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3D Printing of Biomass–Fungi Composite Material: Effects of Mixture Composition on Print Quality

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Citation: Bhardwaj, A.; Rahman, A.M.; Wei, X.; Pei, Z.; Truong, D.; Lucht, M.; Zou, N. 3D Printing of Biomass–Fungi Composite Material: Effects of Mixture Composition on Print Quality. *J. Manuf. Mater. Process.* **2021**, *5*, 112. <https://doi.org/10.3390/jmmp5040112>

Academic Editors:

George-Christopher Vosniakos,
Alborz Shokrani and
George Chryssolouris

Received: 14 September 2021

Accepted: 14 October 2021

Published: 18 October 2021

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Abstract: It is known that 3D printing can facilitate greater design flexibility in the printing of custom shapes for packaging and construction applications using biomass–fungi composite materials. The feasibility of this new method was demonstrated by a preliminary experiment, the results of which were reported in a journal publication in 2020. As a follow-up, this paper reports on an experimental study on the relationship between the mixture composition (i.e., the psyllium husk powder content) and print quality using this new method. Four mixtures were prepared by varying the amounts of psyllium husk powder (in grams) added to 400 mL of water. The ratios (g/mL) of psyllium husk powder weight (w_p) over volume of water (v_w) for the mixtures were 0, 1:40, 2:40, and 3:40. Each mixture also contained 100 g of biomass–fungi material and 40 g of whole wheat flour. The print quality of the samples was evaluated based on the extrudability and shape stability. The results showed that mixtures without any psyllium husk powder were not extrudable. An increase in the ratio of psyllium husk powder to water from 1:40 to 2:40 resulted in improved print quality; however, when the psyllium husk powder to water ratio was increased to 3:40, the extrudability became worse. This phenomenon was explained by analyzing the rheological properties of the mixtures.

Keywords: biomass–fungi composite; mycelium; 3D printing; material extrusion; rheology

1. Introduction

Environmental sustainability is a major concern for the construction and packaging industries. The production of 1 ton of cement generates an average of 0.9 tons of CO₂, resulting in approximately 5–7% of the global CO₂ emissions [1]. Additionally, the packaging industry consumes 38% of the petroleum-based plastics produced globally [2]. It is estimated that with current production and waste-management trends, 12 billion metric tons of plastic waste will be in landfills by 2050 [3].

Sustainable materials such as biomass–fungi composite materials can help reduce the negative impacts of the construction and packaging industries [4–7]. In biomass–fungi composite materials, the biomass derived from agricultural waste (such as rice straw and corn stover) acts as the substrate and a nutrition source for the fungi. The fungi grow as a network of fine white filaments (hyphae), also known as the mycelium. This mycelium binds the biomass together. The mechanical properties of the mycelium are similar to materials such as wood and cork [8]. This is understandable, since fungi are a natural source of structural polymers such as chitin, which are present in the mycelium [9,10].

The advantages of biomass–fungi composite materials include their low cost (estimated cost for raw materials: 0.07–0.17 kg/m³ [9]), biodegradability, and low environmental impacts [11–13]. These materials have found applications in the automotive and

construction industries (e.g., as sound absorption panels and wall panels [14]), the furniture industry, and the packaging industry [5,15]. The production of these parts involves molding-based manufacturing methods [5] that are expensive at low production scales. As such, 3D printing is more economical for producing parts with customized and complex shapes in small quantities than molding-based manufacturing methods. Furthermore, 3D printing can facilitate the fabrication of geometrically complex structures such as sandwich and topology-optimized structures [16–19].

There are many studies on biomass–fungi composite materials. Jones et al. [9] presented a critical review of biomass–fungi composite materials as construction materials. Haneef et al. [13] showed that the mechanical properties of mycelia were associated with the nutrition source for the fungi. Appels et al. [20] reported that factors such as the types of nutrition source, fungal species, and processing (no pressing, cold pressing, heat pressing) affected the physical properties (such as the morphology, density, tensile strength) of the composite. Environmental factors such as the light conditions and CO₂ concentration also impact the fungal density [8]. Attias et al. [7] highlighted the need to develop sustainable production methods for these materials that avoid using plastic molds [7]. Soh et al. [21] developed an extrudable composition for mycelium composites that was tested using a syringe.

Various composites have been printed using the material-extrusion-based 3D printing process [22,23]. Recently, the authors reported their first study on the 3D printing (using material extrusion) of biomass–fungi composites in a journal publication [24]. The study demonstrated the feasibility of the 3D-printing-based method by showing that a printable mixture could be prepared from a biomass–fungi material and that fungi could survive the printing process and grow in the printed sample [24]. This paper reports on a follow-up study to determine the effects of the mixture composition, specifically psyllium husk powder, on the printing quality, as well as the rheological properties of the prepared mixtures. Psyllium husk powder, derived from the seed husk or leaves of the plants of the *Plantago* genus [25], is a source of psyllium polysaccharides that exhibits gelation properties in aqueous solutions [25,26]. It possesses many advantages, such as favorable viscoelastic properties, low cost, biodegradability, and availability [25–27]. While researchers have studied psyllium husk–gelatin blends for bioprinting [25], the role of psyllium in the 3D printing of biomass–fungi composites remains a knowledge gap. This study will reveal the effects of psyllium husk powder on the print quality and rheological properties of biomass–fungi mixtures for material-extrusion-based 3D printing processes. Furthermore, the methodology developed to characterize the print quality and its relationship with the mixture rheology could be extended to the development of new mixtures for extrusion-based 3D printing processes.

