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Biodegradation of poly(ε-caprolactone) in natural water environments

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The environmental degradation of poly(ε-caprolactone)[PCL] in natural fresh water (pond) and in The Baltic Sea is presented in this paper. The characteristic parameters of both environments were measured during experiment and their influence on the biodegradation of the samples was discussed. The loss of weight and changes of surface morphology of polymer samples were tested during the period of incubation. The poly(ε-caprolactone) was more biodegradable in natural sea water than in pond. PCL samples were completely assimilated over the period of six weeks incubation in The Baltic Sea water, but after forty two weeks incubation in natural fresh water the polymer weight loss was about 39%. The results have confirmed that the investigated polymers are susceptible to an enzymatic attack of microorganisms, but their activity depends on environments.

Keywords: biodegradation, poly(ε-caprolactone), fresh water, sea water, pond.

INTRODUCTION

Poly(ε-caprolactone) is a semicrystalline linear aliphatic polyester. This polymer is non-toxic, flexible and hydrophobic[1]. PCL is highly processible as it is soluble in a wide range of organic solvents. It has a low melting point (55–60°C depending upon its crystalline nature of PCL) and glass transition temperature (–60°C) while it having the ability to form miscible blends with wide range of polymers. The number average molecular weight of PCL samples may generally vary from 3 000 to 80 000 g/mol and can be graded according to the molecular weight. The polymer undergoes hydrolytic degradation due to the presence of hydrolytically labile aliphatic ester linkages; however the rate of degradation is rather slow (2–3 years). PCL has low tensile strength (approximately 23 MPa) but an extremely high elongation at breakage (>700%)[2–5].

Poly(ε-caprolactone) is soluble in chloroform, dichloromethane, carbon tetrachloride, benzene, toluene, cyklohexanone and 2-nitropropane at room temperature. It has a low solubility in acetone, 2-butanone, ethyl acetate, dimethylformamide and acetonitrile. This polymer is insoluble in alcohol, petroleum ether and diethyl ether[2, 4].

Biodegradation of poly(ε-caprolactone) can be enhanced by copolymers like polylactic acid and polyglycolic acid. This process is autocatalyzed. Degradation of poly(ε-caprolactone) is a bulk process that can be divided into two phases:

I. Molecular weight loss up to 5 000 due to chain scission. No weight loss is observed during the initial phase of the biodegradation process, which covers a molecular weight range of 200 000 to 5 000.

II. The phase is characterized by decrease in the rate of chain scission and the onset of weight loss. The decrease in the rate of chain scission is associated with an increase in crystallinity, since cleavage takes place in the amorphous region of the polymer. Weight loss has been attributed to an increased chain scission of a low molecular weight (less than 3 000), polymer breakup to produce smaller particles[2, 4, 6].

Biodegradation of polymers leads to fragmentation materials until complete decomposition to CO_2 and H_2O through the action of living organisms.

Enzymatic degradation takes place on the surface of the PCL with the participation microorganisms. PCL hydrolytic degradation takes place very slowly because of its hydrophobic nature (Fig. 1)[2, 7–13].

Poly(ε-caprolactone) can be biodegraded by outdoor living organisms (bacteria and fungi), but they are not biodegradable in animal and human bodies because of the lack of suitable enzymes[2].

Natural water environment is a unique ecosystem with very different microbial activity and sensitivity to pollution. The aim of this study is estimation of biological degradation of PCL in pond (natural fresh water environment) and comparison results with biodegradation PCL in The Baltic Sea (salt water). Furthermore, the PCL samples were located into water (from natural environment) without microorganisms in laboratory to estimate the influence of hydrolytic degradation on PCL.

