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High performance of macrofungi in the production of mycelium-based biofoams using sawdust — Sustainable technology for waste reduction

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ABSTRACT

Expanded polystyrene (EPS) is a synthetic polymer that is widely applicable in the fields of food packaging, equipment protection and civil construction. Aiming to replace EPS, biofoams were developed utilizing mycelia of the macrofungi *Pycnoporus sanguineus*, *Pleurotus albidus* and *Lentinus velutinus* in a medium formulated with sawdust and wheat bran. Sample characterization was obtained by scanning electron microscopy (SEM), thermal analysis and infrared spectroscopy with Fourier Transform; density and compression strength were analysed as mechanical properties. The main results include that the compression strengths of biofoams from *P. sanguineus*, *P. albidus* and *L. velutinus* were 1.3, 0.4, and 1.3 MPa, respectively, which exceed that of EPS (0.4 MPa). The thermal stability of the biofoams was lower than that of EPS; however, they were stable up to 350 °C. Biofoams are denser than EPS, with values of 0.3 and 0.03 g cm^{-3}, respectively. The data obtained for biofoams categorize this material as sustainable substitute for EPS in some applications, while at the same time, reducing the environmental impact caused by sawdust and EPS.

1. Introduction

The growing search for biodegradable materials has shifted the interest of the scientific community towards the development of biodegradable products (Vaisanen et al., 2017). These materials can replace synthetic petroleum-related products, such as expanded polystyrene (EPS), which is used in a huge variety of shapes and applications (Poletto et al., 2011; Song et al., 2009; Treinyte et al., 2018).

Polystyrene is a widely employed polymer (Shimomura et al., 2016; Yousif and Haddad, 2013). In its expanded form, EPS is used in the form of plates or blocks for preparing slabs, as protective packaging for domestic appliances, in crash helmets and as electrical or thermal insulators in homes, among other applications (Chen and Liu, 2004; Haghi et al., 2006). However, there are some drawbacks because it is petroleum-related and is not biodegradable, which means that it takes many years to degrade if left in the environment (Araújo et al., 2008). After its use, EPS is unduly disposed of, piling up in landfills or even in the environment, which compromises the conventional degradation processes because certain compounds present in the polymer chains can act as a barrier against degradation by organic compounds (Araújo et al., 2008). Still, EPS contains benzene, which has been classified as a human carcinogen (IARC group 1) since 1979, with sufficient evidence to support the claim that it causes leukaemia (Loomis et al., 2017). EPS also contains styrene, a substance that can also trigger the development of various neoplasms (IARC Monographs Vol 121 Group).

Various macrofungi develop mycelia of high biological degradation (Espinosa-Valdemar et al., 2011) and mechanical strength and, when dehydrated, besides the low density, become an

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alternative type of innovative technology for producing biodegradable materials that have the potential properties for replacing EPS in at least some applications. A few recent research studies have demonstrated the competitive performances of foam-like mycelium-based composites to EPS (Jiang et al., 2013, 2017; López Nava et al., 2016; Haneef et al., 2017; Girometta et al., 2019).

Aiming at the reduction of environmental impacts caused by the presence of synthetic polymer residues in the environment, there is an incentive to facilitate the development of post-use degradation materials, the by-products of which are not harmful to the ecosystems cycles (Tokiwa et al., 2009; Arifin and Yusuf, 2013). The preparation of biodegradable materials that are environmentally and socially responsible is an appealing alternative to the use of EPS. The preparation of this kind of materials is reached by the metabolization capacity of agroindustrial by-products by some kinds of macrofungi into a more valuable materials. Using this approach, reducing the need for petroleum-related products is emphasized, and consequently, the reduction of waste products, which directly influences the environmental sustainability (De Rosa et al., 2010).

The utilization of lignocellulosic agroindustrial by-products for macrofungi production is expanding worldwide, including in Brazil (Nigam, 2017; Gambato et al., 2016). Lignocellulolytic basidiomycete fungi are capable of degrading a series of persisting organic compounds, such as lignin and several classes of pollutants that have little or no structural homology with lignin. Degradation of lignin and other recalcitrant compounds by basidiomycetes is a co-metabolic process mediated by the coordinated action of an enzymatic system, as well as by various low molecular weight metabolites (Medina et al., 2015; Tuomela et al., 2000). Several macrofungi have the capacity to develop mycelia of high mechanical strength. After being dried, they exhibit low density, becoming an option for preparing biodegradable materials as biofoams.

