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Material Behaviour

Degradation behaviour of PCL/PEO/PCL and PCL/PEO block copolymers under controlled hydrolytic, enzymatic and composting conditions

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ABSTRACT

Short-term hydrolytic and enzymatic degradation of poly(ε-caprolactone) (PCL), one series of triblock (PCL/PEO/PCL) and the other of diblock (PCL/PEO) copolymers, with a low content of hydrophilic PEO segments is presented. The effect of the introduction of PEO as the central or lateral segment in the PCL chain on copolymer hydrolysis and biodegradation properties was investigated. FTIR results revealed higher hydrolytic degradation susceptibility of diblock copolymers due to a higher hydrophilicity compared to PCL and triblock copolymers. Enzymatic degradation was tested using cell-free extracts of *Pseudomonas aeruginosa* PAO1, for two weeks by following the weight loss, changes in surface roughness, and changes in carbonyl and crystallinity index. The results confirmed that all samples underwent enzymatic degradation through surface erosion which was accompanied with a decrease in molecular weights. Diblock copolymers showed significantly higher weight loss and decrease in molecular weight in comparison to PCL itself and triblock copolymers. AFM analysis confirmed significant surface erosion and increase in RMS values. In addition, biodegradation of polymer films was tested in compost model system at 37 °C, where an effective degradation of block copolymers was observed.

1. Introduction

Nowadays, biodegradable polymers have become the subject of a great interest in the fields of biomedical and industrial application as well as in environment protection [1–3]. Among them, biodegradable aliphatic polyesters comprise a group of polymeric materials with a wide range of thermal, mechanical and degradation properties which could be easily tailored to suite particular application, either in medicine or for environment protection. Poly(ε-caprolactone) is one of a few polymeric materials that have been developed and commercialized for application in both fields [4]. Biodegradable polymers can easily be degraded by microorganisms widely distributed in various environments. This ability of some microorganisms may be used as a powerful tool for the treatment and recycling of biodegradable wastes [5]. Poly(ε-caprolactone) (PCL) as one of the most investigated synthetic, aliphatic polyester, has attracted much attention due to its excellent properties and processability [6]. Nevertheless, due to a high crystallinity and hydrophobicity of PCL, its potential application, especially in a biomedical field, could be considerably restricted. It is known that the rate of polyesters biodegradation depends on the chemical composition, molecular weight, hydrophilic/hydrophobic balance, as well as the morphology of the sample, e.g. degree of crystallinity, size of spherulites, surface area of the samples, etc. Biodegradability of PCL can be successfully tuned by copolymerization with poly(ethylene oxide) (PEO) which has outstanding physico-chemical and biological properties including hydrophilicity, absence of toxicity, lack of antigenicity and immunogenicity [7].

Hydrolytic degradation of PCL is a bulk erosion process, accompanied with a decrease in molecular weight. The rate of hydrolytic degradation is influenced by numerous factors, such as pH and temperature, water absorption and crystallinity. The hydrolysis of the ester bonds of PCL chains is autocatalyzed by the carboxylic end groups [8,9]. Different *in vitro* degradation profiles and susceptibility towards hydrolysis dependence on macroscopic shape (homogenous discs or porous scaffolds) or crosslinked PCL networks in phosphate buffer solution have been reported [9,10].

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All types of PCL samples showed relatively low weight loss (<5%) during the first 25 weeks. Hydrolytic degradation could be enhanced by using an acidic or basic medium and copolymerization with more hydrophilic macromonomer [11,12].

Faster degradation of polymers can also be achieved in the presence of lipase-type enzymes [13]. One of the most used lipases is *Pseudomonas* lipase because of the high activity and capability to break ester bonds on hydrophobic substrates [14]. The degradation activity of *Pseudomonas* lipase toward diblock and triblock PCL/PEO copolymers revealed surface erosion mechanism of degradation, followed by complete destruction of samples morphology [15]. In addition, there is a report which confirmed that presence of PEO segments both in diblock and triblock copolymers hardly alter their enzymatic degradation [16]. Degradation of PCL *in vivo* proceeds via random hydrolytic scission of ester bonds, at considerably slower rate compared to other aliphatic polyesters due to its hydrophobicity, high crystallinity and a lack of degrading enzymes [17,18].

PCL can be successfully degraded by various microorganisms like bacteria and fungi which secrete appropriate enzymes (extracellular depolymerases) under different conditions of moisture, temperature and oxygen availability [19]. Therefore, some bacterial strains, such as *Lysunibacillus* sp.70038 degraded PCL film for about 9 wt% after 30 days followed by surface erosion and decrease in molecular weight [20]. Thermophilic bacterium *Ralstonia* sp. MRL-TL was identified as a very active against PCL [21]. Although the mechanism of the degradation process by various bacteria and fungi has not still been highlighted, it is known that enzymatic activity helps bacteria to attach to the polymer surface and degrade it into metabolites. In addition, microbial degradation of PCL caused by bacteria *Alcaligenes faecalis* confirmed preferentially degradation of an amorphous, less ordered, region of polymer than the crystalline part and relatively fast decomposition of polymer films [22].

Biodegradability of PCL in compost has previously been examined under different temperatures. It was concluded that PCL did not show biodegradation at room temperature after 300 days while it showed significantly better degradation ability at higher temperatures [23] and was not affected by the sample shape [24]. However, to the best of our knowledge composting of block copolymers PCL/PEO and PCL/PEO/PCL has not been reported so far.

In our previous study, the synthesis and characterization of two series of diblock and triblock PCL copolymers with a small content of hydrophilic, flexible PEO block was reported [25]. It was shown that a low content of PEO segments and its position as central or lateral segment in copolymer chains could be used as a powerful tool for tuning the thermal, morphological and surface properties of block copolymers. In a present study, the hydrolytic and enzymatic degradation of PCL/PEO and PCL/PEO/PCL copolymers are evaluated in order to gain a better insight into the impact of structure as well as the introduction of a small hydrophilic PEO segment in PCL chain on biodegradability. The major goal of this study was to introduce small hydrophilic PEO into the PCL chains as central or lateral segment in order to improve the degradability of polymeric material, but at the same time to maintain good mechanical and thermal properties inherent to PCL. In the enzymatic degradation experiments *Pseudomonas aeruginosa* PAO1 cell-free extracts containing lipases and other hydrolytic enzymes, were used as a degradation medium for the first time. Finally, degradation of block copolymers in model compost, using *P. aeruginosa* PAO1 cells, completed the investigation of their degradation behaviour. A PCL homopolymer was also included in the research for comparative purposes. In addition, biocomposting of PEO modified PCL copolymers might be the main driving force behind a recycling of biodegradable wastes.

2. Experimental part

2.1. Materials

Homopolymer, PCL and two series of block copolymers, PCL/PEO and PCL/PEO/PCL, were synthesized by ring opening polymerization, ROP, of ε-CL monomer in bulk, initiated either by two hydroxyl terminal groups of PEO or by a hydroxyl terminal group of m-PEO in the presence of $Sn(Oct)_2$ as catalyst, following the synthesis procedure already published in our previous paper [25]. Chemical composition, structure (based on NMR and GPC analysis) and some physical properties of PEO modified PCL copolymers are given in Table 1. Chloroform (Lack-Ner, Czech Republic), $Na_2HPO_4 \times 2H_2O$ (Acros Organics, Belgium), KH_2PO_4 (LachNer, Czech Republic), $CaCl_2$ (Fisher Chemicals, UK), $MgSO_4 \times 7H_2O$ (Acros Organics, Belgium) were used as received.

2.1.1. Preparation of polymer films

The PCL and copolymer films were prepared in glass Petri dishes (diameter 10 cm) from a solution obtained by dissolving preweighted polymer in chloroform (6 wt% solution was prepared by dissolving of 1.5 g of polymer sample in 25 mL of chlorofom) and leaving the solvent to evaporate for 2 days in the air. Obtained films were dried in a vacuum oven, at room temperature, cut into the rectangles (10 × 20 mm, thickness 200 μm, mass about 40 mg) for the degradation experiments.

