Thermally Treated Low Density Polyethylene Biodegradation By *Penicillium pinophilum* and *Aspergillus niger*

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ABSTRACT: Differential scanning calorimetry (DSC), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) were used to determine morphological, structural and surface changes (biodegradation) on thermo-oxidized (80°C, 15 days) low-density polyethylene (TO-LDPE) incubated with *Aspergillus niger* and *Penicillium pinophilum* fungi, with and without ethanol as cosubstrate for 31 months. TO-LDPE mineralization by fungi was also evaluated. Significantly morphological and structural final changes on biologically treated TO-LDPE samples were observed. Decreases to three units on crystallinity and crystalline lamellar thickness (0.4–1.8 Å), and increases in small-crystals content (up to 3.2%) and mean crystallite size (8.4–14 Å) were registered. An oxidation decrease (almost twice) on samples without ethanol with respect to the control was observed, while in those with ethanol it was increased (up to 2.5 times). Double bond index increased more than twice from 21 to 31 months. The higher TO-LDPE changes and fungi-LDPE interaction was observed in samples with ethanol, suggesting that ethanol favors the TO-LDPE biodegradation, at least in case of *P. pinophilum*, probably by means of a cometabolic process. Mineralization of 0.50 % and 0.57 % for *A. niger*, and of 0.64 % and 0.37 % for *P. pinophilum* were obtained, for samples with and without ethanol, respectively. A model to explain morphological and structural changes on biologically treated TO-LDPE is also proposed. © 2002 John Wiley & Sons, Inc. J Appl Polym Sci 83: 305–314, 2002

Key words: polyethylene biodegradation; cometabolism; *Aspergillus niger*; *Penicillium pinophilum*

INTRODUCTION

Polyethylene (PE) is a polymer broadly used for packing and recognized for its resistance to biodegradation. During the last two decades, several groups have studied the biodegradation of polymers such as PE. Hydrocarbon chains with molecular weights smaller than 600 da. can be biodegraded in a relatively short time[1,2] and longer chains are more difficult to biodegradate. Using treatments with photo- and thermo-oxidant agents might increase PE biodegradation rates. These treatments generate free radicals able to oxidize the polymeric molecule resulting in the rupture of chains.[3]

Albertsson et al.[4] mention that it is unlikely that cellulolytic microorganisms can attack polymers with carbonated linear chains such as PE.

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However, some works[5–7] have demonstrated that ligninolytic and cellulolytic fungi (*Phanerochaete chrysosporium*, *Aspergillus niger*, *Penicillium pinophilum*, *Gliocadium virens*, *Paecilomyces variotti*) can degrade oxidized PE products. Pometto et al.[8] found an extracellular enzyme in a *Streptomyces* sp. extract, able to attack and modify thermo-oxidized PE-starch films (70°C, 10 days) after three weeks of treatment.

Results obtained by Potts et al.[1] suggest that enzymes that catalyze *n*-paraffin's degradation by β-oxidation, present in a fungal consortium (*A. niger*, *A. flavus*, *P. pinophilum* and *Chaetomium globosum*) can attack end chains of high molecular weight materials. The polymer biodegradation is begun by extracellular enzymes that break polymeric chains, releasing oligomers and monomers that can be transported into the cell.[9] Many of these fungi also possess highly unspecific oxidative enzymes (oxygenases) that are able to oxidize several substrates, that could also attack polymeric substrates by cometabolic processes. During cometabolism, an organism that grows on an easily assimiled substrate (cosubstrate), and oxidizes a second one which it is unable to use as sole carbon and energy source (e.g., PE).[10,11] All the studies on PE biodegradation have been carried out with thermally or UV treated PE, but there are no reports about PE biodegradation incubated with cosubstrates. The use of alcohols as cosubstrates to induce unspecific enzymes production has been reported for recalcitrant compounds biotransformation.[12]

The objective of this article was to evaluate the effect of a cosubstrate, (ethanol), to induce cometabolic reactions on the morphological, structural and superficial changes (biodegradation) in thermo-oxidized (80°C, 15 days) low density PE (TO-LDPE) incubated with axenic cultures of two filamentous fungi (*Aspergillus niger* and *Penicillium pinophilum*).

MATERIALS AND METHODS

Low Density Polyethylene Thermo-Oxidation

Powdered LDPE (17070, supplied by PEMEX, México City, México) was heated (80°C, 15 days) under dry and dark air atmosphere. Thermally treated LDPE was then cooled by freezing (0°C) and milled in a grinder (particle size <0.542 mm). TO-LDPE was sterilized with UV radiation (~350 $\mu W/cm^2$, 20 h) before incubation with fungi. Untreated milled-LDPE was used as reference in all conditions.

