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Biodegradation Study on Poly(ε-caprolactone) with Bimodal Molecular Weight Distribution

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ABSTRACT: Poly(ε-caprolactone) (PCL) of bimodal molecular weight distribution was exposed to the action of enzymes-lipases from *Aspergillus oryzae* in phosphate buffer at pH 7 and 37°C, and those produced *in situ* by *Bacillus subtilis* in nutrient medium at 30°C for 42 days. The occurrence of biodegradation is proved on the basis of the weight loss, decrease of molecular weight, carbonyl index, crystallinity, and development of cracks on the PCL surfaces. In the case of *Bacillus subtilis*, the degradation (10 wt % loss of PCL) proceeds faster in comparison with lipase from *Aspergillus oryzae* (2.6 wt % loss of PCL), where the degradation process seems to stop during 14 days of experiment. The gel permeation chromatography results reveal that preferential degradation of lower molecular portion did not occur but it is assumed that PCL chains were cleaved in accordance with particular degradation mechanism that depends significantly on biological agent. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: polycaprolactone; degradation; lipase; *Bacillus subtilis; Aspergillus oryzae*; crystallinity; morphology; bimodal molecular weight distribution

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INTRODUCTION

Poly(ε-caprolactone) (PCL) has recently returned back into the arena of smart biomaterials due to its good processability related to its superior rheological and viscoelastic properties over many other aliphatic polyesters and development of novel manufacturing technologies.[1]

Biodegradability of PCL-based materials represents other important feature, for example, for food packaging applications because PCL can be decomposed by the action of microorganisms and their enzymes even when the monomer - ε-caprolactone is obtained by chemical synthesis from fossil resources.[2,3] In most cases the polymer degradation begins with deposition, adhesion, and colonization of microorganisms on polymer surface followed by the formation of a biofilm.[4] Then, an excretion of enzymes-lipases into surrounding as a consequence of the presence of lipidic carbon source proceeds.

Biodegradation initiated by enzyme action is assumed to consist basically of the enzyme adsorption on polymer substrate, followed by the formation of transition-state complex between the polymer and enzyme, which leads to specific chain scission of polymer chains.[5]

Three basic processes participating in enzyme-catalyzed ester bonds cleavage were suggested including random chain scission irrespective of chain lengths, specific chain-end scission, and/or synergic participation of both processes.[6]

Up to now, the degradation mechanisms of PCL were confirmed and discussed in details in case of controlled thermal and nonisothermal degradation studies.[7,8,9,10]

Additional Supporting Information may be found in the online version of this article.

It was also found out that the degradation mechanism of PCL-based implants *in vivo* could be attributed to random hydrolytic chain scission of ester bonds in the first step and was identical with *in vitro* hydrolysis at 40°C.[11]

According to number of studies in a variety of different environments, biodegradation of PCL was postulated to be the surface erosion process accompanied by only minor decrease in molecular weight (MW).[12] Furthermore, the rate and pattern of biodegradation is governed by certain principal factors including PCL characteristics (crystallinity, weight-average molecular weight (M_w), surface morphology), type of microorganism or enzyme and last but not least by mode of pretreatment of polymer specimen.[13,14,15,16]

The initial M_w together with crystallinity (X_c) of studied polymer materials represent an important factor influencing the rate of degradation and therefore, they can serve as parameters indicating the degree of degradation. It was found out that extracellular enzymes as triacylglycerol hydrolases (lipases), secreted by microorganism due to the presence of PCL as the lipophilic substrate, act as depolymerases and reduce polymer chain length to be small enough to penetrate through cellular membranes. The reduction of MW was shown to be the rate-limiting factor of degradation of many polymers.[17] The higher the initial MW of polymer the lower the weight loss as it was clearly observed during degradation of series of PCLs (number-average molecular weight (M_n) = 10.0, 43.0, 80.0 kDa) by the action of *Pseudomonas* lipase.[18] Moreover, the MW value is believed to affect the penetration of water molecules and/or enzymes into the polymer matrix and, thus, to influence diffusion phenomena as well. The dependence of the degradation rate on the MW distribution was already revealed in 1974 when Fields et al.[19] found out direct relationship between the degradation rate and the content of low molecular species in bimodal PCL samples exposed to *Aureobasidium pullulans*.

It was shown that enzymatic degradation of monomodal PCL depends on its initial crystallinity which can decrease[16,20] or increase[15,16] during the degradation process. But the influence of medium without microorganisms or enzymes on X_c should also be taken into account.[21] The change of crystallinity is connected with the mechanism of biodegradation and is usually accompanied by the change of surface morphology[16,22] and/or molecular weight.[20,21]

The aim of this work was to study the biodegradation of PCL with bimodal molecular weight distribution, because up to now, there are only a few studies evaluating the effect of the MW distribution on subsequent degradation of polymer. Bimodal PCL was exposed to *Bacillus subtilis* (*BS*) in nutritious medium containing 1 % (v/v) olive oil and 2 % (w/v) glucose at 30°C. Simultaneously, bimodal PCL was incubated in the presence of commercially available lipase from *Aspergillus oryzae* (*AO*) in phosphate buffer at pH 7 and 37°C. The degradation experiment with commercial *AO* lipase with particular substrate specifity carried out under defined conditions (37°C, pH 7) served as a model system which helped understanding the trends observed in case of complex PCL/*BS*/medium system.

PCL samples were in a form of disks (1 cm in a diameter, 100 μm of thickness) and the degradation experiment was performed in a period of 42 days. The degraded PCL samples were periodically weighted (14, 28, 42 days) and analyzed by differential scanning calorimetry (DSC), gel permeation chromatography (GPC), Fourier transform infrared spectroscopy (FTIR), and confocal laser scanning microscopy (CLSM).