2.2. Hydrolytic degradation experiments

Hydrolytic degradation was carried out in phosphate buffer solution (PBS, pH 7.4, prepared by dissolving 56.8 g $Na_2HPO_4 \times 2H_2O$ and 36.4 g KH_2PO_4 in 1000 mL of distilled water) in order to mimic physiological conditions. Polymer films (in the form of rectangles, dimensions 10 × 20 mm, thickness 200 μm, mass about 40 mg) were introduced into a small vials and 10 mL of PBS was added. The samples were thermostated at 37 °C for 8 weeks. PBS solution was changed every week. For each data point, duplicates were prepared in order to minimize the effect of random errors. After a predetermined period of time, polymer films were taken out from PBS, washed with distilled water and vacuum-dried up to a constant weight.

2.3. Bacterial enzyme degradation experiments

2.3.1. Growth of bacterial culture

Enzymatic degradation of PCL and block copolymers was performed using *P. aeruginosa* PAO1 (ATCC 15692) cell free extract. Strain was grown in MSM medium (Mineral Salts Medium) composed of 9.0 g/L $Na_2HPO_4 \times 12H_2O$ (AppliChem, Germany); 1.5 g/L KH_2PO_4 (Acros Organics, Belgium); 0.2 g/L $MgSO_4 \times 7H_2O$

Table 1
Structure, molecular weight and polydispersity index of PCL/PEO and PCL/PEO/PCL block copolymers.

Sample	Structure PCL_y-PEO_x-PCL_y	*w*(PEO), wt% in copolymer	M_n[a] (g/mol)	M_n[b] (g/mol)	PI[b]
PCL				62970	2.10
PCL/PEO-1	184–23	4.7	20930	30880	1.85
PCL/PEO-2	269–23	3.2	30710	38040	1.70
PCL/PEO-3	381–23	2.5	40450	33330	2.00
PCL/PEO/PCL-1	93-23-93	4.5	22140	34490	1.62
PCL/PEO/PCL-2	123-23-123	3.4	29140	44260	1.60
PCL/PEO/PCL-3	152-23-152	2.8	35670	48170	1.76

[a] Determined by ^{1}H NMR.
[b] Measured by GPC.

(Acros Organics, Belgium); 0.002 g/L $CaCl_2$ (Merck, Germany); 1.0 g/L NH_4Cl (Merck, Germany) and 1 mL salt solution [26], supplemented with casamino acids (0.7%, w/v; Sigma-Aldrich, USA) and olive oil (1%, v/v; Sigma-Aldrich, USA). This culture was used for bacterial enzyme degradation of polymers in liquid medium and under composting conditions.

2.3.2. Preparation of cell free extract for degradation experiments

After 48 h incubation (30 °C, 180 rpm) bacterial culture was centrifuged at 5000 rpm for 10 min (GS-3 rotor, Sorvall Centrifuge, DuPont Instruments, Delaware, USA) and supernatant and pellet were separated. Cell free extract was prepared from pellet using BugBuster Protein Extraction Reagent according to the manufacturer's instructions (Novagen, Wisconsin, USA). Total protein concentration in the supernatant and cell free extract was determined using colouring reagent CBB G-250 (BioRad Protein Assay, BioRad Laboratories, USA) according to Bradford method [27]. In order to determine lipase activity of *P. aeruginosa* PAO1 cell free extract, quantitative assay was carried out using *p*-nitrophenylpalmitate (Sigma-Aldrich, USA) [28].

2.3.3. Enzymatic degradation in liquid medium

Experiments of bacterial enzyme degradation were performed in 20 mM phosphate buffer pH 7.4 (5 mL), with added PCL and diblock and triblock copolymer samples and *P. aeruginosa* PAO1 cell free extract (1.8–2 mg of total protein/mL). Polymer samples (polymer films cut into a rectangles, dimensions of 10 × 20 mm, thickness 200 μm, mass about 40 mg) were incubated at 37 °C with shaking at 180 rpm over four weeks. Polymer films were sterilized with ethanol (70%, v/v) and air-dried before addition to reaction medium. Cell free extracts were added in regular periods (1 mL of a known protein concentration per period): two times per week. At the end of degradation experiments polymer samples were gently wiped with cotton wool and ethanol (70%, v/v). Experiments of enzymatic degradation were done in triplicates.

2.4. Biodegradation of PCL and copolymers under composting conditions

Biodegradation test in compost model system under constant ambiental temperature of 37 °C was carried out with PCL and copolymer films using a commercial mixture of raw materials used for cultivation of white button mushroom [29]. The appearance of the biodegradable polymers before and after the composting test was compared by taking photos. *P. aeruginosa* PAO1 culture used for composting was grown as described in "Growth of bacterial culture", to the exponential phase (10–12 h). The quantity of bacterial cells was calculated to achieve $\log_{10}4$ cells per gram of compost. After addition of bacterial culture, compost was thoroughly homogenized using a sterile spatula. Experiment was set up in glass Petri dish (120 mm diameter, 30 mm height) and 100 g of compost inoculated with *P. aeruginosa* PAO1 was placed into a Petri dish. Polymers were placed inside the compost at a depth of 1 cm. Petri dish was incubated at 37 °C over four weeks. Fresh aliquot of *P. aeruginosa* PAO1 culture was added at the end of week 2 (10 mL), which ensured a constant level of bacterial activity and moisture.

2.5. ATR-infrared spectroscopy (ATR-FTIR) measurements of degraded polymer films

FTIR spectra of the degraded polymer films were recorded using a SHIMADZU (Japan) IR-Affinity spectrophotometer. The number of scans was fixed to 100 with a resolution of 4 cm^{-1} at room temperature. All the scans were carried out within the same predefined range (4000–400 cm^{-1}). Data obtained from these spectra served for estimation of carbonyl index and crystallinity index of degraded samples.

2.6. Gel permeation chromatography (GPC)

The molecular weights of polymer samples before and after the degradation were determined by GPC analysis. The GPC measurements were performed on a Waters 600E apparatus equipped with a Supelco PL-Gel columns and a differential refractometer as a detector. Calibration was performed with poly(styrene) standards. Sample concentration of 1.0 wt% in chloroform solution, injection volume of 200 μL, a flow rate of 1.0 mL/min at 30 °C were used.

2.7. Optical microscopy (OM)

To assess the bacterial degradation rate of PCL and copolymer samples, an optical microscope (Leica DM ILM) with reflected light equipped with a CCD digital camera (magnification of 100 times) was used.

2.8. Atomic force microscopy (AFM) analysis

In order to investigate the surface morphology of the block copolymers and PCL, AFM images of bacterially degraded and non-degraded samples were obtained by atomic force microscopy (AFM) using NanoScope III A (Veeco Digital Instruments, USA) device. Tapping mode AFM images were recorded at room temperature using etched silicon cantilevers with a force constant 60 N/m. Image analysis was done by Nanoscope image processing software. Spherulite size and RMS surface roughness (Rq) were determined on the surface area of 20 × 20 μm^2.

2.9. Water absorption

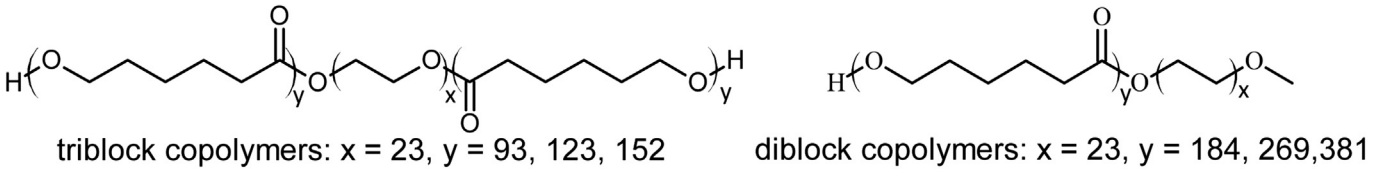
Water absorption of PCL and copolymer films was determined by immersion in phosphate buffer solution (pH 7.4) at 37 °C for 72 h. The water absorption values were averaged on three samples.