Microorganism and Culture Conditions

Two filamentous fungi strains, *Aspergillus niger* ATCC 9642 or *Penicillium pinophilum* ATCC 11797 were used as inoculum. For biodegradation studies, a culture medium with following composition was used (g/L) glucose, 2; LPDE, 20; NH_4Cl, 21.65; KH_2PO_4, 5.6; $MgSO_4$ $7H_2O$, 1.2; $MnSO_4$ $4H_2O$, 0.025; $ZnSO_4$ $5H_2O$, 0.110; $CuSO_4$ $7H_2O$, 0.002; $CoCl_2$ $7H_2O$, 0.001. pH was adjusted to 5 and the medium was sterilized (120°C, 15 min). The culture medium (25 mL) was inoculated (1×10^6 spores/mL) and placed into 125 mL sealed serological bottles under aseptic conditions. In some bottles, ethanol (1% v/v) was added as cosubstrate. Inoculated bottles, at the same conditions without TO-LDPE, were run as controls. Cultures were incubated for 31 months at 30°C, and each study condition was assayed in triplicate.

Gas Chromatography

Once a month the gaseous atmosphere in the sealed bottles under biological treatment was analyzed by gas chromatography for CO_2 and O_2 determinations. A gas chromatograph (GOW-MAC 580) with a thermal conductivity detector (45°C, 150 mA), an Alltech CTR1 column (45°C) and helium as carrier gas (40 mL/min) were used for all determinations. Injector temperature was maintained at 45°C and the injection volume was of 50 mL.

In order to maintain O_2 concentrations at least of 15% (aerobic conditions), the headspace in bottles was completely replaced once per month. Bottles incubated under the same conditions, but in absence of LDPE, were used as controls. Mineralization percentage was estimated by means of a carbon balance, relating the difference between CO_2 produced in samples with LDPE and control samples to the polymer carbon content.[13]

Scanning Electron Microscopy (SEM)

Dehydrated LDPE samples with fungic material adhered were metalized with gold (3 discharges of 40 mA/50 s; each one, argon atmosphere), in a high vacuum metalizator (Bal-Tec SCD 050). Samples were analyzed in an electronic microscope (Zeiss DSM 940A), by means of secondary electrons, with an acceleration voltage of 5 kV

and a work distance between 15 and 17 mm. The images were digitally acquired in a Digital Scanner Microscope.

LDPE Analysis

LDPE samples incubated with *P. pinophilum* or *A. niger* growing in absence or presence of ethanol were taken periodically (3, 7, 11, 16, 26 and 31 months). Samples were vigorously stirred and the biomass was separated by centrifugation (5000 g, 15 min). The floating plastic material was removed and then washed thoroughly with distilled water. The LDPE was then dried (25°C, 24 h) and analyzed by differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR). At the end of the assay, samples were analyzed by scanning electron microscopy (SEM) and X-ray diffraction (XRD).

Differential Scanning Calorimetry (DSC)

LDPE samples were analyzed in a DSC analyzer (910S, TA Instruments) 20–150°C (N_2 atmosphere) with a heating ramp of 10°C/min. Each sample was run twice, and the first run was followed by a final isotherm (150°C, 3 min). Reported data correspond to the second heating and the values given are averages of two measurements. Calibration was made with indium.

Crystallinity ($\%C_{DSC}$), smallest crystallites fraction (*SCF*), melting (T_m) and onset (T_o) temperatures were calculated from thermograms obtained with this technique. {ercent of crystallinity was determined by relating the heat of fusion of LDPE samples and the heat of fusion of 100% crystalline PE (280 J/cm^3).[14] The crystalline lamellar thickness (L_c) was estimated from the melting point (T_m), by Thomson-Gibbs equation.[14] *SCF* was calculated with use of a geometric strategy[7], in which straight lines converging to the apex and being tangent to both sides of the endotherm were drawn and a triangle was completed by prolonging the baseline from the high temperature side. The area of the triangle is assumed to be proportional to the heat of fusion of the biggest and/or the most perfect crystals in LDPE. The first contributions to the melting endotherm come from the smallest or the less perfect crystals. The remaining area of the endotherm was considered proportional to the heat of fusion of the smallest and/or the imperfect crystals, and was related to the total area to obtain the corresponding percentage to the *SCF*.

Fourier Transform Infrared Spectroscopy (FTIR)

Samples were placed on a ZnSe slide and analyzed on a Perkin Elmer 2000 FTIR spectroscope supplied with a Microscope (Perkin Elmer). Relative intensities of carbonyl band at 1715 cm^{-1} and double bond band at 1653 cm^{-1} to that of methylene band at 1465 cm^{-1} were evaluated (carbonyl and double bond index, respectively). Each index is a relative measure of carbonyl groups and double bonds concentration, respectively. An average of 15 LDPE particles was analyzed for each time and for each treatment.

