

Oxo-biodegradable full carbon backbone polymers – biodegradation behaviour of thermally oxidized polyethylene in an aqueous medium

Emo Chiellini*, Andrea Corti, Salvatore D'Antone

Department of Chemistry and Industrial Chemistry, Laboratory of Bioactive Polymeric Materials for Biomedical and Environmental Applications (BIOLAB) – UdR INSTM Consortium, University of Pisa, via Risorgimento 35, 56126 Pisa, Italy

Received 7 February 2007; accepted 5 March 2007

Available online 18 March 2007

Abstract

Several demonstrations of the effective biodegradation in soil of pro-oxidant activated polyethylene (PE) have been reported recently. Nevertheless a comprehensive understanding of the ultimate fate in the environment of the oxidized fragments of oxo-biodegradable polyethylene materials needs the extension of the studies to other natural environments and in particular to aqueous media (river, lake, brackish and marine waters) where accidental plastic littering and the resulting degraded fragments eventually may end up.

In this respect, as part of our continuing activity in the area of oxo-biodegradable polymeric materials, in the present paper we wish to report on the results attained in an ongoing investigation on the biodegradation in a water medium of thermally pre-oxidized low density polyethylene (LDPE) film samples containing pro-oxidant additives.

Thermally oxidized LDPE-film samples and corresponding acetone extractable fractions were submitted to the effect of microorganism flora present in river water. The effective biodegradation was assessed by monitoring the amount of CO₂ developed over time in a respirometer apparatus. Levels of biodegradation up to 12 and 48% for the degraded fragments and corresponding fractions extracted with boiling acetone were detected on a 100-day time frame.

© 2007 Published by Elsevier Ltd.

Keywords: Thermally degradable LDPE; Oxo-biodegradation; Alkane biodegradation; River water

1. Introduction

The extremely low propensity to oxidation and further degradation followed by biodegradation of conventional polyethylene is widely accepted. However, more than 30 years ago it was suggested that pre-aging treatment (UV, heat exposure, oxidation with nitric acid), as well as the addition of pro-degradant systems acting as initiators of thermal and photo-oxidation of polyethylene films promote the fragmentation of the tested samples eventually followed by microbial attack [1–5].

In particular, it was repeatedly demonstrated that abiotic oxidation produces functional macromolecules susceptible to random cleavage with the formation of low molecular weight

oxygenated products including aliphatic carboxylic acids, alcohols, aldehydes and ketones [6–9].

The rate of biodegradation of polyethylene, even under prolonged exposure time (10–32 years) to microbial consortia of soil, was found to be very low, thus accounting for less than 1% carbon mineralization [10,11]. Nevertheless, more recently it has been demonstrated that the use of suitable pro-oxidants as dopants of conventional LDPE or MDPE film induces substantial oxidation of the tested specimen with consequent fragmentation, drop of molecular weight and wettability increases ultimately followed by a fairly-high mineralization extent (60–70%) and fixation of carbon into cell biomass (8–10%) [12] in soil burial tests [13,14].

Most of the studies of the biodegradability of polyolefins containing pro-oxidant have been carried out on complex solid media like soil and compost [13–15], indeed very few studies have been carried out in aqueous media as well as in the

* Corresponding author. Tel.: +39 050 2210301; fax: +39 050 28438.

E-mail address: emochie@dcci.unipi.it (E. Chiellini).

Table 1
Characteristics of thermally degradable PE film samples used in thermal degradation tests [20]

Test sample ^a	Thickness (μm)	Sample code	Mw ^b (kDa)	ID ^b
Lupolen 3026 HK with 15% DCP540 TM	32	LDPE-DCP540	147.7	3.78
French compost bags with 10% ZSK 1314 TM	34	FCB-ZSK10	157.6	4.36
French compost bags with 15% ZSK 1314 TM	34	FCB-ZSK15	157.6	4.36

^a The percent figures (%) are referred to the amount of EPI-masterbatch in the PE granule blends submitted to melt blown extrusion.