EXPERIMENTAL

Materials

Polymer Sample. PCLs were synthesized in the Laboratory of Polymer Synthesis, Brno University of Technology.[23] Two runs were simultaneously performed under the same polymerization conditions to have polymer with tailored bimodal distribution and in a high yield for degradation study. The results of GPC and NMR analyses of PCLs samples denoted as *BS*-PCL and *AO*-PCL are as follows:

GPC characteristics of PCLs

Sample[a]	M_n (kDa)	M_w (kDa)	M_w/M_n
BS-PCL	19.4	132.0	6.8
AO-PCL	25.4	165.0	6.5

NMR spectroscopic analysis confirmed the presence of hydroxylic and benzoxylic end groups, ^{1}H NMR (400 MHz, $CDCl_3$, δ) spectrum in $CHCl_3$ was as follows: 1.37 (m, 2H; CH_2), 1.64 (m, 4H; CH_2), 2.29 (t, J = 7.5 Hz, 2H; CH_2CO), 3.64 (t, J = 6.5 Hz, 2H; CH_2OH), 4.05 (t, J = 6.7 Hz, 2H; CH_2OCO), 5.11 (s, 2H; OCH_2Ph).

PCL powder was processed by dissolving in chloroform (2 wt %). The solution was cast into Petri dishes, air-dried for 2 days and vacuum-dried until constant weight to prepare PCL films. The samples suitable for the degradation study were cut out from PCL films (3 cm in diameter) in a form of circular disks of 1 cm in a diameter and of about 100 μm thickness. All specimens were sterilized by UV irradiation for 30 min in an Aura Mini laminar box, BioTech Instruments, Prague, CZ before degradation experiments.

Biological Agent

BS and culture conditions. The bacterial strain *BS* CCM 1999 was obtained from the culture collection of the Czech Collection of Microorganims, Masaryk University Brno, Faculty of Science. Tested culture was maintained on nutrient agar at 30°C for 3 days before the degradation experiment.

AO. The lipase from *AO* (WE-Nr. 916,028) was obtained from Mucos Pharma.

Assay for lipase activity. Lipolytic activity was determined by *p*-nitrophenyl-laurate (pNPL) as a substrate dissolved in ethanol on UV/VIS HELLIOS spectrophotometer (DELTA Thermospectronic, England).

The reaction mixture consisted of 0.25 mL enzyme solution (0.5 mg mL^{-1} of *AO* lipase), 3.25 mL of phosphate buffer (c = 0.050 in water, pH 7) and 0.25 mL of pNPL (c = 0.0025 in ethanol).

In the case of *BS*, 0.25 mL of the cell-free culture supernatant containing expected extracellular enzymes was added into the reaction mixture.

Hydrolytic reaction was carried out at 37°C for 30 min and afterward 0.5 mL of Na_2CO_3 ($c = 0.1$ in water) was added to stop the reaction. Subsequently, the absorbance was recorded at 420 nm. One unit of lipase activity (U) was defined as the amount of enzyme that caused the release of 1 μmol of *p*-nitrophenol from pNPL in one minute under the test conditions.

The pH optimum of lipase activity was measured using pNPL as a substrate by incubating 0.25 mL of enzyme solution (*AO*) or 0.25 mL of the cell-free culture supernatant (*BS*) with following buffers ($c = 0.050$ in water): citrate-phosphate (pH 5–6), phosphate (pH 7), boric acid-borax (pH 8–9), glycine NaOH (pH 10), and Britton-Robinson (pH 11) at 37°C for 30 min. The temperature optimum was determined under constant pH of 7 at 30, 35, 37, 40, 45, and 50°C.

The lipase thermal stability was determined spectrophotometrically with pNPL as a substrate by measurement of residual activity after 1 h incubation at different temperatures (30, 40, 50, 60, and 70°C) in phosphate buffer ($c = 0.050$ in water, pH 7).

The initial activity of *BS* lipase, independent of PCL studied, was 0.0643 U mL^{-1} of enzyme at pH 7 and 30°C.

The initial activity of *AO* lipase, independent of PCL studied, was 0.0343 U mL^{-1} of enzyme at pH 7 and 37°C.

Degradation Experiment

Degradation experiment with AO lipase. PCL disks were inserted into vials containing 3 mL of phosphate buffer solution (pH 7) and 1.5 mg of *AO* lipase and placed in a thermostat at 37°C. The enzymatic solution was renewed every 3 days to restore the original level of enzymatic activity.

The control samples were treated in the same way but their incubation was carried out in phosphate buffer without enzyme and sodium azide 0.02 % (w/w) was added into the solution to prevent contamination.

Degradation experiment with BS. For inoculum preparation, 1 mL of distilled sterile water was added into nutrient agar inoculated with *BS*. The colonies were rubbed carefully with a sterile vaccination loop and transferred into 100 mL of nutritious medium in a sterile Erlenmeyer flask (250 mL). The nutritious medium (100 mL) was prepared from peptone (3.0 g), yeast extract (1.0 g), NaCl (0.5 g), glucose (2.0 g), olive oil (1.0 mL), and distilled water. The medium was sterilized in an autoclave at 121°C and 103.4 kPa for 20 min. Inoculated Erlenmeyer flasks were dynamically cultivated (90 rpm) for 1 h. Then, PCL specimens were added into it. The medium was renewed every 4 days to restore the original level of lipase activity.

