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Elaboration of a Biomaterial from *Pleurotus ostreatus* Mycelium and Residual Biomass, as an Alternative to Synthetic Materials

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The lack of degradability of polymeric materials has resulted in high levels of environmental contamination and numerous health hazards. The latest UN Environment 2018 report observed that the estimated annual consumption of plastics worldwide is 5 billion, where approximately 10 million are per minute. If this pattern continues, it is expected that by 2030 the planet will produce 619 million t of plastic per year, such as expanded polystyrene (United Nations Organization (UN); United Nations Environment Program, 2018). Different strategies have been developed to obtain biomaterials considering *Pleurotus ostreatus* as mycelium, adapting mixtures of substrates for its preparation. This work evaluates the methodology to obtain a biomaterial that replaces expanded polystyrene through mycelium using wheat bran and malt bran as substrates. An experimental design is proposed, observing that the best results corresponded to the mixture of 75 % malt bran and 25 % wheat bran obtained in 15 days of colonization at 30 °C. After this, the biodegradation was qualitatively by observing its partial decomposition for 48 h, evidencing the degradation of the size of the piece. These results promote the recovery of agro-industrial waste and the circular economy.

1. Introduction

The use of microorganisms in the production of biomaterials, especially in the construction and packaging sector, is the anticipated technology for the near future focused on environmental sustainability. In the packaging area, expanded polystyrene EPS (Expanded Polystyrene Sheets), is a polymer made up of solid plastic particles of polystyrene that have been obtained from styrene monomer. It is distinguished from other materials due to its thermoplastic properties and is widely used because of its low-impact fracture and thermal conductivity (Quintero, 2021). The extensive use of EPS has made this material a worrying source of contamination. In Europe, expanded polystyrene was one of the most demanded plastics with figures close to 6,2 % of the total produced from this type of material by the year 2020 (Plastics Europe, 2022). This plastic generates a series of waste without adequate management and final disposal. In the latest Acoplásticos report of Colombia, polystyrenes had a variation in tons for their use of 4,9 % for 2020 compared to the previous year because packaging and containers make up most of the market with figures close to 55 % tons processed, the rest is used for construction and in the manufacturing and agricultural sectors and for home consumption. (Acoplásticos, 2022). The recycling potential or circularity of the plastic waste is identified based on the "Plastic Pinch Analysis", which determines the ideal maximum external plastic demands with a certain threshold grade of the plastic (Chin et al., 2022).
Some innovative alternatives have been seeking such as the development of biomaterials from fungi and lignocellulosic substrates, which make it possible to obtain a bioproduct. For example, from research it was observed that *Ganoderma lucidum* it's familiar with walnut shells and oak sawdust, showing favorable results in mechanical tests, becoming an alternative to EPS. (Susel et al., 2021), *Trametes versicolor* (Fernández et al., 2020) uses rice hulls and malt bran; and *Pleurotus ostreatus* feeds and grows on the saw dust-coir pith substrate. (Sivaprasad et al., 2021). The composite fabrication for each case is done by selecting a suitable substrate ratio and evaluating the samples based on their biodegradability, compressive strength and other mechanical tests, acoustic performance, thermal conductivity, water absorption properties, among others.

