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Plastics Polymers Degradation by Fungi

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Abstract

The studies on plastic degradation are very important for the development of biodegradable plastics, and for reduction of pollution, since plastic waste can remain in the environment for decades or centuries. We have showed the degradation of oxo-biodegradable plastic bags and green polyethylene by *Pleurotus ostreatus*. This fungus can also produce mushrooms using these plastics. The plastic degradation was possibly by three reasons: (a) presence of pro-oxidant ions or plant polymer, (b) low specificity of the lignocellulolytic enzymes, and (c) the presence of endomycotic nitrogen-fixing microorganisms. In this chapter, the plastic bags' degradation by abiotic and microbial process using the exposure to sunlight and the use of a white-rot fungus will be described. The physical, chemical, and biological alterations of plastic were analyzed after each process of degradation. The degradation of plastic bags was more effective when the abiotic and biotic degradations were combined.

Keywords: oxo-biodegradable, green polyethylene, *Pleurotus* sp., plastic bag, sunlight, landfills

1. Introduction

About 800 million metric tons (Mt) of plastics were produced worldwide in the last 67 years, and 79% of this production is accumulated in the environment [1]. According to these authors, in 2050 is estimated an accumulation of about 12,000 Mt in landfills or in the natural environment that represent an annual accumulation of ~339 Mt. Therefore, the development of efficient degradation process is very important to avoid this annual accumulation.

The human population on the Earth in 2050 will be about 10 billion people [2]. Thus, adequate disposal of plastics wastes is important for the maintenance of the natural resources to supply this population. Furthermore, the development of degradable plastics is necessary to prevent the accumulation of plastic waste in the ocean [3].

In Brazil, the National Solid Waste Policy [4] establishes the selective collection, separation of solid waste, recycling and the shared responsibility for the appropriate management of these wastes among manufacturers, distributors, consumers and the government. However, in these 7 years of law it has observed satisfactory

results only in selective collection. In 2014, with the deadline for replacing dumps to landfills, new deadlines for 2021 are in discussion in the National Congress [5].

Our results of biotic and fungal degradation of oxo-biodegradable plastics and green polyethylene could contribute for development of a process of degradation of these residues using white rot fungi. These microorganisms can grow under adverse temperature, nutrient and moisture conditions that facilitate composting and fermentation processes.

The plastics polymers degradation is analyzed by alterations in mechanical, optical or electrical characteristics, cracking, fission, corrosion, discoloration, phase separation, chemical transformations and formation of new functional groups after degradation process [6].

Unlike of the petroleum-derived synthetic polymers, the biodegradable plastics polymers, when discarded in the environment, can be degraded by non-biological and biological processes [7]. Exposure to ultraviolet light, thermal heating, and treatment with acidic or basic substances function as term initiators or photo-oxidation of polyethylene [6]. After this oxidation fragments of polyethylene are degraded by action of microbial enzymes [7].

Oxo-biodegradable or d2W plastics are polymers that contain a pro-oxidant additive to accelerate photo or thermo-oxidation [8, 9]. So, these polymers when exposed to ultraviolet light or at high temperatures are cleaved in low molecular mass compounds that are assimilated by microorganisms [8]. Several studies have shown the biodegradable plastics degradation, after exposed to ultraviolet light or heat, by bacteria and fungi [10–16].

The plastic bags of green polyethylene are produced using low-density polyethylene (LDPE) and green polymers obtain of sugarcane [17]. We have showed the green polyethylene degradation by *Pleurotus ostreatus* PLO6 [15]. However, little information regarding to the biodegradation of these bags is available.

The microbial enzymes, such as depolymerase, esterase and lignolytic ones, that cleave the polymers in small chain compounds, may be involved in the plastics degradation [6, 13, 18, 19]. Thus, white rot fungi have a great potential, because they are enzymes producers and have shown their ability for treatment of industrial waste [20–22].

The white rot, *P. ostreatus*, is a potent degrader of lignin, cellulose and hemicellulose, which lives as saprophyte in wood. This fungus has also been used in the bioconversion of agricultural residues, in biodegradation of organic pollutants, xenobiotics, and industrial effluents, in the cellulose bleaching and production of food and enzymes [23–25]. We showed that *P. ostreatus* PLO6 are capable to degrade oxo-biodegradable plastics and green polyethylene [13–15]. Furthermore, this fungus form edible mushroom that is source of proteins, fibers, minerals and carbohydrates.

Thus, in this chapter described the plastics bags degradation, by abiotic and microbial process, using the exposure to sunlight and *P. ostreatus*.

