

Fungal biocomposites production from packaging industry residue: PET-coated SBS paperboard

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Abstract

Packaging generates approximately 40 tons/month of waste from Solid Bleached Sulfate (SBS) paperboard coated with Poly (Ethylene Terephthalate) PET. Concerns about environmental sustainability are pushing innovative materials with diverse applications to replace conventional synthetic materials. Biocomposites, which use fungal mycelium as a binder for the particles, are an emerging option in biodegradable and naturally sourced materials. This study aimed to make *Pleurotus sajor-caju* biocomposites, employing SBS paperboard packages coated with PET as the substrate. The study investigated two inoculum fractions (30% and 50%) and two drying methods (conventional and vacuum) at 60 °C. The biocomposites produced with a 50% inoculum and conventional drying displayed favorable characteristics, including a shorter processing time (16 days), a higher drying rate (5.58 g/day), low porosity (21,7%), compressive strength of 0.16 MPa, apparent density of 315 kg/m³ and satisfactory thermal stability.

Keywords: *biocomposites, fungal mycelium, packaging waste, Pleurotus sajor-caju, sustainable production.*

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1. Introduction

The modernization of society, which increasingly depends on packaged products, has increased solid waste generation^[1]. The combination of Solid Bleached Sulfate (SBS) paperboard coated with Poly (Ethylene Terephthalate) (PET) is widely utilized in the production of thermoformed trays for storing partially prepared foods, permitting temperature variation between -40 °C and 220 °C^[2]. It has been estimated that graphic industries alone generate approximately 40 tons of this waste each month^[3]. Treating these residues is challenging due to the polymer coating^[4].

Plastics made from petroleum take decades to decompose, making their replacement essential for environmental conservation. Today, industrial sectors have numerous opportunities to move toward a cleaner, more sustainable, and greener environment. These long-term goals can be achieved using various biopolymers, which offer advantages such as abundant availability, suitable mechanical properties, and easy biodegradability^[5,6]. Then, an alternative use for packaging waste could be as a substrate for producing fungal biocomposites. This involves inoculating a filamentous fungus in a substrate composed of a nutrient material and discontinuous particles. The fungus metabolizes the nutrient material over a sufficient period for the growth of its hyphae,

forming an interconnected mycelial network in and around the residue. This process binds the material particles and assumes the shape of the container in which it will be cultivated^[7], potentially replacing wooden, plastic, foam, and styrofoam packaging^[8]. In the process, the material must be dehydrated to stop fungal growth, unmolded and there must be no active fungal spores that could contaminate the environment. The most commonly used methods for material dehydration are convection drying (conventional drying)^[9] and freeze drying (lyophilization). Nevertheless, in freeze drying, the product must be subjected to freezing and vacuum, which makes the process slower and more expensive^[10]. However, vacuum drying is recommended for temperature-sensitive materials^[11,12] and can be tested.

There is currently research in the literature involving mycelial biocomposites, and each work uses a different production condition for these biocomposites, but all to demonstrate the potential of this material^[13-19].

Amidst the challenges of waste accumulation, sustainable consumption has gained prominence^[20]. Fungi of the genus *Pleurotus* offer a promising solution with their ability to produce white mycelium and degrade both lignin and cellulose. They possess an enzymatic complex with a wide

range of enzymes, such as cellulase, ligninase, cellobiase, laccase, and hemicellulase, enabling them to degrade a diverse array of lignocellulosic residues^[21]. This versatility makes them ideal candidates for the proposed research on waste management.

In an evaluation of the biodegradation of recycled poly (ethylene terephthalate) (PET) by *Pleurotus ostreatus*^[22], a mass loss of 3.3% was observed after 45 days of biodegradation. The production of *P. sajor-caju* mushrooms using PET-coated SBS cardboard packaging waste yielded 47.3%^[23]. Therefore, the potential of SBS paperboard waste coated with PET for *Pleurotus* cultivation and the ability of fungal mycelium to act as a binder for waste particles have been demonstrated.

Based on the above, this work aimed to evaluate the production of fungal biocomposites with *P. sajor-caju* mycelium using waste from the packaging industry, PET-coated SBS paperboard. To seek solutions to environmental problems linked to the accumulation of waste, this work aims to use and add value to waste from the packaging industry, using the abilities presented by fungi, contributing to the reduction of raw material waste and environmental impacts.

2. Materials and Methods

2.1 Microorganisms and maintenance

The fungal species utilized was *Pleurotus sajor-caju*, obtained from the Collection of Cultures of Basidiomycetes at the Instituto de Botânica (São Paulo/SP) under the code CCB 019. The strain was maintained in a WDA medium (Wheat Dextrose Agar)^[24]. The plates were stored in a refrigerator at 4 °C, and replications were conducted every 3 months. The inoculum consisted of wheat grains colonized with *P. sajor-caju* mycelium and refrigerated at 4 °C until use^[25].

