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Development and Characterization of Biomaterials Produced with Fungal Mycelium on Agroindustrial Residues

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Abstract

Production of synthetic plastics has increased substantially and shows no global signs of decline despite environmental concerns; today, annual production exceeds 450 million tons. In the search for more sustainable alternatives, several approaches have been explored, including biomaterials based on fungal mycelium combined with organic agricultural waste. In this study, the influence of two types of agricultural waste on the properties of a mycelium-based biocomposite was investigated. Two experiments were conducted using *Pleurotus ostreatus*, chopped banana leaves, coffee husks, and a 1:1 mixture of these two residues. The fungus colonized the substrates, binding the dispersed particles and giving rise to versatile materials. The colonized samples were then oven-dried, cut to size, and subjected to mechanical testing, swelling tests, scanning electron microscopy, and colorimetric analysis. The resulting materials exhibited low density, compressive strength, Young's modulus, and swelling degree, with values ranging from 130 to 254 kg/m^3, 29 to 268 Pa, 64 to 88 Pa, and 39 to 45%, respectively. The results show that the physical and mechanical properties of different types of agricultural waste strongly influence the properties of the resulting mycelium-based biocomposite, highlighting its potential for customization in sustainable applications as an alternative to synthetic plastics.

Keywords: *Pleurotus ostreatus*; biocomposites; sustainability; coffee husk; banana leaves

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1. Introduction

The increasing production and consumption of petroleum-based plastics have become a major environmental challenge worldwide. Although plastics are indispensable in numerous industrial sectors due to their versatility, durability, and low production cost, their end-of-life management remains inadequate [1]. Currently, less than 10% of the plastics produced globally are effectively recycled, while the majority accumulates in landfills or is released into terrestrial and aquatic ecosystems, where it may persist for

decades or even centuries [2]. This scenario has intensified the search for renewable, biodegradable, and environmentally friendly materials capable of replacing conventional fossil-based plastics [3].

In parallel, the agro-industrial sector generates substantial amounts of lignocellulosic residues, many of which remain underutilized or improperly disposed of, resulting in environmental, economic, and social concerns [4]. Brazil, one of the world's largest agricultural producers, annually generates millions of tons of agricultural by-products and residues during the cultivation, processing, and commercialization of crops [5]. Among these residues, coffee husks and banana biomass are particularly abundant. Coffee processing account for about 45–55% of 1.2 million tons of annual waste, whereas banana cultivation generates considerable amounts of leaves, peels and pseudostems generally discarded after harvesting, representing around 50% of 99.640 tons of annual waste [6,7]. Therefore, the valorization of agro-industrial residues through circular bioeconomy strategies has emerged as an effective approach to reducing waste generation while producing value-added materials as plastic substitutes [4]. These residues are also gaining increasing attention as substrates for fungal cultivation, which provide nutrients and structural support for mycelial growth [8].

In this context, fungi of the *Pleurotus genus* are widely recognized for their ability to grow on a broad range of lignocellulosic substrates, particularly agro-industrial residues [9,10] such as coffee husks [11,12] and banana leaves [13,14]. Previous studies have demonstrated the feasibility of producing mycelium-based composites, mycoblocks, using *Pleurotus ostreatus* cultivated on substrates such as sawdust, sugarcane bagasse, and coffee husk [15]. However, despite these advances, the combination of different substrates for the development of functional materials intended for applications such as thermal insulation panels, packaging materials, and furniture components remains insufficiently explored [16–18]. This underscores the potential of valorizing renewable feedstocks to enhance sustainability in the field of material science [19,20].

Therefore, this study aims to investigate the influence of coffee husks and banana leaves on the development of mycelium-based biomaterials produced using *Pleurotus ostreatus*. By evaluating different substrate compositions, including individual and combined residues, this work assesses their effect on the physical, mechanical, and morphological characteristics of the resulting materials. Ultimately, this research intends to contribute to the advancement of sustainable material development by exploring the potential of lignocellulosic residues as substrates for biodegradable, low-cost, and environmentally friendly alternatives to conventional plastics.

2. Materials and Methods

This study was performed in the Laboratory of Mycorrhizal Association of the Instituto (Bioagro), in the Packaging Laboratory of the Department of Food Technology, and in the Mechanical Testing Laboratory all at the Universidade Federal de Viçosa (UFV).

2.1. Fungal Isolates

Pleurotus ostreatus strain PLO 02 from the fungal collection of the Laboratory of Mycorrhizal Association was used in this study.

Mycelial discs were inoculated from already grown PLO 02 samples onto Petri dishes containing potato dextrose agar (PDA) medium at 25 ± 2 °C for 7 days. Then, the mycelia were divided into eight sections, and each one was transferred to autoclaved glass flasks containing sorghum seeds. These colonized seeds served as solid inocula to produce the biocomposites.

2.2. Mycelium Composite Manufacturing

The substrates used to produce the biocomposites were banana leaves, coffee husks, and 1:1 (*w*/*w*) mixture of these residues. Prior to use, the raw materials were pretreated as follows. The banana leaves were immersed in water for 1 h, rinsed, and centrifuged (CRB1 Britânica) at 1200 rpm for 1 min at room temperature. The coffee husks were immersed and boiled in water for 1 h, rinsed, and then centrifuged to remove excess water. The above-mentioned substrates held no form without fungal colonization; so, after molding them in polypropylene bags and removing them, they fell apart. For this reason, no uninoculated control was carried out, as it would have been impossible to evaluate its mechanical properties under the same conditions.

2.2.1. Experiment 1: Assessment of Growing Parameters and Substrate Usage Viability for Biocomposite Production

This experiment followed a completely randomized design, with 3 treatments (n = 5), totaling 15 samples, in which the independent variable was the substrate type. Accordingly, polypropylene (PP) growth bags with 0.2 µm breathing filters were filled with 500 g of banana leaves (BA), coffee husks (CA), or a mixture containing 250 g of each residue (CB) and sealed. Then, the bags were autoclaved twice at 121 ± 2 °C, for 40 min, with an interval of 72 h between cycles. After sterilization, the substrates were inoculated with 50 g of sorghum seeds colonized with PLO 02 under a laminated flow hood and incubated at room temperature (25 ± 2 °C), in the dark, for 15 days.