2. Methods and Results

The degradation of two plastic polymers used in the production of supermarket plastic bags was evaluated (**Figure 1**, I). The oxo-biodegradable and green polyethylene polymers were submitted the abiotic and biotic degradation (**Figure 1**, II). The oxo-biodegradable bags contain titanium oxide as pro-oxidant additive and low-density polyethylene [14].

The abiotic degradation of the plastic bags was the exposure to sunlight up to 120 days (**Figure 1**, III). This exposure was in the summer time in a green house. In this season, the sunlight is from 6:00 am to 5:00 pm.

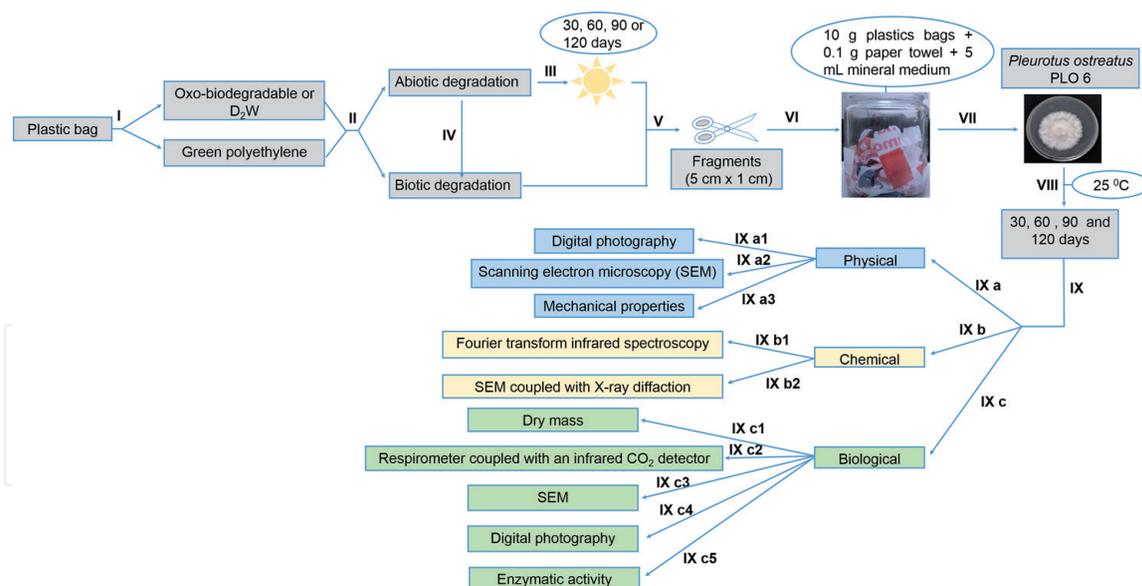


Figure 1. Steps and technics used for monitoring the abiotic and biotic (fungal) degradation of oxo-biodegradable e green polyethylene plastics bags.

For the biotic degradation (**Figure 1**, IV at VIII) the plastic polymers without (**Figure 1**, II) or with the exposure to sunlight (**Figure 1**, IV) was used *P. ostreatus* PLO6 (GenBank accession number KC782771). These polymers were cut in fragments of 5 cm² (**Figure 1**, V) and placed in a glass flask (100 mL) containing paper towel fragments (5–10 cm²) and mineral medium (**Figure 1**, VI). The proportion of plastics and paper towel was of 99:1.

In each glass flask fours discs of agar (6–8 mm) containing the mycelium of *P. ostreatus* PLO6 were inoculated (**Figure 1**, VII). This fungus was cultivated in 20 mL of potato dextrose lignin (0.1%) agar (PDLA) for 15 days. The initial inoculum was obtained from the collection of the Department of Microbiology of Universidade Federal de Viçosa. The stock culture is maintained on PDLA at 4°C.

After inoculation the glass flask were incubated at 25°C for 30, 60, 90 and 120 days (**Figure 1**, VIII).

The alterations in plastic polymers (**Figure 1**, IX) after each time of incubation were performed (**Figure 1**, III, IV at VIII). These alterations were compared with analysis done before of the exposure to sunlight.

Physical alterations (**Figure 1**, IX a), such as wrinkles on the surface, formation of holes and cracks, crumbling, discoloration, were performed by digital photograph and scanning electron microscopy (SEM) with a magnification of 50,000 (**Figure 1**, IX a2). Mechanical properties, such as, energy at break and load at tensile strength were made in universal testing equipment (Instron model 3367) (**Figure 1**, IX a3).

