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Fungal Based Biocomposite for Habitat Structures on the Moon and Mars

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Abstract

One of the key capabilities for long term human exploration missions beyond low Earth orbit is a suitable technology for in-situ resource utilization (ISRU). Indigenous or locally cultivated resources lower the mass and volume of payload that needs to be brought from Earth, and as a result decrease the costs. Therefore, an efficient trade-off space has to be created between robotic ISRU systems brought from Earth, and type of materials used in the process to increase the long term mission sustainability.

The objective of the current study is to investigate the production process and feasibility of fungal based biocomposite material for habitat structures on the Moon and Mars, using automated additive construction technology. Previous studies have shown that certain types of fungi are able to survive in extreme environmental conditions. Experiments with γ-radiation in Co-60 facility at ESTEC showed that the chosen model organism, *Schizophyllum commune,* is able to survive in 20 Gy and 200 Gy dose levels, with the ~30% of colony forming units at 200 Gy. The lower levels of gravity will also not affect the growth of the fungus. Experiments with the random positioning machine (RPM) showed that *Schizophyllum commune* 227 grows even faster in simulated microgravity conditions than at 1G.

The proposed production process of the biocomposite on the Moon or Mars would require cultivating the mycelium of *Schizophyllum commune* (SC) in-situ from a minimum amount of starter culture brought from Earth. This would be combined with locally grown *Azolla filiculoides* (AF), an aquatic fern, with the ability to rapidly increase its biomass growing on water, while fixing nitrogen and carbon directly from atmosphere. For an additive construction experiment with a 6-axis robotic arm a mixture of SC mycelium, AF, water and psyllium was used to generate an extractable paste through a nozzle system to fabricate a number of prototypes with different print parameters.

Keywords: biomaterials, biocomposite, fungi, mycelium, space architecture, in situ manufacturing, robotic manufacturing, additive manufacturing, 3D printing

Acronyms/Abbreviations
ACT - Advanced Concepts Team
ESA – European Space Agency
ISRU – Is situ resource utilization
SC - *Schizophyllum commune*
PO - *Pleurotus ostreatus*
TM - *Trametes multicolor*
AF - *Azolla filiculoides*
SCMM - *Schizophyllum commune* grown on minimal medium
CFU - Colony forming units
RPM – Random positioning machine

1. Introduction

In order to increase the long-term mission sustainability it is necessary to use local resources to produce consumables, such as oxygen and water, and materials for construction. The value of an in situ resource utilization (ISRU) system is highest when the ratio between the mass of materials produced by the system and mass of the system itself, which has to be transported from Earth, is large. To that end, the investment in ISRU includes the costs of (a) prospecting to locate and validate the accessibility of indigenous resources, (b) developing and demonstrating capabilities to extract indigenous resources, (c) developing capabilities for processing indigenous resources to convert them to needed products, and (d) any ancillary requirements specifically dictated by use of ISRU [1].

To date many studies have shown that there are different resources available in the lunar and Martian regolith, which can be used for, for example, the production of surface habitats and infrastructure by various additive manufacturing technologies [2][3][4][5][6][7][8].

Additive manufacturing technology is a very promising technique for utilizing in situ resources on the Moon and Mars. However, when using indigenous resources, it is important to consider the investments needed for (a) locating and validating the accessibility of indigenous resources, and (b) developing and demonstrating capabilities to extract indigenous resources, as mentioned before [1]. To bypass these requirements, in situ manufactured biocomposites might offer a cost effective alternative for the local construction materials. This would require transporting a minimal amounts (i.e. less than 1 mg) of fungal mycelium from Earth that could then be used as an inoculum for continuous in situ production of inocula. The inocula would be used with a local organic material to grow the biocomposite in-space. The production of biocomposite structures could be low cost with limited human assistance, eliminating therefore costly and time consuming processes of locating, validating and extracting of local resources.

Fungal biocomposites are composed of fungal mycelium and a plant waste substrate. Mycelium is a root network of fungus, a vegetative part which consists of thread-like hyphae. Fungus uses mycelium to absorb nutrients from the environment containing carbon and nitrogen in a two-step process. First, enzymes are secreted onto or into the food source by hyphae to break down biological polymers into smaller monomers. These monomers are then absorbed into the mycelium by diffusion and active transport. By consuming plant-based waste products, such as sawdust, mycelium's dense network binds the substrate into a structurally adequate material composite.

Fungal based biomaterials might offer the following advantages over other in situ manufacturing technologies:

1) Lower manufacturing and energy costs due to excluding the costs of (a) locating and validating the accessibility of indigenous resources, (b) developing and demonstrating capabilities to extract indigenous resources, and (c) developing capabilities for processing indigenous resources to convert them to needed products
2) Full manufacturing loop following a cradle-to-cradle principle: the waste of another process (e.g. organic matter from greenhouse) can be used as a basis for fabricating biocomposites, which at the end of their service can be biodegraded and used as a soil for plants
3) Light weight, therefore can be used for creating light weight structures
4) It is non-flammable and has good insulation properties
5) Enables to produce a large variety of materials from transparent films to leather and brick like materials
6) Allows designs with complex geometry

The main limitations are:

1. Needs special environment during the growth period (control of humidity, temperature, light, CO_2 and O_2, therefore energy is needed to sustain that environment)
2. Due to autonomous growing process of a biological material there is a factor of uncontrollability and uncertainty of the final material properties

1.1 Study structure and aims

The two main problems the current study is focusing on are the lack of organic material in space to fabricate the biocomposite and the effect of space environment, such as low-gravity and radiation, on the survivability of fungi. To find the answers to these questions the study is following the structure described below:

1) Matching fungal strains with substrates:
Chosen fungal strains were grown in a biomass substrate. The substrate was inoculated with spawn (wheat grains colonized with mycelium) and grown for 1-4 weeks at 25 °C.