The control samples were treated in the same way but their incubation was carried out in the absence of bacterial strain and 0.02 % (w/w) sodium azide was added into the solution to prevent contamination.

Methods and Testing

Weight loss measurement. PCL specimens were withdrawn after 14, 28, and 42 days from the degradation medium, washed gently with distilled water and vacuum-dried at lab temperature for 1 week. Then the samples were weighted and the values obtained were compared with those of original and control samples.

DSC. DSC measurements were carried out in two heating runs on calorimeter TA Instruments Q 2000 in the temperature range from -80°C to 90°C under nitrogen (50 mL min^{-1}). The test schedule used was as follows: (i) cooling from room temperature to 0°C, (ii) heating from 0°C to 100°C at 10°C min^{-1}, (iii) 5 min isothermal at 100°C, (iv) cooling down to -80°C at 5°C min^{-1}, (v) 5 min isothermal at -80°C, and (vi) heating from -80°C to 90°C at 5°C min^{-1}. The melting temperature was evaluated from the endothermic peak maximum and melting enthalpy was used to determine PCL samples crystallinity (X_c) both from the first and the second heating scans.

GPC. GPC was used for measurement of the relative molecular weight (M_n, M_w) and polydispersity index (M_w/M_n) of PCLs using an Agilent Technologies 1100 Series device equipped with refractive index (RI) detector and two PLgel MIXED Columns 300 × 7.5 mm with particle size of 5 μm. Tetrahydrofuran (THF) was used as the mobile phase at a flow rate of 1 mL min^{-1} at room temperature. Polystyrene standards were used for calibration (Mp = 316500-162).

FTIR. The change of chemical structure in terms of ester bond cleavage in PCL films was evaluated by FTIR measurement on spectrometer Thermo Scientific Nicolet iS10 in a simple transmission mode. The transmission spectra were recorded in the spectral range of 4000–400 cm^{-1} at the resolution 6 cm^{-1} and 128 scans.

Preweighed amount of PCL film was dissolved in dichloromethane to obtain 3 % solution which was cast on KBr disk. The traces of dichloromethane were subsequently removed under vacuum to yield films for FTIR analysis.

CLSM. Morphology of PCL sample surfaces was analyzed by confocal laser scanning microscope Olympus LEXT OLS 3000.

RESULTS AND DISCUSSION

Degradation study of PCL with bimodal distribution by the action of bacterial and fungi lipase was performed. The degradation experiments were carried out under conditions of the optimum action of both types of lipases. The production of lipase *BS* was stimulated with olive oil added into the cultivation medium because the extracellular lipase produced by *BS* is suggested to have inducible character.[24] Degradation process was evaluated from the weight loss, GPC, FTIR, DSC, and CLSM measurements. PCL molecular characteristics before and after the degradation test are summarized in Table I.

The weight loss of the samples exposed to *BS* gradually increased up to 10 wt % during 42 days of experiment. Significant reduction of number-average molecular weight (M_n) as well as weight-average M_w was observed. The reduction of M_n by 45 % can be explained by preferred interaction of the active enzyme site with ester groups situated near PCL chain ends followed by repeated selective abstraction of segments with the same size such as monomer, dimer, or trimer.[22] The decrease of M_w by 70 % indicates scission of the longest PCL chains. Moreover, the shape of the elution curves changed during the degradation period. Besides shifting of chromatograms to lower

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Table I. Characteristics of PCL exposed to biological action

Sample[a]	Period (days)	Weight loss (%)	M_n (GPC)[b] (kDa)	M_w (GPC)[b] (kDa)	M_w/M_n
BS-PCL	0	-	19.4	132.0	6.8
BS-14d	14	0.5	12.0	50.0	4.1
BS-28d	28	5.5	11.7	46.7	4.2
BS-42d	42	10	10.6	37.8	3.2
AO-PCL	0	-	25.4	165	6.5
AO-14d	14	2.6	23.5	142	6.2
AO-28d	28	2.6	22.6	143	7.7
AO-42d	42	2.8	19.0	143	7.5

[a]*BS* labels PCL samples exposed to *Bacillus subtilis* while *AO* labels PCL samples exposed to lipase from *Aspergilus oryzae*; the number after the abbreviation denotes aging time in days.
[b]Determined by GPC using polystyren (PS) standards.

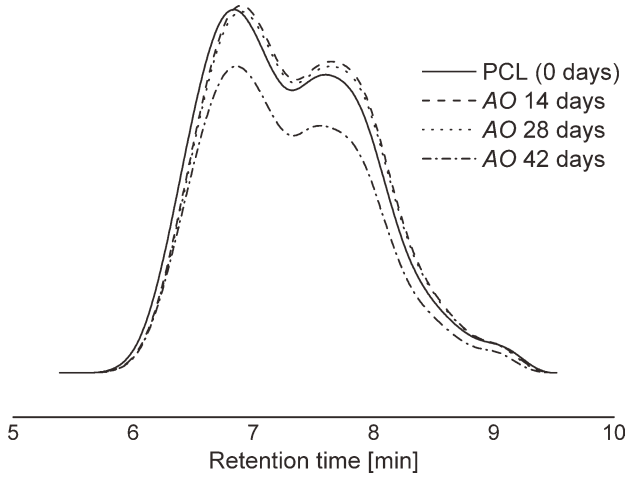

Figure 2. GPC chromatograms of PCLs exposed to *AO* action.

molecular region, the elution curve became almost monomodal with a tail in oligomeric region (Figure 1). The amount of smaller low-mass fraction decreased after 42 days probably as a consequence of the diffusion of low MW species into the degradation medium that corresponds with the weight loss. According to the results, one can suggest the synergic process of the chain-end depolymerization and random scission of ester bonds along PCL chains, irrespective of their lengths.[25,26]