^b Determined by HT-GPC.

presence of selected microbial species. In this respect the assimilation of organic acids derived from polyethylene oxidation by *Arthrobacter paraffineus* was observed by Albertsson et al. [16] as well as the direct assimilation of thermo-oxidized polyethylene by *Penicillium pinophilum*, even though at a very low mineralization rate (0.37% in 31 months) [17], and UV irradiated LDPE by a thermophilic bacterium [18].

The use of selected bacterial strains in liquid cultures demonstrated their capability to biofilm formation on the assayed PE film samples [19,20]. In particular a *Rhodococcus ruber* strain is found to utilize polyethylene films as sole carbon source as established by an 8% weight loss within 30 days of incubation, in a burial test [19]. More recently metabolic studies using ATP/ADP nucleotide assays revealed, after an initial fast growth due to the assimilation of low molecular fractions from thermally oxidized samples of LDPE, that the energetic status of some microbial strains, such as *Rhodococcus rhodochrous* and *Nocardia asteroides*, were compatible with the continuous assimilation of the polymer even though at a fairly low rate [20].

In the present study, aimed at the assessment of the mineralization level in river water medium, LDPE samples doped with pro-oxidant, formerly submitted to a prior investigation of the effects of different degradation conditions in term of either temperature or relative humidity on the oxidation behaviour, were utilized [21]. Accordingly thermally oxidized films at maximum and medium levels of oxidation, as determined by carbonyl index (CO_i), as well as the relevant acetone extractable fractions, were used to feed aqueous cultures of microorganism consortia present in river water. A respirometric procedure [22] based on the determination of the carbon dioxide evolved from the different cultures was utilized to assess the extent of the mineralization of the analyzed films and fractions extracted with acetone.

A fairly high biodegradation of these latter fractions, as well as the positive influence of the achieved oxidation level on the biodegradation propensity of the film samples was clearly demonstrated.

2. Experimental part

2.1. Thermally degradable polyethylene films

Poly(ethylene) film samples containing thermal pro-oxidant additives (LDPE-DCP540, FCB-ZSK15, and FCB-ZSK10) were kindly supplied by EPI Environmental Plastics Inc. (Vancouver, Canada).

Thermal degradation conditions and solvent extraction procedures of the analyzed films have been reported previously [21].

Compositions and characteristics of film samples utilized in accelerated thermal degradation tests are reported in Table 1 whereas in Table 2 is reported the data relevant to the recorded oxidation levels as assessed by the carbonyl index (CO_i) and the amount of the low molecular weight oxidized LDPE fractions as extracted with boiling acetone.

2.2. River water respirometric biodegradation test

The biodegradation tests were carried out in 300-ml Erlenmeyer flasks equipped with a silicone rubber stopper, hanging a 40 ml capacity plastic vial filled with 20 ml of a 0.05 M KOH solution for trapping CO₂ evolved from the microbial culture. Each flask contained 100 ml sterilised low concentration salt solutions having the following composition per litre of distilled water: KH₂PO₄ 85 mg, K₂HPO₄ 218 mg, Na₂HPO₄ 334 mg, (NH₄)₂SO₄ 10 mg, NH₄NO₃ 10 mg, CaCl₂ 36 mg, MgSO₄·7H₂O 23 mg, and FeCl₃·6H₂O 0.3 mg, pH 7.4 ± 0.2 [23].

Table 2
LDPE-film samples thermally treated at 70 °C and relevant fractions extractable with acetone tested in the river water biometer assay

Test sample	Film			Acetone extract			
	CO _i ^a	Mw ^b (kDa)	ID ^b	Film weight (%)	CO _i ^a	Mw ^c (kDa)	ID ^c
LDPE-DCP540	2.3	9.7	2.59	11.3	6.4	1.49	1.41
	4.6	4.5	1.27	21.1	20.7	1.08	1.37
FCB-ZSK15	2.8	7.6	2.44	9.2	6.1	1.67	1.52
	4.4	5.1	1.32	21.8	10.9	1.27	1.42
FCB-ZSK10	2.3	10.1	2.47	10.2	9.5	1.37	1.45
	4.1	4.4	1.25	18.8	12.7	1.27	1.39

^a Evaluated by FT-IR as D_{B1640-1840}/D_{B1435}.