2.2 Preparation of biocomposites

The residue from the thermoforming process of food packaging, composed of SBS (Solid Bleached Sulfate) paperboard with PET coating, was obtained from Baumgarten Gráfica Ltda company, located in the municipality of Blumenau - SC, and used in this work.

The residue with a thickness of 0.4 mm, defined as substrate, was fragmented (2 x 10 mm) and immersed in water for 24 hours (Fig 1a, 1b and 1c). After this period, the excess water was drained, and the moist substrate was packed in polypropylene packages (28 x 40 cm) in the proportion of 150 g (dry mass), and 5% rice bran was added. The packages were sterilized at 121 °C for one hour.

Inoculation was performed in a laminar flow chamber using 30 or 50% of the inoculum relative to the mass of the dry substrate^[18,23]. Incubation occurred in the absence of light, at 30 °C, until the fungal mycelium's complete colonization of the substrate (Fig 1d). The time from inoculation to complete colonization of the substrate by the fungal mycelium was recorded for each inoculum fraction used and defined as mycelial growth time (t_g - days). The substrates colonized with 30% or 50% inoculum were ground in a mini food processor until a homogeneous mixture was obtained^[18]. This mixture was then aseptically introduced and compacted into

cylindrical plastic trays (6 cm Ø) up to a height of 2.5 cm in order to obtain test specimens by NBR 8082^[26]. The trays were sealed and placed in a bacteriological oven without light at 30 ± 2 °C until the fungal mycelium hyphae were recolonized and reestablished (Fig 1e). The duration of this process was recorded for each inoculum fraction used and defined hyphae recolonization time (t_r - days).

2.3 Drying of biocomposites

The test specimens were dried at 60 °C using the conventional and vacuum methods. Eq. (1) obtained the average drying speed (v - g/day), Eq. (2) obtained the initial moisture content (iMo - %), and Eq. (3) obtained the final moisture content (fMo - %).

$$v \left(\frac{g}{day} \right) = \frac{M_i - M_f}{t_s} \quad (1)$$

$$iMo (\%) = \frac{M_i - M_{105^\circ C}}{M_{105^\circ C}} * 100 \quad (2)$$

$$fMo (\%) = \frac{M_f - M_{105^\circ C}}{M_{105^\circ C}} * 100 \quad (3)$$

Where M_i is the initial mass (g - wet mass), M_f is the final mass (g - dry mass at 60 °C), t_s is the final drying time (days - where the test specimens reached a constant mass at 60 °C), and $M_{105^\circ C}$ is the mass without moisture (g - dry mass at 105 °C).

2.4 Parameters evaluated

Adding t_g , t_r and t_s accounts for the total process time (t_t - days) for obtaining the biocomposites test specimens. Measurements were performed to calculate the apparent density (ρ - $\frac{kg}{m^3}$) (Eq. 4), porosity determination (P - %) ^[27] (Eq. 5a and 5b), water sorption (W - %) ^[28] (Eq. 6), air humidity sorption (AirHS - %) ^[18] (Eq. 7), and the compression stress (σ_c - MPa) was calculated at 10% deformation, as gded by NBR 8082^[26] (Eq. 8).

$$\rho \left(\frac{kg}{m^3} \right) = \frac{M_f}{V} \quad (4)$$

$$P (\%) = \frac{\rho_c - \rho}{\rho_c} \quad (5a)$$

$$\rho_c (\%) = \frac{M_f}{V_s} * 100 \quad (5b)$$

$$W (\%) = \frac{M_t - M_f}{M_f} * 100 \quad (6)$$

$$AirHS (\%) = \frac{M_{air} - M_f}{M_f} * 100 \quad (7)$$

$$\sigma_c (MPa) = \frac{F}{A_0} \quad (8)$$



Figure 1. SBS paperboard package coated with PET. (a) before fragmentation; (b) after fragmentation; (c) immersed in water; (d) colonized by fungal mycelium; (e) molds with processed substrate after recolonization and reestablishment of hyphae; (f) biocomposites dried.

Where M_f is the mass of the test specimen immediately after drying, M_t is the mass (g) of the specimen after immersion in distilled water (2, 24, and 48 h), M_{AIR} is the mass (g) measured at each sampling period (58 days), V is the geometric volume of the test specimens after drying, V_s is the skeletal volume of the porous test specimens was measured by volume displacement in water by soaking the samples in water for 5 hours in a graduate cylinder, F is the compression force (N), and A_0 is the cross-sectional area of the test specimen (mm^2).

Thermogravimetric analysis (TGA) was performed using TGA-Q50 equipment (TA Instruments). The heating

rate was $10\text{ }^\circ\text{C}/\text{min}$, starting from 25 to $600\text{ }^\circ\text{C}$ in a nitrogen atmosphere ($60\text{ mL}/\text{min}$ flow rate).