Chemical changes (**Figure 1**, IX b) by Fourier transform infrared spectroscopy (FTIR) (**Figure 1**, IX b1) and SEM coupled with X-ray diffraction (**Figure 1**, IX b2) were determined. These alterations were the disappearance or formation of new functional groups in spectrum of FTIR with scanning of 500 at 4000 cm⁻¹ wave-numbers and the decrease in-oxidant additive concentration by spectrum of X-ray diffraction.

The mycelial growth (**Figure 1**, IX c), the main agent of the biological alterations, was evaluated by dry mass (**Figure 1**, IX c1), respiratory activity (**Figure 1**, IX c2) determined without interruption for 120 days of incubation, electronic micrograph (**Figure 1**, IX c3), digital photography (**Figure 1**, IX c3) and lignocel-lulolytic enzymes activity (**Figure 1**, IX c5).

The capacity of *P. ostreatus* to produce mushrooms in plastics waste may be evaluated under the same growing conditions, steps and procedures shown in **Figure 1**. However, for mushrooms formation it is need, after the mycelial growth (about 20 days), a thermal shock that can be performed by reducing the incubation temperature to 4°C for 24 h and returning to 25°C. During the mushrooms growth, the flasks should be kept in a place at $18 \pm 2^\circ\text{C}$ and a relative humidity of 80%.

A total of 240 days were the time applied to degradation of the plastic bags, being 120 days of exposure to sunlight and 120 days of fungal incubation.

According to the manufacturer, depending on environmental conditions, for example, the exposure to oxygen and outdoor element, oxo-biodegradable plastic bags decompose within a maximum period of 18 months after disposal [26, 27]. They also add that in only 121 days the biodegradability index of d2W plastics was 88.86% [28]. Our time of abiotic degradation is the same those used for calculating the biodegradability index and corresponds to $\frac{1}{4}$ of the required time for the decomposition of these bags. However, after 4 months of exposure to sunlight we did not observe any fragmentation of the plastic bags, only the appearance of small cracks and the bleaching of the film were observed (**Figure 2**). Da Luz et al. [14, 15] also showed changes in mechanical properties of oxo-biodegradable and green polyethylene after 120 days of exposure to sunlight. According to them, this time of exposure is insufficient for other physical or chemical changes, concluding that the mechanical properties alterations, such as the reduction of breaking energy and elasticity facilitated the fungal colonization of plastic waste. The chemical and physical changes in the low-density polyethylene (LDPE) was observed after pretreated of the LDPE sheets with low discharge plasma (O_2 , 3.0×10^{-2} mbar, 600 V) for 6 minutes [29, 30]. According to authors, this pretreated was important by plastics biodeterioration by *P. ostreatus*.

In a new experiment, we observed a fragmentation of oxo-biodegradable plastics bags after 21 months of exposure to sunlight (**Figure 3**). The control samples were cut with a scissors (**Figure 3A**), but after that exposure to sunlight, it was no longer possible to cut the bags. These plastics were easily fragmented using the hand, resulting in a powder (**Figure 3B, C**). This result shows that there are needed more than 18 months of exposure to sunlight to completely degradation of the plastics. However, this result is promising, shows the ability of abiotic degradation of these bags and enables new testing using these bags with exposure to sunlight in a period equal to or greater than 18 months and inoculation of microorganisms to complete degradation of the remaining polymers. Degradation analysis with *P. ostreatus* has not been performed in this experiment.