PCL samples exposed to *AO* action in phosphate buffer revealed the decrease in M_n by 25 % and M_w by only 13 % and hence lower degradation rate as compared with *BS* aged samples. Similarly, lower weight loss being 2.6 wt % after 14 days did not gradually increase during further degradation period. The observed low level of enzymatic degradation could be explained on the basis of both, substrate binding to fungal lipases and their substrate specifity.[27]

The *AO* lipase could initially interact with hydroxyl end group of PCL chains through hydrophilic binding sites and with PCL segments representing fatty acid chains through aliphatic site to direct the substrate into the right binding mode for catalysis. Subsequently, the depolymerization from PCL chains ends with hydroxyl groups toward their centers can be assumed due to the decrease of the area under molecular weight peaks (Figure 2). It is also probable that some conformation defects of PCL segments could terminate the unzipping process after 14 days of the experiment because there was no change in tested parameters at that time observed. Moreover, the acting of *AO* lipase at a specific position on ester bonds of PCL chains, as a consequence of 1,3-regiospecifity of *Aspergillus sp.* lipases reported in literature, could be assumed as well.[28]

It should be mentioned that in the case of *BS* the whole enzyme potential of microbial cells is implemented during the degradation process. This fact together with lower initial molecular weight of studied PCL could contribute to the more rapid degradation process in comparison with lipase from *AO*.

In the case of control samples, the weight loss was negligible, as well as changes in M_w (Figure 3) and thus the occurrence of hydrolytic degradation caused by water solvolysis was excluded. Such finding correlates well with the study of Pitt et al. who did not observe the weight loss until approx. 2.5 years of PCL

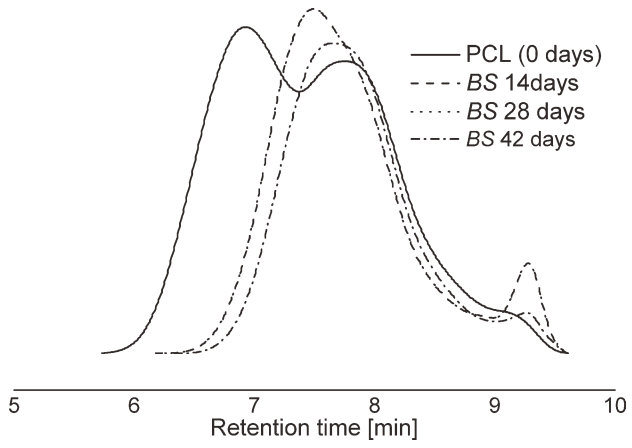

Figure 1. GPC chromatograms of PCLs exposed to *BS* action.

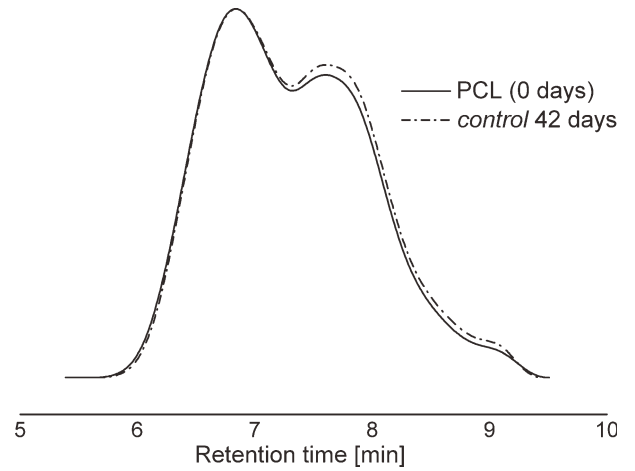

Figure 3. GPC chromatograms of original PCL and PCL immersed in phosphate buffer for 42 days as a control sample (M_n = 23.0 kDa, M_w = 162 kDa, M_w/M_n = 6.9).

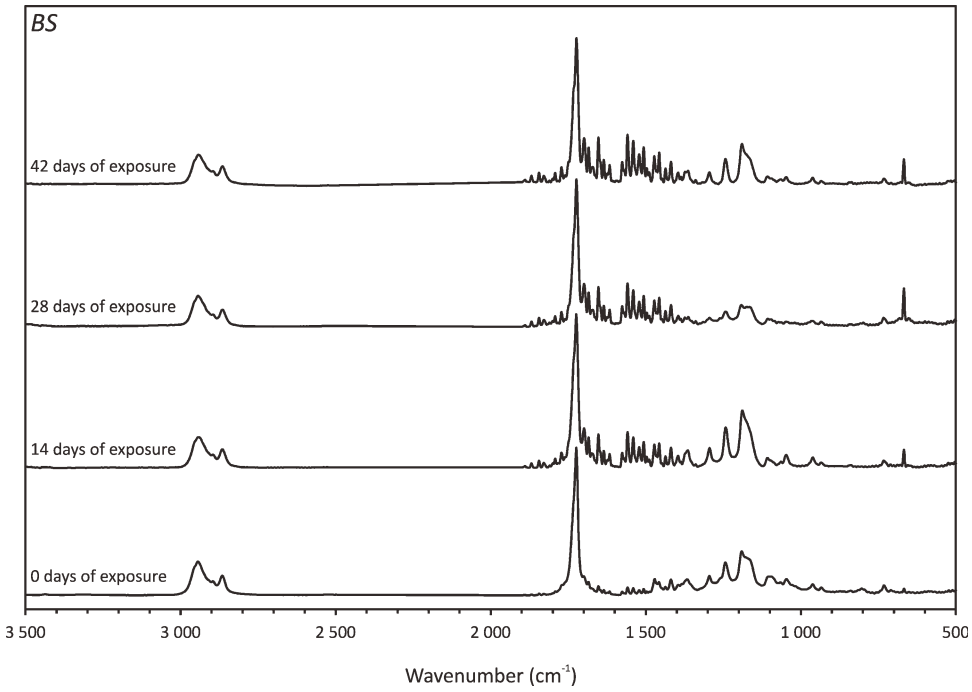

Figure 4. FTIR spectra of PCL films before and after immersion in medium enriched with *BS* in the region of 500–1900 and 2500–3500 cm^{-1} for (a) 0 days, (b) 14 days, (c) 28 days, (d) 42 days.