X-ray Diffraction (XRD)

Changes in the final crystallinity ($\%C_{XRD}$) and final mean crystallite size (L_{110}) were estimated by using the X-ray diffraction technique (XRD). The XRD patterns were recorded with a Philips horizontal goniometer (PW 1380/60) fitted with a scintillation counter, a pulse-height analyzer, and a graphite crystal monochromator placed in the scattered beam. CuKα radiation (λ = 1.5418 Å) was used and the scattered radiation was registered in the angular interval (2θ) from 12–28°. Thus, $\%\ C_{XRD}$ of LDPE samples was calculated by using XRD data, with the following relation:

$$\%C_{XRD} = \frac{A_c^{110} + A_c^{200}}{A_a + A_c^{110} + A_c^{200}} \quad (1)$$

Where A_a and A_c^{hkl} are the areas under the amorphous halo and the *hkl* reflections, respectively. The mean crystallite size (L_{110}) was obtained by XRD data and Scherrer equation, where L_{110} represents the mean crystal dimension normal to the corresponding 110 plane.

RESULTS AND DISCUSSION

Morphological Changes

The morphological changes of thermo-oxidized low density polyethylene (TO-LDPE) due to the biological treatment were evaluated as the changes in percentage of crystallinity ($\%C_{XRD}$ and $\%C_{DSC}$), smallest crystallites fraction (*SCF*) and crystalline thickness (L_c). Significant changes in TO-LDPE crystallinity ($\%\ C_{XRD}$) by biological treatment (BT) with both studied strains were observed at 31 months of incubation (Table I). Samples incubated with *A. niger* presented a significant $\%\ C_{XRD}$ decrease (2.3 and 2.8 units) with-

Table I Final Morphological Changes in Thermally (Control) and Biologically Treated LDPE[a]

Treatment[b]	$\%C_{XRD}$ (%)	*SCF* (DSC) (%)	L_{110} (Å)	L_c (Å)	T_o (°C)
Control	43.4 ± 0.3 A[c]	45.7 ± 0.3 B[c]	112.4 ± 1.2 B[c]	70.3 ± 0.3 A[c]	95.8 ± 0.2 B[c]
A. niger	41.1 ± 0.7 B	47.1 ± 0.4 AB	120.8 ± 1.5 A	69.9 ± 0.4 A	96.5 ± 0.3 AB
A. niger/Et	40.6 ± 0.8 B	48.9 ± 1.1 A	124.5 ± 2.7 A	69.2 ± 0.5 AB	96.4 ± 0.3 AB
P. pinophilum	42.0 ± 0.3 AB	47.9 ± 1.2 A	121.9 ± 6.5 A	68.5 ± 0.7 B	96.8 ± 0.1 A
P. pinophilum/Et	40.4 ± 0.5 B	46.8 ± 1.2 B	126.4 ± 2.3 A	69.0 ± 0.3 AB	96.8 ± 0.1 A

[a] Samples were evaluated by DSC and XRD.
[b] With and without ethanol (Et) as cosubstrate.
[c] Values with the same letter are not significantly different ($\alpha = 0.05$).

out and with ethanol addition, respectively. Samples incubated with *P. pinophilum* have a final $\%C_{XRD}$ reduction of 1.4 and 3.0 units without and with ethanol addition, respectively. This reduction in $\%C_{XRD}$ might be attributed to the smallest and/or imperfect crystallites fraction (*SCF*) increase as shown in Table I. The higher crystallinity decrease, detected in samples incubated with ethanol, can be due to a cometabolic process, where the ethanol in the medium favors the unspecific enzymes production ability to oxidize the LDPE molecule.[11] This result was confirmed by the oxidation increase of TO-LDPE samples incubated with ethanol, detected by FTIR.

As in the results obtained here, Albertsson et al.[15] observed a crystallinity decrease of 2 and 3 units in degradable TO-LDPE samples (100°C) incubated with *Arthrobacter paraffineus* (for 10 months and 3 years, respectively) with respect to an abiotic control. In contrast, Weiland et al.[16] observed a crystallinity increase (9 units) in TO-LDPE samples (70°C) incubated with a fungal consortium (*A. niger*, *G. virens*, *P. variotii*, and *P. pinophilum*) for 21 months. Volke-Sepúlveda et al.[17] found a significant decrease (19-fold) in the heat of fusion of TO-LDPE samples (150°C, 120 h) incubated with *Phanerochaete chrysosporium* for 3 months with respect to an abiotic control. In contrast, Manzur et al.[7] report an increase (30%) in the final value of a relative heat of fusion (measure of higher crystals) in LDPE/sugarcane-bagasse mixture samples (1:1 (w/w), blended at 150°C, 10 min), incubated for 32 days with *P. chrysosporium*. These differences on crystallinity and relative heat of fusion might be dependent on the kind of biological and physicochemical treatment and on the time of incubation.

