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Case Report

Mycoremediation: The case of *Pleurotus ostreatus* on polymers synthetics such as cellulose acetate

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Abstract: Fungi can be used to remove or degrade polluting compounds through a mycoremediation process. Sometimes even more efficiently than prokaryotes, they can therefore be used to combat pollution from non-biodegradable polymers. Cellulose acetate is a commonly used material in the manufacture of cigarette butts, so when discarded, it generates pollution. The fungus *Pleurotus ostreatus* has the ability to degrade cellulose acetate through the enzymes it secretes. The enzyme hydrolyzes the acetyl group of cellulose acetate, while cellulolytic enzymes degrade the cellulose backbone into sugars, polysaccharides, or cellobiose. In addition to cellulose acetate, this fungus is capable of degrading other conventionally non-biodegradable polymers, so it has the potential to be used to reduce pollution. Large-scale cultivation of the fungus has proven to be more economically viable than conventional methods for treating non-biodegradable polymers, which is an additional advantage.

Keywords: fungi; biodegradable; acetylesterase; plastic; cigarette butts

1. Introduction

Fungi are eukaryotic organisms that feed on carbon sources, associated with a high rate of degradation, since in their mycelial stage, they secrete extracellular enzymes and acids that give them the ability to decompose more complex organic compounds [1]. In addition to degrading carbon compounds, they have an important role in environmental biotechnology, thanks to their capacity for leaching, recovery and detoxification of metals, degradation of xenobiotics and organic pollutants [2].

Mycoremediation is a biological treatment that sustainably removes harmful polycyclic aromatic hydrocarbons, halogenated organic compounds, petroleum hydrocarbons, dyes, pesticides, and inorganic compounds through the use of fungi [3]. This biological treatment is highly effective and superior to bacteria in its performance, as they are more competent in co-metabolism and bioaccumulation [4].

A number of fungal strains have been shown to be important pollutant degraders. The degradation of 2,4-dichlorophenol is possible with the fungus *Lentinula edodes* [5]. 2,4-dichlorophenol is ranked 243rd among the most toxic phenolic compounds according to the United States Environmental Protection Agency (EPA) [6], and as a persistent organic compound, its mycoremediation is of great environmental importance.

Mycoremediation of the fungus *Pleurotus pulmonarius* with cellulosic-based radioactive waste has also been studied, showing high degradation rates [7]. Additionally, other post-degradation uses have been found, as indicated in *Cementation of Bioproducts Generated from Biodegradation of Radioactive*

Cellulosic-Based Waste Simulates by Mushroom by Eskander et al. [7], where the degradation products are being studied to generate Portland cement.

Another case studied is the degradation of malachite green, an organic compound used as a dye [8]; the work of Yogita et al. [9] demonstrated the degradation of this dye with the fungi *Jelly sp.*, *Schizophyllum commune* and *Polyporus sp.*, for 10 days and with up to 99.7% effectiveness.

On the other hand, the fungus *Pleurotus pulmonarius* has been characterized as a species capable of degrading petroleum, and therefore is a solution for the bioremediation of soils contaminated by petroleum, having a positive impact on the environment [10]. In a study in 2015, Villacres Manzano demonstrated how the fungus *Trametes versicolor* has generated excellent rates of biodegradation of polycyclic aromatic hydrocarbons (PAHs). These hydrocarbons are a group of more than 100 substances that are formed during the incomplete combustion of coal, oil and gasoline, in addition to other organic substances [11].

It has also been shown that the *Melanized fungi* is capable of absorbing heavy metals and radioactive contaminants from industrial effluents [12]. Likewise, strains of *Pleurotus platypus*, *Agaricus bisporus* and *Calocybe indica* have demonstrated their capacity as bioabsorbents of copper, zinc, iron, cadmium, lead and nickel ions in aqueous solutions [13].

In this way, different fungal strains have been shown to generate benefits in terms of mycoremediation and contribute to existing biological and chemical processes, since they can biodegrade contaminants that had not been possible to remove or reduce by chemical methods or with other organisms such as prokaryotes [14].

2. Degradation of cellulose acetate by *P. ostreatus*

Cellulose acetate (CA) was traditionally considered a non-biodegradable polymer, but its biodegradability was demonstrated in the early 1990s [15,16].

The first step in the degradation of CA, as well as the rate-determining step in this process, is the cleavage of the acetyl group [17]. Acetyl esterases have been recognized as enzymes capable of hydrolyzing this group, specifically acetyl xylan esterases [17]. Xylan and its acetylated form, acetyl xylan, are found in many plants [18]. Xylan is a component of hemicellulose, a compound that saprophytic fungi have adapted to degrade [19], and acetyl xylan shares with CA both the similarity of being a polysaccharide with a glucose backbone esterified with acetyl groups, and also that its acetyl substituents are arranged identically in the equatorial plane [20]. Therefore, enzymes capable of deacetylating this compound are the perfect candidates to catalyze the equivalent reaction in CA, and in fact, this polymer has been reported as a substrate for different acetyl xylan esterases [21].

Once the acetyl group from CA has been removed, the next step is to degrade the cellulose backbone. *P. ostreatus* is known for its ability to degrade cellulose and lignin through the use of extracellular enzymes [22], so this step of the process is faster. These cellulolytic enzymes are cellulases/glucanases, which normally degrade cellulose in plant cell walls [23,24]. Within this category of enzymes are endoglucanases and exoglucanases, the main difference between these two types of enzymes is that endoglucanases randomly cleave cellulose, producing smaller sugars and oligomeric polysaccharides [23], while exoglucanase acts from the reducing or non-reducing ends of a cellulose chain to release cellobiose as the main product [25]. For the degradation of CA, exoglucanase is preferred, although in fact, exoglucanases and acetyl esterases exhibit synergistic properties for the destruction of this polymer [21].

