Evaluation of Metal Biosorption by the Fungus *Pleurotus sajor-caju* on Modified Polyethylene Films

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**Abstract**

Metal ions, such as Fe³⁺, Mn²⁺ and Co²⁺, are present in the formulations of pro-degrading additives and represent a means to accelerate the degradation of post-consumer plastics. When incorporated into the polymer, the pro-degradant additive serves to accelerate the degradation of the polymeric chain through oxidation initiated by light and/or heat, favouring the action of microorganisms. However, the consequences of the disposal of waste containing polymeric pro-oxidants are not yet known and represent an important area of investigation for environmental technology. In this work, we analysed the growth of the edible fungus *Pleurotus sajor-caju* and its ability to biosorb metals in culture media containing polyethylene films with and without a pro-degradant additive. In biosorption process metals, there is a contribution from the pro-degradant additive to the development of mushrooms from *P. sajor-caju* on different substrates. *P. sajor-caju* was effective in removing the following metals: Al, Ca, Cu, Fe, Mg, Mn, K, Na and Zn that were present in the pro-degradant additive and the masterbatch.

**Keywords:** Metals; *Pleurotus sajor-caju*; Pro-degradant additive; Biosorption polyethylene

**Introduction**

The continued use and improper disposal of plastics has compromised the quality of natural resources, often causing serious environmental impacts [1]. When polymeric wastes are generated in large quantities with inadequate environmental management, they can contaminate soil and water, significantly affecting the quality of life and health of humans, in addition to compromising the environment as a whole.

The addition of pro-degrading additives to conventional polyolefins, such as polypropylene (PP), polystyrene (PS) and polyethylene (PE), can alter the degradation process and increase its pace compared to those of the conventional product. This could be a means to reduce the accumulation of waste polymers in the environment [2,3]. The technology of pro-degrading additives consists of adding ions, such as Fe³⁺, Co²⁺ and/or Mn²⁺, to the formulations of polyolefins, with the effect of accelerating oxidation of the polymer when in contact with atmospheric oxygen and energy sources, such as light and/or heat [4].

Currently marketed degradable additives are compounds, such as metallic stearates and stabilisers, typically manganese, which promote the oxidation of the polymer chain when activated by heat, facilitating the access of microorganisms to the polymer chain [5].

The metal released by the degradation of films of polyethylene modified with pro-oxidant additives may pose a threat to the quality of the water and soil when present in high concentrations.

Thus, it is important to know the effect of pro-oxidant additives on the environment to ensure that they do not harm it. Any toxicity may affect animal life present in the soil as well as plant growth and germination. Furthermore, the metals in the additives pose an environmental threat because various metals that are essential to biological systems, such as Fe, Cu, Zn and Mn, can produce toxic effects at high levels, while non-essential metals, such as Cd, Pb and Hg, are toxic in trace amounts [6].

The determination of metals in soil samples is very important in monitoring environmental pollution. Therefore, considerable attention has been applied to reducing metal pollution of the soil through bioaccumulation in edible mushrooms (fruiting bodies) [7-9].

One aspect of biotechnology involves the use of bacteria, fungi, yeasts, algae and other organisms for biosorption, an alternative process for removing metals. These biosorbents have the property of isolating (sequestering) the metal and can be used to decrease the concentration of heavy metal ions in solution to the ppb to ppm level. These microorganisms isolate metal ions dissolved in complex dilute solutions with high efficiency [10].

Mushrooms have been used as biomarkers to determine the heavy metal pollution. Compared with green plants, fungi can accumulate high concentrations of some heavy-metals, such as P, Cd and Hg [6,8].

Fungi are able to accumulate high concentrations of heavy metals [11-13], which can cause serious risks to human health as edible fungi, such as *P. sajor-caju*, can be grown on agroindustrial wastes that have toxic substances, such as heavy metals [14,15]. There are several studies documenting the ability of white rot fungi, such as from the genus *Pleurotus*, to remediate contaminated soil and to reduce toxicity of different types of solid waste [16,17].

In this regard, knowledge of the consequences of degradation of polyethylene films with pro-degradant additives is of fundamental importance to both human health and to develop technologies for metal absorption for bioremediation processes.