The structure of the paper is as follows. Section 2 describes the materials and methods used in the study. Section 3 presents and discusses the experimental results. Finally, in Section 4, conclusions and future research directions are discussed.

2. Materials and Methods

2.1. Biomass–Fungi Material

The biomass–fungi material (its commercial name is “Grow-It-Yourself”) was procured from Ecovative Design, NY. Before the biomass–fungi material was shipped to the authors, it underwent three steps in the factory. First, the biomass material was pasteurized to eliminate harmful microorganisms. Second, the pasteurized biomass material was inoculated with the fungi (belonging to the Basidiomycete group). Thereafter, the fungi colonized the biomass material. Lastly, this biomass–fungi material was dehydrated before being packed. This as-received material was in polypropylene filter patch bags with a filter (1.5 inch square filter with a pore size of 0.2 μm) as part of the bag to facilitate fungi growth. Each bag contained 400 g of the material, as shown in Figure 1. This as-received material was converted into a biomass–fungi mixture that can be 3D-printed by using the material extrusion process. It is noted that the fungi used in this study were no longer alive in the

final products, because the last stage of the 3D printing based manufacturing method for biomass–fungi material is drying; during drying, all fungi are killed. Additional details about this material and 3D printing process can be found in a prior publication [24].



Figure 1. As-received biomass–fungi material in a filter patch bag.

2.2. Experimental Procedure

The experimental procedure is shown in Figure 2. First, the biomass–fungi mixtures were prepared. Thereafter, samples were 3D-printed using these mixtures. Next, the print quality was evaluated based on the extrudability and shape stability. Finally, the rheological properties of the biomass–fungi mixtures were characterized.

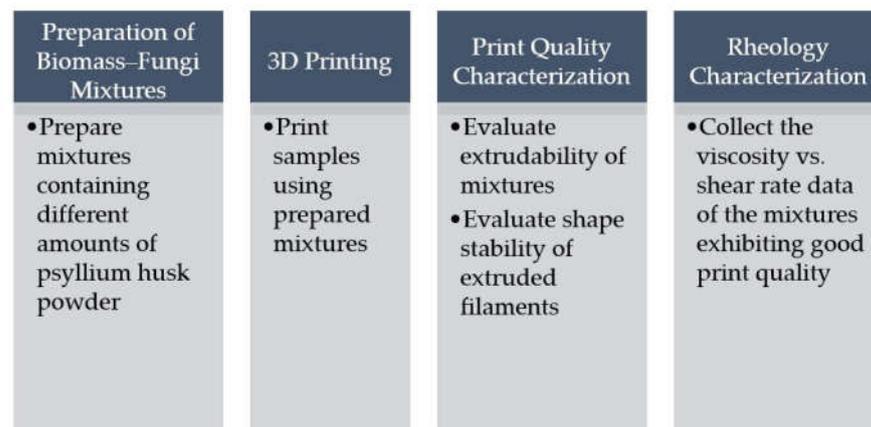


Figure 2. Experimental procedure.

2.3. Preparation of Biomass–Fungi Mixtures

The preparation of the biomass–fungi mixtures comprised two stages.

1. In stage 1, the as-received biomass–fungi material underwent primary colonization. A wheat flour solution was prepared in a beaker by mixing 32 g of wheat flour and 700 mL of water. The mixing was performed manually using a spoon. Thereafter, this solution was poured into the filter patch bag containing the biomass–fungi material. The filter patch bag containing the combination was shaken vigorously by hand for one minute. After this, the filter patch bag was kept in a bucket away from

- sunlight at a temperature of 23 °C for 3–5 days. This stage created a foam-like, dense, primary-colonized, biomass–fungi composite material (see Figure 3a).
- In stage 2, the primary colonized biomass–fungi material was mixed using a commercial benchtop mixer (NutriBullet PRO, Nutribullet). The mixing process was performed in two batches. For each batch, 50 g of the primary colonized material (as shown in Figure 3) was broken off by hand into small chunks. These chunks, along with 200 mL of water (at room temperature) and 20 g of wheat flour, were then mixed in the mixer for 15 s. The mixture container was manually shaken after every 5 s to ensure uniform contact of the mixer blade with the material during the mixing process. Psyllium husk powder (procured from NOW Supplements, USA) was used as an additive to aid printability. After mixing, the primary colonized material, psyllium husk powder, was added into the mixture and mixed manually using a spoon. This mixing process transformed the dense, foam-like primary colonized material into a printable paste-like mixture. Biomass–fungi mixtures with four different compositions were prepared as shown in Table 1.