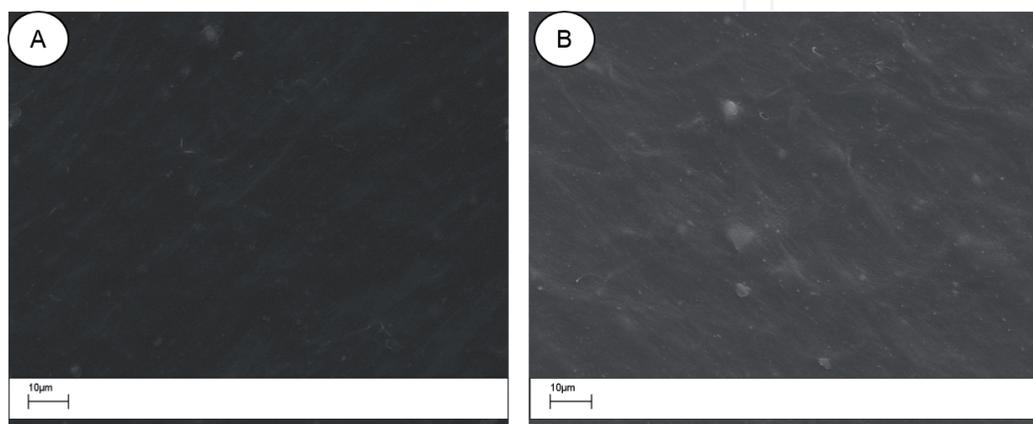
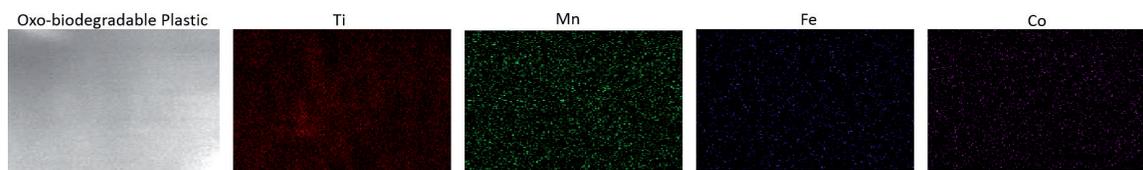


Figure 2. Scanning electron micrograph of oxy-biodegradable plastics before (A) and after 120 days of exposure to sunlight (B).



Figure 3. Oxo-biodegradable plastic bags before (A) and after 21 months of exposure to sunlight (B and C). The letter C shows the fragmented plastic.



Element	Intensity (c/s)	Error	Concentration (% wt)
Si	12.65	2.461	9.402
Ti	76.30	2.946	57.847
Mn	1.45	1.344	1.646
Fe	0.84	1.260	1.027
Co	1.07	1.158	1.494
Cu	7.46	1.253	14.974
Zn	4.78	1.083	11.556
Cd	1.62	2.074	2.055

Figure 4. Spatial distribution and relative concentration of the main elements found in oxo-biodegradable bags. Analysis carried out by a scanning electron microscope coupled to the X-ray diffraction detector.

The oxo-biodegradable polyethylene degradation, assessed by carbonyl index, was observed through exposure to sunlight, up to 90 days, in soil with of moisture and pH control [28]. However, these authors concluded that the polyethylene films without pro-oxidant additive had greater structural and superficial modifications, than the films with the additive. Thus, action of the pro-oxidants by the effect of sunlight depends on e conditions and time of exposure to sunlight.

In the plastic bags the presence of titanium was identified, a component of the pro-oxidant additive (**Figure 4**). This element presents a higher relative concentration than the other elements analyzed and it is uniformly distributed on the surface of the bags. This homogeneous distribution was also observed to manganese, iron and cobalt (**Figure 4**). Furthermore, with the exception of titanium and cadmium, the other elements analyzed are important for fungal metabolism (**Figure 4**). These micronutrients may be elicitors or enzyme cofactors. Thus, the presence of these elements may also have contributed to the *P. ostreatus* growth on the surface of plastic bags.

Mycelial growth of *P. ostreatus* was observed on the surface of the paper towel (**Figure 5A**) and the plastic waste (**Figure 5B**). This figure shows an example of mycelial growth in oxo-biodegradable plastics after 30 (**Figure 5A**) and 90 days

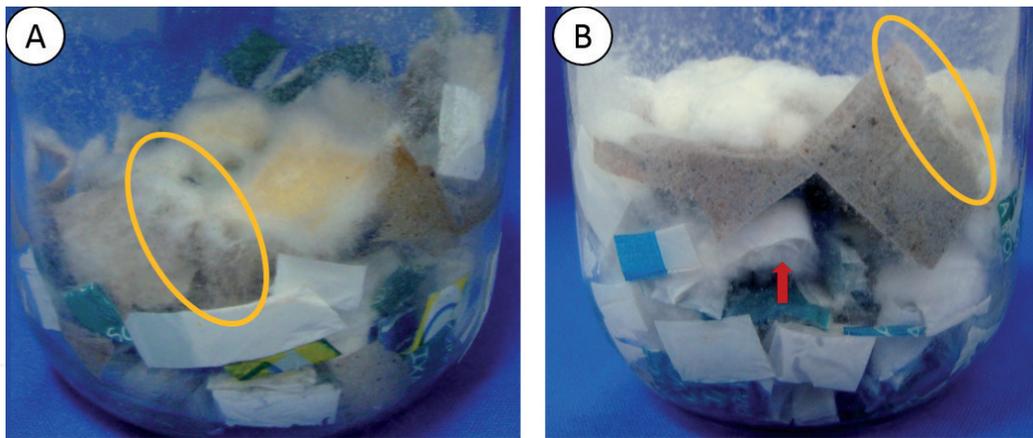


Figure 5. Mycelial growth of *Pleurotus ostreatus* after 30 days of incubation in paper towel and oxo-biodegradable plastic bags after 30 (A) and 90 (B) days of exposure to sunlight. The arrow and circle show mycelial growth.