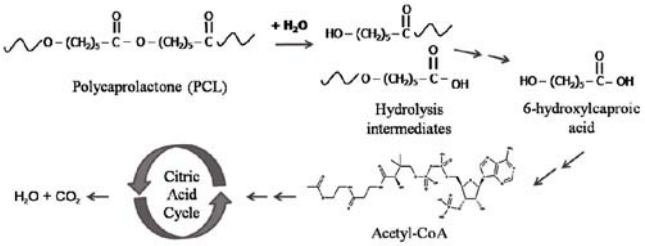

Figure 1. Mechanism of the biodegradation of poly(ε-caprolactone)

EXPERIMENTAL SECTION

Material

Poly(ε-caprolactone) [PCL] samples, the trade name "CAPA 680" (*Mw* = 80 000), were received from Solvay – Belgium.

Polymer films were cut into 150 x 20 mm rectangles. The thickness of polymer samples was 0.06 mm. After incubation in water environments, the samples were left at room temperature and then were taken to investigations.

Environment

Incubation of poly(ε-caprolactone) samples took place in the following environments:

– The Baltic Sea water – under natural conditions;

– sea water with sodium azide (NaN_3) – under laboratory conditions;

– fresh water (pond) – under natural conditions;

– fresh water with sodium azide (NaN_3) – under laboratory conditions.

The incubation of polymer samples in The Baltic Sea water took place in Gdynia Harbour. The samples were located in the special perforated basket at 2 meters depth under the water surface, near a ship of Polish Ship Salvage Company[8–10].

The incubation of polymer samples in fresh water took place in Rumia's ponds in a special basket at 2 meters depth under the water surface.

For comparison the degradation of polymer samples also took place in sea water with NaN_3 (0.195 g/l) in laboratory. The sodium azide was added to sea and fresh water for the purpose of excluding the activity of microorganisms and to evaluate the resistance of the polymer to hydrolysis[14]. The PCL samples were located in the glass aquarium equipped with an aeration pump.

Methods

After incubation the poly(ε-caprolactone) samples were taken out from all environments and washed with distilled water. Then they were dried at room temperature to a constant weight. The changes in weight and surface morphology of polymer films were tested during the experiment.

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

Crystallinity changes [%] – were analysed by the differential scanning calorimetry using Setaram Labsys TG-DTA/DSC Analyzer. The heating scans at the rate of 10°C/min in the temperature range 20–200°C in nitrogen atmosphere, in nitrogen flow 20 cm^3/min were recorded. Based on determined melting enthalpy of PCL samples the percent of crystallinity was calculated[9, 15]. According to the following equation:

$$x\,[\%] = \frac{\Delta H}{\Delta H_{100\%\ PCL}} \cdot 100$$

where:

x – crystallinity (±0.05) [%],

ΔH – melting enthalpy of PCL samples [J/g],

$\Delta H_{100\%\ PCL}$ – 139.5 [J/g], melting enthalpy of 100% crystalline PCL[16].

Macroscopic observations of polymer surface – were analysed organoleptic with a FujiFilm S2500 HD camera. The macrographs were analyzed before and after biodegradation.

Microscopic observation of polymer surface and structure – were analysed with the methalographic microscope ALPHAPHOT-2YS2-H linked to a Casio QV-2900UX camera. The micrographs were analyzed before and after biodegradation.

Scanning electron microscope SEM analysis – micrographs were performed using a FEI Quanta 250 FEG at low vacuum mode (the pressure in chamber was 130 Pa). This mode provides the ability to analyze non-conductive samples without coating the surface layer of the drain electric charge. Imaging was performed with an accelerating voltage of 10 kV.

RESULTS AND DISCUSSION

Environmental parameters

The characteristic parameters of natural fresh water (pond) and sea water under natural and laboratory conditions are presented in Table 1.

Looking at the parameters of natural environments (sea water, pond) in summer months (June–August) we can state that the average temperature 18–19°C was slightly lower than that preferred for enzymatic degradation, which is in the range 20–60°C[17]. In the same time the temperature in laboratory conditions (sea and fresh water with sodium azide) was 21–3°C and it was appropriate for the degradation process.