Biocomposite samples were analyzed using scanning electron microscopy (SEM) to examine their surface morphology. Samples measuring approximately 3 to 5 mm were taken, covered with a conductive layer of gold, applying an electron acceleration voltage of 5 kV and magnifying the images from 25 to 2000 times. The biocomposites produced under the optimal conditions were also analyzed using FTIR (Fourier transform infrared spectroscopy). FTIR spectra were obtained using a Perkin-Elmer Spectrum One B spectrophotometer in 12 scans in the spectral region from

4000 to 650 cm⁻¹, with a resolution of 4 cm⁻¹, using the attenuated total reflectance (ATR) accessory.

2.5 Statistical analysis

The obtained values were submitted to the outlier rejection test (Dixon Q test)^[29] and later to the variance analysis of the mean values of the samples using the Tukey test with a significance level of 5% (ANOVA).

3. Results and Discussions

3.1 Mycelial growth time

The mycelial growth time (t_g – days) for the substrate with 30% inoculum was 14 days, and 7 days for the substrate with 50% inoculum. The time for recolonization and reestablishment of fungal mycelium hyphae (t_r – days) in the trays was 5 and 7 days, respectively, for substrates with 30% and 50% inoculum.

3.2 Drying of biocomposites

The test specimens were subjected to two dehydration conditions to stop fungal growth: conventional oven drying and vacuum drying, both at 60 °C. Figure 2 shows the drying curve behavior of the biocomposites produced with 30% and 50% inoculum and the performance of the two tested methodologies.

The final drying time (t_s – dias) occurred in 48 h (2 days) for all biocomposites (Figure 2), and no statistical difference was observed in the final moisture content (Table 1). The inoculum fraction influenced the initial moisture value and the drying speed, being higher with the inoculum fraction of 50%. The more significant amount of mycelium in biocomposites with 50% inoculum may have influenced these parameters^[14].

Deschamps^[30] produced biocomposites from the brewery residue with banana leaves and 30% *Pleurotus sajor-caju* inoculum; the initial moisture obtained was 78,6%. In the present work, the initial moisture content was 75.82% for biocomposites with 30% inoculum and 92,84% with 50% inoculum (Table 1). The inoculum fraction influenced the initial moisture content, probably due to the greater quantity of wheat grains present.

Rocha et al.^[18] interrupted the fungal growth after 2 days of specimens with an inoculum fraction of 20% and 30% of *Pleurotus sajor-caju* at drying temperatures of 40 and 60 °C,

observing that the inoculum fraction did not influence the drying speed and the final moisture content. The authors obtained a higher drying speed at 60 °C.

In line with Deacon^[31], the moisture at which no fungus can grow is below 14%. However, a slight increase in moisture to from 15 to 16% allows the growth of *Aspergillus* spp., a stress-tolerant fungus. The moisture content in our final product is below 14% (5.51 to 6.23%), effectively inhibiting the growth of fungi. This practical implication makes these biocomposites viable and functional, opening up new possibilities in materials science.

The analysis of the mycelial growth time (t_g – item 3.1), the time for reestablishment of fungal mycelium hyphae (t_r – item 3.1), and the final drying times (Figure 2) reveals an expressive efficiency in the 50% inoculum condition with conventional drying. This condition presented the shortest overall production process time (Table 1), a mere 16 days. Notably, the drying condition in a conventional oven demonstrated the highest average drying speed, 5.58 g/day, further highlighting the efficiency of these production processes.

3.3 Biocomposites analysis

Figure 3 shows the results obtained from the water sorption of the biocomposite specimens.

It is clear from Figure 3 that after 2 hours of immersion, all biocomposites absorbed at least 200% of water regardless of the inoculum fraction and the type of drying. Furthermore, it is observed that the biocomposites dried

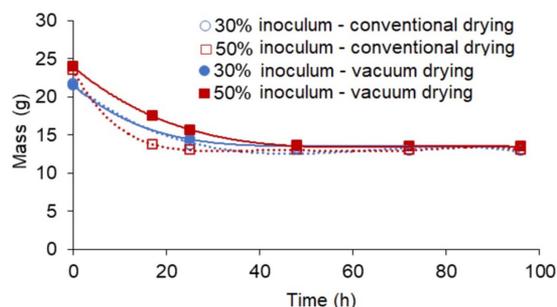


Figure 2. Drying curves, mass (g) x time (h), of the biocomposites produced with 30% and 50% inoculum under conventional and vacuum drying.

Table 1. Average values of initial moisture content (iMo) ± standard deviation (sd) and final moisture content (fMo) ± sd, average drying speed (v) ± sd, final drying time (t_s) and total process time (t_t) of the test specimens produced with 30 and 50% inoculum and dried at 60 °C.