Changes in crystallinity as a function of time are presented in Figure 1. Crystallinity increases in biologically treated LDPE samples between 7 and 16 months can be attributed to the microbial

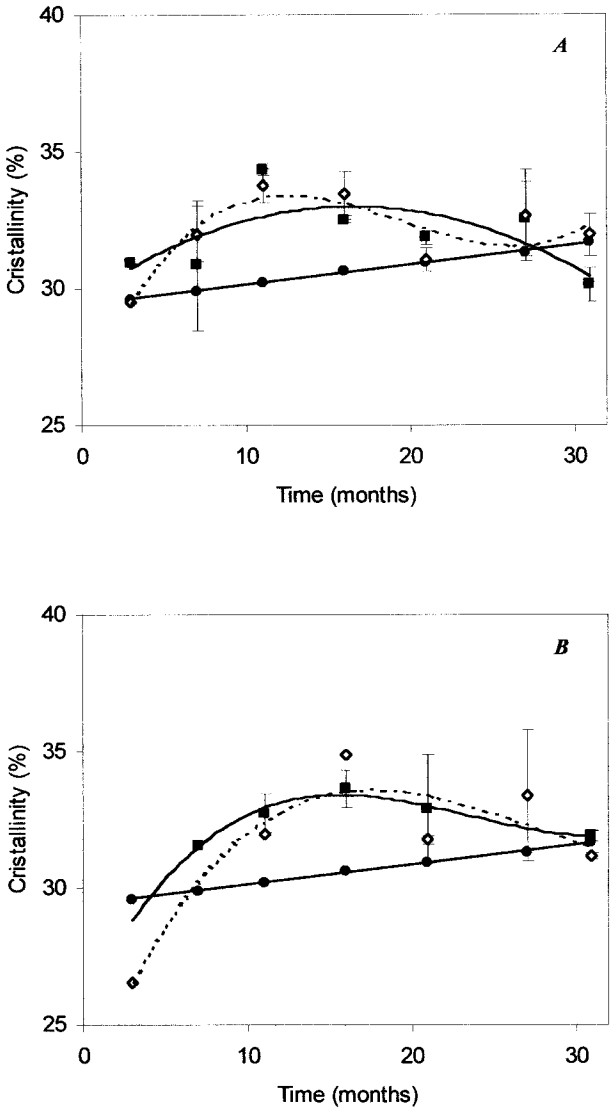

Figure 1 Crystallinity ($\%C_{DSC}$) of LDPE samples incubated for 31 months. (A) *A. niger*; (B) *P. pinophilum*; (●) Control; (■) samples without ethanol; (◇) samples with ethanol.

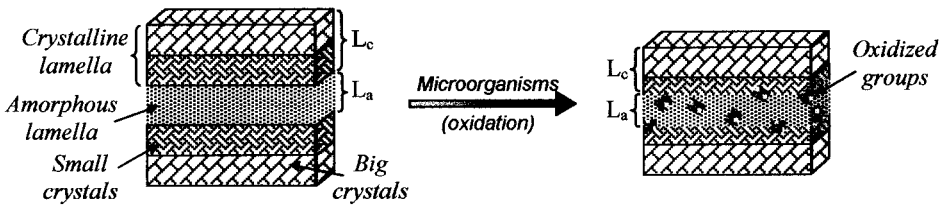

Figure 2 Schematic representation of a proposed model to explain crystallinity and crystalline lamellar thickness (L_c) decrease, and mean crystallite size (L_{110}) increase.

attack on the amorphous fraction, as this fraction is less resistant to enzymatic attack than to crystalline fraction.[18] Manzur et al. proposed that[7] the amorphous fraction has two components: 1) the one that surrounds the crystalline particles, and 2) the one that defines limits and separates crystalline blocks of the crystalline mosaic. The final *SCF* values for all biologically treated samples registered a significant increase (more than 1.1 units) with respect to the TO-LDPE (Table I). This indicates that the small crystals content also increased by the BT effect. The final *SCF* increase for *A. niger* and *P. pinophilum* was 3.2 and 2.2 units, respectively.

Estimation of the final mean crystallite size (L_{110}) demonstrated a significant increase (8.4–14 Å) in this parameter with respect to the control sample (without biological treatment). The increase on L_{110} was higher in cultures with ethanol than on those without. These differences on the final mean crystallite size indicate a higher microbial activity on the amorphous TO-LDPE and on the smaller crystals. Contrary to this study, Albertsson et al.[15] and Manzur et al.,[7] observed an L_{110} decrease (6 and 20 Å, respectively) in TO-LDPE samples incubated with *Arthrobacter paraffineus* for 10 months and with *P. chrysosporium* for 32 days, respectively.

The crystalline lamellar thickness (L_c) was also reduced by the biological treatment of TO-LDPE, and this reduction was independent of the initial ethanol addition to the culture medium. L_c decrease by BT effect can be due to the attack of microorganisms to the amorphous fraction that separates the crystalline particles, which cause an increase in the content of smaller and/or less perfect crystals in crystalline lamellas. This result is confirmed by that on *SCF*.

The onset temperature (T_o) of the final samples was also evaluated in order to determine the morphological changes of TO-LDPE promoted by the biological treatment. In all biologically treated samples, a final T_o increase (0.6–1–C) was observed (Table I). Since T_o represents a measure of crystals that terminate fusion at lower temperatures (smaller and/or more imperfect crystals), the increase of T_o correlates with the increase of L_{110}. The T_o and L_{110} increase observed in this article can be attributed to the gradual degradation of the smallest and imperfect crystals, resulting in a material with bigger or more perfect crystals, which is more resistant to biological degradation.

