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Characterization of 3D Printed Samples from Biomass-Fungi Composite Materials

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Abstract

Biomass-fungi composite materials are derived from sustainable sources and can biodegrade at the end of their service. In these composite materials, the biomass particles (made from agricultural waste such as hemp hurd) act as the substrate, and a network of fungal hyphae grow through and bind the biomass particles together. These composite materials can be used to manufacture products traditionally made from petroleum-based plastics. Potential applications of these products are in the packaging, furniture, and construction industries. Typically, molding-based methods are used to manufacture products using these composite materials. Recently, the authors reported a novel 3D printing-based method using these composite materials. This paper reports a follow-up investigation into the characterization of 3D printed samples from biomass-fungi composite materials. Tensile and compressive testing samples were shaped using molds from a portion of each printed sample. Mechanical properties of these tensile and compressive testing samples and chemical properties of the printed samples were measured. Tensile and compressive testing measurement results showed that, at the significance level of 0.05, effects of 3D printing parameters studied (printing speed and extrusion pressure) were not statistically significant on Young's modulus and ultimate tensile strength of the tensile testing samples, as well as on compressive strength and compressive modulus of the compressive testing samples. Results obtained from the Fourier transform infrared spectroscopy analysis show that chemical composition of printed samples was consistent with the data in the literature.

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Keywords: 3D printing; biomass; characterization; mycelium; composite

1. Introduction

Biomass-fungi composite materials are derived from sustainable sources and can biodegrade at the end of their service. In these composite materials, the biomass particles (made from agricultural waste such as hemp hurd) act as the substrate, and a network of fungal hyphae grow through and bind the biomass particles together. [1, 2]. These biomass-fungi composite materials, serving as sustainable alternatives to petroleum-based plastics, find applications in packaging [3-6], furniture [7], and construction industries [8]. They offer excellent thermal and acoustic insulation properties [9-11] and are inexpensive, with raw material cost ranging from $0.07 to $0.17 per kilogram [10]. In contrast, plastic materials are significantly more expensive. For example, raw material cost ranges from $2.1 to $2.3 per kilogram for polystyrene, from $1.7 to $1.9 per kilogram for phenolic formaldehyde resin, and from $8.2 to $10.2 per kilogram for polyurethane [10]. Moreover, at the end of their service life, these composite materials biodegrade much faster than plastic waste that often lingers in landfills for years [12].

Typically, molding-based methods are used to manufacture products using biomass-fungi composite materials [13-15]. One paper [14] defined different grades of these composite materials and studied the effects of biomass substrate and cultivation time on the mechanical behavior of the materials. There is a strong relationship between the density of the substrate and the mechanical strength. Another article [15] reported morphological and mechanical characterization of biomass-fungi composite materials and a fiber network-based model for mycelium. In another paper [13], it was shown that morphology, density, tensile and flexural strength, moisture, and water-uptake characteristics of the biomass-fungi composite materials could be adjusted by changing the biomass type (such as straw, sawdust, or cotton), fungal species (*Pleurotus ostreatus* or *Trametes multicolor*), and processing method (including no pressing, cold pressing, or hot pressing). It has also been reported that the choice of fungal species influenced the level of colonization and the thickness of the exposed mycelium layer, referred to as the fungal skin. The degree of colonization, skin thickness, biomass type, in turn, influenced the stiffness and water resistance of the resulting materials [13].

3D printing of biomass-fungi composite materials could facilitate the manufacturing of parts with complex shapes (such as sandwich and topology-optimized structures) in art, architecture, interior design, and construction that cannot be easily produced using molding-based or hot-pressing based methods [2, 16, 17]. The use of additive manufacturing has the potential to leverage in situ resources on the Moon and Mars, with the minimal amounts (less than 1 mg) of fungi transported from the Earth to serve as an inoculum for continuous production locally [18-21]. The authors of this paper published the first paper on a 3D printing-based manufacturing method using biomass-fungi composite materials [1]. The six stages of this method are illustrated in Fig. 1 (redrawn from [22]) and briefly described below.

Stage 1 – Preparing biomass-fungi material. Biomass particles are pasteurized at an elevated temperature to kill microorganisms that might compete with the fungi for nutrition. Then fungi spores are added to the biomass particles and fully colonize them. This biomass-fungi material is dehydrated prior to packaging. The resulting biomass-fungi material is packed in filter patch bags and ready for use or shipping.

Stage 2 – Primary colonizing. Water and wheat flour are added in the sterilized filter patch bag that contains biomass-fungi material. The bag is kept in a dark environment for a certain number of days to allow fungi to grow around biomass particles to create a foam-like biomass-fungi composite material.