The incubation process of polymer samples in fresh water (pond) lasted longer than in sea water, so the parameters were measured up to the winter months.

Table 1. Characteristic parameters of natural and laboratory water environments

Incubation time [months]	Fresh water (pond)		Fresh water with NaN_3		The Baltic Sea water*					Sea water with NaN_3	
	Tempeature [°C]	pH	Temperature [°C]	pH	Temperature [°C]	pH	Cl^-content [g/kg]	Oxygen content [cm^3/dm^3]	Salt content [ppt]	Temperature [°C]	pH
June	17.8	9.0	21.7	8.6	17.6	8.5	2.9	7.5	5.4	–	–
July	19.3	8.6	23.4	6.8	20.3	8.2	3.3	7.6	5.6	22.0	7.1
August	18.5	8.4	21.9	7.7	19.3	8.9	3.2	7.4	6.0	21.0	7.8
September	12.3	8.5	19.5	8.2	–	–	–	–	–	–	–
October	10.0	7.7	19.5	7.6	–	–	–	–	–	–	–
November	7.0	9.0	20.3	8.0	–	–	–	–	–	–	–
December	3.8	8.3	19.9	7.1	–	–	–	–	–	–	–
January	5.2	7.8	20.8	8.3	–	–	–	–	–	–	–
February	3.5	7.8	20.4	7.9	–	–	–	–	–	19.0	7.6
March	4.9	8.4	21.5	8.0	–	–	–	–	–	–	–
April	8.5	8.6	21.8	8.2	–	–	–	–	–	–	–
May	11.6	8.8	21.6	8.4	–	–	–	–	–	–	–

Source: own research

*The Baltic Sea water parameters received from Gdynia Management and Meteorology Institute.

In that months the temperature was lower than that preferred for enzymatic degradation.

During all incubation time the average value of pH was alkaline 7–8 and was higher than that preferred for enzymatic degradation 5–8[17]. The best value of pH for growth bacteria in fresh water is 6–8[18]. Water from the pond with a high alkalinity is able to neutralize acidic rainwater supply and may be indicative of conditions in which toxic components are activated[19]. The water in the pond must not contain toxic components, especially since in the pond are bred fishes (trouts).

The water has the capacity to dissolve gases. This solubility decreases with increasing temperature and salinity. Pond power is better than sea water. The amount of oxygen depends on the ability of water to the self-cleaning due to the mineralization of organic substances. Mineralization is done with the help of micro-organisms, especially aerobic bacteria. The average concentration of oxygen in the clear river waters in temperate climates is about 5–7 mg/dm^3 [18].

The presence of salt content in sea water (5–6 ppt) had an influence on development marine organisms which had an influence on degradation of poly(ε-caprolactone).

Abiotic parameters have an influence on development of biotic parameters. Under marine conditions the development of psychrotropic and mesophilic bacteria is observed[8–10]. Algae, heterotrophic and epilitic bacteria are presented in fresh water (pond). The presence of microorganisms in natural water environments has an influence on enzymatic hydrolysis, which takes place on the polymer surface. Only environments in laboratory conditions had not any microorganisms. This is because the sodium azide was excluding the activity of micro-organisms and to evaluate the influence only chemical hydrolysis on polymer degradation.

Biotic and abiotic parameters should have an influence on the degradation process of incubated polymers such as poly(ε-caprolactone).

Weight changes of polymer samples during biodegradation process

The weight changes of poly(ε-caprolactone) after incubation in natural and laboratory water environments are presented in Table 2.

Looking at the results in Table 2 we can compare the weight changes of poly(ε-caprolactone) in four different water environments. The weight changes of poly(ε-caprolactone) samples were higher in natural Baltic Sea water than in natural fresh water environment (pond).

