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Biodegradation of PCL and PVC: *Chaetomium globosum* (ATCC 16021) activity

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Abstract

The increasing use of plastics in human activities has resulted in an enormous amount of residues which became a matter of great environmental concern. Scientific studies on the microbial degradation of natural and synthetic molecules show the potential of fungal application on cleaning technologies. The biodegradation of PCL (polycaprolactone) and PVC (polyvinyl chloride) films by *Aspergillus brasiliensis* (ATCC 9642), *Penicillium funiculosum* (ATCC 11797), *Chaetomium globosum* (ATCC 16021), *Trichoderma virens* (ATCC 9645), and *Paecilomyces variotii* (ATCC 16023) was studied. According to ISO 846-1978—"Testing of Plastics - Influence of fungi and bacteria", samples of the studied polymers were inoculated with a mix suspension of 10^6 fungal inoculum and maintained in moisture glass chambers in a bacteriological incubator at 28 °C for 28 days. The samples were analyzed by means of morphological and color changes, mass loss, optical microscopy (OM), and scanning electron microscopy (SEM) after 28 days of culturing. After the incubation period, visual observations of the PCL films showed many micropores and cracks, pigmentation, surface erosion and hyphal adhesion on the sample surfaces, and a mass loss of up to 75%. On the contrary, there was no evidence of PVC biodegradation, such as changes in color and significant mass loss. *Chaetomium globosum* ATCC 16021 was a pioneer in the colonization and attack of PCL, resulting in significant mass losses. Although PVC was less attacked by the ascomycete, the polymer supported the adhesion and growth of its fertile structures (perithecia), suggesting the fungal potential to degrade both plastics.

Introduction

Plastics are present in many types of products replacing materials such as wood, glass, and metal in different applications. Since they are almost indefinitely persistent in the environment, their accumulation in landfills has vastly increased. Scientific investigations on the microbial ability to degrade plastic waste are considered extremely important and the use of rapidly degrading polymers is strongly recommended.

In a biodegradation process, the enzymes of the biosphere essentially take part in at least one step in the cleavage of the chemical bonds of the material, in a favorable environment, and the biodegradable material may not necessarily degrade within a short time (Singh et al. 2003). The synthetic biodegradable polymer, poly (ε-caprolactone) (PCL), which is a linear, hydrophobic, and partially crystalline polyester, can be utilized slowly by microbes, before its complete assimilation (Calil et al. 2006; Singh et al. 2003). The physical properties and commercial availability of PCL make it very attractive, not only as a substitute for the non-biodegradable polymers in commodities, but also for specific applications in medicine and in agricultural areas (Khatiwala et al. 2008; Singh et al. 2003). Up to now, large-scale use of the PCL has been limited because of their relatively high price, as well as some inferior intrinsic properties. Blending biodegradable polymers with other materials (natural or synthetic) has proved to be an effective and economic way of resolving this problem (Zhao et al. 2008).

PVC has a variety of important technological applications such as in pipes and pipe connections, films for packaging, and window frames (Kamo et al. 2003; Nunes 2006). However, it thus accumulates in landfills in great quantities

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and its incineration is toxic due to the release of hydrogen chloride, which causes serious problems in the treatment of garbage (Pospisil et al. 1999; Veronelli et al. 1999).

International standards suggest the employment of fungal consortia to optimize plastic biodegradation process. Genera of filamentous fungi like *Aspergillus*, *Paecilomyces*, *Penicillium*, *Trichoderma*, *Fusarium*, and *Phanerochaete*, for example, show promising species mainly for the degradation of substrates with large molecules as the plastics (Santaella et al. 2009; Shah et al. 2008). The degradation activity of different types of polymers by *Chaetomium globosum* has also been extensively reported and applied in a diverse number of biodeterioration tests (American Society for Testing and Materials 2002; British Standards Institution 2003; Kim and Rhee 2003; Sowmya et al. 2014; Standards Australia 1999; Von Arx et al. 1986). In this context, our objective was to investigate the biodegradation of PCL and PVC films, obtained by casting, through the analyses of mass loss, color changes, optical microscopy, and scanning electron microscopy (SEM), after exposure to a pool of filamentous fungi recommended by the European Standard Test ISO 846 (1978) (International Organization for Standardization 1978).