^b Determined by HT-GPC.

^c Determined by GPC.

A river water sample collected in the Natural Park of San Rossore (Pisa – Italy) was used as microbial source in 10% by volume ratio to inoculate each test flask.

Film samples, acetone extracts, docosane (C22 linear hydrocarbon) reference material and positive control (sodium acetate) utilized in the tests were added as sole carbon sources at 0.01–0.05% by weight concentration. Test flasks were kept in the dark and incubated at room temperature (20–30 °C) under static conditions.

The biodegradation extent of each test material was calculated as a percentage (corrected for the inoculum endogenous emissions—blank flasks) of the overall theoretical CO₂ production calculated on the basis of the determined carbon content of the samples. The results were reproducible within a confidence of ±2% according to the tests carried out in triplicate.

2.3. Analytical characterization

2.3.1. Spectroscopic characterization

Polyethylene film samples, as well as the relevant extractable fractions were characterized by ¹H NMR and FT-IR, by using a Varian Gemini 200 MHz instrument and a Jasco FT-IR model 410, respectively.

2.3.2. Scanning electron microscopy (SEM)

SEM analyses on polyethylene film samples retrieved from the biodegradation experiments were performed using a Jeol LSM5600LV instrument.

2.3.3. Size exclusion chromatography (SEC)

Molecular weight and polydispersity of the acetone extracted fractions from thermally degraded samples were determined by SEC with a Jasco PU-1580 HPLC pump equipped with two Plgel mixed-D columns (Polymer Laboratories, UK) connected in series, and a Jasco 830RI refractive index detector. Sample elution was carried out with THF at 1 ml/min flow rate. The instrument was calibrated by using polystyrene standard samples.

2.3.4. Elemental analysis

Carbon content of each test sample was determined by elemental analysis by using a Carlo Erba model 1106 elemental analyzer.

3. Results and discussion

The effects of the oxidation level of thermally oxidized LDPE samples (LDPE-DCP540, FCB-ZSK15, and FCB-ZSK10) on the effective biodegradation level recorded in an aqueous medium (river water), were investigated by testing the mineralization level of films having two different carbonyl index (CO_i) values corresponding approximately to an high (4.1–4.6) and a medium (2.3–2.8) degree (Table 2) achieved during thermal oxidation in air at 70 °C [21]. At the same time, the mineralization of the corresponding acetone extractable fractions (Table 2) obtained from the same film at the two

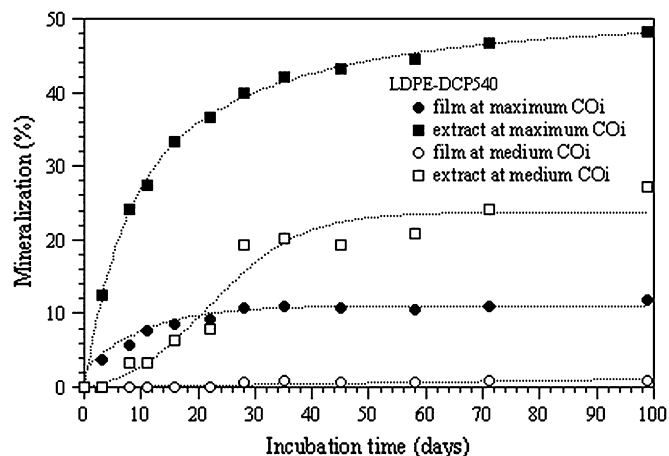


Fig. 1. Biodegradation profiles of thermally treated LDPE-DCP540 films and acetone extracts in river water medium at room temperature.

levels of oxidation (CO_i index) was also assessed. As a reference material C22 solid *n*-alkane (docosane) was used in the respirometric test, whereas the microbial viability was checked by using sodium acetate as a positive control.