2) Preparation of material samples:
Material samples were produced to investigate the feasibility of larger scale growth of mycelium on the chosen substrate and to characterize the material by mechanical testing.

3) Effects of space environment on fungi:
The biomass formation of the fungus was monitored under normal and simulated micro-gravity conditions using random positioning machine (RPM). The survival, viability and melanin production of the organisms was tested in Co-60 facility by using gamma radiation.

4) 3D printing of biocomposite material:
Specially designed extruders were developed for the 3D printing experiment with biocomposite paste. The optimal consistency for the paste, as well as most suitable parameters for printing were studied for the fabrication of prototypes.

2. Fungal biocomposites

From a large group of fungi, Ascomycota and Basidiomycota are considered to be more suitable to create mycelium based materials as they can construct larger and more complex organic structures than other

fungi. From the two, Basidiomycota have two important properties which can make them more suitable for producing biocomposites: septa and anastomosis. Septa, special transverse cell walls, have an opening that can be closed in order to block the draining of a cytoplasma through the rapture when hypha becomes damaged. This will decrease the damage of the colony and therefore will lead to faster colonization of a substrate. Also anastomosis increases the growth speed of mycelium by fusing two different hyphae together when they meet. In addition, it creates a more homogeneous network of mycelium which promotes a fast transport of nutrients [9].

Fig. 1. Pure mycelium materials by M. Montalti, Officina Corpuscoli

Fig. 2. Biocomposite materials by M. Montalti, Officina Corpuscoli

Both, pure mycelium, as well as mycelium combined with a substrate, can be used as materials. The materials containing pure mycelium (Fig. 1) are created by allowing mycelium to completely consume the substrate it is growing in. To that end, it is possible to fabricate rubber-like, paper-like, textile-like, leather-like, and wood-like material out of pure fungal mycelium by varying the environmental growth conditions, and physical and chemical treatments. The main limitation of fabricating materials out of pure mycelium is that the production process takes relatively long and that yield is relatively low, whereas the production process of fungal biocomposites (Fig. 2) by combining mycelium with substrate is fast and yield is high. In order to stop mycelium consuming the substrate completely the biocomposite needs to be treated at 60 ºC to kill the fungus. Depending on the fungus, plant waste substrate and physical and/ or chemical treatments it is possible to produced cardboard-like, softboard-like, hardboard-like, and brick-like biocomposite materials. Jones et al. [10] discuss the exact factors influencing the mechanical performance of the fungal materials, which are the hyphal architecture, cell wall composition, composite constituents and growth kinetics, in more detail. Also Haneef et al. [11] studied the ways to control and tune the physical properties of mycelium materials grown on cellulose and cellulose/ potato-dextrose. As the materials showed different relative concentrations in polysaccharides, lipids, proteins and chitin they concluded that these differences affected the morphology and mechanical properties of the materials.

2.1 State-of-the-art of fungal biocomposites for construction

One of the main benefits of biocomposites is that these materials are renewable and biodegradable, and therefore could be an interesting alternative to various plastics in packaging and insulating materials. To that end Holt et al. [12] developed and evaluated six blends of processed cotton plant biomass materials as a substrate for selected fungi for the fabrication of molded packaging material. They found that the developed biocomposite is a viable alternative to polystyrene foam packaging material by having similar mechanical properties. Jiang et al. [13] investigated a non-traditional approach for fabricating biocomposite sandwich structures based on natural textile fibre, mycelium-bound agricultural waste and bioresin matrix. The study showed that the strength of the panel depends on the type of substrate, degree of colonization within the sandwich skin by mycelium, and bonding between the core and skin. The stiffness was dominated by core strength depending on its thickness. Xing et al. [14] examined the thermal properties of mycelium based bricks as building insulation materials and concluded that the materials exhibited good thermal performance. Jones et al. [15] tested a number of mycelium based biocomposite materials for improved fire safety and found that the biocomposites are very economical alternative to highly flammable petroleum-derived and natural gas-derived synthetic polymers and engineered woods for insulation, furniture and panelling applications.

