Myco-Ex: Evaluation on Additives for Liquid Deposition Modeling of Mycelium-Based Composites and its application toward architecture

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Abstract

Counteracting the usual fabrication techniques of mycelium-based composites (MBC), this bachelor thesis focuses on additive manufacturing with a two-step extrusion process. Since MBCs are based on natural fibers such as hemp, flax, or other agricultural waste products, the thesis aims to prepare an extrudable mixture of pre-grown biomass through additive binding agents. Current MBCs found on the market are used for thermal and acoustic insulation panels or as packaging products, whereas only a few reports on their structural application are used. The improvement of MBC structural behavior is a prerequisite for broadening the range of applications in the building sector. Mechanical properties of natural fiber composites are highly dependent on their components, e.g., fiber length, orientation, and density, and thus can be adjusted to the preferred application and fabrication method. For refinement in print quality to match the required viscosity for the additive fabrication method, psyllium husk and guar gum will be tested. Chitosan is a biodegradable, non-toxic biopolymer that showcases promising application possibilities for the reinforcement of natural fibers. A full-scale prototype for the structural application of MBC will be elaborated on in this study.

KURZFASSUNG

Kurzfassung

Im Gegensatz zu den üblichen Herstellungstechniken myzelgebundener Verbundwerkstoffe (MBC) konzentriert sich diese Bachelorarbeit auf die additive Fertigung mit einem zweistufigen Extrusionsprozess. Da MBC auf Naturfasern wie Hanf, Flachs oder anderen landwirtschaftlichen Abfallprodukten basieren, zielt die Arbeit darauf ab, eine extrudierbare Mischung aus vorgewachsener Biomasse durch additive Bindemittel herzustellen. Die derzeit auf dem Markt befindlichen MBC werden für Wärme- und Schalldämmplatten oder als Verpackungsprodukte verwendet, während es nur wenige Berichte über ihre strukturelle Anwendung gibt. Die Verbesserung des Tragverhaltens von MBC ist eine Voraussetzung für die Erweiterung des Anwendungsspektrums im Baubereich. Die mechanischen Eigenschaften von Naturfaserverbundwerkstoffen hängen stark von ihren Komponenten ab, z. B. Faserlänge, Orientierung, Dichte, und können daher an die bevorzugte Anwendung und das Herstellungsverfahren angepasst werden. Zur Verfeinerung der Druckqualität zur Anpassung an die erforderliche Viskosität für das additive Herstellungsverfahren werden Flohsamenschalen und Guarkernmehl verwendet. Chitosan ist ein biologisch abbaubares, ungiftiges Biopolymer, das vielversprechende Anwendungsmöglichkeiten zur Verstärkung von Naturfasern aufweist. In dieser Studie wird ein maßstabsgetreuer Prototyp für die strukturelle Anwendung von MBC ausgearbeitet.

B.SC THESIS MAI THI NGUYEN

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Abbreviations

MBC(s)	Mycelium-Based Composites
SMS	Spent Mushroom Substrate
MIS	Mycelium inoculated substrate
MBSS	Mycelium-Based sandwich structures
SLM	Selektive Laser Melting
SLS	Selective Laser Sintering
FDM	Fused Deposition Modeling
SLA/STL	Stereolithography
LDM	Liquid Deposition Modeling
MDD	Material Driven Design
HB	Hemp bulk
SB	Straw bulk
SD	Saw dust
PP	Paper pulp
PP GL	Paper pulp Ganoderma lucidum
PP GL MYA	Paper pulp Ganoderma lucidum Malt Yeast Agar
PP GL MYA PHK	Paper pulpGanoderma lucidumMalt Yeast AgarPsyllium husk mixture
PP GL MYA PHK WCE	Paper pulpGanoderma lucidumMalt Yeast AgarPsyllium husk mixtureCellulose powder
PP GL MYA PHK WCE GG	Paper pulpGanoderma lucidumMalt Yeast AgarPsyllium husk mixtureCellulose powderGuar gum
PP GL MYA PHK WCE GG SEM	Paper pulpGanoderma lucidumMalt Yeast AgarPsyllium husk mixtureCellulose powderGuar gumScanning electron microscopy
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PP GL MYA PHK WCE GG SEM DWC HSW DSW DSW DW IBK2	Paper pulpGanoderma lucidumMalt Yeast AgarPsyllium husk mixtureCellulose powderGuar gumScanning electron microscopyDelta Wasp Clay 40100humid single wall sample GG01dry single wall sample GG01double wall sample GG01Institute of Building Construction, Chair 2