Inoculum fraction (%)	iMo (%)	Drying	fMo (%)	v (g/day)	t_s (days)	t_t (days)
30	75.82 ± 8.38 <i>a*</i>	Conventional	5.51 ± 0.84 <i>c</i>	4.53 ± 0.99 <i>d</i>	2	21
30		Vacuum	6.23 ± 1.49 <i>c</i>	4.46 ± 1.23 <i>d</i>	2	21
50	92.84 ± 3.97 <i>b</i>	Conventional	5.79 ± 0.68 <i>c</i>	5.58 ± 0.49 <i>e</i>	2	16
50		Vacuum	5.94 ± 1.23 <i>c</i>	5.18 ± 0.47 <i>f</i>	2	16

*Equal letters in the columns indicate means without significant differences according to Tukey’s test with a confidence level of 95%.

in a vacuum oven did not show a significant difference in water sorption between 2 and 24 hours, showing that they reached saturation in 2 hours. In conventional drying, there is a significant difference between 2 and 24 hours of immersion, indicating that in 2 hours, the biocomposites did not absorb the maximum amount of water, reaching saturation between 2 and 24 hours.

On the other hand, the water sorption behavior was different under conventional and vacuum drying conditions. Reis et al.^[32], when drying eggplant slices in a conventional and vacuum oven at a temperature of 65 °C, they observed different evaporation rates, being 577 g water per day for the convective (conventional) drying process and 513 g water per day in the vacuum drying process. When the eggplant slices were rehydrated, those dried under vacuum demonstrated a greater rehydration capacity (101.68%), while those dried by convection had a lower rehydration content (37.41%). Similar behavior was observed for biocomposites with 50% inoculum. When dried under vacuum, they had a lower drying speed (5.18 g/day - Table 1) and greater rehydration capacity, presenting water saturation in just 2 hours (Figure 3).

Water sensitivity is essential for many practical applications of biocomposites and thus determines their performance in adverse conditions^[33]. For a material to absorb less water,

its density must be increased, or its external surface must be coated with a hydrophobic material^[34].

The greater the material's porosity, the greater its water absorption capacity through water entry into the empty spaces (pores), indicating a lower density^[35]. It is known that the initial amount of inoculum could interfere with the mycelial density and, consequently, the density of the material^[21]. Figure 4 presents the porosity values. Biocomposites with 50% inoculum and conventional drying showed lower porosity, with a statistically significant difference from the other biocomposites. Biocomposites with 50% inoculum and conventional drying reached saturation after two hours of immersion in water (Figure 4), probably due to lower porosity. The same did not happen when 30% of inoculum was used, proving the influence of mycelial density on the density of the material. Furthermore, this behavior only occurred when drying the biocomposites conventionally, as in vacuum drying, the porosity was the same for biocomposites with 30 and 50% inoculum. Vacuum conditions promote higher pore formation than atmospheric pressure drying^[11]. The water vapor liberated from the material expands. This expansion mitigates structural collapse and fosters the formation of pores^[12].

Using scanning electron microscopy (SEM) images of biocomposite specimens, Figure 5a shows the most prominent

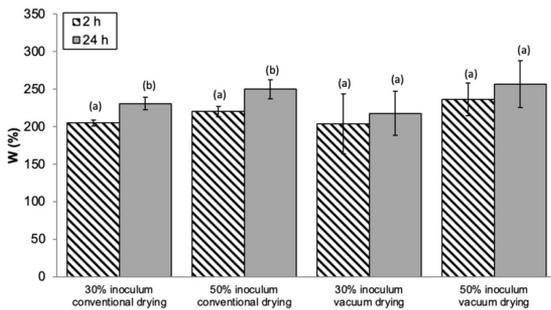


Figure 3. Water sorption (W%) ± standard deviation at 2 and 24 hours of biocomposites immersion with 30% and 50% of inoculum dried in a conventional and vacuum oven. Equal letters between drying conditions (vacuum or conventional) indicate values without significant differences according to Tukey's test with a confidence level of 95%.

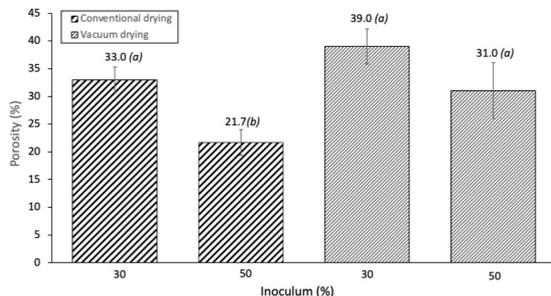


Figure 4. Porosity (%) ± standard deviation values for the biocomposites specimens produced with 30% and 50% inoculum and dried in a vacuum and conventional oven. Equal letters indicate values without significant differences according to Tukey's test with a confidence level of 95%.