2.10. Water contact angle (WCA)

Water contact angle measurements on polymer films were performed on a Krüss DSA100 by using the sessile drop method. A single drop of distilled water with a volume of 20 μL was deposited on the film surface (10 × 20 mm, thickness 200 μm) and the contact angles were measured after 20 s. All measurements were performed in air at temperature of 25 °C. All reported WCA values were an average of five measurements.

3. Results and discussion

Synthesis, detailed structure and properties of PCL and block copolymers were previously described [25]. Diblock (PCL/PEO) and triblock (PCL/PEO/PCL) copolymers' structure with specified block lengths is shown in Scheme 1. While the PEO block length was fixed, the lengths of PCL blocks were varied by changing the molar ratio of CL/EO in the range from 8 to 15. Furthermore, prepolymer, PEO (M_n 1000 g/mol) was incorporated as central, hydrophilic block of PCL/PEO/PCL copolymers, while lateral, hydrophilic segment in diblock PCL/PEO copolymers was m-PEO (M_n 1020 g/mol). Molecular weights of hydrophobic PCL blocks were in the range from 10000 to 40000 g/mol, while the content of flexible hydrophilic PEO segment ranged from 2 to 5 wt%.



Scheme 1. Structure of triblock and diblock copolymers with the specified degree of polymerization for each block.

3.1. Hydrolytic degradation

The hydrolytic degradation of aliphatic polyesters is a very complex process, which involves diffusion phenomena and chemical changes, such as water absorption, ester bond cleavage, diffusion and solubilization of products. Homopolymer PCL, is relatively stable against abiotic hydrolysis, which proceeds by a reduction in molecular weight combined with minor weight loss [30]. Water absorption and water contact angle (WCA) measurements revealed that the hydrophilicity of PCL/PEO and PCL/PEO/PCL block copolymers was slightly improved in comparison to PCL homopolymer (Tables 2 and 3). In addition, WCA values of block copolymers pointed that the hydrophilic PEO block could not demonstrate its properties in such a small content due to synergistic effect of surface morphology and copolymer composition. Furthermore, it could be concluded that the chemical composition of block copolymers, the surface morphology and the roughness of polymer films, which are dictated by polymer film preparation method, have significant impact on wettability and hydrophilicity of the copolymer surface [31].

The hydrolytic degradation of block copolymers and PCL was followed by measurements of weight loss changes after different degradation times over the 8 weeks. Weight loss profiles of diblock copolymers showed significant changes after 4 weeks for different samples. In the first 3 weeks of hydrolytic degradation, only about 2 wt% was lost in the case of all diblock copolymer samples. PCL/PEO-1 sample as the most hydrophilic in the series of diblock copolymers showed greater weight loss after 6 and 7 weeks (in the range of 5–11 wt%), while, diblock copolymers with smaller content of PEO segment (PCL/PEO-2 and PCL/PEO-3) degraded in a lower percentage (5–7 wt%) after 7 weeks (Fig. 1a). Due to a high hydrophobicity, triblock copolymer samples hardly lost a few percentages of their weight. PCL/PEO/PCL-1 sample exhibited a higher weight loss (1–2 wt%) in hydrolytic degradation tests in comparison to other two samples of triblock copolymers (Fig. 1b). When diblock and triblock copolymers are compared, the influence of the position of the flexible PEO segment on hydrolytic degradation is clearly visible. If the PEO block is presented as lateral segment, block copolymer samples degraded more and when the PEO segment is an internal segment, degradation in aqueous media is hardly detectable. The obtained results are not unexpected compared to some literature data where PCL and PCL/PEO block copolymers with longer PEO segment (5000 g/mol) showed a weight loss of about 10 wt%, after 110 weeks [12]. The hydrolytic degradation mechanism of aliphatic polyesters occurs in three phases, which include an incubation period (water uptake), an induction period (molecular weight decrease) and a polymer erosion period (sample weight loss), as mentioned in the literature [32]. Induction period proceeds via random chain scission of the ester bonds in PCL blocks, which preferentially occurred in the amorphous regions, due to a better exposure of ester groups to attack from water molecules. The weight loss of PEO/PCL diblock copolymer samples could be a consequence of a leaching of soluble PEO or PEO-rich polymer chain fragments into the degradation medium

Table 2
Water uptake, weight loss and FTIR results (carbonyl index; CI, crystallinity index) after 7 and 8 weeks of hydrolytically degraded polymer samples.

Sample	Water uptake 72 h (%)	Weight loss, 7 weeks (%)	Weight loss, 8 weeks (%)	CI (7/8 weeks)[a,b]	Crystallinity index[c] (%)
PCL	2.3	0.12 ± 0.2	0.5 ± 0.7	4.2/4.1 (3.9)	58/55 (58)
PCL/PEO-1	4.1	10.9 ± 0.4	6.3 ± 3.0	3.9/3.4 (4.0)	62/64 (60)
PCL/PEO-2	2.9	5.1 ± 2.0	4.4 ± 2.3	3.9/3.6 (4.1)	62/63 (57)
PCL/PEO-3	3.2	7.0 ± 1.0	2.4 ± 0.1	3.9/3.9 (3.9)	61/62 (58)
PCL/PEO/PCL-1	3.8	0.84 ± 0.04	0.87 ± 1.2	4.3/4.0 (4.2)	57/57 (57)
PCL/PEO/PCL-2	2.1	0 ± 0	0.8 ± 0.8	4.2/4.2 (4.0)	57/57 (60)
PCL/PEO/PCL-3	3.3	0.6 ± 0.8	0.6 ± 0.1	4.3/4.2 (4.0)	56/56 (58)

[a] From the ratio of absorbance peaks at 1720 and 1398 cm^{-1}.
[b] In parenthesis the values of control sample are given.
[c] From the ratio absorbance peaks at 1294 and 1167 cm^{-1}.

Table 3
Analysis of enzymatically degraded block copolymer samples: weight loss, WCA and AFM analysis (RMS, diameter) after two weeks of degradation.

Sample	Weight loss (%)	*WCA* glass side (°)	RMS (nm) Glass side[a]	RMS (nm) air side[a]	Diameter (μm)
PCL	4.3	88.6 ± 0.5	130 (100)	82 (70)	80
PCL/PEO-1	11.8	83.2 ± 3.5	158 (120)	175 (166)	100
PCL/PEO-2	5.8	84.7 ± 1.8	200 (113)	189 (160)	100–120
PCL/PEO-3	6.4	84.8 ± 0.4	130 (85)	102 (60)	>200
PCL/PEO/PCL-1	6.2	83.1 ± 0.5	200 (87)	114 (51)	130
PCL/PEO/PCL-2	0.7	84.2 ± 1.6	74 (65)	70 (60)	160
PCL/PEO/PCL-3	0.5	85.5 ± 0.4	80 (77)	88 (73)	130

[a] In parenthesis the values of control sample are given.