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The advantage of these biomaterials over EPS is influenced by the degradation of an ecological material where its bonds are susceptible to microbial attack by different species, facilitating its decomposition; on the other hand, polystyrene can last 500 years and still be in perfect condition.
The nutritional importance of edible mushrooms is mainly based on the quality and quantity of protein (17 - 42 %), low carbohydrate content (37 - 48 %), fat (0,5 - 5,0 %), and considerable fiber content (24 - 31 %) (Bellettini et al., 2019). *P. ostreatus* has a great capacity to produce biomaterials by its vegetative part that grows in the form of branched fibers that adhere to the medium in which it is growing; it is also known for its enzymatic capacity to hydrolyze various complex polysaccharides such as lignin (Zubieta et al., 2022). *P. ostreatus* had been grown in mixtures of sawdust and coconut fiber (Sivaprasad et al., 2021) and mixtures of cotton, hemp, and wheat bran (Sisti et al., 2021) in different proportions. The possibility of adapting with malt bran and wheat bran to obtain a biofoil was evaluated, generating a recovery of these solid wastes. Regarding the bran, the colonization protocol was already known, indicating that the mycelium could grow on this substrate that acted as the main nutrient in the search for lignocellulosic raw material and as filler that provides rigidity to the sheets (Leal and Rodriguez, 2022); regarding the malt bran, (Sisti et al., 2021) after characterization of the material, it was found that it contributes 7,32 % of lignin which is a residue extracted from barley malt and other cereals after the manufacture of beer wort; where according to the American Association of Feed Control Officials (AAFCO) this is a wet by-product that contains approximately 20 - 25 % dry matter of the total produced (Montenegro and Macabares, 2020). The purpose of this investigation is to use wheat bran and malt bran as substrates in solid-state fermentation SSF of *P. ostreatus* to produce a biomaterial. We develop SSF using an experimental design where we use proportions of the substrates in a range of 25 % to 100 % to compare the mycelium growth over the substrates.

2. Methodology

2.1. Materials

Wheat bran and malt bran obtained from a commercial industry were used as a substrate in the SSF.

2.1.1. Microorganism

A commercial strain of *P. ostreatus* to produce orellanas was used. The strain was preserved in a 30 % glycerol solution and Sabouraud broth at 2 ºC.

2.1.2. Propagation of fungal biomass

Activation of the fungi was carried out on wheat bran agar at 30 ºC for 10 days. The medium content used was: 1,25 g/L NH_4NO_3, 2 g/L yeast extract, 10 g/L glucose, 18 g/L agar-agar, 0,10 g/L K_2HPO_4, 0,050 g/L $MnSO_4$, 0,076 G/L $MgSO_4$, 0,076 g/L $CuSO_4$.

2.2. Experimental design

Wheat bran and malt bran were used as lignocellulosic substrates for the elaboration of the biomaterial, it was evaluated by a design of unifactorial experiments with 4 levels as shown in Table 1. Replications of the controls and mixture of substrates were made, where the controls were carried out to have a point of comparison between each substrate at the time of colonization.

Table 1: Experimental design

Factor	Levels	
	Wheat bran [%]	Malt bran [%]
Control	100	
		100
Mixes	25	75
	50	50

2.2.1. Inoculum preparation

For the inoculum preparation, the same medium was used except for agar-agar. Five agar discs were added to 100 mL of medium and incubated at 30 °C and 125 rpm for 10 days.

2.3. Biofoil colonization process

The fungal mycelium obtained was homogenized under sterile conditions to allow better dispersion of the inoculum in each experimental unit and for colonization to be uniform and efficient. The biomass contained in the inoculum was tested. Fungal biomass was centrifuged in the cell suspension for 8 min at 5000 rpm and left in a muffle for 48 h. For the SSF, 100 g of material was mixed with the inoculum and 100 mL of wheat bran broth.

Each experimental unit was arranged in wooden molds (25 cm x 10 cm x 1 cm), to obtain the biofoil for each experimental unit. The fungal biomass concentration in each experiment was 0,82 g/L in dry weight. The material was incubated at 30 °C for 15 days.

2.4. Microbial deactivation

The colonized material was weighed and dried in a muffle at 70 °C for 48 h, to deactivate the growth of the mycelium and prevent the growth of any other microorganisms.

2.5. Biodegradability test

The test to observe the degradation of the biofilms was carried out qualitatively. Pieces (2 cm x 2 cm x 1 cm) were taken from each one and Petri dishes were adapted with 200 mL of water in each one for added pieces; the waiting time was 48 h in a natural environment.

3. Results and discussion

3.1. Obtaining biofoil

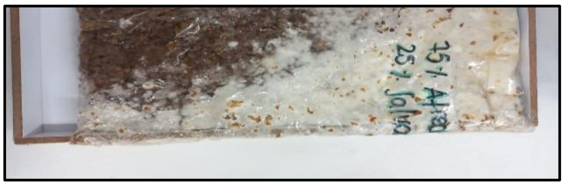
The different proportions of the substrates for obtaining biofoil from *P. ostreatus*, allowed us to identify their effect on the dynamics of biomass propagation as presented in Figure 1 and Figure 2.