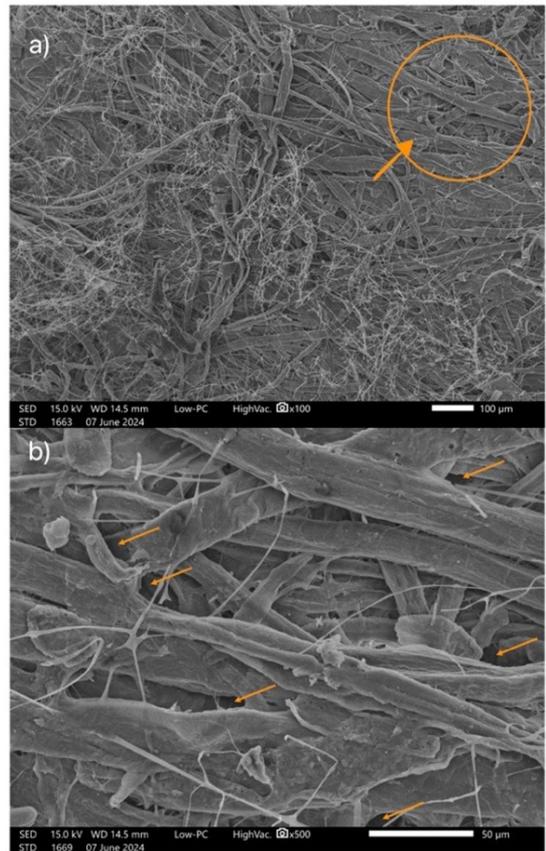


Figure 5. Scanning electron microscopy images of biocomposite specimens at 100x (a) and 500x (b) magnification produced with 50% inoculum and dried in a conventional oven, with arrows highlighting the pores.

structures corresponding to cellulose fibers and the smaller structures corresponding to the mixed and overlapping mycelial and polymeric structures (PET). The polymeric structures are detached from the fibers, probably due to grinding, drying and degradation during the biocomposite production. Figure 5b is an enlarged inset of Figure 5a, showing the pores in the biocomposite with 50% inoculum and conventional drying.

The graph represented by Figure 6 shows the air humidity sorption in biocomposite test specimens, during the 58 days of exposure. The air humidity sorption is an important property that determines the quality and durability of the final product^[36]. This analysis aimed to simulate the product's exposure to ambient conditions.

Analyzing the results in Figure 6, it is observed that all biocomposites, regardless of their inoculum fraction and drying condition, were influenced by the relative air humidity (RairH %) following its daily variation. It is also noteworthy that there was no variation in the behavior of AirHS (%) among biocomposites with the same inoculum fraction, showing coincident curves. An increase in moisture in a product is known to promote the ease of microbial contamination^[31,37], as the humidity at which no fungus can grow is below 14%^[31]. Among the most common contaminants in fungal cultures are the genera *Aspergillus*, *Trichoderma* and *Penicillium*^[31,37]. These fungi exhibit dark sporulation, which is easily detectable by the naked eye^[38]. Despite the sorption of moisture from the air by the biocomposites (Figure 6), no contamination was observed during the 58 days of exposure to ambient air. The moisture content in the final product (Table 1) is below 14% (5.51% to 6.23%). During the 58-day exposure period, the RairH ranged from 40 to 100%, while the moisture sorption from the air by the biocomposites ranged from 0.5 to 6.5%; that is, even the biocomposite with the highest final moisture (6,23%) content did not reach the 14% critical moisture content.

The biocomposites with 50% inoculum fractions exhibited an average AirHS of $4.6 \pm 1.1\%$, while biocomposites with 30% inoculum showed significantly lower air humidity sorption ($3.3 \pm 1.1\%$) according to the Tukey test with 95% confidence.

Rocha et al.^[18] also monitored the air humidity sorption in biocomposites made from mate/guarana waste with 30% *P. sajor-caju* inoculum and obtained a maximum air humidity sorption of 13.1% with a relative air humidity of around 80%. Appels et al.^[14] analyzed the behavior of biocomposites made from beech sawdust, rapeseed straw, and cotton fibers with *P. ostreatus* when exposed to an environment with a relative air humidity of 80% at 40 °C, and the biocomposites exhibited an AirHS of 11.6%. It is evident that humidity sorption depends primarily on the substrate used, but it can be influenced by the fungal species, as some fungi exhibit a hydrophobic nature due to certain proteins found in the mycelium, such as hydrophobins^[39].

Compression stress is an essential property for analyzing the biocomposite's applicability, as higher resistance suggests greater durability^[13]. In Figure 7, the compression strength was not influenced by the inoculum fraction or the drying method, remaining around 0.16 MPa.

