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The biodegradation of amorphous and crystalline regions in film-blown poly(ϵ-caprolactone)

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Abstract

The surface erosion of films of poly(ϵ-caprolactone) (PCL) in compost, in anaerobic sewage sludge and by *Aspergillus fumigatus* was compared with chemical hydrolysis (23 and 50°C). Biodegraded samples displayed grooves or cracks arranged in parallel, whereas samples exposed to an abiotic environment showed no surface changes. Differences in micro-flora together with the initial morphology of the sample resulted in different mechanisms of erosion. The degradation in compost resulted in parallel grooves or cracks, while *A. fumigatus* produced a spherulitic erosion pattern. A preferential degradation in the amorphous parts produced low-molar mass fractions with lengths corresponding to one, two or several times the thickness of the crystal lamellae. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Polycaprolactone; Biodegradation; Surface erosion

1. Introduction

Polymers are frequently used in packaging materials, and they finally end up in the municipal solid waste stream. To reduce the amount of waste, attention has been focused on the development of biodegradable polymers. Poly(ϵ-caprolactone) (PCL) is an aliphatic polyester that is degradable in several biotic environments, including river and lake waters, sewage sludge, farm soil, paddy soil, creek sediment, roadside sediment, pond sediment and compost [1–4]. It has been shown that molar mass and crystallinity are the dominating factors affecting the biodegradability of PCL [1,5]. During composting and degradation in anaerobic sludge, however, it has been shown that temperature plays an important role in the degradation [3,4]. Composting at a higher temperature produced more $^{14}CO_2$ from labelled PCL than composting at a lower temperature [3]. Degradation of PCL in anaerobic sludge produced more gas during treatment at 55°C than at 37°C [4]. It has been suggested that the changes in degradation rate in biological environments at different temperatures are a result of changes in microflora [6]. A wide population of micro- and macro-organisms can exist in a compost. The most abundant are bacteria, actinomycetes and fungi [7].

An abiotic degradation of PCL has also been studied at different pHs and different temperatures [8,9]. Basic pH and high temperature favoured the degradation of PCL in an aqueous environment.

PCL and other aliphatic polyesters degrade by enzymatic and/or chemical hydrolysis. Poly(β-hydroxybutyrate) (PHB) is a naturally produced relative of PCL. Researchers have extensively studied the degradation mechanism of PHB. When PHB single crystals are subjected to enzymatic degradation, the crystals are attacked from the edges with no change in molar mass [10–12]. Marchessault and co-workers explained the degradation mechanism as a combination of *endo*- and *exo*-cleavage, while Doi and co-workers claimed that it is predominantly *exo*-cleavage. This is contrary to the behaviour of PCL, where the molar mass decreases readily during biodegradation [13,14]. The decrease in molar mass is also accompanied by a broadening in molar mass distribution and by the development of low-molar mass peaks [13,14].

While studying the abiotic and biotic degradation of PCL [4], we observed that parallel grooves appeared in the polymer surface. These grooves were found only in polymer film degraded in a biotic environment. It has been shown that the amorphous part of PCL is degraded prior to the crystalline part in a biotic environment [13,15]. Cook et al. [15] observed that, as the degradation of the amorphous parts progressed, radial arms from the spherulites developed in solvent-cast films. The morphology of the blown film, as in our case, is however generally considered to consist of rows

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of lamellae with their normals parallel to the extrusion direction. Keller and Machin have proposed a model (the cylindrite model) for the morphology of the blown polyethylene film [16]. This paper continues the work previously performed at KTH [4], by reporting and discussing why these grooves together with low-molar mass fragments are formed for some, but not all, of the samples degraded in compost and anaerobic sewage sludge and by chemical hydrolysis.

2. Experimental

2.1. Material

The material used in this study was PCL, Tone 787 from Union Carbide. The PCL film was blown with a set-up consisting of two 18 mm Axon single screw extruders, L/D 30, and a 70 mm diameter die producing a two-layer film. The film was approximately 40 μm thick with a blow-up ratio of 2.9–3.4:1. The temperature profile of the extruder was set from 100 to 125°C at a screw speed of 50 rpm. The moulding die was set to 100°C.

2.2. Degradation procedure

2.2.1. Composting

Small-scale composting was performed in a 270 l turnable composting facility over a period of 45 days. The facility was insulated with 5 cm polyethylene foam with no external heating. The garden compost consisted of typical garden waste, such as grass clippings and leaves. The highest recorded temperature inside the compost was 43°C and the moisture content was 60% by weight.