Although generally proposed as a potential replacement for polymer grade foams, a number of studies have also investigated the application of fungal biocomposites in construction as bricks and boards. The main physical requirement for a conventional building brick is its high compressive strength of around 8.6 to 17.2 MPa. To date there are no studies known where such high strengths have been achieved with fungal based materials, which normally have a compressive strength of around 0.5 MPa [10]. Travaglini et al. [16] suggested that natural foams can provide acceptable mechanical properties with the benefits of being lightweight, sustainable and inert. They modelled the mycelium composite in their study as an open-cell foam to assess the performance of the material. The results showed that the compressive strength of the material was almost three times as high as its tensile strength, which is common to open-cell foams. Therefore the mycelium biocomposite was closest to polystyrene foam based on its density and strength properties. Gonzales [17] investigated the possibilities of using the biocomposite material developed by Ecovative Design in creating architectural structures, such as shelters, by shifting the scale and modifying the composition of the material. New York based architecture studio The Living designed a structure for the MoMA competition using mycelium bricks. The structural engineering of the temporary tower 'Hy-Fi' was carried out by Arup and structural testing at Columbia University [18]. The test results showed a very low elastic modulus with increased stiffness at very high stresses, meaning that the material acted more as a foam than a brick and therefore the structure had to be designed accordingly. Lelivelt et al. [19] evaluated the structural performance of different type of mycelium biocomposites. Although the compressive tests showed lower results compared to conventional structural materials, the biocomposites could still potentially offer an interesting alternative in construction when developed further. Heisel et al. [20] designed a load-bearing branching structure made of mycelium building elements. Due to the conservative compressive capabilities of the material the optimal geometry of the structure was found through 3D graphic statics following the compressive loads only. Their study showed that it is possible to achieve structural stability when using weaker materials by utilizing clever geometry and therefore non-standard materials could be used as building materials.

3. Survival of fungi in high radiation environment

One of the show stoppers for the survival of biological organisms in space is the exposure to high radiation levels, such as galactic cosmic radiation (GCR), solar winds and solar particle events (SPEs). Therefore, it is necessary to evaluate whether the chosen model organism is able to survive in such an environment. There is evidence that a specific type of fungi can survive the simulated Martian conditions [21][22] and that the ionizing radiation can even enhance the growth of melanised black fungi [23][24][25]. Onofri, de Vera, Zucconi, et al proved in their Lichens and Fungi Experiment (LIFE) that *Cryomyces antarcticus* and *Cryomyces minteri* are able to survive the simulated martian conditions aboard the Internatinal Space Station for 18 months. They found that more than 60% of the cells and rock communities did not undergo any change due to the exposure [22]. Dadachova, Bryan, Huang, et al studied melanised microorganisms, such as *Cryptococcus neoformans*, *Wangiella dermatitidis* and *Cladosporium sphaerospermum* and found that ionizing radiation changes the electronic properties of the organisms and enhances their growth [24]. In another study, researchers were able to provide clues how melanised black yeast *Wangiella dermatitidis* has adapted the ability to survive or even benefit from exposure to ionizing radiation [22].

3.1 In-space cultivation of organic substrate

To date most fungal biocomposites were made out of higher plants, such as straw and saw dust, as a substrate. However, cultivating higher plants in space is expected to be complicated. Here, mycelium was combined with a lower plant, the aquatic fern *Azolla filiculoides* (AF). *Azolla* is a fern, up to 2.5 cm tall, which floats on water either individually or in mats up to 20 cm thick. It grows in ponds, ditches, water reservoirs, wetlands, channels and slow flowing rivers and therefore does not need soil [26]. A unique property of the genus *Azolla* is its symbiotic relationship with the nitrogen-fixing blue-green alga *Anabaena azollae*. Due to its symbiotic co-evolution with *A. azollae*, the floating fern is able to absorb its nitrogen from air and therefore grow on nitrogen-deficient matter, which makes its cultivation in space less complex. Another benefit is its high distribution rate by being able to double its area in only 7 to 10 days under suitable conditions. AF can also be used as food and feed due to its protein content, as fertilizer for plants and for the biofuel production.

For the cultivation of AF in space a controlled environment has to be created. AF is usually grown in nursery beds of 3 x 4 m and 10 cm deep, in groups of ten or twenty. The atmosphere in the controlled environment has to contain CO_2 and N_2 as the plant needs it for the survival. As the O_2 is released by plant due to the process of photosynthesis the controlled environment should be part of a closed loop system for the gas exchange between the living environment of astronauts and nursery of AF. Also other aspects of the environment have to be controlled, such as the temperature between 15 °C and 26 °C, pH of water

between 4.5 to 7, light intensity between 20 000 to 80 000 lux, nutrient composition and humidity [27].

3.2 In-space cultivation of mycelium

In-space manufacturing of vegetative mycelium, called spawn, would also require a controlled environment. Typically, the process starts with the preparation of pure culture from mushroom spores or tissues. The culture is then added to a nutrient-rich agar or sugar-rich liquid broth on a petri dish. Depending on the type of fungus, after 7 to 21 days of incubation period at 25 °C ± 2 °C, the mycelium has grown out. A part of that mycelium is then transferred to a container filled with cooked and sterilized grains for the production of mother spawn. In our case, the grains would be replaced with the biomass from *Azolla*. The colonization of that biomass would happen after 10 to 21 days of incubation period at 25 °C in dark. The mother spawn can then be used for the further inoculation of the substrate used to prepare the feedstock for 3D printing structural elements [28].

4. Methods

Three different model organisms were chosen for the experiments, *Pleurotus ostreatus* (PO) PC9, *Schizophyllum commune* (SC) 4.39, 4.40 and 227, and *Trametes multicolor* (TM) X. The organisms where chosen based on the previous experience with these type of fungi, the availability of the organisms, and because the fungi are edible (i.e. they do not make toxins) and do not form spores preventing sensitive people to get an allergy.