PCL samples were completely destroyed after 6 weeks incubation in natural sea water, while after 42 weeks of incubation in pond the weight loss was 39%. Weight loss has been attributed to an increased chain scission of a low molecular weight, polymer breakup to produce smaller particles[2, 4, 6]. This is probably a reason of high activity of enzymes in The Baltic Sea water, when presence of psychrotrophic and mesophilic bacteria could be expected[8]. The salinity and water undulation had an influence on degradability of poly(ε-caprolactone) too.

After incubation in laboratory water conditions the weight loss was lower than in natural water environment[10, 20, 21]. There was weight loss about 8.5% after 42 weeks incubation in fresh water with sodium azide (NaN_3) and only 1.4% after 98 weeks of incubation in the same conditions. Salt contents in laboratory sea water probably had an influence on slow chemical hydrolysis of poly(ε-caprolactone) samples.

Degradation process in laboratory conditions might be explained by no enzymatic hydrolytic ester cleavage. The chemical hydrolysis was much slower than enzymatic degradation of all polymer samples in microbially active environments like fresh water and Baltic sea water[10].

Crystallinity changes of polymer samples during biodegradation process

The DSC results of PCL before and after incubation in natural and laboratory water environments, presented in Table 3 and in Figure 2. These results revealed the differences in their crystallinity.

The obtained results indicate significant increase of poly(ε-caprolactone) crystallinity after incubation in

Table 2. Weight changes [%] of poly(ε–caprolactone) samples after incubation in natural and laboratory water environments

Incubation time [weeks]	Environment			
	Fresh water (pond)	Fresh water with NaN_3	The Baltic Sea water	Sea water with NaN_3
1	–1.5	–1.2	–1.7	–0.6
2	–3.2	–1.5	–3.5	–1.2
3	–2.2	–1.5	–10.7	–1.3
4	–2.4	–1.6	–20.7	–1.4
5	–2.5	–2.0	–33.8	–1.5
6	–3.1	–1.9	destroyed	–1.5
7	–4.3	–2.2	–	–
8	–5.5	–2.3	–	–
9	–4.0	–2.4	–	–
10	–4.0	–2.8	–	–
11	–3.8	–2.6	–	–
12	–4.7	–2.7	–	–
15	–5.6	–2.7	–	–
18	–5.4	–3.0	–	–
23	–6.2	–3.6	–	–
26	–7.3	–4.7	–	–
32	–17.6	–5.7	–	–1.4
37	–27.4	–6.9	–	–
42	–39.0	–8.5	–	–
98	–	–	–	–1.4

Source: own research.

Table 3. Changes of crystallinity [%] poly(ε–caprolactone) samples before and after incubation in natural and laboratory water environments

Incubation time [weeks]	Environment			
	Fresh water (pond)	Fresh water with NaN_3	The Baltic Sea water	Sea water with NaN_3
0	51.8	51.8	51.8	51.8
2	–	–	54.7	–
4	56.2	56.3	61.7	55.5
15	60.6	56.9	–	–
37	66.1	60.0	–	–
98	–	–	–	60.9

Source: own research

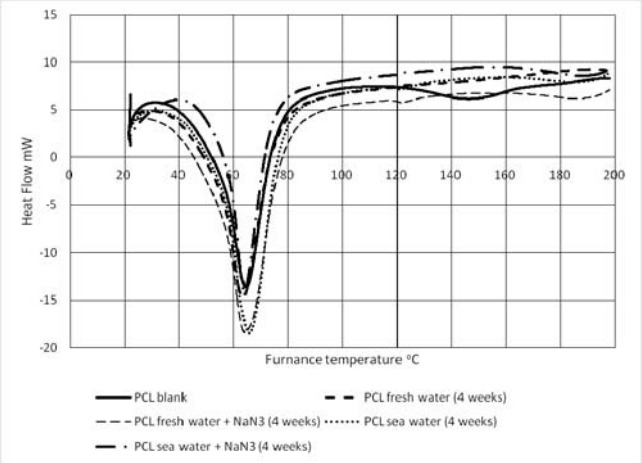

Figure 2. The DSC curves of poly(ε-caprolactone) samples before and after incubation in natural and laboratory water environments