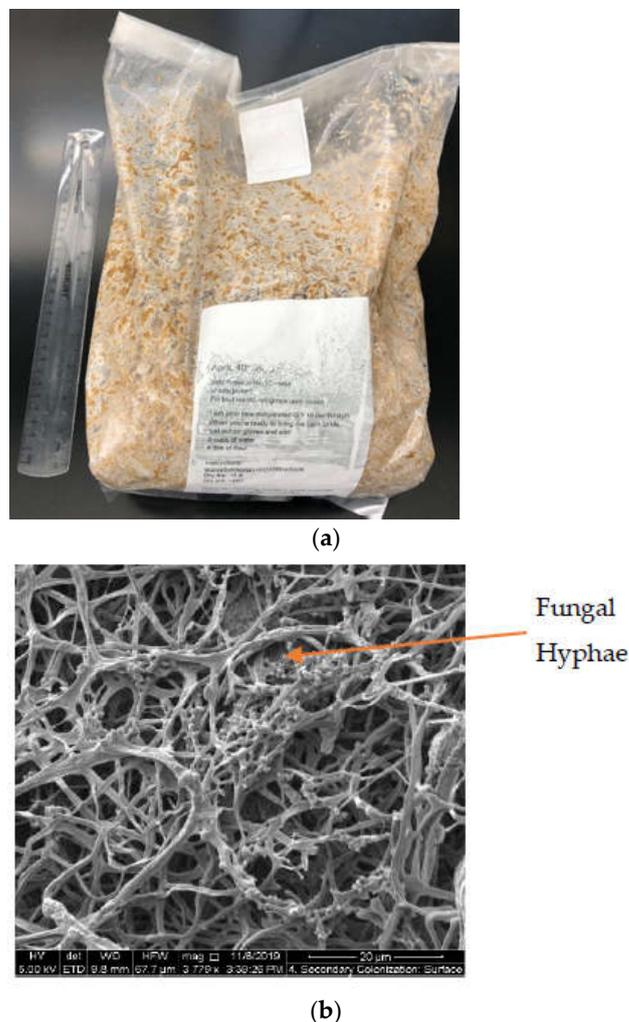


Figure 3. Biomass–fungi material after primary colonization: (a) primary colonized material in filter patch bag; (b) scanning electron microscopy (SEM) image of the surface of a sample after colonization. Scale bar is 20 μm .

To avoid any microbial contamination, the apparatus (such as the 3D printer and beakers) was regularly cleaned with 70 percent ethyl alcohol solution.

Table 1. Mixture compositions.

	Amount of Psyllium Husk Powder (w_p)	Volume of Water (v_w)	Ratio of Psyllium Husk Powder Weight to Volume of Water (w_p/v_w)
Mixture A	0 g		0
Mixture B	10 g	400 mL	1:40
Mixture C	20 g		2:40
Mixture D	30 g		3:40

Primary colonized biomass–fungi material: 100 g; Flour: 40 g.

2.4. 3D Printing

The printer used in this study was a Delta WASP 2040 (Figure 4a) material extrusion 3D printer, which was purchased from Spectrum Scientific (Philadelphia, PA, USA). Material extrusion is defined as a “process in which material is selectively dispensed through a nozzle or orifice” [28]. This printer had a cylindroconical build volume, the cylindrical section of which had a diameter of 20 cm and a height of 40 cm. The conical section was situated on top of the cylindrical section with a base diameter of 20 cm and a height of 30 cm. The printer had a material storage container and an extruder assembly. The mixture used for printing was stored in the material storage container before extrusion and pushed to the extruder assembly using a pneumatically operated piston. The extruder assembly comprised a screw extruder and a casing (Figure 4b). The casing surrounding the screw extruder had a 6 mm nozzle with a square cross-section. Ultimaker Cura software (15.04.2) was used to generate the G-code file used to print the samples.

