds to lower strains (up to 20%), where the cells of the porous structure deformed and compacted but had not yet collapsed. The second region corresponds to higher strains (above 50%), after all the cells had collapsed and the structure gradually compacted. The evaluation of material strength varied depending on the response of the specimen, and two types of stress were chosen. The first evaluation, referred to as the maximum stress, was determined as the highest stress point on the curve. This could correspond to a local peak for specimens that experienced failure, mainly due to transversal splitting. Alternatively, it could represent the maximum stress applied by the dynamometer for specimens that did not fail but instead underwent continuous compaction with the maximum stress achievable. Furthermore, the stress of the samples at a strain of 60%, namely in the compaction zone, was documented.

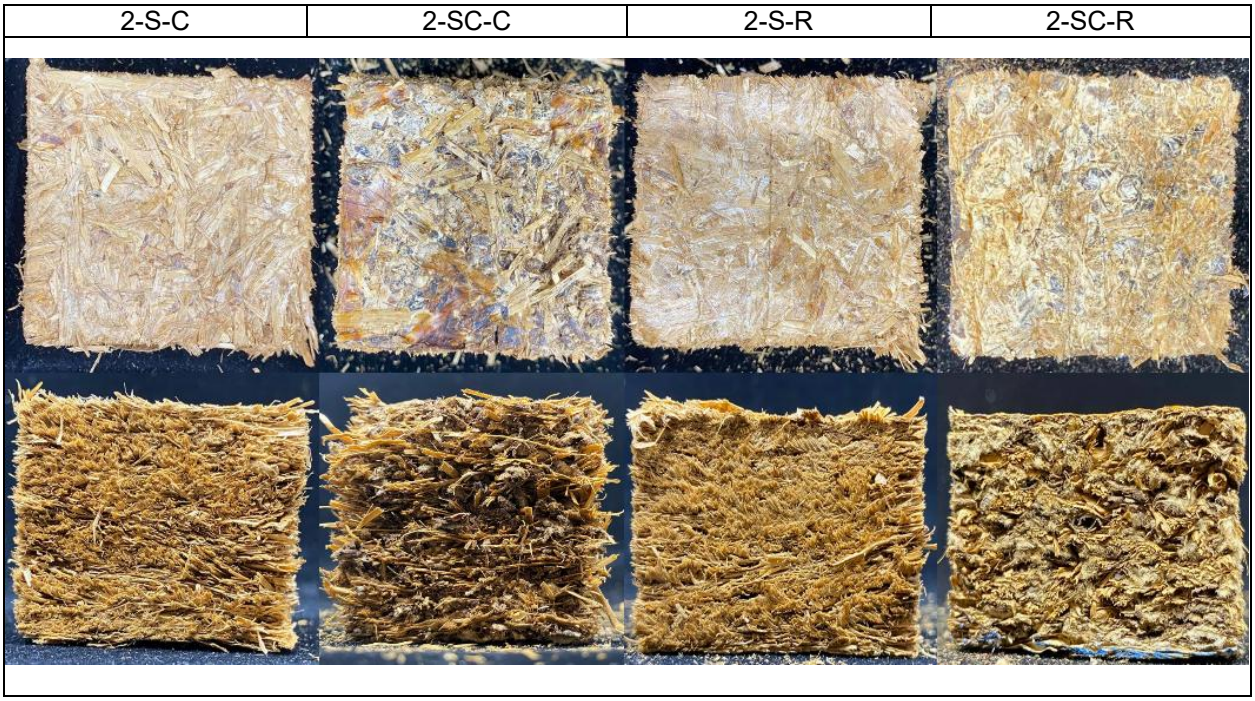

Fig. 3 Mechanical test samples from II flush (50x50x50mm)

4. Results

This study examines the correlation between mechanical properties of MBCs produced using SMS with the substrates Straw (S) and Straw-Cotton (SC) within bags that are shaped Circular (C) and Rectangular (R), and the total mushroom yield. The goal is to analyze the impact of bag shapes and substrate compositions on compressive strength and mushroom yield.

4.1 Edible Mushroom Cultivation: Yield and Growth Time

The production of edible mushrooms was calculated in terms of grams of mushrooms per kilogram of substrate, with production times reported in days, compared across the pre-flush, first flush (I flush), and second flush (II flush) cycles for various treatments, as detailed in Table 1. As the data in Table 1 suggests, the circular bags generally outperform rectangular bags in yield, with the highest values observed for treatments using straw and cotton substrates (1-SC-C and 2-SC-C), followed by those using only straw (1-S-C and 2-S-C). During the first flush, the 1-SC-C treatment achieved a yield of 174 g/kg, followed closely by 1-S-C with a 3% lower yield of 169 g/kg. In the second flush, the 2-SC-C treatment produced 142 g/kg, whereas the 2-S-C treatment yielded 102 g/kg, a 40% reduction.

Table 1. Treatments, quantities of mushrooms yield, peak stress and Young's modulus

Flushes	Substrates	Bags	Abb.	Mushroom yield (g/kg)	Days
Pre-flush	Straw	Circular	0-S-C	0	20
		Rectangular	0-S-R	0	20
	Straw + Cotton	Circular	0-SC-C	0	24
		Rectangular	0-SC-R	0	24
I flush	Straw	Circular	1-S-C	169	27
		Rectangular	1-S-R	157	26
	Straw + Cotton	Circular	1-SC-C	174	34
		Rectangular	1-SC-R	167	39
II flush	Straw	Circular	2-S-C	102	38
		Rectangular	2-S-R	69	40
	Straw + Cotton	Circular	2-SC-C	142	44
		Rectangular	2-SC-R	75	50

In the first flush, the yield differences between circular and rectangular bags were minimal, with 1-S-C producing 8% more than 1-S-R, and 1-SC-C yielding 4% more than 1-SC-R. However, in the second flush, these differences became more pronounced: 2-S-C produced 48% more than 2-S-R, and 2-SC-C yielded 90% more than 2-SC-R, indicating that the rectangular shape for the bags significantly decreases mushroom production. The highest yielding treatments (1-SC-C and 2-SC-C) also required the longest cultivation time, while 1-S-C and 2-S-C took 7 days shorter to cultivate in the first flush and 6 days shorter in the second flush, suggesting a correlation between yield, production duration, and substrate type.

4.2 Mechanical Tests: Compressive Strength

Figure 4 depicts the response of materials to compression testing, showing stress-strain curves for three sets of each treatment during the pre-flush, first flush, and second flush stages. The

maximum stress, stress at 60% strain, and material stiffness for low and high strains were determined from these curves and are presented in Table 2.

Table 2. Comparing the treatments considering the quantities of mushrooms yield, and the peak stress, and peak stress at 60% strain and Young's modulus average among the 3 samples for each treatments.

Flushes	Abb.	Peak stress average (Mpa)	Peak stress at 60% strain average (Mpa)	Young's modulus average (N/mm²)
Pre-flush	0-S-C	18.02	1.43	194.15
	0-S-R	18.03	2.65	162.23
	0-SC-C	10.81	8.16	37.83
	0-SC-R	14.38	9.60	72.13
I flush	1-S-C	18.01	1.68	226.87
	1-S-R	18.01	1.84	181.58
	1-SC-C	9.61	4.03	34.89
	1-SC-R	15.82	6.37	66.71
II flush	2-S-C	18.02	2.70	178.23
	2-S-R	18.01	2.92	160.86
	2-SC-C	18.01	3.48	97.71
	2-SC-R	15.49	10.17	68.88

From figure 4 the stress-strain curves for circular bags made of straw substrates (0-S-C, 1-S-C, 2-S-C) represented in graph "a", show a gradual increase in stress as strain increases. This may be described as the first phase of elastic behavior, where the strain is limited to 20%, and of a subsequent one where the strain increases more rapidly, particularly above 75%. This occurs after the material's pores have collapsed and the cellular structure becomes more compact. In this secondary regime, the denser composition of the material enables a significant ability to bear heavy loads for this substrate arrangement. The greatest stress observed in these samples consistently remains at approximately 18 MPa. This value corresponds to the highest stress that can be measured due to the load cells' capacity, as no failure has been observed in these specimens.

Rectangular bags made of straw substrates (0-S-R, 1-S-R, 2-S-R) represented in graph "c" display a similar trend in stress with strain as circular bags with straw, graph "a". The maximum stress (18MPa) is more homogenously concentrated at strain around 75 -80%, suggesting that the rectangular shape might contribute to similar efficient load distribution compared to the circular bag configuration.

When it comes to circular bags made of straw and cotton substrates (0-SC-C, 1-SC-C, 2-SC-C), graph "b", the initial elastic region is similar. However, there is greater variation among the curves compared to bags made of straw substrates alone (0-S-C, 1-S-C, 2-S-C). This is because the specimens from the pre-flush and the first flush groups (0-SC-C, 1-SC-C) failed due to transversal splitting at strains between 60 and 80%. In contrast, the specimens from the second flush group (2-SC-C) have consistently exhibited no signs of failure. The greatest stress recorded was 18 MPa, occurring at strain levels ranging from 75% to 85%. This indicates that there is a gain in cohesiveness and strength as the manufacturing period lengthens.