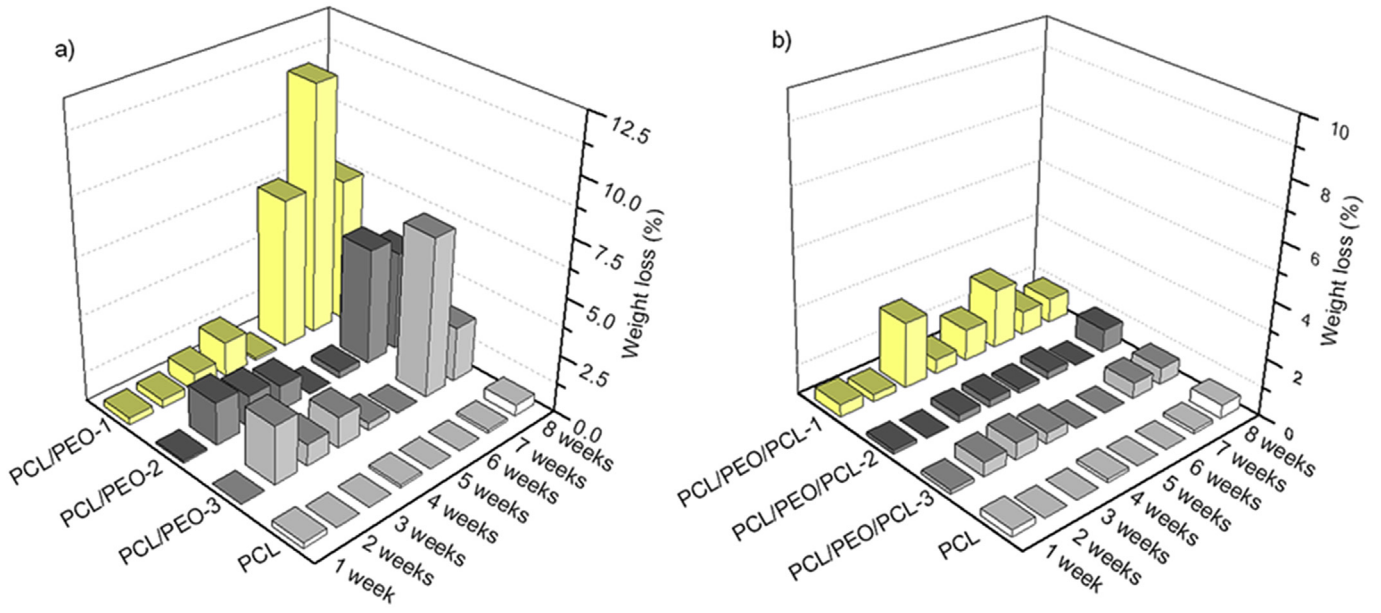


Fig. 1. Weight loss of a) diblock PCL/PEO and b) triblock PCL/PEO/PCL copolymers during hydrolytic degradation in phosphate buffer solution (pH 7.4) up to 8 weeks.

[33].

Two representative FTIR spectra of block copolymers and PCL homopolymer, after 8 weeks of hydrolysis are presented in Fig. 2. The PEO ether band at 1100 cm^{-1} could not be detected due to the small content of PEO segment, while the PCL ester band at 1720 cm^{-1} is apparent. Strong bands coming from the carbonyl stretching around 1720 cm^{-1} and symmetric and asymmetric CH_2 stretching mode around 2943 cm^{-1} and 2864 cm^{-1} can be clearly identified in presented spectra. An absorbance peak at 1294 cm^{-1} is assigned to the C-C and C-O stretching in the crystalline phase and the band at 1164 cm^{-1} is assigned to the C-C and C-O stretching modes in the amorphous phase of PCL. Band at 1239 cm^{-1} is also identified and coming from the asymmetric -COC stretching mode.

Carbonyl index (CI) was calculated from the intensity ratio of the absorbance peak of carbonyl at 1720 cm^{-1} to that of CH_2 at 1398 cm^{-1} [22]. The intensity ratio of absorbance peaks of bands at 1294 and 1167 cm^{-1} were used for calculation of the crystallinity index.

Changes in crystallinity index and carbonyl indexes (CI) of polymer samples after 7 and 8 weeks of degradation in phosphate buffer were monitored by ATR-FTIR analysis (Table 2). The values of carbonyl index from FTIR spectra for PCL and block copolymer samples with small weight loss are almost the same in comparison to control samples. The values of carbonyl index for those samples

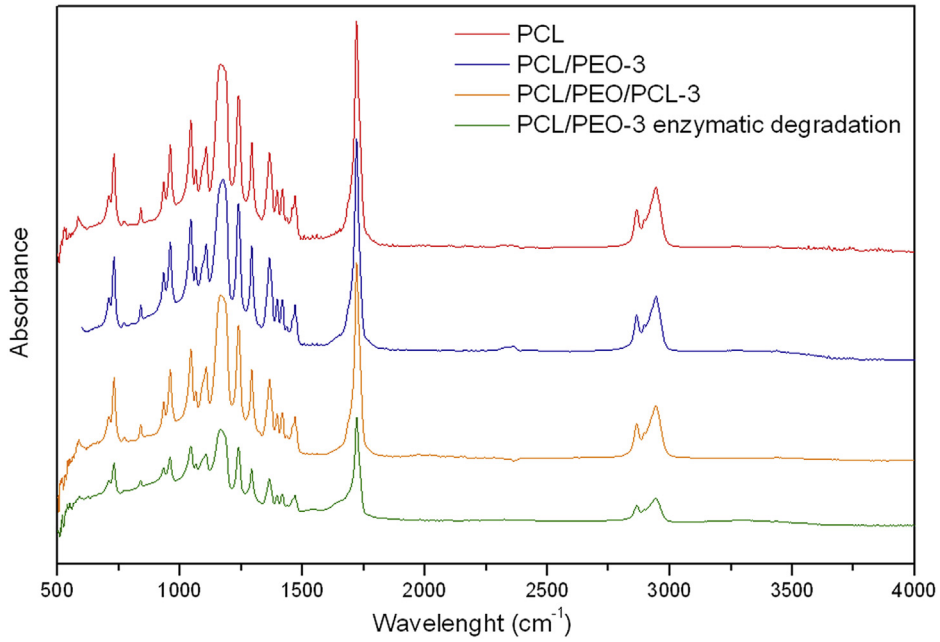


Fig. 2. Representative FTIR spectra of neat homopolymer PCL, diblock and triblock copolymer samples (after 8 weeks of hydrolytic degradation) and PCL/PEO-3 after 2 weeks of enzymatic degradation.

were in the range of 3.9–4.3 which was similar to those before degradation tests, indicating there were no changes in polymer molecular structure. It was further confirmation of small susceptibility to hydrolytic degradation of PCL and those block copolymers with small weight loss under physiological conditions during 7 and 8 weeks. According to the intensity of absorbance peak (at 1398 cm^{-1}), diblock copolymers possessed smaller CI compared to triblock copolymers and homopolymer. Crystallinity index in triblock copolymer series and PCL film slightly decreased during the degradation process while in diblock copolymer series crystallinity index increased. The values of crystallinity index provided additional proof of different degradation behaviour of diblock and triblock copolymers caused by the position of PEO segment and their ability to crystallize during degradation period [34]. Crystallinity index of control samples (that were not exposed to degradation) was very similar for all samples and in the range of 58–60%. Due to a small content of the PEO block as an internal segment, triblock copolymer showed very similar behaviour as homopolymer PCL in degradation media. In the series of PCL/PEO diblock copolymers, slight increase in crystallinity index is observed as a consequence of preferential degradation of the amorphous phase.

3.2. Enzymatic degradation measurements

Enzyme degradation experiments for diblock and triblock copolymers and PCL homopolymer were carried out in PBS (pH 7.4) containing cell-free extract of *P. aeruginosa* PAO1 for the first time. The experiments were performed at 37 °C over four weeks.

Control samples without added enzyme solution showed a negligible weight loss, thus excluding the contribution of hydrolytic degradation or solubilization of degraded species, after two and four weeks. The weight loss of polymer films was more significant compared to the hydrolytic degradation showing that degradation process occurred as a consequence of enzyme action. Given that PCL homopolymer, is known for a poor biodegradation ability [22,35] the measured weight loss of about 3 wt%, after two weeks was in an agreement with previous reports. Differences in degradation ability between diblock and triblock copolymers are shown in Fig. 3. The percentage of weight loss was affected by the position of hydrophilic PEO block, but also by the composition and length of PCL segments in polymer chains. Therefore, diblock copolymer rich in PEO (PCL/PEO-1) showed significant weight loss (about 12 wt%) after two weeks and complete disintegration after 4 weeks, as a result of higher hydrophilicilty and the lowest molecular weight in the series. Weight loss of PCL/PEO-2 and PCL/PEO-3 (about 6 wt% after 2 weeks, and 65 and 36.6 wt% after 4 weeks, respectively) indicated that with the increase of molecular weight of PCL segment, the extent of enzymatic degradation decreased. The same trend like for the diblock copolymer samples could be noticed for the enzyme degradation of triblock copolymer series. The sample with the highest content of PEO segment in triblock series lost about 3–6 wt% but the degradation process hardly happened to the other samples. In addition, there is a significant difference in degradation behaviour of PCL/PEO and PCL/PEO/PCL copolymers. Our experiments indicated better degradation ability of diblock copolymers compared to triblock copolymers, although in some literature data the opposite results can be found [15]. In general, despite the small content of PEO segment in copolymers, considerable difference was noticed.