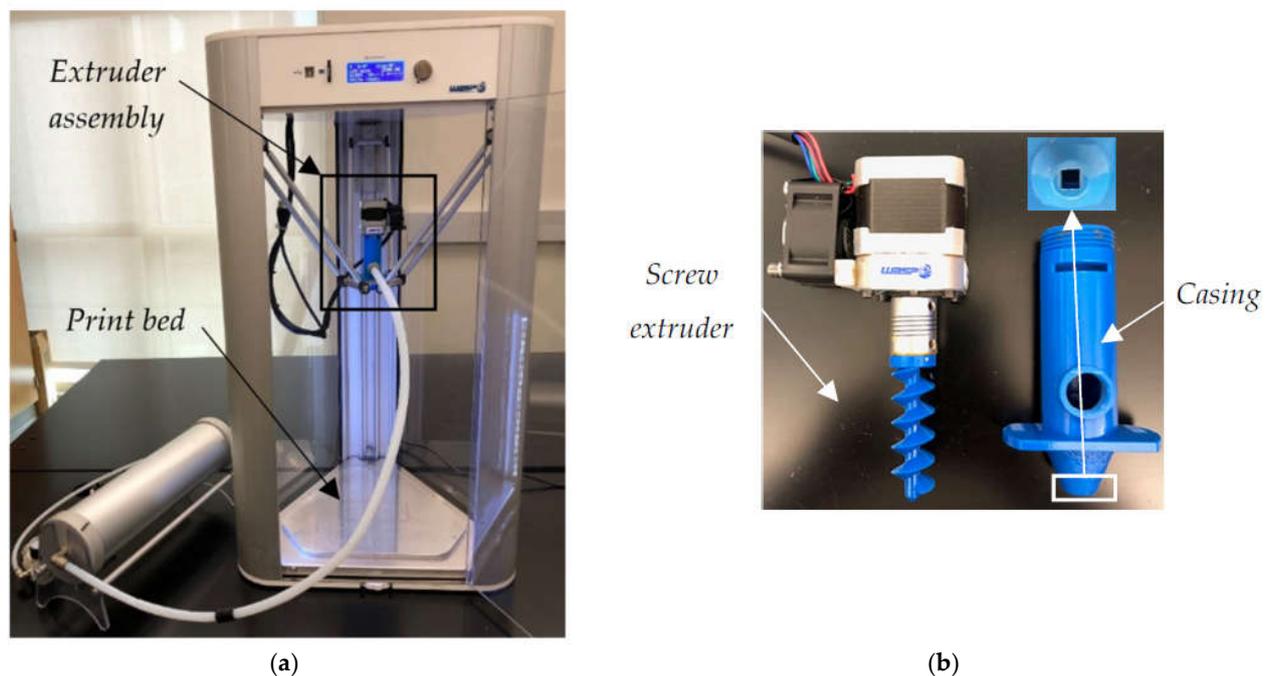


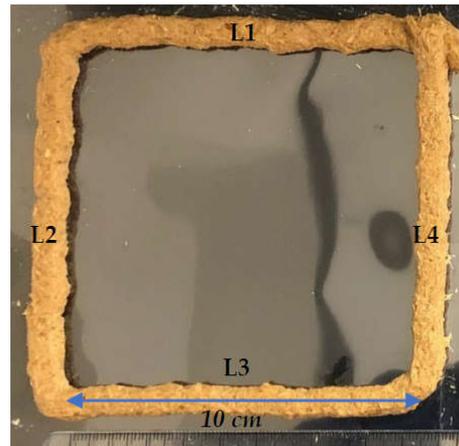
Figure 4. Material-extrusion 3D printer: (a) Delta WASP 2040 printer; (b) extruder assembly, including screw extruder and casing with a square cross-section measuring 6×6 mm.

The print speed was kept constant at 30 mm/s for all experiments. The print speed refers to the speed at which the extruder moves across the print bed (in the X-Y plane) while depositing the material during 3D printing.

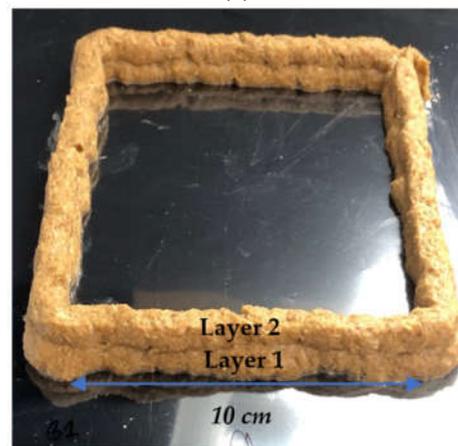
2.5. Print Quality Characterization

In this study, the print quality was assessed using two parameters: extrudability and shape stability. Extrudability is defined as the capacity of the mixture to pass through

the nozzle of the 3D printer [29], which is characterized using two criteria: first, whether the material can be extruded without visible tearing in the extruded filament; second, whether the extruded filament exhibits dimensional consistency. Dimensional consistency was measured by the variation in filament width in the printed sample. A sample was designated to have good dimensional consistency if no more than $\pm 5\%$ variation (from the mean) was observed through the length of the filament [30]. To measure extrudability, two square samples (S1 and S2) were printed. Each sample consisted of four filaments (L1 through L4) with a length of 10 cm each (see Figure 5). Five measurements (width and height) were taken uniformly over the length of each filament.



(a)



(b)

Figure 5. Printed samples used for assessing print quality: (a) extrudability sample; (b) shape stability sample.

In addition to extrudability, the deposited filaments must also possess sufficient strength to support the successive layers during the printing process. The deposited layer is subjected to deformation from three sources: (i) self-weight; (ii) weight of the subsequent layers deposited on top of it; (iii) extrusion pressure [30]. In this study, shape stability refers to the ability of the extruded filaments to resist deformations. (This concept was used by other researchers. For example, Kazemian et al. [30] used shape stability to refer to the ability of the concrete material to resist deformation during layer-wise construction [30]). To quantify the shape stability, two square samples (S3 and S4) containing two layers (10 cm long) were printed. The initial height of layer 1 right after deposition was recorded from samples S1 and S2. For samples S3 and S4, the height of layer 1 was recorded 20 min after the deposition of layer 2 (see Figure 5). The mixture was characterized to have good shape stability if the average height of layer 1 after deposition of layer 2 was within $\pm 5\%$ of

the initial height. The filament width and height were measured using a digital caliper (Mitutoyo, CD-8 ASX; Accuracy: ± 0.03 mm).

