Rapid (bio)degradation of polylactide by mixed culture of compost microorganisms—low molecular weight products and matrix changes

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Abstract

Poly(L-lactide) (PLLA) was rapidly (bio)degraded by a mixed culture of compost microorganisms. After 5 weeks in biotic environment, the films had fragmented to fine powder, while the films in corresponding abiotic medium still looked intact. Analysis of the low molecular weight products by GC-MS showed that microorganisms rapidly assimilated lactic acid and lactoyl lactic acid from the films. At the same time, a new degradation product, ethyl ester of lactoyl lactic acid was formed in the biotic environment. This product cannot be formed by abiotic hydrolysis and it was not detected in the abiotic medium. The degradation of the PLLA matrix was monitored by differential scanning calorimetry (DSC), size exclusion chromatography (SEC) and scanning electron microscopy (SEM). A rapid molecular weight decrease and increasing polydispersity was observed in the biotic environment. In the abiotic environment only a slight molecular weight decrease was seen and the polydispersity started decreasing towards 2.0. This indicates different degradation mechanisms, i.e. preferred degradation near the chain ends in the biotic environment and a random hydrolysis of the ester bonds in the abiotic environment. SEM micrographs showed the formation of patterns and cracks on the surface of the films aged in biotic medium, while the surface of the sterile films remained smooth. The SEM micrographs showed a large number of bacteria and mycelium of fungi growing on the surface of the biotically aged films. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Polylactide (PLA) polymers are perhaps the most frequently used polyesters in biomedical applications due to their many favourable properties, e.g. high strength and biocompatibility. Currently there is an increasing interest in utilising PLA for disposable and (bio)degradable plastic products. PLA meets many requirements as a packaging thermoplastic and may be used as a commodity resin for general packaging applications. Its physical properties and melt processing are similar to conventional packaging resins. Unlike commercially available biodegradable plastic materials synthesised from petrochemicals, e.g. polycaprolactone (PCL) and poly(tetramethylene succinate), PLA is polymerised from lactic acid, which can be prepared effectively by fermentation from renewable resources such as starch and cellulose.

Most of the studies on PLA degradation have concentrated on abiotic hydrolysis [1–3]. The effect of, e.g. residual monomer and other impurities [4,5], molecular weight [6] and copolymerisation [7,8] on hydrolysis rate and properties has been studied. Vert and co-workers [1,2,9] demonstrated in a series of papers that in the case of massive specimens the hydrolysis is faster in the centre than at the surface. They suggested that the hydrolysis products are formed both at the surface and in the inner part, but those localised near the surface dissolve in the ageing medium, while the concentration of carboxylic end groups increases in the centre and catalyses ester hydrolysis, resulting in a surface–centre segregation and multimodal molecular weight distributions for both amorphous and semicrystalline polymers. Degradation of PLA and its copolymers in clinical applications ranging from absorbable sutures, drug delivery systems and artificial ligaments has also been widely studied [10–12].