Finally, rectangular bags with straw and cotton substrates (0-SC-R, 1-SC-R, 2-SC-R), graph "d", show also a similar variability in stress-strain curves as in the circular bags (0-SC-C, 1-SC-C, 2-

SC-C). In particular, the addition of cotton results in higher peak stress values compared to circular bags with straw and cotton (0-SC-C, 1-SC-C, 2-SC-C). However, the strain at failure varies more, with some samples failing at strains around 0.6 mm/mm.

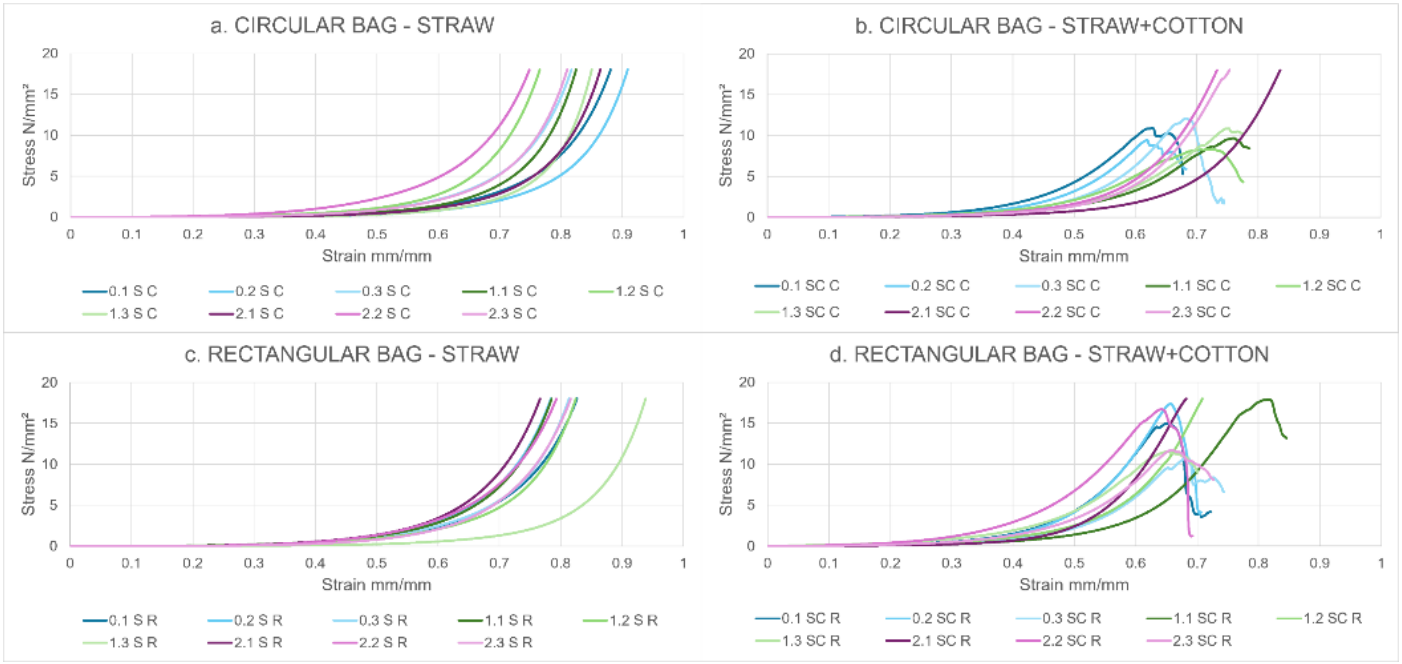

Fig. 4 Compression test – comparing the different treatments: a) circular bags with straw substrates, b) circular bags with straw and cotton substrates, c) rectangular bags with straw substrates, d) rectangular bags with straw and cotton substrates.

The graphs “a” and “b” reported in Figure 5 illustrate the maximum stress and stress at 60% strain for the various systems, highlighting the differences in mechanical performance. Circular bags with straw (0-S-C, 1-S-C, 2-S-C) and rectangular bags with straw (0-S-R, 1-S-R, 2-S-R), not showing failure, maintain consistent peak stress values around 18 MPa, and present a stable value for the stress at 60% strain. The addition of cotton (0-SC-C, 1-SC-C, 2-SC-C and 0-SC-R, 1-SC-R, 2-SC-R), determining the occurrence of failure phenomena, results in greater variability in peak stress, especially in rectangular bags. The graph “c” of Figure 5 presents the stiffness values, range of Young's modulus, showing that circular bags with straw (0-S-C, 1-S-C, 2-S-C) generally exhibit higher stiffness values, around 200 MPa, compared to rectangular bags with straw (0-S-R, 1-S-R, 2-S-R: about 170 MPa). The addition of cotton increases variability in Young's modulus, particularly in rectangular bags, and it is suggested that circular configurations provide a stiffer, more resilient structure, likely due to geometric differences leading to more uniform stress distribution.

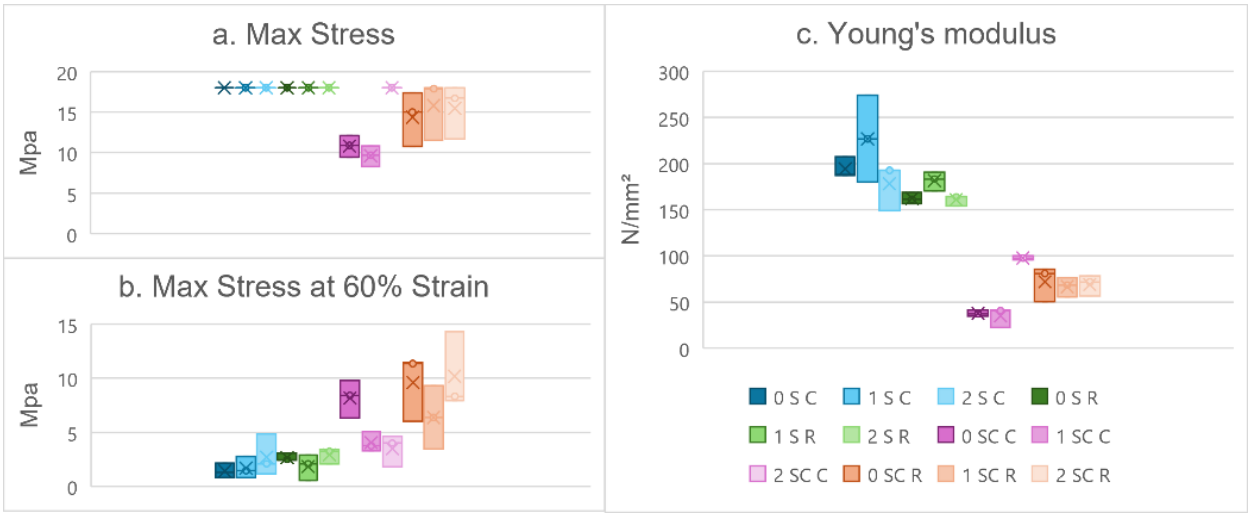

Fig. 5 Comparing the different treatments for a) Maximum Stress, b) Maximum Stress at 60% strain, and c) Young's modulus

Figure 6 compares mushroom yields with peak stress and Young's modulus, elucidating the mechanical properties' influence on mushroom production. The graph "a" shows a relationship between mushroom yield (g/kg) and peak stress (MPa). Treatment 1-S-C exhibits peak stress values around 18 MPa, correlating with the second higher mushroom yield of 169 g/kg during the first flush. During the second flush, circular bags with straw (2-S-C) maintain high peak stress and yield 102 g/kg, while circular bags with straw and cotton (2-SC-C) achieve the highest peak stress and yield of 142 g/kg.

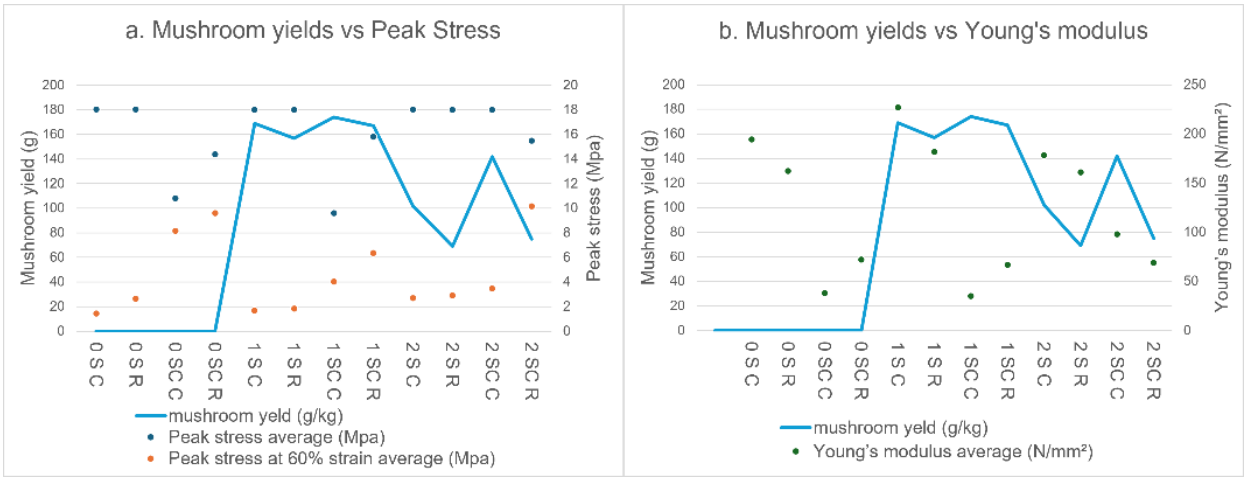

Fig. 6 a) Mushroom Yield vs Peak stress and b) Mushroom Yield vs Young's modulus

The graph "b" highlights the correlation between mushroom yield and Young's modulus (N/mm²). Treatments with circular bags with straw substrates (1-S-C and 2-S-C), exhibit high Young's modulus values exceeding 180 N/mm² in the first flush and 140 N/mm² in the second flush, supporting substantial yields. Conversely, rectangular bags with straw and cotton substrates (0-SC-R, 1-SC-R, 2-SC-R) show lower and more variable Young's modulus values, often below 100 N/mm², corresponding to reduced yields. This emphasizes the importance of substrate stiffness