Subsequently, the polypropylene bags were removed to allow surface mycelium growth, and the samples were kept for 5 additional days under the same conditions. The resulting biomaterials were dried in a forced-air oven (Fanem 315-SE, São Paulo, Brazil) at 100 ± 2 °C for 2 h to inactivate any microorganisms present in the substrate, while preserving the structural integrity of the hyphae [21]. The biomaterials were then stored in polystyrene boxes for 7 days, under laboratory room temperature (22 ± 2 °C) in the dark until further processing. Due to the biological nature of the composite and cultivation method, surface contaminants growth was expected, as discussed later in Section 3.1. After storage, the biomaterials surfaces were lightly scraped using a sterile laboratory spatula to remove superficial contaminants. This was followed by an additional drying step in a forced-air oven for 1 h at 100 ± 2 °C to inactivate any microbial agents that had developed growth on the surface of the biomaterials.

2.2.2. Experiment 2: Evaluation of Physical and Mechanical Aspects of the Biocomposites

Based on the results of Experiment 1, cylindrical polyvinyl chloride (PVC) molds with an internal diameter of 4 cm and a length of 10 cm were filled with the same substrates used previously (n = 7). The molds were sealed with filter paper at the top to allow air flow, with PVC plastic wrap at the bottom, and then placed inside PP bags with 0.2 µm breathing filters, separated by treatment. The molds containing the substrates were autoclaved twice at 121 ± 2 °C for 40 min, with a 24 h interval between cycles. After sterilization, the samples were inoculated with 4 g of inoculum and incubated in the dark at 25 ± 2 °C for 20 days. Subsequently, the biomaterials were removed from the molds and dried in a forced-air oven (Fanem 320-SE), at 70 °C ± 2, for 4 h. The samples were stored in open trays under ambient conditions until further testing.

2.3. Material Characterization

2.3.1. Dimensions and Density

In Experiment 1, rectangular samples (approximately 3 cm × 3 cm × 3 cm), were cut from random specimens of each treatment. For Experiment 2, all specimens from

each treatment were measured with a ruler and caliper. The mass of the samples in each experiment was determined using a precision balance (MARTE AD1000), and the density was subsequently calculated using the following formula:

$$D = \frac{m}{V} \quad (1)$$

where

- D: density (kg/m^3);
- m: mass (kg);
- V: volume (m^3).

2.3.2. Test Method for Measuring Mechanical Properties

Seven samples from each treatment were used to investigate the mechanical behavior through the stress–strain curve. The samples had a cylindrical shape, with a length of 80 mm and a diameter of 40 mm.

Each sample had its dimensions measured to determine the original length and cross-sectional area. To evaluate the mechanical behavior, after a series of preliminary tests, it was decided to use the Standard Test Method for Measuring Compressive Properties of Thermal Insulations, according to ASTM C165-07 [22].

To determine the stress–strain curve of each sample, a monotonic load was applied through a continuous displacement of the machine crosshead at a rate of 7.2 mm/min. To ensure proper load distribution, the samples were accommodated between two plastic caps when placed in the machine. The force response of the sample was measured using an EMIC SV-50 (EMIC (INSTRON), São José dos Pinhais, Brazil) load cell with a capacity of 500 N. The corresponding shortening was measured using an HBM 1-WA/100MM-T displacement transducer, with a stroke of 100 mm (Figure 1). The adopted sampling rate was 5 Hz.



Figure 1. (**1**) Aspect of the mechanical test setup and (**2**) biomaterial sample from Experiment 2 allocated between two plastic caps for better load distribution.

With the initial length of the sample and the cross-sectional area known, the compressive stress and longitudinal strain were calculated using the following equations:

$$\sigma = \frac{F}{A} \quad (2)$$

$$\varepsilon = \frac{\delta}{L_0} \quad (3)$$

where

- L_0: initial sample length;
- A: cross-sectional area of the sample;
- F: applied compressive force;
- δ: shortening;
- σ: normal compressive stress;
- ε: normal strain (change in length over original length L_0).

2.3.3. Colorimetric Assay

The surface color of the samples was measured using a spectrophotometer (ColorQuest XE, HunterLab, Reston, VA, EUA), according to the protocol described in Espitia et al. (2013) [23]. The color attributes—lightness (*L), red–green coordinate (*a), and yellow–blue coordinate (*b)—were determined based on the CIELAB scale (10° observer/D65 illuminant). The total color difference (ΔE) and opacity were calculated using a standard white reference (L^* = 93.44; a^* = −0.63; b^* = 1.21), in accordance with ASTM E308-08 [24].

2.3.4. Scanning Electron Microscopy (SEM)

The surface morphology of the samples was analyzed using an electron microscope (TM3000, Hitachi, Tokyo, Japan). For each treatment, a sample was fixated to a metal support, held by a carbon double sided tape. The images were obtained under vacuum conditions at magnifications of 100× and 1000×. This analysis was conducted at the Laboratório de Embalagens of the Department of Food Technology at the Universidade Federal de Viçosa.

2.3.5. Swelling Degree Measurement

This methodology was adapted from Espitia et al. (2013) [23]. Samples (3 cm × 3 cm × 3 cm) were previously weighed (m_i) and placed in beakers containing 100 mL of ultrapure water, where they were kept for 2 h at room temperature (25 ± 2 °C). Then, excess surface water was gently removed using paper towels, and the soaked samples were weighed (m_f) again. The swelling degree (SD) was determined from the weight measurements before and after submersion, following the formula below:

$$SD = \frac{m_f - m_i}{m_f} \times 100 \quad (4)$$

where

- m_i: initial mass;
- m_f: final mass.