Studies on PLA or PLA oligomers subjected to selected microorganisms [13–18] show in general that the molecular weight decreases initially by abiotic hydrolysis, but after the initial abiotic decrease, the molecular weight for the biotic samples decreases faster than the molecular weight of the abiotic samples. Vert et al. exposed compression-moulded racemic PDLLA to a mixed culture of *Fusarium moniliforme* and *Pseudomonas putida* at 30°C [13]. The polymer initially degraded through chemical hydrolysis followed by
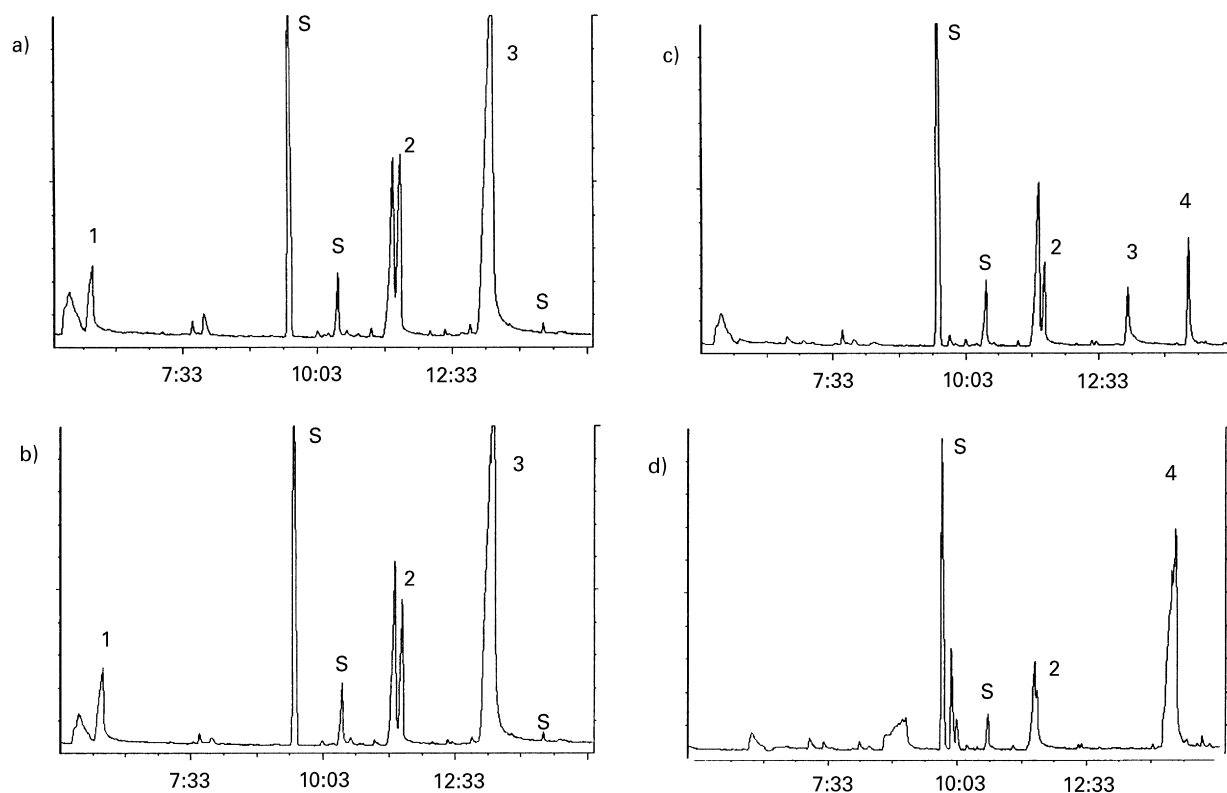
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Fig. 1. The GC–MS chromatograms after extraction from: (a) unaged PLLA film; (b) after 4 weeks in sterile medium; (c) after 2 weeks; and (d) after 4 weeks in biotic medium. Peak 1 = lactic acid, peak 2 = lactide, peak 3 = lactoyl lactic acid, peak 4 = ethyl ester of lactoyl lactic acid and S = system peaks. To show more clearly the changes in the main products, only the beginning of each run is shown. The area with trimer, tetramer and pentamer peaks is not shown, because the amount of these oligomers was very low and remained almost constant.

bioassimilation of the by-products. A mixed culture of *F. moniliforme* and *P. putida* resulted in faster assimilation of oligomers compared to if only *F. moniliforme* or *P. putida* were used [14].

Sixty percent of the PLA film was degraded after 14 days in the liquid culture containing *Amycolatopsis* species isolated from the soil [16]. The film surfaces remained smooth in the sterile controls, but in the inoculated films the surfaces became rough and hemispherical holes were formed. Increased degradation rate was in some cases observed when PLLA was treated with pure enzymes [19–21]. Different additives affect the degradation rate, e.g. addition of $CaCO_3$ to PLA significantly reduced the thermal degradation during processing, but it also reduced the rate of subsequent (bio)degradation in the pure fungal cultures [17].

We have in previous studies identified biotic and abiotic degradation products of, e.g. casein incorporated in building material [22,23], aliphatic polyesters [3,24–26] and polyethylene [27–31]. It is still under discussion whether high molecular weight PLLA is biodegradable or if it degrades by pure chemical hydrolysis. The aim of this study was to show, by comparing the degradation in biotic and abiotic medium, that the presence of microorganisms accelerates the degradation rate of PLLA. Mixed culture of compost microorganisms was chosen as the biotic medium, because

the synergic effect of several cultures enhances the degradation rate compared to degradation in pure cultures. The low molecular weight products were identified and correlated with the matrix changes to clarify the differences in degradation mechanism in biotic and abiotic medium. The changes in the polymer matrix were characterised by size exclusion chromatography (SEC), scanning electron microscopy (SEM) and differential scanning calorimetry (DSC).