4.1 Matching fungal strains with substrates

AF was grown on minimal medium (0.7 mM KNO_3, 0.1 mM $Ca(NO_3)_2$, 0.13 mM KH_2PO_4, 0.1 mM $MgSO_4 \cdot 7H_2O$, 4.7 µM Fe-EDTA, 2.2 µM $MnSO_4 \cdot H_2O$, 0.1 µM $Na_2Mo_4 \cdot 2H_2O$, 8.1 µM H_3B_3, 0.06 µM $CuSO_4 \cdot 5H_2O$, and 3.1 µM $ZnSO_4 \cdot 5H_2O$) refreshing nutrients every week. One third of the biomass of each container (30 x 50 cm) was harvested each week, sterilized for 30 min at 120 °C, and freeze dried resulting in 3.8 g dry weight plant material per container (i.e. a total of 30 g dry weight out of 8 containers on a weekly basis). SC strain 4.8A, PO strain PC9, and TM strain X were grown on AF, either or not homogenized by grinding under liquid nitrogen. Water was added resulting in 12.5 % - 33.3% AF w/v.

4.2 Preparation of material samples

To upscale growth of 227 on AF, we grew the fern in a plastic pool with a volume of 3000 L inside a greenhouse (Botanical Gardens, Utrecht University) with daylight. Air flow in the medium was provided with an aquarium air stone set at regular time intervals. Every week, 33% of the plants were harvested followed by the addition of KH_2PO_4 (123 g/l), K_2SO_4 (150 g/l), $MgSO_4$ $7 \cdot H_2O$ (250 g/l), Fe-DTPA (100 mmol), $CaCl_2$ $2 \cdot H_2O$ (400 g/l), $MnSO_4$ (4 g/l), H_2BO_3 (5.4 g/l), $ZnSO_4$ $7 \cdot H_2O$ (1 g/l), $CuSO_4$ $5 \cdot H_2O$ (0.156 g/l), and Na_2MoO_4 $2 \cdot H_2O$ (0.252 g/l). Nutrients were of agricultural quality. The water and nutrients were completely refreshed once a month. Harvested AF was air-dried in a fume hood or stored in a freezer and later dried in a freeze-dryer. When dried, the water fern was ground in a mortar under liquid nitrogen. Several prototypes were made by growing SC on AF. To this end, 1 volume of water was added to dry ground AF and sterilized at 121 °C for 20 minutes. SC 227 spawn was used to inoculate the AF and pre-grown at 25 °C for 7 days. After that 10% sterile psyllium was added, and the mixture was deposited to fill plastic moulds followed by pressing by hand. The moulds were custom designed for the manufacturing of material samples for mechanical testing. The fungus was allowed to proceed growth for at least 7 days in the dark while covered with cellophane (Fig. 3).

Fig. 3. Preparation of *AF* and *SC* composite in a mould

4.3 Effect of space environment on fungus

In order to evaluate the effect of space environment on the growth of fungus, preliminary tests were done at the facilities of ESTEC with gamma radiation and in simulated micro-gravity conditions.

4.3.1 Irradiation with gamma rays

SC dikaryons 4.39x4.40 and 227 were grown for 6 days at 25 °C in the dark on 20 ml SCMM plates. These cultures were irradiated in Co-60 facility at ESTEC with 20 and 200 Gy level gamma radiation for 3 days. This was followed by macerating the colonies in 50 ml SCMM for 30 seconds at low speed. 0.04 g and 0.004 g of mycelium was plated and colony forming units (CFU) were determined after 3 days of growth at 30 °C in the dark (Table 1).

4.3.2 Micro-gravity simulation

Microgravity was simulated using a random positioning machine (RPM) [29]. SC strains were grown in Petri dishes with 20 ml SCMM + 1.5 % agar on a

perforated poly-carbonate (PC) membrane (0.1 µm pores, 76 mm diameter; Profiltra, Almere). Plates were co-inoculated with 4.39 and 4.40 and 227.1 and 227.2 to enable mating. Cultures were pre-grown at 25 °C in the dark for 4-5 days and transported to ESTEC. Samples (biological triplicates) were placed in the RPM for 3 days, after which dry weight biomass was determined (Fig. 4).