In the United States, a patent by Ecovative Design LLC in 2011 was directed to a fungi-derived biofoam production process. This patent makes use of *Pleurotus ostreatus*, *Fomes fomentarius*, *Ganoderma applantum* and *Inonotus obliquus* fungi, among others, with a substrate of agricultural by-products, such as rice or wheat hulls or cotton seeds (Bayer and McLntyre, 2009).

In this context, the purpose of this study is the development and characterization of biofoams obtained from macrofungi mycelia of South Brazil, including *Pycnoporus sanguineus, Pleurotus albidus* and *Lentinus velutinus*, grown in sawdust and wheat bran, aiming to compare their morphological, thermal, physical and mechanical characteristics to those of EPS. *P. albidus* and *L. velutinus* are edible mushrooms, while *P. sanguineus* is not edible because it has a leather aspect, but is considered as a medicinal mushroom.

2. Material and methods

A flow chart indicating the steps performed for the development of this work is presented in Fig. 1.

2.1. Strains and materials

The fungal strains were from the macrofungi collection at the Laboratory of Enzymes and Biomasses of the University of Caxias do Sul, RS, Brazil, incorporated with the Fungus Collection of the same University (MIUCS)/Herbarium of the University of Caxias do Sul, RS, Brazil (HUCS). In obtaining the biofoams, the macrofungi *Pycnoporus sanguineus* 14G (MIUCS 778), *Pleurotus albidus* 88F.13 (MIUCS 1586) were collected in the locality of São Francisco de Paula, RS, Brazil, and *Lentinus velutinus* 180H.18 (MIUCS 1196) was collected in the locality of Bom Jesus, RS, Brazil. The strains *P. sanguineus* 14G and *P. albidus*88F.13 were selected because preliminary experiments verified that these two strains presented great hyphal formation. *L. velutinus* 180H.18 was selected because it presents a fairly resistant basidioma, resembling leather. The sawdust was from the processed waste of a non-treated *Pinus* sp. wood from Caxias do Sul/Brazil. Wheat, wheat bran and calcium carbonate were acquired in the market.

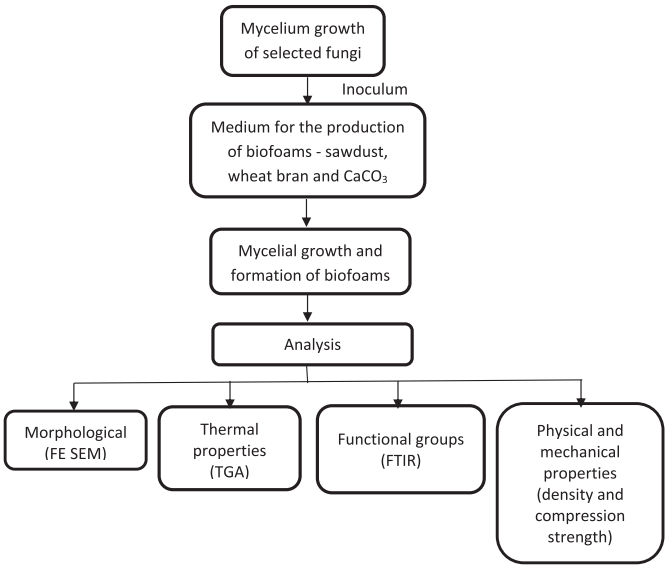

Fig. 1. Flow chart of the activities carried out for the production and analysis of biofoams.

2.2. Culture steps and biofoam preparation

Strains were kept in *Pinus* sp. sawdust agar medium (MS) made of 2% (m/m) milled sawdust (*Pinus* sp.), 2% (m/m) of milled wheat bran, 2% (m/m) agar and 0.2% (m/m) calcium carbonate ($CaCO_3$), dissolved in distilled water. The medium was sterilized in a Phoenix Luferco (Brazil) autoclave for 30 min at 1 atm.

After cooling to ambient temperature ($18 \pm 2\,^{\circ}C$), media was poured into Petri dishes and inoculated with a 1.5 cm disc containing the mycelium of the respective cultures, then incubated in an Oxylab® (Brazil) chamber at 28 °C. After the period of mycelial growth (~10 days), dishes were stored at 4 °C.