2. Experimental

2.1. Materials and degradation procedure

The material was pure poly(L-lactide), PLLA without additives made by Neste Oy, Finland. The 45 μm thick film samples were used. Each series contained about 3 g of the polymer and 800 ml of mineral medium in a 21 Fernbach flask. The mineral medium consisted of per litre of deionised water the following: 5 g $(NH_4)C_4H_4O_6$; 1 g KH_2PO_4; 1 g $MgSO_4 \cdot 7H_2O$; 0.85 ml $FeCl_3 \cdot 6H_2O$ (1% solution) and 7.5 ml $ZnSO_4 \cdot 7H_2O$ (1% solution). A mixed culture of microorganisms from compost was added to the biotic series. NaN_3 (5 ml of 0.02% solution) was added to
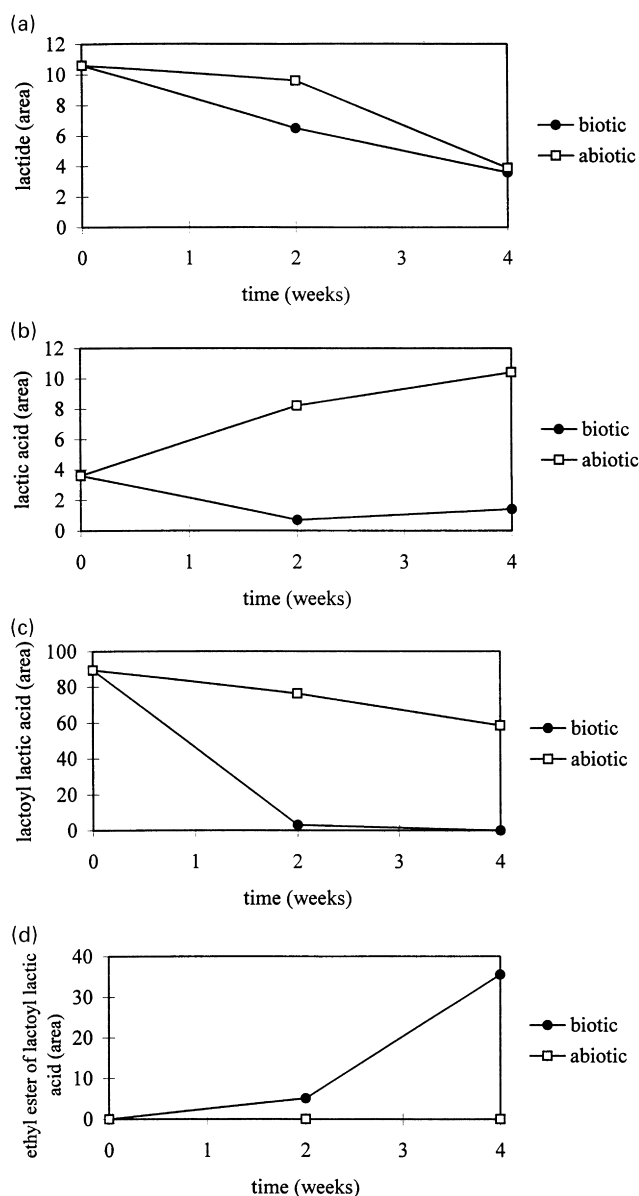
Fig. 2. The relative amounts of lactide, lactic acid, lactoyl lactic acid and ethyl ester of lactoyl lactic acid as a function of degradation time in biotic and abiotic medium.

the abiotic series to prevent microbial growth. The series were held in a shaking oven at 30°C.

2.2. The extraction procedure

After 0, 2 and 4 weeks the low molecular weight products were extracted from the surface of the films. At each time period three 100 mg pieces of polymer were removed from both abiotic and biotic series for the extraction. Each film piece was put into 1 ml of diethyl ether for 1 h to extract the low molecular weight products. At the same time intervals the water soluble products were extracted from the mineral medium. ENV+ solid phase extraction columns (100 mg) from Sorbent AB were used to extract the products from the

buffer solution. The column was first activated with 1 ml methanol and then equilibrated with 1 ml of water pH adjusted to 2 by adding HCl. The pH of the mineral medium with the degradation products was also adjusted to 2 before it was applied to the SPE column. After light drying of the column, the analytes retarded into the column were eluted with 1 ml acetonitrile and analysed with GC-MS. Triple extractions were done from both the films and the mineral medium.