Introduction

1.1. Relevance

The building sector has a significant negative impact on our environment by emitting about 50% of carbon emissions, 50% of total solid waste, and exploiting 20-50% of natural resources worldwide [1]. The constant need for building materials, dwindling resources, and fluctuating raw material prices can lead to severe economic, and social disruptions [2]. Especially the extraction and processing of non-renewable raw materials are often energy-intensive, involve considerable intervention in the natural and water balance, and lead to soil degradation, water scarcity, loss of biodiversity, impairment of ecosystem functions or intensification of climate change can be the result [2,3]. For example, Brazil lost almost 10% of the Amazon rainforest in the period from 2005 to 2015 due to the cleavage of large vast lands. This deforestation is followed by building infrastructure for mining operations and settlement for workers in previously untouched areas [3]. Furthermore, the earth's regenerative capacity is already exceeded in some cases and results in increasing pressure on natural resources and competition for use within a globally growing population [2].

In terms of post-processing of construction materials, the waste is often bulkier, heavier, and more toxic than domestic waste, so their disposal must be carefully managed [4]. Counteracting the linear cradle-to-gate process, the circular production process reimagines waste as a new resource and is only successful if all building components can be disassembled and separately brought back to their original material cycles [5]. To implement sustainability in the building sector, raising awareness of alternative regenerative materials is a prerequisite. Increased research and products of bio-based materials such as bioplastics, and materials out of biomass from bacteria, algae, and fungi can be found recently [6]. Specifically, fungal materials, so-called Mycelium Bound Composites (MBC) are an alternative material solution by being organic in matter and fully compostable. In the building industry, MBC panels are comparable to commercial acoustic tiles [7] or can be applied as insulation panels [8].

1.2. Mycelium-Based Composites in the circular economy

Mycelium-Based Composites mostly consist of fibrous substrate, and lignocellulosic agricultural or forestry by-products. Using MBC in the building industry means a significant increase in mushroom material production which is followed by an increase in Spent Mushroom Substrate (SMS). For global mushroom production of the food industry alone,

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the resulting SMS amount would exceed one trillion kg per year, equivalent to 6 tons SMS km-2 of the global land area [9]. Within the circular economy, SMS can be further processed in multiple applications, e.g. as a source of sugars for bioethanol production [10,11], as a biofertilizer [12], to improve the quality of pig manure-based compost [13], through the extraction of enzymes for environmentally friendly and energy-efficient biofuel or biogas production [14,15] or as feedstock to improve growth of calves by fermenting SMS with lactic acid bacteria [16]. The most sustainable option is dependent on local waste streams, fertilizers, food and feed resources, and its possible integration of SMS in other systems, such as the agri- and horticultural system [9]. Figure 1 describes an integrative approach for several mushroom and mycelium (re-)production processes. It is adapted from Cultivated Building Materials [5] and Mushroom production in the circular economy [9]. Ligno-cellulosic biomass are harvest from agriculture, which can be used as a substrate for mycelium production of MBCs as acoustic tiles, thermal isolation or furniture. By the time of disassembly or disuse, MBCs can be grinded into SMS to either produce MBCs again or to produce fertilizers, feed, etc. as previously explained. Since full degradation of the SMS



Figure 1. Circular Mycelial (Re-)production

takes substantially more time and energy than the mycelial production itself, it is important to address quantitative models predicting the environmental impact and economic viability of mushroom, MBC, and SMS (re-)production processes [9].