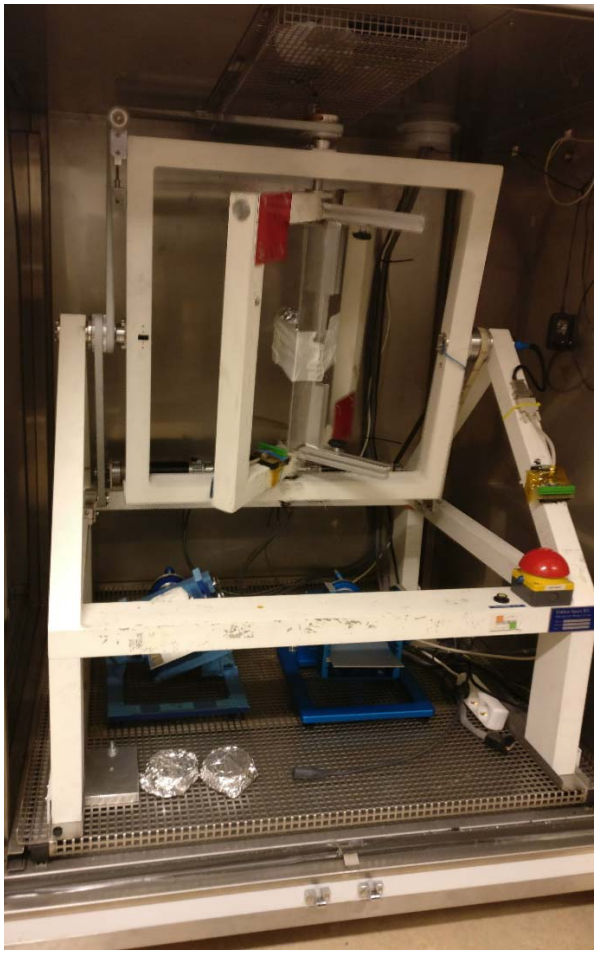

Fig. 4. Simulated micro-gravity test with RPM at ESTEC

4.4 3D printing of biocomposite

The 3D printing study to evaluate the feasibility of printing with biocomposite paste was done in collaboration Officina Corpuscoli, Co-de-iT and digifabTURINg.

4.4.1 Developing extruders

Two typologies of extruding tools have been developed, suitable for being mounted on a robotic arm and specifically addressing the deposition of biomaterials. Extruder I (Fig. 5) has been designed to deposit a mixture of mycelium, plant matter, and

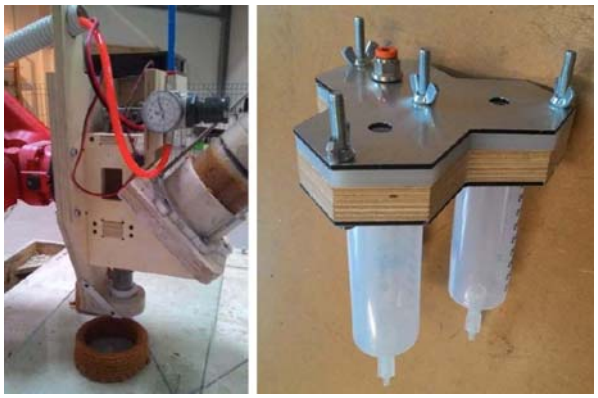

Fig. 5. Bio-material deposition using the paste material extruder I (left) or the water-based extruder II (right) by Officina Corpuscoli, Co-de-iT and digifabTURINg

additives (thickening agents and/or jellifying substances). It is pneumatically and mechanically activated, is able to start and stop extrusion at will, and speed and air pressure can be varied. The material, contained in a transparent tank (2500 cm^3 volume), is transported by air pressure in a Y pipe where a plastic screw, connected to a servo motor, pushes the material through a customized nozzle (with sizes ranging from 3 mm to 18 mm). Extruder II (Fig. 5) that is currently under development has been specifically designed for extrusion and deposition of hydrogels. A pneumatic system controls the pressure needed to activate three different syringes, enabling simultaneous deposition of three different materials (e.g. fungi, substrates, hydrogels).

4.4.2 Defining printing parameters

The custom-made Grasshopper® algorithm in combination with an electronic control system controls which material/syringe to activate and at which deposition speed. The computational design approach allows to digitally approximate, simulate, and render a preview of the material deposition process, to check the coherence of the instruction sequence that is then sent to the system in advance. Parameters involved are related to the material (i.e. type of biomaterial, density and humidity), the robot (arm speed, deposition layer thickness, end-effector spatial orientation) and the extruder (extrusion speed, deposition start and stop). The materials need to be deposited homogeneously without compromising mycelium growth at a later stage (e.g. due to contamination or density issues).

5. Results

5.1 Matching fungal strains with substrates

After 7 days of growth SC had formed most biomass in the 9 cm Petri dish (Fig. 6B), while TM had stopped growing after 3 - 4 days. Strains were also grown on

33% and 5% AF powder (w/v) agar (1.5%) plates. Also in this case, SC had most strongly colonized the substrate after 5 days (data not shown). Notably, 227 showed stronger growth when compared to 4.39 and 4.8A. Again, this shows that the latter strain is the preferred strain for forming bio-composites.

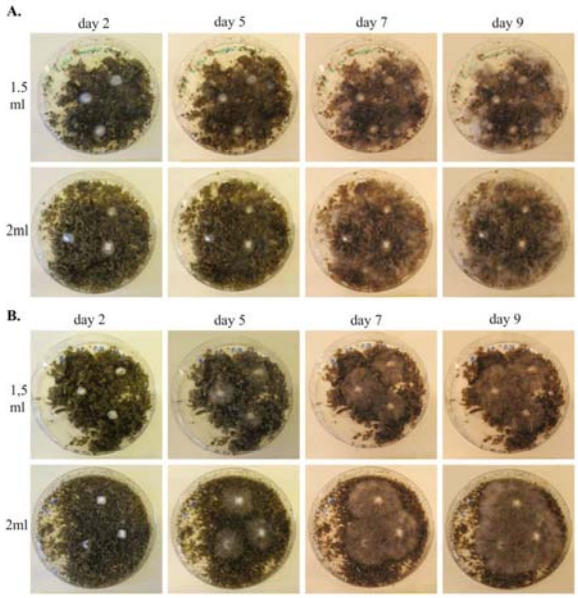

Fig. 6. Growth of PO (A) and SC (B) on freeze dried intact AF after 2, 5, 7, and 9 days of growth (point inoculum) in 9 cm Petri dishes

