Biodegradation of Physicochemically Treated LDPE by a Consortium of Filamentous Fungi

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ABSTRACT: Samples of low density polyethylene previously subjected to physicochemical treatments–thermal treatment (TT) at 105 and 150°C or accelerated aging treatment (AAT)–were subjected to biodegradation by a consortium of four fungi during 9 months. Morphological, structural, and surface changes and mineralization were evaluated. TT samples showed decreases in the onset melting temperature (T_o), melting point (T_m), relative crystallinity (Φ), and mean crystallite size (L_{110}). The degradation products in all treated samples were carbonyl and double bonds groups. The biological treatment (BT) affected the properties of all treated samples. T_o at 3 months decreased with respect to sample at 0 months; the changes were higher in TT samples; the samples then remained without significant changes. Increases in Φ were observed in TT samples within a 3-month BT, after which reductions occurred. After a 9-month BT, increases in L_{110} were registered in all samples (up to 2.6 nm). The highest mineralization value (3.26%) was obtained with the AAT. The reported changes suggested that the fungi mainly digest the amorphous phase of polyethylene in the first stage of the experiment, but later they also digest small crystals. Superficial growth of microorganisms occurred, and penetration of hyphae was observed in most oxidized samples. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 92: 265–271, 2004

Key words: polyethylene; biodegradation; *Aspergillus niger*; *Gliocladium virens*; *Penicillium pinophilum*; *Phanerochaete chrysosporium*

INTRODUCTION

Some synthetic carbon-based polymers of high molecular weight are resistant to microbial attack in the initially produced form. The durable properties in synthetic and natural polymers have attracted more interest during the past decades since environmental problems have increased as a consequence of the accumulation of municipal solid waste generated by the commodity polymers. Some traditional polymers such as polyethylene suffer very slow biodegradation. One of the possible solutions for these problems may be the modification of the polymer to facilitate the biodegradation.

To help the mechanism through which the microorganisms can assimilate the carbon contained in the polyethylene, the polymer must be first transformed to more oxidized compounds of low molecular weight. It is known that the oxidation of polyethylene molecules by means of physicochemical treatments facilitates the action of microorganisms.[1–3] Using treatments with photo- and thermooxidant agents might increase polymer biodegradation rates. These treatments generate free radicals able to oxidize the polymeric molecule, resulting in the rupture of chains.[1] Photooxidation reduces the molecular weight of the polymer and triggers a great variety of physical and chemical changes, favoring the production of carbonyl groups.[2,3] The thermal treatment has been used[4] to make polyethylene more susceptible to biodegradation; at temperatures higher than the melting point the thermal treatment decreases the fusion heat and increases the carbonyl content.[5]

Effects of several filamentous fungi on low density polyethylene (LDPE) and high density polyethylene (HDPE) have been reported. Biological treatment with *Phanerochaete chrysosporium* in a blend of LDPE and sugar cane bagasse (for 32 days) modified the crystalline morphology.[6] *P. chrysosporium* in a thermooxidized (150°C, 120 h) LDPE sample inoculated for 3 months under nonaseptic conditions caused structural and morphological modifications.[7] *Penicillium simplicissimum* grew better on agar plates containing UV-irradiated (500 h) HDPE (which had carbonyl groups) than intact HDPE (which had no carbonyl groups), causing structural changes.[8] *Aspergillus niger* and *Penicillium pinophilum* in thermooxidized (80°C, 15 days) LDPE samples, with and without ethanol as cosubstrate for 31 months, modified the crystalline mor-

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phology and crystalline structure; ethanol favors the biodegradation.[5]

The objective of this work was to evaluate the biodegradation of LDPE by a consortium of four filamentous fungi strains. The polymer was previously subjected to one of the following physicochemical treatments (PCTs): a thermal treatment (TT; 105 and 150°C, 120 h) or an accelerated aging treatment [AAT; 70°C, UV (310 nm), 29 days]. The morphological, structural, and surface changes, as well as the production of CO_2, were evaluated.