1.3. MBC fabrication methods

1.3.1. external molding

During the past years, several application and fabrication techniques of MBCs have been investigated and built. One of the first architectural applications is the *Hy* - *Fi* pavilion [17], which was developed in 2014 for the MoMa PS1 art museum in New York. It consists of 10.000 pre-grown MBC blocks with a dimension of approximately 10x15x23cm. For fabrication, plastic molds were filled with mycelium inoculated substrate to achieve the desired shape. When dried, the bricks are assembled to a 13m tall tower, covering an building volume of 3000m³. The construction proves the structural application of MBCs in a modular brick assembly and is able to resist a wind force of 65mph, however, it has been decided to maintain the supporting sub-structure to minimize the wind-caused movement [18]. After disassembly, the bricks were ground and degraded in soil over a course of 60 days [19].

Another large-scale application is *MycoTree* (2017), a collaboration between the Professorship of Sustainable Construction at Karlsruhe Institute of Technology (KIT) and the Block Research Group at the Swiss Federal Institute of Technology (ETH) Zürich. The spatial structure is self-supported using 3D graphic statics as a design tool to achieve stability through geometry in a compression-only design approach [20]. Digitally prefabricated molds were filled with mycelium-inoculated substrate (MIS) and after the drying phase joined through bamboo plates. The use of steel dowels compensates tensile forces, as opposed to compression-bearing mycelium composites [18].

1.3.2. integrative molding

Grown Structures of student A. Vesaluo from Brunel University in cooperation with architecture firm Astudio (2017) stuffed MIS into tubular cotton bandages. It presents an alternative molding technique by using molds of organic fibers to grow within mycelium. The cotton bandages retain and support the shape of MBC tubes [21].

The additional fiber reinforcement of MBC was investigated throughout a study of J. Liang [22]. The composite is composed by two outer woven or felted natural fibers layers and

one middle core layer of MIS purchased from Ecovative. The sandwich - MBC colonizes all layers successfully and a smooth surface can be achieved [23] in terms of an intense colonization, the final dried product appears stronger. If the interfacial bond between skins and core is sufficiently strong, it can be considered as a thicker core, however bioresin can be additionally added to further improve the layered composites structural capability. The core of mycelium-based sandwich structures (MBSS) has commonly failed in tensile loading, which implies being the weakest part within the composite. Flax MBSS has a tensile strength up to 6MPa, which is ½ compared to resin-infused flax MBSS stength up to 30MPa [22].

The study *Mycomerge* (2021/2) aims to demonstrate the load-bearing potential of mycelium when reinforced with biobased materials, such as long hemp fibers and rattan. The proposed design method aims to guide the growth of the mycelium onto a natural rattan skeleton, which serves as the supporting structure for the mycelium substrate and its fiber reinforcement, where both components merge into a fully biodegradable unit. Due to increasing water content within the rods and wet hemp fibers, the tensile properties of the mycelium-based rattan-reinforced composite have improved, whereas the whole composite performs best under compression.. The funicular multi-layered shell has a thickness of only 2-3cm and is able to bear a load of more than 20 times its own weight of 3.7kg [24].

1.3.3. additive manufacturing

Additive manufacturing is a term for all manufacturing processes in which material is applied layer by layer, enabling a high degree of freedom to design and structure materials in 3-dimensional objects. It is well known as 3D Printing, generative or rapid technology. The 3D printing process is differentiated according to the material used and the form in which it is applied in the process. For printing with powder, widely used methods are for example Selective laser melting (SLM) or Selective laser sintering (SLS); with the use of filaments, a common technique is the Fused Deposition Modeling (FDM); an example for processing fluid materials is the Stereolithography (SLA/STL).

In recent years, a new 3D printing technology arose to print highly viscous materials such as clay or concrete hence upscaling the fabrication of additive manufacturing on a building scale. This process is named Liquid Deposition Modeling (LDM) and is mostly applied by *Wasp* (World's Advanced Saving Project, CSP srl, Massa Lombarda, Italy).

Compared to previously mentioned fabrication examples of MBC, 3D printing offers **auto-mated processing** and thus reduces the effort and time of manufacture.

