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TETIANA POKYNBRODA*[1], NATALIIA KORETSKA[1], AGNIESZKA GĄSZCZAK[2], ELŻBIETA SZCZYRBA[2]

# BIODEGRADATION OF POLYETHYLENE USING SOIL BACTERIA AND RHAMNOLIPIDS

[1]Department of Physical Chemistry of Fossil Fuels of the Institute of Physical-Organic Chemistry and Coal Chemistry named after L. M. Lytvynenko of the National Academy of Sciences of Ukraine, Naukova str, 79060, Lviv, Ukraine
[2]The Institute of Chemical Engineering, Polish Academy of Sciences, Bałtycka 5, 44-100 Gliwice

Seven strains of bacteria were isolated from the landfill. The isolates were co-cultivated with LDPE and rhamnolipids. Changes in the structure of LDPE films after 28 days of exposure to bacteria were confirmed by FTIR spectroscopy. The toxicity of plastic biodegradation products in a liquid nutrient medium was investigated and their safety for plants was shown. However, these biodegradation products have acute lethal toxicity for the crustacean *Daphnia magna*.

*Keywords:* biodegradation, polyethylene, rhamnolipids

## 1. INTRODUCTION

Plastic has been with us since the beginning of the 20th century. It's considered an affordable, versatile, and durable material, hence, the worldwide production of plastic is still rising. Over the past 10 years, the global production of plastic has increased by 97 Mt and is 367 Mt. Now, the world is producing twice as much plastic wastes as two decades ago. The bulk of them ends up in landfill, incinerated, or leaking into the environment, and only 9% are successfully recycled, according

* ✉ pokynbroda@ukr.net 

to a new OECD report. Today, hundreds of polymers are produced on an industrial scale [1]. These are mainly inexpensive thermoplastic polymers: polyethylene terephthalate (PET), polyethylene (High Density Polyethylene (HDPE), Low Density Polyethylene (LDPE), and Linear Low Density Polyethylene (LLDPE)), polyvinyl chloride (PCV), polypropylene (PP), and polystyrene (PS). These materials were created as an invention that was supposed to improve the quality of human life. However, the misuse of plastics and a lack of moderation have made these materials ubiquitous, causing serious challenges for society and the economy, and above all for the environment. Disposable plastic protective equipment and plastic packaging lead to a steady increase in solid waste and pollution on soil, they affect the natural ecosystem and people's health [2]. Mindless, uncontrolled use of them may lead to a global environmental catastrophe with consequences that we cannot fully predict. A promising strategy to overcome the accumulation of plastics and their derived in the environment is their biodegradation. It is a natural process that keeps our planet clean and healthy. Unfortunately, this environmentally friendly way of pollutant utilization is not a fast process, and the rate at which wastes are produced far outpaces the rate of natural biodegradation, leading to an unsustainable state.

The most popular plastic is polyethylene, which accounts for almost 30% of all polymers produced. Polyethylene (PE) has a semi-crystalline structure and it is composed of a linear chain of carbons held together by hydrogen bonds. Although this plastic is resistant to biodegradation due to its structure and lack of reactive functional groups, high molecular weight, and strong hydrophobic properties, many microorganisms have developed the ability to use them as a source of carbon and energy. Biodegradation of the polyethylene was a major subject of several research and reviews [3-5]. Microorganisms capable of hydrolyzing PE were isolated from soil, seawater, compost, activated sludge [6], the gut of the greater worms *Galleria melonella* [7], *Tenebrio molitor* [8], *Plodia interpunctella* [9].

Pre-treatment is often necessary to facilitate the decomposition of polymers by microorganisms. The purpose of this procedure is to reduce the average length of the polymer chain or to modify its surface (reducing the hydrophobicity of the polymer, introducing carbonyl or hydroxyl groups). The most commonly used pre-treatment techniques are UV radiation, gamma radiation, high temperature, and nitric acid treatment [11]. Bacterial species *Bacillus* spp. [10], *Rhodococcus* spp. [12], and *Pseudomonas* spp. [13], and fungi *Aspergillus* and *Fusarium* [14] were depolymerizing PE after pretreatment. Untreated PE was also degraded by a few other bacteria: *Acinetobacter pittii* IRN19, *Micrococcus luteus* IRN20 [15], different species of *Pseudomonas* (*Pseudomonas putida* IRN22, *Pseudomonas aeruginosa*

PAO1, *P. aeruginosa* ATCC, and *Pseudomonas syringae*, *Pseudomonas* sp. E4 [16,17] and bacteria from the genera *Delftia*, *Comamonas*, and *Stenotrophomonas* [18].