2.2.2. Anaerobic degradation in sewage sludge

Anaerobic degradation was performed in oxygen-free flasks containing 0.8 g PCL and inoculum. Control flasks contained only the inoculum. The experiment was performed at two temperatures, 37 (mesophilic) and 55°C (thermophilic). The production of gas was measured with a syringe. The volume of gas is defined as the gas volume produced in the flask with the samples minus the gas volume from the control samples.

2.2.3. Chemical hydrolysis

The hydrolysis was performed in a solution containing distilled water or 0.01 M phosphate buffer solution at pH 10.5. Both solutions were kept at two temperatures, 23 and 50°C. NaN_3 (0.02% (w/w)) was added to avoid the growth of micro-organisms at 23°C. pH was adjusted to the initial values after 3 weeks.

2.2.4. Degradation by pure microbial culture

The microbial degradation was performed in 250 ml Erlenmeyer flasks containing salt media were the polymer samples constituted the main carbon source. Each flask contained 0.3 g of polymer film ($50 \times 50\ mm^2$) and 150 ml of salt medium. The salt medium contained per litre the following: 5.0 g $(NH_4)_2C_4H_4O_6$; 1.0 g KH_2PO_4; 1.0 g $MgSO_4 \cdot 7H_2O$; 0.8 ml of a 1% solution of $FeCl_3 \cdot 6H_2O$; and 8 ml of a 1% solution of $ZnSO_4 \cdot 7H_2O$. The samples were inoculated at 23 and 30°C during a period of 49 days with *A. fumigatus* after the medium had been adjusted to pH 5.5 by the addition of HCl. After sampling, the polymer film was allowed to dry at 23°C after sterilization with 70% (v/v) ethanol in water. Sterile control consisted of 0.3 g of polymer film ($50 \times 50\ mm^2$) and 150 ml of salt medium (described above), pH adjusted with HCl and with 5 ml 0.02% (w/w) NaN_3-solution added three times during the experiment.

2.3. Etching

The PCL film was etched in a 40% water solution of methylamine (Merck) for 48 h.

2.4. Scanning electron microscopy (SEM)

The surface changes were observed with a JEOL scanning microscope model JSM-5400 using an acceleration voltage of 15 kV. The samples were gold plated with a Denton Vacuum Desk II cold sputter etch unit for 2×30 s.

2.5. Size exclusion chromatography (SEC)

The molar mass was determined with a chromatography system consisting of a Waters 510 pump, a Waters WISP 712 and a Waters 410 RI detector. The system was equipped with three PLgel 10 μm mixed-B columns ($300 \times 7.5\ mm^2$) from Polymer Laboratories. The solvent used during the analysis was tetrahydrofuran (THF). The sample injection volume was 200 μl. Calibration was performed with polystyrene standards in the molar mass range 2000–1 950 000 g/mol. Each sample was analysed four times to give an appropriate average of the molar mass.

2.6. MALDI-TOF analysis

The samples were dissolved in THF (5 mg/ml). The solution was diluted five times with the matrix (2,5-dihydroxybensoic acid), and approximately 0.5 μl of the solution was analysed with a HP G2025 A LD TOF system.

2.7. Differential scanning calorimetry (DSC)

The thermal properties of PCL samples were studied by a Perkin–Elmer DSC-7 at a heating rate of 10°C/min. The apparatus was calibrated with an indium standard. The values of the degree of crystallinity are obtained from the first scan, and the melting temperature from the second. The mass crystallinity was estimated using the heat of fusion (ΔH_f^0) of the totally crystalline PCL: 139.5 J/g [17].