First known publication for the development of extrudable MBC-paste is from Soh et al. (2020), using bamboo fibers and chitosan. The fibers with a particle size of 500μ m are used as substrate, whereas chitosan is applied as a rheological modifier for extrusion. *Ganoderma lucidum* (GL) was chosen in this study and purchased as sawdust spawns. 3wt% Chitosan with medium molecular weight (200–800 cP viscosity for 1 wt% chitosan in 1% acetic acid, acetylation degree 75–85%) was stirred over a course of 48h in 1% acetic acid solution and is then added to grown MIS of bamboo fibers. The resulting paste is extruded into 5 cm long struts manually using 6 mm tip diameter serological syringes. The mycelium struts were set to grow for 20 days at a temperature of 23 ± 0.5 °C and 65-70 % humidity. Fiber size of 500µm in a ratio of 60:40, 70:30 with 3wt% chitosan solution (pH 6) was giving the best results. Chitosan did not limit mycelial growth, but compression tests indicate an **unexpected decrease** in mechanical properties of 240kPa without chitosan to 40kPa with chitosan [26].

Pulp-Fiction from Godeia et. al (2020) uses fine wood chips, paper pulp as substrate mixed with kaolin clay, water as a bonding agent, and an unknown thickening agent to ensure stable printing. Three tests are carried out: pulp with no fungal strain, strain from *Byssomerulius corium*, and lastly, strain from *Gloeophyllum sp*. The paper pulp mixture shows the best results with *Gloeophyllum sp*. in a bending test to evaluate stiffness, a test for dispersion in water, and a test for water absorption [27]. Extrusion takes place trough *Vormvrji Lutum v4* by air pressure and a rotating auger screw. The pulp mixture is printed with settings for a nozzle diameter of 3.5mm and a layer height of 1.5mm.

Chen. et al (2021) outline the production of paste, consisting of *Fomes fomentarius* mycelium, alginate, and water with 71 wt.% mycelium in the solid content. Through a combination of freeze drying and cross-linking with calcium, the printed samples could remain stable in the presence of water and show low bulk densities of 0.12 ± 0.01 g/cm3 with interconnected macropores [28]. The samples were printed in 10, 20 and, 30 layers with a nozzle diameter of 1.6 mm, a layer height of 1.3 mm, and a printing speed of 10 mm/s).

Titled Arch from Modanloo et. al (2021) examines sawdust, paper pulp, and wheat bran as substrate materials and compares arabic gum to guar gum as binding agents. Best results were achieved by using paper pulp and wheat bran as a base substrate for oyster mushroom and guar gum as binding additive with water. The paste mixture is filled into a customized tank with a diameter of 30cm, and final printing nozzle of 9mm, a speed of 100mm/s, and air pressure of 1.7bar. Extrusion by air pressure was sufficient due to the continuous printing path. The reported extruded material results in double diameter as the nozzle of 8mm to a printed diameter of 16mm and after drying to 14mm [6].

Elsacker et. al (2022) tested several binding additives (psyllium husk, corn starch, xanthan gum, paper cellulose, guar gum, and locust bean gum) for extrusion of MIS using beechwood sawdust (0.2–1.25 mm) inoculated with Ganoderma lucidum. The ratio of water and additive is crucial for mycelial growth as in psyllium husk and locust bean results in intense hyphae formation [29]. Psyllium husk was the most promising additive for extrusion and biocompatibility with *Ganoderma lucidum*. Using KUKA KR 15/2 6-axis industrial robot and a customized extruder, final nozzle diameter of 5mm was chosen and air pressure of 2 bar at a speed of 45mm/s.

1.4 Scope

This Bachelor Thesis focuses on evaluating binding additives for the LDM printing process. The goal is to promote the growth for full coverage of mycelium since it has hydrophobic and tensile properties [30]. In addition to growth, the critical factors influencing the printing results are also observed. The project questions the strength of MBCs and aims to find alternative methods to improve its mechanical properties by either the fabrication method itself or in presence of fiber-reinforcement qualities of additives like chitosan.

Materials and Methods

2.1. Methodology

The Material Driven Design process describes the comprehension of the specific material as the starting point for the design, followed by creating an experienced landscape, material manifestation and lastly product design [30]. Working with new bio based materials, specifically MBCs, it needs continuous feedback loops until material selection, fabrication methods and parameters as well as design possibilities are aligned [6]. All steps are codependent on the results of the other.