A model to understand the morphological changes on TO-LDPE by BT effect is proposed in Figure 2. In accordance with this model, LDPE is initially composed of amorphous and crystalline sheets (composed of big and small crystals). Once the polymer is exposed to the BT, the fungi attack mainly the amorphous sheets, producing an initial crystallinity increase (7–16 months). Later, with the attack on the smaller size crystals that are located in the amorphous-crystalline interface, an increase in the amorphous fraction and a decrease in the crystalline one are detected. By microbial attack on the smaller crystals initially contained in crystalline sheets, a decrease is registered in crystallinity, L_c, and *SCF*, and an increase in L_{110}.

Structural Changes

Fourier transform infrared (FTIR) is an effective method to quantify the content of carbonyl, double bond, and other functional groups during the TO-LDPE degradation. Changes in biologically treated TO-LDPE samples were analyzed by FTIR over the entire incubation period (31 months). Although the biological treatment of TO-LDPE with both strains (*A. niger* and *P. pinophilum*) did not have a significant effect on the carbonyl index (CI), the addition of ethanol on the culture media had an important effect on the increase (up to three times) of these groups (Fig. 3). The biological TO-LDPE oxidation started after 8 months of incubation and reached maximal val-

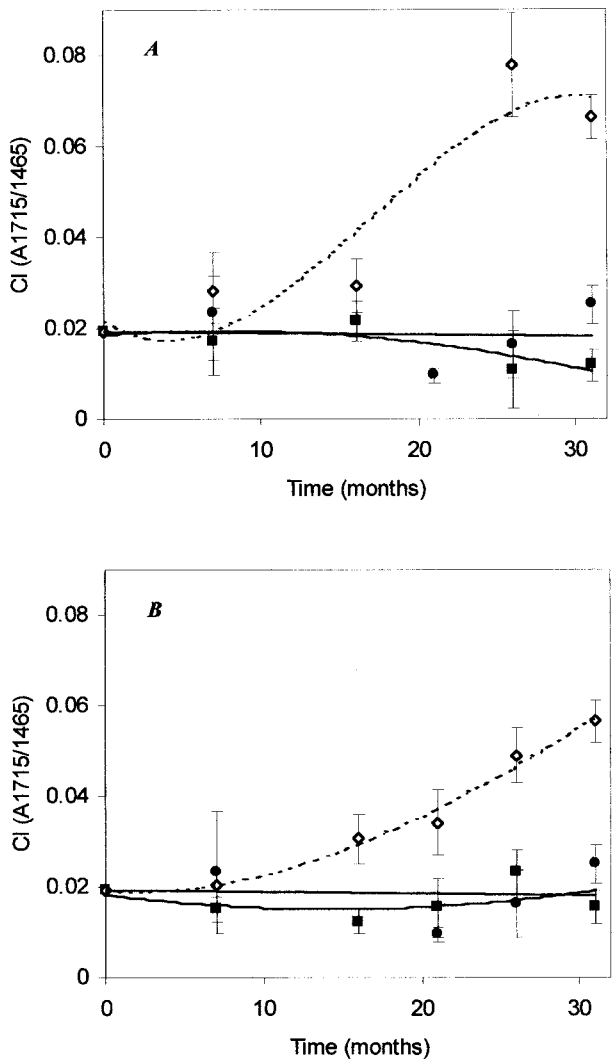

Figure 3 Carbonyl index (CI) of LDPE samples. (A) *A. niger*; (B) *P. pinophilum*; (●) Control; (■) samples without ethanol; (◇) samples with ethanol.

ues of 0.062 and 0.056 for the samples inoculated with *A. niger* and *P. pinophilum* growing in media containing ethanol. FTIR spectra of TO-LDPE inoculated with *A. niger* in presence of ethanol showed that the CI increase was due to carbonyl groups content present in both ketones (1715 cm^{-1}) and acyclic esters (1740 cm^{-1})[19] (Fig. 4). Similar behavior was observed in samples inoculated with *P. pinophilum* with ethanol (results not shown).