for enhancing production. The observed variability in peak stress and Young's modulus, especially in the Straw-Cotton substrate treatments, underscores the complex interactions between bag shape, substrate composition, and mechanical performance. These findings are crucial for optimizing substrate mixtures and container designs in mushroom farming to achieve optimal mushroom yields and mechanical strength for biomaterial production**.** These results indicate that circular bag shapes generally provide better mechanical performance under compression, particularly when produced with straw only substrates. The addition of cotton introduces variability among the samples, especially in rectangular bags.

5. Conclusions

This study demonstrates the possibility of simultaneously producing food (mushrooms) and MBCs. By analyzing the effects of substrate composition and bag shape on mechanical properties and mushroom yield, the research concluded that circular bags, particularly those with straw substrates, offer superior mechanical performance and good mushroom yields. This insight is crucial for optimizing mushroom cultivation to obtain SMS that can be used to produce MBCs with high mechanical strength and mushroom yield. The MBCs obtained are comparable to foams and structural expanses, which are used in a wide range of applications due to their properties of lightness, thermal and acoustic insulation, and impact resistance. The positive correlation between mechanical strength and mushroom yield suggests that robust cultivation systems can enhance both mushroom productivity and the quality of SMS for further use as MBCs. This research bridges agricultural productivity and material science, supporting the sustainable use of SMS in developing innovative, eco-friendly biomaterials. This material could also be conceived for “single use” applications to create temporary structures because as it is 100 % natural it could be easily disposed of as compost without ever generating waste. Despite the material not being as robust as traditional building materials, we can design geometries that compensate for its weaker properties. Building on the outcomes of this initial experimental phase, the next chapter further investigates the acoustic properties of SMS-based MBCs, culminating in the development of a prototype wall presented in Chapter 7.

Note: This chapter are based on the conference paper “Mush2Biom: From Edible Mushrooms to Sustainable Biomaterials”, by Chiara Dognini, Seyedeh Aisa Shams Hojjati, Laura Eleonora Depero, John Pecchia, and Benay Gürsoy. The paper was presented at the SIGraDi 2024 Conference and published in the official proceedings, available at https://sigradi.org/sigradi2024. The content has been revised, expanded, and reorganized to better align with the structure and objectives of this dissertation.

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Chapter 6

Acoustic Characterization of SMS-Based MBCs

1. Objectives

This chapter continues the investigation presented in Chapter 5, which introduced the integrated dual-production process combining edible mushroom cultivation and MBCs fabrication. While the earlier analysis focused on assessing the mechanical performance of the MBCs and their correlation with mushroom yield, the present chapter shifts the focus toward the acoustic characterization of the resulting materials. The primary objective here is to evaluate the sound absorption capabilities of the dehydrated SMS-based MBCs, with particular attention to their potential application in interior partitioning and noise mitigation. Given the material's fibrous structure, high porosity, and absence of additional processing, it is hypothesized that the MBCs may naturally exhibit favorable acoustic behavior, particularly at mid- to high-frequency ranges. This acoustic assessment complements the mechanical analysis and contributes to a more holistic understanding of the MBC's performance within a circular production model. By validating both structural and functional properties, the chapter aims to confirm the technical viability of MBCs as sustainable, multifunctional building components for temporary or low-impact architectural applications.

2. Introduction

To demonstrate the acoustic effectiveness of the SMS-based MBCs developed in Chapter 5, acoustic absorption coefficients were measured using samples collected at different stages of the edible mushroom cultivation process, as described in Chapter 5, specifically, before fruiting, during the first fruiting cycle, and after the second fruiting cycle. This methodology also aligns with the approach presented in Chapter 2, based on the study by Dognini et. al. 2023 [1] in which the acoustic absorption properties of mycelium growing in the GPW substrate were similarly evaluated by time. In this study it was also compare the acoustic absorption of two substrates: straw (S) and straw with cotton (SC), alongside comparing with rectangular- and circular-shaped bags. The results indicate that the acoustic biomaterial reaches absorption coefficients close to 1 at frequency ranges around 1000 Hz, achieving almost complete acoustic absorption. Altogether, most samples exhibit strong absorption in the higher frequencies of 2000 – 6000 Hz, with most coefficients exceeding 0.5, validating MBCs cultivated on SMS as an acoustic biomaterial. By repurposing SMS into MBCs with effective acoustic performance, these composites emerge as a viable and sustainable alternative to conventional acoustic panels. Such innovative approaches are essential for advancing sustainable solutions within the expanding bioeconomy. In this context, the study utilizes SMS requiring only minimal post-processing through. Once harvested, the remaining colonized substrate can be directly valorized through dehydration or heat treatment and subsequently transformed into MBCs with acoustic absorptive properties. This valorization pathway is particularly innovative, as it enables the direct conversion of agricultural waste into high-performance acoustic and sustainable building materials, while simultaneously optimizing and streamlining the mushroom cultivation process itself. Moreover, the investigation into the acoustic performance of SMS-based MBCs further expands the material's potential applications, building upon the foundational developments introduced in Chapter 5.

3. Methodology

The methodology for edible mushroom cultivation and sample preparation follows the procedure outlined in Chapter 5, which is based on the protocol described by Dognini et al. [2]. The objective of this study is to evaluate the acoustic performance of SMS-based MBCs through sound-absorption tests. The dehydrated MBC material used for the samples prepared in Chapter 5 was used to prepare the acoustic samples that were cut into circles of 100 mm and 29 mm diameters to fit in the impedance tube for acoustic measurements using a band saw and sanded using a belt sander for a tight fit. The thickness of the SMS samples was maintained to be approximately at 40 mm after heat-pressing. Close-up images of the Flush II straw-cotton samples from the circular bag (SC-C) used in the impedance tube is depicted in Fig. 1.

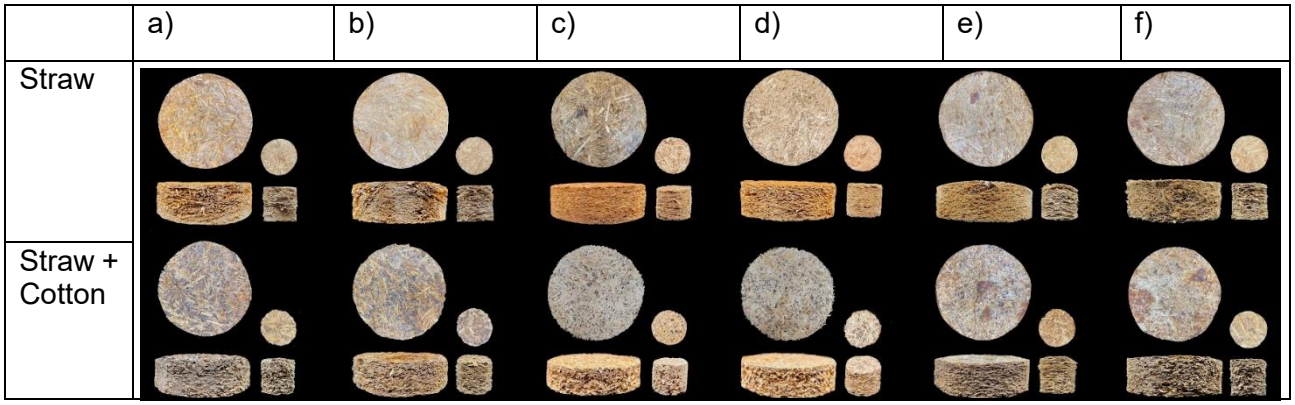

Fig. 1 Top and side view images of the Large (diameter:100 mm) and Small (diameter:29 mm) acoustic samples: a) Pre-flush Circular shape; b) Pre-flush Rectangular prism shape; c) Flush I Circular shape; d) Flush I Rectangular prism shape; e) Flush II Circular shape; f) Flush II Rectangular prism shape

3.2 Acoustic Absorption Testing Procedure

Acoustic tests were performed to measure the absorption coefficients (α) of the samples. Absorption coefficients (α) were measured using a Brüel & Kjær Type 4206 Impedance Tube as shown in Fig. 2, in accordance with the ASTM E1050-19 standards [3].

