Microbial Degradation of Thermo-Oxidized Low-Density Polyethylene

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ABSTRACT: Thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and Fourier transform infrared spectroscopy (FTIR) were used to determine the relative heat of fusion, crystallinity, and composition of thermo-oxidized low-density polyethylene (LDPE) inoculated with *Phanerochaete chrysosporium* and incubated for 3 months at 30°C under nonaseptic conditions. DSC analysis of thermo-oxidized samples (150°C, 120 h) followed by microbiological treatment showed a remarkable reduction on the heat of fusion (36%) compared to that of untreated LDPE. TGA allowed determination of changes due to the biological activity but not those that occurred by thermal treatment. A significant increase in the carbonyl index of the thermally treated (150°C, 120 h) LDPE samples was observed due to the increase in the oxidation level during this treatment. In contrast, the double-bond index (evaluated by FTIR) increased considerably (23%) as a result of the microbial treatment. The observed structural and morphological modifications of the biologically treated LDPE indicate that the reduction of the chain size may be carried out by scission mechanisms. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 73: 1435–1440, 1999

Key words: polyethylene biodegradation; *Phanerochaete chrysosporium*; thermal analysis; Fourier transform infrared spectroscopy

INTRODUCTION

Synthetic plastic polymers have been extensively used due to their structural stability and properties such as chemical stability, low weight, hydrophobicity, versatility, and resistance to chemical and biological deterioration.

World-wide production of plastics is more than 78 million tons per year; almost half of that is discarded within a short time, remaining in garbage deposits and landfills for decades (more than 30 years). Synthetic plastics accumulate in nature at a rate of 25 million tons per year.[1] Polyethylene represents 64% of the produced synthetic plastics. It is produced in approximately equal amounts of high-density polyethylene (HDPE) and low-density polyethylene (LDPE). These are mainly used for containers, which are discarded within a short time (plastic bags, bottles, disposable products). An alternative to dispose recalcitrant plastics such as polyethylene, polystyrene, and polyvinylchloride is biodegradation, a biological process where certain microorganisms degrade them to obtain energy for their growth.[2] The degradation of plastics in nature is a very slow process that involves environmental

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factors such as temperature, humidity, pH, and solar energy, followed by the action of wild micro-organisms. The primary biotic mechanism for the degradation of high molecular weight polymers, such as polyethylene, is the hydrolysis by extracellular enzymes produced by micro-organisms. The hydrolytic enzymes degrade the main polymeric chain and result in polymers of lower molecular weight with modified mechanical properties, making it more accessible for the microbial assimilation.[3]

Plastics disposal by conventional techniques (incineration, pyrolysis, recycling, and landfill disposal) involves technical and economical problems. Polymeric materials are not easily biodegraded. Efforts have been directed to develop mild physicochemical procedures (thermal and radiation treatments)[2] to facilitate the biodegradation process and eliminate them from nature in shorter periods of time. Moreover, the microbial biomass produced can be easily disposed of by conventional methods (compost), or used as feedstock. The major problem in polyethylene biodegradation is its high hydrophobicity level and high molecular weight. Therefore, thermal or radiation treatments on polyethylene reduced the polymeric chain size and form oxidized groups (carbonyl, carboxyl, and hydroxyl). These treatments modify the properties (crystallinity level, morphological changes) of the original polymer and facilitate the polymer biodegradation.[1] Plastics treated by radiation might be more recalcitrant than the thermally treated plastics, indicating structural differences in the residual plastics.

Growth of micro-organisms on plastic polymers can be related to their capability to cause changes in the polymer molecular weight and on some of their measurable physical and chemical properties.[2] To detect morphological and structural changes in polymers, some physicochemical (thermal analysis, X-ray diffraction, gel permeation chromatography) and spectroscopic (infrared and Raman spectroscopy, nuclear magnetic resonance and mass spectroscopy) methods can be used.

In the present work, low-density polyethylene (LDPE) was treated thermally and then used for biodegradation studies. This structural and morphological changes on the treated LDPE at 150°C for 8 h and 120 h inoculated with *P. chrysosporium,* and further incubated for 3 months, were determined by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and Fourier transform infrared spectroscopy (FTIR). Modification observed in LDPE properties during the physicochemical and microbial degradation are also discussed in this article.