3. Results and Discussion

3.1. Composite Growth

3.1.1. Experiment 1: Assessment of Growing Parameters and Substrate Usage Viability for Biocomposite Production

This experiment involved the production of biocomposites composed of coffee husks, banana leaves, and a mixture of those two residues, bound by *Pleurotus ostreatus* mycelium. Rapid fungal growth was observed, resulting in the progressive colonization of all substrates. By the fourth day of incubation, all treatments showed healthy mycelial growth, although treatments CA and CB (Figure 2(2)) exhibited more advanced mycelia colonization compared to treatment BA, likely due to differences in substrate distribution affecting the nutrient availability, porosity, and lignocellulosic accessibility [25]. It is also important to note that this study did not conduct experiments to address specific substrate chemical

composition; however, coffee husks and banana leaves are mostly composed of cellulose, hemicelullose and lignin, with a few trace elements (K, Ca, Fe) [26,27] which may vary according to the environment conditions in which the plants were cultivated. The growth rate shown, as well as the fungus adaptability to the different substrates was expected, as *Pleurotus ostreatus* is categorized in the white-rot fungus ecological group, characterized by the synthesis of lignocellulolytic enzymes that generate white-colored cellulosic remnants after substrate degradation [28,29], as seen in Figure 2.

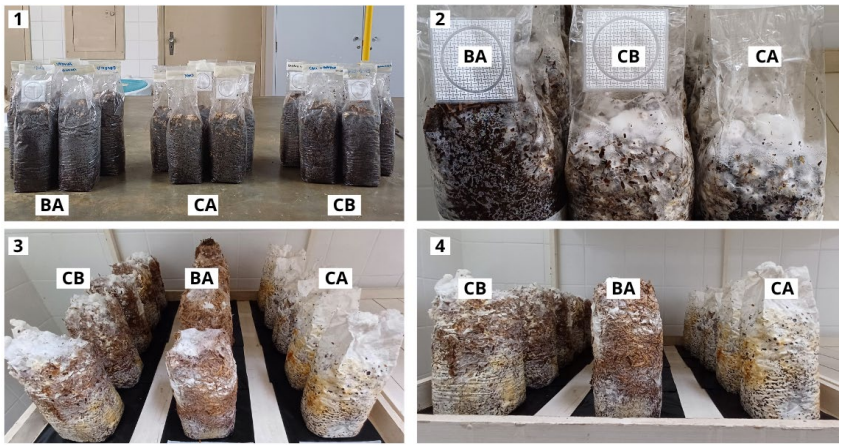


Figure 2. Experiment 1 biocomposites made with *P. ostreatus* and coffee husks (CA), *P. ostreatus* and banana leaves (BA) and *P. ostreatus* and a mixture of the previous residues (CB). (**1**) Biocomposites on the first day of incubation; (**2**) early colonization, fourth day after inoculation; (**3**,**4**) biocomposites after PP bag removal, top and front view respectively. Moreover, expressive mushroom production occurred, especially in treatments CA and CB (Figure 3). The fruiting bodies were removed, and the biocomposites were dried. Mushroom development occurred probably due to mechanical and thermal stress caused by the removal of the PP bags and the abrupt change in environmental conditions outside the incubation chamber.

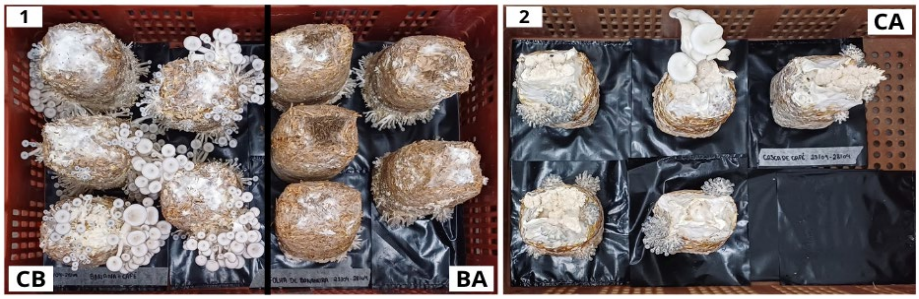


Figure 3. Primordia development on Experiment 1 samples (n = 5). (**1**) *P. ostreatus* and the mixture of both substrates (CB), *P. ostreatus* and banana leaves (BA) and (**2**) *P. ostreatus* and coffee husks (CA).

Complete substrate colonization was achieved by day 16 in all samples, with CA showing the most vigorous mycelia development, which was assessed visually and by handling the samples. At this stage, primordia formation was also observed (Figure 2(3)), indicating the transition from vegetative growth to reproductive development under favorable environmental conditions [30]. After removal of the PP bags, the substrates were incubated for additional five days. Although no further superficial mycelia expansion was observed, primordia development became evident (Figure 2(4)).

Reproductive structures' growth in *P. ostreatus* cultivation is often induced by different types of stress, such as temperature drops, and although there were no major temperature fluctuations, the composite handling and removal of polypropylene bags might have been a source of enough mechanical stress to induce mushroom development (Figure 3). This

aspect can be further confirmed when we address the literature on mushroom cultivation, as it is a common practice to wait for full substrate colonization and remove the containment bags before fruiting body development [12,25,31]. Mushroom formation is not ideal for the development of a final biocomposite based product, as the organism diverts energy from vegetative growth (mycelium growth) to the development of fruiting bodies, which may result in a less robust colonization that might culminate in less structurally sound material.

Seven days after the inactivation of *P. ostreatus*, the biocomposites absorbed moisture uptake from the environment, likely driven by their intrinsic hygroscopic nature and exposure to ambient relative humidity. This moisture accumulation may have create favorable conditions for secondary microbial colonization on the material surface (Figure 4). The filamentous fungi observed were identified as *Penicillium*, *Mucor*, *Rhizopus* and *Aspergillus*, genera commonly associated with organic matter decomposition, contamination and spoilage in edible mushroom cultivation and lignocellulosic substrates under similar environmental conditions [31–33]. At this stage of the production process while designing a shelf stable product, growth of the cited fungal genera is not acceptable. However, it also highlights one of the advantages this material has over traditional fossil fuel-derived plastics, which is its rapid degradability in the environment, making it a good sustainable alternative if undesirable fungal growth is halted for the duration of the product life cycle.