2.6. Rheological Characterization

The rheological characterization of the biomass–fungi mixtures was conducted using a TA Instruments DHR-2 rheometer. The gap between the base Peltier plate and the flat plate (8 mm in diameter) was 500 μm (see Figure 6). The shear rate was changed from 0.01 to 2000 s^{-1} . The shear thinning behavior of the mixtures was characterized by plotting a viscosity vs. shear rate curve. The shear elastic modulus (G'), shear loss modulus (G''), and loss tangent (i.e., $\tan(\delta)$) were also measured, where δ is the phase angle of the material. The loss tangent ($\tan(\delta)$) is the ratio of the shear loss modulus (G'') to the shear elastic modulus (G'), as shown in Equation (1). This ratio determines the liquid-like or solid-like nature of a mixture [25,31]. Mixtures behaving in the same manner as solids would fail to extrude through the printer nozzle. Alternately, mixtures exhibiting dominant liquid characteristics would not be printable for multiple layers, as the extruded layers would collapse as soon as the second layer was printed on top of them.

$$\tan(\delta) = \frac{\text{Shear Loss Modulus, } G''}{\text{Shear Elastic Modulus, } G'} \quad (1)$$

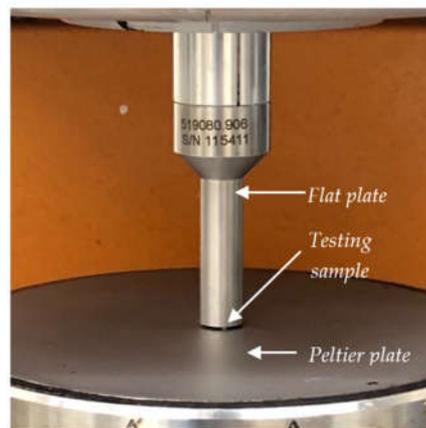


Figure 6. Apparatus used for rheological characterization.

Frequency sweep testing ranging from 0.1 to 100 rad/s was used for the measurement of these moduli and loss tangent parameters. All measurements were conducted at 25 $^{\circ}\text{C}$. At least five test runs were performed to characterize the rheological properties of each mixture (for each composition). In this paper, the average of the five test runs is reported for each mixture.

3. Results and Discussion

3.1. Effects of Psyllium Husk Powder Content on Extrudability

Mixture A (without any psyllium husk powder) was not extrudable. As shown in Figure 7a, the piston in the material container pushed the water out of the biomass–fungi mixture. This water was deposited on the print bed. The non-printable biomass–fungi mixture was left as a dry puck in the material storage container, as shown in Figure 7b.

Figure 8 shows the printed samples using mixture B (wp/vw = 1:40), mixture C (wp/vw = 2:40), and mixture D (wp/vw = 3:40). The extruded filaments of mixtures B and C, as shown in Figure 8a,b, did not show tearing. The average filament width was 9.57 mm for mixture B, while this was 8.68 mm for mixture C. Filaments from both mixtures exhibited good dimensional consistency as well. The average variations in filament width were 1.87% for mixture B and 2.5% for mixture C. These variations were within the $\pm 5\%$ variation

range defined for good extrudability. The detailed data for the width measurements of samples S1 and S2 are shown in Table 2.

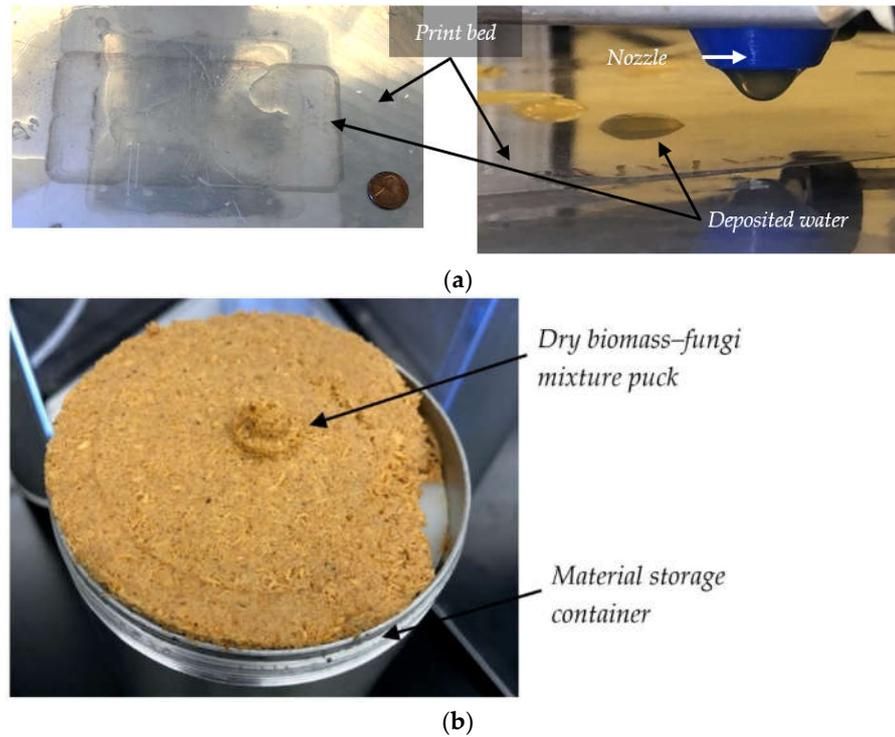


Figure 7. Printing results for mixture A: (a) only water from the biomass–fungi mixture was deposited during the printing process; (b) non-extrudable, dry biomass–fungi mixture puck left behind.