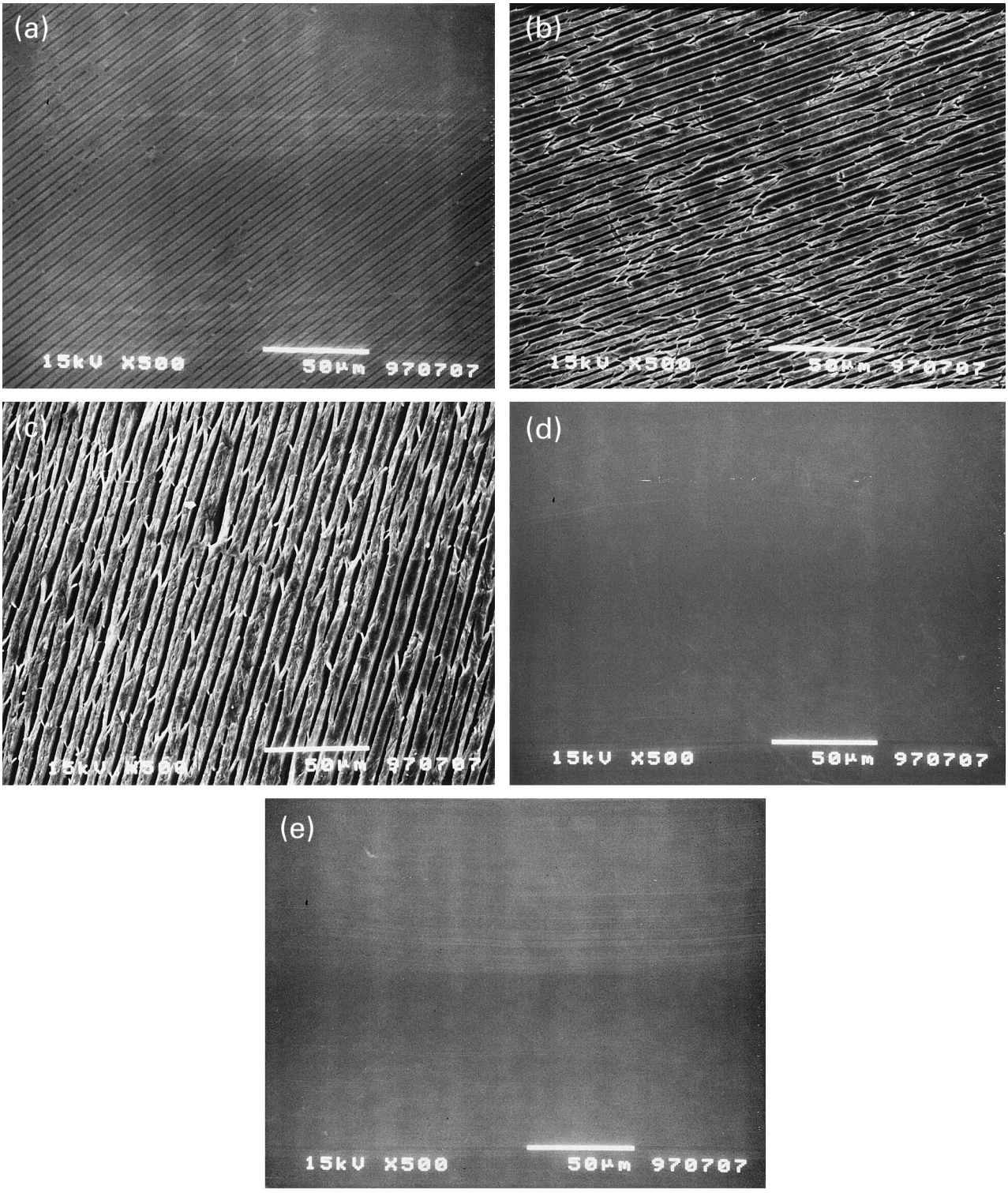

Fig. 1. SEM micrographs of the film-blown PCL samples degraded in compost for (a) 4, (b) 16 and (c) 36 days, and degraded by chemical hydrolysis at pH 7 at (d) 23 and (e) 50°C.

3. Results and discussion

3.1. Texture

The film-blown PCL samples exposed to compost were investigated with SEM. Fig. 1 shows that the biodegraded samples displayed parallel grooves or cracks arranged perpendicular to the machine direction. The samples degraded for 4 days exhibited grooves on some parts of the surface (Fig. 1(a)). As the degradation proceeded, the

