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Biodegradation of Modified Poly(ε-caprolactone) in Different Environments

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Abstract

The comparison of degradation of modified poly(ε-caprolactone) film in different environments is presented. Poly(ε -caprolactone) was incubated in a natural environment - sea water (the Baltic Sea), in buffered salt solution, in liquid medium containing sea water with NaN_3 and in compost with plant treatment activated sludge (under natural and laboratory conditions). The characteristic parameters of sea water and compost with active sludge are described. Their influence on degradation of modified poly(ε -caprolactone) is discussed.

Weight changes, viscosity, tensile strength, DSC parameters of melting and crystallization and changes in morphology of polymer surface were tested during the period of biodegradation.

In liquid medium containing sea water with sodium azide and in buffered salt solution the slow hydrolysis process of poly(ε -caprolactone) was observed.

In natural environments the poly(ε -caprolactone) film was completely assimilated after seven weeks of incubation. This means that poly(ε -caprolactone) modified by slip and anti-block one additives was very sensitive to an enzymatic attack by microorganisms present in the Baltic Sea water and in the compost with activated sludge under natural conditions.

Keywords: modified poly(ε -caprolactone), sea water, compost, biodegradation

Introduction

Biodegradation is a desirable long-term future solution for the disposal of used materials and requires intensive research and development before it becomes practical.

Aliphatic polyesters presently constitute the most attractive class of artificial polymers, which can degrade in contact with living organisms. According to the literature degradation of aliphatic polyesters in a living environment can result from simple chemical hydrolysis of ester bonds or from enzymatic attack or both [1-4].

Poly(ε -caprolactone) is an important member of the aliphatic polyester family known as materials susceptible to microbial degradation [5-6].

Poly(ε -caprolactone) is degradable in several biotic environments, including river and lake waters, sewage sludge, farm soil, paddy soil, creek sediment, roadside sediment, pond sediment, and compost [7-9]. It has been reported that esterase and other kinds of lipase could degrade poly(ε -caprolactone) [10-12].

The degradation times of poly(ε -caprolactone) varied with molecular weight, crystallinity degree and morphology [5].

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According to the literature polymer morphology plays a critical role in degradation phenomena. It is well known that degradation of semicrystalline polymers occurs in two stages. The first stage consists of degradation of the amorphous phase, resulting in an increase in crystallinity of polymer. The second stage starts when most of the amorphous regions are degraded; subsequently the crystalline phase is degraded [5, 13-16].

Our earlier studies confirmed the biological degradation of poly(ε-caprolactone) in natural environments [17-19].

Poly(ε-caprolactone) modified by slip and anti-block processing additives was the subject of these studies. The comparison of degradation of modified poly(ε-caprolactone) film in different natural and laboratory environments is presented.

Experimental

Material

Poly(ε-caprolactone) (PCL) used in this work was kindly supplied by the Royal Institute of Technology in Stockholm.

The film-blown PCL consisted of:

- 90% pure poly(ε-caprolactone) (from Union Car bide, trademark Tone 787 with reported MW = 80.000 and p (23°C) = 1.145 g/cm^3);
- 10% OE 5440 (Ethylene Butyl Acrylate (EBA) copolymer containing 17% Butyl Acrylate (BA) with anti oxidant) - slip and anti-block commercial additive from Neste.

PCL film was cut into 15 x 2 cm rectangles with 0.06 mm thickness. The samples were left at room temperature and weighed with a precision balance. Average weight of each PCL sample was 0.13 g.

Environment

Biodegradation of PCL samples took place in the following environments as:

- compost with activated sludge in natural environment
- compost in laboratory environment
- the Baltic Sea
- sea water with sodium azide under laboratory condi tions
- buffered salt solution.

The compost pile 1.5 x 2 x lm (width, length and height) was prepared in the natural conditions of a sewage farm. It consisted of the sample of activated sludge from sewage treatment plant, burnt lime, and straw. Burnt lime (0.45kg CaO/lkg dry mass of compost) was added to ravage phatogenic bacterium and egg parasites, to deacidificate activated sludge and to convert active sludge to compost. The straw was added to maintain the higher temperature of the compost pile [20]. We can expect that in the compost pile prepared under natural conditions the types of living microorganisms are dependent on the location of pile. There were conditions from aerobic (upper part of pile), microaerophilic (in the middle) and facultative anaerobic (at the bottom).