In the biocomposites of yerba mate and guaraná, using *P. sajor-caju*, Rocha et al.^[18] obtained a compression strength of 0.094 MPa. Ghazvinian et al.^[16], cultivating *P. ostreatus* with straw substrate achieved 0.02 MPa. Meanwhile, Bruscatto et al.^[15] obtained a higher compression strength value (0.4 MPa) in biocomposites with sawdust and wheat bran using *P. albidus*.

The compression strength found in the present study falls within the range of values reported in the literature, which does not rule out the possibility of improving the production conditions of this biocomposite for packaging applications. The mechanical properties of the biocomposites can be enhanced by incorporating cold or hot pressing into the process. With applied pressure, material porosity is reduced, material density increases, and fibers are reoriented within the material plane^[17,40].

One of the leading products used for packaging is expanded polystyrene (EPS), commonly known as Styrofoam®, a trademark of the Knauf company. The compressive strength of EPS type 1 to 6, resulting from the polymerization of styrene in water, must be between 0.035 and 0.173 MPa, with a density of 10 to 30 kg/m³^[41]. Therefore, the compression strength obtained for this studied biocomposite is similar to this type of EPS.

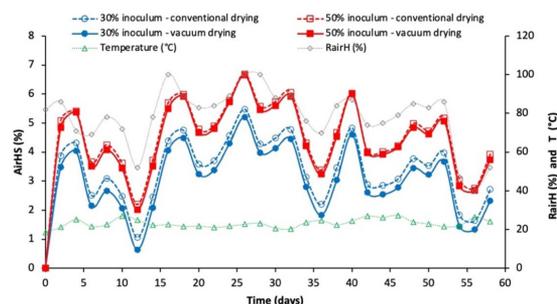


Figure 6. Air humidity sorption (AirHS %) per exposure time (days) in biocomposites produced with 30% and 50% inoculum and dried in 60 °C in a conventional and vacuum oven. The gray and green lines refer to the measurement of relative air humidity (RairH%) and ambient temperature (°C), respectively, at the time of weighing.

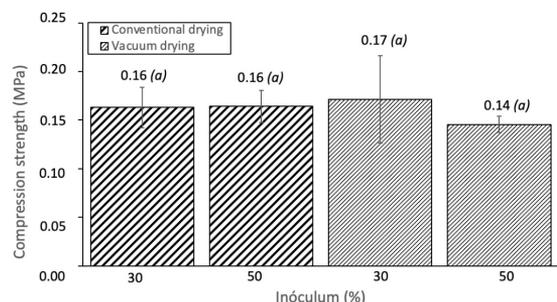


Figure 7. Compression strength (MPa) ± standard deviation for the test specimens of biocomposites produced with 30% and 50% inoculum and dried in vacuum and conventional ovens. Equal letters indicate values without significant differences according to the Tukey test with a confidence level of 95%.

Figure 8 presents the apparent densities of the biocomposites. It is observed that inoculum fractions of 30% and 50% dried in a vacuum oven showed no statistically significant difference, remaining around 300 kg/m³. However, the densities of the biocomposites dried in a conventional oven are statistically different, with 315 kg/m³ for the 50% fraction and 274 kg/m³ for the 30% fraction, being in agreement with the lower porosity (Figure 4) of biocomposites with 50% inoculum and higher for those with 30%, as according to Thibault et al.^[42], materials with more pores have lower density.

The density of biocomposite materials varies depending on the applied substrate. Girometta et al.^[36] discuss in their work that the density of mycelium alone ranges from 30 to 50 kg/m³. Biocomposites produced from agricultural residues have lower density (60–130 kg/m³) compared to those produced with forest residues, such as sawdust (87–300 kg/m³), for different fungal species^[17], poplar sawdust (220 kg/m³ for *Pleurotus ostreatus*)^[19], hemp (170–260 kg/m³ for *Coriolus versicolor*)^[43]. The biocomposites in the present study, which used cardboard with PET, resemble the biocomposite of the literature in terms of density (274 to 325 kg/m³ - Figure 8). Despite having a high density compared to EPS (which typically ranges from 10 to 30 kg/m³), biocomposites are still lighter than other types of biocomposites. According to

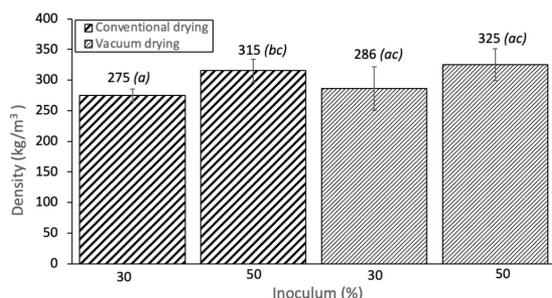


Figure 8. Apparent density (kg/m³) ± standard deviation for the specimens of biocomposites produced with 30% and 50% inoculum and dried in a vacuum and conventional oven. Equal letters indicate values without significant differences according to Tukey's test with a confidence level of 95%.

