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Bending and Compressive Behavior of a Co-cultivated Mycelium-Bacteria Based Composite

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Abstract

This research investigates compressive and flexural behavior of a composite formed through a double binding process given by co-cultivation of a bacterial and fungal strain. Co-cultivation in this research refers to the simultaneous inoculation of the bacterial partner Sporosarcina Pasteurii and the fungal partner Ganoderma Lucidum into one composite. The paper describes the investigation of the impact of microbially induced calcium carbonate precipitation (MICP) as source of mineral substrate. The motile bacterial cells are using the fungal mycelium as a network of transportation, therethrough spreading an enzyme, which catalyses a chemical reaction, resulting in homogeneous creation of micro-scale calcium carbonate minerals attached to and in between mycelium hyphae. The research compares three compositions of substrate mixture and drying methods of co-cultivated species against a monocultivation of G. Lucidum and investigates the microbial morphology, and the influence of the double binding process on flexural and compressive strength. Through microscopic imaging, increased density of fungal growth in co-cultured specimen was observed. Additionally, higher mechanical properties in both bending and compression have been found in testing specimen with MICP as secondary binding process, with the highest flexural modulus of 6,94 MPa and compressive modulus of 7,27 MPa in pressed specimen.

Keywords: bacteria-fungi interaction, mycelium-bacteria based composite, co-cultivation, MICP, compressive behavior, flexural behavior, mechanical properties

1. Introduction

Global material use has risen to “more than three times over the last 50 years” and continues to increase by 2,5% yearly. In the published report “Global Resources Outlook 2024” by the International Resource Panel of the UN Environment Programme, the IRP urges for consequent change to avoid another 60% increase of material consumption from 2020 to 2060. With the built environment being one of the main consumers and contributors to further rising of material extraction and simultaneously culpable for 35% of global landfill through construction and demolition waste, desperate change is needed within architecture, engineering and construction (AEC) industry [1], [2]. In their 2023 report on “Building Materials and Climate: Constructing a New Future”, the UN Environment Programme challenge the use of raw materials for the built environment amongst those, while still stated as ongoing research to further survey all related emissions and consumptions, the growing of material through filamentous fungal organisms is mentioned as potential source of building materials [3]. The binding of loose ligno-cellulosic substrate by the vegetative network, mycelium, of filamentous fungi challenges the need for synthetic binders. Here a purely microbially produced form of binder combines particles into a firm solid. Additionally, the composition of purely organic nature of mycelium based composites (MBC), offers the potential to degrade and partly disintegrate over time at the end-of-use [4].

MBC have already proven successful acoustic insulation [5] or foam-like packaging material [6]. While synthetic binders can be exchanged through microbial ones, the challenge of load bearing capacity of such composites remains. Currently, basic research on improvement of mechanical properties of the material are ongoing, experimenting with different substrate compositions and post-processing methods [7], [8], [9]. This research investigates the mechanical properties of a composite material, formed through

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a co-cultivated and living community of bacteria and fungi. Each partner of this microbial community brings forward one method of substrate binding, presenting a double binding process within a single composite, as previously described in [10]. The two binding activities are catalysed by the bacterial partner *Sporosarcina Pasteurii* through bio-cementation and the mycelium, the vegetative network of the fungus *Ganoderma Lucidum*. The paper outlines the protocols of experiment setup and results for flexural and compressive strength test, comparing mechanical properties of the different compositions substrate compositions, post processing methods of the co-cultured community of microbes and the mono-cultured pure fungal composite. The experiments investigate if the co-cultivation affects negatively the flexural strength of the composite due to mineral particles and if compressive strength can be increased through the double binding process.

2. Background:

The bacteria used, S. Pasteurii is a soil borne bacteria utilised for research on self-healing concrete [11] and biocementation of loose soil [12] and solidification of soft materials [13]. A study has showed to increase the compressive strength of a paper waste based foam when undergoing MICP, decreasing deflection from a range between 12,7-19,8 to a range between 6,9-9,7 mm under a load of 800N for respectively uncalcified and triple calcification processes [14]. Another research has found that mechanical properties of rammed earth can be improved by introducing the bacteria to sandy soil if large and connected voids between the particles are possible and if the composite was cured at high humidity. If compression of the earthen material was too high, water filled voids were rarer, restricting cell mobility and confining them to the unconnected void [15]. However, while this method offers promising reinforcement of material, it is also linked to the release of ammonium during the transformation of urea for the activation of MICP, costly use of nutrient media and loss of cell movement when not fully immersed in liquid which reduce the microbe´s viability at larger scale [12], [15].