Materials and methods

Molar mass of polymers

Poly (ε-caprolactone) (PCL): (Solvay-K6800) - M: 85,000 g/mol
Poly (vinyl chloride) (PVC): (Sigma-P-9401) - M: 73,491 g/mol

Film preparation

The films were prepared by casting of 1,2-dichloroethane (dicet) solution made by mixing 0.2 g of PCL or PVC in 15 mL of dichloroethane, stirred for 30 min at 60 °C. Each solution was placed in Petri dishes for drying in an oven at 45 °C and 0.5 mmHg vacuum and during 2 h for evaporation of the solvent. The films obtained were 40–50 μm thick, which were kept for 2 days in a vacuum desiccator and cut so as to obtain four samples.

Fungal strains

The biodegradation of the samples was evaluated according to ISO 846-1978 standard test, which analyzes fungal growth and the fungistatic effect of the film. Firstly, the strains were incubated on medium with no other carbon source but the polymer (named incomplete agar). In a second treatment, the strains were grown in complete agar (glucose as C source), to confirm that the film composition did not inhibit the fungal development. The standard strains used were as follows:

Aspergillus brasiliensis ATCC 9642; *Chaetomium globosum* ATCC 16021; *Penicillium funiculosum* ATCC 11797; *Paecilomyces variotii* ATCC 16023; and *Trichoderma virens* ATCC 9645. The cultures were obtained from the André Tosello Culture Collection, Campinas, São Paulo, Brazil.

Nutrient-salt dispersant solution

In a volume of 1000 mL of distilled water, the following reagents were dissolved: 0.3 g K_2HPO_4, 0.7 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 2.0 g $NaNO_3$, 0.5 g KCl, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.1 g Tween 80. The solution was sterilized by autoclaving at 121 °C, 101,325 kPa for 15 min.

Test preparation

Growth test: The incomplete agar was prepared adding 2% (v/v) agar into nutrient-salt solution and the complete agar was complemented with 3% (v/v) glucose. Both culture media were sterilized at 121 °C, 101,325 kPa for 15 min.

Spore suspension preparation

All cultures were grown on slants of storage medium for 15 days at 28 °C. Fungal colonies were then covered with 5 mL of dispersant mineral salt solution. To test the fungistatic effect of the polymers, the strains were grown in complete agar, and 5 mL of the same salt solution containing glucose was used. Delicate scrapings with a sterile platinum loop were done to resuspend the cells. The concentration of 10^6 spores/mL was set by counting in Neubauer chamber. This operation was repeated for each fungal strain, before getting a final 25 mL mix suspension.

Sample sterilization

With the aid of a sterile needle, the polymers were heat labeled, estimated on analytical scale, and sterilized with 3% hypochlorite solution for 30 min, according to ASTM D-6288-98 (American Society for Testing and Materials 1998).

Biodegradation in agar medium

A sample of each polymer was placed in a Petri dish with complete agar and a second one on the incomplete medium. A spare sample was kept as control, inside an envelope in the dark. Individual samples of polymers (PCL and PVC) were immersed in 25 mL mix suspension with a concentration of 10^6 spores/mL, for 15 s. To track possible hydrolysis, samples of polymers were also immersed in sterile distilled water, for 15 s, and inoculated on both media tested. The assay was made in triplicates. They were placed in two humidity glass chambers: (a) control and (b) pool, both sealed with

PVC film Magipack®, incubated in a bacteriological incubator at $28 \pm 1\,°C$ and not less than 85% relative humidity for 28 days. During the experiment, temperature and humidity were checked three times a day with the help of a digital thermo hygrometer.