The samples of PCL were put into a special basket and buried at lm deep within the compost pile.

In the laboratory the compost consisted of only active sludge and the same amount of burnt lime. The compost was located in a closed polypropylene container 0.5 x 0.5 x 0.75 m (width, length and height). We suppose that in laboratory compost there were rather microaerophilic conditions.

The characteristic parameters of both composts were measured during the degradation time of PCL.

The incubation of PCL in the Baltic Sea water took place in Gdynia Harbour. The samples of PCL were located in the special basket at 2 meters depth under the water surface, near a ship of Polish Ship Salvage Company. For comparison the degradation of PCL samples also took place in sea water with NaN_3 (0.195 g/11) in laboratory. The sodium azide was added to sea water for the purpose of excluding the activity of microorganisms and to evaluate the resistance of the polymers to hydrolysis.

The hydrolytic degradation of PCL was also studied in a buffered salt solution (pH 7.2) at 37°C [21]. The composition of the buffered salt solution is presented in Tab. 1.

Table 1. Composition of the buffered salt solution.

Salt	Concentration [mg/l]
NaCl	95
KCl	5
$NH_2C(CH_2OH)_3$	30
$Ca(CH_3COO)_2H_2O$	4
$MgSO_4 \cdot 7H_2O$	2
$Na_2HPO_4 \cdot H_2O$	2
NaN_3	3

Methods

Investigation of Compost

Dry mass in compost was determined according to Polish Standard [22]. The samples of compost were dried at 105°C until a constant weight was obtained. The average samples of approximately 10 g required drying for at least 24 hours. When percent of dry mass in compost was known, moisture content was calculated.

PH of compost was determined using a pH-meter [22]. To each 50 g sample of compost, 100 cm^3 of distilled water was added. Samples were homogenised for 30 min. and were put aside for 1 hour; then the pH of the extract was measured using a Teleko model N 5172f pH-meter.

Activity of dehydrogenases was measured by spectrophotometric method with TTC (triphenyltetrazolium chloride). This method is based on the dehydrogenation of

glucose added to the compost and transfer of hydrogen to the colourless biological active compound of TTC, which goes through a reduction to TF ($TTCH^{32}$, red compound). The intensity of this colour is measured using "Specol" colorimeter (490 nm).

This is the method for an estimation of biochemical activity of microorganisms in the activated sludge by oxidation process of organic compounds [22, 23].

Investigation of PCL Samples

After incubation, the samples were taken out from the environment and washed with distilled water and dried at room temperature to a constant weight.

The changes in weight, tensile strength, intrinsic viscosity, crystallization and surface morphology of PCL film were tested during the experiment.

Weight changes (%) were determined using a Gibertini E 42s electronic balance. The weight of clean and dried samples of PCL after biodegradation was compared with that before biodegradation. Weight loss was calculated and expressed in a percentage (%). The average from 3-5 PCL samples was the final result of the investigation.

Intrinsic viscosity of PCL was performed at 25°C by means of an Ostwald's viscometer. Flow times of $5 \cdot 10^{-4}$, $10 \cdot 10^{-4}$, $15 \cdot 10^{-4}$, $20 \cdot 10^{-4}$, $25 \cdot 10^{-4}$, $30 \cdot 10^{-4}$ [g/dl] solutions of PCL in benzene were measured. The variations of specific viscosity as a function of concentration (c) were extrapolated to c=O and finally the values of intrinsic viscosity were calculated [24].

Tensile strength of PCL was measured at room temperature by means of a Tensile Tester Type Fu 1000e made by VEB Thuringer Industriewerk Rauenstein. Tensile testing was performed using rectangular strips of investigated polymer film (20 mm x 150 mm x 0.06 mm), in accordance with Polish Standard [25]. Tensile strength was calculated as tensile force [N] to cross-sectional area [mm^2]. The results for three specimens were averaged for each value determined.

The phase transition of PCL was investigated by means of a differential scanning calorimeter Perkin Elmer DSC7/ Unix type in the temperature range from -20°C to 160°C under nitrogen as the purge gas. The heating rate was 20°C/min and the cooling rate was 10°C/min. Sample weight was 8-12 mg.

