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Pilot study of the influence of thermoplastic starch based polymer packaging material on the growth of diatom population in sea water environment

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Novel polymeric packaging materials susceptible to environmentally friendly decomposition appeared on the global market. The paper is devoted to an investigation of the impact of degradable polymer packaging on marine life. The chosen polymer was a commercial packaging based on thermoplastic starch (TPS over 85%). The microorganism chosen was *Phaeodactylum tricornutum* diatom (identified in many aquatic reservoirs, with a tendency to flow with seawater plankton in places of great saline oscillation). The packaging material was incubated both in natural seawater and in the presence of diatom population. The *chlorophyll* a content was determined as the criterion of diatom growth in the presence of tested polymers. The polymer surface and the colour changes in the diatom culture were recorded photographically. The presence of polymeric samples significantly changed the kinetic of diatom growth in seawater during incubation affecting its biological balance. During the experiment in seawater, diatom adhered to polymer surfaces and the polymer stimulated their growth.

Keywords: thermoplastic starch, packaging, biodegradation, diatom, seawater environment.

INTRODUCTION

Nowadays, many food products and everyday use commodity are packed into laminated polymers, the so-called packaging foils. All kinds of plastic bags are applied during various transport issues. Consequently with technical improvement in packaging field, its advanced technology and economic development, the quantity of waste being generated and stored by society is also rising, causing an enormous demand for its proper disposal. Polymers left on the beach or dropped into the sea can cause a destructive impact on biodiversity and the quality of seawater.

Global packaging market is currently evolving, introducing the 2nd-generation polymer-packaging materials. Those new materials are able to compost/biodegrade in a biological degradation process being an environmentally friendly way of waste decomposition. The following examples include: cellulose, starch and their derivatives[1]; polyhydroxybutyrate (PHB, Biopol), as well as various modifications of the 1st- generation polymers used in packaging (polyethylene, polypropylene, etc.). Not only are new polymers said to minimize the difficulty of traditional polymer waste storage but they also decrease undergoing decomposition into simple, nontoxic compounds introduced into native environment.

Biodegradation tests of these polymers in accordance with ASTM D 5209-91 standard[2] were performed by Biatoli et al. in aerobic conditions in the presence of municipal sewage sediments. The authors tested starch composites (like Mater-Bi). The presented outcome confirmed their biodegradable character. Another regulation that verifies the compostable/biodegradable character of the 2nd-generation polymers is the European standard EN13432:2000. In accordance with this standard the starch composites were classified as the fully biodegradable polymer in the manner of microbiological composition. Starch is the most susceptible to degradation and microorganism's attack.

Rutkowska et al.[3] was involved in the research of material based on thermoplastic starch (TPS) and cellulose derivatives from their natural origin, such as biodegradation under natural weather conditions in the compost with sewage sludge. Degradation tests were performed at a waste treatment plant and under laboratory conditions at stable temperature. Changes in the weight and the morphology of the polymeric material were tested. As the final result enzymatic degradation was proved through dehydrogenases. Y class Mater-Bi lost more than 90% of weight after 4 months when disposed of in the standard environment under controlled conditions. On contrary in the investigated natural, weather-dependent, composting conditions this material lost about 20% of weight after the same period of the incubation.

Mezzanotte et al.[4] conducted a series of biodegradation tests according to the standard test method ISO 14851 in order to compare the performance of different activated sludge inocula on different plastic materials (polycaprolactone (PCL) and starch-based material). The inocula were derived from two municipal wastewater treatment plants and from the treatment plant of a pharmaceutical company. The starch-based material was degraded to similar or higher extents than PCL in the municipal sludge. Industrial sludge gave good results with PCL and starch-based materials (PCL = 100%; starch-based material = 89% weight loss), but turned out to be less active towards cellulose.

Alvarez et al.[5] studied degradation of TPS reinforced with sisal fibre composite in the soil with the contribution of bacterial micro-flora and fungus. The tested materials consisted of 38% of thermo-plastified starch, 38% of cellulose derivatives and 22% of additives (i.e. natural plasticizers). The test was performed for 400 days. The authors observed water absorption by the polymer as the first step of its changes due to the biodegradation process. Water sorption results indicated that the composites absorbed less water than the matrix (pure starch). Further-

more, trapped water facilitated the microorganism attack, through bond hydrolysis. In the soil environment degradation of composites with natural fibres and matrix proceeded on a similar level. After 12 months' incubation period, filamentary microorganism on the starch polymer surface, as fungus *Actinomycetes* were noticed.