The production of surfactant compounds by bacteria is key to bacterial degradation of PE, as biosurfactants reduce interfacial tension and facilitate bacterial adhesion to PE surfaces. Biosurfactants increase the water bioavailability of hydrophobic water-insoluble substances and change the properties of the bacterial cell surface. Because of their potential advantages, biosurfactants are widely used in many industries such as food production, chemistry, cosmetics, pharmaceutics, and agriculture. Various surfactants (both synthetic and biogenic) are used for the biodegradation of plastics.

The addition of the nonionic surfactant Tween-80 (0.5%) to LDPE resulted in a higher biodegradation rate as observed by ATR-FTIR, DSC, and CL than in LDPE without surfactant [19]. Light microscopy showed a larger number of microorganisms on LDPE films with Tween-80 than on films without this surfactant. Tribedi and Sil [20] showed that the addition of mineral oil to the *Pseudomonas* sp. strain AKS2 culture enhanced bacterial attachment to the LDPE surface. It resulted in increased biofilm formation and enhanced polymer degradation (5% of the original PE material for 45 d). In the same studies, Tween 80 was found to have an adverse effect on biofilm formation.

Karlsson et al. exposed low-density polyethylene samples to UV radiation for 27 days [21]. Thereafter they were added to biotic and abiotic environments. The experiment lasted for 10 years. The addition of a surfactant (5% palmitate iron carboxylate-Fe(III)hydroxide) to a nutrient solution containing PE resulted in an increased degradation rate. The capabilities of a microbial consortium, composed of *Penicillium raperi*, *Aspergillus flavus*, *Penicillium glaucoroseum*, and *Pseudomonas* spp., for biodegradation of ultraviolet (UV)-pretreated and un-pretreated mixed plastics (polyethylene, polystyrene foam, and polyethylene terephthalate) in the presence of biosurfactant (rhamnolipid, 0.016%) was examined [22]. The utilization of biosurfactant had negative effects on biodegradation and wettability of PE, due to the consumption of rhamnolipid as a carbon source rather than the plastic itself. The chemical transformation of PE remained unchanged in all conditions except "UV-pretreated & bio-treated". Biodegradation of polyethylene was carried out using *B.subtilis* [23]. The addition of biosurfactant (surfactin, 0.03 %) helped in the attachment of microbes to PE films and thereby enabling them to use polymer as a carbon source at a faster rate.

In this study, we focused on the biodegradation of low-density polyethylene as the most common polymer used for single-use packaging. Rhamnolipids are a class

of biosurfactants that contain rhamnose as the sugar moiety linked to β-hydroxylated fatty acid chains. They are well-known biosurfactants that are usually used for the bioremediation of contaminated soils or water [24] and in many industries including petroleum, food, agriculture, etc. Rhamnolipids have no significant hazardous impact on the biotic constituents of the environment such as insect larvae, mammalian cells, and skin tissues [25].

In our opinion, to solve the problem of plastic pollution in the environment, we should focus on the isolation of microorganisms that are able to degrade plastic and synthesize biosurfactants. Therefore, the aim of the work was the isolation of microorganisms - potential destructors of plastic, and the study of the effect of rhamnolipids on the process of polyethylene biodegradation.

## 2. METHODS

LDPE-degrading bacteria were obtained using plastics as the sole carbon source in the culture medium. The bacteria were isolated from the soil at the landfill (Bytom, Poland). First, soil samples were taken from the landfill at a depth of 20 cm. Impurities were removed from the samples and then weighed. About 10 g of soil was mixed with 90 ml of sterile water.