5.2 Preparation of material samples

60 kg of wet weight AF used to fabricate larger scale samples resulted in 3 kg dry weight material. Several prototypes were made by growing SC on AF (Fig. 7). Mechanical pressing was unsuccessful and resulted in a very brittle material (not shown). During drying of the colonized substrate the composite bended and shrunk

Fig. 7. Prototype of *AF* and *SC* composite after waterjet cutting

(shrinkage in width, length, height, volume: 14%, 13%, 33 %, 49,5%). Drying in a vacuum oven (ESTEC) did only partly prevent bending of the material (not shown). Bending was abolished by drying gradually in a circulated fumehood using a weight and metal grid. Still, materials were brittle and cracked during drying. Cracking was not observed when we used *Azolla caroliniana* as a substrate (Aquaplantsonline, The Netherlands) (note PCR indicated that this species was in fact *Azolla cristata*). This is probably due to the lower content of phenolic material enabling more efficient colonization, thereby creating a more homogenous material. The fabricated panels were sent for the mechanical testing at ESTEC.

5.3 Irradiation with gamma rays

Both strains of SC survived the radiation even at 200 Gy dose level, although viability of the strain 439x440 was 3-fold lower when compared to untreated mycelium (Table 1). This is more than 60 x the lethal dose for humans [30]. To assess whether melanin production is induced by radiation the medium of the colonies was set to pH 10 with 1 M NaOH and sterilized at 121 °C for 20 min. Samples were centrifuged for 5 min at 6000 g at 4 °C. The pH of the supernatant was set at pH 2 with 18.5% HCl to precipitate melanin. After 48 hours of incubation at 4 °C, the melanin was pelleted by centrifuging 10 min at 6000 g at 4 °C followed by washing with water chloroform, ethyl acetate, and ethanol [31]. Samples were washed three more times with water and dissolved in 1 ml 50 mM borate buffer (pH 8.0) followed by quantification of melanin at 200 – 800 nm using a DU-800 UV/VIS spectrophotometer (Beckman Coulter). Results showed no differences between non-irradiated and irradiated strains, implying the melanin production is not induced by gamma rays.

Table 1. Colony forming units (CFU) and dry weight of cultures resulting from 0.04 and 0.004 g SC mycelial macerate after irradiation

Strain	Irradiation (Gy)	CFU (0,04 gr inoculum)	CFU (0,004 gr inoculum)
439x440	0	606-800	30-119
439x440	20	504	32-56
439x440	200	130-323	15-47
227	20	10	2-4
227	200	52	2

5.4 Micro-gravity simulation

The experiments in simulated micro-gravity conditions with RPM showed that SC strain 4.39x4.40 had no significant change in biomass formation, while 227 was shown to grow faster in these conditions ($p = 0{,}0131$) (Fig. 8).

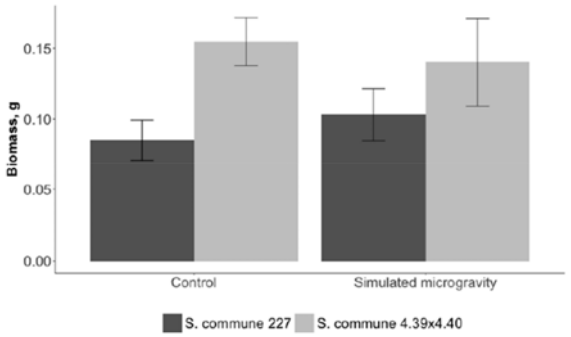

Fig. 8. Effect of simulated microgravity on the biomass of SC 4.39x4.40 and 227 strains (including the weight of the PC membrane)

5.5 3D printing of biocomposite

A wide number of tests were performed to identify the optimal composition of the substrates for 3D printing, and fungal growth. Sawdust has been selected as the model substrate because of previous experience and the fact that particle size can be adapted. The tests had as objective the identification of protocols for creating materials characterized by an optimal viscosity, preventing collapse of the structure before full fungal

Fig. 9. 3D printing test and objects by Officina Corpuscoli, Co-de-iT and digifabTURINg

colonization will have taken place and at the same time allowing the mycelium to effectively penetrate the substrate. Agar, alginate, corn starch, guar gum, arabic gum, and psyllium (and combinations thereof) have been used as thickening agents and / or jellifying agents.

Following the deposition, growth tests with PO and TM have been performed in order to verify the suitability of the viscous substrates compositions for promoting fungal colonization. The preliminary studies showed that the combination of saw dust (68%), psyllium (8%), water (24%) is most successful for printing and fungal growth (Fig. 9 and 10). Preliminary studies have also been executed by combining psyllium with azolla. Growth studies of mycelium deposited on such mix are currently taking place.