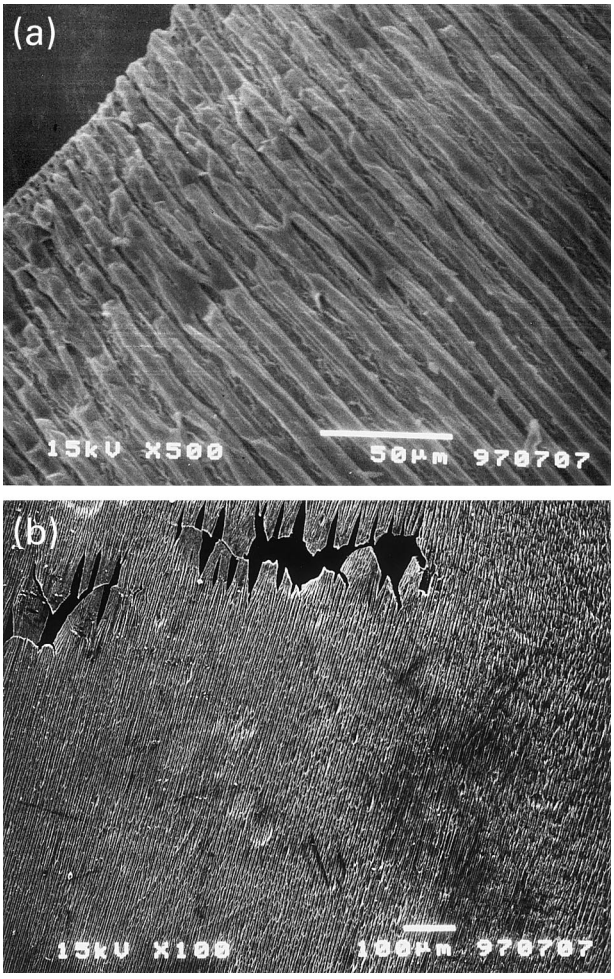

Fig. 2. SEM micrographs of the film-blown PCL sample degraded in (a) thermophilic anaerobic sludge for 29 days and (b) in compost for 36 days.

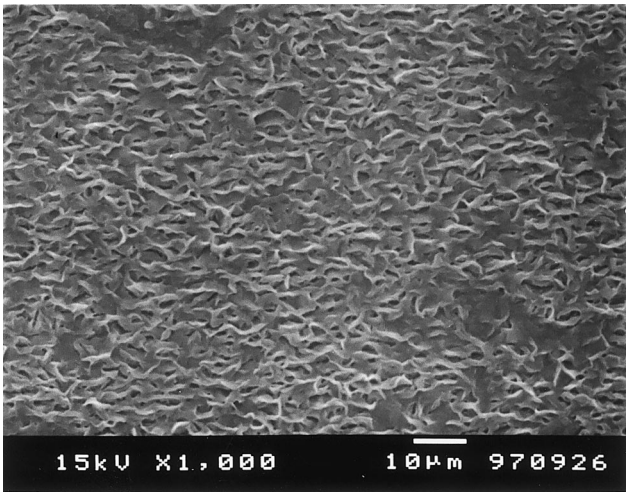

Fig. 3. SEM micrograph of the film-blown PCL exposed to *A. fumigatus* at 30°C for 7 days.

number of grooves increased and the ridges between the grooves became wider, while the widths of the grooves remained approximately unchanged (Fig. 1(b) and (c)). The samples exposed to hydrolysis at pH 7 at either 23 or 50°C showed no changes on the surface (Fig. 1(d) and (e)). The degradation thus seemed to be homogeneous in the abiotic environment. The samples were degraded evenly. In the biotic environment, on the other hand, the degradation took place mainly in regularly spaced areas of the samples. The biodegraded samples were also investigated with ESEM. The grooves were also present in the ESEM micrographs (not shown), and were thus not caused by vacuum during sputtering of the samples.

Fig. 2(a) shows the edge of a sample subjected to thermophilic anaerobic sludge for 29 days. The area at the edge was smooth, which showed that the ridges eventually eroded. At later stages of the biodegradation, holes appeared and the grooves around the holes became less visible. Fig. 2(b) shows that the area surrounding the holes was thinner and exhibited less grooves than other parts of the sample, suggesting that the sample became more smooth at later stages of the bio-erosion. This has also been observed on poly(3HB-co-3HV) exposed to fungi [18]. The results shown in Fig. 2 indicate that the bio-erosion proceeds more rapidly from the edges of the sample as well as around holes.

Samples of the blown PCL films exposed to *A. fumigatus* at 23°C without agitation displayed grooves in a pattern resembling that observed on composted samples, but the blown films exposed to *A. fumigatus* at 30°C exhibited a surface structure resembling elongated spherulites, as shown in Fig. 3. These different degradation patterns and different degradation mechanisms may be caused by different extracellular enzymes excreted at different temperatures by *A. fumigatus*.

It has been shown that when thick PCL samples are exposed to *A. flavus* and *Penicillium funiculosum*, the amorphous parts degrade first, leaving the crystalline material as radial spherulite arms. The crystalline parts were then degraded [19]. Film-blown LDPE samples, 15 μm thick, were exposed to *A. niger*, *Gliocaldium virens*, *Paecilomyces variotii* and *P. funiculosum* for eight months after an initial thermo-oxidation. Long parallel grooves were seen on these samples [20], similar to those seen in the present study. Previously, uniaxially drawn (600%) PCL exposed to a *Fusarium* species exhibited a periodic surface structure perpendicular to the direction of orientation. The authors suggested that the observed structure was due to the selective degradation of amorphous regions between lamellae, lying perpendicular to the direction of stretching [21].