Microscopic observations of PCL surface were analysed with the optical microscope ALPHAPHOT-2YS2-H linked to a Nikon F90X camera. Final magnification was 1:270. The surface was analysed before and after biodegradation.

Results and Discussion

The characteristic parameters of both composts with plant treatment activated sludge under natural and laboratory conditions are presented in Tab. 2.

Table 2. Parameters of compost in natural and laboratory environments.

Parameter	Month					
	August		September		October	
	N	L	N	L	N	L
Temperature [°C]	28.6	22.7	19.1	22.1	15.1	17.6
pH	7.5	7.4	7.8	7.6	7.1	7.1
Moisture content [%]	61.7	46.8	59.7	49.1	40.5	39.9
Activity of dehydrogenases [mol TF/mg of dry mass]	0.0644	0.0555	0.0443	0.0551	0.0393	0.0484

N – compost under natural conditions
L – compost under laboratory conditions

Looking at the parameters of both composts (temperature, pH, moisture content and activity of dehydrogenases) we can state that slightly different conditions were present in both composts, which can influence the development of living microorganisms and the process of biological degradation of PCL samples. The average temperature in both composts was on the same level (~21°C) - preferred for enzymatic degradation [26]. In the laboratory the temperature was more stable than in the compost under natural conditions. The average pH of composts was near (~7.4) under natural and laboratory conditions. This means that in this case (temperature about 21°C and neutral pH) psychrotrophic and psychrophilic microorganisms could play the main role in the experi-

Table 3. Weight loss (%) of PCL after incubation in compost.

Environment	Weight loss [%]						
	Incubation time [week]						
	1	2	3	4	5	6	7
	August	September				October	
Compost in natural environment	4.6	6.0	15.7	29.5	51.7	72.6	–
Compost under laboratory conditions	3.2	34.5	47.2	51.1	57.8	–	

[–] – complete disintegration

ment. Taking the conditions of both composts into consideration we can expect that the microaerophilic conditions resulting from the activity of living organisms were at the end of the composting process.

It is known that a large population of micro- and macroorganisms can exist in compost. According to the literature the most abundant are bacteria, actinomycetes and fungi [27]. The activity of dehydrogenases depends on the degree of the growing microorganisms of the populations, which produce enzymes involved in the biodegradation process.

The activity of dehydrogenases in compost at a sewage farm changed a lot during the experiment. At the beginning of the experiment (in August) the activity of dehydrogenases was the highest in natural compost, because of high temperature (28°C) or other factors which were not analysed. At the end of the experiment the activity of dehydrogenases decreased by about 40%.

The activity of dehydrogenases in compost in laboratory was more stable.

The results of weight changes of PCL sample during experiments in composts are presented in Tab. 3.

A decreases of PCL weights were observed in both composts studied but the course of changes vs. time was different.

At the beginning of the experiment (one-week of incubation) PCL samples exposed to both composts showed weight loss. The weight loss of samples incubated in the natural environment for one week in August was higher than in the laboratory, because the temperature, moisture content and activity of dehydrogenases in compost under natural conditions were higher than in the laboratory.

After two weeks it is clearly seen that in laboratory compost the weight was decreasing dramatically. During this time (September) the parameters of the laboratory compost such as temperature and activity of dehydrogenases were higher than in natural terms. This intensive biodegradation process could also be accelerated by activity of microaerophilic microorganisms living in laboratory compost. After six weeks of incubation in this compost the PCL samples were completely disintegrated. Biodegradation of modified the PCL in compost at the sewage farm was slower because the temperature and activity of dehydrogenases decreased during the period of investigation. The disintegration of modified PCL samples was noted after seven weeks of incubation in compost in natural environment.

The characteristic parameters of water in the Baltic Sea according to Gdynia Water Management and Meteorology Institute and sea water with sodium azide are presented in Tab. 4.

The characteristic parameters of sea water in natural environment presented in Table 4 indicate that the average temperature (~19°C) of the Baltic Sea water was slightly lower than that preferred for enzymatic degradation, which is in the range 20-60°C [26].

The average value of pH in sea water was alkaline (8.5) and was higher than that preferred for enzymatic degradation (5-8) [26]. The temperature of the Baltic Sea and slight alkalinity of water had an influence on development of psychrotrophic and mesophilic bacteria.

Looking at the results in Table 5 we can compare the weight changes of modified PCL incubated in three different water environments.