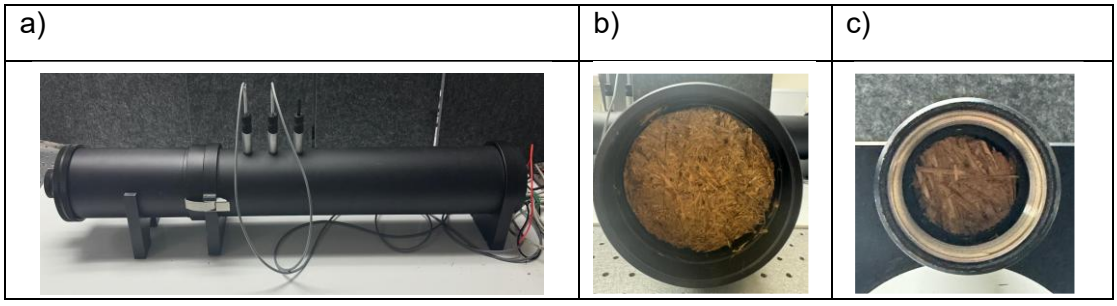

Fig. 2 Image of a) Brüel & Kjær Type 4206 Impedance Tube used for the tests, b) 100mm Straw-Cotton, Pre-flushed (S-C, Pre-flush) sample inside the large impedance tube, and c) 29mm Straw-Cotton, Pre-flushed (S-C, Pre-flush) sample inside the small impedance tube

SMS samples were classified into four main categories: circular straw (S-C), circular straw-cotton (SC-C), rectangular straw (S-R), and rectangular straw-cotton (SC-R). For each sample group, two types of circular specimens were created, each with four replicates: 100 mm diameter specimens for low-frequency absorption tests and 29 mm diameter specimens for high-frequency absorption tests, as shown in Table 1. The bulk densities were found by using a mass-to-volume ratio method and the static airflow resistivity was found by using the back-cavity-length-based impedance transfer function method [4].

Table 1. Acoustic samples for sound absorption test with large and small replicates for each treatment

Sample Name	Substrate Type	Bag Shape	Flush Type	Number of Large Replicates (100 mm)	Number of Small Replicates (29 mm)	Thickness (mm)	Bulk Density ($\mathbf{kg/m^3}$)	Flow Resistivity ($\mathbf{Pa\ s/m^2}$)
S-C Pre-flush	Straw	Circular	Pre-flush	4	4	40	210	4970
S-C Flush I	Straw	Circular	Flush I	4	4	35	250	2595
S-C Flush II	Straw	Circular	Flush II	4	4	35	225	3895
SC-C Pre-flush	Straw-Cotton	Circular	Pre-flush	4	4	35	390	3550
SC-C Flush I	Straw-Cotton	Circular	Flush I	4	4	55	300	2405
SC-C Flush II	Straw-Cotton	Circular	Flush II	4	4	40	275	3210
S-R Pre-flush	Straw	Rectangular	Pre-flush	4	4	40	200	3590
S-R Flush I	Straw	Rectangular	Flush I	4	4	35	200	4675
S-R Flush II	Straw	Rectangular	Flush II	4	4	30	225	4490
SC-R Pre-flush	Straw-Cotton	Rectangular	Pre-flush	4	4	45	365	3695
SC-R Flush I	Straw-Cotton	Rectangular	Flush I	4	4	40	285	2895
SC-R Flush II	Straw-Cotton	Rectangular	Flush II	4	4	35	380	3235

The frequency range for the low-frequency absorption tests ranged from 50 Hz to 1.6 kHz and the high-frequency absorption tests ranged from 2 kHz to 6.4 kHz. The four replicates in each sub-category were averaged to determine the sound absorption coefficient of the sample category. Acoustic absorption coefficients were compared by separating broadband frequency results into both one third-octave and octave band frequencies. One-third octave band frequencies ranges from 63 Hz to 6300 Hz, while the octave band frequencies are 125 Hz, 250 Hz, 500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz. The frequencies of interest for the low-frequency absorption tests with the large impedance tube ranged from 63 Hz to 1600 Hz, and the high-frequency absorption tests with the small impedance tube ranged from 2000 Hz to 6300 Hz. Since the MBCs grown in the same bags were utilized in the low and high-frequency impedance tube tests, a full set of one-third octave band absorption coefficients, octave band absorption coefficients, and the Sound Absorption Average (SAA) were determined for each MBC group. SAA is the arithmetic average of twelve one-third octave bands of absorption coefficients ranging from 200 Hz to 2500 Hz. In addition, a Noise Reduction Coefficient (NRC) rating was calculated. NRC is a single number rating for sound-absorbing efficiency in mid-frequencies, where human speech is most commonly heard, and often used in evaluating the effectiveness of materials in reducing reverberation in rooms such as offices, classrooms, and auditoriums [5]. The NRC value can be found by $NRC = (\alpha_{250} + \alpha_{500} + \alpha_{1000} + \alpha_{2000})/4$, where α_{250}, α_{500}, α_{1000}, and α_{2000} represents the absorption coefficient values at 250, 500, 1000, and 2000 Hz, respectively. Similar to acoustic absorption coefficients, the higher the NRC value, the better the acoustic absorption performance of the material in the speech range. The mean sound absorption coefficients of the sample groups at the given frequency levels were compared using the Mann–Whitney U test with the SPSS software (IBM Corp. Released 2015. IBM SPSS Statistics for Mac, Version 29.0. IBM Corp: Armonk, NY, USA). Similar to a previous study by Walter and Gürsoy [6], the Mann–Whitney U test was implemented to find whether the flush stages and the bag shapes made an impact on the acoustical properties. A *p*-value of <0.05 was considered statistically significant.

4. Results

4.1 Acoustic Absorption Test Results

Fig. 3 illustrates the third-octave band results of the impedance tube testing consisting of both the large (100 mm) samples and small (29 mm) samples, respectively. As shown in Fig. 3, many of the absorption coefficients (α) peak at around 600 Hz to 800 Hz, with most of the samples reaching past absorption of 0.7 in these peaks. In Fig. 3b and 3d, it can be observed that most of the straw-cotton samples, both circular and rectangular, achieve absorption coefficients near 1 at their peaks. It is also evident that past 2000 Hz, most of the absorption coefficients plateau above 0.5, and some around 0.7.