Characterization of PCL and PVC films

The fungal attack examination was carried out under a dissecting microscope. By means of optical microscopy, comparisons between the control samples and biotreated ones were made. The samples were analyzed according to change of color to the naked eyes. The images were recorded through digital photos. The mass loss of the films was measured by analytical weighing (CHYO JK 200 ± 0.0001 g) before and after the microbial treatment. After biodegradation, the fungi were cleared and the specimens were washed and dried. The mass loss of specimens (M) was measured according to the mass of specimens before (M_0) and after biodegradation (M_1).

$$M\ (\%) = \frac{(M_0 - M_1)}{M_0} \times 100\% \qquad (1)$$

Optical microscopy with camera

Comparative optical micrographs of PVC films and PCL untreated and biotreated were performed by microscope Olympus CX21. The pictures were recorded by digital camera Canon PowerShot A570 IS.

Scanning electric microscopy

This measure was performed to detect the change in morphology of the polymer film surface of PCL and PVC after biotreatment. The surfaces of the samples were coated with the gold using a Balzers MED 010 sputter coater, after which they were mounted onto a sample holder and their morphology was examined under a scanning electron microscope (Zeiss DSM 940-A), 5 kV.

Statistical analysis

To evaluate the efficiency of degradation by consortium of fungi compared to strains isolated and also the different culture media used had to be based on the data of mass loss. The comparison between the experimental groups was performed using the nonparametric Kruskal-Wallis test, using the statistical program BioEstat, version 5 (2008), considering significant differences when the p value was less than 0.05 ($p < 0.05$).

Results and discussion

Fungal growth on the polymers

Fungal growth pigmented the agar of all complete medium plates, both for PVC and PCL samples; however, filamentous growth on the surface of the films showed to be weak and mainly restricted to the heat-marked areas and up to the edge of the films (indicated by arrows, Fig. 1a, b). Concerning fungal development on incomplete medium, PVC showed no growth evidence in the middle or inside the film but there was a marked adhesion of perithecia *Chaetomium* on the edges of the film (Fig. 1c). Already PCL samples supported the specific growth of *C. globosum* (Fig. 1d).

Among all reference strains recommended by the European Standard Test ISO 846-1978, *Chaetomium globosum* was the only one able to use the PCL as the carbon source, using it as substrate of adhesion and degrading it; in some assays, the sample was completely degraded after 28 days. Compared to PVC, PCL presents better conditions for fungal growth due to its structure and hydrophilicity. PVC has negative charge macromolecules owing to the presence of the chlorine atom in its chain, making dipole-dipole interactions (Grisa et al. 2011; Martins-Franchetti and Marconato 2006).

Hydrophobic and electrostatic interactions have also been suggested to be of great importance for the adhesion of fungal reproductive structures although this is questioned by some authors (Jones 1994). Microorganisms have a negative surface potential, as well as the surface to be colonized, which could lead to an electrostatic repulsion between them. However, it is known that factors such as hydrogen bonding and hydrophobic interactions may counteract such forces of electrostatic repulsion, thus favoring microbial adhesion (Jones 1994).

Scanning electron microscopy

The morphological changes on the surface of PVC and PCL films due to the action of fungal consortium were analyzed after incubation period of 28 days. Figure 2a shows a SEM image of the surface of the untreated PVC film. As seen, the polymer showed pores (indicated by arrows) formed due to the output hydrochloric acid polymer surface. The PVC film biotreated for 28 days showed superficial erosion with wrinkle aspect (Fig. 2b, c), as also reported by Shah and co-workers (2011) (Shah et al. 2008). The susceptibility of the polymer surface to microbial action is shown by the presence of fungal reproductive structures (perithecia) and hyphae of *C. globosum* (Fig. 2d) and spores (Fig. 2e, f).