Figure 2. Methodoly diagram

2.2. Materials

Hemp bulk (HB) was purchased from Hanffaser Uckermark . Straw bulk (SB) was purchased from Cordes and must be shredded to a particle size of 2-6mm. Sawdust (SD) leftovers were delivered from a wood workshop nearby. Paper (PP) is reused from packaging insulation material to be shredded and used as a substrate supplement. Three different mixtures of fibers were set as substrate base materials. HB-SB in a ratio of 1:1, HB-SB in 2:1, and SD-SB (2:1). Substrate material compositions as listed in Table 1. and are autoclaved for 20 minutes at a temperature of 121 degrees. The sterilized substrate bass must cool down at room temperature before inoculation.

Ganoderma lucidum (GL) was supplied by IBBS University of Stuttgart and further cultivated on Malt Yeast Agar (MYA) petri dishes, later in a lignocellulosic substrate. Optimal growing conditions are a pH value of 6 and a temperature of 30 degrees. SD-SB substrate did not show growth, whereas HB-SB 2:1 resulted as best growing substrate material and is there chosen as base substrate for MBC paste.

	HB	SB	2.5g CH₃COONH₄
	(ml)	(ml)	2.4g CaSO4 +
			H ₂ O (ml)
HB-SB 1:1	240	240	250
HB-SB 2:1	320	160	250
SD-SB 2:1	320	160	250

Table 1. substrate mixtures

2.3. Production of MBC paste

Following additives are used in this project: Psyllium husk (PHK), kaolin clay powder (KCP), cellulose powder (WCE), guar gum (GG), and chitosan (200-600mPa·s, 0.5% in 0.5% Acetic Acid at 20°C) from TCI Chemicals was used. All additives must be autoclaved without adding water. High temperatures combined with water mixed with additives would result in a strong bulky mass. The first step of MBC paste is to shred or scrumble MIS into fibers. Paper pulp is then added to the MIS fibers in a ratio 2:1. Additives and solution content is adapted according to its bonding capacity and is listed in Table 2. PHK, KCP, GG are added to MIS fibers and mixed evenly before adding solution of ammocium cetate and calcium sulfate.

Using serological syringes with diameter of 4mm and 6mm, the mixtures extrudability are tested. Kaolin clay powder was not succesfully exruded because of high water release while manually extruding KCP mixture. Cellulose Flour without guar gum or psyllium husk was not extrudable thus can be considered as filler material but is not necessarily required. PHK and GG01 mixtures were successfull extrudable via 6mm syringe nozzle and further tested for LDM printing. To prepare the GGCH mixture, 1%wt chitosan is dissolved in a 10% acetic acid solution for 20 minutes resulting in a pH value of 3.5.

	Additive (g)	MIS (ml)	PP (ml)	2.5g CH₃COONH₄
				2.4g CaSO4 +
				H ₂ O (ml)
PHK	10	100	50	85
WCE	10	100	50	50
KCP	20	100	50	50
GG01	10	100	50	50
GG02	10	100	50	35
	1%wt chitosan			
GGCH	100ml 10%acetic	100	50	25
	acid solution			

Table 2. paste mixtures

2.4. Scanning Electron Microscopy

SEM Images are carried out by Prof. M. Schweikert at IBBS, University of Stuttgart.

<u>2.5. Mechanical test</u>

Compression tests are carried out by Dr. M. Hörning at IBBS, University of Stuttgart.

2.6. Hardware

The large-format printer Delta Wasp Clay 40100 (DWC) is used in this project. It has a printing volume of a maximum diameter of 400mm and a max. height of 1000mm. It does not use linear motion to place the material, instead, it controls the movements by calculating the coordinates using three arms as parallelograms. By movements on the vertical axes, the angles of the parallelogram are changed accordingly. Control and mechanics are installed at one platform at the top part of the printer, which makes a lighter printhead compared to classic cartesian printheads. This reduces inertia enabling quick movements. DWC has a maximum printing as well as travel speed of 150mm/s. The used printhead is the LDM Wasp Extruder XL, which comes in nozzle sizes of 3mm,6mm, and 8mm and is the first extruder with flow control while printing dense fluid materials. A gear stepper motor attached to an auger screw out of POM plastic, a semi-crystalline thermoplastic with high mechanical strength and stiffness, which offers to print harder mixtures.