Four plastic samples of LDPE, HDPE, PP, and PET and corresponding thicknesses of 0.14 mm, 0.3 mm, 2 mm, and 0.24 mm were purchased locally. Three sets of plastic samples were produced as 30 mm×30 mm films. Before use, the plastics were disinfected with 75% ethanol for 1 hour, washed 3 times with sterilized distilled water, and dried in an oven at 60 °C overnight. Films were first disinfected and weighed (± 0.02). Then they were added to culture flasks with sterile mineral salts medium (MSM). Mineral salts media was prepared as 0.7 g of $KH_2PO_4$, 0.7 g of $K_2HPO_4$, 0.7 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of $NH_4NO_3$, 0.005 g of NaCl, 0.002 g of $FeSO_4 \cdot 7H_2O$, 0.002 g of $ZnSO_4 \cdot 7H_2O$, and 0.001 g of $MnSO_4 \cdot H_2O$ per 1000 mL of sterilized water [26] and autoclaved at 121 °C for 20 min. These culture flasks were incubated at 30 °C with rotation at 130 rpm for 21 days. A total of 7 different strains of bacteria were isolated. Bacterial strains were stored on nutrient agar.

The determination of microbial physiological and biochemical indicators was carried out using the method of bacterial identification in “Berger’s Bacterial Identification Manual” (eighth edition). Gram staining method [27] and biochemical tests [28] were used for the identification of microorganisms.

Catalyze test: A culture sample was placed on the slide and then hydrogen peroxide was added to the slide drop by drop. Samples that showed bubble formation indicated positive results.

Oxidase test: The smear of a colony of the isolated bacterium was introduced on a filter paper containing a few drops of oxidase reagent (0.1g of tetramethyl-p-phenylenediamine dihydrochloride in 100 ml of distilled water). The colony having oxidase activity gave a characteristic color.

Motility test: The mobility test of both isolated strains was performed in a semi-solid agar medium containing 0.4 g agar, 2 mg peptone, and 1 g NaCl in 200 ml of distilled water. The sterilized straight wire was used to inoculate the culture in a single stab centrally from top to bottom of the medium. The incubation of the tube was carried out at 30ºC overnight. A diffused hazy growth represented a motile bacterium, spread throughout the medium.

Casein hydrolysis: Bacteria were inoculated on nutrient agar with milk. Culture agar plates were kept in the incubator for one or two days. The transparent zones around the bacterial growth lines testify to their ability to hydrolyze casein.

Gelatin hydrolysis: The overnight culture suspension was pipetted into two tubes that contained a gelatin agar base. Both of these tubes were kept in an incubator at 37ºC. One tube was tested after 4 hours and another one was tested after 24 hours.

Starch hydrolysis test: The tested bacteria were grown on agar plates containing starch. Since no color change occurs in the medium when organisms hydrolyze starch, an iodine solution was added as an indicator to the plate after incubation. While the non-hydrolyzed starch forms dark blue color with iodine, its hydrolyzed end products do not acquire such dark blue color with iodine.

Lecithinase test: In egg yolk agar, the lipoprotein component lecithovitellin can be split by lecithinase into phosphorylcholine and an insoluble diglyceride, which results in the formation of a precipitate in the medium. Microorganisms that possess the enzyme lecithinase break down lecithin to insoluble diglyceride and phosphorylcholine, which results in a white opaque zone of precipitation that spreads beyond the edge of the colony.

Pathogenicity test by potato: first, the potato tubers were sterilized and cut into slices (1.0 cm in thickness) with a sterile blade. Those slices were inoculated and incubated for 24-48 h at 28 ± 2ºC in Petri plates with sterile filter paper at the bottom. Tuber slice wetted with sterile water in one Petri plate was kept as control. Softening of the inoculated tuber slices was taken as a positive reaction [29].

The capability of a microbial consortium, composed of 7 strains, for biodegradation of polyethylene (LDPE) in the presence of biosurfactant

(rhamnolipid) was examined. Rhamnolipids were obtained during the cultivation of the strain *Pseudomonas* sp. PS-17 [30]. The biodegradation test was initiated after adding the established consortium of seven strains (to a final optical density of 0.1 (λ=550 nm)) to each flask with 250 mL of MSM and a biosurfactant solution (0.1 g/L). Each flask contained six pieces of pre-disinfected 30 x 30 mm PE plastic. All flasks were incubated at 30°C, 130 rpm for 28 days. All experiments were performed in duplicate, and results were expressed as mean ± standard deviation.

The change in the number of cells of microorganisms was determined by the spectrophotometric method by measuring the turbidity of the culture liquid at 550 nm (spectrophotometer HACH Lange DR 3900).

The chemical transformation of plastic samples was examined by the ATR-FTIR technique (PerkinElmer, USA). The clean and disinfected plastic films were scanned on both sides, 8 times at a resolution of 4 $cm^{-1}$ from 4000 to 400 $cm^{-1}$.