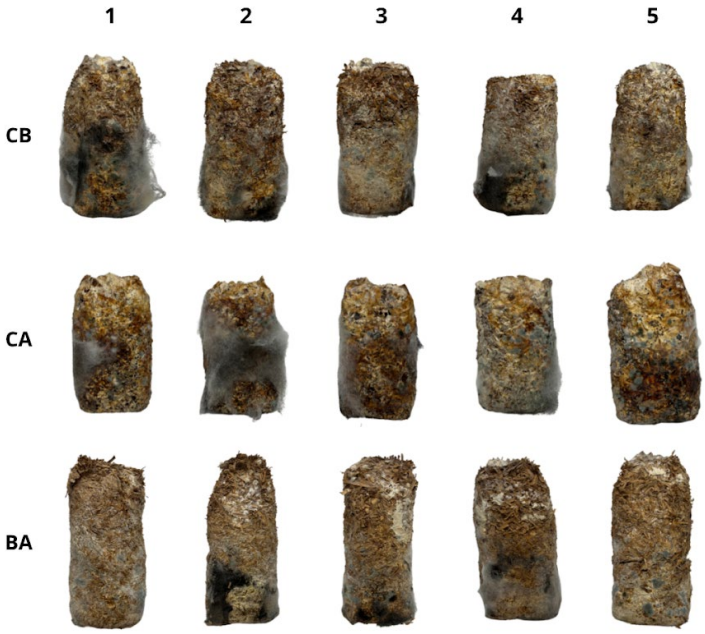


Figure 4. Contaminant growth on the surface of the biocomposites samples (**1**–**5**) from Experiment 1. *P. ostreatus* and banana leaves (BA), *P. ostreatus* and coffee husks (CA) and *P. ostreatus* and a mixture of the previous residues (CB).

Overall, these results demonstrate the dual nature of mycelium-based biocomposites, combining promising bioconversion of agro-industrial residues with pronounced responsiveness to environmental conditions, which must be carefully controlled to ensure material stability and performance. Based on these findings, the growth and drying conditions were changed to primarily avoid mushroom development and surface contaminant growth.

3.1.2. Experiment 2: Evaluation of Physical and Mechanical Aspects of the Biocomposites

For Experiment 2, the growth parameters were adjusted based on our findings with Experiment 1, avoiding fruiting body formation and contaminant growth. *P. ostreatus* colonization of the substrates was monitored for 20 days (Figures 5 and 6), followed by a drying protocol and mechanical testing. Visually, colonization followed a similar pattern to

that observed in Experiment 1, although with a more heterogeneous distribution across the substrates. In all samples, the upper region, which was in direct contact with the inoculum, was rapidly colonized, while hyphen propagation toward the lower portions occurred more slowly and was only completed toward the end of the incubation period.

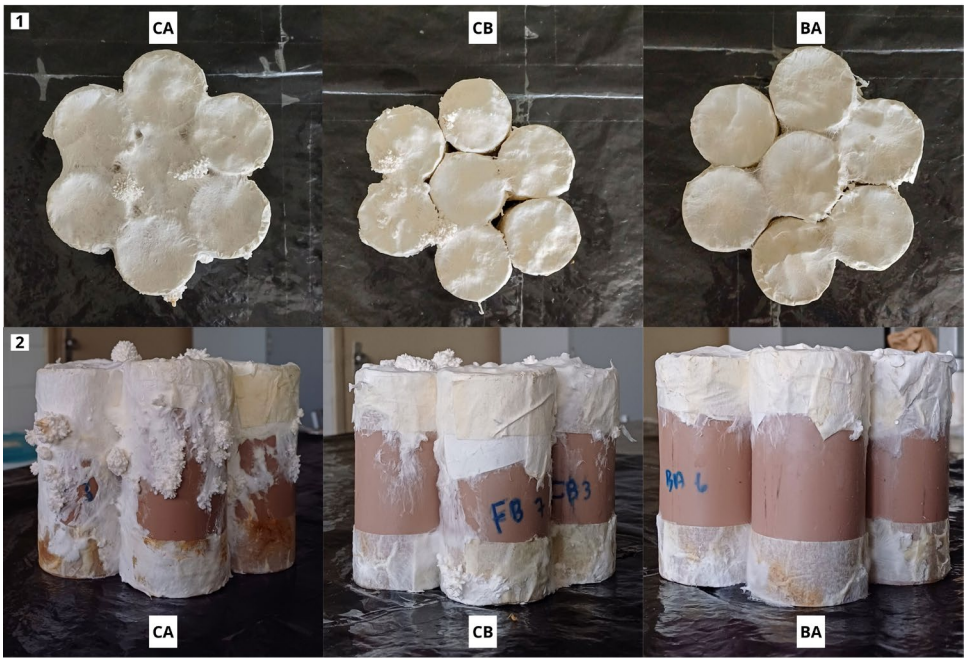


Figure 5. (**1**) Transversal and (**2**) frontal view of the molds from Experiment 2 (n = 7). *P. ostreatus* and banana leaves (BA), *P. ostreatus* and coffee husks (CA) and *P. ostreatus* and a mixture of the previous residues (CB).

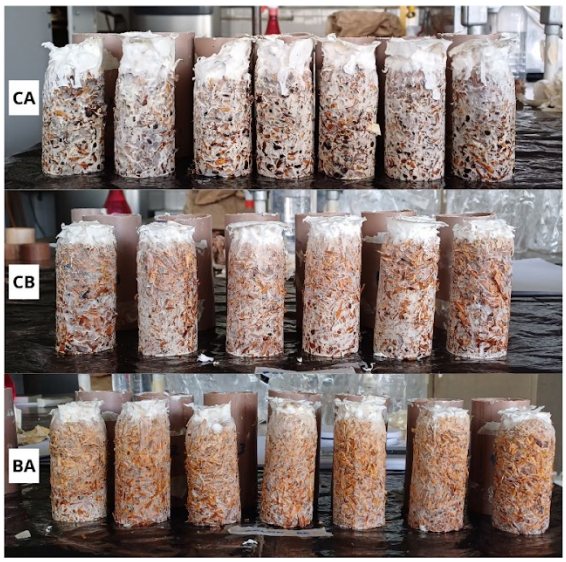


Figure 6. Side view of the samples from Experiment 2 after taking them out of the molds. *P. ostreatus* and banana leaves (BA), *P. ostreatus* and coffee husks (CA) and *P. ostreatus* and a mixture of the previous residues (CB).