2.3. Gas chromatography–mass spectrometry

The low molecular weight products were identified by Finnigan GCQ gas chromatograph–mass spectrometer. The GC was equipped with a RTX-5MS column. Helium was used as a carrier gas. The column was first held at 40°C for 1 min and then programmed to 250°C at 8°C/min and finally held at 250°C for 13 min. The injector temperature was held at 225°C.

2.4. Size exclusion chromatography

The change in the molecular weight during the inoculation was analysed by SEC. The instrument was equipped with Waters 6000A pump, a refractive index detector, a PL-EMD 960 light scattering evaporative detector and two PL gel 10 μm mixed-B columns (300 × 7.5 mm^2) from Waters. Dimethylformamide (DMF) was used as mobile phase. The flow rate was 1 ml/min. Polyethylene oxide standards in the molecular weight range 10 600–645 600 g/mole were used for calibration. Triplicate samples were analysed each time.

2.5. Scanning electron microscopy

The surface structures of the films after culture were observed by JEOL scanning electron microscope model JSM-5400 using a acceleration voltage of 15 kV. The samples were gold–palladium sputtered with a Denton Vacuum Desk II cold sputter etch unit for 2 × 30 s.

2.6. Differential scanning calorimetry

Melting behaviour of the samples was studied using a Mettler Toledo DSC 820 Calorimeter at a heating rate of 10°C/min in a nitrogen atmosphere. The values of heat of fusion originate from the first scan, and the melting temperatures from the second. A reference value of 93.6 J/g was kept for heat of fusion in calculating crystallinity. The apparatus was calibrated with Indium standards.

3. Results and discussion

3.1. Low molecular weight products

Several low molecular weight products, e.g. lactic acid, lactide, the acyclic dimer lactoyl lactic acid and trace
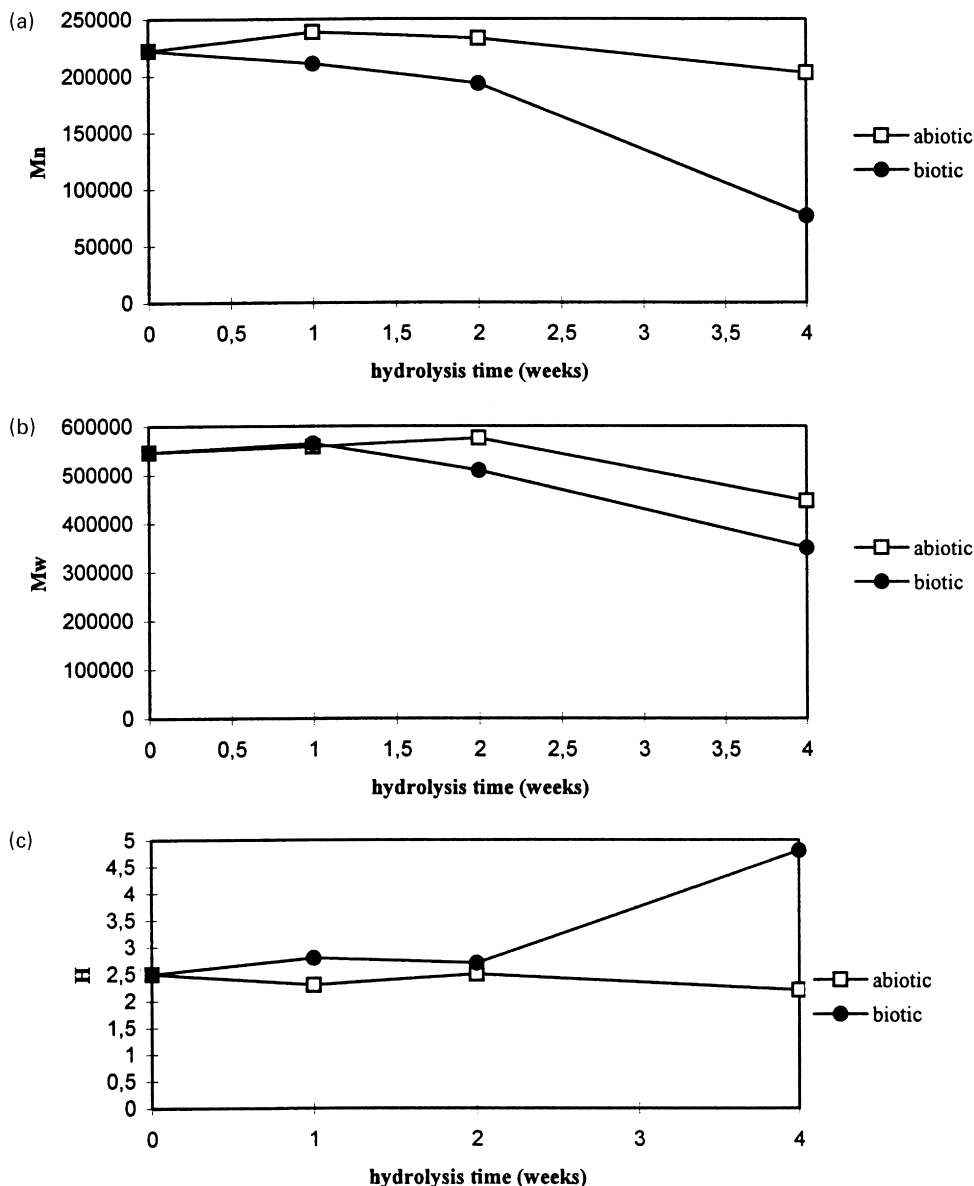
Fig. 3. The changes in (a) number average molecular weight, (b) weight average molecular weight and (c) polydispersity as a function of degradation time in biotic and abiotic environment.