Wettability. The water contact angle technique was used to assess the hydrophobicity changes of plastic samples. To define LDPE hydrophobicity changes static contact angle variation at room temperature was measured, between a drop of distilled water and the LDPE surface by musing sessile drop method and recording with a video camera (JVC™ GZ-EX355 Everio). To this end, 50 μL deionized water was placed on top of the sheets, and photographs were captured at three points of the sample.

Weight loss was examined on plastic films. After incubation for 28 days, the LDPE films were removed and measured to determine weight loss. The plastic films (were gently washed 3 times with 75 % ethanol, sterilized water, and then immersed in 30 mL 10 % SDS solution for 24 h. Plastic films were then oven-dried at 65 °C for 24 h and their weight was measured by a 6- digit balance. Weight loss was calculated as:

$$\Delta m = \frac{m_0 - m}{m_0} \cdot 100\%$$

The phytotoxicity of cell-free culture supernatant was evaluated in a static test [31]. Seeds were purchased from a local company. Their germination potential was examined at 22 ± 2°C in darkness, prior to the assays as a control for the (90% guaranteed) viability of the seeds. The static test was based on root elongation and seed germination of *Lepidium sativum* and *Triticum aestivum* L. 10 seeds were placed on each plate (diameter 75 mm) to the filter paper and 4 mL of the cell-free culture supernatant or water was added. The plates were maintained at room temperature (22 ± 2ºC). Seed germination and root elongation (≥5 mm) were

determined after 5 days of incubation in the dark. Relative seed germination, relative root length, and germination index were then determined as seen below;

$$\text{Relative seed germination} = \frac{\text{number of seeds germinated in the supernatant}}{\text{number of seeds germinated in the control}} \cdot 100\%$$

$$\text{Relative root length} = \frac{\text{mean root length in the supernatant}}{\text{mean root length in the control}} \cdot 100\%$$

Toxicity studies were performed in a fermented medium without bacterial cells (centrifugation at 4°C, 15 min, 5000 g, Eppendorf AG 22334 Hamburg ). Toxicity tests using the microcrustacean *Daphnia magna* were performed on organisms aged from 6 to 24 hours. Toxicity was measured by the effect on mortality after 24 and 48 hours of exposure [32].

All experiments were performed in duplicate, and results were expressed as mean ± standard deviation. All the experiments with plants and microcrustaceans were carried out in five replicates.

## 3. RESULTS

The first task was to isolate bacteria - potential destroyers of plastic. An extract was prepared from the soil from the landfill, which, together with polyethylene film, was introduced into a solution of mineral salts. No other carbon source was introduced. The flasks were placed in an incubator for three weeks. After this time, bacteria were sown on nutrient agar. At the end, seven different strains of bacteria - potential destroyers of plastic - were isolated. Their morphological features, such as size, shape, structure, edge contour, and color of the colony, were established (Table 1).

Gram staining was performed. Among the bacterial isolates from the soil, five were gram-negative and two were gram-positive. Another element of bacterial identification is the biochemical profile, which also helps to determine the possibilities of wider application of the tested bacteria. A test for catalase and oxidase activity was performed. It was established that catalase is produced by four bacterial isolates. Five isolates demonstrate oxidase activity.

Table 1. Morphological features of bacterial isolates from the soil

| Bacterial isolates | Morphology | Pigmentation | Diameter, mm |
|---|---|---|---|
| 1 | Colonies are round, flat-convex, flat, transparent, shiny; the contour of the edge is even; the structure is uniform; the consistency is paste-like | light cream | 5 |
| 2 | Colonies are round, flat-convex, opaque, smooth, and shiny; the contour of the edge is even; the consistency is paste-like | light yellow | 1-2 |
| 3 | Colonies of irregular shape, cloudy, flat-convex, the surface is radially striated; the contour of the edge is jagged; the consistency is paste-like. | cream | 5-8 |
| 4 | Colonies are round, flat-convex with a raised center, the surface is rounded, shiny with a shine, and transparent; the contour of the edge is wavy; the structure is uniform; the consistency is paste-like | yellow | 1-2 |
| 5 | Colonies are round, drop-shaped, smooth, shiny, and opaque, the contour of the edge is even; the consistency is pasty | orange | 1-2 |
| 6 | Colonies are rhizoidal, bent, not smooth, opaque, the contour of the edge is wavy; the consistency is brittle, dry | white | 5-6 |
| 7 | Colonies are round, convex, smooth, shiny, and opaque, the contour of the edge is even; the consistency is pasty | brown | 1 |

The ability to hydrolyze casein and gelatin, as well as lecithinase activity, were also tested. Gelatin and casein are hydrolyzed by five isolates, and lecithinase is produced by only two. The amylase activity test showed that only two isolates hydrolyzed starch. Studying the phytopathogenicity of bacteria, it was established that one type of microorganism can cause potato diseases. Table 2 summarizes the results of all conducted tests.