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Efforts have been made to assess the possible damage to human health from the ingestion of mushrooms [8,18]. Iano and Rajaratnam [19] found that P. sajor-caju and Pleurotus flabellatus, when compared with other basidiozymes of the genus Pleurotus sp., are collectors of heavy metals and also have high rates of degradation of the medium where they are growing. The mycelium of fungi can absorb heavy metals, which can accumulate and move to the other parts of the fungal body in concentrations, and are sometimes far higher than that of the medium [19].

In this context, the aim of this study was to evaluate the growth and capacity for the biosorption of metals by the fungus P. sajor-caju PS2001 on solid culture medium containing PE films with an added pro-degradant (used in the amounts indicated by the additive supplier). The parameters evaluated were the biomass of P. sajor-caju produced and the content of metals present in the fruiting bodies and in the fungal culture media.

Experimental

Polyethylene films

Polyethylene films were used either without the presence of a pro-degradant additive known (PE) or with 1.5% of the blue pro-degradant additive (PEOX). The blue pigment was used at a concentration of 18.6% in the masterbatch (mixture of pigments, dyes or additives dispersed in a polymeric resin). Its heat resistance is 260°C, and its melt index 25 ± 3 g, 10 min⁻¹.

The blue PE films were produced in an industrial single-screw extruder double fillet, with a diameter/length (D/L) of 1.30, a screw diameter of 45 mm and a temperature profile of 142, 195, 195, 183 and 160°C. The film thickness was 5 microns.

Strain

The fungal strain used was P. sajor-caju PS 2001 from the collection of microorganisms of the Enzymes and Biomass Laboratory, Institute of Biotechnology, University of Caxias do Sul, Rio Grande do Sul, Brazil.

Metal biosorption tests with Pleurotus sajor-caju

To evaluate the biosorption of metals by the fungus P. sajor-caju, different culture media were prepared containing PE and PEOX films, as shown in Table 1.

The moisture content of the culture medium was maintained at levels close to 66%, values considered ideal for fungal growth. The media (MPM, L and L-MPM) listed in Table 1 were divided into nine packages wrapped in polypropylene film.

To each package, 400 g (wet weight) of culture medium was added. The packages containing the media were sterilised by autoclaving at 120°C and 1 atm for two hours [20]. After sterilisation, all containers were inoculated with 5% (w/w) of P. sajor-caju spawn. For each medium, three packs were prepared without the addition of polyethylene films, as controls. To the rest were added 2% (w/w) of PE film (no pro-oxidant additive) or 2% (w/w) PEOX film (with pro-oxidant additive).

The cultures were incubated at 24 ± 3°C and 90% humidity until the substrate was colonised. Then, the packaging was perforated, and the cultures were transferred to an incubation room, with the same conditions of humidity and temperature for the formation of fruiting bodies, which were harvested weekly, and evaluated for dry matter.

Determination of metals

Determination of metals in the fruiting bodies and in the culture media before and after the experiment was performed. The following metals were quantified: Al, Ba, Cd, Ca, Pb, Co, Cu, Cr, Fe, Mg, Mn, Ni, K and Na. The analysis was performed using an atomic absorption spectrometer (Varian Model 250 Plus SPECTRaa) according to METHOD 3050B (Method 3050B, 1996).

Results and Discussion

Assays of metal biosorption by the fungus Pleurotus sajor-caju

In this work, it was found that when the fungus P. sajor-caju was grown in the culture medium MPM, composed mainly of sawdust, mushroom production was higher compared to that in other culture media (L or L-MPM) (Table 1 and Figure 1). This finding demonstrates the potential of mushrooms in the bioconversion of lignocellulosic residues [21], even in the presence of additives from polyethylene films and not with pro-oxidant additives.

Figure 1 illustrates the growth process and fruiting of fungi P. sajor-caju during the period of the experiment on different substrates.