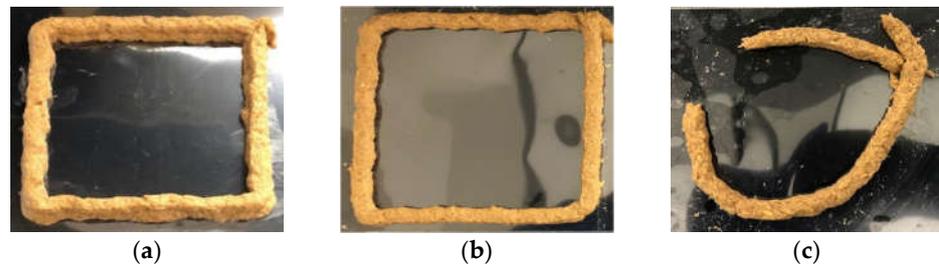


Figure 8. Printed samples using three mixtures with different levels of psyllium husk powder: (a) mixture B (wp/vw = 1:40); (b) mixture C (wp/vw = 2:40); (c) mixture D (wp/vw = 3:40).

3.2. Effects of Psyllium Husk Powder Content on Shape Stability

Figure 9 shows height data for layer 1 after depositing layer 2 for samples printed using mixtures B and C. The error bars indicate the standard deviation of the respective samples in Figure 9. The reductions in height for layer 1 were 11% for the samples printed using mixture B and 5% for the samples printed using mixture C. Samples printed using mixture C showed much better shape stability than samples printed using mixture B.

To summarize, mixtures A and D performed poorly in the extrudability test; therefore, they did not present shape stability data. Mixture C exhibited the best print quality in terms of both extrudability and shape stability. Mixture B displayed good extrudability, although its shape stability was not as good as mixture C.

Table 2. Filament width data for samples S1 and S2 printed using Mixtures B and C.

Mixture B (wp/vw = 1:40)									
Test Run	S1				S2				Average
	Filament Width (mm)				Filament Width (mm)				
	L1	L2	L3	L4	L1	L2	L3	L4	
1	8.30	10.44	9.15	9.77	10.69	10.12	9.19	9.96	
2	9.56	9.50	9.18	9.78	10.04	9.10	9.24	9.72	
3	9.82	10.50	8.91	9.65	9.93	10.6	8.81	9.13	
4	9.94	9.45	9.16	9.26	9.89	8.95	9.68	9.16	
5	10.66	9.41	9.95	9.61	8.78	8.71	9.38	9.72	
Average Width (mm)	9.66	9.86	9.27	9.61	9.87	9.50	9.26	9.54	9.57
Variation from Average Width (%)	0.90	3.03	3.13	0.46	3.09	0.77	3.24	0.33	1.87

Mixture C (wp/vw = 2:40)									
Test Run	S1				S2				Average
	Filament Width (mm)				Filament Width (mm)				
	L1	L2	L3	L4	L1	L2	L3	L4	
1	8.60	9.13	8.97	9.41	8.31	8.00	8.13	8.20	
2	8.56	8.66	8.21	9.80	9.04	8.49	8.71	8.54	
3	9.19	8.88	8.61	8.84	9.55	8.36	8.01	8.59	
4	8.56	8.46	8.87	9.10	8.01	8.47	8.31	8.84	
5	9.20	9.09	8.64	8.98	8.32	8.72	7.80	8.88	
Average Width (mm)	8.82	8.84	8.66	9.23	8.65	8.41	8.19	8.61	8.68
Variation from Average Width (%)	1.64	1.89	0.23	6.29	0.39	3.13	5.62	0.81	2.50

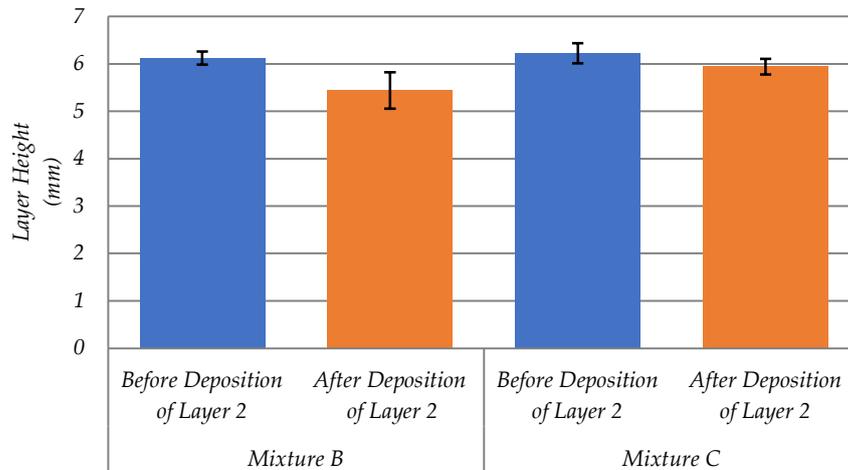


Figure 9. Height changes in layer 1 after depositing layer 2.