in vitro degradation test, when the M_w of PCL was reduced to 5000 and thus, PCL oligomers started to diffuse from tested material bulk.[29]

The degradation process through ester bonds cleavage was further confirmed by the decrease of the intensity of the band at 1726 cm^{-1} detected in drop-cast FTIR spectra of incubated PCLs (Figures 4 and 5). The decrease was pronounced in the case of *BS* incubation in accordance with observed higher weight loss. In a similar way, the reduction of carbonyl index calculated as a ratio of 1726/1398 cm^{-1} [15,30] documented different rates of degradation taking place in *AO* and *BS* incubated PCL samples (Table S1, Supporting Information).

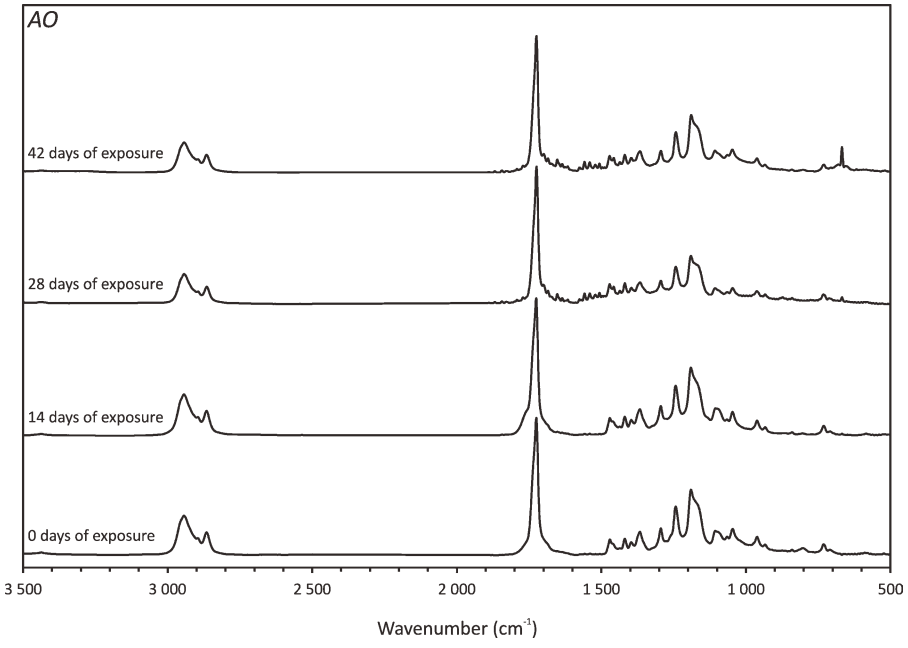

Figure 5. FTIR spectra of PCL films before and after immersion in phosphate buffer enriched with *AO* in the region of 500–1900 and 2500–3500 cm^{-1} for (a) 0 days, (b) 14 days, (c) 28 days, (d) 42 days.

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Table II. DSC characteristics of PCL exposed to biological action

Sample[a]	Period (days)	$^{1st}X_c$ (%)[b]	$^{2nd}X_c$ (%)[b]	$^{1st}T_m$ (°C)[c]	$^{2nd}T_m$ (°C)[c]
PCL	0	64.1	50.7	63.5	55.8
BS-14d	14	60.8	49.0	63.5	54.1, 56.2
BS-28d	28	59.1	48.5	62.7	54.2
BS-42d	42	53.8	41.1	61.6	52.3, 55.5
AO-PCL	0	63.6	52.2	63.5	55.9
AO-14d	14	53.4	44.7	63.6	56.5
AO-42d	42	54.7	42.1	64.7	56.7

[a]*BS* labels PCL samples exposed to *Bacillus subtilis* while *AO* labels PCL samples exposed to lipase from *Aspergilus oryzae*; the number after the abbreviation denotes aging time in days.
[b]X_c labels crystallinity calculated according to the $\Delta H_m/\Delta H_m^0$ ratio where, ΔH_m^0 of PCL is 139.5 J g^{-1}. 1st, 2nd labels the first and second heating, respectively.
[c]T_m represents melting, temperature, 1st, 2nd labels the first and second heating, respectively.

DSC data confirmed the degradation process of bimodal PCL both by *BS* bacteria and *AO* lipase although the degradation mechanism was evidently different.