EXPERIMENTAL

Physicochemical treatments

Commercial low density polyethylene was used (17,070, produced by Pemex, México). Its density and flow index values were 0.917 g/cc and 7 g/10 min, respectively. To induce changes in its structure and to facilitate the different microorganisms' actions, the polyethylene was first subjected to two PCTs. The polyethylene was subjected to AAT in a weathering tester aging chamber at 70°C and UV radiation (310 nm) for 29 days to simulate its degradation of 10 years under environmental conditions. For the TT two temperatures (105 and 150°C) were used for 120 h using an oven in the presence of air atmosphere. The thermally treated plastic was then cooled quickly in liquid nitrogen. After these two PCTs the polyethylene was milled in a grinder and sifted until reaching a diameter smaller or equal to 0.54 mm. The polyethylene was sterilized with UV radiation (460 $\mu W/cm^2$, 14 h) before incubation with fungi. Untreated milled-polymer (U-LDPE) was used as reference.

Biological treatment and culture conditions

The biological treatment (BT) was carried out in a liquid culture medium using sealed serologic bottles (125 mL). The medium with the following composition (in g/L), per liter of distilled water, was used for biodegradation studies: NH_4NO_3, 1.51; KH_2PO_4, 1.35; $MgSO_4 \cdot 7H_2O$, 1.48; $MnSO_4 \cdot 4H_2O$, 0.021; $ZnSO_4 \cdot 7H_2O$, 0.007; $CuSO_4 \cdot 5H_2O$, 0.006; $CoCl_2 \cdot 6H_2O$, 0.006. The pH was adjusted to 5, and the medium was sterilized at 120°C for 15 min. A mass of 0.5 g of sterilized polyethylene (see above) was added to 25 mL of culture medium and further inoculated (1×10^6 spores/mL of each strain) with a consortium of four filamentous fungi strains (*Aspergillus niger* ATCC 9642,[7] *Gliocladium virens* ATCC 9645,[9] *Penicillium pinophilum* ATCC 11,797,[7] and *Phanerochaete chrysosporium* H289[6,7]). The cultures were incubated at 29°C for 9 months and each study condition was assayed in quadruplicate. Inoculated bottles at the same conditions without LDPE were run as controls. To maintain O_2 concentrations above 18% (aerobic conditions) in the gas phase into the serological bottles, the headspace was completely replaced after CO_2 analysis under aseptic conditions in a laminar flow chamber.

Twenty serological bottles were used for each LDPE treatment. LDPE analysis was carried out from three serological bottles for each LDPE treatment every 3 months. The polyethylene was separated from the biomass first with vigorous agitation using a stirrer and then a Hettich Universal centrifuge at 8,200*g* for 10 min. The floating plastic material was removed and washed with distillated water. Afterward, it was dried at room conditions.

Differential scanning calorimetry (DSC)

The thermal analysis of the polyethylene samples was carried out in a DSC analyzer (910S TA Instrument). LDPE samples were encapsulated in standard aluminum pans and heated at a rate of 10°C/min, in a nitrogen atmosphere (50 mL/min), at a temperature interval from 20 to 150°C. Each sample was run twice, and the first run was followed by a final isotherm at 150°C for 3 min. The unit was calibrated using indium. The reported values represent the average for three specimens.

Relative crystallinity (Φ), melting (T_m), and onset melting (T_o) temperatures were calculated from thermograms obtained with this technique. The value of T_m was taken as the temperature at the apex of the endotherm. The quantities Φ and T_o were calculated with the use of a geometric strategy. The baseline is drawn between 40 and 113°C, which correspond to the temperatures of start and end of fusion; a straight line is then drawn from the apex and tangent to the low temperature side of the endotherm. These two lines with the high temperature side of the endotherm form a triangle. The area of this triangle is assumed to be proportional to the heat of fusion of the biggest and/or the most perfect crystals in LDPE since the first contributions to the melting endotherm come from the smallest or the least perfect crystals. The area of the endotherm outside the triangle was considered proportional to the heat of fusion of the smallest and/or imperfect crystals. In this way the relative crystallinity was calculated as the heat of fusion corresponding only to the biggest crystallites (triangle area), and the intersection of the tangent line to the endotherm (at the low temperature side) with the baseline was defined as the onset melting temperature (T_o).