Three other relevant classes of enzymes present in *P. ostreatus* with degradation capacity are: lignin peroxidases, manganese-dependent peroxidases, and laccases [26]. Their main mechanism of action is the creation and use of free radicals to destroy synthetic polymers such as CA [26].

3. Use of *P. ostreatus* for the degradation of other type of polymers

It's worth clarifying that the industrial use of the *P. ostreatus* fungus is nothing new, as several companies have worked on polymer biodegradation using the fungus's chemical and biological properties. One example is a Mexican company responsible for collecting and degrading cigarette butts. In Mexico alone, 5 billion cigarette butts are discarded each year, equivalent to 22,150 tons of garbage, according to the Ministry of Health. This waste represents between 30% and 40% of the waste collected during urban and coastal cleanup activities [27].

Now, expanding the use of the fungus in the degradation of other polymers, research has been carried out with Green Polyethylene, made from ethylene obtained from sugarcane. da Luz et al. explain that *P. ostreatus* can degrade and produce enzymes using oxo-biodegradable plastic waste without prior physical treatment [28]. It is also explained that *P. ostreatus* is a lignocellulolytic fungus that can use lignin, cellulose and hemicellulose as carbon and energy sources [29,30]. This fungus has been used in the degradation of agro-industrial waste [29,32], the bioremediation of pollutants [31] and pulp bleaching [32]. The ability of lignocellulolytic fungi to degrade a wide range of compounds is related to the high efficiency of their enzymatic system.

Trying to relate the properties of lignocellulolytics with the degradation of oxodegradable plastics and how these processes occur, one argument is based on the action of this group of enzymes in a cometabolic process [28]. Cometabolic processes are commonly used in the treatment of recalcitrant compounds, where a carbon source is added to induce the synthesis of certain enzymes, which degrade natural substrates as well as contaminants or unwanted residues [33]. Another type of polymer that has been studied to verify its effectiveness in terms of its degradation is expanded polystyrene (EPS), which is a thermoplastic used in the industry for construction, thermal and acoustic insulation, and packaging of objects and food [34]. All these polymers share similarities in their properties that make them degradable in contact with the fungus, however, it should be noted that factors such as temperature, humidity, time, among others, intervene that can modify the metabolism of the fungus on the polymer.

4. Environmental and economic impact on the degradation media of *P. ostreatus*

The degrading effect of *P. ostreatus* on cellulose acetate in cigarette butts is of great relevance since these represent an environmental problem whose magnitude is not appreciated [35]. A single one can contaminate up to 50 liters of water and it is estimated that they can take up to 25 years to degrade [36]. The impact on the rice agriculture industry is highlighted by the use of *P. ostreatus* as a microorganism for fungal pretreatment for the delignification of lignocellulose polymer (polymer that makes up rice husk) promoting the modification for the conversion of biomethane from lignocellulosic biomass as one of the largest sources of renewable energy from biomass in the world [37,38]; this is because factors have been found that affect the enzymatic hydrolysis of lignocellulose [39], causing rice waste to not be used at the industry/economy level and causing pollution at the environmental level [40]. Likewise, the green polyethylene industry has used the organism *P. ostreatus*, since although these plastics are easily degraded, there is no information available on the half-life or the degradation rate when they are discarded in the environment and in turn, it has been shown to facilitate the mineralization of the polymer [41], due to the complex of lignocellulolytic enzymes produced by the fungus based on the same principle of cellulose acetate degradation [42,43].

5. Environmental sustainability-economics of *P. ostreatus* cultivation

According to Gonzalez [14], the difference in application costs between conventional treatment methods and those related to biological organisms is quite considerable, in addition to implying a lower economic investment, the protection of the quality of the environment is guaranteed, to operate within the limits of the ecosystem.

In addition to its environmental benefits, the *P. ostreatus* fungus is economically viable for large-scale cultivation for its use. Referring to research in 2019 [44], it is indicated that an internal rate of return (IRR) of 18% was obtained, giving favorable results with respect to the investment and pointing towards sustainable economic viability.

Currently, some companies use systems that generate economic profits through practices that help reduce pollution. In the case of cigarette butts, different business models are used. The first is voluntary collection, where multiple establishments organize to place waste containers, in addition to coordinating their collection, obtaining benefits such as certifications and being identified as a "responsible company" with their waste. A second line of business is the sale of waste containers to companies, government institutions, among others, with the installation and collection service. A third line is the sale of products from biodegradable cellulose pulp, such as flower pots (made with 25 butts), pencils (10 butts), sheets of paper and even jewelry. From this idea, a fourth line of business emerged with a B2B model: the sale of cellulose pulp to paper and cardboard mills, which has become the most profitable aspect, since it is offered as a raw material and on a large scale [27].

6. Final considerations

There is sufficient reported evidence of *P. ostreatus*'s ability to degrade polymers such as CA. This fungus's mycoremediation capacity and economic viability give it the potential to be used to degrade waste and biopolymer pollution. Integrating living organisms like the fungus *P. ostreatus* into various industrial sectors opens up new opportunities to implement biological processes that benefit the environment. Additionally, in the field of enzymology, further research is important to better understand the biological degradation of synthetic polymers.

Conflict of interest: The authors declare no conflict of interest.

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