The mineralization profiles of the analyzed samples recorded within 100 days incubation time are reported in Figs. 1–3, whereas in Fig. 4 the biodegradation curves of the reference materials recorded in the same incubation time frame, are also reported.

In all cases the acetone extractable fractions obtained from the corresponding LDPE films at the highest CO_i degree underwent fairly rapid mineralization, reaching about 42–48% biodegradation at 100 days of incubation (Figs. 1–3).

In particular the mineralization profile of the acetone extract derived from LDPE-DCP540 sample at maximum CO_i was found to start without any apparent induction phase, thus following an exponential phase within 1–2 days of incubation (Fig. 1). The corresponding biodegradation curve was also matched by a double exponential fit reaching 48% mineralization, with a positive slope, after 3 months of incubation. A similar behaviour was also observed in the case of the acetone

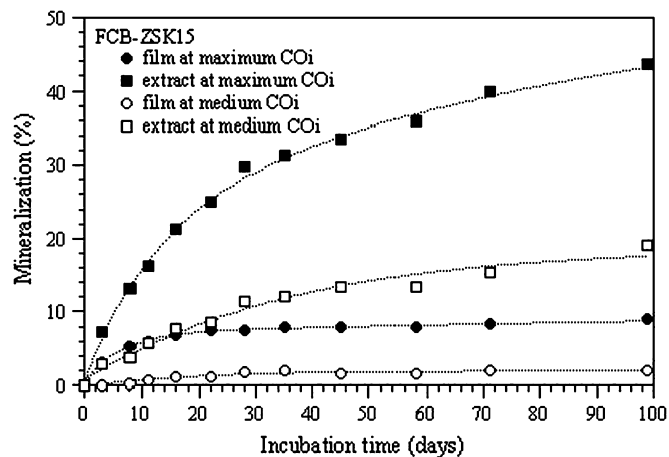


Fig. 2. Biodegradation profiles of thermally treated FCB-ZSK15 films and acetone extracts in river water medium at room temperature.

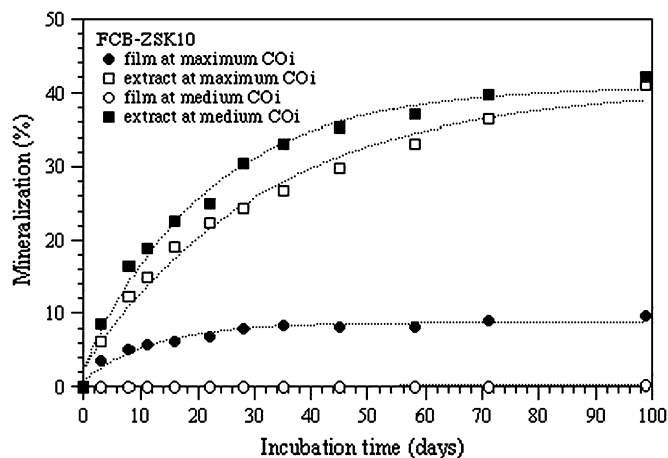


Fig. 3. Biodegradation profiles of thermally treated FCB-ZSK10 films and acetone extracts in river water medium at room temperature.

extractable fractions derived from the FCB-ZSK15 and FCB-ZSK10 samples at the maximum level of oxidation (Figs. 2 and 3), whose maximum extent of biodegradation was however a little lower (42–44%) than that recorded in the case of the acetone extract from LDPE-DCP540 sample at maximum CO_i.

It is worth mentioning that the extent and the profile of the biodegradation curves of these extracts were also comparable with the biodegradation profile recorded for the docosane sample (Fig. 4), thus suggesting a substantial similarity in the type of microbial metabolism most likely attributable to the chemical nature of aliphatic compounds. We have to remark that the docosane has a molecular weight (310 Da) substantially lower than any of the fraction extracted with acetone even though it did not contain any carbon atom in a formal oxidation stage higher than -2. The reliability of the respirometric test was also confirmed by the extent of mineralization (98%) reached at 100 day incubation by using sodium acetate as a positive control (Fig. 4).