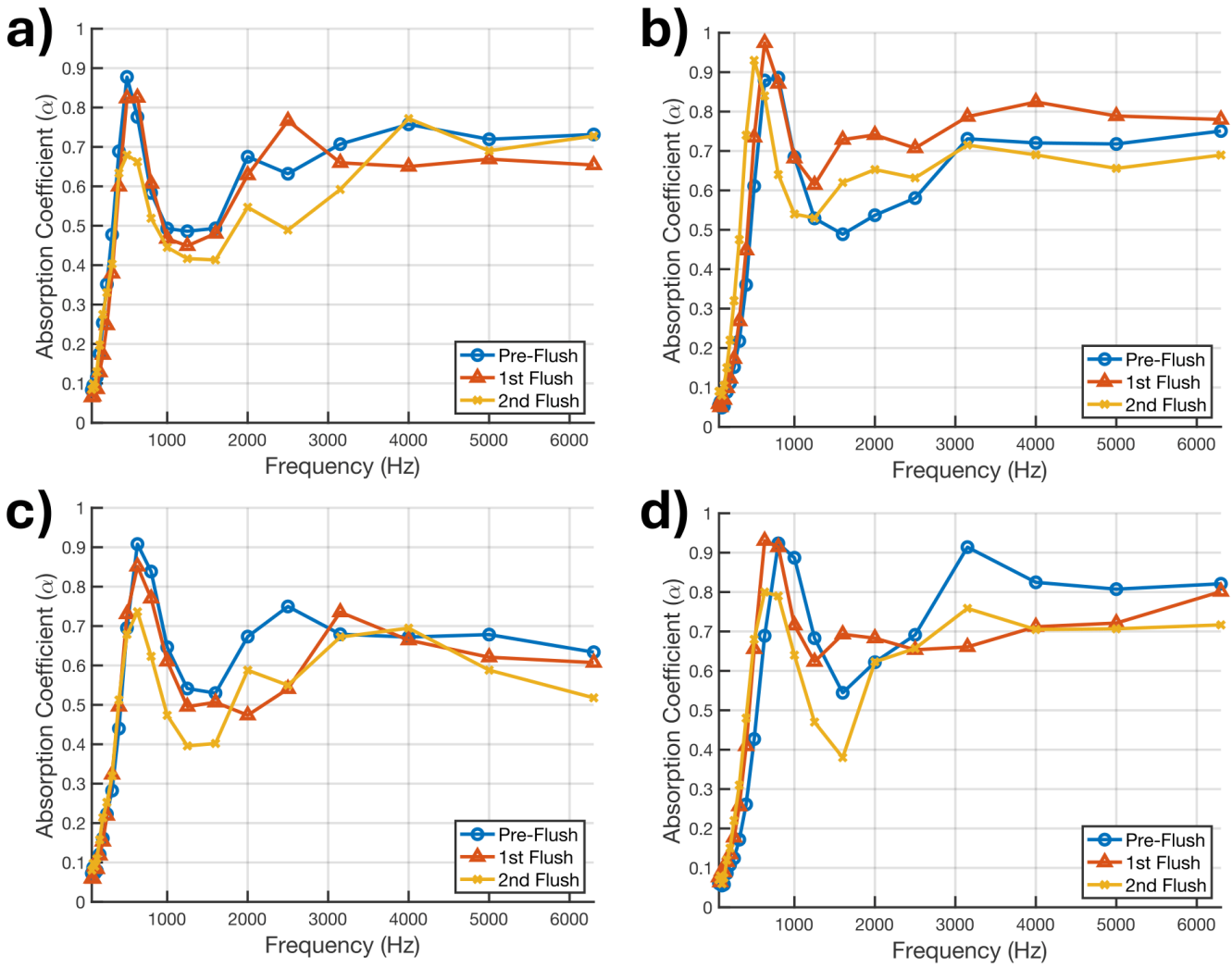

Fig. 3 Averaged third-octave frequency sound absorption coefficient (α) measurements from 63 Hz to 6300 Hz of Pre-flush, Flush I, and Flush II samples for a) circular straw (S-C), b) circular straw-cotton (SC-C), c) rectangular straw (S-R), and d) rectangular straw-cotton (SC-R)

The measured absorption coefficients of octave band frequencies, SAA, and NRC values for MBC samples are presented in Table 2. The results cover three conditions: Pre-flush, Flush I, and Flush II, with corresponding samples and octave band center frequencies from 125 Hz to 4000 Hz. In the Pre-flush condition, the S-C sample showed relatively higher absorption at 500 Hz of 0.87 and at 4000 Hz with 0.76, with an overall average absorption coefficient of 0.57. In the Flush I condition, absorption values were similar, with the SC-C and SC-R samples reaching an SAA value of 0.59 and 0.57, respectively, while the other samples ranged between 0.51 to 0.54. Flush II conditions showed some variation, particularly for SC-C, which recorded an absorption coefficient of 0.93 at 1000 Hz and an SAA of 0.59. All samples have absorption coefficients above 0.6 in the 4000 Hz range. Additionally, it can be seen that the NRC values of these samples range from 0.5 to 0.6.

Table 2. Absorption coefficients (α) for octave band frequencies: for circular straw (S-C), circular straw-cotton (SC-C), rectangular straw (S-R), and rectangular straw-cotton (SC-R), with Sound Absorption Average (SAA) and NRCs

		Octave Band Center Frequencies (Hz)						SAA	NRC
	Sample	**125**	**250**	**500**	**1000**	**2000**	**4000**		
Pre-flush	S-C	0.12	0.35	0.87	0.49	0.68	0.76	0.57	0.60
	SC-C	0.06	0.15	0.61	0.68	0.54	0.72	0.50	0.50
	S-R	0.08	0.22	0.70	0.65	0.67	0.67	0.56	0.55
	SC-R	0.06	0.12	0.43	0.89	0.62	0.82	0.51	0.50
Flush I	S-C	0.09	0.25	0.82	0.47	0.63	0.65	0.54	0.55
	SC-C	0.07	0.17	0.73	0.68	0.74	0.82	0.59	0.60
	S-R	0.09	0.22	0.73	0.61	0.47	0.66	0.51	0.50
	SC-R	0.09	0.18	0.66	0.72	0.68	0.71	0.57	0.55
Flush II	S-C	0.14	0.33	0.68	0.45	0.55	0.77	0.48	0.50
	SC-C	0.11	0.32	0.93	0.52	0.65	0.69	0.59	0.60
	S-R	0.12	0.25	0.68	0.47	0.59	0.69	0.48	0.50
	SC-R	0.08	0.22	0.68	0.64	0.62	0.70	0.52	0.55

4.2 Mann-Whitney U Tests of the Acoustic Absorption Tests

Tables 3 and 4 present the outcomes of the Mann-Whitney U Tests, with the first test assessing if the flushes have a statistically significant effect on acoustic absorption, and the second assessing if the bag shape has a statistically significant effect on acoustic absorption. In the first Mann-Whitney U test two independent groups (Pre-flush vs. Flush I and Pre-flush vs. Flush II) are compared across different octave band frequencies. This test is non-parametric, meaning it does not assume a normal distribution of the data, and it is used to determine if there is a significant difference between the pairs. A *p*-value less than 0.05 is considered statistically significant, meaning that there is a strong indication that the difference between the two groups is not due to chance. As shown in Table 3, for the Pre-flush vs. Flush I groups, across the octave band frequencies, none of the *p*-values fall below the 0.05 threshold, meaning that there are no statistically significant differences between the Pre-flush and Flush I conditions for any of the frequencies. For the Pre-flush vs. Flush II groups, however, at 125 Hz ($p = 0.012$), 250 Hz ($p = 0.021$), and 1000 Hz ($p = 0.006$), the *p*-values are below 0.05, indicating statistically significant differences between the Pre-flush and Flush II conditions at these frequencies. This suggests that the flush significantly impacted the acoustic behavior in these frequency bands. The other frequencies (500 Hz, 2000 Hz, 4000 Hz) do not show significant differences, as their *p*-values are above 0.05. Overall, these tests suggest that Flush II has a greater impact on acoustic absorption in certain frequency bands than Flush I.

Table 3. Calculated *p*-values for Pre-flush vs. Flush I and Pre-flush vs. Flush II of all samples

	Octave Band Frequencies (Hz)					
	125	**250**	**500**	**1000**	**2000**	**4000**
Pre-flush vs. Flush I	0.305	0.287	0.119	0.086	0.44	0.104
Pre-flush vs. Flush II	0.012	0.021	0.224	0.006	0.46	0.367

Additionally, Table 4 presents a comparison between circular and rectangular bags. The results indicate that for the straw (S) samples, there is a significant difference at 250 Hz (p = 0.02), suggesting that the shape (circular vs. rectangular) affects acoustic performance in this frequency band. There is no significant difference at other frequencies, although 125 Hz (p = 0.057) is close to the threshold. Conversely, in the straw and cotton (SC) samples, statistically significant differences are observed in the middle-frequency ranges of 500 Hz (p = 0.045) and 1000 Hz (p = 0.045), highlighting the influence of bag shape on acoustic properties. At other frequencies, including 125 Hz, 250 Hz, 2000 Hz, and 4000 Hz, no significant differences were found between the two shapes. Overall, the results suggest that the shape (circular vs. rectangular) can affect acoustic performance in certain frequency bands, depending on the material (straw or straw with cotton).

Table 4. Calculated *p*-values of the circular vs. rectangular samples of straw (S-C vs. S-R) and straw with cotton (SC-C vs. SC-R) from all flush cycles

	Octave Band Frequencies (Hz)					
	125	**250**	**500**	**1000**	**2000**	**4000**
S-C vs. S-R	0.057	0.02	0.101	0.101	0.316	0.118
SC-C vs. SC-R	0.932	0.219	0.045	0.045	0.697	0.58

4.3 Microstructural Characterization

Fig. 4 presents field emission scanning electron microscopy (FESEM) images taken from the surface of a circular straw-cotton (SC-C) sample, showing the hierarchical structure integrating pores, fibrous network and the straws. In Fig. 4a, pore-like channels are observed, resembling a hyphal network. Fig. 4b and Fig. 4d further reveal an interconnected fibrous network embedded within a bulk matrix. Fig. 4c depicts a straw component located on the surface of the SC-C sample.