The relative number average molecular weights $M_n/M_{n,o}$ and weight average molecular weights $M_w/M_{w,o}$ were determined for both degraded (2 and 4 weeks) and non-degraded polymer samples (Fig. 4). A decrease of the average molecular weights expressed as relative to the starting ones: $M_n/M_{n,o}$ and $M_w/M_{w,o}$ for all copolyesters as a function of time is observed. As expected, the least affected in terms of molecular weight change was PCL. After four weeks of degradation, triblock copolymer sample PCL/PEO/PCL-1 exhibited a significant decrease in both M_n i M_w by 25.1% and 15.4%, respectively. The number average molecular weight of the samples PCL/PEO/PCL-2 and PCL/PEO/PCL-3 decreased 19% and 15.5%, respectively, while M_w remained unchanged. On the other hand, diblock copolymer sample PCL/PEO-1 showed a considerable decrease in molecular weight, M_n by 34.5% and M_w by 21.5% with an increase in the polydispersity index from 1.67 to 1.97, after two weeks of enzymatic degradation. After 4 weeks of degradation the PCL/PEO-1 (the copolyester which was fragmented and this further promoted its degradation) exhibited the highest decrease in M_n (66%) and M_w (42%). The slightly higher decreases in M_n are

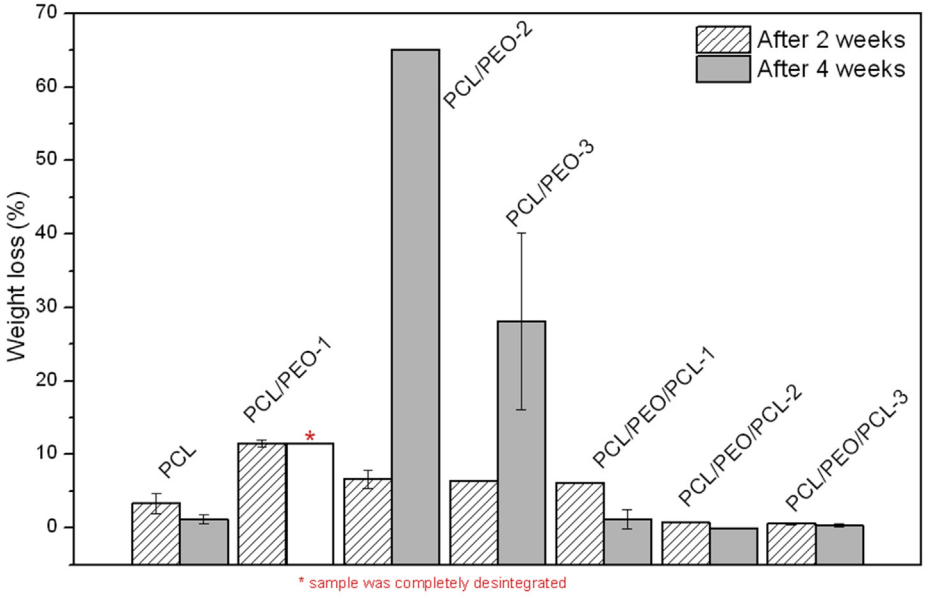


Fig. 3. Weight loss of PCL, diblock and triblock copolymers during two and four weeks of enzyme degradation at 37 °C in a phosphate buffer solution (pH 7.4).

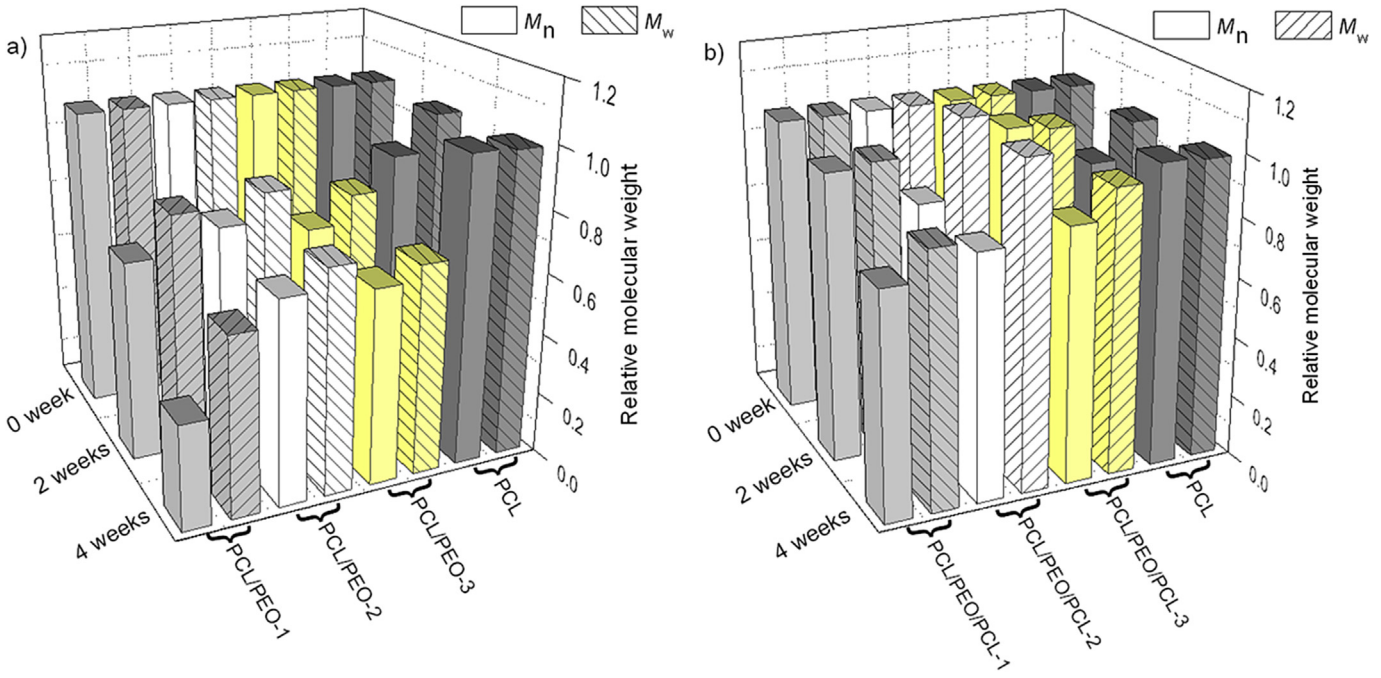


Fig. 4. Changes in molar masses of block copolymer samples after two and four weeks of enzymatic degradation.

associated with the increase of the fraction of lower molecular weight chains compared to the original sample [36,37]. This is in agreement with a fact that erosion degradation of PCL took place in the vicinity of polymer chain ends [37] and that hydroxyl-end groups catalyzed hydrolysis. The hydrolysis step of aliphatic polyesters could occur either as a random reaction or by end-initiated unzipping reaction. It could be considered that the surface degradation is the main degradation mechanism of PCL based copolymers, which is in accordance with optical, AFM and FTIR analysis. The presence of hydrophilic PEO segments could increase the hydrophilicity of the polymer matrix, thus accelerating polyester hydrolysis in enzyme medium, which is in accordance with the decrease of molecular weight with respect to neat polymer.