Stage 3 – Mixing. The biomass-fungi material prepared in Stage 2 is mixed with additives (such as water, wheat flour, and psyllium husk) to prepare biomass-fungi mixture for 3D printing.

Stage 4 – 3D printing. Samples are printed using the biomass-fungi mixture prepared in Stage 3.

Stage 5 – Secondary colonizing. The printed samples are kept in a sterilized environment away from direct sunlight for fungi to further grow in 3D printed samples.

Stage 6 – Drying. The printed samples are dried (for example, in a conventional oven) to kill all the fungi in these samples. If the fungi are not killed, they may restart growing when the printed samples are exposed to suitable humidity conditions.

The authors' preliminary experimental results [1] showed that fungi could survive the mixing and printing stages and continue growing in the printed samples. The authors also investigated the effects of mixture composition on rheological properties of biomass-fungi mixtures prepared for 3D printing [22]. Additionally, they investigated the effects of waiting time (between the time the mixture was prepared and the time of 3D printing using the mixture) on mechanical properties and rheological properties of the biomass-fungi mixtures, as well as the minimum printing pressure required for continuous extrusion during 3D printing, and the quality of the printed samples (characterized by layer-height shrinkage and filament-width uniformity) [23]. Other researchers also published papers involving extrusion-based 3D printing of biomass-fungi composite materials [24, 25]. One research group developed a dedicated extrusion system for robotic printing, and investigated the rheological and biological behavior of the

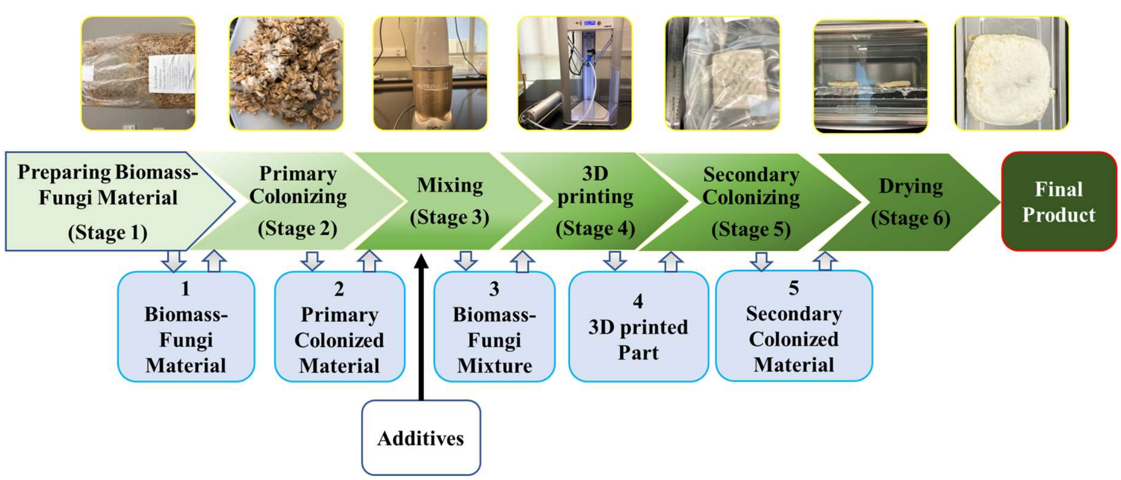

Fig. 1. Six stages of the 3D printing-based manufacturing method using biomass-fungi composite materials.

biomass-fungi materials [24]. Another research group investigated 3D printing workflows and extrudable mixtures with mycelium-based composite materials that were cultivated on wastepaper and waste cardboard. They also investigated ways to mitigate contamination [25].

To the authors' best knowledge, there is no study on mechanical properties and chemical properties of 3D printed samples from biomass-fungi composite materials. This paper aims to fill this gap by presenting an investigation of mechanical (tensile and compressive testing) properties of molded samples prepared from 3D printed samples, and chemical properties of 3D printed samples from biomass-fungi composite materials. These results were compared with data in the existing literature on molding-based manufacturing methods using biomass-fungi composite materials.

2.Materials and Methods

2.1. Materials

The biomass-fungi material was procured from GROW.bio (Green Island, NY, USA) and was received in a polypropylene bag with a square-shaped filter (1.5 × 1.5 inch in size) having the pore size of 0.2 µm. The biomass particles were from hemp hurd [3]. Fig. 2 shows the biomass-fungi material. Wheat flour (all-purpose flour: Great Value, Walmart, Bentonville, AR, USA) and psyllium husk (NOW Supplements, Bloomingdale, IL, USA) were purchased from a local Walmart store.