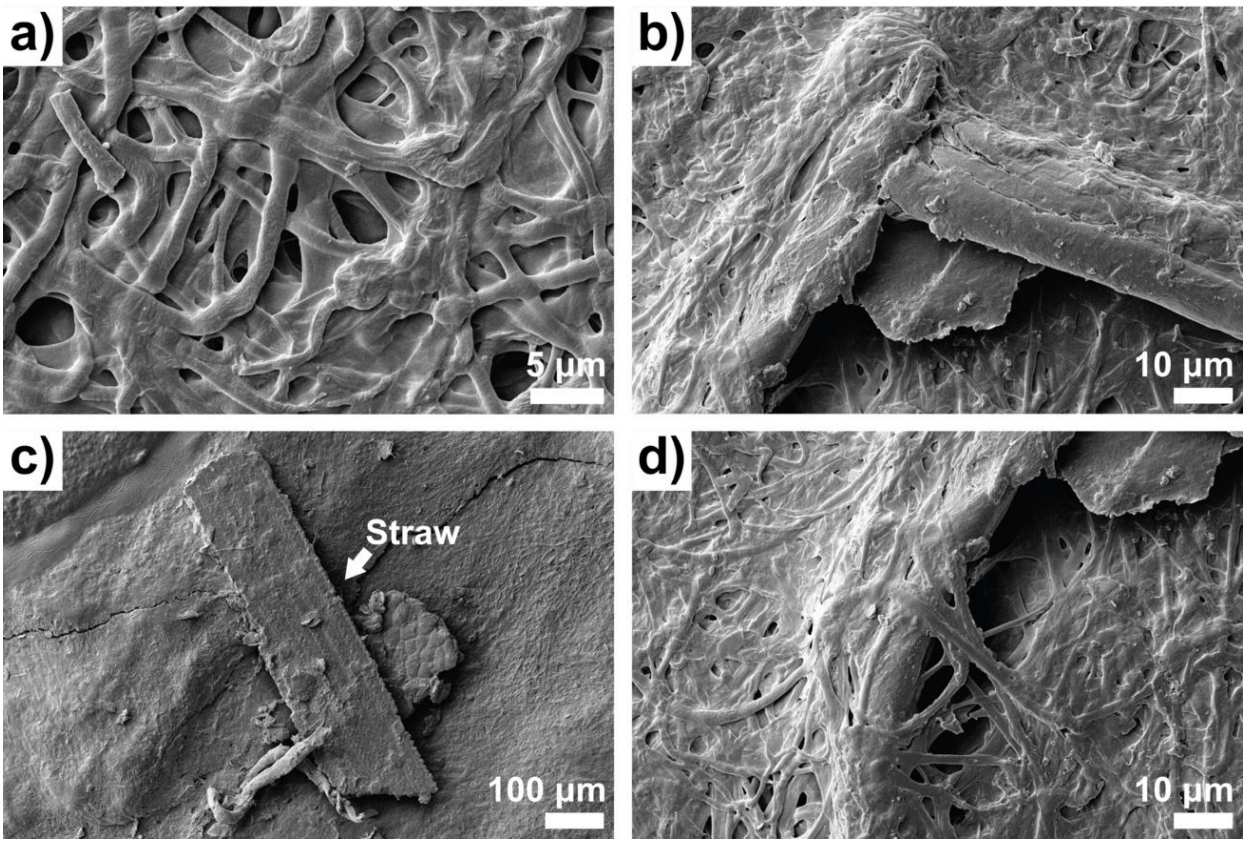

Fig. 4 Surface FESEM images of the Circular Straw-Cotton (SC-C) sample at different magnifications. Images were recorded on a ThermoFisher Helios microscope operated at 5 kV and 25 pA. Prior to imaging, the sample surface was sputter-coated with a gold film of 20 nm thickness.

5. Discussion

In this work, it is demonstrated that SMS-based MBCs can be used as acoustic absorptive material. A material is considered a sound-absorbing material when the average sound absorption coefficient (α) of the octave band frequency results is larger than 0.2. As shown in Table 2, the average coefficients are around the 0.5 range for all samples. Additionally, Fig. 3 shows that all samples resulted in absorption coefficients above 0.5 for frequencies of 600 Hz and above. These absorption coefficients from MBCs are desirable as they achieve absorption better than certain acoustic materials. The sound absorptiveness arises from the composite's porous morphology as seen in Fig. 4 and from the internal air gaps. It can be hypothesized that the incident sound waves are attenuated through multiple reflections within the interconnected pore network. Additionally, as seen in Table 1, the density values are higher with the straw-cotton substrates. These straw-cotton substrates with higher density also generally yielded more acoustic absorption in the 1 kHz to 2 kHz range. It is also generally known that the increase of flow resistance results in higher sound absorption performance, due to the loss of propagation when the sound travels through the material. However, there were no apparent correlating results to the absorption tests. This may be because mycelium growth is sporadic and inconsistent with various growth and cycles.

Observing the statistical analysis results in Table 3, in the lower frequencies of 125 Hz and 250 Hz, *p*-values of 0.012 and 0.021, respectively, indicate significant differences between Pre-flush and Flush II. At 1000 Hz, the difference is more significant between Pre-flush and Flush II with a *p*-value of 0.006, showing a substantial effect of the flushing process on the acoustic characteristics at this mid-frequency. However, no substantial differences can be found between Pre-flush and Flush I, because the materialistic properties in Pre-flush are similar to Flush I compared to Flush II.

Bag shape also influenced absorption behavior, as seen in Table 4. For the Straw (S) samples, the comparison between circular (C) and rectangular (R) bags shows statistically significant differences at lower frequencies, particularly at 125 Hz (p = 0.057) and 250 Hz (p = 0.02), suggesting that the shape of the bags substantially influences the absorption behavior in the lower frequency range. For the Straw and Cotton (SC) samples, these results shift to the middle frequencies, with significant differences observed at 500 Hz (p = 0.045) and 1000 Hz (p = 0.045). Overall, these results suggest that the shape of the bag plays a role in the absorption behavior at certain frequency ranges.

6. Conclusion

SMS-based MBCs show substantial promise as effective acoustic absorption materials. They achieve near-total sound absorption at specific frequencies with a thickness of 40 mm, offering acoustic performance comparable to conventional materials. Additionally, these materials are functional, sustainable, and economically beneficial, repurposing mushroom waste into potentially marketable acoustic panels. The transition from mushroom cultivation to acoustic material production can occur at any fruiting cycle, with expected costs lower than current acoustic absorbers in the market. This combination of affordability and sustainability can lead to increased profitability for producers while promoting eco-friendly agricultural solutions. The scalability of this process could open new market opportunities, making it attractive to a broader range of producers and potentially driving growth and innovation within the industry.

However, challenges remain, including understanding the sustainability and longevity of the material, fungal resistivity, and the carbon footprint and climate impact of large-scale commercialization. It also important to note that despite that the mycelium composites are generally fire-resistant [7], [8], the fire-resistance properties of the SMS samples still require further investigation and represent a clear direction for future research. With further research, SMS-based MBCs could become a viable and sustainable option for sound absorption in building applications. In conclusion, this study marks the final stage of the research phase on SMS-based MBCs and has enabled the construction of a wall prototype, designed for use in a trade fair exhibition. The prototype serves as a tangible demonstration of the feasibility of reusing SMS as a temporary material within the construction sector. This prototype is presented in detail in the following chapter.

Note: This chapter are based on the manuscript “From Edible Mushrooms to Sustainable Materials: Sound Absorption of Mycelium-Based Composites Cultivated on Spent Mushroom Substrates”, by Jee Woo Kim, Chiara Dognini, Seyedeh Aisa Shams Hojjati, John Pecchia, Yingxin Zhu, Yang Yang, Yun Jing, and Benay Gürsoy. The paper is currently under peer review for publication in the journal Applied Acoustics (Elsevier, https://www.sciencedirect.com/journal/applied-acoustics). The content has been revised, reorganized, and adapted to fit the structure and scope of this dissertation. The author gratefully acknowledges the valuable collaboration and shared contributions of all co-authors, with special thanks to Professors Benay Gürsoy and Yun Jing for their expert guidance and support throughout the development of this research. Their interdisciplinary insight was instrumental in advancing the acoustic characterization presented in this chapter.

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Chapter 7

Mush2Biom Prototype

1. Objective

This final chapter concludes the research by evaluating the mechanical performance and material consistency of the Mush2Biom prototype, a modular wall system composed of MBCs obtained from SMS from Edible Mushroom's cultivation Farms. Following the development of the dual-production process and the construction of a full-scale demonstrator, this chapter focuses on validating the technical feasibility of the material for architectural applications. The main objective is to investigate the compressive properties of the SMS specifically their peak compressive strength and Young's modulus, in order to determine whether the material is suitable for use in temporary building systems. The chapter also explores the relationship between the bio-composite's mechanical behavior and its inherent characteristics, such as low density, fibrous structure, and absence of compaction or chemical additives. These features, while being limited from a structural perspective, are crucial to the material's environmental benefits and biodegradability. Together, these evaluations provide a comprehensive validation of the Mush2Biom system, confirming its potential as a circular, low-impact construction solution and bringing the research project to its conclusion.