The pre-inoculum medium utilized for the development of the fungal strains was constituted by adding wheat grains and calcium carbonate (1%) in glass jars that were previously sterilized for 180 min at 1 atm. Once the medium was cool, the jars were inoculated by placing around 50% of the agar medium containing the previously grown mycelium on the surface of the medium in the respective glass jars. Incubation lasted for 15 days at the temperature of $24 \pm 2\,^{\circ}C$.

The culture media for mycelium development and for biofoam production were prepared from the basic culture medium (Gambato et al., 2016) formulated with 94% (w/w) milled *Pinus* spp. sawdust, 5% (w/w) milled wheat bran and 1% (w/w) calcium carbonate ($CaCO_3$), with the humidity adjusted to 66% (Camassola et al., 2013). The medium was placed in plastic 10 cm × 6 cm (diameter × height) moulds and sterilized for 180 min at 1 atm. After cooling, the medium was inoculated by adding 5% (m/m) of the mycelium-containing pre-inoculum and incubating at $24 \pm 2\,^{\circ}C$ until complete colonization of the medium occurred. The biofoams were prepared so that the obtained material had the same thickness (25 mm) as that of commercial EPS. The biofoam products were then dried at 80 °C for 24 h.

2.3. Morphological analysis of biofoams

Morphological analysis was conducted with the aid of a TESCAN (MIRA3) field emission scanning electron microscope (FE SEM) from the 'Prof. Israel Baumvol Center for Micrography' at the University of Caxias do Sul/RS/Brazil. All the samples were covered by a thin gold layer to avoid distortion due to charging effects, and an acceleration tension of 15 kV was used.

2.4. Determination of thermal properties

Thermal properties were assessed by thermogravimetric analysis (TGA) using a Shimadzu instrument (TGA-50, Japan) at a heating rate of 10 °C.min^{-1}, for a temperature interval of 28 °C to 810 °C, in a nitrogen (N_2) atmosphere. The mass for each sample was approximately 10 g.

2.5. Assessment of samples' functional groups

The assessment of the functional groups making up the biofoams was obtained by Fourier Transform Infrared Spectroscopy (FTIR) using a Nicolet instrument (IS10, Thermo Scientific, USA). The wave number range was 400–4000 cm^{-1}, with the aid of attenuated total reflectance (ATR).

2.6. Assessment of physical and mechanical properties

Sample densities were calculated based on Equation (1) and were expressed on the basis of the average value of three test specimen from each biofoam, compared to the EPS density.

$$d = \frac{m}{v} \quad (1)$$

where d is the density in g.cm^{-3}, m the mass of the composite in g and v the sample volume in cm^3.

Assessment of biofoam compression strength was carried out based on test specimens of 25 × 25 × 25 mm (length × width × height), in a universal testing machine (model EMIC DL-2000, Brazil), at a speed of 50 mm min^{-1}, with a charge cell of 100 kg. For the sake of property comparison, EPS was prepared with the same dimensions as those set for the biofoams.

3. Results and discussion

3.1. Morphological characterization of biofoams

The biofoams produced from the regional macrofungi *P. sanguineus*, *P. albidus* and *L. velutinus* can be observed in Fig. 2. In Fig. 2A and C, which corresponding to *P. sanguineus* and *L. velutinus* biofoams, the formation of a mycelial 'surface layer' around the material can be observed. This is in contrast to Fig. 2B, which depicts *P. albidus* with hyphae entanglements in the colonized material.

For the three macrofungi, biofoams contained compacted hyphae among sawdust and wheat bran particles, with thick growth between the interstices of the substrates and a homogeneous, dense morphologies (Fig. 3).

Fig. 3 shows SEM micrographs of the biofoams obtained from *P. sanguineus*, *P. albidus*, *L. velutinus* cultures, as well as EPS micrographs. Fig. 3B shows a higher number of hyphae adhered to the substrate than adhered to each other, while Fig. 3A and C exhibited elongated, well-distributed hyphae, both in the substrate particles and their interstices, providing a matrix and compacting the material. In regard to cell characteristics, EPS had cells; however, biofoams contained an entanglement of microfibrils with spaces among them, which were responsible for the characteristic lightness of this material.