The micrograph results of PCL films biotreated for 28 days showed layer erosion on the polymeric surface (Fig. 3b) and spherulitic degradation (Fig. 3c), different from the untreated sample (Fig. 3a). Microbial adhesion was observed by

Fig. 1 PVC (**a**) and PCL (**b**) films on complete agar. PVC (**c**) and PCL (**d**) on incomplete agar showing adhesion of *C. globosum* perithecia, biotreated for 28 days. Arrows indicate fungal growth
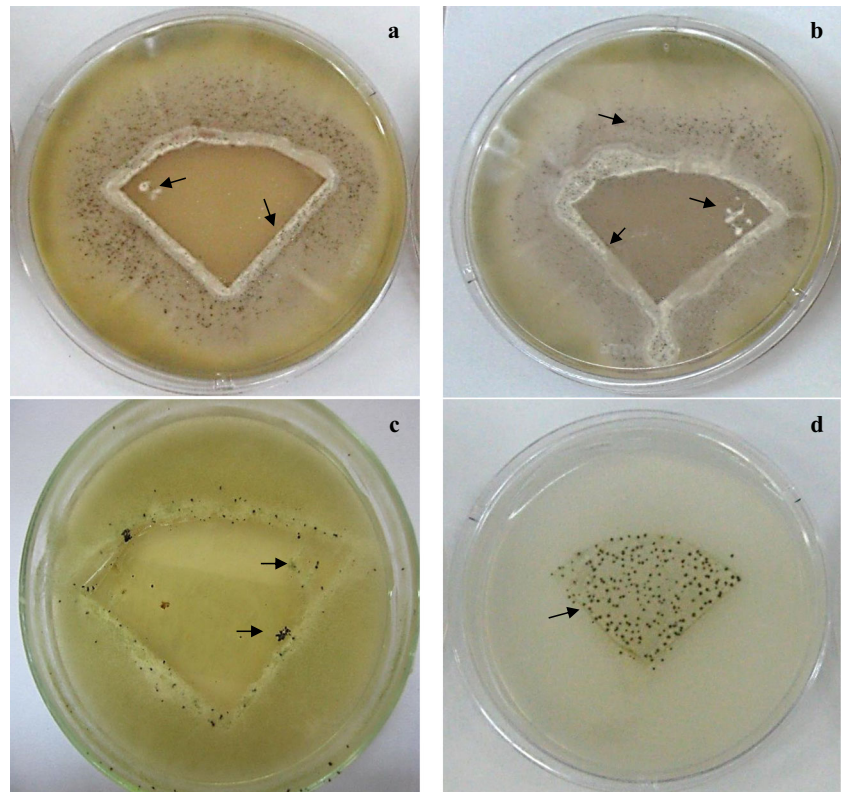

presence of spores and hyphae (Fig. 3d–f), which are typical events associated with the PCL biodegradation process.

The experiment showed that both hydrophilic (PCL) and hydrophobic (PVC) polymers were subjected to fungal colonization and subsequent biodegradation. It is known that most synthetic plastics are resistant to microbial attack due to its hydrophobic characteristic, which inhibits the adhesion and enzymatic microbial activity. Usually, the surface hydrophilicity of the polymers need modification to allow