Wide angle X-ray scattering (WAXS)

Changes in the mean crystallite size (L_{110}) were estimated in the standard way[10] by using the WAXS technique. The patterns were recorded with a Phillips

TABLE I
Melting Temperature (T_m, °C) of Samples Subjected to BT

Sample	0 months	3 months	6 months	9 months
U-LDPE	105.3 ± 0.3	105.4 ± 0.3	104.4 ± 0.5	104.8 ± 0.3
TT/105	102.2 ± 0.4	101.0 ± 0.3	101.5 ± 0.5	101.6 ± 0.3
TT/150	104.5 ± 0.1	103.7 ± 0.1	103.9 ± 0.3	103.6 ± 0.3
AAT	105.4 ± 0.2	104.6 ± 0.1	104.1 ± 0.5	104.3 ± 0.2

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 was used and the scattered radiation was registered in the angular interval (2θ) from 5 to 45°. A current of 30 mA and a voltage of 45 kV were used.

Fourier transform infrared spectroscopy (FTIR)

A Perkin–Elmer 2000 FTIR spectroscope supplied with a microscope (Perkin–Elmer) was used. The evolution of the changes in chemical structure was carried out every 3 months. The polyethylene particles were first flattened and then placed on a zinc selenure crystal for their infrared analysis. An average of 18 particles was analyzed for each one of the three specimens used each time. The intensity of the band at 1,715 cm^{-1} attributed to the carbonyl group (C=O) and that at 905 cm^{-1} attributed to the vinyl groups (–CH=CH_2) were both related to that of the methylene (–CH_2) band at 1,460 cm^{-1}. These relative quantities are the carbonyl and vinyl index, respectively. Each index is a relative measure of the group's concentration. The methylene band was used as reference since it does not change during the degradation of the polyethylene.[3]

Scanning electron microscopy (SEM)

Dehydrated LDPE samples with adhered fungic material were metalized with gold (3 discharges of 40 mA/50s each one, argon atmosphere), in a high vacuum metalizator (Bal-Tec SCD 050). Samples were analyzed in a microscope (Zeiss DSM 940A), by means of secondary electrons, with an acceleration voltage of 5 kV. The analysis was carried out at 6 and 9 months of the biological treatment.

Gas chromatography (GC)

The CO_2 concentration at the gaseous atmosphere (100 mL) into the sealed bottles under biological treatment was analyzed once per month by GS. 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 50 μL. After sampling, the headspace was completely replaced and analyzed for CO_2 concentration to determine the CO_2 produced by the action of the inoculated fungi.

RESULTS AND DISCUSSION

Thermal and crystalline changes

The untreated polyethylene (U-LDPE) was used as the reference sample for the evaluation of the changes produced by the physicochemical and biological treatments. The values of T_m and T_o as a function of time are listed in Tables I and II. The results in both runs of DSC had similar trends, although different values; those presented in these tables corresponded to the first run. The effects of the PCTs are presented as values at 0 months. The samples subjected to thermal treatments (0 months) presented decreases in the melting point (up to 3.1°C in TT/105) and in the onset melting temperature (up to 8.7°C in TT/105). The difference between T_m and T_o values can be taken as a measure of the polydispersity in the crystallites size. Sample TT/105 presented the highest polydispersity.

The AAT sample at 0 months did not show significant changes in T_m and T_o, but showed a slight increase in the crystallinity (Φ) (Fig. 1) compared to the U-LDPE. This increase in the crystallinity agrees with the reported results.[11,12] The TT samples presented decreases in the crystallinity, whose values depend on the temperature used in the thermal treatment. The TT/150 sample presented the highest decrease. TT samples have different behavior because, in addition to the different cooling rates to which they were subjected after the thermal treatment, they suffered different structural changes, detected by FTIR as seen below. Since T_o represents the temperature at which the bigger or less imperfect crystals start to melt, these

TABLE II
Onset Melting Temperature (T_o, °C) of Samples Subjected to BT

Sample	0 months	3 months	6 months	9 months
U-LDPE	94.2 ± 0.6	94.2 ± 0.2	93.4 ± 0.3	93.5 ± 0.4
TT/105	85.5 ± 0.1	85.2 ± 0.4	84.0 ± 0.4	85.2 ± 0.5
TT/150	93.5 ± 0.6	92.5 ± 0.2	93.2 ± 0.1	93.1 ± 0.2
AAT	93.7 ± 0.3	93.2 ± 0.5	93.0 ± 0.4	93.7 ± 0.3