Differences in PI values were detected for PCL/PEO-2 and PCL/PEO-3 samples and showed broader molecular weight distribution after degradation as a result of the larger decrease in M_n in comparison to M_w. Better degradation ability of diblock copolymers could be attributed to lower molecular weight and lateral position of the PEO block.

Changes in crystallinity index and carbonyl index after enzymatic degradation of homopolymer and diblock copolymer samples was also identified using FTIR analysis. Representative FTIR spectra of non-degraded and degraded PCL/PEO-3 samples are shown in Fig. 2. Samples subjected to enzyme degradation showed completely different intensity of characteristic bands in the FTIR spectra, which further affected the values of CI and crystallinity index [38]. As the intensity ratio of bands used for calculations decreased, the CI and crystallinity index decreased and increased, respectively (Fig. 5). For the sample with the highest weight loss in series of diblock copolymers, PCL/PEO-1 (11.8 wt%), changes in

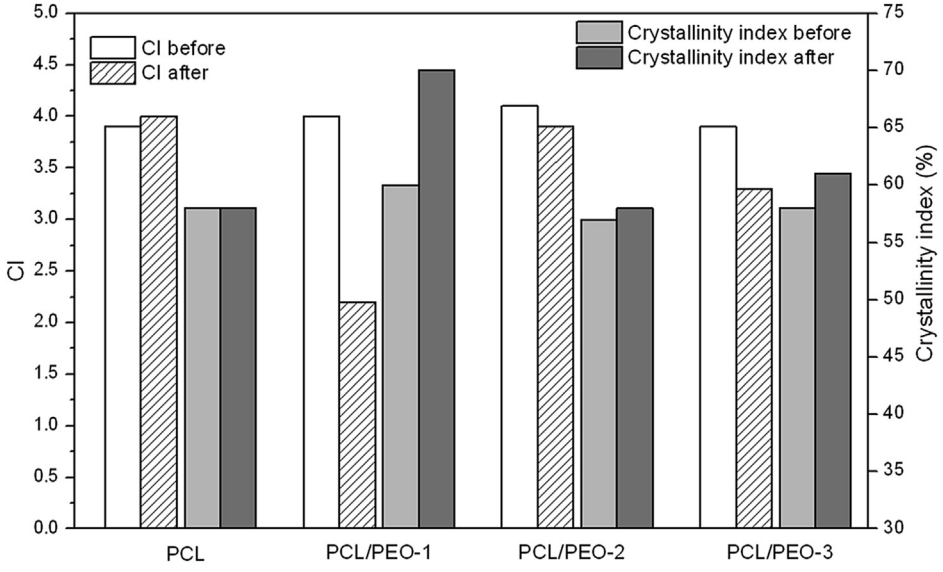


Fig. 5. Carbonyl index, CI, and crystallinity index change of diblock copolymer samples after two weeks of enzymatic degradation.

carbonyl index were the most significant when compared to other samples and decreased from 4.0 to 2.2. Also, a significant change in the crystallinity index (from 60 to 70%) was detected for this sample. The crystallinity index increase was expected, as it was proven in the number of studies that the degradation process starts from the amorphous parts of polymers, so after degradation crystalline part is what mostly remains. In addition, a smaller change in CI and crystallinity index for samples with lower weight loss could be observed. In comparison to PCL sample which hardly showed any changes in CI and crystallinity index, FTIR analysis confirmed that, together with the weight change of copolymer samples, structural changes also took place and were more pronounced for those one with higher weight loss.

Optical microscopy is a good method for visualization of enzymatically degraded polymer samples when enzymatic degradation proceeds via surface erosion mechanism [10,38]. The investigated polymer samples, block copolymers and PCL, crystallized in the form of spherulites, with a difference in spherulites' size. The crystallization process started from the center (nucleation sites) and proceeded with a radial lamellar growth in order to form spherulites. As PEO with a molecular weight of about 1000 g/mol is not able to crystallize, the crystallization of synthesized block copolymer samples originates from PCL blocks. Differences in a spherulite diameter between PCL and diblock and triblock copolymers could be explained by the contribution of the PEO segment, but PCL segment length also affected samples morphology. The significant difference was observed between two sides depending on their exposure to air or to the glass (Petri dish) which has been explained in our previous study [25].

Changes in surface morphology of polymer films (PCL and PCL/PEO-1 and PCL/PEO/PCL-1) after two weeks of enzymatic degradation are presented in Fig. 6. Although polymer films exhibited different surface morphology at the different faces, both air and glass faces underwent enzymatic degradation equally, which is in agreement with already reported results [14,16]. It is important to notice that if the weight loss of samples was above 5 wt%, changes in surface morphology could be visible by optical microscopy. Furthermore, in the case of those strongly degraded samples, surface of polymer films also exhibited some holes and cavities.

In the series of triblock copolymers, only for the most hydrophilic sample (PCL/PEO/PCL-1, weight lost about 6 wt%) significant surface erosion was recorded. For other two triblock copolymer samples, PCL/PEO/PCL-2 and PCL/PEO/PCL-3, slight erosion was detected. It was also observed that before degradation, spherulites had clearly distinguishable boundaries and after degradation those boundaries were less visible. For the most degraded sample, after the exposure to enzymatic degradation, the majority of spherulites could not be clearly observed any more.

The surface morphology of diblock copolymer films that showed better degradation capabilities was completely destroyed and spherulite morphology could be hardly detected, which is in agreement with the weight loss profiles (Table 3). Diblock copolymer surface was strongly eroded and the boundaries between the spherulites disappeared. However, for the sample PCL/PEO-3, more or less degraded spherulites were still present indicating the smaller degradation extent in comparison to the other diblock copolymer samples. It can be assumed that the amorphous parts of the samples are degraded first and remaining crystalline spherulites are still visible.

Surface erosion observed by optical microscopy after enzymatic degradation was further quantified by AFM analysis thought the assessment of changes in surface roughness (Table 3.). It was found that PCL and block copolymer films showed different surface morphology after degradation (Fig. 7 and Fig. 8). Before degradation, perfect spherulites' lamellar structure was clearly visible. The surface roughness of PCL and block copolymers was determined by the diameter of the spherulites, therefore the larger spherulites contributed to the smoother surface. Sample PCL/PEO-3, which had the biggest spherulites diameter (~200 μm), possessed small RMS value before degradation (85 nm) compared to other diblock