Figure 1: Biofoil with 15 d of colonization, 100 % wheat bran.

(a)

(b)

(c)



(d)

(e)

(f)

Figure 2: Biofoil with 15 d of colonization. (a) 100 % R1 malt bran; (b) 100 % R2 malt bran; (c) 75 % malt bran and 25 % R1 wheat bran; (d) 75 % malt bran and 25 % R2 wheat bran; (e) 50 % malt bran and 50 % R1 wheat bran; (f) 50 % malt bran and 50 % R2 wheat bran. The notation, R(n): Replica(n).

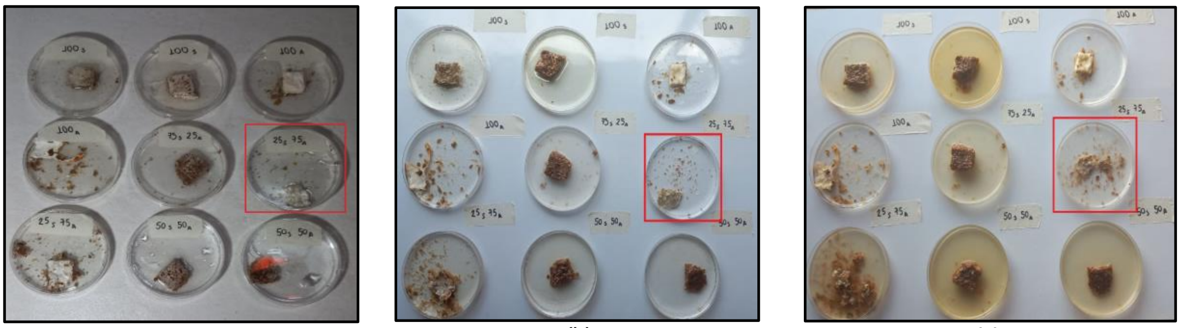
There was greater colonization with proportions of 100 % wheat bran (see Figure 1), considering that this substrate acts as a positive control; however, the mechanical analysis carried out by (Leal and Rodríguez, 2022) on biofoil of 100 % wheat bran, allowed to observe that despite the microbial growth, the structure of the material does not conform to a rigid material. Excess moisture was also observed in Figure 1(a)-(b). that correspond to the malt bran controls, where it is observed that after 15 days of incubation, the colonization of the mycelium occurred in less than half of the sheet, which indicates that the growth was superficial and should proceed at a certain time longer for colonization or seek to remove moisture from the substrate to obtain an assembled biofoil. Regarding the proportions of 75 % of malt bran and 25 % of wheat bran, it was the one that best-achieved colonization by the fungus for both replicates (see Figure 1 (c) - (d)); unlike the other mixtures, this could be favored by the amount of moisture provided by the malt bran, where from a characterization of this, it was observed that it has moisture of 80 %. Finally, when looking at Figure 1. (e) - (f), corresponding to the mixtures of 50 % malt bran and 50 % wheat bran, it is evident that the fungus managed to colonize just a little more than half of the biofoil, probably because more incubation time was required and variables such as humidity were not controlled. Fernández et al., 2020 evaluated the micellar growth of *P. ostreatus* on 3 different substrates, including malt bran, and indicated that it was propitious to perform drying before using the residue and thus favor hydration and softening where the inoculation stage was carried out at a rate of 10 % of the wet mass of said substrate; likewise, he recommends using fluorescent lamps once you begin to observe the development of the fruiting bodies. Bohórquez, 2021 indicates that when making use of brewing residues it is necessary to carry out a pre-treatment to the substrate to favor the growth of the mycelium and given the percentage of macronutrients which are found in proportions of 51 % carbon and 5 % nitrogen, with a relationship carbon/nitrogen (C/N) of 10:20, it is necessary to add some supplement to increase them. this research strengthens the reason mycelium growth was arrested for the 100 % malt bran blocks.