(**Figure 5B**) of exposure to sunlight and 30 days incubation with the fungus. The yellow circle and the red arrow show, respectively, the mycelial growth on the paper towel and plastic. This paper was added in the culture medium to retain moisture and to be an inducer of fungal growth and stimulates the synthesis of lignocellulolytic enzymes that degrades the paper itself and the plastic (**Figure 5**).

The respiratory activity of *P. ostreatus* was influenced by time exposure to sunlight. This result confirms that the physical changes caused by sunlight contributed to the fungal growth in plastic bags. Furthermore, we did not observe reduction of this activity until 90 days of incubation showing a cellular activity for a long period. The activity of lignocellulolytic enzymes, like laccase, cellulase and xylanase, during 45 days of incubation of *P. ostreatus* in oxo-biodegradable plastics was observed [13].

The *P. ostreatus* growth on the surface of oxo-biodegradable plastic was also observed by SEM (**Figure 6**). In this micrograph, the red arrows show hyphae in plastic waste after 60 days of exposure to sunlight and 30 days of incubation. Da Luz et al. [13–15] showed the formation of mycelium on the surface of d2w plastic and green polyethylene with different time of exposure to sunlight and of fungal incubation. They also reported the morphological characteristics of the mycelia of *P. ostreatus* PLO6.

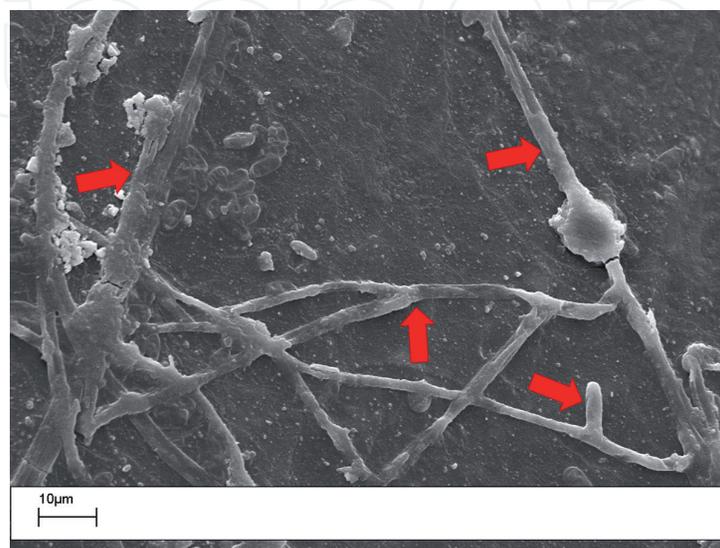


Figure 6. Scanning electron micrograph of oxo-biodegradable plastics after 60 days of exposure to sunlight and 30 days of incubation with *Pleurotus ostreatus*. The arrows show the hyphae.