Fig. 10. 3D printed objects in different stages of colonization: just printed, after 3 days and after 1 week of growth by Officina Corpuscoli, Co-de-iT and digifabTURINg

6. Concept for robotic manufacturing of biocomposite structures

The proposed manufacturing sequence includes four phases:

1) A temporary inflatable structure is installed with the specific internal environment needed for fungal growth
2) A robotic arm is placed inside the inflatable to print the structure
3) After printing, the system is left untouched until the mycelium has fully grown through the substrate to form biocomposite
4) The growth of mycelium is stopped by removing the temporary inflatable structure and exposing it to very low or very high temperatures

Based on the final capabilities of the developed printing paste two options for the fabrication of biocomposite structures can be envisaged:

1. In the first case the temporary inflatable structure acts as a workshop for the production of biocomposite elements, such as bricks or panels. The elements can take any necessary form based on the specific mould applied. The inflatable structure ensures the right environmental conditions for the continuous production of components for the construction. The finished building elements are then assembled into bigger structures by using robotic arm.

2. In the second case the temporary inflatable structure provides the necessary environment for the 3D printing of a biocomposite structure. In this case the viscosity of the printed substrate is thick enough to be printed without a mould and supporting materials. Therefore a variety of complex shapes can be produced with the help of a robotic extruder. After the completion of the printing process the structure is left untouched until the mycelium has grown through the substrate. The growth is stopped by removing the inflatable structure.

7. Next steps

One of the following steps of the study is to evaluate mechanical properties of the developed material. Therefore compression and 4-point bending tests are planned at the facilities of ESTEC in the near future. In addition, further steps have to be taken to improve the mechanical properties of the material by possibly reinforcing it with fibres or combining the biocomposite with other suitable additives. The developments in 3D printing study aim to evaluate the *Azolla* based printing paste for its printing and structural properties. If successful, tests on larger scale structures need to be conducted.

8. Conclusions

Fungal based biocomposite materials might offer a cost-effective alternative to in-situ manufacturing of habitat structures and elements based on indigenous resources, mainly due to the lower manufacturing costs, recyclability and light weight. Many studies about terrestrial applications of fungal biocomposites have already shown the possibilities of using the material in construction. Although, at the current state with much weaker mechanical properties than conventional building materials, it has successfully been demonstrated that the material can be used in load-bearing structures when structurally optimised to compression strengths only. Future developments in the field of biocomposites promise improvements in the mechanical properties of the material, therefore opening up new areas for the application possibilities.

In our study we aimed to study the feasibility of using biocomposite material for space applications. We evaluated the possibilities of in-space manufacturing of newly developed fungal biocomposite material by conducting preliminary experiments on matching plant based substrate with fungus, effects of space environment on fungal growth and 3d printing of biocomposite structures. To that end we were able to identify the best performing fungus that grows on *Azolla filiculoides* (AF) substrate. We found that *Schizophyllum commune* (SC) grows better on AF when compared to *Pleurotus ostreatus* and *Trametes multicolor*. Tests showed that growth is possible at micro-gravity conditions and growth of SC may even be stimulated by micro-gravity. We were able to confirm that growth is possible at high radiation levels. SC was able to survive even at 200 Gy dose level of gamma radiation for 3 days. In addition, small scale AF/SC biocomposite panels have been produced for the mechanical testing at ESTEC. In the framework of 3d printing study tools for extrusion of paste-like biocomposites have been developed, computational design strategies have been implemented for controlling robotic devices and for consequent deposition of mycelium based pastes and different shaped 3D printed mycelium composite objects have been created.

References

[1] Rapp, D., 2008. Human Mission to Mars. Enabling Technologies for Exploring the Red Planet. Springer
[2] Cowley, A., Imhof, B., Teeney, L., Waclavicek, R., Spina, F., Canals, A., Schleppi, J., Lopez Soriano, P., 2016. An ISRU-Based Architecture for Human Habitats on Mars; the 'Lava Hive' Concept. Acta Futura
[3] Anand, M., Crawford, I. A., Balat-Pichelin, M., Abanades, S., Westrenen, W. van, Pe´ raudeau, G., Jaumann, R., Seboldt, W., 2012. A brief review of chemical and mineralogical resources on the Moon

and likely initial in situ resource utilization (ISRU) applications. Planetary and Space Science 74, p. 42–48

[4] Cesaretti, G., Dini, E., De Kestelier, X., Colla, V., Pambaguian, L., 2014. Building components for an outpost on the Lunar soil by means of a novel 3D printing technology. Acta Astronautica 93, p. 430–450

[5] Montesa, C., Broussarda , Gongreb, M., Simicevicb, N., Mejiac, J., Thamd, J., Allouchea, E., Davisa, G., 2015. Evaluation of Lunar Regolith Geopolymer Binder as a Radioactive Shielding Material for Space Exploration Applications. Advances in Space Research 56.5, p. 1212-1221

[6] Tang, Y., Fuh, J.Y.H. , Loh, H.T. , Wong, Y.S., Lu, L., 2003. Direct laser sintering of a silica sand. Materials and Design 24, p. 623–629

[7] Khoshnevis, B., Thangavelu, M., Yuan, X., Zhang, J., 2013. Advances in Contour Crafting Technology for Extraterrestrial Settlement Infrastructure Buildup. AIAA SPACE 2013 Conference and, September 10-12, 2013, San Diego, CA

[8] Howe, A.S., Wilcox, B., McQuin, C., Townsend, J., Rieber, R., Barmatz, M., Leichty, J., 2013. Faxing Structures to the Moon: Freeform Additive Construction System (FACS). AIAA SPACE 2013 Conference and Exposition, September 10-12, 2013, San Diego, CA.