The fungi incubated in culture medium MPM-PEOX and L-MPM-PEOX (culture medium containing PE films with additives) exhibited similar fruiting as their respective culture media controls (MPM) (Figure 2), which confirms that the presence of the pro-degradant additive does not interfere in the development of fungi. Instead, the first appearance of the fruiting bodies was in media supplemented with a pro-degradant additive, suggesting that the metals present in the films of PE induced a stress situation that can promote the appearance of the fruiting bodies or may have contributed to the availability of nutrients in the media.

The fact that the culture medium MPM-PE and L-MPM-PE, which both contained polyethylene films without pro-oxidants, showed lower mushroom production, indicating that the presence of PE films interfere with P. sajor-caju mushroom production. However, cultures grown in the presence of films containing a pro-degradant additive (PEOX) showed an increase in mushroom production compared with cultures grown with PE film. This result supports the hypothesis that stress or nutrient supplementation by PEOX films in the medium enhances the growth of mushrooms. It is possible that the presence of micronutrients in low concentrations allowed better development of macrofungi, such as P. sajor-caju. However, higher concentrations of micro-nutrients may inhibit the development of fruiting bodies. These nutrients are important in the formation of the structure to be essential metabolic processes. Thus, the presence of certain ions may inhibit the effect of other ions, either by their toxic effects or low concentration.

Medium L did not support the development of P. sajor-caju, demonstrating the difficulty of using the fungus P. sajor-caju in soil bioremediation processes.

Because there was more mushroom biomass in the MPM cultures

| Culture medium | Composition |
|----------------|-------------|
| MPM (mushroom production medium) – Control | 94% (w/w) Pinus sp. sawdust, 5% (w/w) wheat bran, 1% (w/w) calcium carbonate |
| L (land) | 100% land |
| L-MPM | 50% (w/w) land, 50% (w/w) MPM |

Table 1: Composition of culture medium for the incubation of the fungus Pleurotus sajor-caju.
As observed in Table 2, Ba initially present in the masterbatch was observed post-harvest in the media MPM-PEOX and MPM-PE. However, Ba was not observed in the fruiting bodies, indicating that the fungus *P. sajor-caju* did not absorb this metal.

Comparing the concentration of the metals Zn, Na, Fe, Al and Ca in fruiting bodies from MPM-PEOX and those from the MPM-Control, there was an increase in the concentration of these metals. This increase was not observed in mushrooms from MPM-PE compared with the MPM-Control. Cu and Mn in the MPM-Control showed a decrease compared with MPM-PE and MPM-PEOX.

Analysis of metals present in the fruiting bodies produced in (MPM-control, MPM-PE, MPM-PEOX) (Figure 2), analysis for biosorption of metals was performed on the fruiting bodies produced on these media.

Figure 1: Growth and fruiting of the fungus *Pleurotus sajor-caju* during the experiment (a) incubation in the incubator (overview); (b, c, d) the culture in MPM culture medium; (e) the culture in Land culture medium; (f) *P. sajor-caju* spawn (“seeds”).

Figure 2: Quantity of fruiting bodies (biomass of mushrooms) of dehydrated *Pleurous sajor-caju* obtained after growth on different substrates (MPM, and L), control, unmodified and modified with pro-oxidants. The values indicate the percentage composition of the medium.
the culture media MPM-PEOX demonstrated an increase in the concentration of the metals Al, Ca, Cu, Fe, Mg, Mn, K, Na and Zn compared with the fruiting bodies produced in the MPM-Control and MPM-PE, indicating the possibility of using *P. sajor-caju* for biosorption of these metals.

Analysing the data presented in Table 2, although it was verified that the fungus *P. sajor-caju* produced in the media MPM-PEOX was efficient in the removal of Al, Ca, Cu, Fe, Mg, Mn, K, Na and Zn, which were originally observed in the pro-degradant additive and masterbatch.

The metals Al, Ca, Cu, Fe, Mg, Mn, Na and Zn that were initially present in the sawdust (control) showed an increase in concentration in all of the treatments and fruiting bodies.