In order to investigate the cause of the different erosion patterns, melt-pressed films were prepared and subjected to *A. fumigatus* and composting. After degradation by *A. fumigatus*, the films displayed a spherulitic surface structure resembling those previously reported [15]. However, when the films were subjected to composting, randomly directed cracks appeared at the surface.

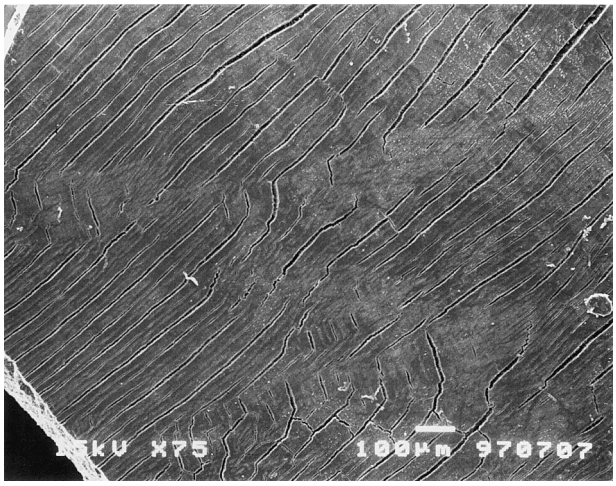

Fig. 4. SEM micrograph of the film-blown PCL etched for 48 h in methylamine.

The difference in the degradation pattern between the melt-pressed and the film-blown samples can in part be attributed to the difference in morphology caused by the processing. The melt-pressed film has a non-oriented spherulitic structure. The film-blown samples are oriented and a morphologic model based on cylindrites has been proposed [16], as discussed above. Wide-angle X-ray studies of the blown film showed that the films were oriented in the machine direction [22], which indicated that the grooves were situated between the crystalline cylindrites. Fig. 4 illustrates the cylindrite structure of the film-blown samples by showing an etched PCL sample.

The erosion mechanism of the film-blown PCL differed between the composted samples and the samples subjected to *A. fumigatus* at 30°C. An inoculum from the compost was placed in the same mineral medium (30°C) as was used in the *A. fumigatus* experiment and the blown film was added. SEM micrographs of the film taken after degradation showed that grooves appeared on the surface. Consequently, not only the initial morphology of the sample but also the type of micro-organism influences the bio-erosion pattern.

3.2. Molar mass measurements

A decrease in molar mass was observed for the samples during the degradation. The composted samples showed the fastest molar mass reduction followed by the samples in thermophilic anaerobic sludge [4]. The degradation in the biotic environments resulted in a faster reduction in $\bar{M}_n$ than in $\bar{M}_w$. The faster reduction in $\bar{M}_n$ was explained as being due to a preferred degradation near the chain ends [23], which are in most cases situated in the amorphous part of the material. The shape of the SEC changed during degradation. Fig. 5 shows the SEC curve for the PCL degraded in different environments. Low-molar mass tails appeared in the chromatograms during degradation in the thermophilic anaerobic sludge and in the compost. The formation of low-molar mass fractions did not start until after 10 days of degradation. As the degradation proceeded, the amount of low-molar mass fraction increased. Smaller low-molar mass fractions have been reported by Lefebvre et al. [24]. This phenomenon, however, was neither observed during the abiotic hydrolysis at 50°C or for samples degraded in the mesophilic anaerobic sludge, nor did these samples display any grooves on the surface.

To achieve a more precise determination of the low-molar mass tails, MALDI-TOF anlayses were carried out.