The structure change of PCL samples exposed to *BS* bacteria was manifested by the gradual decrease of crystallinity (X_c) that achieved 16 % after 42 days and by the slight decrease of melting (T_m) together with crystallization temperature (T_c; Table II). DSC data of control samples were nearly without change in time suggesting no significant impact of PCL conditioning in nutrient medium without bacterial strain on its structure. It can be seen that the first heating endotherms split from one peak to double one during the second heating, whereas no split was observed for control samples (Figures 6 and 7). It is evident that the original peak gradually shifted to lower T_m and only small peak remained on its place after 42 days. It means that crystallites were predominantly

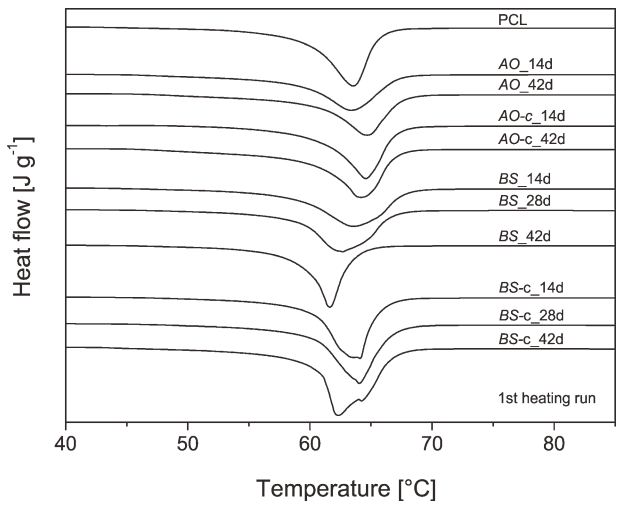

Figure 6. DSC first heating curves of all PCL samples including the control ones (PCL denotes the original polymer, AO_14d denotes PCL exposed to *AO* for 14 days, BS-c_42d denotes control sample for bimodal PCL sample exposed to *BS* for 42 days).

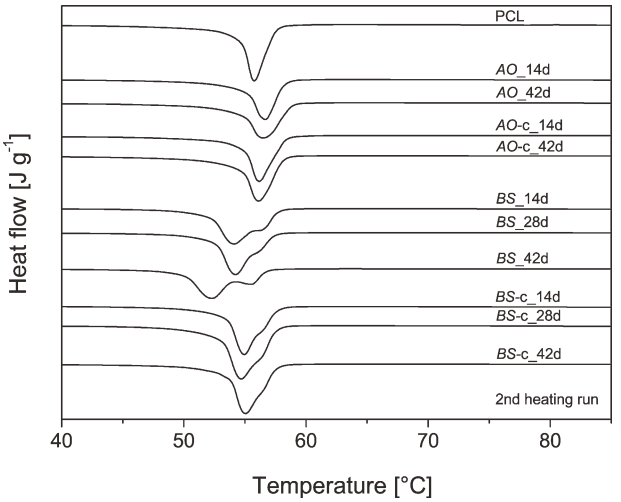

Figure 7. DSC second heating curves of all PCL samples including the control ones (the meaning of DSC curves titles is the same as in Figure 6).

formed by shorter chains produced by scission of the longest ones, as revealed by GPC. Simultaneously, the endotherm shape turning from the narrow one for the original PCL through formation of a shoulder up to double peak reflected gradual bond scission. The scission of PCL bonds was also confirmed by the decrease of the second heating X_c in time with respect to the original PCL, because no decrease of the second heating X_c was measured for control samples. The trend of the second X_c was the same as of the first one (Table II). Thus, the DSC data support the occurrence of enzyme-catalyzed chain cleavage as proved by FTIR measurement.

DSC data of PCL samples exposed to *AO* lipase are summarized in Table II. The samples were measured after 14 and 42 days of experiment. It was shown insignificant change of T_m during 42 days and no change in T_m was observed for control samples. The same situation was observed after the second heating both for degraded and control samples, where no change in endotherm shapes occurred (Figures 6 and 7). These facts imply that the longest PCL chains were not broken and oligomeric species were not formed. However, both the first and the second crystallinity decreased by about 16 % even after 14 days with respect to control PCL and no decrease of X_c was observed for control samples both after the first and the second heating during the same period. Based on the results obtained, the enzyme-catalyzed PCL chain cleavage occurred but under different mechanism compared with the action of *BS* bacteria. The X_c did not change within period 14–42 days which together with the weight loss reflected action of *AO* lipase on PCL substrate only during the first 14 days, even if the *AO*/buffer system was renewed every 72 h. It is supposed that the enzyme-catalyzed cleavage of PCL chains occurred also in crystalline region because both the surface of PCL samples was not amorphous and the original X_c was relatively high (64 %).

The decrease of crystallinity of PCL with monomodal molecular weight distribution exposed to phosphate buffer solution containing lipases was reported by Gan et al. and Sekosan et al.[16,20]