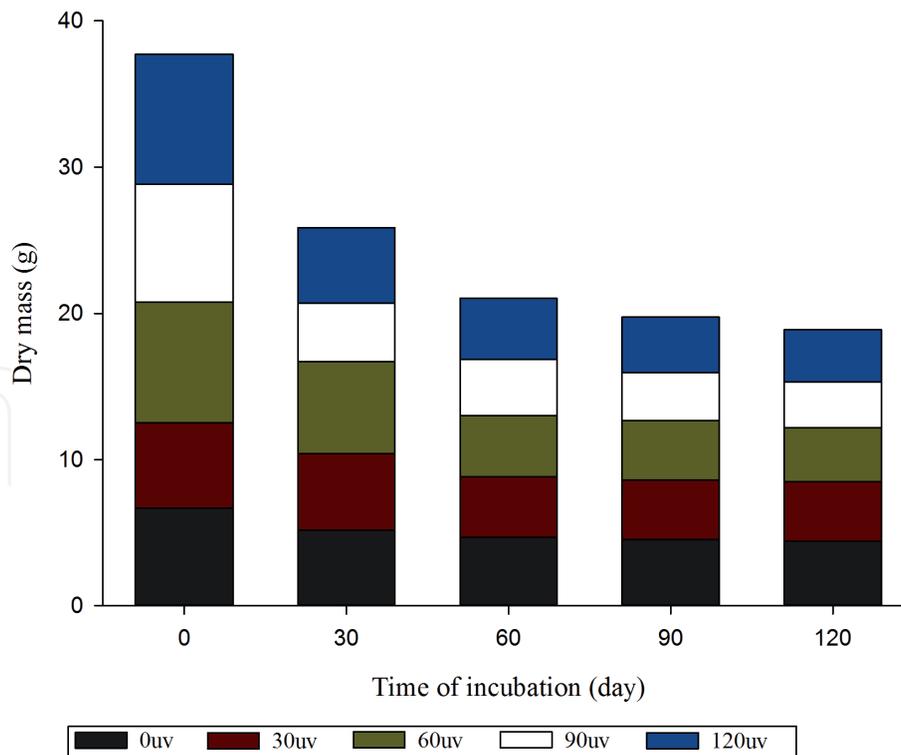


Figure 7. Loss of dry mass plastic after inoculated with *Pleurotus ostreatus* in oxo-biodegradable plastic bags before (0uv) or after 30, 60, 90 and 120 days of exposure to sunlight.

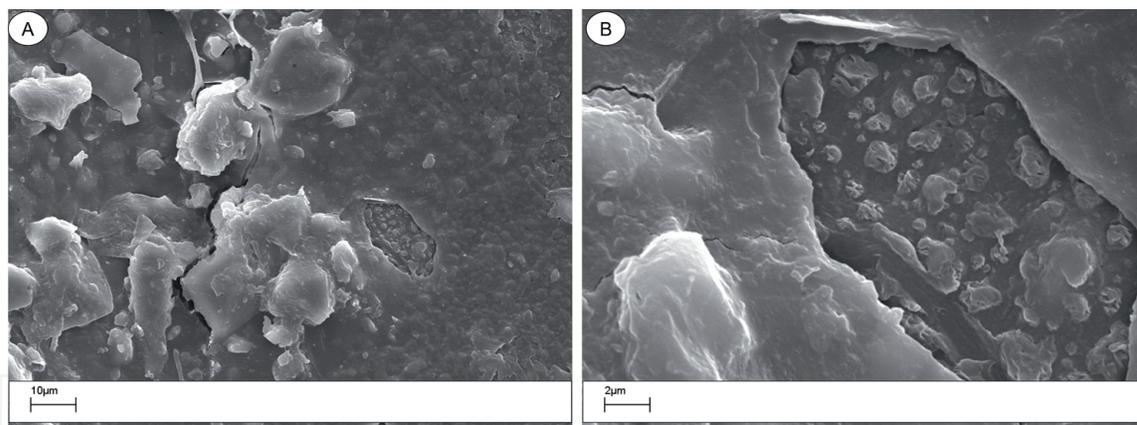


Figure 8. Scanning electron micrograph of oxo-biodegradable plastic bags after 30 days of exposure to sunlight and 30 days of incubation with *Pleurotus ostreatus*. Micrograph without (A) and with a scale of 100 folds (B).

The loss of plastic dry mass was influenced by the time of exposure to sunlight and fungal incubation (**Figure 7**). Fungal growth was lower in plastic polymers without exposure to sunlight than in others with different time of exposure to sunlight. This result shows that *P. ostreatus* can grow in plastic waste without or with exposure to sunlight. However, this exposure facilitates the fungal growth, as shown by Da Luz et al. [14, 15]. Thus, the combination of abiotic and biotic processes shows to be more efficient in the oxo-biodegradable plastic and green polyethylene degradation. In addition, the presence of other carbon sources from marine sediments and lack of abiotic degradation as the initiator were the main factors of the lack of biodegradation of polyethylene and biodegradable plastic bags after 100 days of incubation with benthic microbes [3].

In this study, we observed the formation of cracks and holes in oxo-biodegradable plastics and green polyethylene after fungal growth (**Figures 8 and 9**).