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Table 2. Biochemical characterization of bacterial isolates from the soil

| Test | Bacterial isolates from the soil | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Gram test | - | + | + | - | - | - | - |
| Motility test | + | - | - | + | + | - | - |
| Catalase test | - | - | + | + | - | + | + |
| Oxidase-test | + | + | + | + | - | - | + |
| Casein hydrolysis test | - | - | - | + | + | + | + |
| Gelatin hydrolysis test | - | - | - | - | + | + | + |
| Amylase test (starch hydrolysis test) | - | - | - | - | + | + | - |
| Lecithinase test | - | - | + | - | - | - | - |
| Phytopathogenicity test | - | - | - | + | - | - | - |

Plastic is not eternal, but the actual biodegradation of polymers to $CO_2$ and water in the environment can take hundreds of years. Thus, the use of microorganisms as a means of recycling plastic will only make practical sense with a "reasonable" time perspective. This makes it necessary to look for solutions that significantly speed up this process. It is known that the rate of biodegradation depends on the properties of the polymer, such as molecular weight, the content of crystalline and amorphous phases, porosity, hydrophobicity, or the type of functional groups present, as well as on environmental conditions, such as pH, temperature and humidity.

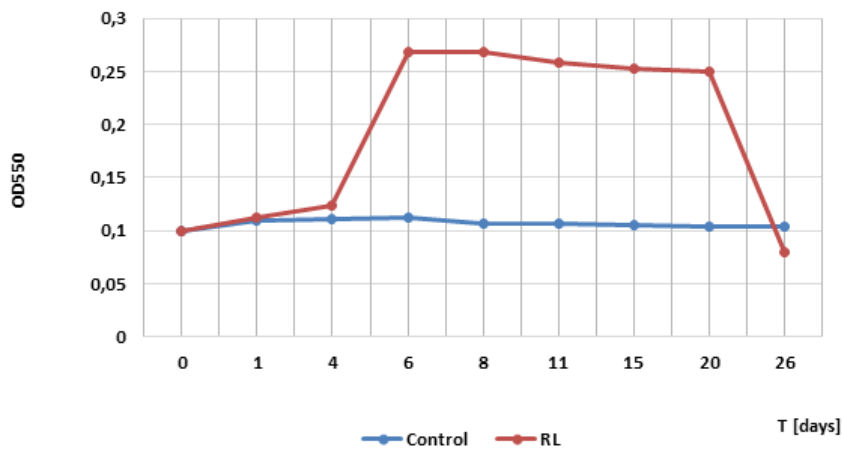


Fig. 1. Biomass growth bacterial isolates with rhamnolipids (0,1 g/l) during biodegradation of LDPE

Chemical or physical degradation, such as UV radiation or oxidation, can prepare the material for microbial degradation. Experiments on the degradation of polyethylene films by isolated strains of bacteria were conducted. To increase the rate of degradation in laboratory conditions, bacteria were grown together with plastic and biosurfactants (rhamnolipids, 0.1 g/l), which should contribute to the hydrophilization of plastic. The experiment lasted 28 days. Comparing the absorption of culture with LDPE film and LDPE film with rhamnolipids (Fig.1), the former showed only slight fluctuations in turbidity over twenty-six days, i.e., no increase in biomass was obtained. When rhamnolipids were added to improve the degradation of polyethylene films, a dramatic increase in substrate turbidity was observed between days four and six. This indicates the adaptation of bacterial

metabolism to biosurfactants and the use of rhamnolipids as a carbon source. After the twentieth day of growth, a sharp decrease in the turbidity of the substrate was observed, which is a consequence of the formation of clumps of biomass.

The ability of microorganisms to use plastic as a carbon source is determined by the characteristics of biofilm formation, weight loss, changes in the surface or structure of the material, as well as changes in mechanical and thermal properties.