At the first week (in June) of incubation of PCL samples in Baltic Sea water small changes of weight were observed because of the low temperature (~17°). In the next few weeks the weight changes were higher in natural environment than in laboratory-sea water with NaN_3 and buffered salt solution.

The weight losses of the sample incubated in sea water with sodium azide were not significant (Table 5.). These changes might be explained by nonenzymatic hydrolytic ester cleavage. In buffered salt solution the changes were higher than in sea water with sodium azide, because there was higher temperature and more favourable pH for hydrolytic degradation [4], but the hydrolysis was much slower than enzymatic degradation of PCL in the Baltic Sea.

If we compare the results of weight loss of PCL samples in Tables 3 and 5, we could state that after seven weeks of incubation of modified PCL samples under natural weather, depending on conditions the samples were completely disintegrated.

The changes of intrinsic viscosity of modified PCL after

Table 4. Parametres of the Baltic Sea water and sea water with NaN_3.

Parameter	Month									
	June		July		August		September		September (next year)	
	N	L	N	L	N	L	N	L	N	L
Temperature [°C]	17.6	–	20.3	22.0	19.3	21.2		21.0		19.0
pH	8.5	–	8.2	7.1	8.8	7.8		8.1		7.4
Cl content [g/kg]	2.92	–	3.28	–	3.21		–		–	
Oxygen content [cm^3/dm^3]	7.55	–	7.57	–	7.42		–		–	
Salt content [ppt]	5.36	–	5.58	–	6.01		–		–	

N – the Baltic Sea water
L – sea water + NaN_3

Table 5. Weight loss *(%)* of PCL after incubation in aqueous environment.

Environment	Weight loss [%]							
	Incubation time [week]							
	1	2	3	4	5	6	7	64
	August	September				October		
Sea water	2.4	8.0	20.6	38.9	74.4	89.7	–	
Sea water with NaN_3	0.8	1.0	1.0	1.1	1.1	1.2		1.7
Buffered salt solution		2.57		8.6		11.0		

[–] – complete disintegration

degradation were giving the information about changes of molecular weight. The characteristic parameters are presented in Tab. 6.

Table 6. Changes of intrinsic viscosity (g/dl) of PCL after incubation in different environments.

Environment	Intrinsic viscosity [g/dl]			
	Incubation time [week]			
	0	2	4	6
Sea water	1.22	0.84	0.64	–
Sea water with NaN_3		1.61	1.09	0.90
Compost in natural environment		1.12	0.80	–
Compost under laboratory conditions		1.06	0.71	0.49

Longer incubation time in a microbially active environment shows higher decrease of viscosity of biodegraded PCL was observed. The decrease of intrinsic viscosity could indicate a decrease of molecular weight of PCL. However, the relationship between changes of intrinsic viscosity and molecular weight had only a quality character, and should be confirmed by other analytical techniques. We supposed that lower changes of intrinsic viscosity after treatment of PCL in both composts could be caused by enzymatic degradation on the polymer surface. While more visible changes of intrinsic viscosity of PCL after incubation in Baltic Sea water could be explained by degradation of PCL in this biotic environment as a result of chemical and enzymatic hydrolysis of ester bonds. The data of tensile strength at break of PCL samples before and after biodegradation are shown in Table 7.

The decrease in tensile strength at break of PCL sample after biodegradation was observed explicitly and was associated with weight changes. Tensile strength at break was reduced as weight loss increased.

At the beginning of the experiment both in abiotic and biotic environments a decrease in tensile strength at break was seen; even weight changes after biodegradation in abiotic environments were not significant. Surprisingly, after a longer time of incubation in sea water with sodium azide we observed improvement in that mechanical property of PCL.