Final significant increases on the double bond index (DBI) were observed for *P. pinophilum* with and without ethanol (0.029) and for *A. niger* with ethanol (0.029), as compared with the final value obtained for the biologically untreated TO-LDPE sample (0.012). For samples incubated with *A. niger* without ethanol, a slight increase (0.016) was detected. Similarly, Volke-Sepúlveda et al.[17] observed a significant DBI increase (more than two-fold) in TO-LDPE samples (150°C, 120 h), by the effect of the *P. chrysosporium* incubation for three months. This result is attributed to an enzymatic scission mechanism and to assimilation of the low molecular weight chains. DBI increase can be related to a material weight loss, explained by the incorporation of the polymeric fragments to the microbial biomass.[20]

The CI decrease in biologically treated LDPE samples without ethanol, and DBI increment in all samples with BT, can be explained according to a proposed mechanism for PE biodegradation.[20] According to this mechanism, once formed carbonyl groups along the polymeric chain (abiotic factors), these can be microbially attacked (CI decrease), and lead to insaturate chains release (DBI increase). Oxidized PE molecules can be hydrolyzed by extracellular enzymes. The molecule is then transformed to a fatty acid, activated by CoA-SH and metabolized by means of the β-oxidation.[21] The CI increase in samples incubated with ethanol suggest a cometabolic transformation of TO-LDPE (oxidized compounds production), and simultaneously an attack on the oxidized fragments is carried out, liberated unsaturated chains (DBI increase). The LDPE molecule is biologically co-oxidized by extracellular unspecific enzymes induced by the ethanol presence.[11] The presence of unspecific enzymes that act on *n*-al-

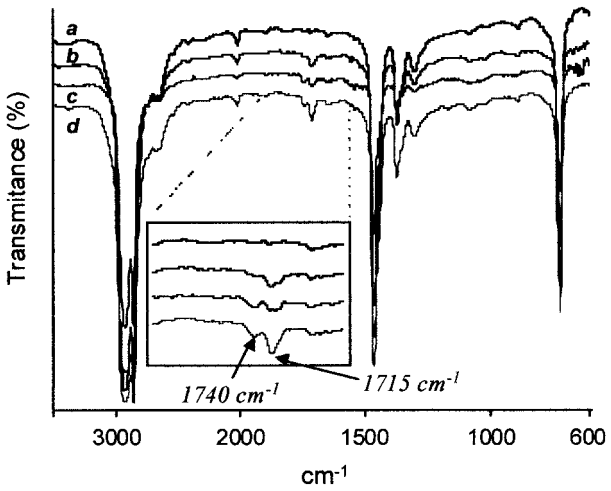

Figure 4 Carbonyl groups bands evolution in TO-LDPE samples incubated with *A. niger* in the presence of ethanol. (a) control (TO-LDPE); (b) 16 months; (c) 21 months; (d) 31 months of incubation.

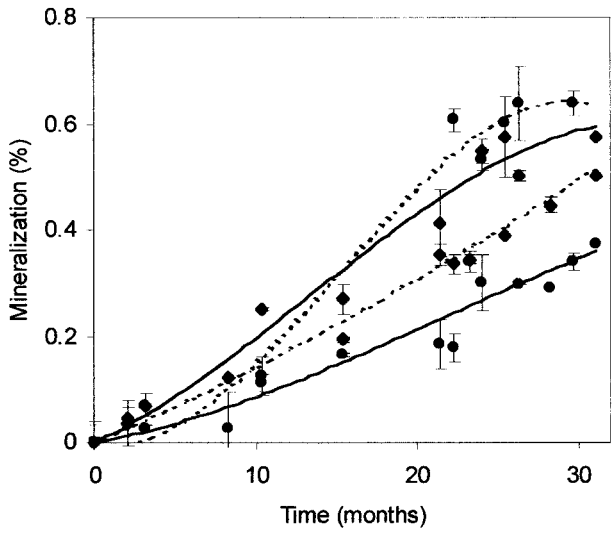

Figure 5 Mineralization percentage of TO-LDPE samples biologically treated with (♦) *A. niger*; and (●) *P. pinophilum*; (—) with; and (——) without ethanol as cosubstrate.

kanes degradation was demonstrated in our laboratory for both strains (results not shown).

The higher structural and morphological changes detected between the 11 and 20 months of incubation suggest that the polymeric matrix biodegradation began during this period.

LDPE Mineralization

The degradation process of a polymer can be continually monitored by the CO_2 produced and the O_2 consumed during culture. Additionally, the CO_2 production with TO-LDPE as sole carbon source is a direct measure of the mineralization, which indicates the fraction of the polymer carbon content that is converted to CO_2, as a final product of the microbial respiration.[3,9,13]

Figure 5 shows the mineralization values for all the TO-LDPE biological treatments. TO-LDPE mineralization started after 3 months of culture and it presented a maximum rate of 0.016% per month, (after 10 months of culture), for samples inoculated with *P. pinophilum* in presence of ethanol, which corresponds to a CO_2 production rate of 0.207 mmol of CO_2 per month. Mineralization was markedly favored when ethanol was added to the culture medium inoculated with *P. pinophilum* (0.64% and 0.37% for samples with and without ethanol, respectively). However, ethanol addition did not stimulate the TO-LDPE mineralization by *A. niger* (0.50 % and 0.57 % for samples with and without ethanol, respectively).