For mycelium based composites mainly compressive and flexural strength is researched [16]. Currently used to replace fossil-based foams, advances in strengthening mycelium-based composites and therethrough their feasibility in architecture are being undertaken. Amongst those are genetic modification of the organisms [17], methods of densification after fungal growth through either heat or cold pressing methods, flexural strength and modulus for three-point testing methods ranging from 0,06-0,29 MPa and 1-9 MPa without pressing, 0,21-0,24 MPa and 12-15 MPa after cold pressing and 0,62 – 0,87 MPa and 34-80MPa after heat pressing [7]. While these pressing methods add a step and more energy embodiment to the process, mechanical properties have also been investigated through substrate adjustments. Here one study adds between 1% and 5% of nanoclay (NC) to the composition and reports a similar flexural strength and flexural modulus for samples of different composition, all post-processed through heat-pressing at 30kN and 200degC for 1h. The flexural strength lies between 1,47 MPa (with NC) and 1,46 MPa (control) and flexural modulus of 0,19 GPa and 0,22 GPa respectively. In the same study, compressive properties were tested with the same substrate compositions, but with non-pressed samples. Compressive stiffness was increased to a negligible degree with values of the Compressive Young´s modulus ranging from 0,45 and 0,54 MPa (NC reinforced) and 0,34 MPa (control) [8]. Another study determined an increase of compressive strength and modulus when adding between 0 and 37,5% of natural reinforcement particle (NRP) of mineral nature to the growth substrate. On samples that have not been pressed, they measured compressive strength from 26 kPa to 127kPa (varying between fungal strains) and compressive modulus between 7,3 MPa and 30,3 MPa without NRP to the highest compressive strength of 508kPa and modulus of 48,5 MPa with 37,5% NRP [18]. Another method of sandwiching materials and integration of other materials to form anisotropic composites have been tested for compressive strength by Rigobello et al. [19]. Here different methods have been tested and compared to a pure mycelium control sample with a mean Young´s modulus of 1,79 MPa. Jacketing with a hessian textile and adding of rattan or reed fibres in parallel or coaxially to the load with overall results of Young´s Modulus for compression ranging from 0,66 to 9,21 MPa with the highest value for samples with reed in a coaxial position to the load.

This research relies on the co-cultivation and the therethrough enabled method of bacterial mobility throughout the organic substrate through the fungal highway method. This microbial interaction between

S. Pasteurii and *G. Lucidum*, allowing for a double binding process of the composite has been previously described in [10].

3. Method:

Lab environment and microbial strains:

The research has been developed in a DIY lab environment, where sterile environment was given through a self-built laminar flow hood and sterilisation of tools and media in a pressure cooker with maximum 118,6 degC and 95kPa pressure for 60 minutes. Sporosarcina Pasteurii, a soil borne bacteria which produces urease enzyme and catalyses the microbial precipitation of calcium carbonate (MICP) [20] and Ganoderma Lucidum, a white rod, filamentous and ligno-cellulosic decaying fungus are used in this research.

Fungal Strain pre-culture:

G. Lucidum was first grown on a CYM-Agar medium [21], which after 5 days was used to inoculate rye berries. The rye berries were first soaked for around 3h in warm tap water then drained and sterilised in the pressure cooker and left until completely cooled down. The rye was inoculated with the full contents of the 90mm diameter agar plate that was completely covered with G. Lucidum and inside a microfiltration bag (purchased from [22]) placed inside an incubator at 28degC and in darkness.

After 5 days and visibly full covering of the berries by the fungus in white mycelium, forming one solid package, the rye berries were used to inoculate beech wood sawdust. The sawdust was purchased from Dansk Træmel, which is sold for the smoking of food. It is free of other substrates and untreated, with grain sized varying in length from 1-3mm. The wood was prepared with 70% volume/weight tap water and then sterilized in a closed high temperature resistant polypropylene bag. After the wet substrate had fully cooled down, the fungal rye berries were mixed into the mass. Everything was homogeneously mixed by hand inside of a micro-filtration bag and closed with a polypropylene gastight clamp for bags. The inoculated wood mass was then stored in an incubator for 20 days at 28degC in darkness.

Bacterial strain pre-culture:

S. Pasteurii was transferred from its storage medium Tryptic Soy Agar with a 2% urea concentration into the same medium without agar, providing a liquid medium. The transferred culture is grown overnight in constant shaking and at room temperature, resulting in a liquid pre-culture to be used for inoculation of the composite.

Preparation of testing specimens:

Table 1: *Composite mixtures*: (1) G. Lucidum, Beech Wood 70% v/w tap water; (2) G. Lucidum, S. Pasteurii, Beech Wood, 70% v/w tap water; (3) G. Lucidum, S. Pasteurii, Beech Wood, 70% v/w tap water with 2% urea concentration; (4)) G. Lucidum, S. Pasteurii, Beech Wood, 70% v/w tap water with 2% urea and 1% Calcium Chloride ($CaCl_2$)concentration; *dimensions:* Mould A: 160x40x40mm; Mould B: 160x40x60mm; *MICP activation:* (1) no MICP expected; (2) (3) $CaCl_2$ bath; (4) $CaCl_2$ included in the composite mixture; *Drying:* (a) Low temperature – 40degC 24h; (b) High Temperature – 70degC 24h; (c) Heat pressed 200degC 15min. *It was not possible to keep the mould at constant temperature while pressing after the pressing duration of 15 min, the temperature of the mould initially at 200degC had decreased to about 80degC.

Drying treatment / mixture	AD - Low temperature Dried 40 degC	OD - High temperature Dried	P - Heat pressed 200 degC*
1 – GL – BW	Mould A – no $CaCl_2$ bath	Mould A – no $CaCl_2$ bath	Mould B – no $CaCl_2$ bath
2 – GL – SP – BW	Mould A –$CaCl_2$ bath	Mould A –$CaCl_2$ bath	Mould B –$CaCl_2$ bath
3 – GL – SP – BW – UR	Mould A –$CaCl_2$ bath	Mould A –$CaCl_2$ bath	Mould B –$CaCl_2$ bath
4 – GL – SP – BW- UR – $CaCl_2$	Mould A – no $CaCl_2$ bath	Mould A – no $CaCl_2$ bath	Mould B – no $CaCl_2$ bath

For the mechanical properties testing four composite mixtures were prepared and three different sample drying methods were used. Each sample was replicated four times, of which three were chosen for testing. This was decided to account for either contamination of samples or mechanical accidents like breaking while demoulding. The end size of the samples to undergo testing were 160x40x40mm. Two sets of moulds were created, each accumulating the four replicates, mould A provided the dimensions previously stated while mould B was 160x40x60mm to be compressed to 40mm height. The composite mixture was prepared according to the following table, preparing approximately 80g of dry material per sample. For all samples tap water was used and sterilised in sealable containers in the pressure cooker. After cooling down below 40degC microfiltered (0,2 µm through syringe filtration), premixed solutions of 40% urea and 20% Calcium Chloride ($CaCl_2$) were added respectively to reach 2% urea and 1% $CaCl_2$ concentrations. The pre-grown G. Lucidum spawn was blended to a homogeneous flaky substrate and each composition was homogenously mixed by hand in a large container.