biocompatibility and cell adhesion (Cheng and Teoh 2004; Kumar et al. 1983; Zhang et al. 2001). When this stage is reached, fragments of molecules are metabolized by the fungi and craters and erosions appear on the material surface as a result of microbial activity. Since the microorganisms use the polymeric surface components and products of its own metabolism, they release organic acids as aggressive metabolites and esterases, growing deeply in the material, resulting in enlargement to the damaged area and thus promoting the
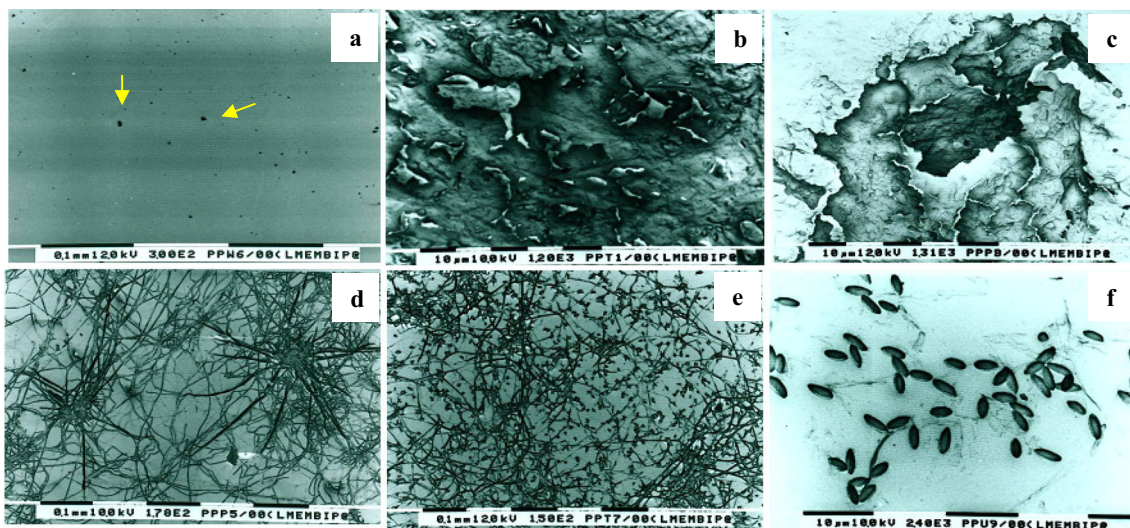
Fig. 2 Comparative SEM micrographs of PVC untreated film (**a**); biotreated for 28 days with superficial erosion (**b**); widened fissure, leading to the formation of cavities with wrinkles (**c**); *Chaetomium* perithecia, (**d**); and hyphae and spores under polymeric surface (**e**, **f**)
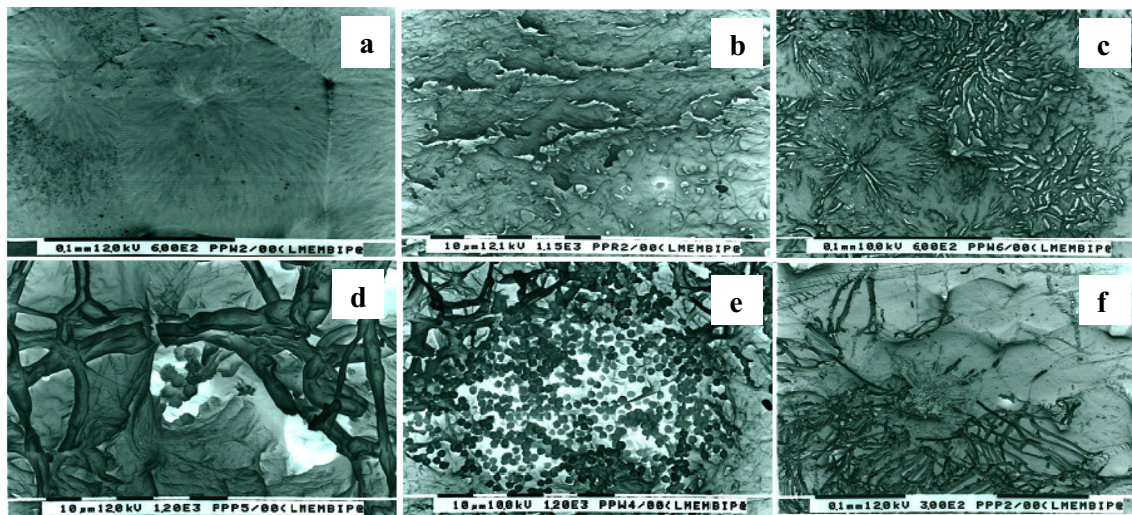
Fig. 3 Comparative SEM micrographs of PCL (**a**) original and biotreated during 28 days (**b**) with superficial layer erosion and (**c**) spherulitic degradation, (**d**) biophysical action of hyphae forming pores, (**e**) adhesion of spores, and (**f**) hyphae presence, evidencing the spherulites

degradation (El-Aghoury et al. 2006; Lugauskas et al. 2003; Webb et al. 1999).

Thermoplastics, like PVC, are inert and resistant materials to biodegradation because of its high molecular mass, long carbon chain backbone, three-dimensional structure, hydrophobic nature, and lack of functional groups recognizable by existing microbial enzyme systems (Chiellini et al. 2003; Hadad and Sivan 2005; Watanabe and Kawai 2011).