Albertsson and Karlsson[22] studied the ^{14}C-LDPE biodegradation in soil, over 10 years. They observed that microorganisms used between 0.2 and 5.7% of polymeric carbon for CO_2 production. The maximum CO_2 production value detected from untreated PE incubated with *Fusarium redolens* or in soil, was around of 0.2% per year (w:w). At the same time, in UV-treated PE (42-day) samples, it was used between 1.3 and 5.7% of the polymeric carbon for CO_2 production. While in PE irradiated for 7 days, the degradation rate was 0.042% per month. PE degradation thus depends on the content of the additives and on the degree of molecular oxidation. Furthermore, the polymer carbon content can be used for the production of CO_2, biomass, and other intermediary products (e.g., short chain hydrocarbons, alcohols, acids, ketones and aldehydes).[15] However, during co-metabolic processes of this kind of substrates, there is an accumulation of intermediary compounds that are not metabolized.[23]

Superficial Changes

Until now, thermal (DSC) and spectroscopic (FTIR) methods have been used to evaluate the TO-LDPE biodegradation. However, because the initial attack generally begins with a surface colonization, scanning electronic microscopy (SEM) allows direct observation of this kind of degradation.[24,25] The microorganisms' adhesion to the polymeric surface is a fundamental step in order for biodegradation to take place.[26,27]

Figure 6 shows SEM micrographs of *A. niger* (a and b) and *P. pinophilum* (c and d) growth on TO-LDPE surface at 31 months of culture. A *A. niger* hypha adhered or even penetrating in TO-LDPE surface, in a sample without ethanol is presented in Figure 6(a). In these samples, an increase in the hyphae diameter (6–8μm) was observed. In samples incubated with ethanol, the micelial growth onto polymeric surface was abundant and had fewer spores than in samples grown without ethanol. This can be attributed to the biomass production derived from ethanol. Hyphae diameter observed in these samples was significantly smaller (3–4 μm) than in samples without ethanol, and some hyphae were clearly penetrating the polymeric surface (Fig. 6b).

In TO-LDPE incubated with *P. pinophilum* without ethanol, the micelial growth on the polymeric surface was higher than in *A. niger* strain. Hyphae penetration in the polymeric structure and changes on the superficial appearance of