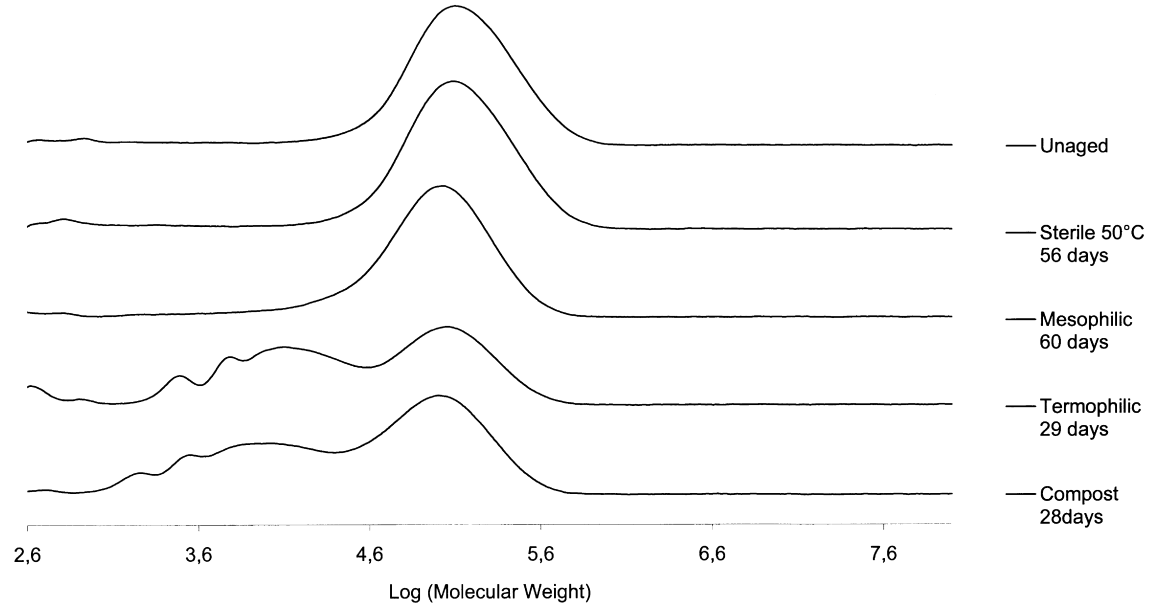

Fig. 5. SEC curves for the film-blown PCL degraded in compost for 28 days: in thermophilic anaerobic sludge for 29 days; in mesophilic anaerobic sludge for 60 days; and in an abiotic environment at 50°C (pH 7), and for an undegraded sample.

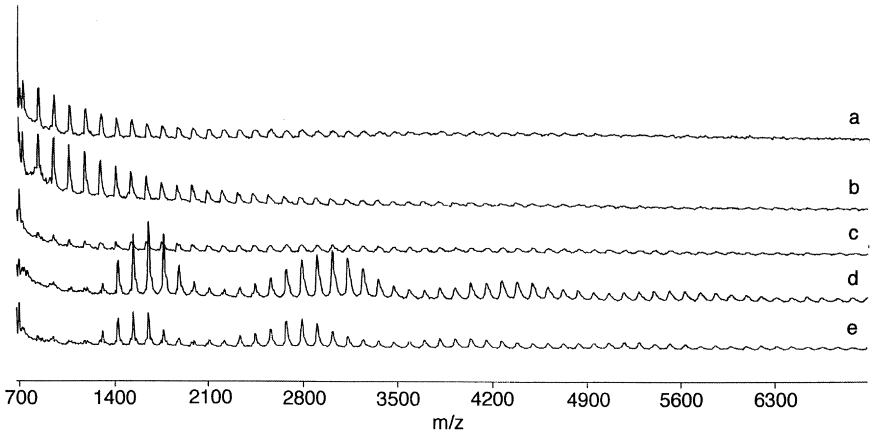

Fig. 6. MALDI-TOF spectras of PCL samples: (a) unaged; (b) abiotic environment 50°C, pH 7, 56 days; (c) anaerobic mesophilic sludge, 60 days; (d) anaerobic thermophilic sludge, 29 days; and (e) compost, 28 days.

Fig. 6 shows the MALDI-TOF spectra of PCL degraded in different environments. Due to the resolution of the MALDI-TOF technique, the molar mass of only the last three fractions could be determined. The differences between the peaks of the fractions were found to correspond to 10 repeating units of PCL, i.e. that the second fraction from the end contains 10 more repeating units than the first fraction, and the third contains a further 10 repeating units. SEC analysis did not allow higher molar mass fractions to be well resolved from the subsequent fraction containing another 10 repeating units. The fractions with the higher molar mass are therefore less resolved than the lower molar mass fractions. The higher molar mass fractions are however present in a higher concentration (Fig. 5).

The amorphous part of the material was degraded more rapidly than the crystalline part, as mentioned earlier, and this was also shown in the pattern of the SEM micrographs. In the crystalline part of the material, the molecules are regularly arranged in lamellae, which consists of folded molecules. Some parts of a molecule that has crystallised can be in the amorphous zone, such as chain ends, tie-chains and loops. Since the amorphous part is degraded faster than the crystalline part, it is mainly the crystalline part that remains after degradation. The crystalline part of the molecule consists of either one length of repeating units of PCL, i.e. as long as the lamella is thick, or two or three or more. The molar mass of the fragment increases with the number of repeating units corresponding to the thickness of the lamellae multiplied by the number of times the polymer chain is folded before it enters the amorphous phase. This explains the low-molar mass fractions shown in Figs. 5 and 6.