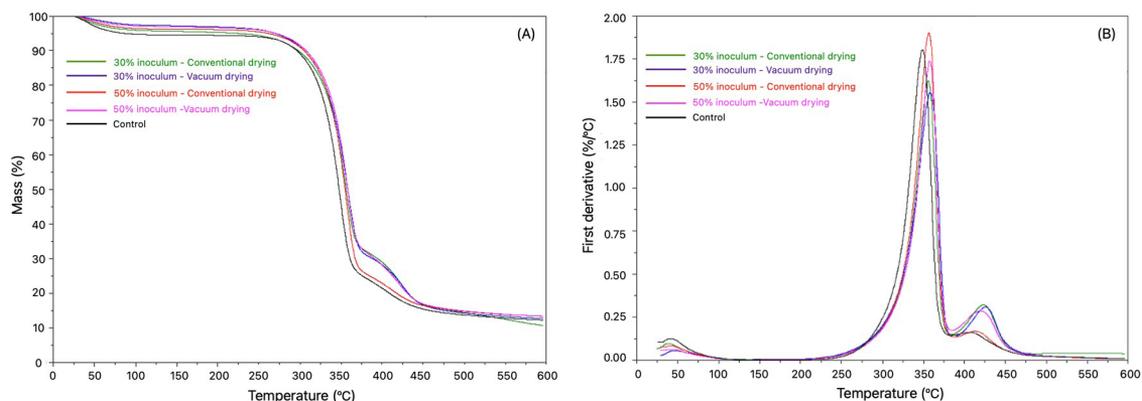


Figure 9. Thermogravimetry (TG)(A) and derivative thermogravimetry (DTG)(B) curves for the biocomposites produced with 30% and 50% inoculum of and dried in conventional and vacuum ovens. The control is only cardboard substrate coated (SBS) with PET (SBS + PET).

López-Nava et al.^[44], these biocomposites are lightweight enough to be utilized in a variety of applications, including food and appliance packaging.

Regarding the thermal performance of the biocomposites, 3 stages of mass loss were observed for the biocomposites produced on SBS paperboard coated with PET (Figure 9 and Table 2). According to Mano et al.^[45], this analysis aids in determining the maximum processing temperature limit that can be used without material decomposition.

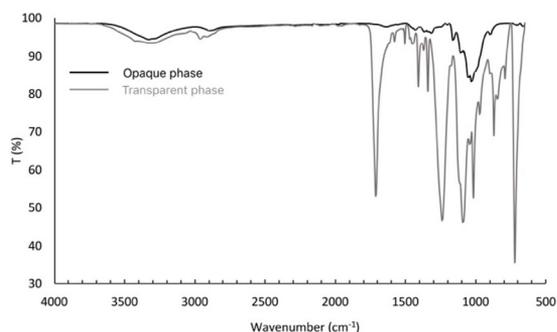
The first stage can be related to water loss, which occurs from 25 to 150/200 °C (Figure 9). For the biocomposite with a 30% inoculum fraction dried in a vacuum oven, this first thermal event had a lower percentage of mass loss (2.730% - Table 2) than in the subsequent events, indicating a lower initial moisture percentage in the cultivated biocomposite. This corresponds to the loss due to the evaporation of surface water.

The second stage characterizes cellulose and hemicellulose degradation^[46]. The condition of vacuum drying and 50% inoculum stood out with temperatures 331.94 and 357.52 °C followed by 331.04 and 356.46 °C for conventional oven drying, showing more excellent thermal stability than the control (without fungal mycelium), indicating that the fungal mycelium influences this stability^[47]. The third event is associated with a residue of approximately 13% for the biocomposite with 50% inoculum and around 11% for biocomposites with 30% inoculum. Bruscato et al.^[15] with pure EPS, analyzed only one stage of thermal manipulation, with T_{onset} at 318 °C, T_{max} of 440 °C and a residue percentage of nearly null, showing that this polymeric material is more stable than the biocomposites here studied. However, Jones et al.^[17], in a *Trametes versicolor* biocomposite, obtained 25% residue formed at 500 °C and demonstrated that at temperatures higher than this, there are insignificant drops in mass loss for mycelium-based materials.

The biocomposites with 50% inoculum and conventional drying were produced in 16 days. They presented an average drying speed of 5.58 g/day, a compressive stress of 0.16 MPa, an apparent density of 315 kg/m³, low porosity (21,7%) and satisfactory thermal performance.