every water environments. These results confirm that the hydrolytic degradation of PCL (the hydrolytic chain cleavage proceeds in amorphous regions) lead therefore to an increase of the polymer crystallinity. This is the first stage degradation of PCL. This situation starts from the second week of incubation of PCL in the marine environment and from the fourth week of incubation of PCL in the pond and in laboratory conditions. The second stage begins when most of the amorphous regions are degraded and subsequently the crystalline phase is degraded[22–25]. This phase should start after 4 weeks incubation of PCL in The Baltic Sea and after 37 weeks of incubation of PCL in the natural pond. Samples from laboratory conditions need more time to degradation. The degree of crystallinity from DSC results increases faster during degradation of poly(ε-caprolactone) in The Baltic Sea water environment. This research confirms the previous weight changes results.

Macroscopic observations of the surface of polymer samples during biodegradation process

The macroscopic observations were in good agreement with changes of the weight of polymer samples.

The macroscopic changes on the surface of PCL before and after incubation in natural and laboratory water environments indicate on their enzymatic degradation (Fig. 3).

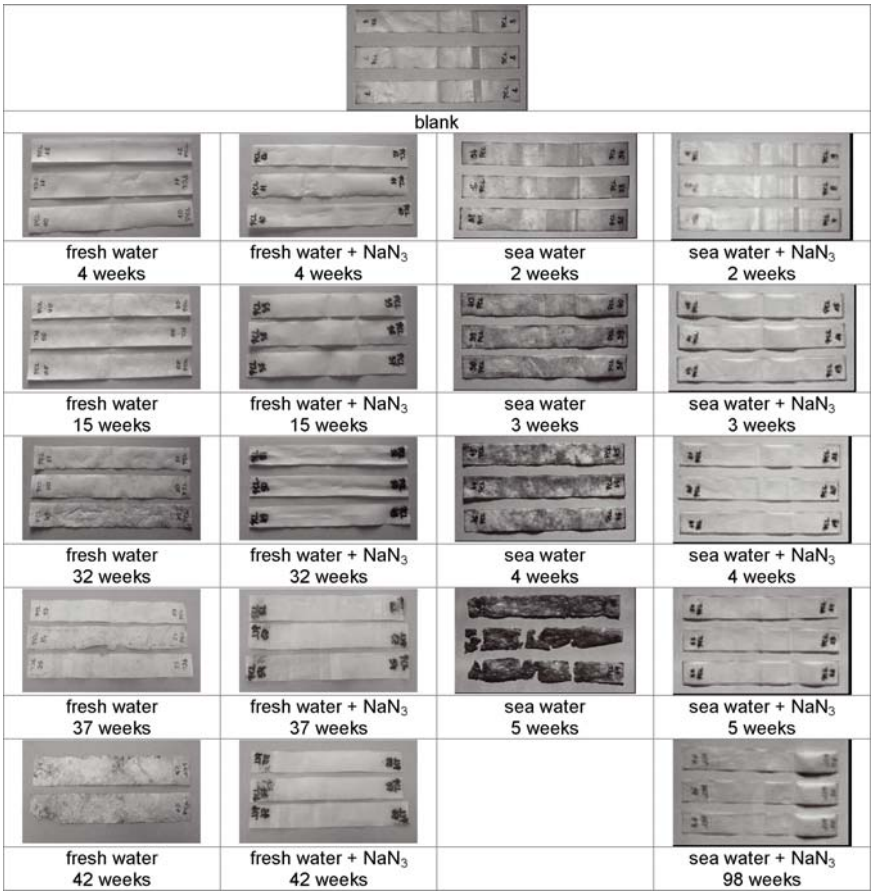

Figure 3. Macroscopic observations of the surface of poly(ε-caprolactone) samples before and after degradation in different water environments

Surface degradation or erosion involves the hydrolytic cleavage of the polymer backbone only at the surface. This situation arises when the rate of hydrolytic chain scission and the production of oligomers and monomers, which diffuse into the surroundings, is faster than the rate of water intrusion into the polymer bulk[2].