The effect of various additives on the 2nd-generation polymers biodegradation rate under natural conditions (in the compost with activated sludge and in seawater) was investigated by Rutkowska et al.[6]. The tests included starch, chalk or processing additives (as poly(ethyl-butyl acrylate) with oxidant, PE-LD, oleic acid amides) modified PCL. Moreover, the researchers examined the environmental degradation of polymer materials like TPS, and modified cellulose in The Baltic Sea at Nordic Wharf of Gdynia harbor[7]. The degradation process was also tested in laboratory conditions in seawater with sodium azide (eliminating the microorganisms activity) added. It was found that in the natural environment of seawater the enzymatic hydrolysis of the tested materials occurred (clear erosion of the surface and weight loss). The samples of the modified cellulose were more susceptible to the attacks of microorganisms living in seawater than the samples of the thermoplastic starch.

Based on the literature data, it can be stated that starch-based polymers are biodegradable packaging polymeric materials in various environments to the different extent. However, there have been no tests undertaken so far to follow the sea life balance in case the polymer degradable packaging materials appeared in the sea environment. The aim of this paper was to investigate the influence of commercial packaging polymeric material based on TPS on the seawater environment. The growth of marine diatom population in natural seawater in the presence of the polymer material was verified. Diatom was used as a biological pilot sensor (one of the suggested by the authors marine indicators) to assess the impact of polymer debris on the condition of natural seawater. Generally, diatoms are broadly used for water quality testing. They are very sensitive to any pollution in seawater environment[8, 9, 10].

MATERIAL AND METHODS

Material

As the tested material, commercial packaging bags (polymer composite with over 85% content of TPS) used by Carrefour Network were chosen. The bag is stamped as compostable in accordance with EN13432:2000.

Polymeric samples cut into stripes (20x150mm), were divided between two experimental systems. For setting the A system the stripes were sterilized in the UV light (immersed in 3% H_2O_2 for 30 min) before the experimental system was set up and for system B the stripes were not subjected to any sterilization process.

Environments

Natural seawater, which constitutes the experimental medium for both systems, was taken from the Baltic Sea (Sopot) on 28 July 2008.

System A

The water was filtered and sterilized to remove suspension and microorganisms. Afterwards, the water was enriched with biogenic salts – microelements, macroelements and vitamins. Finally the algae inoculums was added into a defined amount rich with nutrients seawater.

The algae tested was an axenic culture of the diatom *Phaeodactylum tricornutum* Bohlin (SAG 1090-la) obtained from the German Collection of Göttingen University[11]. The organism was kept in the Biochemical Laboratory Algae Collection of Polish Academy of Sciences Oceanology Institute in Sopot. Cells were grown in original F/2 medium and in the nutrient-enriched Baltic seawater (1.5g dm^{-3} $NaNO_3$ and 0.04 g dm^{-3} K_2HPO_4). The *P. tricornutum inoculum* was incubated for 2 weeks in the original and 24 weeks in the modified medium- enriched seawater (in the presence and without the presence of polymer samples).

The water-inoculum set-up was stirred magnetically. The medium with a defined initial pH, salinity and *chlorophyll* a content was used in the experimental system.

The sterilized polymer samples were placed in the 500 cm^3 Erlenmeyer flasks (previously cleaned, degreased and sterilized) and covered with 300 cm^3 sea medium containing diatom. At the same time 3 repeats and 3 blank samples (controls containing only medium plus diatom) were prepared. The Erlenmayer flasks were stoppered with sterile cotton plugs. These operations were carried out in a laminar flow cabinet under a UV lamp.

The experimental system was incubated at a constant temperature 24±0.5°C for 24 weeks under constant daylight lamps providing an irradiance of 7.63 $\mu M/m^2/s$ (LiCor equipped with a spherical sensor SPQA2005 and undirectional sensor Q21859). The closed system was stirred manually from time to time throughout the analysis.

The initial polymer concentration in the experimental flask was ca. 0.001g/cm^3 (2 stripes per flask). The whole system was controlled in respect of the sterility. The sterility of the inoculum and the polymer samples was checked 7 days after the system had been set up. All the tests proved its sterility.

System B

The incubation of the tested polymer samples took place in the natural seawater under laboratory conditions at a stable temperature. The seawater medium was not modified and enriched, its content was not changed.