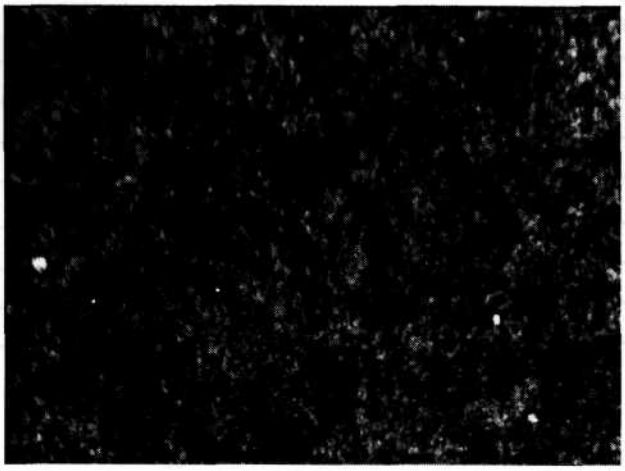
Under natural conditions in our experiment enzymatic degradation caused surface erosion. Swelling and bursting of growing cells of the invading macro- and microorganisms might cause mechanical damage to the surface of PCL. This was a reason for the dramatic decrease in tensile strength after incubation in the Baltic Sea water and in composts. The tensile strength of PCL samples incubated three weeks in sea water was very low (δ_B = 1.7 MPa). After the same time of incubation in compost, the measurement of tensile strength was impossible because samples were torn to pieces.

The microscopic observations were in good agreement with changes of weight of PCL samples. While studying

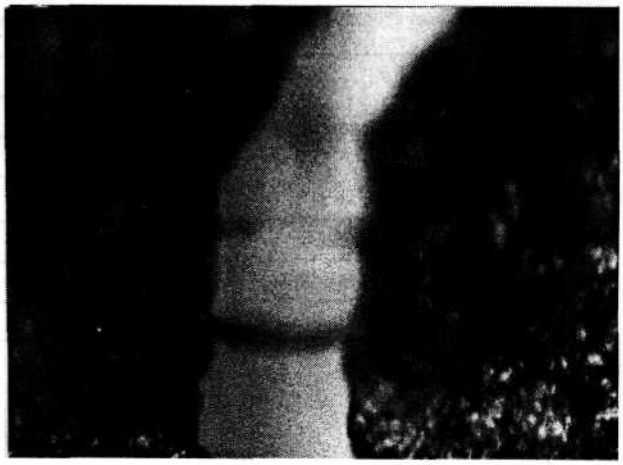
Table 7. Tensile strength δ_B[MPa] of PCL after incubation in different environments.

Environment	Tensile strength δ_B [MPa]							
	Incubation time [week]							
	0	1	2	3	4	5	6	64
Sea water	20.2	14.6	12.1	1.7	1.2	–	–	
Sea water with NaN_3		16.9	24.8	27.7	24.9	24.1	27.5	16.2
Buffered salt solution			11.1		8.4		7.5	
Compost in natural environment		7.1	3.2	–	–	–	–	
Compost under laboratory conditions		6.5	–	–	–	–	–	

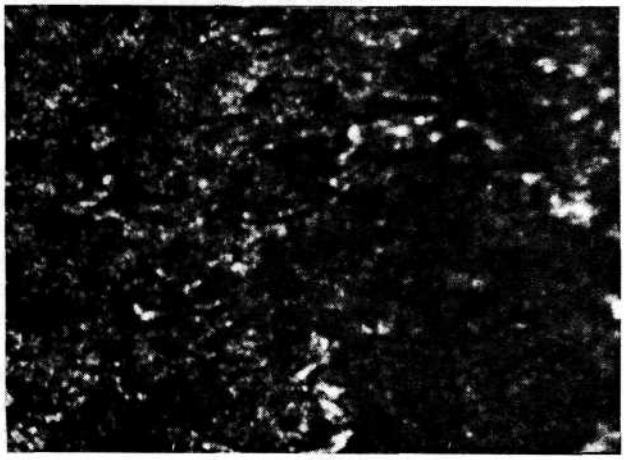
[–] – samples were torn up in pieces



a) blank

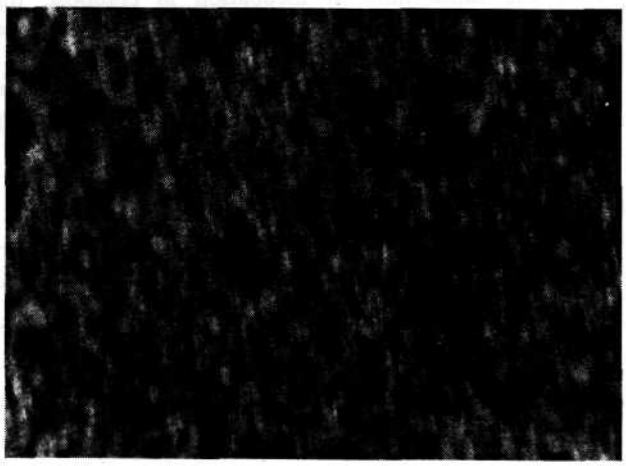


d) sea water 6 weeks



b) sea water 2 weeks

e) compost 6 weeks



c) sea water 4 weeks

Fig. 1. Micrographs of PCL sample before (blank) and after incubation in a natural environment.