Biodegradation process of poly(ε-caprolactone) was very fast in The Baltic Sea. After 3 weeks incubation there were the dark brown places on poly(ε-caprolactone) which are a consequence of microorganisms activity (the weight loss was –10.7%). After 4 weeks the samples had a lot of small holes (the weight loss was –20.7%) and after 5 weeks the samples were break up into pieces (the weight loss was –33.8%). The mechanism of PCL degradation could be attributed to random hydrolytic chain scission of the ester linkages, which caused a decrease in molecular weight[2].

After first weeks incubation in fresh water (pond) the white tarnished places on the PCL surface could be observed, which were become more visible after longer incubation time. The first holes were noticed after 32 weeks biodegradation in fresh water (the weight loss was –17.6%). After 37 and 42 weeks incubation in fresh water the PCL samples were more thin and led to disruption (the weight loss was from –27.4% to –39%).

Because of the absence of microorganisms in laboratory conditions (fresh and sea water with sodium azide) at the end of incubation the samples were in good condition. Only chemical hydrolysis could be expected[8].

Microscopic observations of the surface of polymers during biodegradation process

Studying the biotic degradation of poly(ε-caprolactone) we observed that under natural water conditions the samples could degrade enzymatically, leading to polymer surface erosion.

The microscopic changes on the surface of poly(ε-caprolactone) after incubation in four different water environments are presented in Figure 4. After incubation in natural environments the samples were not homogenously destroyed and there were different images depending on where the picture was taken.

The surface of blank PCL sample, observed under metallographic microscope, consisted of two phases: crystalline (bright) and amorphous (dark). After 2 weeks incubation in The Baltic Sea and 4 weeks incubation in pond we observed an increase in crystallinity.

According to literature semicrystallinity of poly(ε-caprolactone) plays a critical role in degradation phenomena, because the amorphous phase is degraded first and as a result an increase in crystallinity of polymer occurs. After that the crystalline phase is degraded too[22–25].

The microscopic observations confirmed that the amorphous phase was degraded first. That was the first stage of biodegradation of PCL and it began from 2 weeks