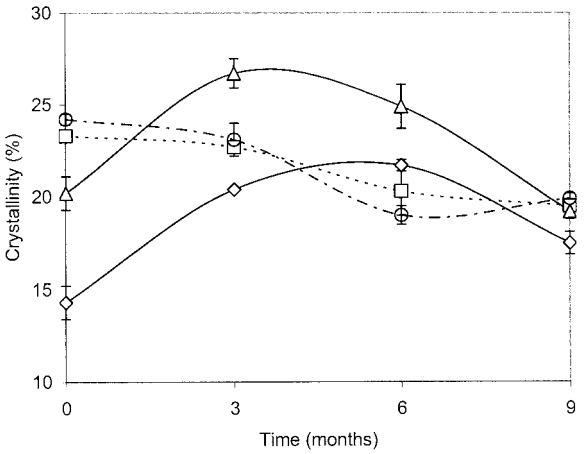

Figure 1 Kinetics of crystallinity changes of physicochemical-treated LDPE subjected to biological treatment. □ U-LDPE, △ TT/105, ◇ TT/150, ○ AAT.

decrements of T_o and T_m may show a relationship with the decrements of Φ.

As an effect of the BT, all treated samples presented slight decreases of T_m and T_o at 3 and 6 months with respect to the samples without BT; after these time periods the values increase slightly or remain without changes over the time scale of the biodegradation experiment. The samples subjected to TT showed the highest changes. The decreases in T_m after the BT indicate the presence of smaller or imperfect crystals compared to the originally existing ones.

The crystallinity of the samples subjected to biological treatment (Fig. 1) presented significant increases at 3 and 6 months in TT/105 and TT/150 samples, respectively, (6.5% in sample TT/105 and 7.4% in TT/150), in comparison to the same samples without biological treatment. After these time periods a decrease was observed in both TT samples. These changes suggest a two-stage mechanism: a crystallinity increase at the first stage due to the microbial attack on the amorphous fraction, and a further stage represented by the attack on smaller crystals. This could explain the increase and subsequent decrease in crystallinity. The AAT sample presented a 5.2% decrease at the 6 months while the U-LDPE sample presented a delayed smaller decrease.

The estimation of the mean size of the crystalline particles was made by using the Scherrer equation, [10] which is given by

$$L_{hkl} = \frac{K\lambda}{\beta\cos\theta}$$

where L_{hkl} represents the mean crystal dimension normal to the corresponding *hkl* plane, K is a constant of the order of 1, λ stands for the radiating wavelength, 2θ is the scattering angle, and β is the half-height width of the scattering peak. This equation does not consider instrumental broadening corrections, but, for the purpose of this study, it is used as a practical reference to estimate the average dimension of the crystalline particles. With $\lambda = 1.5418$ Å, $2\theta = 21.5°$ corresponding to the 110 plane and β measured from the angular position of this peak, the crystal mean size was estimated before and after the biological treatment.

The initial values of L_{110} (0 months) for U-LDPE, TT/105, TT/150, and AAT samples were 7.4, 7.0, 6.8, and 6.5 nm, respectively. The reduction of these values with respect to the reference sample (U-LDPE) represents an increase in the number of the smallest crystals due to the physicochemical treatment. This is in concordance with the results obtained by the DSC technique in which decreases in the T_m, T_o, and Φ values were observed. The L_{110} values at 9 months for U-LDPE, TT/105, TT/150, and AAT samples were 9.2, 9.5, 7.2, and 6.6 nm. These larger values for the respective samples mean a decrement in the number of the smallest crystals due to the action of the microorganisms.

The final mean crystallite size values for all biologically treated samples registered a significant increase. These values indicate a high microbial activity on the amorphous phase and on the smaller crystals. Increases in L_{110} (0.84–1.4 nm) were reported[5] in thermally treated (80°C, 15 days) LDPE incubated with *A. niger* and *P. pinophilum* fungi. However, L_{110} reductions of 0.6 nm with *Arthrobacter paraffineus*[13] and 2.0 nm with *P. chrysosporium*[6] growing on LDPE samples were previously reported. These differences on mean crystallite size might be dependent on the kind of biological and physicochemical treatments and on the time of incubation.