MATERIALS AND METHODS

Low-Density Polyethylene Thermo-oxidation

Powdered LDPE (17070, supplied by PEMEX, Mexico) was heated in an oven at 150°C under a dry and dark air atmosphere for 8 and 120 h. This treatment oxidized the LDPE. The treated plastic was then cooled in liquid nitrogen for getting the amorphous fraction of this material. Afterwards, these treated materials were milled in a grinder (particle size lower than 0.542 mm). Untreated LDPE was used as a reference in all the studies.

Micro-organism and Culture Conditions

The filamentous fungus *Phanerochaete chrysosporium* (H289) strain was used as inoculum. The medium with following composition was used for fermentation studies (all in g/L): glucose, 2; LDPE, 20; NH_4Cl, 21.65; KH_2PO_4, 5.6; $MgSO_4 \cdot 7H_2O$, 1.2; $MnSO_4 \cdot 4H_2O$, 0.025; $ZnSO_4 \cdot 5H_2O$, 0.110; $CuSO_4 \cdot 7H_2O$, 0.002; $CoCl_2 \cdot 7H_2O$, 0.001. Ethanol (1% v/v) was also added as cosubstrate and the initial pH was adjusted to 4.5. The culture (size 1×10^6 spores/mL) was inoculated in 25 mL of the above medium and kept in 100-mL sealed bottles under nonaseptic conditions; these were incubated for 3 months at 30°C. The cultured bottles were aerated after every 15 days to maintain aerobic conditions.

LDPE Analysis

At end of fermentation, the LDPE samples were stirred vigorously, and the biomass was separated by centrifugation (5000 rpm, 15 min). The floating plastic material was removed and then washed thoroughly with distilled water. Afterwards, material was dried at room temperature (25°C), and further analyzed in triplicate by DSC, TGA, and FTIR techniques.

Differential Scanning Calorimetry (DSC)

Samples were analyzed on a thermal analyzer (910S, TA Instruments) from 20 to 150°C, using an nitrogen atmosphere (50 mL/min) and a heating ramp of 10°C/min. Each sample was run twice, the first run was followed by a final iso-

Table I Reduction (%) on the Relative Heat of Fusion of LDPE Thermally Treated (TT) During 8 and 120 Hours, Without and With Microbial Treatment (mo)

Treatment	Change in $\Delta H_f'$ (%)
LDPE without treatment	0
TT (8 h)	0
TT (8 h) + mo	1.65
TT (120 h)	4.85
TT (120) + mo	31.46

therm at 150°C for 3 min. Reported data correspond to the second heating of the DSC analysis. Calibration was made with Indium.

Thermogravimetric Analysis (TGA)

Samples were analyzed on a TG analyzer (DuPont Instruments 951) from 20 to 500°C under a nitrogen atmosphere (50 mL/min), using a heating ramp of 10°C/ min.

Fourier Transform Infrared Spectroscopy (FTIR)

Samples were placed on a ZnSe slide and analyzed on a Perkin-Elmer 2000 spectroscope supplied with a FTIR Microscope (Perkin-Elmer). Relative intensities of the carbonyl band at 1715 cm^{-1} (carbonyl index) and the double-bond band at 1650 cm^{-1} (double bond index) to that of the methylene band at 1465 cm^{-1} were evaluated.[4]

RESULTS AND DISCUSSION

Differential Scanning Calorimetry (DSC)

This technique measured the change in heat of fusion in the samples treated physicochemically and biologically. The heat of fusion is directly related to the degree of cristallinity of the polymeric sample.[5] The results obtained are listed in Table I. The values of heat of fusion of samples were normalized with the reference untreated LDPE ($\Delta H_f'$). Thermal treatment of samples at 150°C could not decrease the heat of fusion until 8 h, but a remarkable decrease in this variable was observed after 120 h.