Fungal strains used in the consortium proposed by ISO-846 were selected based on their enzymatic ability to decompose and assimilate polymeric material as their sole carbon source. Our results showed a significant adhesion of perithecia of *Chaetomium* in PCL films and in some samples of PVC. *C. globosum* (Ascomycota, Pezizomycotina, and Sordariales) is the commonest and most cosmopolitan species among all, frequently associated with decomposition of plant remains, paper, and other cellulosic materials (Domsch et al. 1993). Is was also found to degrade polyethylene with an important role of its peroxidase production, considered a key enzyme for the degradation process (Sowmya et al. 2015).

It is assumed that the morphology of *C. globosum* may also be important for the adhesion of the fungus on the polymer surface. Their larger sexual spores (8.5–11.0 μm × 7.0–8.5 μm × 6.5–7.5 μm) compared to those of *P. funiculosum*, for example, 2.5–3.5 μm × 2.0–2.5 μm, and the production of ascomata with roughened lateral hairs which help the attachment of the structure to the substratum showed to be useful for mycelial growth. Large spores have wider contact angles with the surface and adhesion strength, whereas small conidia present smaller contact angle. Besides, *A. brasiliensis*, *P. funiculosum*, *Paecilomyces variotii*, and *T. virens* produce spores of very smooth wall which can be easily removed from a surface (Domsch et al. 1993). The adhesion of spores in polymeric materials also depends on the water contained

therein; larger spores may reserve more water (Semenov et al. 2003). Thus, the SEM observations suggested a considerable superficial deterioration of polymer by the virtue of consortium action, with emphases to *C. globosum* activity.

Mass loss

The determination of the mass loss is the most commonly method used and relatively sensitive to determine changes caused by microbial action in the polymers (Flemming 1998). Control samples (without fungal inoculation) showed no mass loss (results not shown). There was no statistical difference with respect to the mass loss of samples of PVC exposed to microbial treatment in the complete and incomplete medium ($p = 0.1571$). In accordance with our results, Campos (2008) studied PVC films treated with micro-organisms from soil and slurry and did not report any significant mass loss of PVC films studied. Based on our results, one may suggest that PVC degradation could achieve notable levels if an increase of the porosity and hydrophobicity of the polymer could be reached and raising the incubation time of the treatment. In longer periods of exposure, fungal consortium produces more damage to PVC films (Campos 2008).

The degradation of PCL film consortium was more efficient for tests in incomplete medium ($p = 0.0003$) than in complete medium ($p = 0.0008$). These values show that the fungal species of ISO 846-1978 were able to assimilate this polymer as the sole carbon source. The mass loss obtained by the action of the consortium in our study was 75% for PCL films and 9% for PVC films, both in incomplete medium and biotreated for 28 days. Massardier-Nageotte et al. (2006) observed a complete PCL colonization by micro-organisms, getting 35% of degradation after 28 days under aerobic conditions. Another study with the same polymer showed losses

of 30% in 60 days using *Bacillus* sp. PG01 (Massardier-Nageotte et al. 2006; Wu et al. 2007).

In some PCL films biotreated in incomplete medium, which was only observed the adhesion of *C. globosum*, the biodegradation of the sample was complete. Campos (2008) found significant mass loss, especially when the PCL films were treated for 60 days in soil, reaching 90% of mass loss. With 90 days of treatment, the PCL films were completely biodegraded. Therefore, the mass loss was an important parameter to measure the degradation of PCL films at the present work.

Conclusions

The ascomycete *Chaetomium globosum* (ATCC 16021) was considered a strain with potential to degrade poly (ε-caprolactone) and poly (vinyl chloride). Statistically significant mass losses were observed in the films of PCL on incomplete medium, which proves the use of the polymer by the consortium as a nutrient, since it was the only carbon source available. The microbial adhesion occurred both in the hydrophilic polymer (PCL) and hydrophobic (PVC) polymers, which is the first step in the degradation process. The biodegradation of the samples subjected to biotreatments was dependent on the contact and adhesion of the fungus to the surface of the polymers. Morphological changes as well as mass loss in the PVC as in PCL were important for the evaluation of the biodegradation process.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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