amounts of cyclic trimer, tetramer and pentamer were already present in the unaged PLLA films. Lactide and the acyclic dimer, lactoyl lactic acid, were the major products in the chromatograms followed by lactic acid. Lactic acid and the acyclic dimer, lactoyl lactic acid, were both rapidly assimilated by the microorganisms. Already after 2 weeks the amount of lactic acid and lactoyl lactic acid had decreased to almost zero. After 4 weeks in the biotic environment a small amount of lactic acid was still detected, but the lactoyl lactic acid peak had totally disappeared. The reason why after 4 weeks in biotic environment we could still see some lactic acid, but no lactoyl lactic acid is explained by the sterile controls, which showed that the amount of lactic acid increases in the abiotic medium. This means that new lactic acid is formed during the experiment

due to abiotic hydrolysis of the PLLA. This abiotic hydrolysis is even going on in the biotic environment and the amount of lactic acid present in the biotic environment depends on the ratio between the rate of formation of new lactic acid and the rate by which it is removed by the microorganisms.

The microorganisms were not as effective in utilising the cyclic lactide. The decrease in lactide concentration in both biotic and abiotic medium is explained by hydrolysis to lactic acid or dissolvation into mineral medium. The amount of the cyclic trimer, tetramer and pentamer remained practically constant. These oligomers are probably formed during the processing of the films as cyclic oligomers are known thermal degradation products of polylactide [32,33].

A new degradation product, identified as ethyl ester of lactoyl lactic acid, appeared in the biotic chromatograms
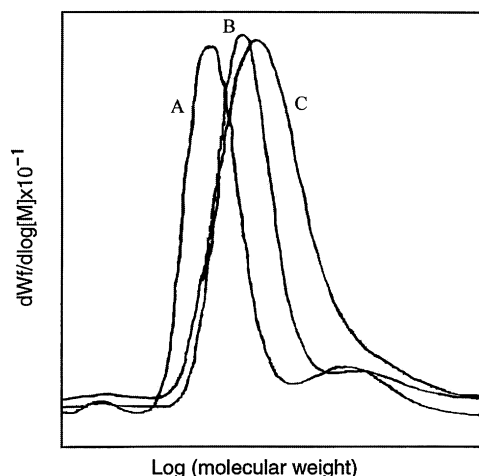
Fig. 4. The shape of the SEC curves changed during the ageing. Originally there was a shoulder on the low molecular weight side of the curves (A curve). After 4 weeks in abiotic medium the low molecular weight shoulder had decreased in size but was still clearly distinguishable (B curve). This shoulder had totally disappeared and the overall curve had broadened after 4 weeks in biotic medium (C curve).

after 2 weeks. The amount of ethyl ester of lactoyl lactic acid increased rapidly between 2 and 4 weeks. The ethyl ester of lactoyl lactic acid cannot be formed by abiotic hydrolysis and it was not detected after the abiotic ageing. We have in earlier studies shown that the amount of carbonyl groups in the polyethylene decreases during biodegradation [27,29]. The consumption of the carbonyl groups in the polylactide chain could also explain the formation of ethyl ester of lactoyl lactic acid in the present study.