ATR-FTIR analysis was performed. The spectra obtained for the primary foil (black color) and films taken from 28-day-old isolates of the consortium cultures with and without rhamnolipids (red and blue colors, respectively) are presented in fig.2.There was a significant change in the heights of the peaks for wave numbers 2850 and 2920 (corresponding to C-H bonds) and the appearance of a slight perturbation for 2350. The spectra of LDPE films, which are degraded by bacteria in the presence or absence of biosurfactant, indicate that there is no effect of rhamnolipid on the biodegradation of this material. During weighing, a slight decrease in the weight of the films was recorded in both cases. The mass loss of films cultured without and with rhamnolipids was 0.39% and 0.94%, respectively. In the control variants (without bacteria), the mass of the films slightly decreased - 0.17% in the presence of rhamnolipids and 0.14% without them. The decrease in weight in the control variants indicates that partial hydrolysis of polyethylene also takes place in abiotic conditions, and it increases in the presence of biosurfactants. However, even with a decrease in weight, the structure of the plastic does not change according to the data of the FTIR spectra. The duration of the experiment (28 days) is not sufficient. In order to verify the effectiveness of the selected bacteria for the biodegradation of plastic, the biodegradation time should be increased to 1 year.

In all cases (both for the film degraded by rhamnolipids and without them), a decrease in the water contact angle was observed after 28 days of cultivation, which means that the polyethylene films became more hydrophilic and susceptible to biodegradation. The water contact angle of the initial polyethylene film was 94°. When the film was cultivated with bacteria and in the presence of rhamnolipids, the angle was 81° and 78°, respectively. In flasks that were shaken abiotically, the wetting angle of the control polyethylene films did not change, while that of the film with rhamnolipids decreased and was 80°. That is, rhamnolipids contribute to the partial hydrophilization of the surface of the polyethylene films.

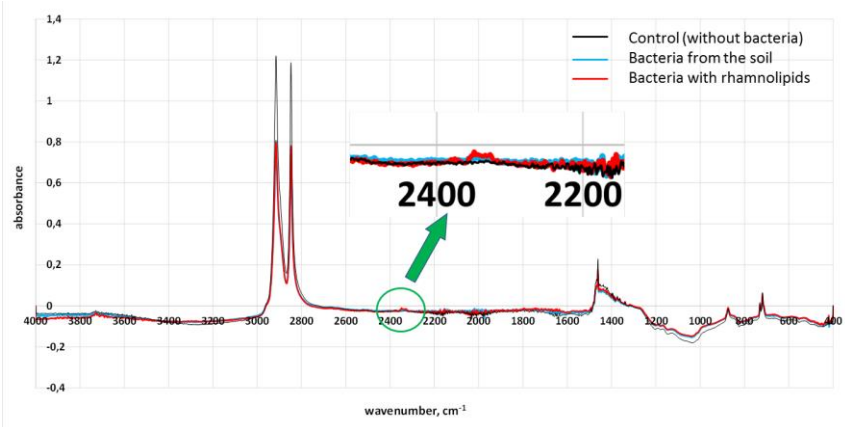


Fig. 2. The FTIR spectra of degraded LDPE films by bacterial isolates from the soil with rhamnolipids (0,1 g/l)

For the first time, the toxicity and phytotoxicity of the biodegradation products of polyethylene and bioplastics in the culture medium were investigated and compared (Table 3). For this purpose, under similar conditions (OD 0.1, 28 days, 30°C, 130 rpm), bacteria were cultivated in the presence of plastic and bioplastic (bags for organic waste made of starch, 7P0595). After centrifugation, the toxicity of the supernatant was determined.

The safety of polyethylene biodegradation products for monocot (wheat *Triticum aestivum* L.) and dicot (cress *Lepidium sativum*) plants has been demonstrated, and the degree of their toxicity does not exceed 20%. However, for the aquatic environment, the products of biodegradation of polyethylene are dangerous and have acute toxicity for the crustacean *Daphnia magna* (51% for polyethylene and 68% for polyethylene with rhamnolipids). This indicates that not only plastic is a water environmental hazard, but also its decomposition products. Unlike polyethylene, biodegradation products of bioplastics are safe both for plants (for example, wheat and watercress) and for the crustacean *Daphnia magna* (7% toxicity).