Table 2. Thermogravimetric analysis data of biocomposites cultivated on the cardboard substrate coated (SBS) with PET (SBS + PET) with 30% and 50% inoculum and dried in conventional and vacuum ovens.

Biocomposites	Weight loss 1 (%)	Weight loss 2 (%)	T_{2onset}	Weight loss 3 (%)	T_{3max}	Residue (%)
			T_{2max} (°C)		$T_{3endset}$ (°C)	
Control (SBS + PET)	5.44	69.47	323.21	12.84	410.41	12.23
30% - conventional	4.35	63.82	348.71	21.21	446.22	10.63
			328.95		423.43	
30% - vacuum	2.73	67.65	355.66	16.91	442.18	12.68
			328.56		426.56	
50% - conventional	3.75	71.56	358.37	11.34	449.83	13.33
			331.04		416.65	
50% - vacuum	3.02	65.81	356.46	17.85	448.30	13.32
			331.94		422.13	
			357.52		447.47	

**Figure 10.** Infrared spectroscopy of opaque and transparent phase of biocomposites produced with 50% inoculum and dried in conventional oven.

Thus, the condition with 50% inoculum and conventional drying was the best for producing biocomposites from cardboard substrate coated (SBS) with PET (SBS + PET). FTIR analyses were performed to better understand this biocomposite's chemistry (Figure 10).

The opaque phase of the material shows a spectrum characteristic of cellulose, indicating the presence of O-H at 3300 cm^{-1} and the set of bands between 1200 and 1000 cm^{-1} . The band near 1030 cm^{-1} , related to C-O stretching in carbohydrates, stands out.

The infrared spectrum of the transparent phase of the composite corroborates the presence of bands characteristic of polyethylene terephthalate (PET), namely, those that identify the presence of the ester group and, in this case, the aromatic ester. Consequently, the PET spectrum exhibits an intense band at approximately 1720 cm^{-1} , which is attributed to the stretching of the carbonyl group (C=O). In addition, two other bands are observed at 1245 cm^{-1} and 1100 cm^{-1} , which relate to C-C-O and aromatic O-C-C stretching, respectively^[48]. The prominent band at 720 cm^{-1} , attributed to out-of-plane C-H deformation of the aromatic ring, is also included.

Thus, it appears that although fungi of the genus *Pleurotus* have an enzymatic complex capable of degrading PET^[22],

Figure 10 confirms what had already been observed in Figure 5, PET is still present in biocomposites. However, the degree of PET degradation by *P. sajor-caju* must be verified^[4].

4. Conclusions

Biocomposites with 50% inoculum demonstrated superiority over those with 30% inoculum because they were produced in a shorter time (16 days) and exhibited a higher average drying speed (5.58 g/day conventional drying) and had low porosity (21.7%). With the density (315 kg/m^3), these biocomposites are light enough to be used in various applications, including food packaging and household appliances, as they have satisfactory thermal performance. Despite absorbing more humidity from the air than the biocomposites with 30% inoculum, no contaminations were observed during the 58 days of exposure to ambient air. About the drying method, no advantage was observed when using vacuum drying, as conventional drying was sufficient to achieve satisfactory results.

Although fungi of the genus *Pleurotus* have an enzyme complex capable of degrading PET, this material remained present in the biocomposites, verified by FTIR analyses. However, verifying the extent of PET degradation by *P. sajor-caju* is necessary.

It is interesting to see how waste generated by the packaging industry can be transformed into something useful. Combining these residues with the fungal specie *P. sajor-caju* is an excellent step towards solving environmental issues related to improper waste disposal. This approach can get a biomaterial (fungal biocomposite) that can substitute materials like expanded polystyrene (EPS) due to its superior compressive strength and similar thermal performance.

5. Author's Contribution

- **Conceptualization** – Elisabeth Wisbeck; Nicole Fernanda Souza
- **Data curation** – NA

- **Formal analysis** – Denise Abatti Kasper Silva; Elisabeth Wisbeck; Josiane Costa Riani
- **Funding acquisition** – Elisabeth Wisbeck
- **Investigation** – Elisabeth Wisbeck; Mariane Bonatti-Chaves; Nicole Fernanda Souza
- **Methodology** – Elisabeth Wisbeck; Mariane Bonatti-Chaves
- **Project administration** – Elisabeth Wisbeck
- **Resources** – Denise Abatti Kasper Silva; Josiane Costa Riani
- **Software** – NA
- **Supervision** – Elisabeth Wisbeck; Mariane Bonatti-Chaves
- **Validation** – Elisabeth Wisbeck; Nicole Fernanda Souza
- **Visualization** – Elisabeth Wisbeck; Nicole Fernanda Souza
- **Writing – original draft** – Elisabeth Wisbeck; Nicole Fernanda Souza
- **Writing – review & editing** – Elisabeth Wisbeck; Mariane Bonatti-Chaves

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