Table 2: Ingredient compositions for different samples.

1 – GL – BW	2 – GL – SP – BW	3 – GL – SP – BW – UR	4 – GL – SP – BW- UR – $CaCl_2$
860g Beech sawdust	860g Beech sawdust	860g Beech sawdust	860g Beech sawdust
672g Tap water	672g Tap water	672g Tap water 2% urea	672g Tap water 2% urea, 1% $CaCl_2$
100g G. Lucidum spawn	100g G. Lucidum spawn	100g G. Lucidum spawn	100g G. Lucidum spawn
	672µL S. Pasteurii preculture	672µL S. Pasteurii preculture	672µL S. Pasteurii preculture

The moulds were sprayed and wiped with 70% ethanol, then placed in microfilter bags and filled with the inoculated substrate mixtures. Using a flat piece of wood in the same dimensions of the mould, light pressure was applied manually until light resistance was felt throughout the sample. The microfilter bags were closed either with a gastight clamp or with a piece of tape, sealing the full length of the opening. Then they were carefully placed in the incubator chamber, set at 28degC in darkness for 8 days. In non-sterile conditions, only working surfaces and gloves wiped with 70% ethanol solution, the samples were demoulded, by gently cutting lose the sides and pushing the fragile composites out of the mould. Air exposed sides of the samples had grown far more mycelium then the ones touching the mould, additionally those less covered in mycelium showed loss of humidity. Sterile tap water was sprayed onto the samples before placing them in alcohol wiped PP microfiltration boxes (purchased from [23]). The closed boxes were placed back into the incubator at 28degC and in darkness for 6 days. During this time, consumption of humidity was noticed and each day dried spots were sprayed with sterile tap water if necessary to improve the growth and full coverage of the samples by G. Lucidum and with the fungal network, the bacteria S. Pasteurii. After another 4 days of growth, it was noticeable that the bottom and top side of the sample had been growing the fastest, forming a completely closed outer skin of mycelium. The samples were therefore rotated 90deg around the longitudinal axis to increase growth on the remaining two long sides and achieve a homogenous and closed mycelium outer skin.

On the 6th day after unmoulding (14 days of total growth), set 2 (GL-SP-BW) and 3 (GL-SP-BW-UR) were submerged in a calcium rich bath to activate the biocementation process. The liquid for set 2 was sterile tap water with a 2% urea and 1% $CaCl_2$ concentration. Set 3 was sterile tap water with 1% $CaCl_2$ concentration, where the additives were mixed in after cooling down of the water. The samples stayed in the bath for 15 min, weighted with sand buckets to ensure full submersion. All the samples were then stored in a drying chamber at 40 degC with air ventilation on for 3h. This full cycle was repeated 3 times. After the last bath, the 4 low temperature (AD) samples of each composition were stored in the drying chamber at 40degC, with ventilation and in darkness for 24h. Here, the temperature of 40degC was chosen to dry at low temperature, which increases the drying speed compared to room temperature but decreases the energy consumption compared to high temperature drying and is below the inactivation temperature for the chosen fungus [24], [25]. 4 of each composition were placed into a 70degC (OD) chamber, where they stayed in darkness for 24h, until complete dehumidification. This temperature is above the 60degC limit, after which only a small number of specimen have been found to be

reactivatable [26], however reducing energy use by keeping the temperature low. The third drying method pressed the specimen (P), compressing by 30% of the initial height (60mm to 40mm). As no heat press was available for this testing series, a custom steel mould was built to fit four specimen. The mould was composed of three pieces, one bottom sheet, one centre part, with 60mm wall height where the samples were placed in between and a last piece to press down. All three parts were heated in an oven until reaching 200degC, then the samples were fitted into those, and pressed down to 35mm height, compressing with 1 ton. After 15min the compressive force was released and the samples sprang back to around 40mm. During demoulding the samples of composition 4 were damaged and therefore eliminated from testing. All pressed samples were also added to the 70degC chamber for 24h and removed when all samples were completely dehumidified.