3.3. Effects of Psyllium Husk Powder Content on Rheological Properties

Figure 10 shows viscosity versus shear rate plots for all the mixtures. These plots show that the mixtures had shear thinning behavior. Shear thinning refers to the behavior of non-Newtonian fluids, whose viscosity decreases with increasing shear rate. For mixtures A, B, and C, increasing their psyllium husk powder content led to a reduction in the viscosity of the mixtures. Not much difference was observed in the viscosity of mixtures C and D.

As the psyllium husk powder content was increased, the elastic modulus of the biomass–fungi mixtures was reduced for mixtures A, B, and C (see Figure 11). Further addition of psyllium husk powder resulted in a 37% average increase of G' for mixture D as compared to Mixture C.

The shear loss modulus (G'') represents the viscous nature of a mixture. An increase in the psyllium husk powder content in the mixtures resulted in decreased G'' for mixtures A, B, and C (see Figure 12). Mixture D showed a loss modulus similar to that of mixture C

This was expected, since mixtures C and D had similar viscosity characteristics, as shown in Figure 10.

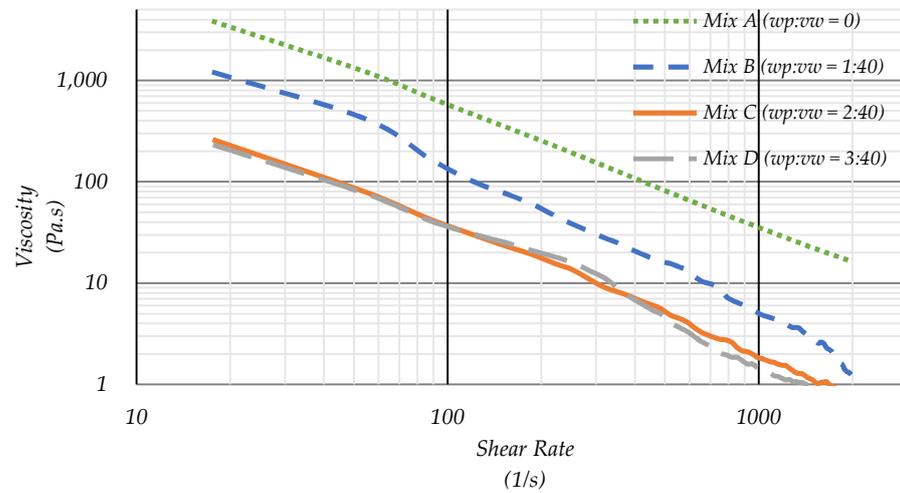


Figure 10. Effects of psyllium husk powder content on the rheological behavior of the biomass–fungi mixtures.

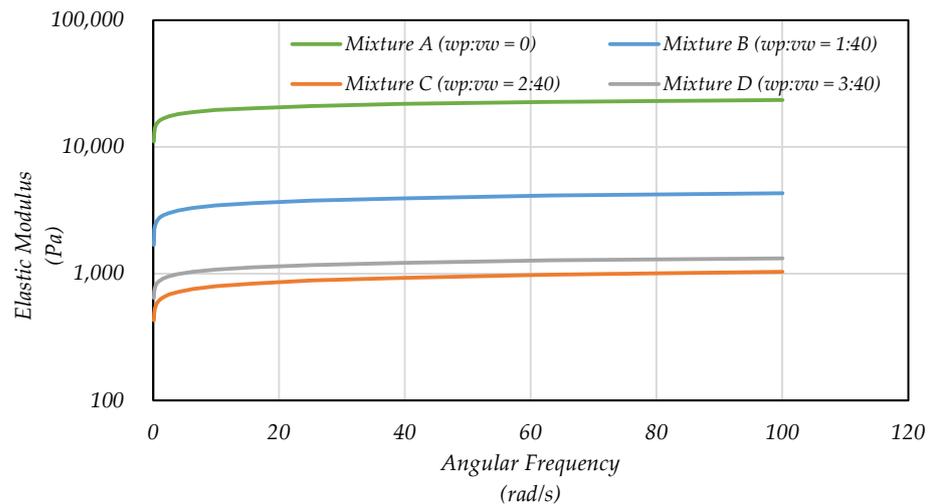


Figure 11. Relationships between elastic modulus (G') and angular frequency for four biomass–fungi mixtures.

Loss tangent $\tan(\delta)$ values less than 1 are related to solid-like properties, while values greater than unity are associated with liquid-like properties. Increasing the psyllium husk powder content increased the loss tangent $\tan(\delta)$, with values of 0.19, 0.26, and 0.28 for mixtures A, B and C, respectively (see Figure 13); therefore, as more psyllium husk powder was added, the mixture went from being non-extrudable (mixture A) to printable (mixture C). Further addition of psyllium husk powder resulted in a reduction of the $\tan(\delta)$ value to 0.23, which represented the increased solid-like behavior of mixture D as compared with mixtures B and C. This led to the observation of tearing in the extruded filament for mixture D. While mixture D was extrudable, mixture A was not. This could be attributed to the fact that mixture D had a higher $\tan(\delta)$ value (0.23) than mixture A (0.19).