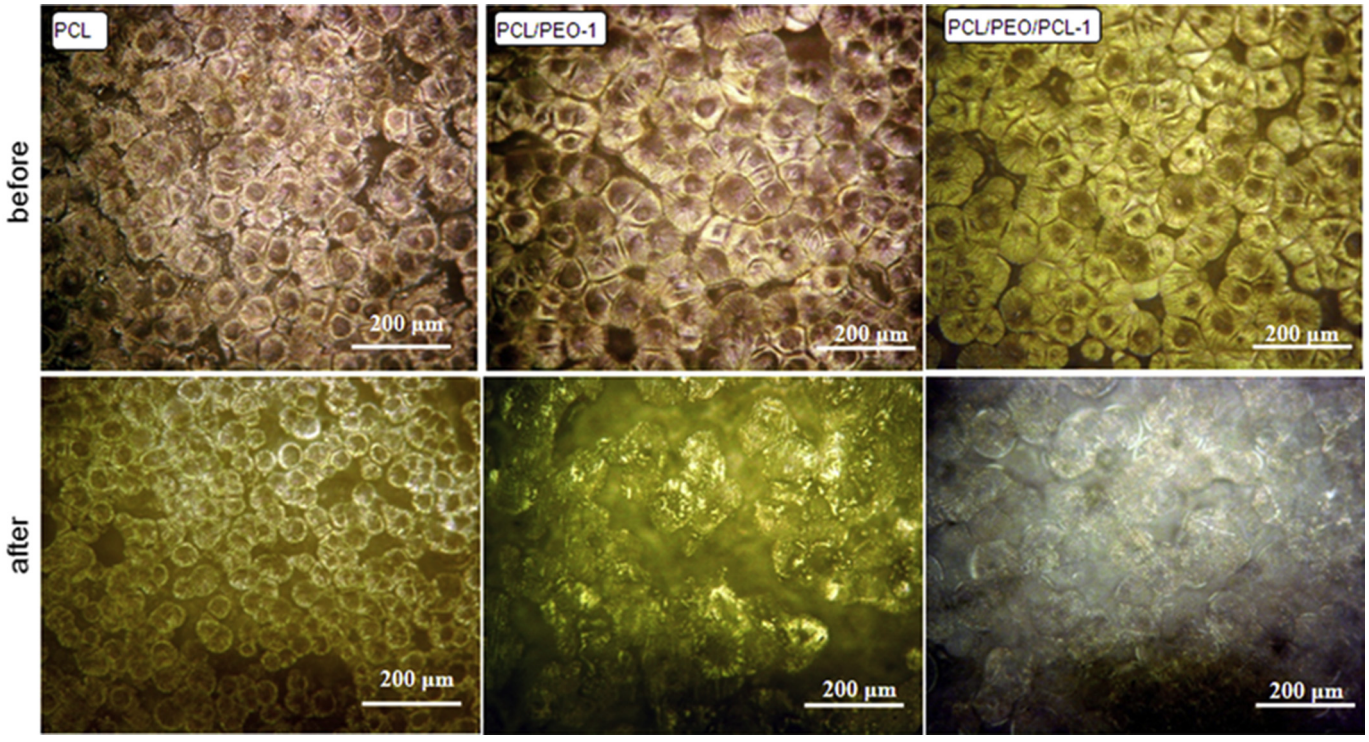


Fig. 6. Optical microscopy of PCL, PCL/PEO-1 and PCL/PEO/PCL-1 copolymer samples before and after enzymatic degradation.

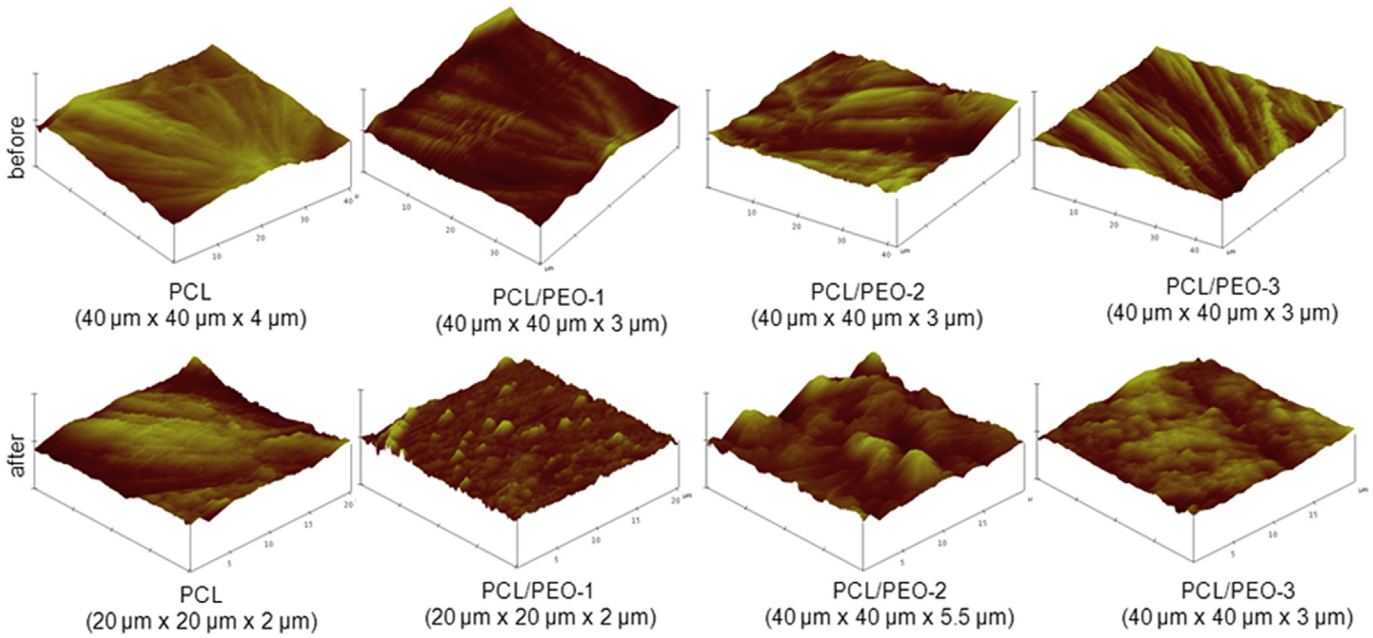


Fig. 7. AFM micrographs of PCL and PCL/PEO diblock copolymer samples before and after degradation.

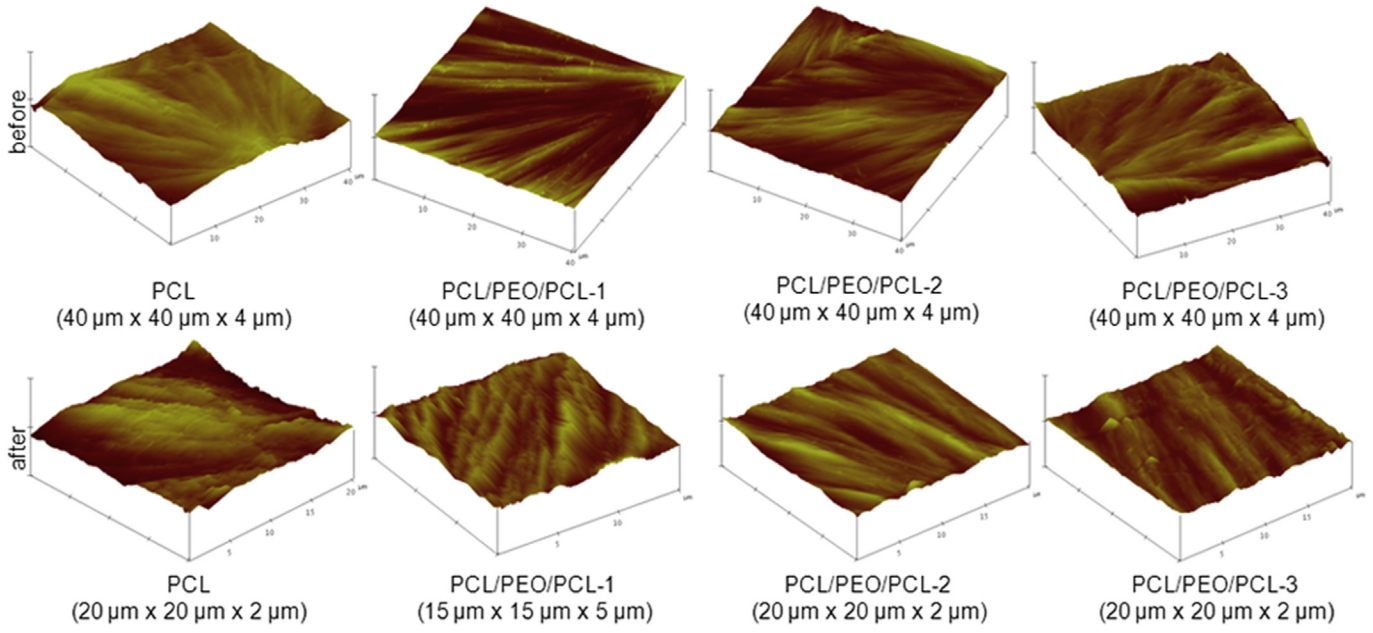


Fig. 8. AFM micrographs of PCL and PCL/PEO/PCL triblock copolymer samples before and after degradation.