Fig. 1 shows chromatograms obtained after extractions from biotically and abiotically aged films. To more clearly show the changing amounts of the main products, e.g. lactic acid, lactoyl lactic acid, lactide and ethyl ester of lactoyl lactic acid, only the beginning of each run is shown. It was not of interest to show the whole chromatograms, because the amount of trimer, tetramer and pentamer was very low and remained almost constant. Fig. 2 shows the relative amounts of lactide, lactic acid, lactoyl lactic acid and ethyl ester of lactoyl lactic acid as a function of ageing time in biotic and abiotic medium. No products were detected in the extractions from the mineral medium. In the biotic medium the absence of the products is explained by the removal and assimilation of the low molecular weight hydrolysis products, e.g. lactic acid and lactoyl lactic acid directly from the films. In the abiotic medium the degree of degradation in the matrix was very low as seen by, e.g. SEC results.

3.2. Molecular weight changes

Fig. 3 shows the changes in number average molecular weight (M_n), weight average molecular weight (M_w) and the polydispersity (H) as a function of ageing time. The number average molecular weight started decreasing slightly during the first 2 weeks in biotic environment and decreased then rapidly between 2 and 4 weeks. The weight average molecular weight started decreasing after 2 weeks in biotic environment. A much more moderate molecular weight decrease was seen in the films aged in abiotic medium.

In the biotic medium the M_n decreased faster than M_w and the polydispersity of the samples increased from 2.5 to 4.8 during the first 4 weeks. In the abiotic medium the polydispersity of the samples decreased from 2.5 to 2.2 during the same time period. These differences in the polydispersity show that the degradation mechanism differs in biotic and abiotic medium. The faster reduction in M_n compared to M_w in biotic medium is explained by a preferred degradation near the chain ends [34], while decrease of polydispersity towards 2 in the abiotic medium indicates random hydrolysis of the ester bonds.

Fig. 4 shows the change in the shape of the SEC curves. Originally there was a shoulder in the low molecular weight side of the curves. This shoulder and the shape of the curves remained almost the same in the abiotic environment, but after 2 weeks in the biotic environment the shoulder had almost melted into the main peak and it could only be seen as a slight broadening on the right side of the peak. After 4 weeks in the biotic medium the shoulder had totally disappeared, but the shape of the overall peak had further broadened. After 5 weeks the films in the biotic environment had completely fragmented to small pieces, while the films in the sterile mineral medium still looked intact.

3.3. Changes on the surface of the films

Fig. 5 shows the scanning electron micrographs of the surfaces of the samples aged for 28 days at biotic and abiotic environments. Initially the surface of PLLA films was smooth. However, the pictures reveal that the surface of the samples subjected to microorganisms was significantly eroded after 28 days. Both bacteria and fungi was seen on the surface of the films (Fig. 5). Fig. 5(a) and (b) shows the large number of bacteria cells growing on the surface of the films. We also observed growth of mycelium of fungi on the surface (Fig. 5(c) and (d)). Washing with ethanol before the SEM analysis was not enough to remove these bacteria and fungi from the films. Surface erosion and patterns and cracks after mycelium were also seen (Fig. 5(d) and (e)). The surface of the films remained smooth and almost intact during the abiotic hydrolysis (Fig. 6).