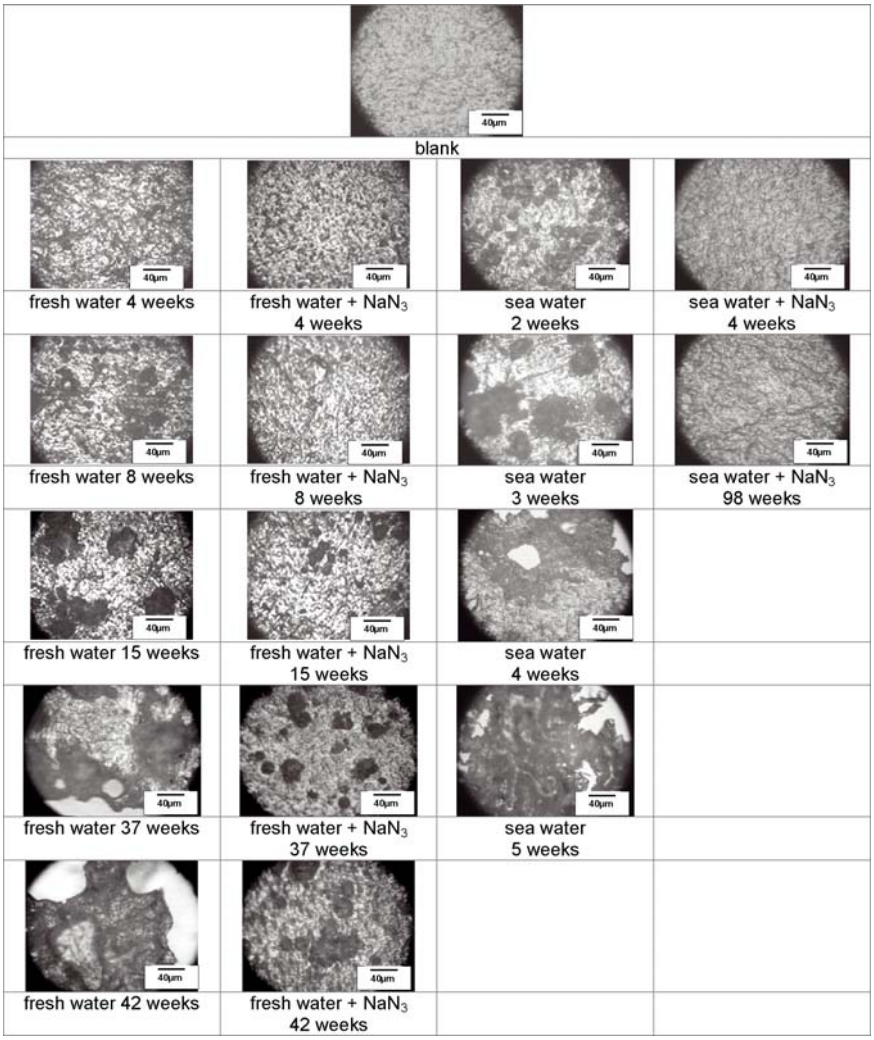

Figure 4. Microscopic observations of the surface of poly(ε-caprolactone) samples before and after degradation in different water environments

incubation in The Baltic Sea and 4 weeks incubation in pond. After 4 weeks incubation in natural sea water and 37 weeks incubation in pond the decrease of birefringent element was observed, crystalline phase began to degrade. That was the second stage of biodegradation of PCL. There were black areas on the surface of samples, which represented an agglomeration of microorganisms. There were probably psychrotropic and mesophilic bacteria under marine conditions, and there were presumably heterotrophic and epilitic bacteria in the pond. The kind of environments and their abiotic parameters indicate on the presence of microorganisms. Because the biodegradation process of poly(ε-caprolactone) in The Baltic Sea was very fast, we could observed holes on polymer film after 4 weeks incubation in that environment. The connection of holes to each other brought to fragmentation of polymer samples (it was after 5 weeks incubation).

Biodegradation process of poly(ε-caprolactone) in fresh water (pond) was more slowly. There were holes on the polymer film after 37 and 42 weeks of incubation in fresh water (pond).

Degradation process of PCL samples in laboratory fresh water (with NaN_3) was very slowly. The increase in crystallinity of polymer sample was visible after 8 and 15 weeks incubation in laboratory. There were not any wholes on the polymer films. These changes indicate only on chemical hydrolysis and PCL samples require more time to degradation.

There were no evident changes on surface of poly(ε-caprolactone) samples after 98 weeks incubation in sea water with sodium azide, which indicate on resistance of PCL to chemical hydrolysis, because there were salinity and absence of microorganisms.

Scanning electron microscope SEM analysis of polymers during biodegradation process

The microscopic changes on the morphology of poly(ε-caprolactone) samples under SEM after incubation in four different water environment are presented in Figure 5.

The morphology of blank poly(ε-caprolactone) samples were without orientation. Micrographs have shown the increase of small bright elements on the surface of PCL samples during all incubation time. It might be an evidence of increase in crystallinity as a result of degradation amorphous phase.

After 4 weeks incubation in natural sea water and also after 15 and 37 weeks incubation in pond we observed changes on the PCL morphology, which indicated on the enzymatic hydrolysis, which might be an evidence of presence of the microorganisms activity.

The changes on the PCL morphology after 37 weeks incubation in laboratory fresh water indicated only on chemical hydrolysis.

Degradation process of poly(ε-caprolactone) samples in sea water with sodium azide in laboratory was not very significant, which indicate on resistance to chemical hydrolysis.