The water-polymer set up was settled in 10 l aquarium. The whole system was aerated by aquarium pomp to keep a similar condition as in natural aerobic environment. The changes of weight loss were monitored after 12, 24 and 36 weeks. That kind of test appeared to be useful to estimate the degradation progress of starch-based materials[3, 6, 7].

Methods

Investigation of diatom growth:

– *Visual observation* was performed during which diatom culture colour changes and polymer surface view were registered. The photographs were taken at fixed intervals. The colour intensity of the samples with polymer strips was compared with that of the control samples.

– *The content of chlorophyll* a (Jeffrey and Humphrey 1975) was measured spectrophotometrically to assess the growth of *P. tricornutum* (HITACHI U-2800 UV-VIS – at λ=750 nm, λ=665 nm, λ=630 nm and λ=480 nm) per

unit of culture volume (turbidity was indentified on λ=750 nm, as the reference 90% acetone was used).

Investigation of polymer weight loss

After the proper incubation time, the samples were removed from the seawater environment, washed with distilled water and dried at room temperature to a constant weight.

– *Weight changes* (%) were determined using an analytical balance RadWag (WAS110/X). The polymer samples were weighed before and after the degradation process.

RESULTS AND DISCUSSION

Visual observation

In Fig. 1 the image of system A after the 1st week of the incubation was presented (3 samples of diatom culture with the tested polymer samples – 1M, 2M, 3M).

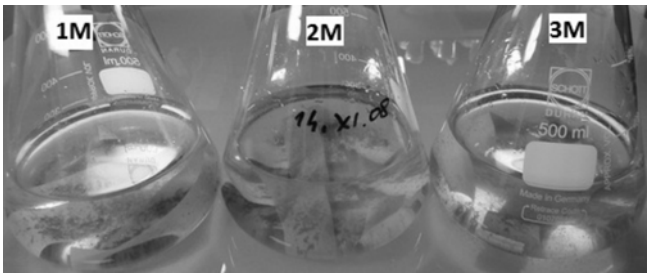

Figure 1. The view of *P. tricornutum* culture after 1st week of the incubation in the presence of polymer samples (M1, M2, M3)

After the 1st week the colour of the system became more intense than initially (t = 0). At that time, there were no visible differences between the controls and the samples of diatom culture incubated with polymer stripes. Based on the visual estimation, the growth of the diatom cells was on the same level in all flasks.

In comparison with the beginning, after the 2nd week of the incubation extensive cell growth was observed (both in the controls and in the samples with polymeric material). *P. tricornutum* culture created brownish colour of the solution which is characteristic of this algae. The surface of the tested polymer has not changed till that time.

After the 12th week, it was noticed that the growth of the diatom culture in the presence of the polymer stripes was still rising (the colour was intense brownish). Therefore, the gain of the mass of the selected bio-indicator was observed. The diatom adhered to the polymer surface. However, the manual active shaking of each flask broke this bonding. The diatom growth was visibly greater for the samples with polymeric material than for the controls. The colour of the solution of diatom culture in the controls also changed comparing with the 2nd week, however the change was not so obvious as it was for the samples with the polymeric material. The surface of the tested polymer still has not changed.

After the 16th week of the incubation the system colour (both in the controls and in the samples with the polymer material) became pale (comparing with the 12th week). The bio-indicator mass was reduced in all the experimental flasks. Therefore the seawater became an unfavourable environment for diatom growth both in the controls and in the samples with the tested polymer. The surface of the tested polymer did not change.

Fig. 2 shows the image of the system A after the 24th week of TPS composite incubation with *P. tricornutum* culture and control sample. The opaque brownish colour of the solution was observed for all the flasks.

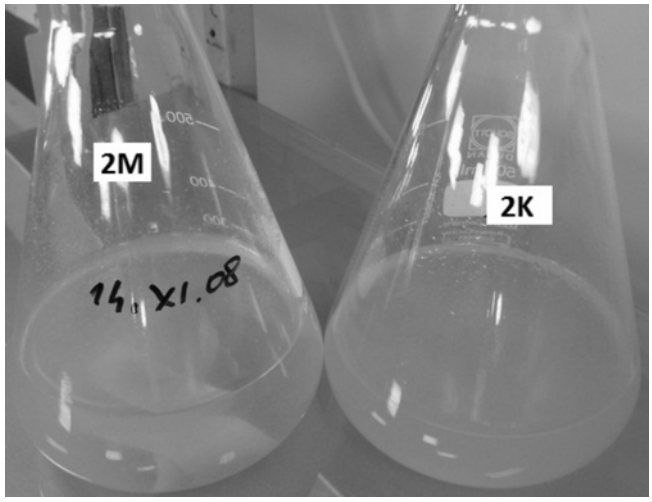

Figure 2. The view of *P. tricornutum* diatom culture starting from the left with the polymer sample and control in 24th week of the incubation in seawater