[9] Lelivelt, R.J.J., 2015. The mechanical possibilities of mycelium materials. Eindhoven Univeristy of Technology

[10] M. Jones, T. Huynh, C. Dekiwadia, F. Daver, S. John. 2017. Mycelium Composites: A Review of Engineering Characteristics and Growth Kinetics. Journal of Bionanoscience 11, p. 241-257

[11] M. Haneef, L. Ceseracciu, C. Canale, I. S. Bayer, J. A. Heredia-Guerrero, A. Athanassiou. 2016. Advannced Materials from Fungal Mycelium: Fabrication and Tuning of Physical Properties. Scientific Reports 7, 41292

[12] G. A. Holt, G. McIntyre, D. Flagg, J. D. Wanjura, and M. G. Pelletier. 2012. Fungal Mycelium and Cotton Plant Materials in the Manufacture of Biodegradable Molded Packaging Material: Evaluation Study of Select Blends of Cotton Byproducts. Journal of Biobased Materials and Bioenergy 6, p. 431-439

[13] L. Jiang, D. Walczyk, G. McIntyre, R. Bucinell, G. Tudryn. 2017. Manufacturing of Biocomposite Sandwich Structures Using Mycelium-Bound Cores and Preforms. Journal of Manufacturing Systems

[14] Y. Xing, M. Brewer, H. El-Gharabawy, G. Griffith, P. Jones. 2018. IOP Conf. Series: Earth and Environmental Science 121, 022032

[15] M. Jones, T. Bhat, T. Huynh, E. Kandare, R. Yuen, C. H. Wang, S. John. 2018. Waste-derived low-cost mycelium composite construction materials with improved fire safety. Fire and Materials, p. 1-10

[16] S. Travaglini, J. Noble, P. G. Ross, C. K. H. Dharan, 2013. Mycology Matrix Composites. Proc. of the American Society for Comp., p. 517-535

[17] E. M. Gonzales. 2009. Growing architecture through mycelium and agricultural waste. Columbia University, GSAPP

[18] S. Saporta, F. Yang, M. Clark. 2015. Design and Delivery of Structural Material Innovations. Structures Congress

[19] R. Lelivelt, G. Lindner, P. Teuffel, H. Lamers. 2015. The production process and compressive strength of mycelium based materials. First Int. Conf. on Bio-based Building Materials

[20] F. Heisel, K. Schlesier, J. Lee, et al. 2017. Design of a load-bearing mycelium structure through informed structural engineering. Seoul Biennale of Archtecture and Urbanism

[21] Scalzi, G., Selbmann, L., Zucconi, L., Rabbow, E., Horneck, G., Albertano, P., Onofri, S., 2012. LIFE Experiment: Isolation of Cryptoendolithic Organisms from Antarctic Colonized Sandstone Exposed to Space and Simulated Mars Conditions on the International Space Station. Origins of Life and Evolution of Biospheres 42, p. 253-262

[22] Onofri, S., Vera, J.-P., de, Zucconi, L., Selbmann, L., Scalzi, G., Venkateswaran, K.J., Rabbow, E., Torre, R., de la, Horneck, G., 2015. Survival of Antarctic Cryptoendolithic Fungi in Simulated Martian Conditions On Board the International Space Station. Astrobiology 15 (12), p. 1052-1059

[23] Zhdanova, N.N., Tugay, T., Dighton, J., Zheltonozhsky, V., Mcdermott, P., 2004. Ionizing radiation attracts soil fungi. Mycological Research 108 (9), p. 1089–1096

[24] Dadachova, E., Bryan, R.A., Huang, X., Moadel, T., Schweitzer, A.D., Aisen, P., Nosanchuk, J.D., Casadevall, A., 2007. Ionizing Radiation Changes the Electronic Properties of Melanin and Enhances the Growth of Melanized Fungi. Plos One (5), e457

[25] Robertson, K.L., Mostaghim, A., Cuomo, C.A., Soto, C.M., Lebedev, N., Bailey, R.F., Wang, Z., 2012. Adaptation of the Black Yeast Wangiella dermatitidis to Ionizing Radiation: Molecular and Cellular Mechanisms. Plos One 7 (11), e48674

[26] A. Hussner, 2010. NOBANIS – Invasive Alien Species Fact Sheet – Azolla filiculoides. From Online Database of the European Network on Invasive Alien Species – NOBANIS. www.nobanis.org, date of access x/x/201x.

[27] http://www.plantsrescue.com/tag/azolla-filiculoides/

[28] https://radicalmycology.com/educational-tools/cultivation-info/fungi-101/

[29] Van Loon, JJWA. 2007. Some history and use of the random positioning machine, RPM, in gravity related research. Advances in Space research 39.7:1161-1165

[30] Levin, SG, Young RW and Stohler RL. 1992. Estimation of median human lethal radiation dose computed from data on occupants of reinforced concrete structures in Nagasaki, Japan. Health physics 63.5:522-531

[31] Pal, AK, Gajjar, DU, Vasavada, AR 2014. DOPA and DHN pathway orchestrate melanin synthesis in Aspergillus species. Medical Mycology 52:10–18.