3.3. Thermal analysis

The degree of crystallinity increased from 54 to 65%

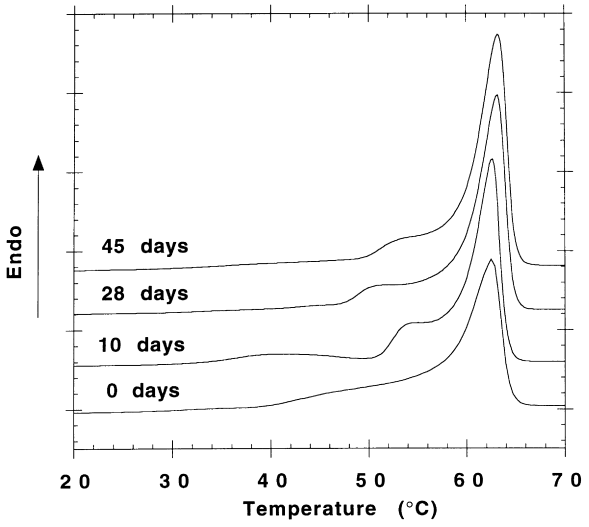

Fig. 7. DSC curves from the first scan for the film-blown PCL degraded in compost for 0, 10, 28 and 45 days; first heating.

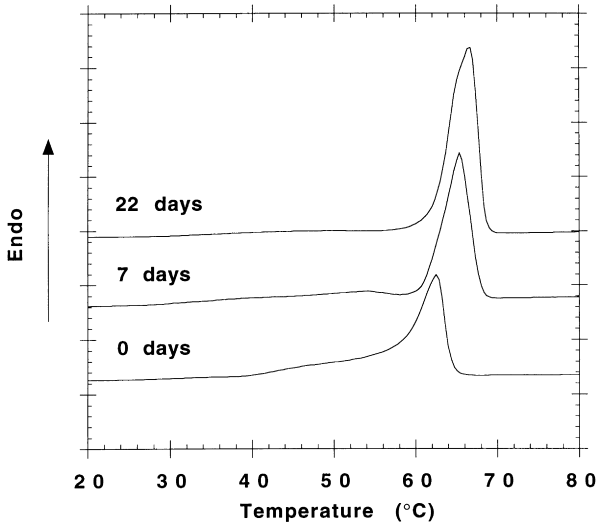

Fig. 8. DSC curves from the first scan for the film-blown PCL degraded in the thermophilic anaerobic sludge for 0, 7 and 22 days; first heating.

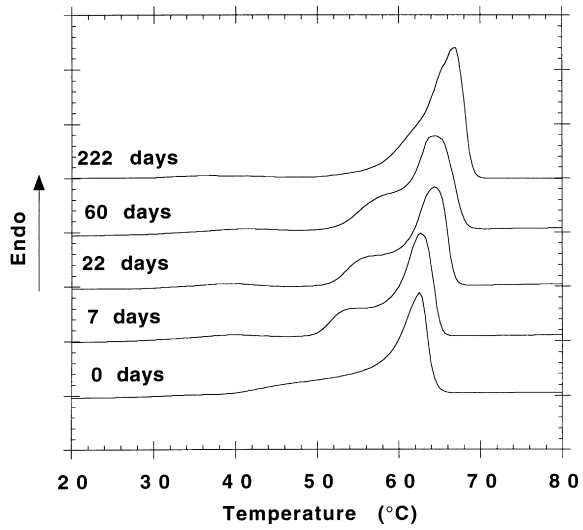

Fig. 9. DSC curves from the first scan for the film-blown PCL degraded in the mesophilic anaerobic sludge for 0, 7, 22, 60 and 222 days; first heating.

during composting, as described previously [4]. Fig. 7 shows that a shoulder was detected on the low-temperature side of the main melting peak in the first heating after 10 days in compost. The appearance corresponds to the time of formation of the low-molar mass fractions seen in the SEC chromatograms. The shoulder extended to lower temperatures with increasing degradation time. A plausible explanation is that lamellae thinner than the average thickness are formed out of the low-molar mass polymer chains formed by chain scission. In addition, a broad region with low melting temperature was observed between 35 and 50°C after 10 days. It was not however observed at longer degradation times. We also observed that the temperature of the main melting peak increased due to annealing of the samples during composting. The thermograms during the second heating contain no low-temperature melting regions and the melting temperature was unaffected during degradation.