Similarly, Haneef et al. (2017) also verified changes in the morphology of hyphae according to the fungal species. These authors produced advanced materials from fungi, combining *Ganoderma lucidum* and *Pleurotus ostreatus* mycelium with polysaccharide-based substrates of different compositions, including cellulose and cellulose/potato-dextrose, and observed changes in hyphae morphology that were more pronounced for *P. ostreatus* grown in media with different nutrient sources.

It should be noted that the initial moisture of the samples was 69.5 ± 0.51%, and was 57.3 ± 0.46% after mycelial growth. However, for the storage of this material, moisture needs to be removed through dehydration, and according to the application, it is also important to waterproof the structure.

3.2. Characterization of thermal properties

Thermal analysis data by thermogravimetry for *P. sanguineus*, *P. albidus* and *L. velutinus* biofoams and EPS are shown in Fig. 4.

Biofoams of the different macrofungi exhibit similar behaviours. Three mass loss events were identified for the biofoams. In the first event, the maximum degradation rate (T*max*) at approximately 65 °C and the mass loss value was 10%. This event was attributed to the evaporation of water released from the biofoams as a function of the rise in temperature. The low observed value for T*max* was attributed to water removal (Joseph et al., 2003).

In the second mass loss event, T*onset* values were identified for *P. sanguineus*, *P. albidus* and *L. velutinus* at 362, 355 and 360 °C and T*max* values were 378, 382 and 379 °C, respectively. This event was associated with lignocellulosic components (i.e. cellulose, hemicellulose and lignin) thermal degradation, which occurs between 220 and 310 °C. Between 310 and 410 °C, fibre cellulose degrades, while lignin is degraded in a complex and slow manner throughout the whole temperature range (Borsoi et al., 2013;). Therefore, these events can be attributed to hemicellulose and cellulose degradation and to low lignin degradation (De Rosa et al., 2010; Kim et al., 2006).

The third event was associated with a 20% residue for biofoams at approximately 810 °C, the final degradation products of lignocellulosic fibres comprising non-degraded wastes and impurities

Fig. 2. Photographic record of the biofoams obtained from macrofungi cultures. *(A) Pycnoporus sanguineus*, (B) *Pleutorus albidus* and (C) *Lentinus velutinus*.