The effect of the microorganisms in semicrystalline polymers is expected to occur in the amorphous phase rather than in the crystals because the latter are more resistant to enzymatic attack.[14] It has been proposed[6] that the amorphous fraction has two components: 1) the one that surrounds the crystalline particles, and 2) the one that defines the limits of crystalline blocks of the crystalline mosaic. The results presented here suggest that, once the polymer is exposed to the BT, the fungi first attack mainly the amorphous phase, causing the crystallinity increase and the separation of crystalline blocks of the crystalline mosaic, in this way smaller crystals are obtained. Later, with the attack on the smaller size crystals located in the amorphous–crystalline interface or formed during the first stage, an increase in the amorphous fraction and a decrease in the crystalline one are produced. By the microbial attack on the smaller crystals, a decrease in crystallinity and an increase in L_{110} is obtained. Similar behavior was observed using two fungal strains under cometabolic conditions.[5]

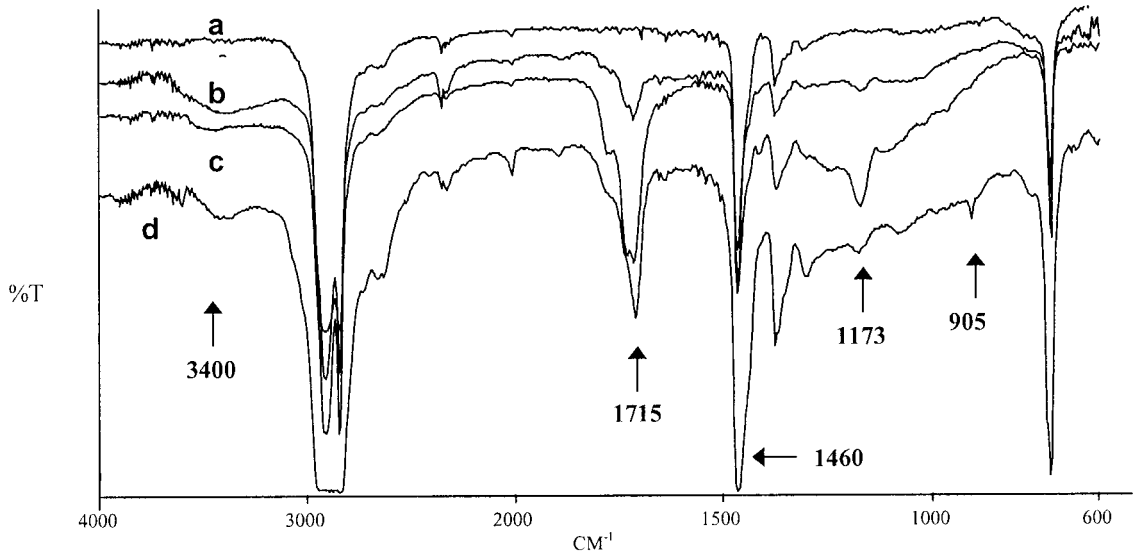

Figure 2 FTIR spectra of polyethylene with and without PCT. From top to bottom: polyethylene without PCT, with TT at 105 and 150°C, and with AAT. The hydroxy, carbonyl, methylene, and vinyl bands are indicated.

Structural changes

It is well established that carbonyl groups are the main products in thermo- and photooxidation treatments. The spectra of U-LDPE and those of samples with PCT are presented in Figure 2. The appearance of the carbonyl group was observed in all physicochemical-treated samples; this band is absent in U-LDPE. The PCT samples presented the appearance of hydroxy groups (3,400 cm^{-1}) and a peak in the band at 1,173 cm^{-1}, which is also related to carbonyl groups. The simultaneous exposition of a polymer to heat and oxygen carries it to thermooxidative degradation; the result includes the formation of oxidized groups.[15] In addition to this type of degradation, the AAT sample suffered a photooxidation reaction causing an increase in the groups with double bonds (905 cm^{-1}). The appearance of these groups has been reported[16] during the photooxidation of polyethylene after 180 h of UV radiation (330–360 nm). Hydroxyl and carbonyl groups increased after photoirradiation of LDPE in an accelerated weathering chamber (290 nm).[17] In contrast to the thermooxidation, the products of the photooxidation in the polyethylene are mainly composed of a carbonyl group as well as vinyl groups.[18] The greater oxidation level after the PCT was presented in the AAT sample. Furthermore, a threefold increase of the vinyl index (VI) was also observed.