2.2. Preparation of biomass-fungi mixtures for 3D printing

700 mL of autoclaved water and 32 g of autoclaved wheat flour were added in the filter patch bag that contained the as-received biomass-fungi material, as shown in Fig. 2 (a) and (b). The bag was shaken vigorously by hand for one minute. This bag was then stored in a closet at 23 °C for five days for fungi to grow (i.e. primary colonizing). Fig. 2 (c) shows some biomass-fungi material after primary colonizing.

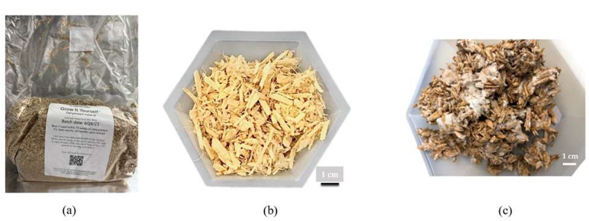

Fig. 2. Biomass-fungi material: (a) in a polypropylene bag; (b) in a plastic bowl, (c) after primary colonizing.

Then 50 g of primary colonized biomass-fungi material were broken into small pieces by hand. These small pieces, 200 g of autoclaved water, and 20 g of autoclaved wheat flour were mixed using a commercial mixer (NutriBullet PRO: Capital Brands, Los Angeles, CA, USA). The mixing mode used was intermittent and mixing time was 120 seconds. For the intermittent mixing mode, the mixing was performed for a period of five seconds. Afterwards, the mixing container was shaken manually twice to ensure good contact of the mixture with the blades. Then the mixing started again. Thereafter, 10 g of psyllium husk powder was mixed into this mixture manually using a spatula. The resulting biomass-fungi mixture was ready for 3D printing.

2.3. 3D printing experimental setup and conditions

The WASP Delta 2040 (WASP, Massa Lombarda RA, Italy) 3D printer shown in Fig. 3 was used for the printing experiments. This printer was equipped with an LDM (Liquid deposition modeling) extruder kit [22, 23] and a material storage container (with a storage volume of 3L). A pneumatically operated plastic piston was used to push the biomass-fungi mixture through the nozzle assembly. The custom-built nozzle assembly comprised a screw extruder and a casing with a 6-mm opening.

It is essential to understand how process parameters affect printed samples. Based on their previous studies [22, 26, 27], the authors have selected extrusion pressure and printing speed as input process parameters in this study. Printing speed determines the productivity [28, 29], and extrusion pressure affects the rheological behavior of mixtures prepared for 3D printing [26].

Table 1 shows two printing parameters (printing speed and extrusion pressure) and their values used in this study.

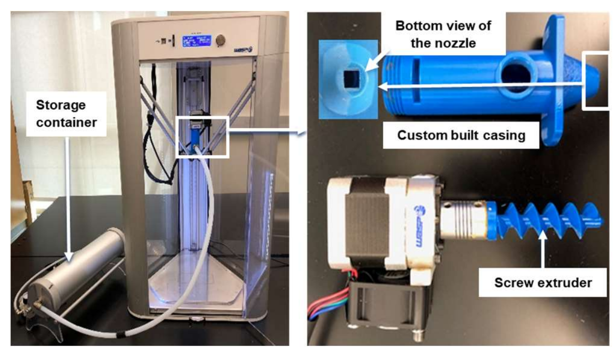

Fig. 3. Delta WASP 2040 3D Printer and its nozzle assembly.

Table 1. Printing parameters and their values used in this study

Parameter	Value
Printing Speed (mm/s)	30, 120
Extrusion Pressure (Bar)	2, 6

2.4. Preparation of tensile and compressive testing samples

Samples with the dimension of 80 mm × 80 mm × 30 mm were printed with 30% infill at different levels of printing speed and extrusion pressure as listed in Table 1. Then a portion was cut from each printed sample and shaped into tensile testing samples using a rectangular mold and compressive testing samples using a cylindrical mold, as illustrated in Fig. 4. The tensile testing samples and compressive testing samples were prepared this way (using molding) because of the following reasons. (1) In the biomass-fungi composite material, the mechanical properties (strength, etc.) are primarily determined by the binding of biomass particles by fungi hyphae. After printing, the printed samples (or molded samples) will need to go through secondary colonizing (so fungi can grow through the biomass particles and bind them). (2) To prepare testing samples with needed dimensional accuracy, molding is more suitable than extrusion-based 3D printing. Tensile testing samples had the dimension of 4.8 cm × 1.1 cm × 1.3 cm (Fig. 4) and compressive testing samples were 1.5 cm in diameter and 1 cm in height (see Fig. 4). Thereafter, these molded tensile and compressive testing samples were kept on slotted base (Fig. 4) for five days for fungi to grow (i.e. secondary colonization). These molded samples were flipped after 2.5 days to facilitate fungal growth on the bottom side of the samples. After another 2.5 days, these samples were dried in countertop convection oven (Black+Decker TO3250XSB, New Britain, Connecticut, USA) at a temperature of 95°C for 6 hours. Resin (UV curable) was applied on both ends of the tensile testing samples to facilitate a good grip by the clamps when putting them on the tensile testing machine. This resin was cured using a UV flashlight (395-405 nm).