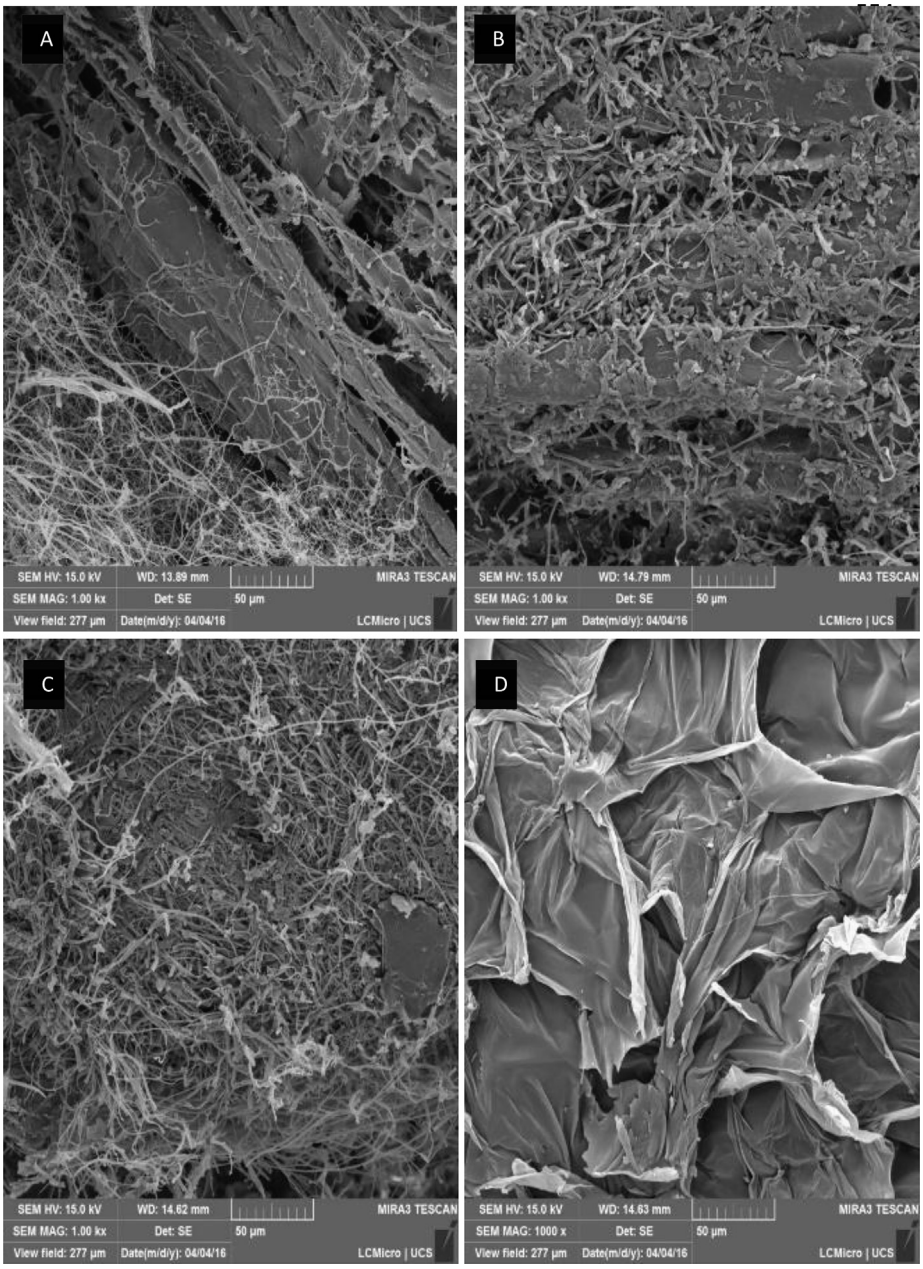

Fig. 3. FE SEM Micrographs of (A) *Pycnoporus sanguineus*, (B) *Pleutorus albidus*, (C) *Lentinus velutinus* biofoams and (D) of EPS.

(Hammoui et al., 2015). Natural fibres consist of a mixture of organic materials, which have a variety of chemical and physical changes when submitted to thermal treatments (Morán et al., 2007).

In the case of EPS, only one event was identified, with T*onset* at 318 °C, T*max* of 440 °C and a residue percentage of nearly null. The maximum mass loss was observed at 475 °C, with a value of 420 °C for total EPS degradation being reported in the literature (Al-Hosney and Grassian, 2005; Taghian Dinani et al., 2015).

T*max* values were similar for the biofoams, but different relative to EPS, indicating that the latter is endowed with a higher thermal stability. The difference between the biofoam curves and the one for EPS could be attributed to reactions occurring in the macrofungi structures, as well as the sawdust, which provided changes to the polymer matrix thermal stability (Ghaffar and Fan, 2015).

3.3. Characterization of the functional groups of the samples

Infrared spectra for both substrates and biofoams are shown in Fig. 5. The infrared absorption spectra of the biofoams are associated with the biomolecules present in the medium and in the mycelia, e.g., polysaccharides (1200–900 cm^{-1}), nucleic acids (1255–1245 cm^{-1}), proteins (amide I at 1700–1600 cm^{-1}, amide II and III at 1575–1300 cm^{-1}) and lipids (3000–2800 cm^{-1}, ~1740 cm^{-1}) (Haneef et al., 2017) (see Fig. 5).

Fig. 5A identifies the bands related to vibration frequencies of the bonds associated with the CO_3^{2-} anion (1441, 1085, 870 and 720 cm^{-1}) of $CaCO_3$ (Chupin et al., 2013). For wheat bran, the bands relate to the frequencies of the C-C, −OH and C-H groups of the ring, whereas the side groups of cellulose and hemicellulose (3285, 2924 and 995 cm^{-1}) (Castro-Alves et al., 2017) are identified. For the