the biotic degradation of PCL samples we observed that under certain circumstances PCL could degrade enzymatically, leading to polymer surface erosion. This polymer erosion was found only in polymer film degraded under natural conditions. Microscopical observations of PCL surface are presented in Fig. 1.

The surface of blank sample of PCL when observed under optical microscope consisted of two phases (bright - crystalline and dark - amorphous). Both phases were slightly oriented in the same direction (Fig. la). The observation with the use of crossed polarizers did not reveal any characteristic birefringent structure. After treatment in natural environments (sea water and compost) the samples were not homogeusly destroyed and there were different images depending on where the picture was taken.

Generally, after two weeks of biodegradation under natural conditions we observed an increase of orientated birefringence elements, which might be evidence of an increase in crystallinity (Fig. lb).

According to literature semicrystallinity of PCL plays a critical role in degradation phenomena, because the amorphous phase is degraded first and as a result an increase in crystallinity of polymers occurs, when most of the amorphous phase is degraded, subsequently the crystalline phase is degraded [5, 13-16].

The microscopic observations could confirm that the amorphous phase was degraded first. After four weeks of experiment the decay of birefringent element was observed, crystalline phase began to degrade (less orientated birefringence elements) (Fig. lc). At the end of the experiment (six weeks) we could see very distinctly the black area on the surface of film studied, which represented an agglomeration of microorganisms (Fig. Id, e), and observation of PCL film morphology was unable to follow.

The change of crystallinity degree after incubation was confirmed by DSC measurement. The microscopic observations were in good agreement with changes of weight and DSC of PCL samples. Melting peaks occur in the temperature ranges from 62°C to 69°C on DSC curves, obtained at 2nd heating runs, were due to the melting of crystallites. The changes of melting enthalpy indicated changes of crystalline degree of PCL. In Table 8 melting enthalphy of crystalline phase is presented.

Table 8. The changes of melting enthalpy ΔH [J/g] of PCL after incubation in different environments.

Environment	Melting enthalpy ΔH [J/g]			
	Incubation time [week]			
	0	2	4	6
Sea water	37.5	44.4	40.4	38.1
Compost in natural environment		39.4	42.4	40.3
Compost under laboratory conditions		43.7	42.6	39.3 (5 weeks)

The increase in crystallinity was confirmed by an increase of melting enthalpy of the crystalline phase of PCL samples after two-week degradation in all environments, because the amorphus phase was degraded first.

During the next period of incubation in Baltic Sea water and compost in laboratory the crystalline phase was degraded and the enthalpy decreased. Changes of melting enthalpy of the crystalline phase of PCL samples after biodegradation in compost in the natural environment confirmed slower biodegradation rates, because after four weeks of incubation we could still observe an increase of melting enthalpy. This means that the amorphous phase degraded slower in the compost under aerobic conditions (sewage farm) than under the anaerobic - in laboratory.

At the end of the experiment (six weeks) we could see the decrease of melting enthalpy in all biotic environments, which indicates the degradation of crystalline phase of PCL.

Conclusion

Biodegradation of modified poly(ε-caprolactone) in sea water and compost with activated sludge was very fast. The film was completely assimilated over the period of seven weeks. The obtained results indicate that modified poly(ε-caprolactone) was very sensitive to enzymatic attack of microorganisms in living environment and rather resistant to chemical hydrolysis.

Microscopic observations and DSC studies of poly(ε-caprolactone) incubated in sea water and compost with plant treatment activated sludge leads to the conclusion that the biodegradation of poly(ε-caprolactone) in a microbially active environment occurred in two stages. The first stage consisted of the degradation of amorphous phase, resulting in an increase in crystallinity of the polymer. The second stage started when most of the amorphous regions were degraded; subsequently, the crystalline phase was degraded. The polymer became prone to fragmentation and enzymatic surface erosion proceeded.

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