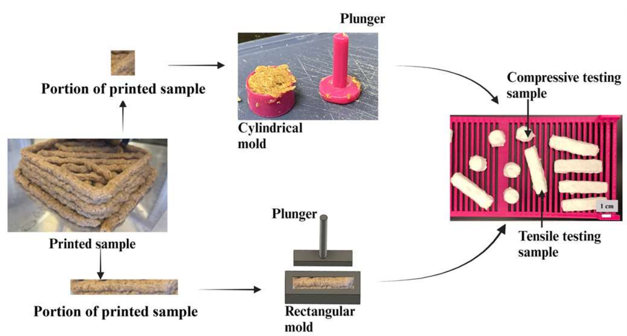

Fig. 4. Preparation of tensile and compressive testing samples.

2.5. Mechanical Characterization

2.5.1. Tensile testing

The tensile testing was conducted on the Instron 68TM-5 Universal Testing Machine. A load cell of 500 N and a loading rate of 10 mm/min were used. 5 tensile testing samples were prepared for each printing parameter combination (low printing speed and low extrusion pressure, and high printing speed and high extrusion pressure).

2.5.2. Compressive testing

The compressive testing was conducted on the Instron 5943 system equipped with compression platens. Each sample was compressed to 75% of its initial height with a 0.05 mm per second rate. 3 compressive testing samples were prepared for each print parameter combination (low printing speed and low extrusion pressure, and high printing speed and high extrusion pressure).

2.6. Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) in Attenuated Total Reflection (ATR) mode was carried out in an iD7 ATR with Nicolet™ iS™ 5 Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) in the spectral range of 500–4000 cm^{-1} and resolution of 16 cm^{-1} for identification of distinct functional groups in the 3D printed biomass-fungi composite materials.

2.7. Statistical analysis

The differences in tensile strength between molded samples prepared with the printing condition of low printing speed and low extrusion pressure and the molded samples prepared with the printing condition of high printing speed and high extrusion pressure were examined by t-test using Minitab software (Minitab, USA). The significance level was set as 0.05 [30].

3. Results and Discussion

3.1. Mechanical characterization

3.1.1. Tensile testing

Fig. 5 shows the stress-strain curves of the tensile testing for two sets of samples. One set of samples were prepared from the printing condition of low printing speed and low extrusion pressure and the other set of samples were prepared from the printing condition of high printing speed and high extrusion pressure printed samples. The shaded region denotes the standard deviation.

The differences in Young's modulus and ultimate tensile strength were not statistically significant (p-value $\geqslant 0.05$ obtained from two tailed t-test) between the molded tensile testing samples prepared from the printing condition of low printing speed and low extrusion pressure and the molded tensile testing samples prepared from the printing condition of high printing speed and high extrusion pressure. A possible explanation for this observation ould be as follows. The stress-strain relationship of the molded samples prepared from 3D printed samples is primarily determined by the binding of biomass particles by fungi hyphae. After the molded samples went through secondary colonizing for 5 days (so fungi could grow through the biomass particles and bind them), any differences caused by the differences in printing conditions became negligible. In authors' previous paper, they have shown that printing parameters did not have statistically significant effects on fungal growth in printed [27].

The values of yield stress and ultimate tensile strength obtained for tensile testing samples in this study were similar to those reported in the literature for molded biomass-fungi composite materials [13, 15, 31]. The average value of Young's modulus of the molded tensile testing samples prepared from printed samples was 55.58 MPa and Young's modulus reported in the literature ranges from 20 to 80 MPa for different molded mycelium-based biofoams and mycelium-based composites [13, 31].

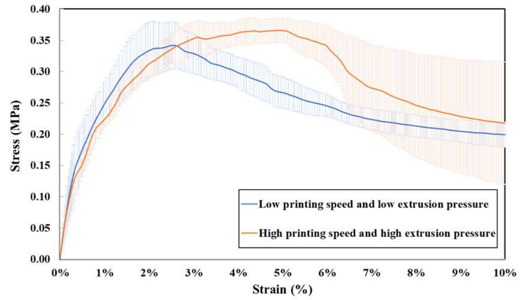

Fig. 5. Stress vs. strain graphs obtained from tensile testing.