With regard to the results presented in Fig. 1 and Fig. 2 it was concluded that colour system was changing during the whole experimental time. Initially (up to 12th week) the colour was darkening, which indicated that diatom biomass was rising. In the terminal period (from the 16th to the 24th) the colour of the controls and the samples with polymer became pale (diatom population was mortified in all the experimental flasks).

In the last period of the experiment, after the 24th week of the incubation, it was noticed that the polymer colour was also changed from vivid green into greenish. Discoloration and light deposits could be observed on their surface, which proved that its morphology changed.

Content of chlorophyll a

Table 1 lists the results of photosynthetic pigments absorbance measurement in a given wavelength (λ=750 nm, 665 nm, 480 nm and 630 nm) for terminal system samples (controls and samples of diatom culture incubated in the presence of the polymer).

Table 2 lists the chlorophyll a content (*chlorophyll a* active and total) for terminal system samples (the controls and the samples of diatom culture incubated in the presence of the polymer). The content of each experimental flask was measured three times. The given data were submitted to statistical analysis using Dixon's Q-test. The values that differ considerably from the majority of the rest were rejected. Then the average chlorophyll a contents with standard deviation was calculated for control samples and *P. triconrmutum* culture with polymer samples.

From the results gathered in Table 1 and 2, it can be seen that production of *chlorophyll a* in the *P. tricornutum* culture incubated in the presence of polymers in seawater was higher than for control.

A higher chlorophyll content compared to the reference sample (%K) was found for the samples incubated in the presence of TPS composite (251% for total *chlorophyll a* content and 268% for active one). The presence of starch

Table 1. Absorbance quantity ABS (for λ=750 nm, 665 nm, 480 nm and 630 nm) for 24th week of *P. tricornutum* culture incubated in the presence of the polymer samples/acetone extracts.

Tested sample	Sample identification	Absorbance quantity for different wavelengths [nm]			
		665 before acidification [ABS665-ABS750]	665 after acidification [ABS665-ABS750]	480	630
CONTROL (K)	$1K_1$	0.058	0.035	0.171	0.015
	$1K_2$	0.064	0.039	0.187	0.017
	$1K_3$	0.066	0.041	0.191	0.016
	$2K_1$	0.058	0.035	0.166	0.015
	$2K_2$	0.057	0.035	0.158	0.014
	$2K_3$	0.064	0.038	0.185	0.017
	$3K_1$	0.051	0.031	0.146	0.013
	$3K_2$	0.067	0.043	0.196	0.023
	$3K_3$	0.051	0.031	0.148	0.013
TPS - rich packaging material (M)	$1M_1$	0.092	0.054	0.237	0.022
	$1M_2$	0.153	0.09	0.390	0.040
	$1M_3$	0.157	0.09	0.394	0.038
	$2M_1$	0.139	0.081	0.365	0.034
	$2M_2$	0.14	0.082	0.365	0.035
	$2M_3$	0.146	0.084	0.374	0.035
	$3M_1$	0.143	0.083	0.351	0.034
	$3M_2$	0.16	0.101	0.387	0.037
	$3M_3$	0.158	0.089	0.391	0.037

ABS_{750} – turbidity
ABS_{480}, ABS_{630} – absorbance before acidification after subtraction of turbidity quantity

Table 2. Comparison of *chlorophyll* a content in *P. tricornutum* culture incubated with and without tested polymer