Similar results indicating that LDPE treated at 70 and 100°C did not suffer significant changes in its crystallinity were reported elsewhere.[6] According Weiland and David,[7] the crystalline lamellae have a very low permeability to oxygen,

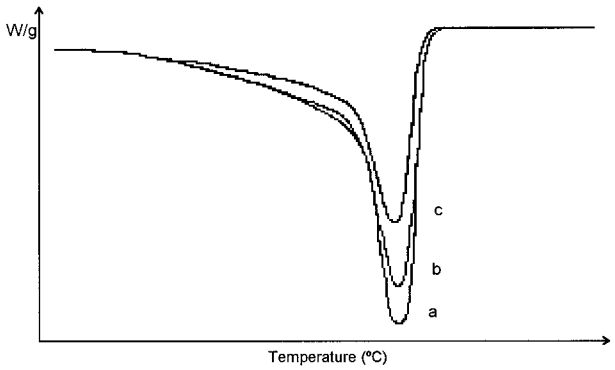

Figure 1 DSC endotherms of LDPE samples: (a) without thermal treatment; (b) thermally treated at 150°C for 120 h; (c) thermally treated at 150°C for 120 h, incubated with *P. chrysosporium*.

and are insensitive to thermal oxidation below the melting point, oxidation, thus, being mainly restricted to the amorphous interlamellar phase.

The change in the $\Delta H_f'$ of thermally treated samples for 8 h was low when these were further treated biologically (Table I). However, this was considerably decreased (19-fold) when biological treatment of the 120-h thermally treated samples was carried out. Weiland and David[7] in their studies observed an increase (9%) in the level of crystallinity of thermo-oxidized LDPE (70°C) treated microbially for a period of 3 months. In contrast, Albertsson and coworkers[6] reported a decrease (2%) in the crystalline fraction of thermo-oxidized LDPE (100°C) further treated biologically using *A. paraffineus* for 10 months. However, in the present work, more than a 9% decrease in cristallinity (calculated as in Caro[5]) of thermo-oxidized LDPE (150°C, 120 h) treated biologically using *P. chrysosporium* for 3 months was observed (Fig. 1). This remarkable decrease in the crystallinity level could observed because of the temperature selected (150°C) for the present

Table II $T_{98\%}$ Values of LDPE Thermally Treated (TT) During 8 and 120 Hours, Without and With Microbial Treatment (mo)

Treatment	$T_{98\%}$ (°C)
LDPE without treatment	382
TT (8 h)	371
TT (8 h) + mo	324
TT (120 h)	369
TT (120 h) + mo	321

Table III Carbonyl [A1715/1465] and Double Bond [A1653/1465] Indexes of Thermally Treated LDPE (150°C for 8 and 120 Hours), Without and With Microbial Treatment (mo)

Treatment	A1715/1465[1]	*	A1653/1465[2]	*
LDPE without treatment	0.0053	C	0.00242	C
TT (8 h)	0.0292	B	0.00023	C
TT (8 h) + mo	0.0313	B	0.00001	C
TT (120 h)	0.0556	A	0.02941	B
TT (120 h) + mo	0.0611	A	0.06797	A

* Values with the same letter are not significantly different
[1] Variance analysis: $R^2 = 0.7865$; $VC = 26.3905$
[2] Variance analysis: $R^2 = 0.9479$; $VC = 28.2760$

work, which is far above the LDPE melting point (120°C). A reduction of the crystal size and the oxidation of LDPE molecules could improve the microbial degradation.

Thermogravimetric Analysis (TGA)

Because changes in the weight of a given compound at defined temperatures and time intervals are accurately detected, TG analysis allows determination of its thermal stability.[5] The weight reduction rate under defined conditions is inversely proportional to the size of the polymeric chains.

In this work, the temperature at which a 2% reduction of the mass weight was observed ($T_{98\%}$) was used to compare the thermal stability of the different thermal and biologically treated samples. Results are shown in Table II. $T_{98\%}$ values were 11 and 13°C lower for the thermal-treated samples during 8 and 120 h, respectively, than the $T_{98\%}$ value obtained for the nontreated LDPE.