2. Introduction

This final chapter introduces the Mush2Biom prototype, an innovative wall system designed for temporary architectural applications such as fairs, exhibitions, and pavilions. The project addresses a critical environmental challenge faced by the mushroom industry: the disposal of large volumes of SMS. By offering a circular and sustainable alternative, the prototype showcases a novel approach to both waste valorization and ecological construction. As previously noted, mushroom cultivation generates approximately 5 kilograms of SMS per kilogram of edible mushrooms produced [1, 2], presenting a significant environmental issue [3], particularly in terms of disposal and its ecological implications [4, 5]. Martín et al. report that improper handling of SMS during storage can trigger anaerobic digestion and the emission of greenhouse gases [6]. Moreover, SMS can generate unpleasant odors and leachates that pollute water bodies, contributing to eutrophication [7]. The European Union banned landfilling of SMS in 1999 (European Council, 1999) [6, 8], removing a previously common disposal route and intensifying the pressure on the mushroom sector to find sustainable alternatives. Traditional mushroom cultivation, especially for Pleurotus species, usually ends after three harvesting cycles [9], as further flushes result in declining yields and increased costs. This inefficiency contributes to the underutilization of SMS and highlights the broader waste issue within the industry. The Mush2Biom prototype represents a significant step toward circular economy practices in construction. Rather than treating SMS as waste, this research repositions it as a valuable resource, proposing a model of industrial symbiosis between the agri-food and building sectors. In collaboration with CAUTO (a social cooperative), A.F. Serre Srl, and Società Agricola Mancon S.s., SMS was collected and transformed into mycelium-based composite (MBC) panels, suitable for use in modular wall systems. A full-scale 3×3-meter wall was constructed using these panels, demonstrating both the technical feasibility and scalability of the solution. The result is a lightweight, biodegradable wall system with excellent adaptability. Thanks to its low weight and modularity, the system is well-suited for multiple applications, including insulating panels, surface

cladding, and eco-conscious architectural elements. This prototype proves that SMS-derived MBCs can substitute conventional, resource-intensive materials in both temporary and, potentially, permanent construction. In this specific implementation, we focus on temporary uses such as trade fairs, exhibitions, and pavilions contexts that often generate large volumes of non-recyclable waste. The Mush2Biom wall offers a zero-waste solution: once its function has ended, the panels can be composted or converted into biogas, closing the loop without adding to landfill pressure. By demonstrating the successful use of MBCs in real-world, temporary structures, the Mush2Biom prototype redefines sustainable construction and event design, aligning with the principles of ecological responsibility and circular economy. This research positions SMS-based materials as viable, scalable alternatives to conventional construction products, with potential applications in thermal and acoustic insulation, biodegradable emergency structures, and sustainable design. As the construction industry increasingly moves toward low-impact materials, Mush2Biom offers a compelling opportunity for companies, designers, and investors to actively participate in the transformation of the built environment.

3. Materials and methods

The SMS used in this study was sourced from mushroom farms following the third and final harvest cycle. As previously discussed, *Pleurotus* cultivation typically concludes after three flushes due to the decline in productivity, rendering further cycles economically unviable at an industrial scale. Each SMS block had approximate dimensions of 500×350×250 mm and an initial weight of around 10 kg. The substrate primarily consisted of straw colonized by mycelium, as shown in Figure 1. Upon collection, the SMS blocks underwent a dehydration process at 24 °C to reduce moisture content. Drying continued until the mass stabilized, indicating complete water loss. The total weight reduction was approximately 55%, with each block decreasing from an initial 10 kg to about 4 kg after dehydration. Once dehydrated, five SMS blocks were selected for mechanical characterization. Mechanical tests were performed using an electromechanical dynamometer (Instron, Mod. 3366) by compressing each sample to 35% of its initial height with a 0.05 mm per second rate to study its behavior under uniaxial compression. The results of these tests were used to assess the feasibility of employing SMS-based MBCs in construction applications, particularly for modular wall systems.

Fig 1 SMS-based MBC specimens used for compression testing (sample 1-5). Samples were used as cultivation substrate for edible mushroom production and then transformed into MBCs measuring 500×350×250 mm.

4. Results

The mechanical behavior of the five samples was evaluated under uniaxial compression up to 35% strain. The stress–strain curves (Fig. 2) revealed a non-linear response typical of cellular and fibrous bio-based materials, with all samples displaying gradual strain hardening and no sudden collapse. This behavior is consistent with a progressive compaction of the internal fibrous network, which enables energy absorption across a wide strain range. Peak compressive strength values ranged from 0.028 MPa to 0.048 MPa, while Young's modulus varied between 0.07 MPa and 0.14 MPa (Table 1). The average peak stress was 0.036 MPa (SD: 0.008 MPa, CV: 24.54%), and the average Young's modulus was 0.100 MPa (SD: 0.029 MPa, CV: 29.15%). These levels of variability are reasonable given the natural origin of the substrate and the minimal processing involved. The low mechanical values observed are expected and coherent with the material's physical characteristics. The SMS-based MBCs were not subjected to any compaction, chemical treatment, or additive reinforcement, their structure derives solely from the natural colonization of straw by mycelium during the edible mushroom's cultivation and the dehydration process. As a result, the internal matrix remains highly porous, with relatively weak inter-fiber bonding and significant void content. This leads to lower density around 90–100 kg/m³ and reduced mechanical stiffness compared to densified or engineered composites.

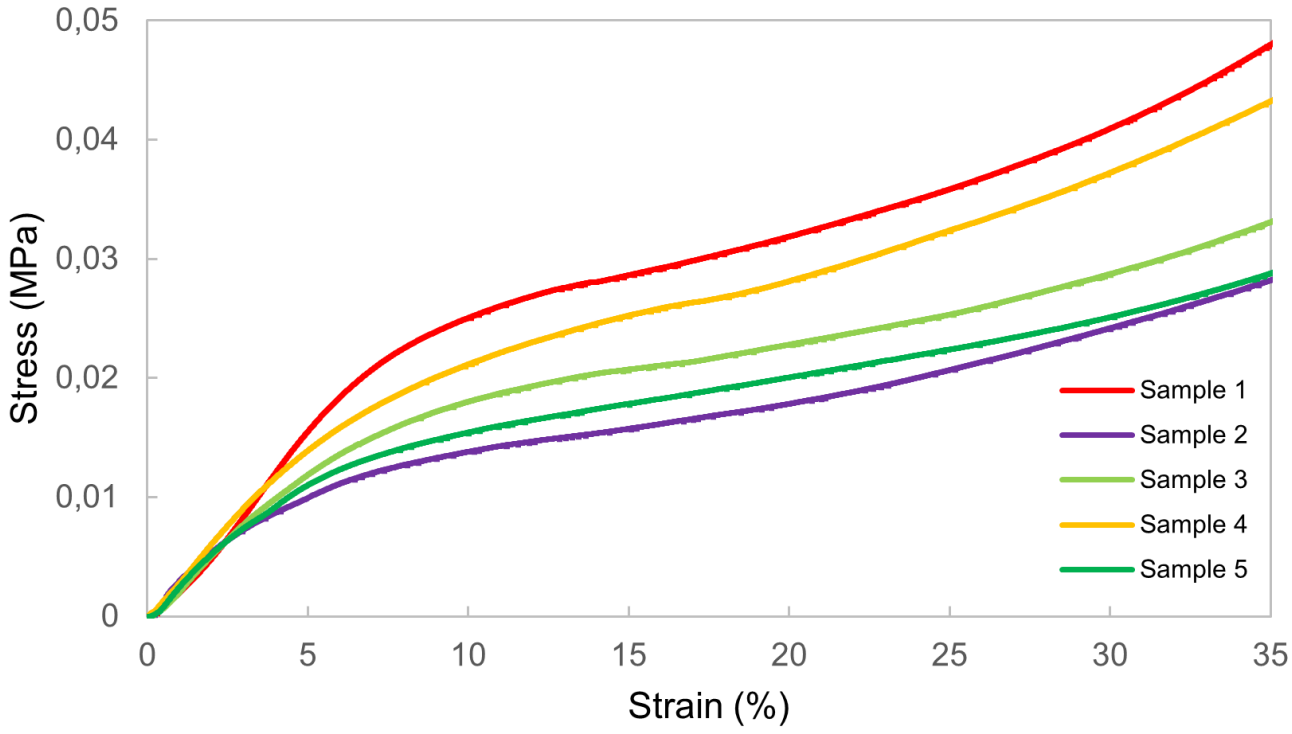

Fig 2 Mechanical compression test of the 5 samples 1-5 tested using 500×350×250 mm specimens. Dashed lines represent the individual replicates, while solid lines indicate the median curve.

Table 1 Peak stress (MPa), Strain where the Peak Stress occurred (mm/mm) and Young's modulus (N/mm^2) of the replicates 1-4 occurred into the range of 0.35 Reported values include the mean, standard deviation (SD), and coefficient of variation (CV) for each mechanical parameter.

Replicates	Dimensions (mm)	Weight (g)	Peak Stress (MPa)	Strain (%)	Young's Modulus (N/mm^2)
1	500*350*250	4459.20	0.048	35	0.14
2	500*350*250	4335.98	0.028	35	0.08
3	500*350*250	4655.21	0.033	35	0.09
4	500*350*250	4357.90	0.043	35	0.12
5	500*350*250	4673.88	0.029	35	0.07

However, this trade-off between weight and mechanical performance is advantageous for certain applications. The material's low density ensures ease of handling and installation, while its ability to deform gradually under compression without brittle failure makes it suitable for non-structural elements such as partition walls, acoustic buffers, or temporary installations. The progressive shape of the stress–strain curves also suggest that, despite the modest peak strength, the material can sustain load redistribution over time without sudden loss of integrity. The statistical overview of the results is summarized in Table 2 and visually presented in Fig. 2. The bar chart illustrates the mean and standard deviation for both parameters, underlining the disparity between stiffness and strength but also confirming the overall repeatability across samples. Taken together, these findings demonstrate that even without intensive processing, SMS-based MBCs can deliver a stable and predictable compressive response, particularly for short-term or reusable construction applications where full structural performance is not required.