The presence of lactic acid and lactoyl lactic acid in the films probably have an important part in initiating the (bio)degradation of the films by enabling the colonisation and multiplication of cells of microorganisms on the film surfaces. This lead to continued growth of fungi and bacteria on the films also after assimilation of the initially present lactic acid and lactoyl lactic acid, as most of the lactic acid and lactoyl lactic acid were removed after 2 weeks, but most of the microbial growth on the films took place between 2 and 4 weeks as shown by SEM.
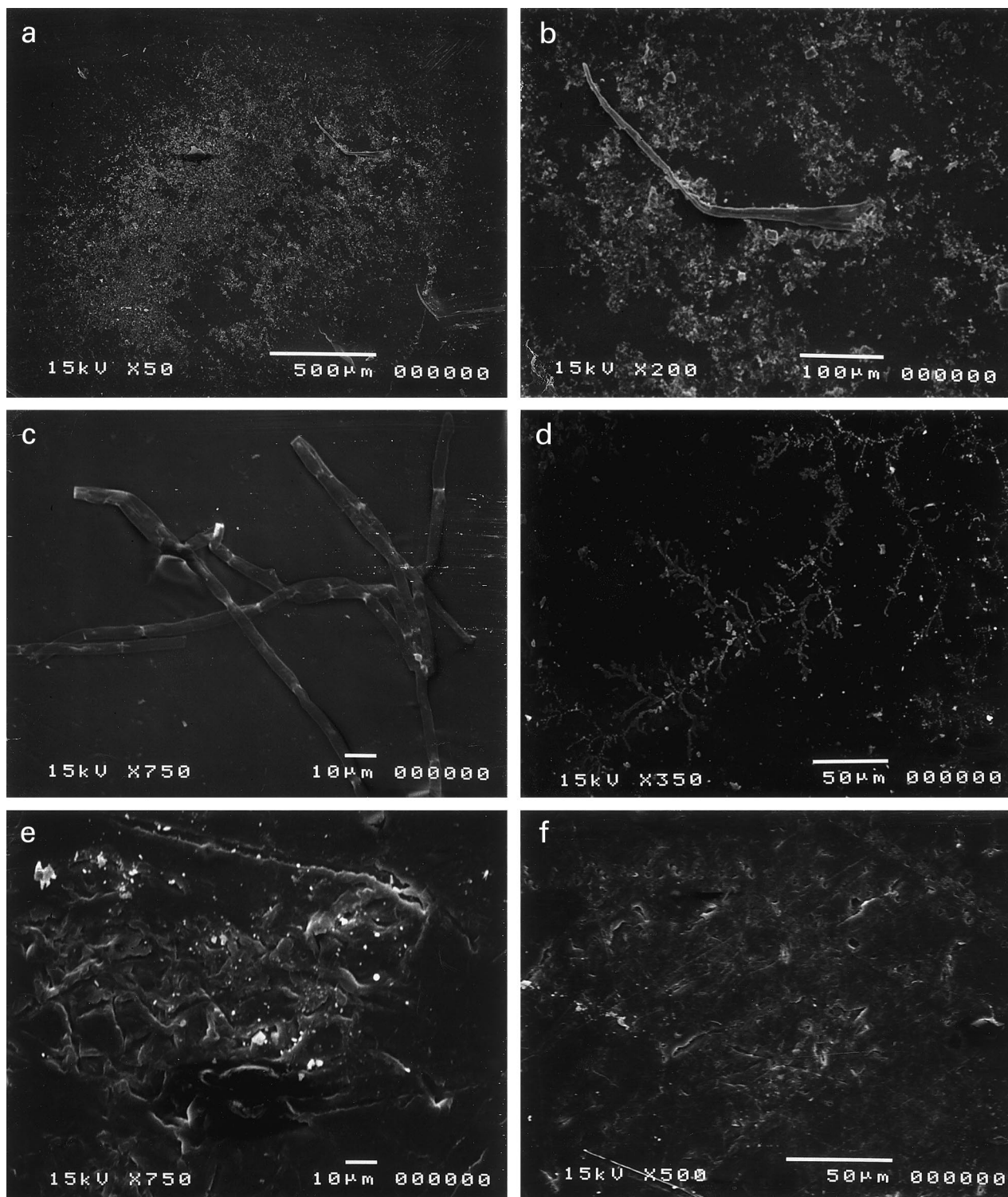
Fig. 5. SEM micrographs showing (a) and (b) the large amount of bacteria, (c) and (d) the growth of mycelium on the surface of the films and (e) and (f) surface erosion of the (bio)degraded films. All the pictures are taken after 28 days in biotic medium.

3.4. Thermal analysis

Table 1 gives the degree of crystallinity, T_g and T_m, for the biotic and abiotic samples. The degree of crystallinity measured as the heat of fusion during the first heating increased during both biotic and abiotic hydrolysis, but the increase was larger for the biotic samples. After 4 weeks the melt temperature had decreased from 175 to 169.5°C for the biotic samples. The main decrease in melt temperature took place between 2 and 4 weeks in the

Table 1
Changes in T_g, T_m and degree of crystallinity for the biotically and abiotically aged samples as measured by DSC

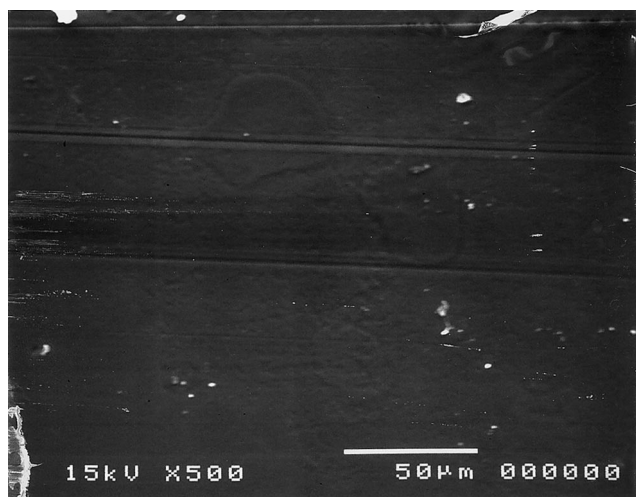| No. of days	T_g (°C)		T_m (°C)		Degree of crystallinity (%)	
	Biotic	Abiotic	Biotic	Abiotic	Biotic	Abiotic
0	63.3	63.3	175.1	175.1	50.2	50.2
14	63.1	63.8	173.8	174.4	54.2	52.5
28	60.5	63.8	169.5	174.3	57.7	54.5