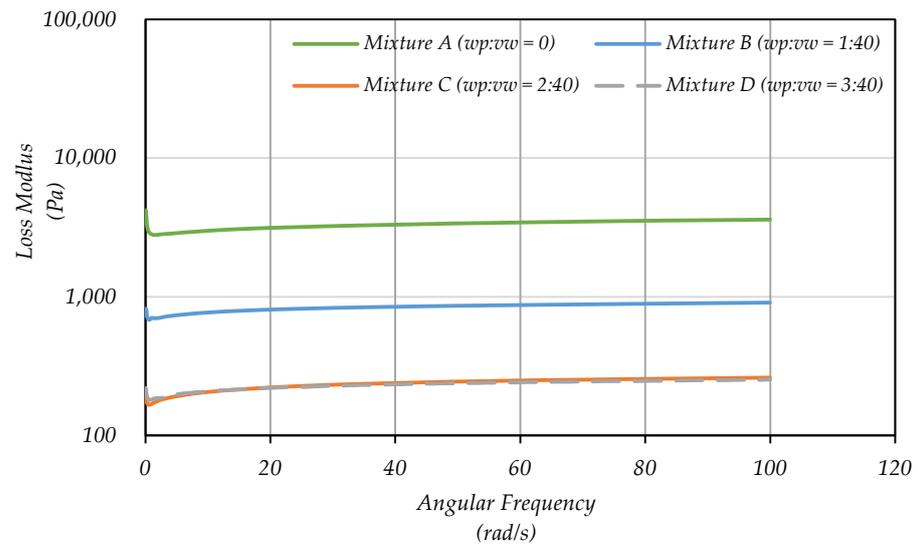


Figure 12. Relationships between the loss modulus (G'') and angular frequency for four biomass–fungi mixtures.

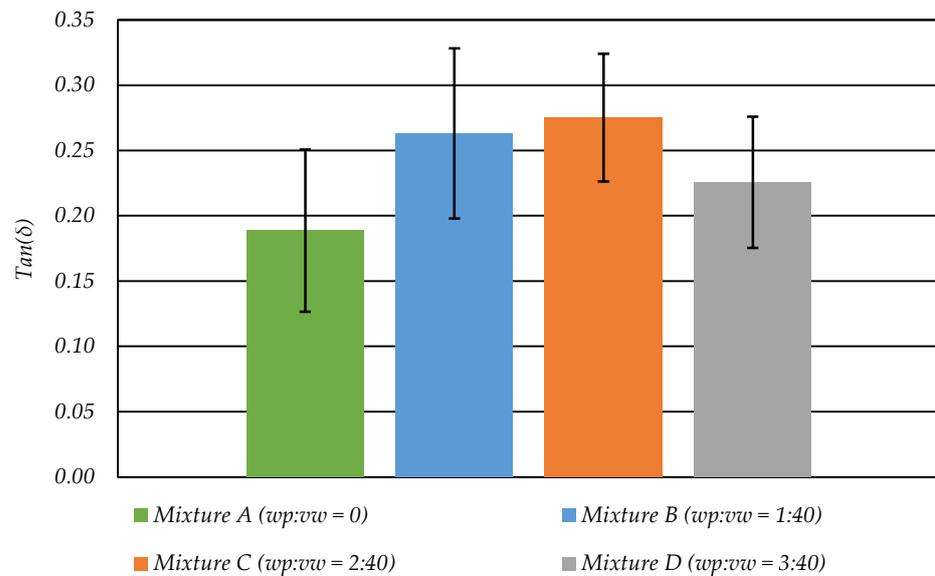


Figure 13. Loss tangent values for the mixtures.

4. Concluding Remarks

This paper has reported on an experimental study of 3D printing of a new class of sustainable materials for applications in the construction and packaging industries. Specifically, the study was focused on the effects of the mixture composition (the content of psyllium husk powder) on the print quality and rheological behavior. The main conclusions are as follows:

- The mixture containing no psyllium husk powder could not be extruded;
- Mixtures containing wp/vw ratios of 1:40 and 2:40 exhibited good extrudability (i.e., deposited filaments showed no tearing);
- The mixture containing wp/vw = 2:40 was the only mixture that met the shape stability criteria for print quality;
- When the wp/vw ratio was increased to 3:40, the mixture did not show good extrudability (printed filaments were not continuous). This performance was reflected in the increase in solid-like behavior of the mixture as observed in the loss tangent, i.e., $\tan(\delta)$ data;

- Mixture B (wp/vw = 1:40) showed higher viscosity than mixture C (wp/vw = 2:40). Both mixtures showed shear thinning behavior.

Future studies will include SEM characterization of the biomass–fungi mixtures and printed samples. Furthermore, the effects of elapsed time after mixture preparation on mixtures' rheology and printability will also be analyzed.

Author Contributions: Methodology, A.B., A.M.R. and X.W.; investigation, A.B., A.M.R. and D.T.; writing—original draft preparation, A.B.; writing—review and editing, A.B., Z.P., A.M.R., X.W., D.T., N.Z. and M.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The authors confirm that the data to support the findings of this study are available within the article or upon request to the corresponding author.

Acknowledgments: Use of the Texas A&M University Soft Matter Facility are acknowledged.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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