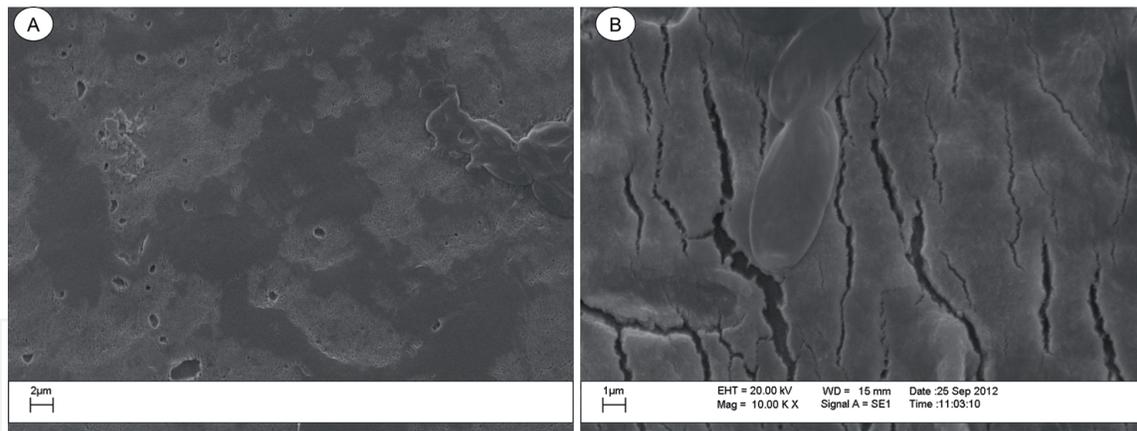


Figure 9. Scanning electron micrograph of green polyethylene plastics bags after 30 days of exposure to sunlight and 30 days of incubation with *Pleurotus ostreatus*. Micrograph without (A) and with scaling of 100 fold (B).

Comparing the **Figure 2B** and **8** it is observed that these changes in plastic polymers were caused by *P. ostreatus* growth. The fungi, *Penicillium oxalicum*, *Penicillium chrysogenum*, *Myceliophthora* sp., *Phanerochaete chrysosporium*, and *Trametes versicolor*, also exhibit the ability to degrade polyethylene [16, 31, 32].

In a simulation according to ASTM G160–03 of polyethylene films degradation with and without pro-oxidant additive through the exposure to sunlight on the soil, different genera or microbial groups, *Geothrichum* spp., *Mucor* spp., *Rhizopus* spp., *Thichoderma* spp., *Penicillium* spp., *Aspergillus* spp. and Zygomycota, were identified [28]. These results indicate that (1) the plastic films did not alter or inhibit the development of the microbial community of the soil, since these microorganisms are part of the natural microbial community of the soil or (2) the growth of these microorganisms was due to the use of the films as source of carbon and energy. According to the authors, the biodegradation of polyethylene without or with pro-oxidant additive can be shown by the adhesion and surface erosion of the films, microbial colonization and presence of fruiting bodies and hyphae on the plastic surface.

The **Figures 8** and **9** show the plastic degradation with 30 days of exposure to sunlight and 30 days of incubation with different scale enlargements. According to Da Luz et al. [14], the low specificity of the lignocellulolytic enzymes and presence of pro-oxidant ions and endomycotic nitrogen-fixing microorganisms were the main reasons for the biotic degradation of oxo-biodegradable plastics. Gómez-Méndez et al., [29] observed activities of laccase, manganese peroxidase (MnP) and lignin peroxidase during *P. ostreatus* growth in plasma pretreated Low-density polyethylene (LDPE) sheets. These authors showed that LDPE biodeterioration was due to activities of these fungal enzymes. Furthermore, the LDPE after mycelia fungal may be used by biochar production [30].

The laccase produced by the fungus *Cochliobolus* sp. isolated from plastic dumped soils showed capacity for polyvinyl chloride degradation [33]. This enzyme produced by *Myceliophthora* sp. was also able to degrade polyethylene [30] and polyurethane [34]. Manganese peroxidase from white rot fungi, *Phanerochaete chrysosporium*, is involved in the degradation of nylon and polyethylene [35]. The laccase and manganese peroxidase activity of *Penicillium* sp. are responsible by degradation of polyethylene [31, 36] and natural rubber [37]. These studies confirm the low specificity of these enzymes to the substrate.

After 120 days exposure to sunlight, no changes in the FTIR spectrum of oxo-biodegradable plastics was observed. This result shows that pro-oxidant oxidation by sunlight was not sufficient for cleavage of the polymer chain or it there is no oxidation thereof. However, in a previous study, a reduction of the relative

concentration of titanium on the surface of oxo-biodegradable plastics wastes after exposure to sunlight was observed [14]. According to these authors, the oxidation of the pro-oxidant may have occurred initially by sunlight and then by co-metabolism with the extracellular fungal enzymes. The authors concluded that the presence of this pro-oxidant proved to be important to cause the breakage of this chain in fragments that were used as a source of carbon and energy by fungus.