3.1.2. Compressive testing

Fig. 6 shows the stress-strain curves in the compressive testing for two sets of samples: the samples prepared from the printing condition of low printing speed and low extrusion pressure and the samples prepared from the printing condition of high printing speed and high extrusion pressure printed samples. The shaded regions indicate the standard deviation for the respective samples.

The differences in compressive strength and compressive modulus were not statistically significant (p-value $\geqslant$ 0.05 obtained from two tailed t-test) between the molded compressive testing samples prepared from the printing condition of low printing speed and low extrusion pressure and the molded compressive testing samples prepared from the printing condition of high printing speed and high extrusion pressure. A possible explanation was the same as that for the results of tensile testing results. In one of the authors' previous papers [27], they did not find statistically significant effects of printing parameters on fungal growth in printed samples. The stress-strain relationship of the molded samples prepared from 3D printed samples is primarily determined by the binding of biomass particles by fungi hyphae. After the molded samples went through secondary colonizing for 5 days (so fungi could grow through the biomass particles and bind them), any differences caused by the differences in printing conditions became negligible.

The values of compressive strength obtained in this study were similar to those reported in the literature for molded biomass-fungi composite materials [14, 31]. The average value of compressive strength was 0.81 MPa for the molded compressive testing samples prepared from the printed samples. In one paper [14], reported values of compressive strength for molded mycelium-based composite material were in the range of 0.1 to 0.5 MPa. In another paper [31], reported values of compressive strength for mycelium-based biofoam were in the range of 0.1 to 0.9 MPa.

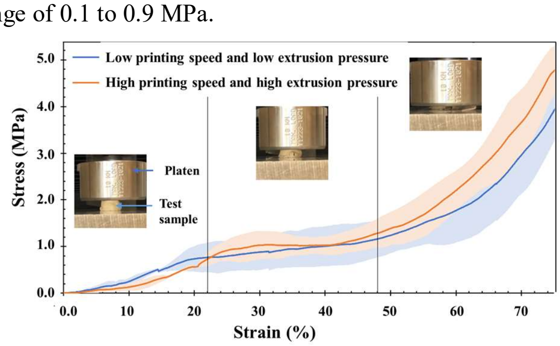

Fig. 6. Stress vs. strain graphs obtained from compressive testing.

3.2. Fourier Transform Infrared (FTIR) Spectroscopy

Fourier transform infrared (FTIR) spectroscopy was utilized to identify functional groups located on the surface of printed samples. This technique enables the detection of unique energy absorption bands associated with each specific functional group [32, 33]. The Fourier transform infrared (FTIR) spectrum of the secondary colonized sample (**Error! Reference source not found.**) displayed a prominent band (3286 cm^{-1}) within the range of 3500-3100 cm^{-1}. It is possible that the N-H and O-H stretching vibrations overlap in this region [34, 32, 35, 36]. The absorption band observed at 3286 cm^{-1} is likely indicative of the stretching vibrations of N-H or O-H functional groups. The fungi cell wall consists of various biopolymers, including chitin, chitosan, glucan, mannan, as well as proteins and lipids [32]. Due to the complex composition of the cell wall, it was challenging to pinpoint the exact group responsible for the observed stretching vibration. The bands observed in the range of 3000-2850 cm^{-1} were indicative of alkyl chains (2924 and 2870 cm^{-1}). The amide I band, centered at 1635 cm^{-1}, primarily represented the stretching mode of the C=O bond, while the amide II band, centered around 1543 cm^{-1}, was a combination of N-H bending and C-N stretching. Furthermore, the bands in the regions of 1200-984 cm^{-1} and 634-450 cm^{-1} 1041 cm^{-1} indicated the presence of phosphate groups (1041, 632, 594 cm^{-1}) in the biomass. Therefore, the spectral data confirmed the presence of amine, hydroxyl, carboxyl, and phosphate groups in the biomass [37-39]. The spectra revealed lignin bands at 1532 cm^{-1}, and 1450 cm^{-1}. It also revealed chitin bands at 1373 cm^{-1}, and 1226 cm^{-1}. Typical bands assigned to cellulose were observed at 897 cm^{-1}. The absorption band at 897 cm^{-1} was assigned as C-O-C stretching vibration of glycosidic bonds in polysaccharides. The results obtained from the FTIR analysis of 3D printed samples were not significantly different from the data reported in the literature on the molded samples of biomass (hemp hurd)-fungi composite materials [37, 29, 35, 38, 39].

- literature for the molding-based hemp biomass-fungi composite materials.

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