Comparable lower extents of biodegradation were instead observed in the cultures supplemented with the acetone extractable fractions obtained from the LDPE films at the medium level of oxidation (Figs. 1–3). In all cases not more than 20% mineralization was observed after 3 months of incubation. Therefore, the lower propensity to biodegradation of these kinds of extracts in the presence of river water microorganisms could be attributed to limitations imposed by the higher molecular weight of the fractions extracted from the LDPE films at medium CO_i with respect to the Mw of the fractions obtained from the acetone extraction of LDPE films at maximum CO_i (Table 2), as well as to the differences in the amount of oxidized functional groups as qualitatively evidenced in NMR spectra recorded for the two fractions (Fig. 5). In fact the NMR spectrum of the acetone extracted fraction at the medium CO_i is characterized by the presence of absorption signals attributable almost exclusively to aliphatic carbonyl groups (Fig. 5a), whereas for the corresponding fraction at maximum level of oxidation, typical absorption signals of protons bonded to the carbon in ester groups were observed in a larger relative amount with respect to the aliphatic proton signals (Fig. 5b).

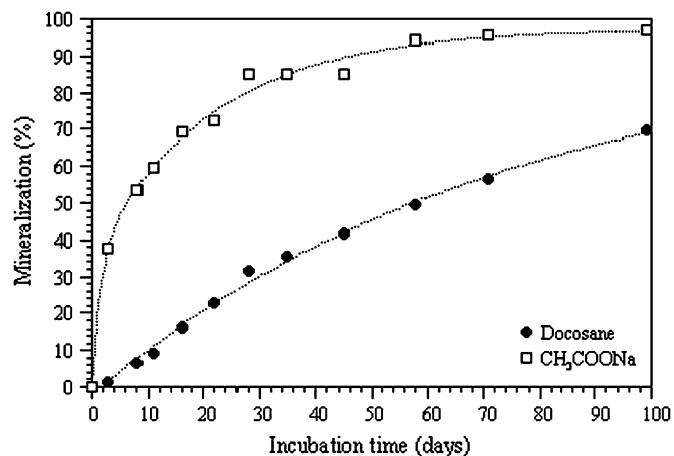


Fig. 4. Biodegradation profiles of docosane and sodium acetate reference materials in river water medium at room temperature.

The presence of larger amounts of oxidized functional groups in the acetone extracts obtained from the films at maximum CO_i was quantitatively confirmed by FT-IR analysis (Table 2, Fig. 6).

It is also worth noting that the propensity to biodegradation of the acetone extracts was positively influenced by the corresponding CO_i (e.g. amount of oxidized groups). In this respect, the highest degree of mineralization (48.3%) was recorded in the case of the extract having 20.7 CO_i obtained from the LDPE-DCP540 film at maximum level of oxidation (Fig. 1), whereas minor extents of biodegradation (19.1–41.0%) were recorded in the cultures supplemented with acetone extracts having comparable lower CO_i values and comprised between 6.1 and 12.7 (Figs. 1–3).

The biodegradative propensity of thermally degraded LDPE films was also markedly affected by the level of oxidation. In fact only the films characterized by higher CO_i values underwent a significant mineralization. The discrimination between samples at high and medium levels of oxidation was even evident at low levels of mineralization whereas the mediumly oxidized samples did not experience any apparent microbial attack (Figs. 1–3). The higher propensity to biodegradation of the tested materials as a function of the effective degree of oxidation under the adopted conditions was also indirectly confirmed by the higher microbial colonization of the surface of the films with maximum CO_i with respect to the corresponding specimen at lower value of functionalisation (Fig. 7).

Finally, as repeatedly reported [1,16,18,20], the microbial consumption of oxidized fractions present in the thermally degraded films was also confirmed by the decrease (30–35%) in the CO_i values of the films submitted to the biodegradation test with respect to the starting materials.