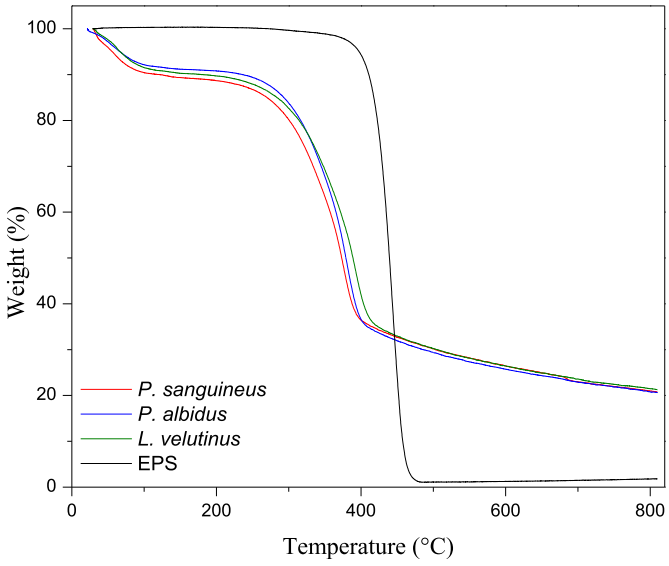

Fig. 4. TGA curves for biofoams from *Pycnopoprus sanguineus*, *Pleurotus albidus*, *Lentinus velutinus* and of EPS.

sawdust of *Pinus* spp. one single band is observed at 1028 cm^{-1}, which is related to the C-O bond stretching of the cellulose structure (Mohan et al., 2006).

Based on the literature, macrofungi used in this study have some polysaccharides, and therefore, biofoams also exhibit them (Fig. 5B). The base spectrum of all the biofoams is exactly the same. In the region between 1600 and 1100 cm^{-1}, several bands are observed that can be attributed to noise or contaminants. Table 1 lists the assignment of the FTIR bands for the biofoams.

In a study by Castro-Alves et al. (2017), the authors observed the profile of the polysaccharides, which make up the fungus species *P. albidus*, and obtained the FTIR spectrum for the extracted polysaccharides. Data presented in this study are like those found in the literature, with the bands identified in the fungi species studied aligning with the main bands that were reported.

On the other hand, in Fig. 5A the characteristic wheat bran bands could not be observed, probably because it was consumed by the macrofungus or due to overlap with bands belonging to cellulose, hemicellulose or lignin. Additionally, the band related to $CaCO_3$ (1441 cm^{-1}) was not identified in any biofoam sample, evidencing its small level in the material.

In the work of Haneef et al. (2017) comparing the spectra of the mycelium species, independently of the feeding substrates, the *G. lucidum* showed a higher contribution of lipids, whereas *P. ostreatus* showed relatively more intense bands related to polysaccharides. When comparing these data with those obtained in this study, it is clear that the chemical nature of the feeding substrates is also responsible for distinct changes in the infrared spectra of the fungal biomaterial.

3.4. Characterization of physical and mechanical properties

To produce mushroom/mycelium-based foam, the challenge of maintaining a consistent density with a raw material that is a living organism has to be overcome (Abhijith et al., 2018). The density values of *P. sanguineus*, *P. albidus*, *L. velutinus* and EPS were 0.32, 0.30, 0.35 and 0.03 g cm^{-3}, respectively (Fig. 6). The biofoams were denser, probably due to the composition of the obtained foams. Despite not being very significant, the value obtained for the *P. albidus* foam was lower. This can be attributed to the fact that its