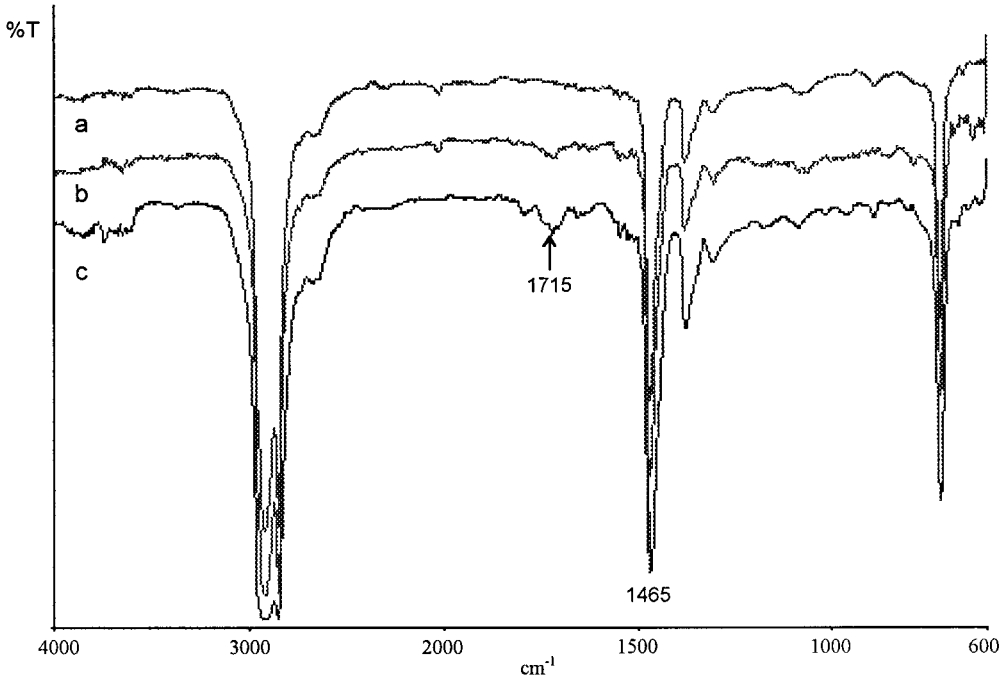

Figure 2 Infrared spectrum of LDPE samples: (a) without thermal treatment; (b) thermally treated at 150°C for 8 h; (c) thermally treated at 150°C for 120 h. Peaks detected at 1715 cm^{-1} and 1465 cm^{-1} are shown.