4. Conclusions

The biodegradation propensity of thermally degradable (oxo-biodegradable) polyethylene films previously shown in solid incubation media (natural soil and compost) has been confirmed also in a relatively simple environment such as that represented by river water containing wild microorganism consortia.

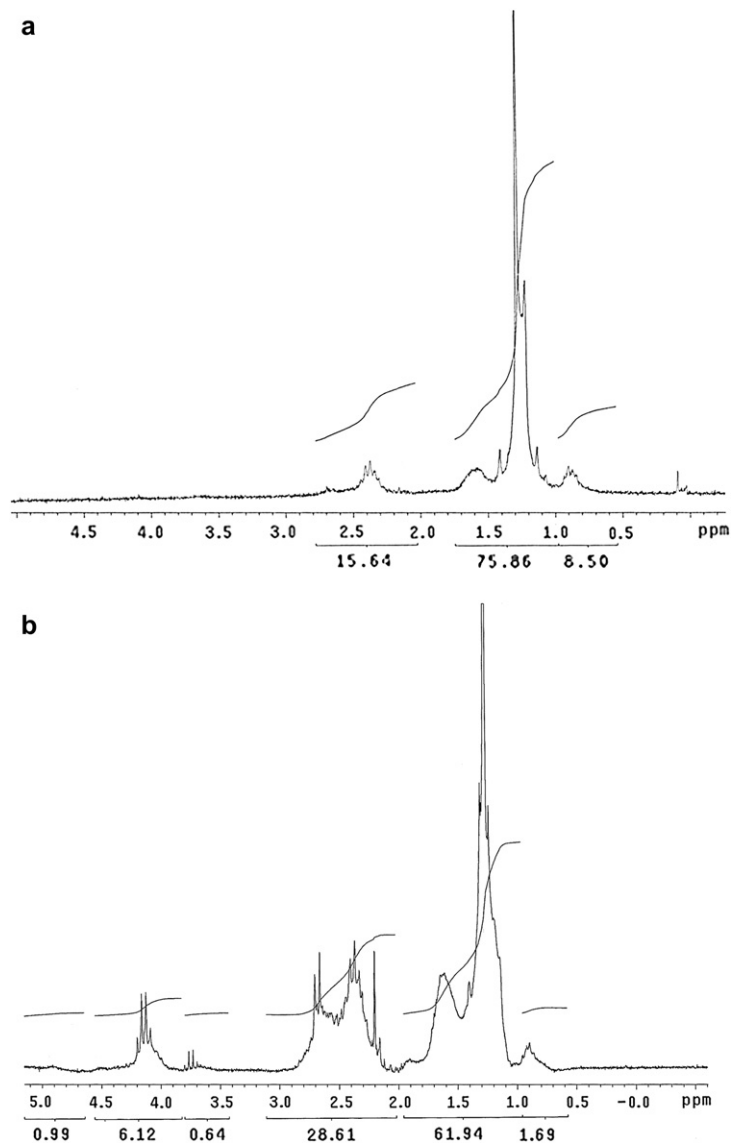


Fig. 5. ^1H NMR spectra of acetone extractable fractions from thermally treated LDPE-DCP540 films at medium (a) and maximum (b) COi.

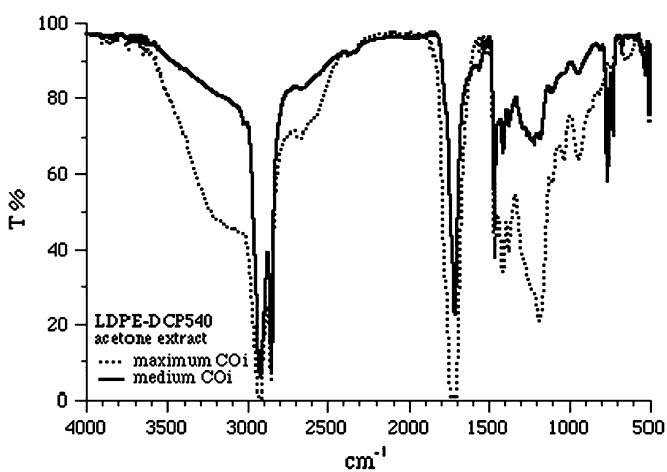


Fig. 6. FT-IR spectra of acetone extractable fractions from thermally treated LDPE-DCP540 films at maximum (a) and medium (b) COi.