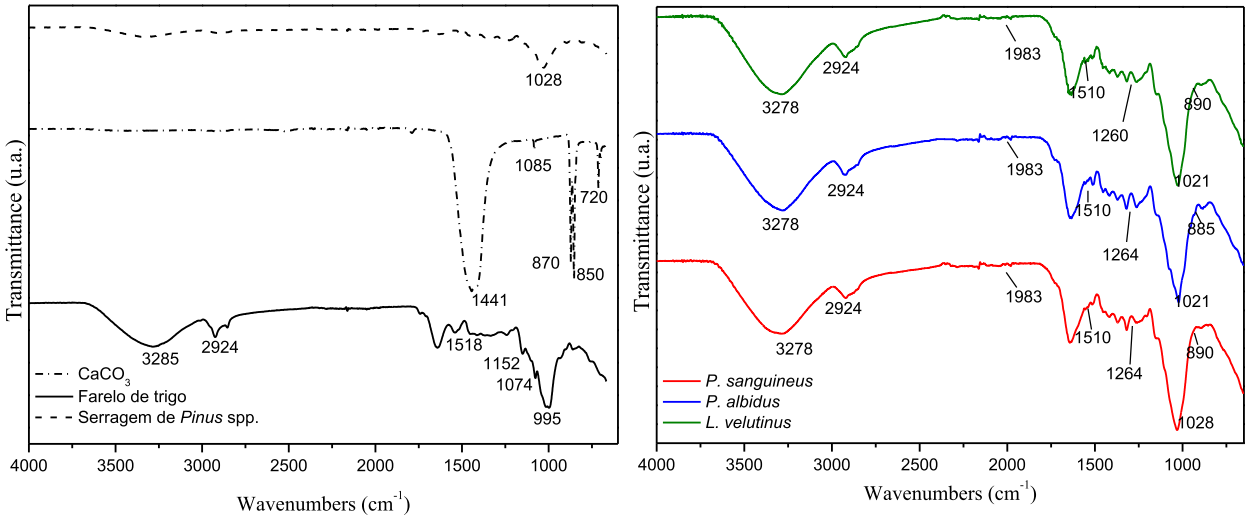

Fig. 5. Infrared spectra (A) of $CaCO_3$, wheat bran and sawdust substrates and (B) biofoams samples from *Pycnoporus sanguineus*, *Pleurotus albidus*, *Lentinus velutinus*.

Table 1
FTIR bands assignment for the biofoams of *Pycnoporus sanguineus*, *Pleurotus albidus* and *Lentinus velutinus*.

Attribution	Vibrational frequency (cm^{-1})		
	P. sanguineus	*P. albidus*	*L. velutinus*
O-H stretching vibrations	3278	3278	3278
C-H stretching vibrations	2924	2924	2924
Between 915 and 1110 cm^{-1}: assigned to the D-Glcp unit.	1028	1021	1021
β-configuration, of the sugar units in the polysaccharide	890	885	890

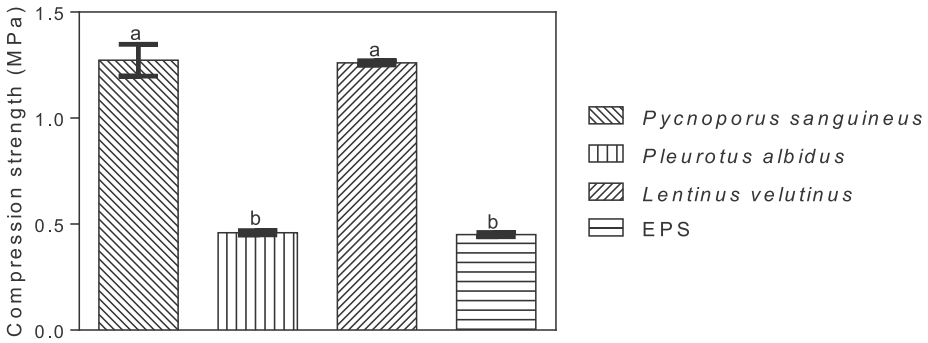

Fig. 6. Compression strength for biofoams of *Pycnoporus sanguineus*, *Pleurotus albidus*, *Lentinus velutinus* as compared with EPS [a, b, c]. Values were determined in triplicate. Same letters mean there are no significant differences at 5% ($p < 0.05$ for the Tukey test).

development was more accentuated among the interstices around the sawdust particles and the wheat bran than in the housing of the overall agglomerate, with no formation of a mycelium envelope as was observed for the remaining fungi (Fig. 2A and C).

All biofoams were obtained for the same substrate colonization period. Because the macrofungi are different, *P. albidus* can demand a longer growth period for providing the mycelial envelope relative to the other macrofungi. However, it is important to note that there was no contamination of any microorganism specimen during the biofoam production process. This is due to the controlled conditions for the manipulation and incubation of the substrates and inocula used for the biofoam production process, although there are reports in the production literature of substances with potential antimicrobials produced by *P. sanguineus* (Jaszek et al., 2015), as well as fungi of the genus *Pleurotus* (Ćilerdžić et al., 2015).

Comparing the data obtained in this work to density to the data obtained by López Nava et al. (2016) (0.057–0.209 g cm^{-3}) and Holt et al. (2012) (0.066–0.224 g cm^{-3}), it is clear that biofoams produced with sawdust have a higher density, but as concluded by López Nava et al. (2016) it is still light enough to be applied as food packaging or domestic electrical device packaging material.