It confirms results of DSC analysis and microscopic observations under methalographic microscope.

CONCLUSIONS

Abiotic parameters (temperature, pH, salinity) and biotic parameters (algae, bacteria) had an influence on the biodegradation process. Different kind of microorganisms in natural water environments differently influenced on the degradation process.

Biodegradation of poly(ε-caprolactone) in The Baltic Sea was faster than in pond environment. The samples

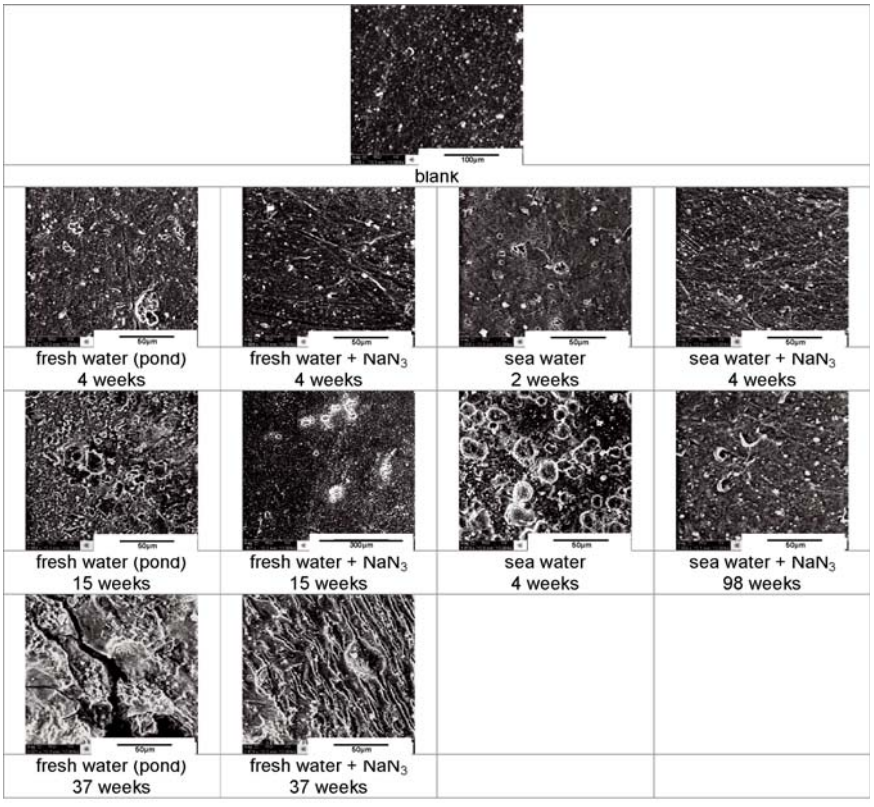

Figure 5. Microscopic observations of poly(ε-caprolactone) samples under SEM after degradation in different water environments

were completely assimilated after 6 weeks incubation in natural sea water. Weight loss has been attributed to an increased chain scission of a low molecular weight. During degradation process, changes observed for poly(ε-caprolactone) in both natural environment (fresh water and sea water) were as a result of an enzymatic hydrolysis, but in laboratory conditions there were only chemical hydrolysis. The obtained results indicate that poly(ε-caprolactone) was sensitive to enzymatic attack of microorganisms in natural environments and rather resistant to chemical hydrolysis.

Microscopic observations of the surface of poly(ε-caprolactone) incubated in natural environments lead to conclusion that the biodegradation process this polymer occurred in two stages. The first stage consisted of the degradation of amorphous phase, resulting in an increase in crystallinity of the polymer. The decrease in the rate of chain scission is associated with an increase in crystallinity, since cleavage takes place in the amorphous region of the polymer. The second stage started, when most of the amorphous regions were degraded – next, the crystalline phase was degraded. The polymer became prone to fragmentation and enzymatic surface erosion proceeded.

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