A positive influence on the level of oxidation achievable during the pre-aging treatment of thermally degradable poly(ethylene) on both the rate and extent of biodegradation has been also demonstrated by comparing the behaviour of polymer films and the corresponding acetone extractable fractions containing significantly different amounts of oxygenated groups.

In this connection, the structural characterization of thermally oxidized degradable polyethylene fragments could be used for an effective prediction of their ultimate fate when ending eventually in river aqueous environment.

The rate of degradation is very much dependent upon the molecular weight and level of oxidation of the samples submitted to a biodegradation test.

Finally we can conclude that full carbon backbone oxo-biodegradable PE samples as widely demonstrated also in the case of poly(vinyl alcohol), that can be considered as linear PE regioselectively oxidized in 1,3-position, can experience oxo-degradation and hence bio-degradation in water media.

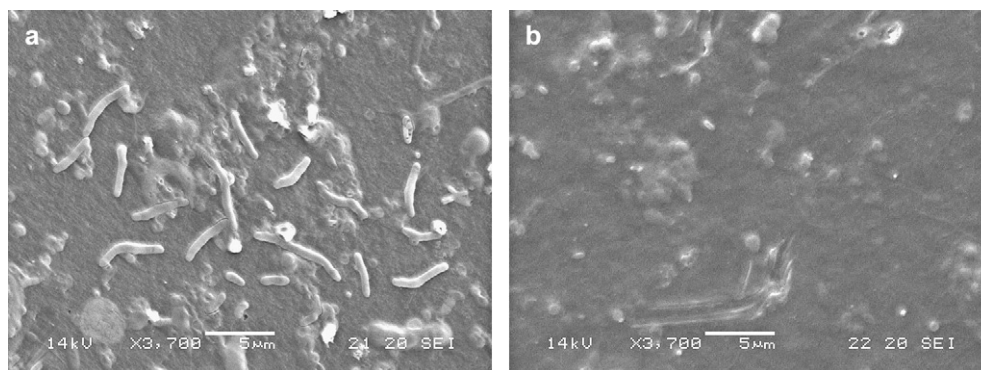


Fig. 7. SEM micrographs of thermally treated LDPE-DCP540 films at maximum (a) and medium (b) CO_2 withdrawn after 35 days incubation in the presence of river water medium at room temperature.

The extension of the undertaken investigation is also in progress for other aqueous media including marine and civil-industrial waste water from treatment plants.

Acknowledgements

The project was developed within the framework of design and preparation of new polymeric materials for environmental and biomedical applications PRIN 2004-512206070. The partial financial support by EPI Environmental Plastics Inc. (Canada) is gratefully acknowledged.