Figure 4. illustrates the operation principle of DWC. The material is filled into a tank with a volume of 5L. Material is compressed by an input air pressure depending on the consistency of the mixture. Out of the tank, the material moves downwards through a pipe into the extruder head, where the auger screw and motor are attached to. The resulting extrusion is only performed via an auger screw, allowing the material to be held back in order to print overhangs or models with gaps. The 2-step extrusion process enables the material to be pre-compressed in the tanks before extrusion, avoiding air bubbles within the mixture.

2.7. Software

Design and geometry explorations are scripted in Grasshopper 3D, a Plug-In for modeling software Rhinoceros 3D computer-aided design application (CAD). Grasshopper is a visual programming language and environment for modeling generative or complex geometries through specific parameters. Parametric design is decisive when working with unpredictable new materials and fabrication methods. It is possible to integrate certain algorithms and scripts to match the design goal and help to include all outputs of MDD criteria. To set the print parameters (slicing) for the 3D model into readable G-code, Cura 4.13.1 is used. One must create a custom printer profile for WDC, as Cura is normally not applied for LDM printing. WDC is reading Marlin G-code flavor to control the machine, whereas its core functions are receiving commands to be executed (G-codes) from a connected computer or SD card, planning and controlling operations on all axes, and monitoring the work process, the movement of the print head and temperature control. In addition, one can set custom material profiles to calculate the printed material volume and the resulting printing time span.



Figure 4. Two Step extrusion process

Results

<u>3.1. Growth</u>

Tested samples from manual extrusion show varying growth duration and intensity (Figure. 5). PHK01 resulted in uneven growth along struts, mycelial growth was only partially visible. The total duration was 10 days. Best growth results were obtained by GG01 struts with a total growth period of 5 days. Homogenous growth throughout the GG01 growth process was observed and resulted in a fully white outer coverage.



Figure 5. Results of extruded struts from mixture left to right PHK, GG01, GGCH

Through the growing success of GG01, a further sample of GGCH was run to test the compatibility of mycelium with 1%wt chitosan in 10% dissolved acetic acid solution. GGCH strands have the longest growth period of 20 days, from which it can be considered that the pH value of 3.5 slows down mycelial growth. As shown in Figure 5 (r), mycelium grew along the surface of GCCH, starting at one tip. GG01 and GGCH show great results of aerial mycelium, but especially for GGCH growth within the strut is due to visual observation questionable. Therefore, SEM images of cross-sections of all three samples were taken to examine mycelial growth within the extruded struts. In further material tests, only 2/5 GCCH samples were successful.

3.2. Cross section

Figure 6. shows the cross sections of a) PHK, b) GG01, and c) GGCH. The differences in the thickness of the outer mycelial layer are visible at a magnification ranging from 57x-73x. GGCH shows the thickest outer layer with approximately 800μ m up to 1000μ m, followed by GG01 with a thickness of $200-300\mu$ m, mycelial skin from PHK results in the thinnest

with 20-50 μ m. Images of mycelium growth within the struts are taken at a magnification ranging from 351x to 368x. PHK shows again the lowest density of mycelium hyphae in between fibers resulting in uncovered areas of 50-100 μ m², but notably more growth along the fibers. GG01 is showing the best results of hyphae formation between fibers, followed by GGCH. Images from GGCH show some less intensely grown gaps of approximately 50 μ m. SEM and resulting images were conducted by Prof. Schweikert at IBBS.



Figure 6. SEM images of cross-sections from a) PHK b) GG01 c) GGCH

3.3. Printing mixtures, parameter and quality

PHK, GG01, and GGCH mixtures, and later GG02 (Table 2) were set for automatic extrusion via WDC. Nozzle diameters of 3mm, 6mm, and 8mm are tested, whereas only GG01 was extrudable with all nozzle diameters. Printing speed at 20mm/s, 10mm/s, and 5mm/s and layer heights of 2mm, 4mm, and 6mm were set as parameters to evaluate the printing quality.