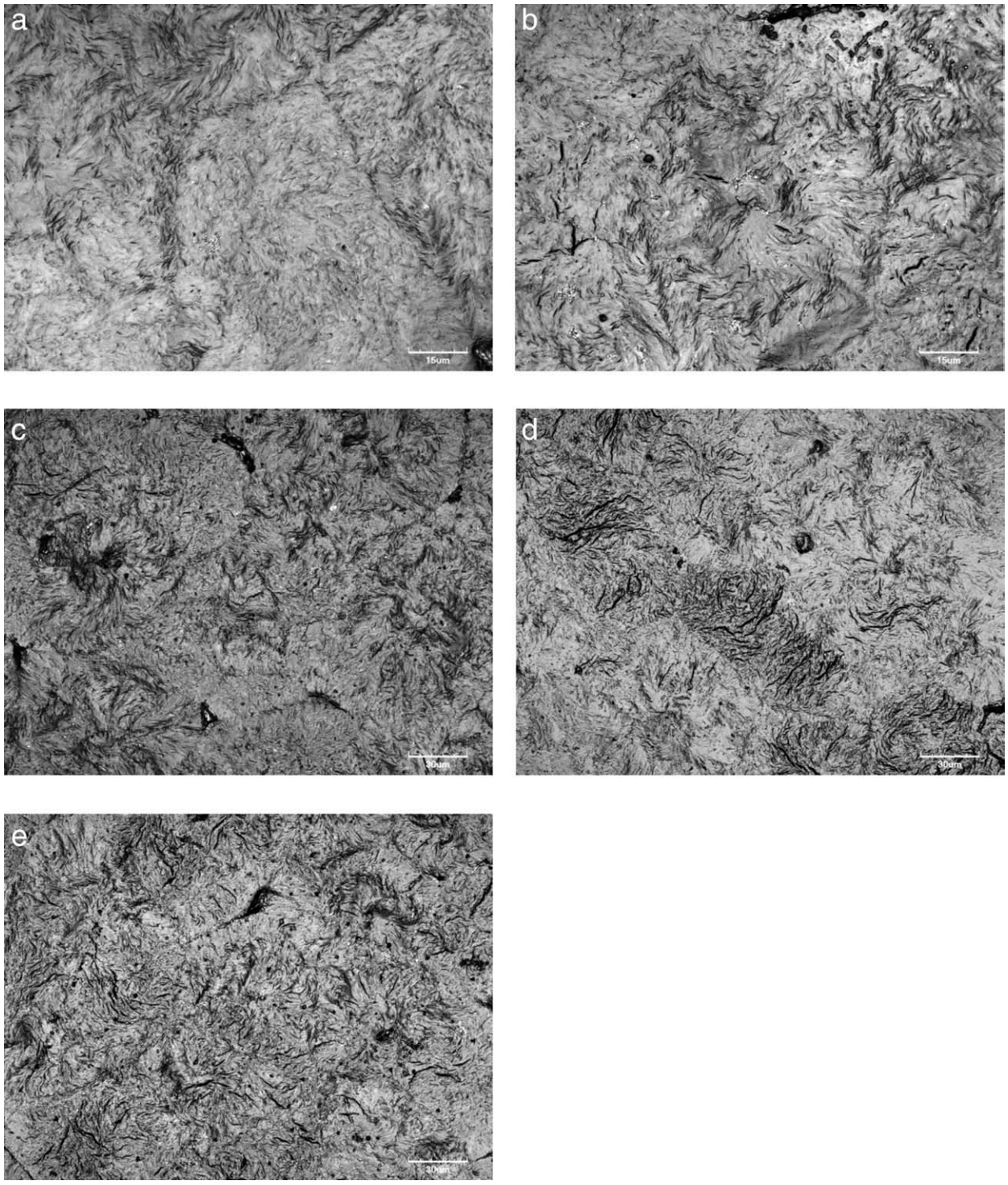

Figure 8. CLSM micrographs of PCL sample surfaces exposed to *BS* (a) original PCL film (scale bar is 15 μm), (b) PCL film aged for 14 days (scale bar is 15 μm), (c) PCL film degraded for 14 days (scale bar is 30 μm), (d) PCL film degraded for 28 days (scale bar is 30 μm), and (e) PCL film degraded for 42 days (scale bar is 30 μm).

Unfortunately, the first did not explain degradation in detail and there are also no data of PCL crystallinity. The latter stated that biodegradation occurred also in crystalline region if X_c of the studied polymer was originally high (more than 45 %). On the other hand, the crystallinity increased during the degradation of PCL with originally low X_c. Yoshioka et al. observed the increase of X_c of monomodal PCL immersed only in phosphate buffer solution by 16 % in 12 weeks without the weight loss and decrease of M_W, although the original X_c of PCL was high (56.5 %).[21] The reason was the fact that amorphous chains were