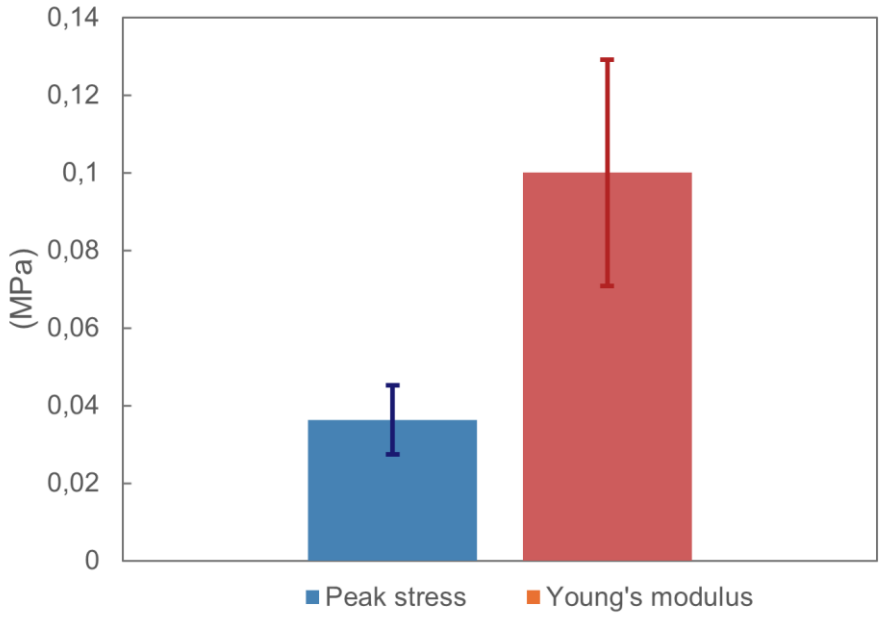

Fig 2 Average values of *Peak Stress* and *Young's Modulus* obtained from the four replicates 1-4 based on the compression tests. Vertical error bars represent the **standard deviation**, indicating the variability among the samples.

Table 2 Peak stress (MPa), Strain where the Peak Stress occurred (mm/mm) and Young's modulus (N/mm^2) of the replicates 1-4 occurred into the range of 0.35 Reported values include the mean, standard deviation (SD), and coefficient of variation (CV) for each mechanical parameter.

Parameter	Mean (MPa)	Std.Dev. (MPa)	CV (%)
Peak stress	0.036	0.008	24.54
Young's modulus	0.100	0.029	29.15

5. Conclusions

The experimental results presented in this chapter demonstrate that SMS-based MBCs can exhibit reliable mechanical behavior under compression, even in the absence of mechanical compaction or chemical treatments. Although both peak compressive strength and Young's modulus were relatively low, averaging 0.036 MPa and 0.100 MPa, respectively, these values are consistent with the physical nature of the material: lightweight, fibrous, and highly porous. The mechanical limitations observed are not a weakness of the material, but rather an expected consequence of its minimal processing. The absence of external pressing or synthetic binders preserves the biodegradability and environmental neutrality of the material, making it particularly suited for low-impact, short-lifespan applications. In this context, low stiffness and strength are offset by benefits such as low weight, ease of handling, and post-use compostability. Importantly, all five tested samples exhibited consistent stress–strain behaviour, with no sudden failure or collapse. This indicates a high degree of material continuity and internal cohesion, even in the presence of natural variability. The moderate coefficients of variation (24.54% for peak stress, 29.15% for Young's modulus) confirm a satisfactory level of reproducibility for bio-fabricated materials grown under non-industrial conditions.

The Mush2Biom prototype thus proves the feasibility of integrating MBCs into architectural elements for temporary or modular uses, such as interior partitions, exhibition structures, or sustainable pavilions. A full-scale demonstration wall measuring 3×3 meters was assembled using the SMS-based MBCs, as shown in Figure 3, highlighting the material's ability to form self-supporting vertical assemblies with minimal auxiliary structure. The prototype visually confirms the mechanical cohesion observed in laboratory testing and reinforces the practical viability of the system in real-world architectural contexts.

In conclusion, SMS-based MBCs offer a promising alternative to conventional building materials in contexts where structural demands are low but environmental sustainability is paramount. Further improvements in performance could be explored by adjusting density and post-treatment process such as compaction, dehydration and beyond, but even in their untreated form, these composites provide a functional, zero-waste solution for eco-conscious design and temporary construction.

Fig. 3 Mush2bioM full-scale prototype wall (3 × 3 m) constructed using dehydrated SMS-based MBCs. The modular assembly demonstrates the material's ability to form self-supporting vertical structures without additional chemical treatments or compaction. The system highlights the potential of MBCs for temporary, low-impact architectural applications such as fairs trade, exhibition walls and pavilions.

Fig. 4 This Mush2Biom full-scale prototype wall (3 × 3 m) was built and exhibited at FUTURA EXPO, Italy, using dehydrated SMS-based MBC panels. The modular system demonstrates the material's capacity to form self-supporting vertical structures without chemical treatments or mechanical compaction. The prototype showcases the potential of these biomaterials for temporary, low-impact architectural applications such as trade-fair installations, exhibition walls, and lightweight pavilions.

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Conclusions

This doctoral research set out to address a dual challenge: the pressing environmental impact of conventional building materials and the unresolved issue of managing SMS in the edible mushroom industry. Through an interdisciplinary approach that bridged microbiology, environmental engineering, material science, and industrial design, the study proposed, developed, and validated an integrated dual-production system capable of yielding both edible mushrooms and MBCs within a single circular framework. The research was articulated in progressive stages, beginning with a comprehensive state-of-the-art analysis of MBCs and culminating in the construction of a full-scale prototype, the Mush2Biom wall, made entirely from dehydrated SMS. Each experimental phase contributed critical insights: from methodological replication using new substrates, to mechanical and acoustic characterization, and ultimately, to the definition of a zero-waste process that transforms agricultural byproducts into functional, biodegradable building materials. The results confirmed that SMS, typically considered waste after three mushroom harvests, can be effectively repurposed without the need for further fungal growth or chemical treatment. Mechanical testing demonstrated sufficient compressive strength and stiffness to support self-standing construction applications such as wall panels, acoustic baffles, and exhibition structures. These values, although lower than those of traditional structural materials, are entirely consistent with the lightweight, fibrous, and uncompressed nature of the SMS blocks. Notably, the production method required only dehydration, with no compaction or binder additives, ensuring the full biodegradability of the final product. The acoustic testing further revealed that MBCs produced via the dual system exhibit sound absorption properties suitable for interior environments, particularly in contexts where noise control and sustainability are equally prioritized. Statistical analyses across multiple samples demonstrated acceptable reproducibility in both mechanical and acoustic performance, reinforcing the material's viability in real-world scenarios. The culmination of this research was the Mush2Biom prototype, presented in Chapter 7, a 3×3-meter wall constructed and exhibited during FUTURA EXPO (March 7–9, 2025, Brescia, Italy). The prototype served not only as a proof of concept, but also as a public demonstration of how circular design, regenerative production, and sustainable architecture can converge in a single application. The positive reception from stakeholders confirmed the growing interest in bio-based alternatives while validating the practical implementation of the proposed system. Overall, this thesis demonstrates that integrating edible mushroom cultivation and MBC fabrication into one closed-loop process is not only biologically and technically feasible but also scalable and economically attractive. By valorizing waste, simplifying production, and reducing environmental impact, this dual-production model represents a promising strategy for advancing sustainability in both agriculture and the built environment.

Importantly, the outcomes of this work also establish a solid foundation for future sustainability assessments, including Life Cycle Assessment (LCA), Techno-Economic Analysis (TEA), and Social LCA (SLCA), aimed at quantifying the broader environmental and economic advantages of MBC-based systems. Furthermore, the demonstrated biodegradability and organic composition of SMS-derived composites open additional research avenues related to their end-of-life valorization, such as their potential reuse as feedstock for biogas production, as bio-adsorbent materials for water decontamination, or as soil-improving amendments within regenerative agricultural practices. In conclusion, this research lays the groundwork for future studies and industrial applications that may expand the reach of biomaterials while addressing the urgent need for regenerative strategies in construction, agriculture, and waste management.

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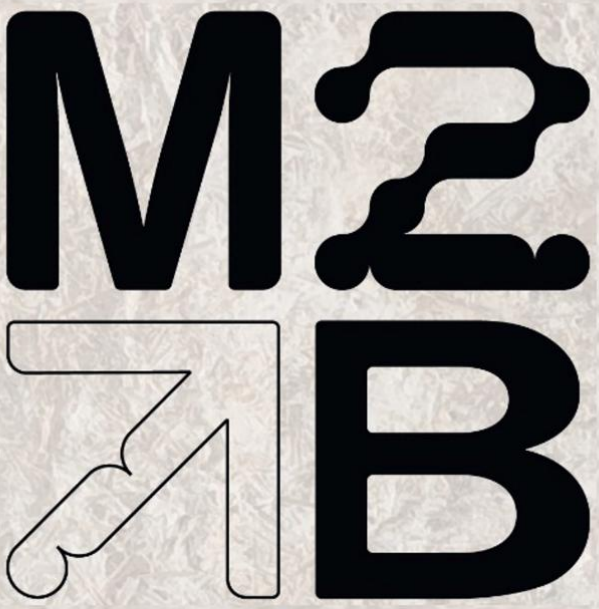

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