The carbonyl index (CI) was calculated from the band at 1,715 cm^{-1}. The CI values as a function of time for samples with biological treatment are presented in Figure 3. The TT/105 and AAT samples presented increases of 62% at 3 months and of 28% at 6 months, respectively; after these times the CI content remained practically unchanged until 9 months. The quantitative analysis of this degradation product revealed for both samples an increase in the amount of CI with the total exposure time, although their values were different according to the treatment used in each sample. In contrast, the TT/150 sample presented a decrease of 40% in this variable at 3 months of incubation with the fungi mixture; it then remained practically constant. This decrease in the carbonyl group was observed for photoirradiated LDPE under composting conditions.[17]

The VI for samples subjected to BT is presented in Figure 4. The U-LDPE did not present significant changes, but the TT/150 sample presented an increase of this group from the 3rd month of incubation. At 9 months of BT, an increase was observed in the TT/105 (of the double) and AAT samples (of the 45%). The segments formed during the chains ruptured by the effect of BT could cause the formation of the vinyl group.

The decreases in the CI indicated an attack by action of the microorganisms in the more oxidized chains; these results are in accordance with the mechanism of biodegradation proposed by Albertsson et al.[3] The

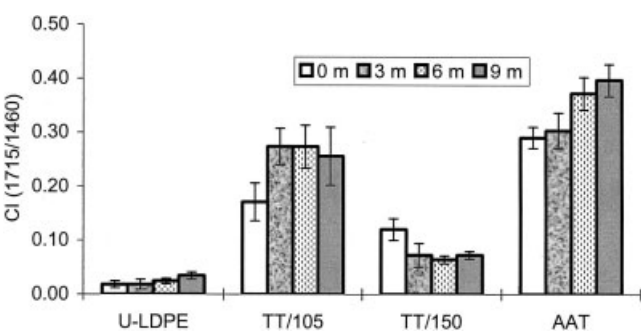

Figure 3 Carbonyl index of the polyethylene samples subjected to BT during 9 months.

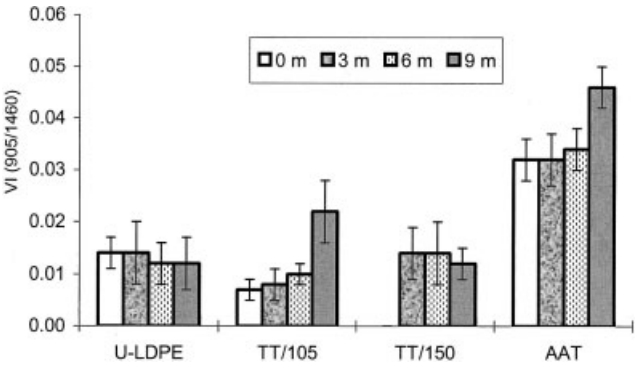

Figure 4 Vinyl index of the polyethylene samples subjected to BT during 9 months.

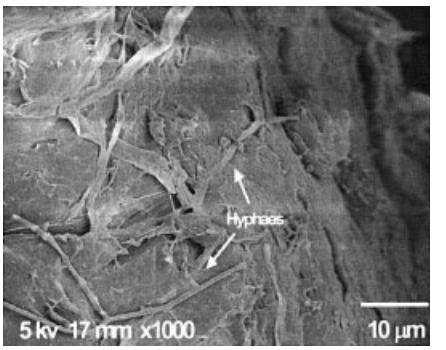

Figure 6 SEM micrographs of the thermally treated polyethylene (TT/150) showing superficial growth at 9 months of BT.

oxidized group is transformed to a carboxylic acid (1,700–1,725 cm^{-1}) and then is metabolized through the route of the β-oxidation.

The increase in the CI could be explained by the microbial oxidation of short chains. Schlegel[19] presented a mechanism for the oxidation of alkanes of high molecular weight. In this mechanism the participating enzymes caused the oxidation of the chain of the alkane to give as a final result the formation of carboxylic acids. In Figures 3 and 4 can be observed that the effect of the BT caused an increase or decrease in the CI and increases or the appearance in the VI, which could indicate the coexistence of two chemical reaction mechanisms during the polyethylene biodegradation.

Production of CO_2

The data of the production of CO_2 from all samples during the biological treatment are presented in Figure 5. The CO_2 produced by the microorganisms (by endogenous metabolism) from samples without LDPE was subtracted from the CO_2 produced in samples with polyethylene. For that reason the results shown in the figure represent the CO_2 produced only from the carbon contained in the polyethylene.