Mechanical Properties Method:

Of each set three specimen were chosen for testing at the Technological Institute according to the test method *DS/EN 1015-11:2019 Methods of test for mortar for masonry – Part 11: Determination of flexural and compressive strength of hardened mortar* [27] using a Shimadzu AGS-J testing setup [fig 1]

Figure 1 Testing setup for (left) flexural tests, (right) compressive tests

Determination of Flexural Strength and Modulus:

Flexural tests were executed using a 3-point method, with two supporting steel rollers (50mm length and 10mm diameter) spaced with 100mm in between and a third roller in the same dimensions placed in the centre between those. All tests except the first specimen, which was tested at 1mm/min, were testes with a 5 mm/min load rate and experiments were ended at failure or stopped when the samples were too flexible and the centre touched the bottom part of the testing set-up. This was mainly seen in the low-temperature dried samples and was possibly caused by remaining humidity inside the composite. Flexural Strength was calculated according to DS/EN 1015-11:2019 [27]:

$$f = 1{,}5\,\frac{\mathrm{F}\cdot\mathrm{l}}{\mathrm{b}\cdot\mathrm{d}^2}$$

Where F is the maximum load applied to the specimen [N], l is the distance between the centres of the support rollers [mm], b is the width of specimen [mm], d is the depth of the specimen [mm]. As no formula for the determination of the flexural modulus is stated in this first standard, the one from ISO *16978:2003 Wood-based panels — Determination of modulus of elasticity in bending and of bending strength* [28] was applied.

$$E = \frac{(F_2 - F_1)\cdot \mathrm{l}^3}{4\cdot \mathrm{b}\cdot \mathrm{d}^3\cdot (a_2 - a_1)}$$

Where l, b and d are the same as previously defined. $F_2 - F_1$ is the increment of lead on the first straight line portion of the load-deflection curve and $a_2 - a_1$ is the increment of deflection corresponding to $F_2 - F_1$.

Determination of Compressive Strength and Modulus:

Uni-axial compression tests were performed using two 50x50mm steel plates as pressing and supporting structure for the specimen. While 36 samples were broken in the flexural strength tests, both sides of the broken samples were used (72 specimen) and were tested for compressive strength with a load rate of 10 (mm/min). As failure was not detectable through a numerically identified breaking point,

compression was tested in proportion to strain. The tests were ended when reaching a material strain of approximately 0,5 (measure as linear deflection of 20mm) or the maximum load capacity (10kN) of the machine setup (occurring sometimes just before reaching a strain of 0,5). Compressive strength for each strain recorded is calculated according to DS/EN DS/EN 1015-11:2019:

$$f_c = \frac{F}{A}$$

f_c being the stress, F the load [N] and A the cross-sectional area of the bearing plate, perpendicular to the load. The compressive modulus is determined from the slope of the straight-line portion of the stress-strain curve.

Density:

Is calculated from weight and dimensions after the drying process.

Morphology:

The outer surface of (AD) and (OD) specimen of composition 1 and 4 had a white velvety outer skin. (AD) and (OD) of composition 2 and 3, which underwent several solution baths, were rough and grainy. The heat pressed specimen resembled wood particle board, with a smooth and homogeneously bound outer surface. While composition 2 and 3 showed collapsed hyphae on the inside and small distributed formations of $CaCO_3$, composition 4 showed the densest distribution of fungal hyphae on the interior of the samples, comparing to all, even the control monocultured samples.

When analysing the exterior of (OD) of composition 1 with a digital microscope skin at approximately 800x magnification looks very dense and spatial. The interior, although seemingly very little mycelium has grown at bare eye, shows that hyphae has spread in low density all throughout the specimen [fig 2].

Figure 2 1OD (a) interior – (b) exterior

In composition 2 the hyphae has collapsed onto itself and is holding $CaCO_3$ particles in between and on top of the material. Instead of spatial distribution of loose strands, the hyphae in these specimen has formed a more dense but thinner layer around the composite [fig 3].

Figure 3 (a) 2AD exterior - (b) (c) 2OD interior

The interior of composition 3 shows a much denser distribution of the fungus within the composite and an increased formation of $CaCO_3$ minerals. The hyphae look partially like the skin on the outer side of the specimen, collapsed and attached to other strands, however, it is less collapsed and shows a more

spatial configuration. Attached to the hyphae and to the wood particles are mineral assemblies of $CaCO_3$, which are in much higher density and larger assemblies as seen in specimens of composition 2 [fig 4].

Figure 4 3OD (a) (b) (c) interior

Specimen of composition 4 show the most abundant mycelium growth inside and outside. $CaCO_3$ particle can be found mostly inside the sample. While less visible due to non-collapsed hyphae, small and large formations of $CaCO_3$ have formed and attached in between the hyphae and onto the wood particles [fig 5].

Figure 5 4AD (a) exterior - (b) ((c) interior

4. Results:

In this research it has been found that the co-cultivation of the two microbes and thus the creation of a double binding process have led to higher flexural properties. This is especially visible in composition 2 and 3, which have been submerged to the liquid bath to enhance the biocementation reaction and in high temperature and pressing methods of drying. Values in the category of low temperature drying (AD) for those bath activated specimen are listed in the list of results (table 3) and graphs, but have been declared unreliable due to remaining interior humidity at start of testing process. Additionally, pressed (P) specimen of composition 4 were left out of the experiment due to damage to pieces while demolding.

Flexural properties:

The flexural strength of the tested specimen ranges from (AD) 0,26 – 0,38 MPa, (OD) 0,24 – 0,27 MPa and (P) 0,26 – 0,32 MPa and flexural moduli from (AD) 1.97 – 2,61 MPa, (OD) 1,14 – 5,00 MPa and (P) 0,99 – 6,94 MPa [fig 6]. When comparing the flexural moduli, it becomes visible, that all (P) values are higher when in co-cultivation. In composition 2 and 3 (OD) specimen also showed a higher modulus than in the control sample. The double-binding method through both microbes seems to contribute to an improved bending stiffness of the composite material.

Table 3: Values from flexural and compressive testing.