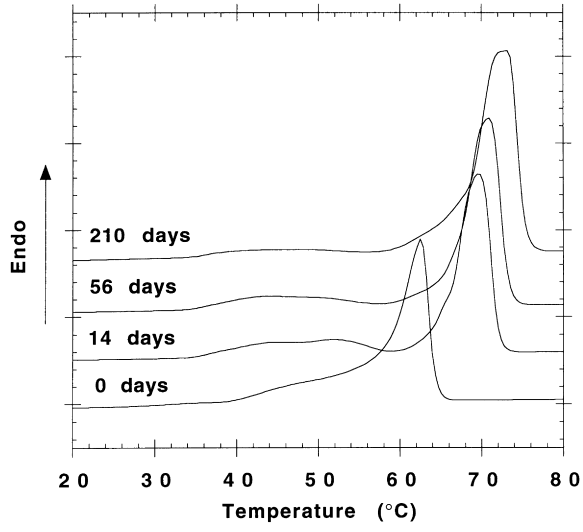

Fig. 10. DSC curves from the first scan for the film-blown PCL exposed to chemical hydrolysis at pH 7 50°C for 0, 14, 56 and 210 days; first heating.

During degradation in anaerobic sludge at 55 (thermophilic micro-organisms) and 37°C (mesophilic micro-organisms), the degree of crystallinity increased from 54 to 68 and 62%, respectively [4]. Fig. 8 shows the thermograms for the samples degraded in the thermophilic sludge. The melting region increased in both temperature and intensity but no shoulder was observed on the low-temperature side of the main endotherm. Again, a broad region of low-melting temperature was noticeable between 30 and 55°C after 7 days. Fig. 9 shows the melting endotherms for the samples biodegraded in the mesophilic sludge. The endotherm increased in temperature with increasing degradation time, and after 7 days a shoulder developed on the low temperature side of the main melting peak similar to those described previously for the composted samples. In a recent publication by Gan et al., a similar effect was seen in DSC-traces of the solvent-cast PCL films degraded by lipases in phosphate buffer [25]. In contrast to our results, the degree of crystallinity decreased during degradation due to the simultaneous consumption of amorphous and crystalline regions. Fig. 9 also shows that at longer degradation times, i.e. 222 days, the low-temperature shoulder is integrated into the main melting peak. This is probably due to the lamellae perfection.

Fig. 10 shows thermograms of PCL films subjected to abiotic degradation at 50°C and pH 7. The degree of crystallinity increased continuously, from 54 to 68%, with increasing degradation time, and a low-temperature melting region, 35–55°C, was apparent after 14 days of degradation. As with the samples exposed to thermophilic sludge, no shoulder on the melting endotherm was detected. A possible explanation for the absence of the low-temperature shoulder is that at higher temperatures (50 and 55°C), more rapid crystal rearrangements are possible. The samples degraded in an abiotic environment at 50°C for 14 days exhibited higher melting temperatures than the samples degraded for 22 days in the thermophilic sludge at 55°C. Differences in the average molar mass yield thinner lamellae, which melt at a lower temperature, and $\bar{M}_n$ of the latter sample was very low (30% of the original) compared with the former (unchanged).

4. Conclusion

During the biodegradation of film-blown samples of PCL, both in compost and in thermophilic anaerobic sludge, regularly spaced grooves developed on the film surface. Such grooves were not seen in the case of samples degraded in an abiotic environment. The width of the grooves increased with increasing time of biodegradation. This was interpreted as indicating a preferred degradation of the amorphous part of the material, which is situated in long rows perpendicular to the machine direction in the film-blown samples.

The molar mass of the samples decreased as a result of the biodegradation and the molar mass distribution became multi-modal. Analysis of the low-molar mass peaks with MALDI-TOF revealed that there was a difference in molar mass between the peaks corresponding to 10 repeating units. This was explained by a preferential degradation of the amorphous part, which includes loops and tie-chains from the crystalline part. This eventually leads to molecules with a length approximately equal to x times the lamella thickness.

The crystallinity increased in the biodegraded samples. The composted samples developed low-temperature melting shoulders corresponding to the low-molar mass parts observed with SEC and MALDI-TOF. The low-temperature melting shoulders were not seen in the thermophilic samples. A possible explanation for this is, crystal rearrangements occurring at the higher temperature (55°C) during the thermophilic conditions.

Acknowledgements

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