This vertical gradient in colonization may be attributed to limitations in oxygen diffusion, moisture distribution, and nutrient accessibility within the compacted substrate matrix, which are known to influence mycelial growth dynamics in solid-state systems [31,33]. Similar spatial heterogeneity has been reported in mycelium-based composites and mush-

room cultivation systems, where colonization is typically initiated at inoculation points and progresses according to local environmental conditions and substrate porosity [34].

Moreover, the external mycelial growth of *P. ostreatus* was observed to bind the molds together, suggesting active hyphen extension beyond the substrate surface. This behavior suggests that the fungus was capable of partially degrading the cellulose-based filter paper used as a sealing material, consistent with the known enzymatic capacity of *P. ostreatus*. This phenomenon was more pronounced in the CA treatment (Figure 6), possibly due to enhanced enzymatic activity associated with the substrate composition and higher fungal vigor [34]. These results demonstrate that both the substrate architecture and environmental limitations critically shape mycelial growth behavior, including its ability to extend beyond the substrate and interact with surrounding materials.

3.2. Density

Treatment CA presented the lowest density (190.72 kg/m^3), followed by CB (202.47 kg/m^3) and BA (254.35 kg/m^3). The higher density observed in BA may be associated with its higher moisture content. As banana leaves tend to occupy a larger volume, the resulting biomaterials were larger in size compared to the other treatments, which may have led to non-uniform drying and, consequently, higher residual moisture and density. Additionally, differences in the particle packing and substrate porosity may have influenced the water retention and evaporation rates during drying, directly affecting the final density values [30]. In contrast, Experiment 2 resulted in lower density values, with mean densities of 163.36 kg/m^3, 137.81 kg/m^3 and 130.15 kg/m^3 for the CA, CB and BA treatments, respectively. This reduction may be related to improved colonization homogeneity and structural rearrangement of the mycelial network, which can increase the porosity and reduce the bulk density after drying [20,35]. However, these results (Figure 7) are within the range reported in the literature.

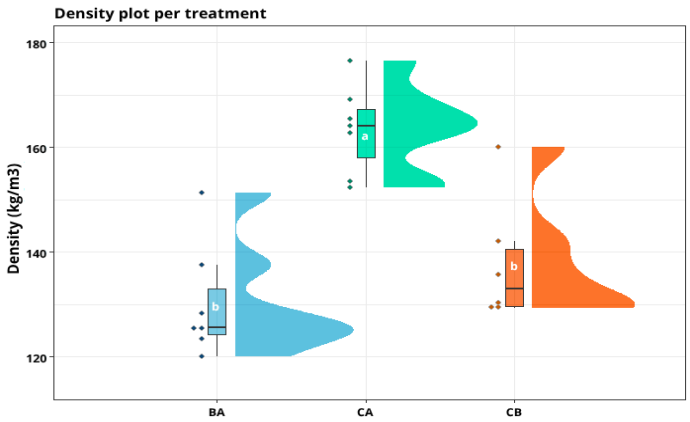


Figure 7. Density plot for the samples of experiment 2 (n = 7). *P. ostreatus* and banana leaves (BA, in blue), *P. ostreatus* and coffee husks (CA, in green) and *P. ostreatus* and a mixture of the previous residues (CB, in orange). Significance value: 0.05, two-way ANOVA, letters **a** and **b** indicate the statistical difference.

For instance, Holt et al. (2012) [21] evaluated biomaterials produced with *Ganoderma lucidum* using both liquid and solid inocula and substrates composed of cotton-derived agricultural residues, starch, and gypsum, reporting densities varying from 66.5 kg/m^3 to 224 kg/m^3; with higher values associated with solid inoculum. Yang et al. (2017) [36] reported density values for biomaterials produced using a white-rot fungus endemic to Alaska, cultivated under different conditions, including variations in substrate type, packing density, growth time, and moisture content. For samples grown over two weeks,

density values ranged from 240–265 kg/m^3 (densely packed samples) to 165–195 kg/m^3 (loosely packed samples). Additionally, Alemu, Tafesse, and Deressa (2022) investigated the supplemented growth of *P. ostreatus* on coffee husks, sugarcane bagasse, and sawdust, yielding biomaterials with densities of 292, 311, and 334 kg/m^3, respectively [14]. As demonstrated, the results obtained in this study (130–254 kg/m^3) are consistent with the literature. Although mycelium-based biomaterials are considered lightweight, their density remains higher than those of ultra-light [37] materials such as expanded polystyrene (EPS, 12–48 kg/m^3) [38]. However, this higher density may be offset by the low cost of the raw materials, which positively affects the overall product cost, productivity, and performance as discussed in subsequent sections. Furthermore, the differences observed between the two experiments conducted in this study, despite being consistent with the literature, highlight the complexity of biomaterial development processes.

3.3. Mechanical Behavior

The mechanical properties of the biomaterials were evaluated through compressive stress–strain analysis, providing insights into their structural behavior and potential applications. The stress–strain curves corresponding to the samples of the CA series (Figure 8). The mycelium–coffee husk composite exhibited linear stress–strain behavior.

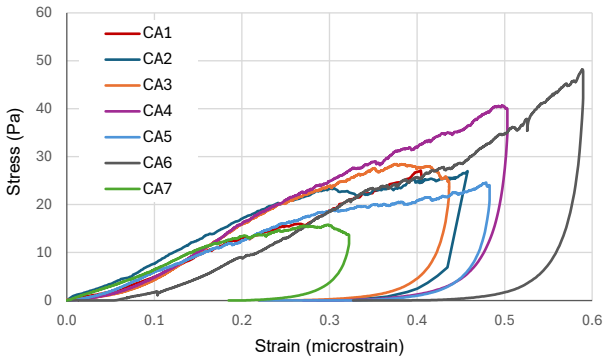


Figure 8. Stress–strain curves for the CA (coffee husks and *P. ostreatus*) samples.

The mechanical properties measured for the CA samples, which are essential for determining the material's functionality, durability, and potential industrial application, are shown in Table 1. The strain energy density was obtained by computing the area under the stress–strain curve.

Table 1. Mechanical properties for CA samples.