Label	Dry Density [kg/m³]	Flexural Strength [MPa]	Flexural Modulus [MPa]	Compressive Strength at strain [MPa]	Strain [%]	Compressive Modulus [MPa]
1-GL-BW (AD)	268,31 ± 11,14	0,35 ± 0,10	2.08 ± 0.16	0,89 ± 0,22	0,51 ± 0,01	1,10 ± 0,14
2-GL-BW (OD)	228,83 ± 12,30	0,24 ± 0,06	1.14 ± 0.19	0,81 ± 0,08	0,50 ± 0,02	0,89 ± 0,15
3-GL-BW (P)	468,77 ± 5,02	0,32 ± 0,06	0.99 ± 0.15	1,64 ± 0,72	0,48 ± 0,01	2,60 ± 1,29
4-GL-SP-BW (AD)	321,85 ± 15,57	0,34 ± 0,01	2.34 ± 0.41	1,27 ± 0,61	0,53 ± 0,05	1,18 ± 0,14

5-GL-SP-BW (OD)	255,39 ± 15,07	0,27 ± 0,04	4.54 ± 1.11	0,94 ± 0,17	0,53 ± 0,02	1,12 ± 0,25
6-GL-SP-BW (P)	326,95 ± 2,43	0,26 ± 0,04	6.94 ± 0.62	5,91 ± 0,21	0,48 ± 0,00	7,26 ± 0,46
7-GL-SP-BW-UR (AD)	409,58 ± 27,72	0,26 ± 0,04	1.97 ± 0.28	0,92 ± 0,12	0,49 ± 0,01	1,27 ± 0,23
8-GL-SP-BW-UR (OD)	268,40 ± 6,72	0,27 ± 0,00	5.00 ± 1.64	1,39 ± 0,79	0,54 ± 0,04	1,26 ± 0,48
9-GL-SP-BW-UR (P)	345,74 ± 8,33	0,29 ± 0,10	5.64 ± 0.13	4,80 ± 0,65	0,52 ± 0,01	5,68 ± 1,27
10-GL-SP-BW-UR-$CaCl_2$ (AD)	256,61 ± 12,30	0,38 ± 0,03	2.61 ± 0.87	1,203 ± 0,44	0,53 ± 0,07	1,12 ± 0,21
11-GL-SP-BW-UR-$CaCl_2$ (OD)	234,86 ± 11,22	0,37 ± 0,00	2.21 ± 0.41	0,859 ± 0,13	0,51 ± 0,03	1,01 ± 0,27
12-GL-SP-BW-UR-$CaCl_2$ (P)	396.76 ± 73,15	NA	NA	NA	NA	NA

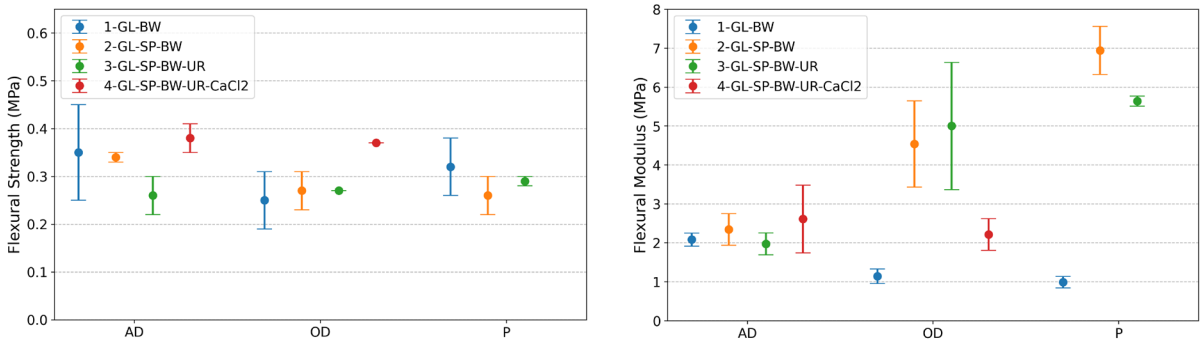

Figure 6 (left) Flexural Strength in MPa, (right) Flexural Modulus in MPa

Compressive properties:

At approximately 20 mm deflection, load was measured between 972 N up to 10512 N. Compressive strength is determined at that fixed highest deflection with values for (AD) 0,89 - 1,27MPa, (OD) 0,81 - 1,39 MPa and (P) 1,64 - 5,91 MPa. From the linear portion of the stress strain curve fig[7], the slope was used to determine the compressive modulus with values ranging from (AD) 1,09 - 1,23 MPa, (OD) 0,84 - 1,38 MPa and (P) 2,26 - 7,27 MPa. The lowest value for all drying methods can always be found in the control, monocultured mycelium based composites. While, the moduli are increasing with MICP reinforcement, this increase is negligible in (AD) and (OD). Only in our (P) specimen, the moduli are varying between more significant values within the different compositions. When plotting the compressive moduli to density in the Ashby chart fig[8], their compressive moduli are within the category of foams, while their density is within the lower end of natural materials.