PHK was only hardly extrudable with a nozzle diameter of 8mm by manually rotating the auger screw with a pressure of 3bar and a flow rate of 1.1 (110%). During the printing process, PHK struts could not be placed at a speed of 10mm/s and causing clogging issues. As well described by Elsacker et al. (2022), the accurate ratio of water and psyllium husk is hard to match. Upscaling PHK mixture resulted in a bulky mass due to high swelling behavior of psyllium husk powder. An extrudable mixture with psyllium husk for LDM printing could not be reached. PHK mixture was not successful for the automized printing process but can find its application for example in manual modeling or forming of MBCs.

GG01 was extrudable with all nozzle sizes, but printing with 3mm diameter caused clogging, in some cases also with 6mm, therefore air pressure must be slightly increased to 2.5 bar. Best results could be achieved by using an 8mm nozzle, a layer height of 4mm, and a speed of 10mm/s. The speed at 5mm/s was showing better results in quality and should be adjusted when printing complex structures. A comparably low flow rate was set to match the mixture due to its high-water content of only 0.1 (10%).

GGCH could be printed with a 6mm and 8mm nozzle, but with an air pressure of 2.5 bar. Speed and flow rate were set the same as for GG01.

For each mixture hollow cylinders with dimensions of 37.5mm in height and 75mm in diameter with a wall thickness of 8mm, and full cylinders with the same height and diameter were tested. For hollow cylinders a layer height of 4mm shows the best results with a tolerance of +2mm in wall thickness, resulting in a thickness of 10 mm instead of 8mm. Layer height must be adjusted for full cylindric samples to 6mm, otherwise, the cylinder diameter would expand horizontally with a tolerance of 20-30mm.

A very high-water release of GG01 was observed, which is why the mixture is adjusted as GG02. GG02 was well extrudable with a small increase of air pressure at 2.2 bar. A speed at 10mm/s and/or 5mm/s, flow rate of 0.1 (10%) and a layer height of 4mm. A comparison of GG01 and GG02 results is shown in Figure 7 visualizing the importance of water content in the mixture. High water content as in GG01 results in slight instability while printing,

but high deformation while growth process, and high shrinkage after drying process.



Figure 7. GG01 (top), GG02 (bottom) while printing, printed result, after drying

3.4 Growth of printed samples

GG01 and GG02 show both great results in the formation of aerial mycelium, but in G001 samples no growth was visible at the bottom part of the sample, because it was standing in 1cm high water. By removing water from its grow box, mycelium could then grow further, covering the whole sample. No such water release was taken place during the growth process of GG02 samples. GG01 and GG02 were turned around so that mycelium could grow on the surface touching the bottom on day 5/6 and were taken out of growing boxes on day 8. The mycelial outer layer with a thickness of approximately 0.5-1cm, could be seen by turning the sample 180 degrees.

GGCH did not show any growth within the course of 12 days and was not further investigated throughout the given time frame.

3.5 Drying process

All samples were baked at 85 degrees in a regular oven for 5 hours. Due to the strong deformation of GG01 during the growth and drying process, the samples result in an average diameter of 88mm despite the given diameter of 75mm. The average height of GG01 samples is 24mm after baking hence means a tolerance of -11mm. GG02 average di-

mensions after dyring process are 29mm in height with diameter of 77mm. GG02 mixture result in less deformation, but still shows high shrinkage. Printed GG02 hollow cylindric samples before growth and drying process were measured 86mm in diameter meaning the shrinkage in diameter considered 10.46%.GG02 single wall cylinders result in a final weight of 23.5g (before 125.3g) thus total weight loss of 81%. GG02 full solid cylinders result in a final weight of 66.3g (before 270.8) thus total weight loss of 75%.

3.6 Mechanical test

Three samples were tested for compression: a) humid single wall GG01 (HSW), b) dry single wall GG01 (DSW), c) double wall GG01 (DW). Compression tests were conducted by M. Hörning at IBBS using Zweck/Roell Z010. Due to samples uneven surface, the measurements wer calculated in between compression of 20%-40%. HSW has a Young's e-modulus of 338KPa < DSW with a result of 868KPa < DW of 1,44MPa. Results show that dried samples has a considerable higher strength compared to humid samples. As expected sample c) with 2 wall layers reached higher values. In comparison to Elsacker et. al (2019), DW has higher results than non-compressed samples ranging from 0,1MPa to 1,5MPa, whereas DW result in lower compared to pre-compressed molded samples with highest value of 2,3MPa [31]. However, samples from Elsacker et. al (2019) were measured as full solid cylinders compared to DW as hollow cylinder.