Tested sample	Sample identif.	chlorophyll a concentration [mg/dm^3]									
		total[1]					active[2]				%K
		X	$\overline{X}_1 \pm$ SD	x	$\overline{X}_2 \pm$ SD	%K	x	$\overline{X}_1 \pm$ SD	x	$\overline{X}_2 \pm$ SD	
CONTROL (K)	$1K_1$	0.34	0.36 ± 0.02	0.34	0.34 ± 0.04	100	0.31*	0.33 ± 0.00	0.31	0.31 ± 0.03	100
	$1K_2$	0.37		0.37			0.33		0.33		
	$1K_3$	0.38		0.38			0.33		0.33		
	$2K_1$	0.34	0.34 ± 0.02	0.34			0.31	0.32 ± 0.03	0.31		
	$2K_2$	0.33		0.33			0.29		0.29		
	$2K_3$	0.37		0.37			0.35		0.35		
	$3K_1$	0.29	0.29 ± 0.00	0.29			0.27	0.27 ± 0.00	0.27		
	$3K_2$	0.38*		0.38			0.32*		0.32		
	$3K_3$	0.30		0.30			0.27		0.27		
TPS - rich packaging material (M)	$1M_1$	0.53	0.77 ± 0.21	0.53*	0.86 ± 0.05	251	0.51	0.75 ± 0.21	0.51*	0.83 ± 0.06	268
	$1M_2$	0.88		0.88			0.84		0.84		
	$1M_3$	0.91		0.91			0.89		0.89		
	$2M_1$	0.80	0.82 ± 0.02	0.80			0.77	0.77 ± 0.00	0.77		
	$2M_2$	0.81		0.81			0.77		0.77		
	$2M_3$	0.84		0.84			0.83*		0.83		
	$3M_1$	0.82	0.89 ± 0.05	0.82			0.80	0.84 ± 0.07	0.80		
	$3M_2$	0.92		0.92			0.79		0.79		
	$3M_3$	0.91		0.91			0.92		0.92		

$\overline{X}_1$ – the average value taken from 3 repeats
$\overline{X}_2$ – the average value taken from 9 repeats
SD - standard deviation
* result skip in Dixon's Q-test with probability 95%,
– *chlorophyll a* total[1] – in accordance with Jeffrey and Humphrey (1975)
– *chlorophyll a* active[2] – in accordance with Lorenzen (1967)

stimulated the growth of diatom culture in the seawater environment.

Weight changes (%)

Fig. 3 presents the results of weight changes recorded after the 12th, 24th and 36th week of the incubation in the natural seawater for the investigated TSP samples.

After 12 weeks of the incubation in natural seawater, the decrease of polymer weight was observed on the level of 4.4%. Finally, after 36 weeks the weight dropped up to 5%. Those results might be a possible indication of the beginning of polymer degradation in the natural seawater. According to the literature, mentioned previously, the simple weight changes tests are adequate for the registra-

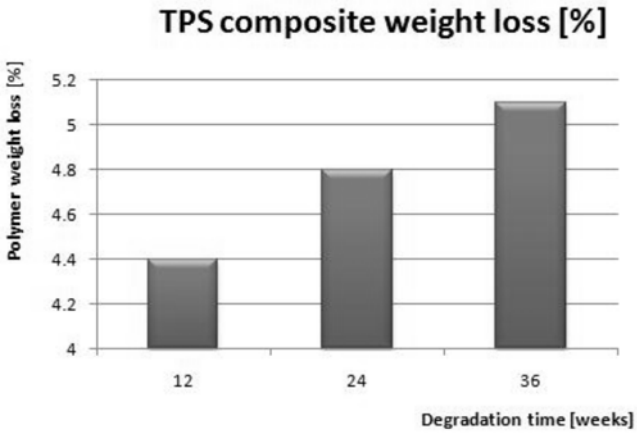

Figure 3. Weight loss analysis (%) for TPS – rich packaging material incubated in natural seawater (for 12, 24, 36 weeks)

tion of the degradation degree of starch-based materials in the seawater.

CONCLUSION

Macroscopic observations proved the influence of the TPS polymer on *P. tricornutum* marine diatom growth. During the experiment in system A the change of the seawater medium colour was observed. The most intensive colour system was seen for *P. tricornutum* culture incubated with the tested polymer. The more intensive colour of the system, the higher number of cells in the inoculum is detected. Throughout the incubation period diatom *P. tricornutum* cells adhered into TPS composites polymer surface and its growth was stimulated. The tested polymer was capable of interfering the balance of marine environment being a source and energy for *P. tricornutum*. Polymer degradation products could be the reason of the higher degree of diatom growth in comparison with the blank samples. Further research is going to be taken to follow that observation to a more detailed examination.

The increase of diatom biomass was in correlation with the increase of pigment content. More than 100% *chlorophyll* a gain in samples with polymer material was observed comparing with the control ones. It was proved that the presence of polymeric sample significantly changed the kinetic of diatom growth in the seawater after 24 weeks of the incubation.

From the test carried out in system B it was stated that in natural seawater (without *P. tricornutum* diatom) only partial destruction of the tested polymer took place, resulting in around 5% polymer weight loss after 36 weeks of the incubation.

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