Sample	P_{max} (N)	δ_{max} (mm)	σ_{max} (Pa)	E (Pa)	Strain Energy Density (N/m^2)
CA1	29.60	35.30	27.1	66.8	5.08
CA2	28.60	29.10	23.4	90.6	9.05
CA3	30.94	37.67	28.5	90.2	7.34
CA4	46.42	40.86	40.7	77.3	10.73
CA5	29.30	40.90	24.6	68.5	7.04
CA6	57.00	45.80	48.2	84.2	11.70
CA7	18.50	28.00	15.8	71.2	3.36
Mean	34.34	36.80	29.8	78.4	7.76
σ	11.97	6.02	10.2	9.31	2.76
C.V.	34.9%	16.4%	34.3%	11.9%	35.5%

P_{max} = maximum force resisted by the sample; δ_{max} = shortening corresponding to the maximum stress; σ_{max} = maximum stress reached during the test; E = Young's modulus.

The mycelium composite containing banana leaf (BA and CB series) exhibited a nonlinear stress–strain behavior, well represented by a fifth-degree polynomial (Figure 9).

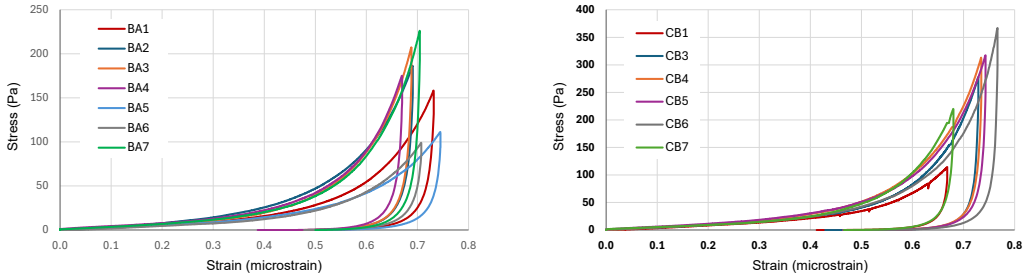


Figure 9. Stress–strain curves for BA (banana leaves and *P. ostreatus*) and CB (mixture and *P. ostreatus*) samples.

The mechanical properties measured for BA and CB samples are shown in Table 2. Since the modulus of elasticity varied nonlinearly, it was evaluated at a strain of 0.6 microstrain ($\varepsilon = 600 \times 10^{-9}$) to enable comparison among the samples.

Table 2. Mechanical properties for BA samples.

Series	Sample	P_{max} (N)	δ_{max} (mm)	σ_{max} (Pa)	E_{06} (Pa)	Strain Energy Density (N/m^2)
BA	BA1	174.80	59.90	158.3	10.5	23.97
	BA2	209.30	60.10	186.5	91.7	28.68
	BA3	232.90	59.90	207.5	88.1	26.66
	BA4	191.40	59.90	175.1	88.8	24.34
	BA5	108.40	59.80	111.4	41.9	19.59
	BA6	107.00	60.10	99.0	43.2	15.84
	BA7	247.10	59.90	226.1	84.0	29.66
	Mean	181.56	59.94	166.3	64.0	24.11
	σ	51.76	0.10	43.7	29.6	4.58
	C.V.	28.5%	0.2%	26.3%	46.3%	19.0%
CB	CB1	146.60	58.10	114.3	65.2	18.78
	CB2	damaged sample				
	CB3	340.40	64.80	277.8	81.8	36.36
	CB4	368.20	64.80	313.0	101.4	44.16
	CB5	346.80	64.80	317.3	96.6	46.00
	CB6	412.00	49.80	367.1	76.0	46.27
	CB7	256.10	60.10	220.0	104.7	29.89
	Mean	311.68	60.40	268.3	87.6	36.91
	σ	87.23	5.41	82.0	14.3	10.01
	C.V.	28.0%	9.0%	30.6%	16.4%	27.1%

Table 3 provides the mean values of the measured parameter for each composite, providing a comparative overview of their physical and mechanical properties.

The mycelium-based composite containing a mixture of coffee husk and banana leaves showed the highest compressive strength (σ_{max} = 268 Pa) and the highest stiffness (E_{06} = 87.6 Pa).

The mycelium–coffee husk composite exhibited the lowest compressive strength (σ_{max} = 29.8 Pa) and an intermediate stiffness among the three tested materials (E_{06} = 78.4 Pa).

The mycelium–banana leaf composite showed intermediate compressive strength (σ_{max} = 166 Pa) and the lowest stiffness among the three tested materials (E_{06} = 64 Pa).

Table 3. Mean ± standard deviation (SD) values of the physical and mechanical properties determined for each composite.

Property	CA	BA	CB
Pmax (N)	34.34 ± 11.97	181.56 ± 51.76	311.68 ± 87.23
δmax (mm)	36.80 ± 6.02	59.94 ± 0.10	60.40 ± 5.41
σmax (Pa)	29.8 ± 10.2	166.3 ± 43.7	268.3 ± 82.0
E_{06} (Pa)	78.4 ± 9.31	64.0 ± 29.6	87.6 ± 14.3
Density (kg/m^3)	163.1 ± 8.55	130.1 ± 9.98	137.8 ± 10.14
Strength/density	18.1 ± 6.27	129.8 ± 39.34	194.3 ± 55.14
Strain energy density (N/m^2)	7.76 ± 2.76	24.11 ± 4.58	36.91 ± 10.01

Values are expressed as mean ± standard deviation (SD). CA and BA: n = 7; CB: n = 6 for the mechanical properties because one specimen was damaged before testing.

The superior mechanical performance of the composite containing mixed substrates suggests a synergistic effect between coffee and banana leaves. While coffee husks contribute to the stiffness, banana leaves improve the structural integrity and stress distribution, resulting in more cohesive and mechanically efficient material. In contrast, the single-substrate composites exhibited limitations related to either insufficient bonding (coffee husks) or excessive porosity and flexibility (banana leaves).

The comparison of the densities and strength-to-density ratio for all samples is displayed in Figure 10. The strain energy density obtained was 7.76, 24.11, and 36.91 N/m^2 for the CA, BA, and CB composites, respectively. The incorporation of banana leaves resulted in higher average strength-to-density ratio and strain energy density values, demonstrating their improved mechanical efficiency compared with composites produced solely from coffee husks. These results indicate that the addition of banana leaves contributes to enhanced load-bearing capacity while maintaining a favorable density, improving the overall performance of the composites.