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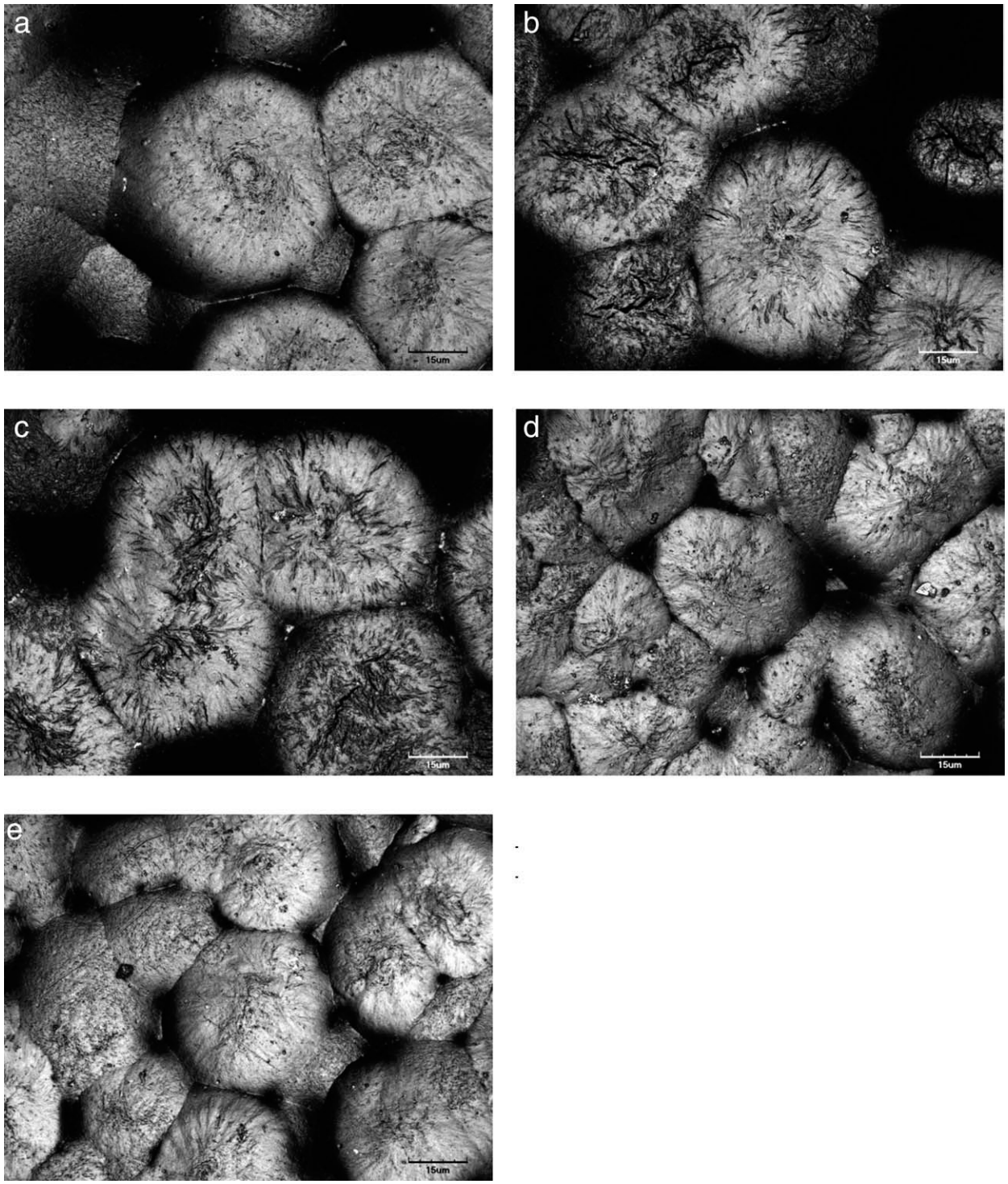

Figure 9. CLSM micrographs of PCL sample surfaces exposed to *AO*. (a) Original PCL film (scale bar is 15 μm), (b) PCL film degraded for 14 days (scale bar is 15 μm), (c) PCL film degraded for 42 days (scale bar is 30 μm), (d) PCL control sample immersed in phosphate buffer for 14 days (scale bar is 30 μm), and (e) PCL control sample immersed in phosphate buffer for 42 days (scale bar is 30 μm).

in rubber-like state under conditions of experiment, and, therefore, able to crystallize.

In this work, PCL amorphous phase was also in rubber-like state but crystallinity of PCL samples degraded by *AO* and *BS* action decreased. It is assumed that the main reason was relatively high initial X_c of the PCL material, which did not increase under given experimental conditions of degradation test. This statement is supported by the fact that in control samples no change of X_c was observed.

It is supposed that microbial biodegradation occurs in amorphous phase. Nevertheless, the ability of microorganism

adhesion on polymer surface and its influence on subsequent biodegradation pattern should be taken into account as well. It is also assumed that the released enzymes will act on available substrate in immediate vicinity. Because of the solution casting, PCL surfaces were formed by spherulites. This means that PCL surface was not amorphous and plain because spherulites have 3D structure, where lamellar units are tilted under different angles. The cracks distributed in certain directions (certain places) were observed on PCL surfaces exposed to *BS* action by CLSM [Figure 8(a–e)]. They could be the consequence of the preferential bacteria adhesion to pits and grooves of 3D surface, where they were protected against friction and shear forces.[31] As the *BS*/degradation medium was renewed every 4 days the compact biofilm was not observed and hence, bacterial adhesion supposed to take place via reversible physicochemical interaction between bacteria and PCL surfaces. Subsequently, the release of depolymerizing enzymes should occur. Based on the results obtained, one can suggest that the enzyme-catalyzed chain cleavage occurred also in crystallites because the polymer crystallinity decreased. However, this statement probably involves the cleavage of bonds in amorphous phase of crystallites.

The cracks were observed on *AO* degraded sample surfaces using CLSM as in previous case reflecting the enzyme action, whereas no cracks were observed on control sample surfaces [Figure 9(a–e)]. Because spherulites covered surfaces of PCL samples the enzyme could attack also crystallites, which corresponds with the decrease of X_c. It is evident that number and size of cracks did not change after 14 days which corresponds well with the observed trends in FTIR spectra, weight loss and molecular weight. It is worth mentioning that both the number and size of cracks were much higher as compared with *BS* exposed samples. The reason could be the synergic action of phosphate buffer solution, where the osmotic inflow of water could accelerate degradation process.

Thus, with no doubt, the biodegradation is a complex process, where both initial characteristics of polymer specimen and its processing, biological agent, degradation medium, and conditions have specific influence.

CONCLUSIONS

PCL of bimodal molecular weight distribution was exposed to the action of enzymes-lipases from *AO* and those produced *in situ* by *BS*. The occurrence of biodegradation was proved on the basis of the weight loss, decrease in molecular weight (M_n, M_w), carbonyl index, and crystallinity together with cracks observed on PCL sample surfaces in comparison with control samples. Random chain scission dominated during the exposition of PCL to *BS* bacteria in nutrient medium. On the contrary, the action of *AO* lipase in phosphate buffer lead to the degradation of PCL chains by the unzipping mechanism. It is assumed that scission of PCL bonds occurred also in amorphous regions of crystallites due to the high crystallinity of the original PCL. This is also supported by crystallinity decrease during degradation and the presence of the cracks observed in spherulites.

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