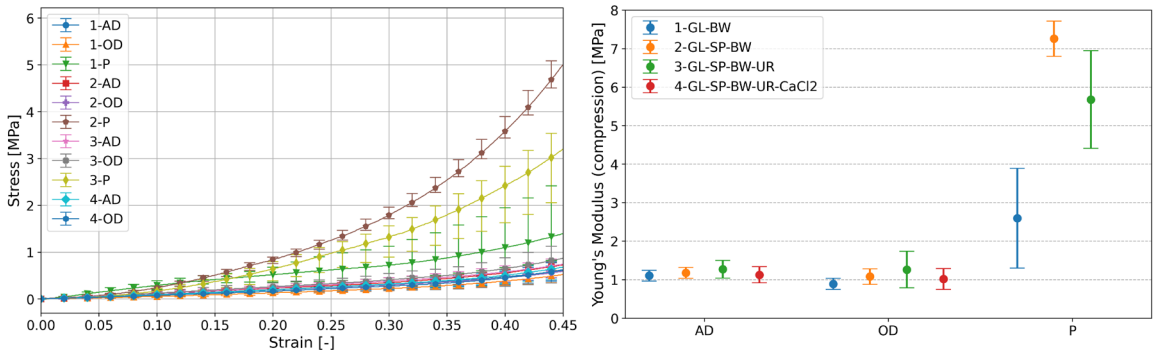

Figure 7 (left) Complete measured Compressive Stress-Strain diagram, (right) Compressive Modulus in MPa

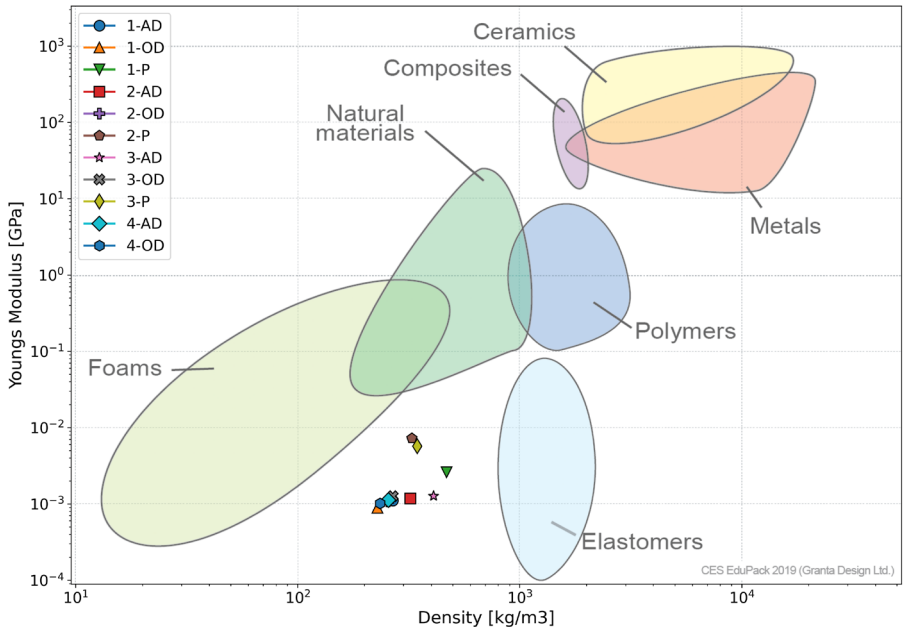

Figure 8 Compressive Modulus Ashby Plot, over chart created using CES EduPack 2019, ANSYS Granta © 2020 Granta Design

5. Discussion:

Experiment Limitations:

While the microscopic imaging of a series of spots within and outside of the specimen informs about the distribution and size of MICP formations, a missing indicator in this experiment is the quantification of mineral particle development. This could further inform the relationship to mechanical properties. Additionally, the manual filling and pressing of moulds with inoculated substrate, leads to minimal inconsistencies in material density, which in return could affect growth and testing results.

Test results of (AD) of composition 2 and 3 have been affected by insufficient drying time. While these underwent the bathing technique, the timespan of drying was not long enough at 40degC to completely dry out the specimen towards the core. This affects the weight and therefore the density calculation, but potentially also the testing results, which often did not fail but instead touched the lower part of the testing setup.

Morphology:

S. Pasteurii is known to have a faster growth rate and higher biomineralization activity when in urea rich environments [29]. This has also been noticed in compositions 3 and 4 in this testing series. When examining the interior morphology of the composite, it became clear that while they all showed growth of both fungal and bacterial partners, a much higher growth rate could have been achieved. This is assumed to be connected to a loss of humidity when growing of the samples in the although coated, wooden moulds. After unmoulding, each sample checked at a daily basis for lack of exterior humidity and sprayed with sterile tap water as necessary. However, it seems that this extra humidity did not fully sink in to the centre part of the samples and as visible fungal growth took place on the outer skin, less dense growth took place within them. Additionally, the samples could have stayed in the incubator for at least 7 days longer to wait for a more abundant growth of the microbes. While this applies to all testing sets, compositions with urea (2,5%) dissolved in the water content when inoculation of the microbes and in co-cultivation show a visibly more abundant fungal growth. While composition 4 also containing $CaCl_2$ (1%), which enabled MICP to take place during composite growth, a higher development is assumed if the sprayed water during the growth process would have contained urea and $CaCl_2$. Here $CaCO_3$ particle assemblies have formed mainly in the core of the samples and a potential increase of strength is assumed at higher calcification rate on the exterior of the samples.

Flexural behavior:

Almost all invalid flexural tests due to bending until touching the lower part of the setup occurred in (AD) dried specimen. In this research it has been found that the double binding process of the two microbes increases flexural behaviour in both categories (OD) and (P). This is assumed to be linked to the increased density and reinforcement within and of the skin of the composite, the interior mechanical jamming of the mineral particles and a higher density of fungal growth when in co-cultivation.