In polyethylene green, which contain none pro-oxidant additive, no changes in the FTIR spectrum after exposure to sunlight was observed.

The formation of bands of the bonds oxygen-hydrogen and carbon-hydrogen at $3500\text{--}3000\text{ cm}^{-1}$ and carbon-oxygen and ether or peroxide at $1500\text{--}1000\text{ cm}^{-1}$ were the main changes in the FTIR spectra observed in plastic waste after *P. ostreatus* growth. The carbon-hydrogen bond band may be evidence of the fragmentation of the polyethylene chain. The other bands observed indicate that an oxidation has occurred, which may have contributed to the fungal colonization in the plastic polymers (13–15).

In studies on the plastics degradation for *P. ostreatus*, the authors also observed chemical and physical changes similar to the observed in our study [29, 30].

The intensity of the degradation was higher in the green polyethylene than in the oxo-biodegradable polymers (**Figures 8 and 9**). The green polyethylene degradation by fungus was possible due to the presence of sugarcane polymers in the composition of the bags, low specificity of the lignocellulolytic enzymes and presence of endomycotic nitrogen-fixing microorganisms. In addition, Da Luz et al. [15] was observed mineralization in green polyethylene with longer times of exposure to sunlight and fungal incubation.

Similar to Da Luz et al. [13], during the time of incubation we also observed the mushrooms formation in the plastic (**Figure 10**). The conversion of plastic waste into fungal biomass and mushrooms would be a very important biotechnological innovation for plastic waste degradation that has been increased by millions of tons in recent years [1, 3, 16] and for environmental sustainability. However, the presence of toxic compounds and heavy metals, and also due to the low productivity and high costs are the main limitations to mushrooms production. Productivity in mushrooms can be increased by altering the composition of substrate, as for example, adding different proportions of agroindustrial residue and plastic.

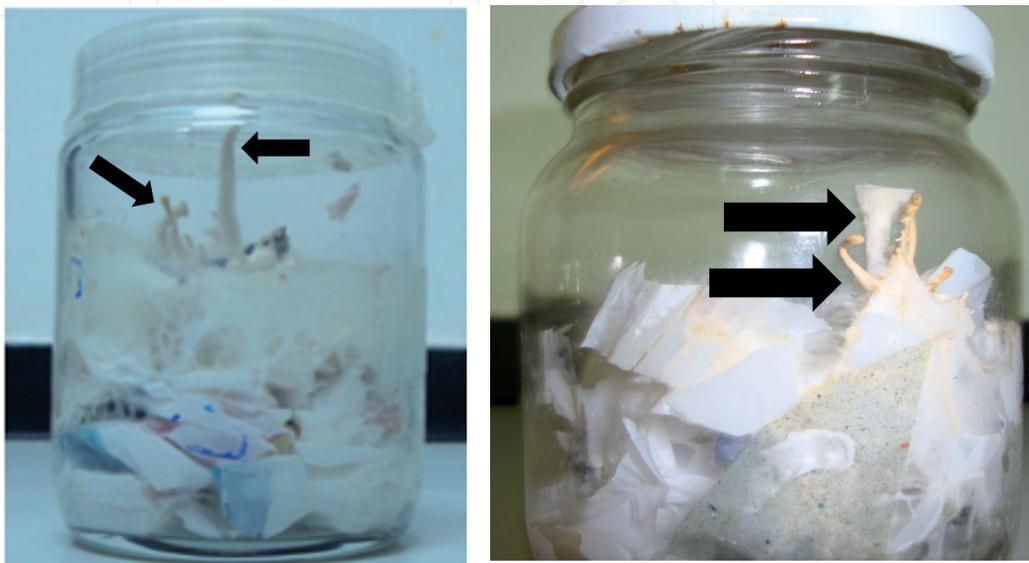


Figure 10. Mycelial growth and *Pleurotus ostreatus* mushrooms (arrows) formation in substrate containing oxo-biodegradable plastics and paper towels (99: 1 m/m).

3. Conclusions

The exposure to sunlight up to 120 days is insufficient to initiate degradation of oxo-biodegradable and green polyethylene plastic bags. However, this exposure is important for *P. ostreatus* growth. Therefore, these plastics degradation occurs more efficiently with the combination of abiotic and biotic process. These plastics degradation may be due to the activities of lignocellulolytic enzymes that are produced during fungal growth on the plastics sheets.

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Conflict of interest

The authors declare no conflict of interest.

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