It is also important to emphasize that, changes in the density of biofoam material will occur, due to the fact that biofoams are produced with substrates (lignocellulosic) that present variations in their compositions, as well as different concentrations of sawdust that can be used in combination with other materials, such as wheat bran.

Based on Fig. 2A and C, which correspond to the *P. sanguineus* and *L. velutinus* biofoams, these fungi produce a more compact material than that of *P. albidus* (Fig. 1B), indicating a lower compression strength of the latter, relative to the other two biofoams.

As already identified, mycelial growth behaves as an agglomerating agent for the wheat bran and sawdust components, providing material compaction and structuration. This, in turn, influences the compression strength in the tests applied to each sample and compared to EPS (Fig. 5).

Fig. 5 shows that a compression strength of 1.3 MPa was attained in tests with *P. sanguineus* and *L. velutinus* biofoams, which did not differ significantly one from the other ($p < 0.05$ for the Tukey test). This result was nearly 60% higher than the value of 0.4 MPa obtained for the *P. albidus* biofoams, which was similar to that of EPS, both without significant difference ($p < 0.05$ for the Tukey test). The highest compression forces observed for the *P. sanguineus* and *L. velutinus* fungi should relate to the shape of the basidiomas produced by these fungi, which are leathery. *Pleurotus* basidiomas, however, are fragile, easily broken.

The data obtained in relation to compression strength were also corroborated by López Nava et al. (2016) in a material developed using crop residues (*Triticum* sp.), fungi (*Pleurotus* sp.) and edible films (carrageenan, chitosan and xanthan gum). The compressive strength of the fungal material samples showed a resistance of 42 kPa, while for EPS type XI the resistance was 35 kPa.

These values are associated with the sawdust in the obtained biofoams, which increase the compression strength properties due to cellulose, hemicellulose and lignin being the main components of natural fibres (Mohan et al., 2006). The adhesion force between cellulose fibres and lignin is magnified by the presence of covalent bonds between lignin chains and the constituents of cellulose and hemicellulose (John and Thomas, 2008). When producing biocomposites based on *L. edodes* macrofungus and palm wastes, Tavares et al. (2013) reported having carried out compression tests; however, the values obtained in the analysis were not specified.

This study identified the presence of a higher amount of hyphae resulting from fibre degradation. Under these conditions, the development of macrofungi was favoured as a function of access to the micronutrients in the medium, providing higher mycelial density. Other lignocellulosic materials also have potential for the production of biofoams, such as sludge based on below paper (Sepehri and Sarrafzadeh, 2018). In addition, in order to increase resistance and extend the range of applications of these fungal biocomposites/biofoams, the use of bioresins is an important and interesting strategy (Jiang et al., 2019).

The global EPS market size was estimated at 6.62 million tons in 2016, with the packaging industry dominated its use and accounting for 42.5% of total volume (Research and Markets, 2017). If 50% of this packaging market were replaced by mycelium-based biofoams, it would reduce the EPS in the environment by 1.41 million tons by year. Additionally, when deposited in landfills, this biofoam contributes to the acceleration of the degradation process of other materials, since its composition facilitates the development of other microorganisms; it can even can be used as fertilizer for the soil. Even if discarded irresponsibly, such as along rivers, this material decomposes rapidly, since it is completely biodegradable (Lelivelt, 2015). In addition, as sawdust and other lignocellulosic materials are used to convert to biofoams, they result in less of an environmental impact and less consumption of petroleum products.

4. Conclusion

The biofoams were fabricated using three types of white rot fungi can be a realistic alternative to petroleum-based plastics. The most promising results were obtained from the macrofungi biofoams *P. sanguineus* and *L. velutinus* due to their increased compression strength and the matrix behaviour providing a compact, resistant biofoam. For the various biofoams, the analysed values and behaviour were similar in respect to the

thermogravimetric profile, with a lower thermal stability relative to EPS, but remaining stable at temperatures up to 350 °C. The compression strength of macrofungus biofoams is 60% greater than that of EPS, and their biodegradable properties directly influences the consideration of this material as an alternative to EPS in some applications. Sustainable materials are a strategy to reduce environmental pollution and this biofoams proposed herein strongly support this strategy, since are natural polymers, require minimum energy for production.

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