Fig. 6. SEM micrograph showing the smooth surface of the sterile control after 28 days of abiotic hydrolysis.

correlation with the rapid molecular weight decrease during this time. In the case of the abiotic samples the melt temperature remained almost constant. Fig. 7 shows the DSC curves from the first heating for the unaged PLLA and PLLA after 28 days in abiotic and biotic medium. The transition of both T_g and T_m towards lower temperature in the case of the biotically aged samples is clearly seen.

4. Conclusions

The presence of microorganisms accelerated the degradation of polylactide compared to the degradation in corresponding sterile mineral medium. The changes in polydispersity indicate different degradation mechanisms in biotic and abiotic mediums, i.e. preferred degradation near the chain ends in the biotic medium and random hydrolysis of the ester bonds in the abiotic medium. Microorganisms rapidly utilised lactic acid and lactoyl lactic acid from the biotically aged films, but they were not as effective in assimilating the cyclic lactide. A new degradation product, ethyl ester of lactoyl lactic acid, was formed in the biotic environment. No products were detected in the extractions from the mineral medium. This is explained by the rapid utilisation of lactic acid and lactoyl lactic acid directly from the films. The presence of easily assimilated low molecular weight products probably had an important part in enabling the initial growth of microorganisms on the film surface. However, the SEM micrographs showed that the amount of bacteria and fungi on the surface of the films continued
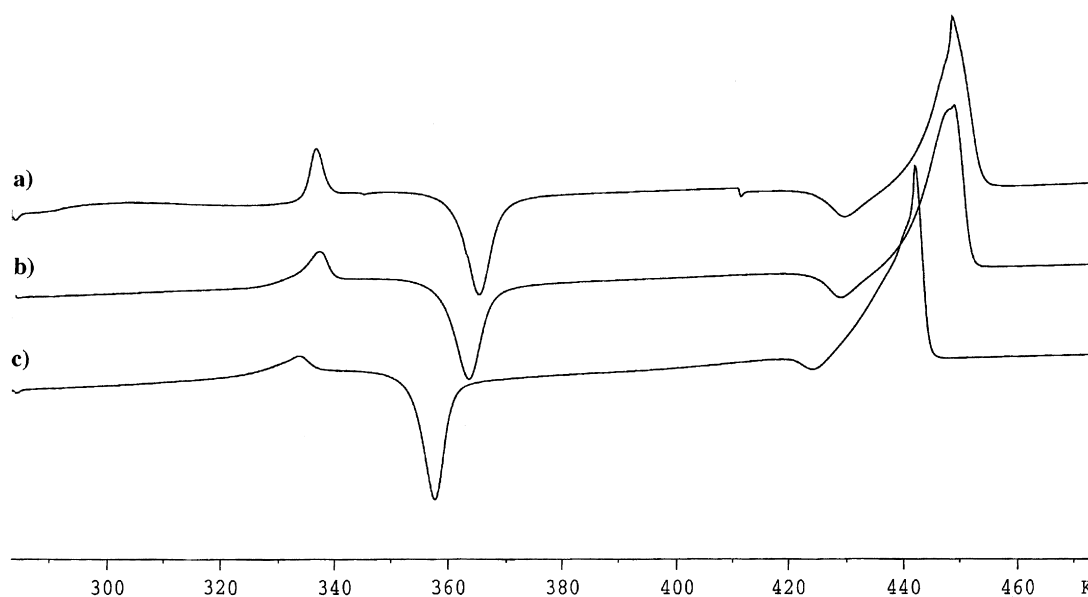

Fig. 7. DSC curves from the first heating for: (a) the unaged sample; (b) after 28 days in abiotic medium; and (c) after 28 days in the biotic medium.

to increase after the initial lactic acid and lactoyl lactic acid had been utilised.

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