copolymer samples. In the series of diblock copolymers, spherulite diameter depended on the molecular weight of copolymer and as the molecular weight increased, the spherulites enlarged. Although different diameters of spherulites were measured, in the case of triblock copolymers, spherulite size had no impact on surface roughness. Triblock copolymers showed hardly detectable difference in RMS values before degradation, smaller than those for diblock copolymers and similar to homopolymer PCL. It should be also pointed out that the different way of crystallization of air oriented and glass oriented polymer films affected RMS. Generally, for the glass side of polymer films lower RMS values were detected. According to the surface erosion mechanism of enzymatic degradation, changes in morphology and RMS values were in a line with a degradation extent of copolymer films. The lamellar structure of PCL and block copolymer films was destroyed and the intensity of morphological change was in agreement with degradation extent: the higher weight loss of samples the more intensive changes in lamellar structure. Therefore, RMS value of PCL/PEO/PCL-1 sample increased from 87 nm to 200 nm on glass side i.e., from 51 to 114 nm on the air side, which indicated strongly degraded surface morphology. Other two samples from the triblock copolymer series, which lost less than 1 wt% showed smaller changes in RMS values. Due to a higher degradation extent of diblock copolymers, surface erosion of those samples was more

intensive, and consequently higher RMS values after degradation were observed. It was interesting that for diblock copolymer PCL/PEO-1, which lost about 12 wt% of the weight, difference in RMS value before and after degradation was only about 30 nm in comparison to sample PCL/PEO-3 (weight loss 6.4 wt%) with RMS detected change of almost 60 nm. This could be explained by the spherulites' diameter of PCL/PEO-3 polymer which possessed smaller starting RMS value due to very large spherulites (200 μm). Because of the relatively small surface roughness of this diblock copolymer, compared to other samples, the enzymes presented in a cell-free extract could easily attack polymer surface. Finally, the RMS values for both sides of polymer films were equally changed as the surfaces became rougher in both cases.

3.3. Degradation in compost

Biodegradation of block copolymers and PCL was tested in model compost containing a commercial mixture used for cultivation of white button mushroom and supplemented with culture of *P. aeruginosa* PAO1, at 37 °C. During four weeks of composting, some of the samples exhibit significant surface degradation followed by roughing and polymer disintegration (Fig. 9). Homopolymer PCL exhibited the smallest level of degradation in comparison to block copolymers. For those samples which showed higher degradation extent in compost, some areas were more affected than others, indicating that degradation in compost is carried out through an inhomogeneous mechanism. According to literature [36], this behaviour is attributed to better contact of the compost in more degraded areas. Also an inhomogeneous growth of the microorganisms on the polymer film could be an additional explanation of the inhomogeneous degradation. There are some reports about influence of specific surface area of the specimens on biodegradation process in compost where biodegradation can be accelerated by enlarging surface area of samples exposed to microorganism action [24].

As can be seen from the photographs, even after four weeks in compost, PCL showed the best resistance to microorganisms with a few degraded domains only, while the film was mostly compact. The highest disintegration upon degradation was observed for PCL/PEO/PCL-1 and PCL/PEO-1 block copolymers due to higher content of hydrophilic PEO, but also to the smallest molecular weight in the series. For those block copolymers in compost strong disintegration of films was recorded, after only one week. Biodegradation process took place at different extents on polymer film surface leading to entirely disintegration of samples and migration of degradation products to compost. It is important to highlight that with the increase of molecular weight, polymers showed lower degradation. Therefore, triblock copolymer PCL/PEO/PCL-3 and diblock copolymer PCL/PEO-3 were barely damaged in terms of their compactness. However, as degradation preceded from the surface to the interior of the sample, surface morphology changes were noticed. Finally, biodegradation in compost revealed the possibility of analyzed samples to be degraded in a natural environment by activity of some microorganisms normally present in nature.

4. Conclusions

Short-term hydrolytic and enzymatic degradation of PCL, triblock and diblock copolymers (with a low content of hydrophilic, flexible PEO segments) was analyzed based on weight loss as well as by using FTIR, GPC, AFM and optical microscopy. PCL/PEO copolymers exhibited higher degradation extent in phosphate buffer in comparison to PCL and PCL/PEO/PCL copolymers, according to carbonyl and crystallinity index values and weight loss. Enzymatic degradation of polymer samples in the presence *P. aeruginosa* PAO1 cell free extract indicated higher degradation compared to hydrolytic degradation. Optical microscopy and AFM revealed the strong changes in surface morphology, and assessment of RMS values after enzymatic degradation confirmed surface erosion process. FTIR analysis of enzymatically degraded polymer films also indicated significant changes in carbonyl and crystallinity indexes while GPC analysis revealed higher decrease in molecular weight of diblock copolymers. This leads to conclusion that the degradation mechanism of PCL and their block copolymers with PEO occurs through surface erosion accompanied with small decrease in molecular weight. Degradation tests in compost showed significant level of

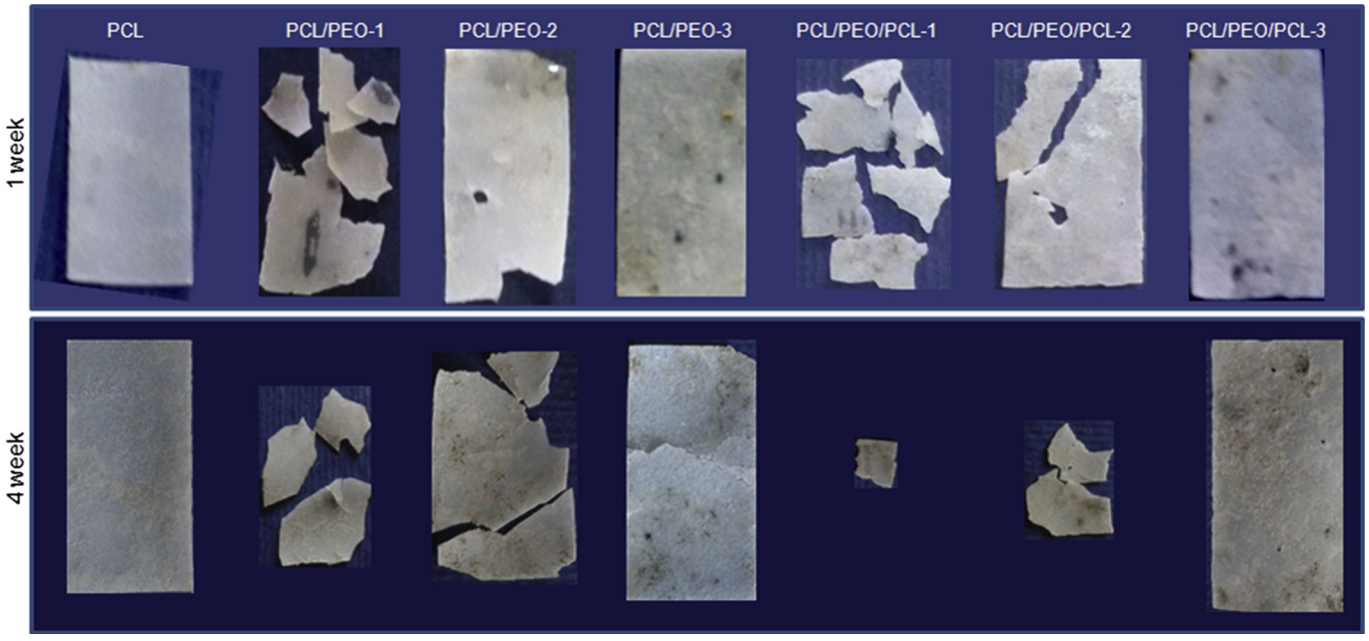


Fig. 9. PCL, triblock and diblock copolymer samples photographed after 1 week and 4 weeks of composting experiment.

destruction of block copolymer films in comparison to PCL sample. It was shown that both, PEO segment content and molecular weight of polymer chains have significant impact on the biodegradation process. In addition, although the degradation process followed surface erosion mechanism, it was noticed that some regions were eroded more than others indicating an inhomogeneous nature of the degradation process. Finally, we have confirmed that the addition of PEO hydrophilic segment into PCL chains greatly affect bulk and surface properties which is beneficial for hydrolytic, enzymatic as well as environmental film degradation.

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