Compressive strength:

In this research, we have found that while different compositions only lead to a negligible increase of compressive moduli when in co-culture compared to the control mono-culture composites when dried at low or high temperature. A much larger difference in compressive modulus, can be seen between compositions when specimen have been pressed. As we did not have access to an actively heated press with constant temperature a DIY version was developed. For this, a custom steel mould is heated in an oven until 200degC and then used to press the samples within a hydraulic press. However, as the temperature drops within minutes during pressing, due to heat dissipation, this pressing method lies in between previously stated cold or heat pressed techniques. We expect that only the outer skin has reached 200degC for a short time whereas the core did not reach this high temperature, due to the volume of the samples. This pressing method produced samples with an outer fibre board like appearance, where the layers of the mycelium are densified and lignin of the sawdust softened and therethrough formed a binding action. This is reported to happen at temperatures around 160degC [30]. The increased compressive behaviour is expected to be related to the mechanical jamming and densification of the additional MICP reinforcement, decreasing void spaces within the composite.

Future investigations will study the differences of cold and heat pressing for this composite, examining if heat pressing is a necessary step. As constant heating adds to the energy consumption during production and leads to deactivation of the microbes, it could be questioned if cold-pressing and improvement of bonding through fungal and bacterial activity can be a sufficient technique for an increase of compressive behaviour. Additionally, it needs to be verified if a fully enclosing skin would affect the tests, compared to the specimen used in this experiment. The specimen to test the compressive behaviour of the composite were taken from the already halved pieces from the flexural bending tests.

6. Conclusion:

This research has examined the flexural and compressive strength of a co-cultivated composite material. While mycelium-based composites are currently heavily studied for application in architecture, low compressive strength is still presenting a major challenge. Although the testing samples in this research have not grown as abundantly as they could have, due to time limitation and non-homogeneous distribution of humidity within the samples, the compressive strength at maximum 50% deflection and compressive modulus with maximum values of 5,91 MPa and 7,27 MPa, measured for (P) specimen, are relatively high compared to state-of-the-art research. Comparing the MICP reinforced co-cultivated samples to monocultured pure mycelium compositions, the values have been found to increase both in compression and in bending stiffness. The overall highest values have been found in pressed and co-cultivated specimen.

The sharing of space by the bacterial and fungal partner additionally eliminates the necessity for high grade nutrient media for the bacterial partner in the composite and drastically decreases the economic impact for this composite. Additionally, the enhanced density of fungal growth, the movement of bacterial cells over the fungal network and the increased mechanical values demonstrate a promising potential to be explored and produced at larger scale at similar cost and labour but improved properties when compared to monocultured mycelium based composites. While this method needs to be further studied, this paper has contributed with new data on mycelium based composites and presented a new study on microbial co-cultivation for enhanced mechanical properties of a microbially formed composite for architectural application.

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References

[1] I. R. P. United Nations Environment Programme, 'Global Resources Outlook 2024 - Bend the trend: Pathways to a Liveable Planet as Resource Use Spikes', Feb. 2024, [Online]. Available: https://wedocs.unep.org/20.500.11822/44901

[2] K. Kabirifar, M. Mojtahedi, C. Wang, and V. Tam, 'Construction and demolition waste management contributing factors coupled with reduce, reuse, and recycle strategies for effective waste management: A review', *Journal of Cleaner Production*, vol. 263, p. 121265, Apr. 2020, doi: 10.1016/j.jclepro.2020.121265.

[3] Y. C. for E. + A. United Nations Environment Programme, 'Building Materials and the Climate: Constructing a New Future', Sep. 2023, [Online]. Available: https://wedocs.unep.org/20.500.11822/43293

[4] A. Van Wylick, E. Elsacker, L. Yap, E. Peeters, and L. De Laet, *Mycelium Composites and their Biodegradability: An Exploration on the Disintegration of Mycelium-Based Materials in Soil*. 2022. doi: 10.4028/www.scientific.net/CTA.1.652.

[5] Mogu, 'Mogu Wave', mogu. Accessed: May 29, 2023. [Online]. Available: https://mogu.bio/acoustic-collection/mogu-wave/

[6] 'Compostable Packaging - Earth-friendly Styrofoam® Alternative | Ecovative'. Accessed: Mar. 18, 2024. [Online]. Available: https://www.ecovative.com/pages/packaging

[7] F. V. W. Appels *et al.*, 'Fabrication factors influencing mechanical, moisture- and water-related properties of mycelium-based composites', *Materials & Design*, vol. 161, pp. 64–71, Jan. 2019, doi: 10.1016/j.matdes.2018.11.027.

[8] E. Elsacker, L. De Laet, and E. Peeters, 'Functional Grading of Mycelium Materials with Inorganic Particles: The Effect of Nanoclay on the Biological, Chemical and Mechanical Properties', *Biomimetics*, vol. 7, no. 2, Art. no. 2, Jun. 2022, doi: 10.3390/biomimetics7020057.

[9] A. Ghazvinian and B. Gürsoy, 'Mycelium-Based Composite Graded Materials: Assessing the Effects of Time and Substrate Mixture on Mechanical Properties', *Biomimetics*, vol. 7, no. 2, Art. no. 2, Jun. 2022, doi: 10.3390/biomimetics7020048.

[10] L. H. Kieffer, 'Bacteria-Fungi Interaction for Material Formation', *Research Directions: Biotechnology Design*, Apr. 2024.

[11] H. M. Jonkers, 'Limestone-producing Bacteria: Self-healing Concrete', in *Cultivated building materials: industrialized natural resources for architecture and construction*, D. Hebel and F. Heisel, Eds., Basel ; Boston: Birkhäuser, 2017.