References

- [1] Brown BS, Mills J, Hulse JM. Chemical and biological degradation of plastics. *Nature* 1974;250:161–3.
- [2] Albertsson A-C. Biodegradation of synthetic polymers. 2. Limited microbial conversion of C^{14} in polyethylene to CO_2 - C^{14} by some soil fungi. *J Appl Polym Sci* 1978;22:3419–33.
- [3] Cornell JH, Kaplan AM, Rogers MR. Biodegradation of photooxidized polyalkylenes. *J Appl Polym Sci* 1984;29:2581–97.
- [4] Albertsson A-C, Erlandsson B, Hakkarainen M, Karlsson S. Molecular weight changes and polymeric matrix changes correlated with the formation of degradation products in biodegraded polyethylene. *J Environ Polym Degrad Stab* 1998;6:187–95.
- [5] Contat-Rodrigo L, Ribes Greus A. Biodegradation studies of LDPE filled with biodegradable additives: morphological changes. *J Appl Polym Sci* 2002;83:1683–91.
- [6] Scott G. Environmental biodegradation of hydrocarbon polymers: initiation and control. In: Doi Y, Fukuda K, editors. *Biodegradable plastic and polymers*. Amsterdam: Elsevier; 1994.
- [7] Albertsson A-C, Barenstedt C, Lindberg T, Karlsson S. Degradation product pattern and morphology changes as means to differentiate abiotically and biotically aged degradable polyethylene. *Polymer* 1995;36:3075–83.
- [8] Karlsson S, Hakkarainen M, Albertsson A-C. Dicarboxylic acids and ketoacids formed in degradable polyethylenes by zip depolymerization through a cyclic transition state. *Macromolecules* 1997;30:7721–8.
- [9] Khabbaz F, Albertsson A-C, Karlsson S. Chemical and morphological changes of environmentally degradable poly(ethylene) films exposed to thermo-oxidation. *Polym Degrad Stab* 1999;63:127–38.
- [10] Albertsson A-C, Karlsson S. The influence of biotic and abiotic environments on the degradation of polyethylene. *Prog Polym Sci* 1990;15:177–92.
- [11] Otake Y, Kobayashi T, Ashabe H, Murakami N, Ono K. Biodegradation of low density polyethylene, polystyrene, polyvinyl-chloride, and urea-formaldehyde resin buried in soil for over 32 years. *J Appl Polym Sci* 1995;56:1789–96.
- [12] Chiellini E, Corti A, D'Antone S, Billingham NC. Microbial biomass yield and turnover in soil biodegradation tests: carbon substrate effects. *J Polym Environ*, submitted for publication.
- [13] Chiellini E, Corti A, Swift G. Biodegradation of thermally-oxidized, fragmented low-density polyethylenes. *Polym Degrad Stab* 2003;81:341–51.
- [14] Jakubowicz I. Evaluation of degradability of biodegradable polyethylene (PE). *Polym Degrad Stab* 2003;80:39–43.
- [15] Alariqi SAS, Pratheep Kumar A, Rao BSM, Singh RP. Biodegradation of γ -sterilised biomedical polyolefins under composting and fungal culture environments. *Polym Degrad Stab* 2006;91:1105–16.
- [16] Albertsson A-C, Erlandsson B, Hakkarainen M, Karlsson S. Molecular weight changes and polymeric matrix changes correlated with the formation of degradation products in biodegraded polyethylene. *J Environ Polym Degrad* 1998;6:187–95.
- [17] Volke-Sepulveda T, Saucedo-Castaneda G, Gutierrez-Rojas M, Manzur A, Favela-Torres E. Thermally treated low density polyethylene biodegradation by *Penicillium pinophilum* and *Aspergillus niger*. *J Appl Polym Sci* 2002;83:305–14.
- [18] Hadad D, Geresh S, Sivan A. Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. *J Appl Microbiol* 2005;98:1093–100.
- [19] Gilan I, Hadar Y, Sivan A. Colonization, biofilm formation and biodegradation of polyethylene by a strain of *Rhodococcus ruber*. *Appl Microbiol Biotechnol* 2004;65:97–104.
- [20] Koutny M, Sancelme M, Dabin C, Pichon N, Delort A-M, Lemaire J. Acquired biodegradability of polyethylenes containing pro-oxidant additives. *Polym Degrad Stab* 2006;91:1495–503.
- [21] Chiellini E, Corti A, D'Antone S, Baciù R. Oxo-biodegradable carbon backbone polymers – oxidative degradation of polyethylene under accelerated test conditions. *Polym Degrad Stab* 2006;91:2739–47.
- [22] Chiellini E, Corti A. A simple method suitable to test the ultimate biodegradability of environmentally degradable polymers. *Macromol Symp* 2003;97:381–95.
- [23] ISO 14593. Water quality – evaluation of the ultimate aerobic biodegradability of organic compounds in aqueous medium – method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test); 1999. www.iso.ch/iso/en.