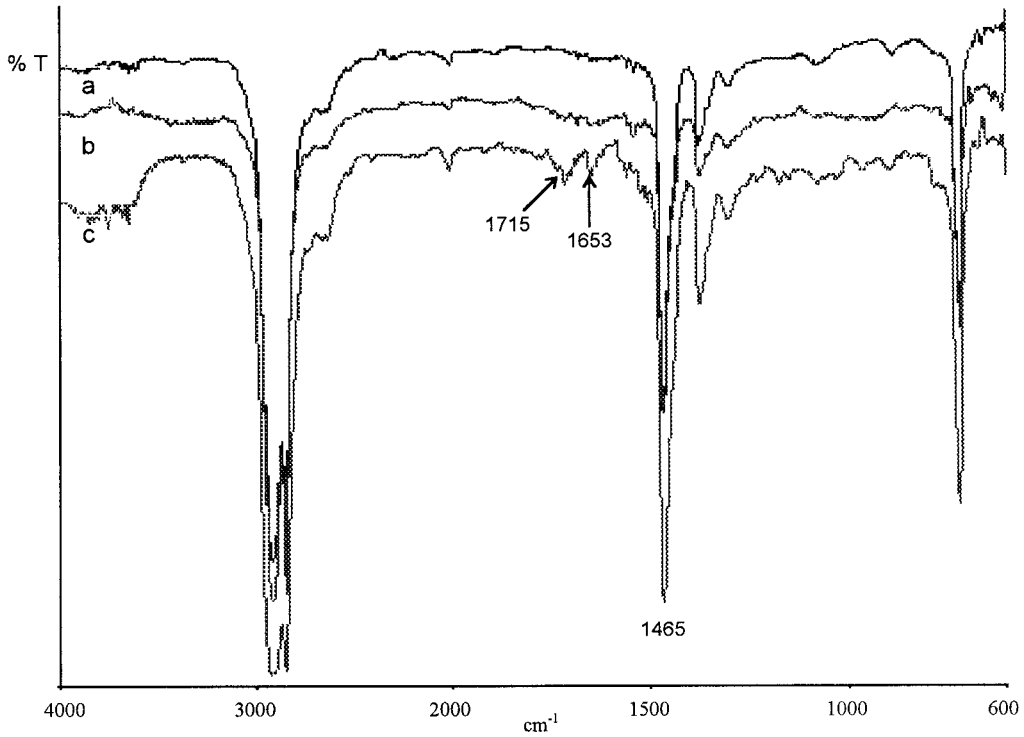

Figure 3 Infrared spectrum of LDPE samples: (a) without thermal treatment; (b) thermally treated at 150°C for 8 h, incubated with *P. chrysosporium*; and (c) thermally treated at 150°C for 120 h, incubated with *P. chrysosporium*. Peaks detected at 1715 cm^{-1}, 1653 cm^{-1}, and 1465 cm^{-1} are shown.

The further microbial treatment caused a reduction of the $T_{98\%}$ values to 47 and 48°C, for samples treated for 8 and 120 h, respectively. Although the higher reduction in $T_{98\%}$ value was due to the microbial treatment, these results reflected reduction in the size of the polymeric chains due to both treatments, thermal and biological. Duration of the thermal treatment at the studied conditions apparently did not have a significant effect on the $T_{98\%}$ value.

Fourier Transformed Infrared Analysis (FTIR)

Structural changes, such as an oxidation level of LDPE due to thermal and biological treatment, can be accurately detected by FTIR.[8] To obtain quantitative information of the structural modification of the treated LDPE, the carbonyl and double bond indexes were estimated (see Materials and Methods section). Use of these indexes compensate differences due to the sample thickness.[4]

Table III shows the carbonyl and double-bond index values of the untreated LDPE and the thermal and microbially treated LDPE. Statistical analysis of the carbonyl index values showed significant increases in thermally treated samples at 8 and 120 h. However, the additional microbial treatment did not modify this index value. Increase of the thermal treatment duration led to a significant increase in the carbonyl group concentration (Fig. 2) due to a higher oxidation of the molecule.

The increase in the carbonyl band (1715 cm^{-1}) is characteristic of the thermo-oxidative degradation.[9–13] Biodegradation of pure LDPE requires reduction of molecular weight and introduction of oxidized groups into the chain. Biotic reduction of the carbonyl groups abiotically introduced to the LDPE was previously reported.[4,6] In the present work, a modification of the previously formed carbonyl group was not detected due to the short incubation period with micro-organisms (90 days). Recent studies (unpublished results) demonstrated that carbonyl index of thermo-oxidized LDPE followed by a biotic treatment decreased more than 50% after 180 days of incubation. Analogously, Weiland and David[6] observed a reduction in the carbonyl group concentration after 150 days of incubation with a mixed fungal culture.[6]

Results of the modification of the double-bond band, expressed as the double-bond index, are presented in Table III. Samples thermally treated for 8 h did not present changes in the double-bond index without and with micro-organisms. However, this index significantly changed in thermally treated samples for 120 h without and with micro-organisms (Fig. 3). The highest increase in the double-bond index was obtained in the samples that were biologically treated. Formation of —CH═CH— groups due to microbial activity has also been previously reported. The increase of the double-bond index in thermo-oxidized LDPE samples biotically treated for 10-year-[4] and 32-year-old materials taken from a bioactive soil[14] was previously reported. Similar trends on the —CH═CH— group formation were found in the present work in thermo-oxidized samples treated by micro-organisms in only 90 days of incubation, indicating that thermo-oxidized LDPE modifications may be carried out by a direct mechanism of enzymatic scission and assimilation of the low molecular weight chains.[15]

CONCLUSIONS

Thermal analysis (DSC and TGA) of the thermo-oxidized samples did not allow determination of significant morphological changes in the thermally treated LDPE. However, structural and morphological changes for biologically treated samples were observed for the three techniques used (DSC, TGA, and FTIR). Oxidation due to the thermal treatment at 150°C for 120 h was accurately detected by FTIR. Carbonyl index levels were not modified during the 90 days of microbial treatment. The increase on the double bond index and the structural modifications observed by DSC in the biotically treated samples allow stating that these modifications were carried out by a reduction of the chain size by microbial scission mechanisms.

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