[12] A. I. Omoregie, E. A. Palombo, and P. M. Nissom, 'Bioprecipitation of calcium carbonate mediated by ureolysis: A review', *Environmental Engineering Research*, vol. 26, no. 6, 2021, doi: 10.4491/eer.2020.379.

[13] B. Beyer, D. Suárez, and N. Palz, 'Microbiologically Activated Knitted Composites - Reimagining a column for the 21st century', in *Proceedings of the 37th eCAADe and 23rd SIGraDi Conference*, Porto, Sep. 2019. doi: https://doi.org/10.52842/conf.ecaade.2019.2.541.

[14] A. Mosse, V. Rennie, Q. Poudoulec, and D. Suárez Zamora, 'Paper waste grown as a biocalcified foam: perspectives from a bacterial and design viewpoint'. Accessed: Mar. 21, 2024. [Online]. Available: https://www.researchgate.net/publication/377315575_Paper_waste_grown_as_a_biocalcified_foam_perspectives_from_a_bacterial_and_design_viewpoint

[15] E. Bernat, L. Gil, C. Escrig, J. Barbé, and M. Cortés, 'Effect of Sporosarcina Pasteurii on the strength properties of compressed earth specimens', *Materiales de Construcción*, vol. 68, p. 143, Feb. 2018, doi: 10.3989/mc.2018.12316.

[16] A. Rigobello, C. Colmo, and P. Ayres, 'Effect of Composition Strategies on Mycelium-Based Composites Flexural Behaviour', *Biomimetics*, vol. 7, no. 2, Art. no. 2, Jun. 2022, doi: 10.3390/biomimetics7020053.

[17] F. V. W. Appels, J. Dijksterhuis, C. E. Lukasiewicz, K. M. B. Jansen, H. A. B. Wösten, and P. Krijgsheld, 'Hydrophobin gene deletion and environmental growth conditions impact mechanical properties of mycelium by affecting the density of the material', *Sci Rep*, vol. 8, no. 1, p. 4703, Mar. 2018, doi: 10.1038/s41598-018-23171-2.
[18] L. Gou, S. Li, J. Yin, T. Li, and X. Liu, 'Morphological and physico-mechanical properties of mycelium biocomposites with natural reinforcement particles', *Construction and Building Materials*, vol. 304, p. 124656, Oct. 2021, doi: 10.1016/j.conbuildmat.2021.124656.
[19] A. Rigobello and P. Ayres, 'Compressive behaviour of anisotropic mycelium-based composites', *Sci Rep*, vol. 12, no. 1, p. 6846, Apr. 2022, doi: 10.1038/s41598-022-10930-5.
[20] J. Qiu, S. Cook, W. V. Srubar, M. H. Hubler, J. Artier, and J. C. Cameron, 'Engineering living building materials for enhanced bacterial viability and mechanical properties', *iScience*, vol. 24, no. 2, p. 102083, Jan. 2021, doi: 10.1016/j.isci.2021.102083.
[21] ATTC Medium 987, 'Search'. Accessed: Jun. 27, 2024. [Online]. Available: https://www.atcc.org/search
[22] 'Home', MycoGenetics Pilz-Shop. Accessed: Mar. 19, 2024. [Online]. Available: http://www.pilzzucht-shop.de/
[23] 'Mushroom Shop - Tyroler Glückspilze'. Accessed: Mar. 20, 2024. [Online]. Available: https://gluckspilze.com/home
[24] H. Vašatko, L. Gosch, J. Jauk, and M. Stavric, 'Basic Research of Material Properties of Mycelium-Based Composites', *Biomimetics*, vol. 7, no. 2, Art. no. 2, Jun. 2022, doi: 10.3390/biomimetics7020051.
[25] I. S. Santos, B. L. Nascimento, R. H. Marino, E. M. Sussuchi, M. P. Matos, and S. Griza, 'Influence of drying heat treatments on the mechanical behavior and physico-chemical properties of mycelial biocomposite', *Composites Part B: Engineering*, vol. 217, p. 108870, Jul. 2021, doi: 10.1016/j.compositesb.2021.108870.
[26] E. Elsacker, M. Zhang, and M. Dade-Robertson, 'Fungal Engineered Living Materials: The Viability of Pure Mycelium Materials with Self-Healing Functionalities', *Advanced Functional Materials*, vol. 33, no. 29, p. 2301875, 2023, doi: 10.1002/adfm.202301875.
[27] 'DS/EN 1015-11:2019'. Accessed: Apr. 12, 2024. [Online]. Available: https://webshop.ds.dk/standard/M319511/history
[28] E. Standards, 'DIN EN 310', https://www.en-standard.eu. Accessed: Jul. 01, 2024. [Online]. Available: https://www.en-standard.eu/din-en-310-wood-based-panels-determination-of-modulus-of-elasticity-in-bending-and-of-bending-strength-german-version-en-310-1993/
[29] L. Ma, A.-P. Pang, Y. Luo, X. Lu, and F. Lin, 'Beneficial factors for biomineralization by ureolytic bacterium Sporosarcina pasteurii', *Microbial Cell Factories*, vol. 19, no. 1, p. 12, Jan. 2020, doi: 10.1186/s12934-020-1281-z.
[30] X. Y. Chan, N. Saeidi, A. Javadian, D. E. Hebel, and M. Gupta, 'Mechanical properties of dense mycelium-bound composites under accelerated tropical weathering conditions', *Sci Rep*, vol. 11, no. 1, Art. no. 1, Nov. 2021, doi: 10.1038/s41598-021-01598-4.