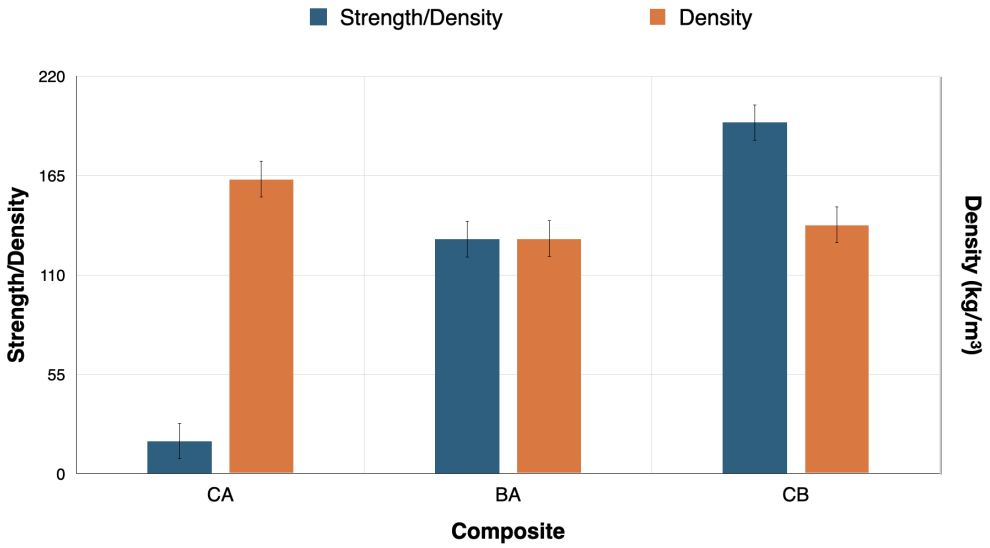


Figure 10. Mean density and strength-to-density ratio of the mycelium-based composites produced with coffee husks (CA), banana leaves (BA), and a mixture of coffee husks and banana leaves (CB). Results are expressed as mean ± standard deviation (SD) (CA and BA, n = 7; CB, n = 6, because one specimen was damaged before mechanical testing).

The variability observed among specimens may be attributed to the heterogeneous nature of mycelium-based composites, including differences in the substrate particle distribution, packing, porosity, residual moisture, and the extent of mycelial colonization. Although the CB composite exhibited the highest strength-to-density ratio, the variability

represented by the SD error bars indicates differences among specimens. Therefore, the apparent improvement associated with the mixed substrate should be interpreted cautiously, and further process standardization and a larger number of specimens will be required to confirm this behavior.

The two-banana leaf-based composites exhibited a stress–strain response well described by a fifth-degree polynomial, demonstrating a strong constitutive nonlinearity, possibly indicating hyperelastic-like behavior. Materials with this characteristic are particularly suitable for applications requiring an adaptive mechanical response, combining initial flexibility with progressive stiffening underload.

Such materials are highly effective for energy dissipation and can be applied in impact protection systems (e.g., helmets, insoles, and packaging), energy-absorbing structures in automotive engineering, and mechanical barriers or dampers. The low initial stiffness enhances comfort, while the increased stiffness at higher strains provides protection against severe loading conditions.

Additionally, these materials may be suitable for applications in which the mechanical response depends on deformation or vibration amplitude, such as "smart" vibration isolation systems, adaptive structural supports, and devices analogous to nonlinear tuned mass dampers [39,40]. Under low-amplitude vibrations, the material remains compliant, whereas under high-amplitude vibrations, it becomes stiffer, thereby mitigating excessive deformation and instability.

Furthermore, these composites may also offer opportunities in biomedical engineering, particularly as lightweight cellular structures for prosthetic components, orthotics devices, and human–machine interfaces. Similar deformation mechanisms have been associated with improved stress distribution, enhanced conformability to complex anatomical geometric, and greater comfort in prosthetic, orthotic, and wearable biomedical devices manufactured from auxetic materials [41,42].

3.4. Color Analysis

In general, the biomaterials were predominantly white, due to the presence of *P. ostreatus* mycelium, with a slight brownish hue associated with the substrates. Data acquired by a colorimetric assay (Table 4) from samples of Experiment 1 confirm these visual observations, which are relevant aesthetic attributes to the biomaterials. Furthermore, the highest opacity was observed in the CB treatment, followed by the BA and CA groups, (160.64; 50.0 and 49.28, respectively). A higher opacity may be advantageous for food packaging applications, as it can provide protection against UV radiation. Additionally, BA treatment exhibited a higher whiteness index (17.36), probably due to higher local mycelial coverage in the analyzed region, compared to those measured for CB (8.54) and CA (6.96), despite their overall higher colonization levels.

Table 4. Colorimetric analysis data from Experiment 1 samples. *P. ostreatus* and banana leaves (BA), *P. ostreatus* and coffee husks (CA) and *P. ostreatus* and a mixture of the previous residues (CB).

	Treatments		
	BA	**CA**	**CB**
*L**	48.71	31.72	35.07
*a**	9.32	5.52	6.48
*b**	20.82	10.76	11.58
Y White	17.36	6.96	8.54
Y Black	8.68	3.43	13.71
Opacity	50.00	49.28	160.64

Lightness (*L), red–green coordinate (*a), and yellow–blue coordinate (*b).

3.5. Microstructural Surface Scanning

Scanning electron microscope images of all biocomposites showed hyphae of *P. ostreatus* intertwined with the substrates, acting as binding networks (Figure 11B). Reproductive structures of *Penicillium* sp. were also observed (yellow arrow "b"), which are commonly reported in mushroom fruiting blocks [15]. All treatments exhibited highly irregular surface morphology (yellow arrow "c"), with *P. ostreatus* hyphae functioning as a natural bio-adhesive, filling voids and connecting different parts of the substrate. Hyphae anastomosis was also observed (yellow arrow "d"), indicating the formation of an interconnected network. The thicker and more robust hyphae observed in the CB treatment may have contributed positively to its mechanical behavior, enhancing its comprehensive strength and stiffness.