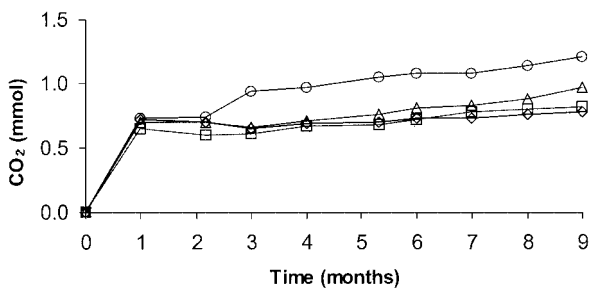

Figure 5 Production of CO_2 in samples of polyethylene incubated 9 months with the fungi consortium. □ U-LDPE, △ TT/105, ◇ TT/150, ○ AAT.

In the 1st month an increase in the production of CO_2 due to the microbial attack on the more accessible part in the polyethylene was observed. This CO_2 production could be also due to the biodegradation of additives present in the original LDPE. However, during the next 8 months the samples remained without significant changes, except the AAT sample, which showed an increase from the 3rd month of incubation, reaching a final mineralization level of 3.26% (expressed as C − CO_2 divided by the total C-LDPE in samples). The sample with accelerated aging presented the greater production of CO_2, possibly because this sample presented the greatest oxidation level by the effect of the PCT (Figs. 3 and 4). The presence of carbonyl groups facilitated the microbial action on the polyethylene, in agreement with results reported.[3,17]

Surface changes

The superficial growth of hypha was increased with the temperature used in the TT. Superficial growth of hyphae for thermally treated samples is presented in Figure 6, the SEM micrograph corresponded to 9 months of BT. The superficial growth of hyphae was a function of the oxidation level of each sample. Ohtake et al.[20] observed superficial growth of hyphae on the most degradated part of polyethylene.

The biodegradation of the polyethylene was evidenced through two effects shown in Figure 7: the formation of cavities on the surface and the penetration of hyphae in the material. The presence of small and large cavities on the surface was previously observed[17] and suggested that these cavities may be due to the absence of a uniform distribution of short branches or photodegradable products in the polymer matrix. The AAT sample presented the greater number of penetrations of hyphae. These observations suggest that, due to the lack of a uniform distribution, there are hard and soft zones near the polyethylene

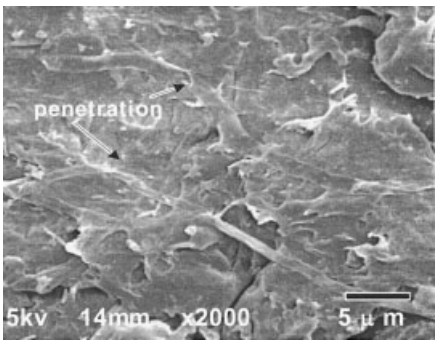

Figure 7 SEM micrographs of the polyethylene with AAT. The cavities and the penetration of hyphae are observed.

surface. Probably the penetration of hyphae occurred in the soft zones.

CONCLUSION

The observed changes indicated modifications in the original crystalline structure of the polyethylene due to the PCT. The decreases in T_o, T_m, and crystallinity give evidence of the presence of smaller crystals. The increase in vinyl groups and carbonyl groups indicated the oxidative degradation of the polymer. It was observed that the TT favored the formation of smaller or imperfect crystals in addition to the formation of carbonyl groups. For the case of the treatment with accelerated aging there were other series of reactions that caused the increase in the carbonyl groups to be greater than in the TT samples.

During the BT a mechanism with two stages was observed. The increase and later the decrease of crystallinity observed during the BT indicate a predominant stage at the beginning, where the microorganisms attack mainly the amorphous phase of the LDPE, and then followed by another stage, where the smaller or imperfect crystals also suffer the microbial attack. This effect was confirmed by the increase of the mean size of the crystalline particles after BT because of the decrease of the number of the smallest crystals.

The decrease in the CI showed that microorganisms prefer oxidized chains, but the increase in this index indicated a microbial oxidation possibly on the shortest chains. This suggests the coexistence of two chemical mechanisms during the biodegradation of the polyethylene. The TT favored the polyethylene biodegradation as seen through the morphological changes. The higher oxidation level of the AAT sample favored the mineralization (by the production of CO_2) of polyethylene.

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