3.7 Architectural application

Additive manufactured MBC could be applied as facade paneling, interior wall division, or acoustic panels due to its lightweight properties. An important fact of the two-step extrusion process is the pre-compressed material with 2-2.5 bar. The density changes significantly in the presence of binding additive guar gum, which indicates better results in compressive structures. A structural lightweight application is possible e.g. column of a pavilion. The design results from a) material composition, b) fabrication method and area, and c) application. A column is chosen as a prototype and its goal is to preserve moisture content during the growth process enhancing mycelial growth on the outer surface. Parameters such as size, and number of edges define the base geometry. "Pockets" are generated by a solid body collision algorithm using Kangaroo Physics (Plug-In for GH, Rhino3D). These can be adjusted by parameters such as number of divisions of each polygon side, the radius of colliding points, and repulsion strength. Multiple variations (Figure 8.) can be generated and analyzed to fit the required angles, and radii for LDM printing, supporting mycelial growth. The base of a design variant is printed in a 1:1 scale with a 6mm nozzle at a speed of 5mm/s but must be stopped due to clogging issues after 1h:40mins. A nozzle diameter of 8mm is recommended.



Figure 8. Generated design possibilities



Figure 9. Printing of GG02 illar bottom

Discussion

The extrusion of MBCs for their possible application in the construction sector is achievable with GG02 paste. Compared to Modanloo et. al (2021), GG02 show less tolerance in printed results of only +2mm, but its printing speed is significantly lower. Elsacker et. al (2022) printed at higher speed with a smaller sized nozzle of 5mm using a substrate with smaller particle size and psyllium husk as binding agent. Although GG02 has a good printing quality and growth result, the paste composition can still be adjusted as the flow rate is compared to clay very low. The viscosity plays a major role affecting the flow rate and other printing paramters e.g. speed hence must be measured. Further GG mixtures needs to be tested observing the change in growth behaviour accordingly.

It should be highlighted, that the material is pre-compressed within the LDM process at 2-2.5 bar, wherefore the density of the composites should be measured for GG02 samples a) not pre-compressed, b) compressed with 2bar, c) after the growth phase, and d) after drying phase.

Since no framework for mechanical testing of MBCs is given, there is no reliable comparison with former projects possible. Further, MBC hollow cylinder samples should be compared: a) non -compressed, b) compressed molded, c) compressed molded with additives, and d) 3d printed with GG02 to evaluate different mechanical behavior concerning fabrication method and density.

Hydrophobicity was not tested accurately in this project and therefore not mentioned. The drying process is questionable due to drying the samples in an oven at 85 degrees, which has an irreversible effect on hydrophobins of GL. Hydrophobic tests of samples a) drying at room temperature, c) drying >40 degrees, or other non-temperated drying methods must be carried out.

Solid geometries are not recommended for LDM printing due to the long drying phase and high deformation during the growth process.

GGCH samples failed in this project, possible reasons can be that: a pH value of 3.5 due to dissolving chitosan in 10% acetic acid harms mycelial growth. Also chitosan with too high viscosity was purchased and may have not impregnated the fibers.

Acknowledgements

This bachelor thesis was carried out in the Department of Architecture and Urban Planning at IBK2 (Institute of Building Construction, Building Technologies and Design) in cooperation with the Department of Plant Biotechnology at IBBS (Institute of Biomaterials and Biomolecular Systems) under tutoring of Eliza Biala and supervision of Prof. M. Ostermann and Prof. A. G. Heyer.

I am grateful for the opportunity to freely choose this bachelor thesis and work in the environment of the IBK2 at Future Material Lab and I want to thank all of those who have contributed to this work, escpecially from IBBS for consultations of Prof. A. G. Heyer, Gisela Fritz and Johannes Hauser; SEM images of Prof. Schweikert; and mechanical tests of M. Hörning.

Lastly, I want to thank my family and friends who supported me throughout the past years, who has encouraged me to archieve my dreams and aims.

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