There were never signs of cross-contamination with another microorganism, nor the presence of a decomposition odor for the malt bran despite being stored for so long and with a high percentage of moisture, which indicated that the colonization protocol for this substrate with other mixtures is viable and must be evaluated in longer growth times where other variables could intervene, such as the humidity control and particle size. Faced with all the above, it is evident that malt bran is the substrate that mainly provides moisture to the mixture, being 80 % and a percentage of lignin of 7,32 %, together with cellulose and hemicellulose of 26,10 %. and 21,80 % respectively. For each conformed biolayer, a Scanning Electron Microscope (SEM) test could be performed, as indicated by (Wösten, 2019), (Susel et al., 2021) and (CampardellI et al., 2021) where in their research they consider the heterogeneity of the hyphae and basidia and the variation of micellar growth between each substrate. The SEM test will allow for determining the mycelia extending throughout the substrates and their mixtures and identifying the blinding between the biofoil with the highest and weak growth density.

This project was focused on the recovery of solid waste for malt bran, which is why, from the beginning of the investigation, a mixture that had less than 50 % of this substrate was not considered. In the same way, it must be taken into account that adequacy of substrates with a percentage of less than 50 % malt bran would not allow the correct growth of the mycelium since the bran, due to its high water retention capacity, would make it term the number of available nutrients is diminished in all the extension of the mixture; for this reason, most authors prefer to make a biofoil with proportions even lower than 20 % of wheat bran (Joshi et al., 2020).

3.2. Biofoil degradation

A qualitative test was carried out without any type of accelerator to demonstrate the degradation process in an environment with high humidity content in a period of 48 h.



(a) *(b)* *(c)*

Figure 3: Biodegradability test. (a) Chunks at time 0; (b) Pieces at 24 h; (c) Pieces at 48 h. Note. Pieces with uniform sizes (2 cm x 2 cm x 1 cm), qualitative follow-up for a total of 48 h.

Seeing Figure 3. (a) and Figure 3. (c), the decrease of the biomaterial in an aerobic environment rich in humidity can be appreciated, since for the test, 200 mL of water were added in a conventional petri dish and specimens of each biolayer of (2 x 2 x 1) cm were added in order to have homogeneity inside the dish. On the other hand, it was ensured to have all the time individually, it is observed that for the 75:25 mixture of malt bran and wheat bran in its replicates, there is initially a rigid and compact material, however, it dissolves easily in water. On the other hand, in the mixture where each substrate is found equally, there is a clear indication of water resistance, which preliminarily could indicate how the proportions affect the biodegradation parameter.
In the case of this research, it is hasty to mention the total degradation time, however, with the previous results in mixtures with 75:25 of malt bran and wheat bran respectively, a degradation time of 2 days was specified, considering the composition of the malt bran, which facilitated a partial disintegration of the biomaterial. However the biodegradability of this kind of biocomposites not only depends on the physico-chemical structure of the polymer but also influenced by environmental conditions such as temperature, pH, humidity, and oxygen content (Olivares et al., 2021).

4. Conclusions

The propagation conditions for *P. ostreatus* biomass were established, achieving the colonization of blocks of 100 g of net material. This colonization was carried out at an inoculum concentration of 0,82 g/mL in 15 days of incubation using wheat bran and malt bran. In the same way, it is established that the propagation of the mycelium under only malt bran is not viable due to the contribution of the C/N ratio of 10:20, which must be supplemented with wheat bran. Obtaining biofoil as a potential substitute for expanded polystyrene is viable under proportions of 75:25 of malt bran and wheat bran respectively, managing to be colonized throughout the biomaterial homogeneously. Subsequently, the biodegradation of the biomaterial was evaluated qualitatively identifying the partial decomposition of the material in a period of 48 h, evidencing the differences between the first and the last day in terms of the degradation and visual reduction of the considerable size of the piece under observation, the 75:25 mixes stand out for their degradation and the 50:50 mixes for their water resistance.

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