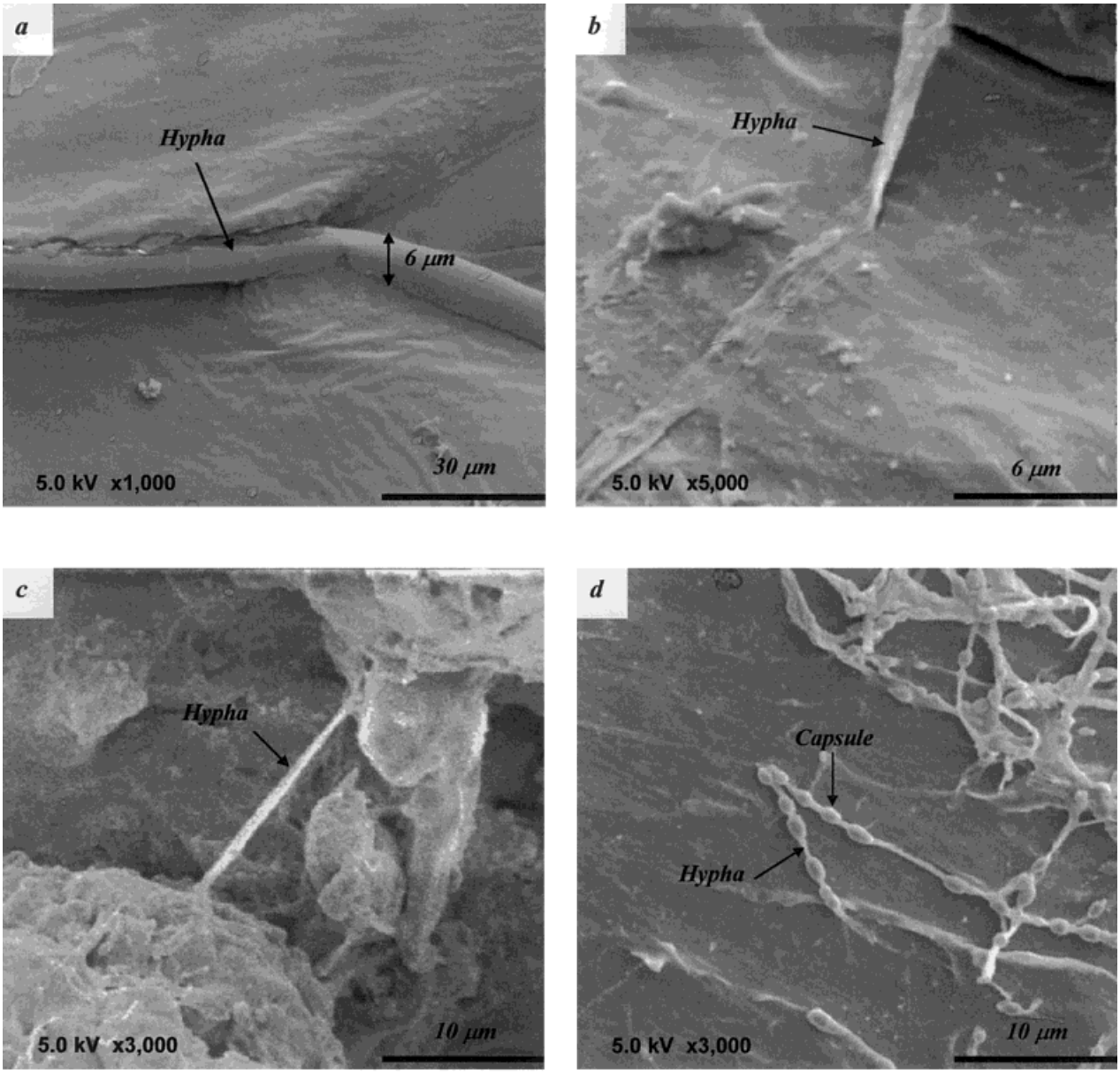

Figure 6 SEM micrographs of fungal growth on TO-LDPE surface. (a) and (b) *A. niger* hyphae adhered to TO-LDPE surface in samples without and with ethanol, respectively; (c) *P. pinophilum* hypha penetrated in TO-LDPE surface in samples incubated without ethanol; and (d) in samples with ethanol.

samples was also observed (Fig. 6c). In samples incubated with ethanol, an abundant superficial growth was observed, as well as a greater number of hyphae penetrating the polymeric surface, in comparison to samples incubated in absence of ethanol. Hyphae aspect observed in these samples were different than that of samples without ethanol. Numerous capsulated structures were also observed along the whole hypha (Fig. 6d).

Moriyama et al.[26] studied the superficial corrosion on plasticized poly(vinyl chloride) (PPVC) films by fungi for four years. They observed a similar appearance on the polymer surface to that shown in Figure 6(c). This result was attributed to the fungic growth from the plasticizer used in PPVC. The rough appearance observed in some areas of TO-LDPE might be due to a process of superficial corrosion caused by the polymer biodegradation by *P. pinophilum*.[1,27]

Similar capsular structures to those observed in *P. pinophilum* samples were also observed by Milstein et al.[27] in white rot fungi mycelium (*P.*

chrysosporium, *P. ostreatus* and *T. versicolor*) incubated with a polystyrene-lignin copolymer for three weeks. Microorganisms that colonize the polymer surface can probably adhere to this due to extracellular polymer production, mainly constituted by polysaccharides. Part of these polysaccharides (mainly glucane and quitine degraders), synthesized by several fungi groups, form a kind of capsule or sheath that is covalently bonded to the polymer wall and plays an important role in supporting and transporting depolymerizant enzymes during polymeric surface attack.[27]

Some reports indicate that microorganisms that grow on hydrocarbons, and can adhere to them by the production of superficial cellular components. It was suggested that specialized structures are required for hydrocarbon penetration through microbial cellular wall.[28] Cundell and Traxler[29] demonstrated that a *Penicillium sp*. strain grew with sheathed structures in hyphae holes, around hydrocarbons drops.

Apparently, in samples incubated with *P. pinophilum* without ethanol, the contact and interaction of fungic mycelium with LDPE was less effective than in samples incubated with ethanol. Thus, ethanol seems to have a positive effect in TO-LDPE biodegradation, at least for the *P. pinophilum* strain.

CONCLUSIONS

The TO-LDPE biodegradation (quantified as morphological and structural changes) and its mineralization during initial stages are attributed to the presence of oxidized low molecular weight fragments that initially increase the polymer susceptibility to microbial attack.

P. pinophilum incubated with and without ethanol showed a higher TO-LDPE biodegradation efficiency (estimated as changes on DSC, XRD and FTIR) than did *A. niger*. Mineralization was also higher for *P. pinophilum* with the addition of ethanol. The lower mineralization level by *A. niger* could be due to the use of a polymer carbon fraction for biomass and/or to intermediary oxidized metabolite production by the fungi. In samples incubated with ethanol, this response could be a sign of a cometabolic process, characterized by the accumulation of intermediary compounds that are not metabolized.

The incubation of both strains with ethanol in the medium caused significant morphological and structural changes in TO-LDPE samples, which provide a strong evidence of a cometabolic process favoring the LDPE oxidation and degradation.

Differences in analyzed variables by effect of *A. niger* or *P. pinophilum* incubation can be due to differences in enzymatic systems and in attack mechanisms on the LDPE. It is probable that *P. pinophilum* strain possesses extracellular enzymes with a certain degree of unspecificity, able to depolymerize lignocellulosic materials that can act on polymers.

The importance of this study is the presentation of LDPE biodegradation by a cometabolic process, which represents a potential option for the degradation of this type of molecules. Also, a model is proposed that can shed light on understanding the mechanisms that cause morphological and structural changes in biologically treated TO-LDPE.

The use of these strains, together with other microorganisms, as a consortium, can be a potential option for the degradation of recalcitrant molecules as PE.

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