Increases in the concentration of metals in the substrate after the harvest period can be related to the exchange of metals between the substrate and the fruiting bodies. Purkayastha [22] in experiments with *P. sajor-caju* using radioactive isotopes 

| Component | Control – without modified polyethylene films and before harvest | After harvest | *P. sajor-caju* - Fruiting body | After harvest | *P. sajor-caju* - Fruiting body | After harvest | Limit of detection |
|-----------|----------------------------------------------------------------|--------------|--------------------------------|--------------|--------------------------------|--------------|-------------------|
| AI        | 11.41                                                          | 9,693.00     | 298.40                         | 635.62       | 25.02                          | 518.97       | 15.69             | 678.14            | 36.29             | 0.1                 |
| Ba        | < L.D.                                                         | < L.D.       | < L.D.                          | < L.D.       | 21.66                          | < L.D.       | 17.35             | < L.D.            | 0.1               |
| Cd        | < L.D.                                                         | < L.D.       | ND                             | ND           | ND                             | ND           | ND                | ND                | 0.02              |
| Ca        | 63.70                                                          | 131.134.20   | 4,189.40                       | 6,756.58     | 206.44                         | 5,966.49     | 239.61            | 6,897.18          | 412.93            | 0.01               |
| Pb        | < L.D.                                                         | < L.D.       | ND                             | ND           | ND                             | ND           | ND                | ND                | ND                | ND                  |
| Co        | < L.D.                                                         | < L.D.       | ND                             | ND           | ND                             | ND           | ND                | ND                | ND                | ND                  |
| Cu        | 2.27                                                          | < L.D.       | 2.8                            | 4.25         | 4.15                           | 3.73         | 3.82              | 3.6               | 4.28              | 0.01               |
| Cr        | < L.D.                                                         | < L.D.       | < L.D.                         | ND           | ND                             | ND           | ND                | ND                | ND                | ND                  |
| Fe        | 44.40                                                          | 248.28       | 58.50                          | 1,442.65     | 89.00                          | 1,338.82     | 58.65             | 2,004.98          | 98.13             | 0.04               |
| Mg        | 15.54                                                          | 356.40       | 658.40                         | 1,554.44     | 1,704.49                       | 1,373.61     | 1,328.80          | 1,394.86          | 1,819.39          | 0.01               |
| Mn        | 1,717.71                                                      | 27.99        | 64.60                          | 142.82       | 9.05                           | 130.73       | 6.52              | 133.84            | 9.39              | 0.01               |
| Ni        | < L.D.                                                         | < L.D.       | < L.D.                         | ND           | ND                             | ND           | ND                | ND                | ND                | ND                  |
| K         | < L.D.                                                         | < L.D.       | 5.99                           | 31,830.38    | 5.86                           | 32,465.85   | 0.01              |
| Na        | 1,267.34                                                      | 376.20       | 237.70                         | 587.80       | 235.73                         | 728.77       | 0.01              |
| Zn        | 1.29                                                          | 2,439.92     | 15.20                          | 5.26         | 54.79                          | 25.02         | 14.71             | 28.22             | 0.01              |

* L.D.: below the detection limits  
ND: not determined  

Table 2: Concentration of metals determined in the components of culture medium and in the different culture media.

cause severe changes in their physiological processes, and in certain circumstances, it may even kill the mycelium. Therefore, fungi have defence mechanisms to mitigate the toxicity of metals. These defence mechanisms are generally based on using extracellular and intracellular immobilised metal chelation compounds. In some fungal groups, the heavy metals are chelated intracellularly by peptide compounds of low molecular weight [27].

In addition to chelation, metals such as copper and cadmium tend to be retained in the mushroom by binding to aromatic amino acid residues. Metals bound to amino acids can also cause damage to proteins by induction of oxidative stress associated with the production of reactive oxygen species, such as superoxide and hydroxyl radicals [28].

Another factor that may contribute to the biosorption of metals is the presence of chitin in the fungal cell wall, an amino polysaccharide, which can sequester metals.

Conclusions

The present results indicate that the fungus *P. sajor-caju* has the potential to be used in the biosorption of metals, with biosorption differing between the different metals present in polyethylene films with pro-degradant additives. Furthermore, it was found that the presence of polyethylene films without pro-degradants in the culture media interfere with the production of mushrooms. These results also indicate that further studies should be performed to evaluate the effects of metals present in oxi-biodegradable films after their degradation in the environment.

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