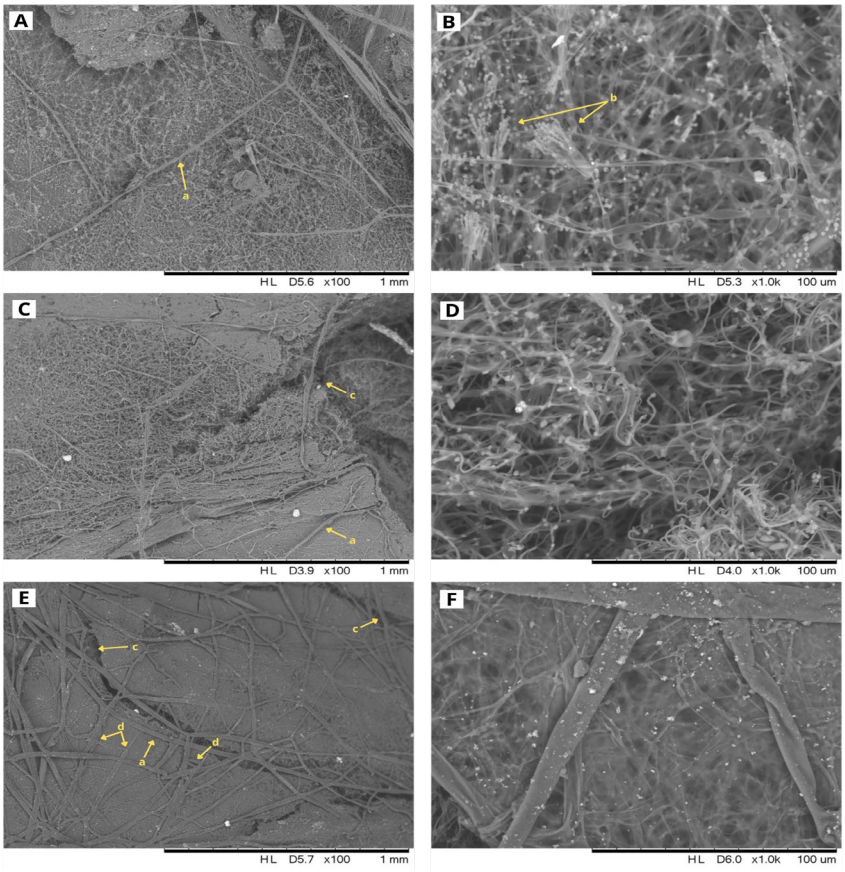


Figure 11. Scanning electron microscopy images of *P. ostreatus* mycelium on banana leaf substrates (**A**,**B**), coffee husk (**C**,**D**), and a mixture of leaf and husk (**E**,**F**). Yellow arrows indicate hyphae of *P. ostreatus* (a), *Penicillium* reproductive structures (b), gaps on the substrate held by mycelium (c) and fusion of hyphae (d).

3.6. Swelling Degree

The swelling degree reflects the material's capacity for water absorption. For this parameter, the CA treatment exhibited the highest value (45.4%), followed by BA (40.3%) and CB (39.4%) (Table 5).

These results suggest that coffee husks have a higher affinity to water uptake, which may favor the biodegradability of the material, as higher water availability promotes microbial decomposition activity. Tajvidi et al. (2019) [39] investigated the development of mycelium-based biocomposites using two distinct processing protocols with the same raw materials. Their findings demonstrated that prior fungal colonization through a pre-inoculation step reduced the water absorption by approximately 40%. These results,

consistent with those observed in the present study, indicate that both the substrate composition and processing condition play a significant role in determining the material's water absorption capacity and degradation behavior. Additionally, the water in which the samples were submerged developed a reddish hue, initially observed in the CA and CB treatments and, after 2 h of immersion, also in the BA treatment (Figure 12).

Table 5. Swelling degree (SD) of Experiment 1 samples. *P. ostreatus* and banana leaves (BA), *P. ostreatus* and coffee husks (CA) and *P. ostreatus* and a mixture of the previous residues (CB).

Treatment	Initial Weight (g)	Final Weight (g)	SD
BA	13.49	22.58	40.3%
CA	7.61	13.95	45.4%
CB	8.68	14.32	39.4%

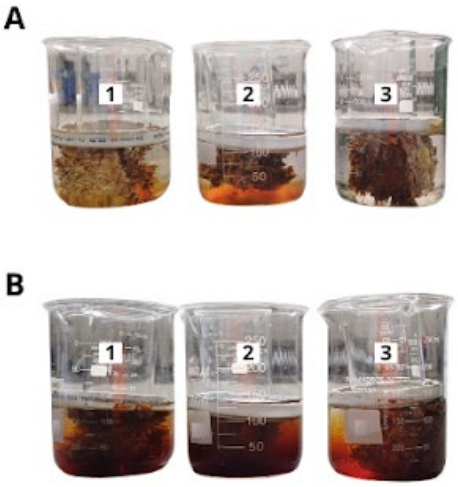


Figure 12. Submerged samples of blocks colonized by *P. ostreatus* (**A**), and samples after 2 h of submersion (**B**). (1) Banana leaf + coffee husk; (2) coffee husk; (3) banana leaf.

This observation suggests the release of soluble compounds (exudates) into the medium. Further investigation is required to determine the chemical nature of these compounds and whether they originated from the substrates themselves or from partial decomposition mediated by *P. ostreatus*.

4. Conclusions

This study demonstrated that mycelium-based biocomposites produced from agricultural residues are highly moldable materials whose properties are strongly influenced by production parameters. The different residues used in this research demonstrated that the biocomposite behavior directly depends on the substrate composition, which also affects the mycelial growth patterns. Rigid residues, such as coffee husks, contributed to the increased stiffness of the biocomposites, whereas more flexible residues, such as banana leaves, enhanced the strength and deformation capacity. The mycelia of *P. ostreatus* effectively acted as a natural binding agent, integrating the organic residues into a cohesive structure capable of maintaining its integrity under high stress and allowing for large deformations. Overall, mycelium-based biocomposites derived from agricultural residues have strong potential for the development of sustainable materials, with possible applications in environmentally friendly products.

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