Table 3. Phytotoxicity and toxicity of cell-free culture supernatant after biodegradation of polyethylene with and without rhamnolipids

| Samples after biodegradation | Phytotoxicity | | | | Toxic effect |
|---|---|---|---|---|---|
| | Wheat (*Triticum aestivum* L) | | Cress (*Lepidium sativum*) | | *Daphnia magna* |
| | Relative root length, [%] | Relative seed germination, [%] | Relative root length, [%] | Relative seed germination, [%] | Degree of toxicity, [%] |
| Cell-free culture supernatant (polyethylene) | 82 | 81 | 83 | 92 | 51 acute lethal toxicity |
| Cell-free culture supernatant with rhamnolipids (polyethylene) | 83 | 95 | 85 | 94 | 68 acute lethal toxicity |
| Cell-free culture supernatant (bioplastic) | 91 | 96 safe | 93 | 97 | 7 safe |

## CONCLUSIONS

- Seven types of bacteria were isolated from the landfill. It was shown that these isolates are able to survive in an environment with LDPE as the only carbon source. Their morphological and biochemical characteristics were established for further identification.
- When the isolates were co-cultured with plastic and rhamnolipids, the rhamnolipids were mainly used as a carbon source and did not significantly contribute to the biodegradation process.
- Changes in the structure of LDPE films after 28 days of exposure to bacteria and rhamnolipids were confirmed by FTIR spectroscopy.
- For the first time, the toxicity of plastic biodegradation products in a liquid nutrient medium was investigated and their safety for plants was shown.

However, these biodegradation products have acute lethal toxicity for the small planktonic crustacean *Daphnia magna*.

## SYMBOLS

*ATR-FTIR* – spektroskopia osłabionego całkowitego odbicia w podczerwieni z transformacją Fouriera
attenuated total reflectance Fourier-transform infrared spectroscopy

*CL* – katodoluminescencja
cathodoluminescence

*DSC* – skaningowa kalorymetria różnicowa
differential scanning calorimetry

*FTIR* – spektroskopia w podczerwieni z transformacją Fouriera
Fourier-transform infrared spectroscopy

$m$ – masa końcowa folii [g]
residual weight of the plastic film [g]

$m_0$ – masa początkowa folii [g]
initial weight of the plastic film [g]

$OD_{550}$ – gęstość optyczna
optical density

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TETIANA POKYNBRODA, NATALIIA KORETSKA, AGNIESZKA GĄSZCZAK, ELŻBIETA SZCZYRBA

# BIODEGRADACJA POLIETYLENU Z WYKORZYSTANIEM BAKTERII GLEBOWYCH I RAMNOLIPIDÓW

Polietylen to tworzywo termoplastyczne o niezwykle szerokim zakresie zastosowań, m.in. w produkcji opakowań jednorazowego użytku. Jest uważany za jeden z najczęściej produkowanych polimerów syntetycznych na świecie. Poszukiwania skutecznych drobnoustrojów-destruktorów tworzyw sztucznych prowadzone są od wielu lat w różnych środowiskach, takich jak gleba z namorzynów, wysypiska śmieci, woda morska, larwy i in. Wyizolowano kilka rodzajów grzybów i bakterii rozkładających różne tworzywa sztuczne, ale stopień degradacji wciąż jest niezadowalający. Biodegradacja plastików jest bardzo powolna ze względu na ich hydrofobowość, która utrudnia mikroorganizmom przyczepienie się do ich powierzchni.

Pierwszym zadaniem była izolacja bakterii – potencjalnych destruktorów plastiku. Z gleby skażonej odpadami (wysypisko śmieci) sporządzono ekstrakt, który wraz z folią polietylenową (jako jedynym źródłem węgla) wprowadzono do roztworu soli mineralnych. Po trzech tygodniach inkubacji rozpoczęto testy skriningowe bakterii. Ostatecznie wyizolowano siedem różnych szczepów bakterii i oznaczono ich cechy morfologiczne. Ustalono, że wśród izolatów bakteryjnych pięć jest gram-ujemnych, a dwa gram-dodatnie. Cztery z nich wytwarzają katalazę, pięć hydrolizuje żelatynę i kazeinę, dwa hydrolizują skrobię, a dwa produkują lecytynazę (Tabela 1). Badając fitopatogenność bakterii ustalono, że jeden rodzaj drobnoustrojów jest zdolny do wywoływania chorób roślin (ziemniaka).

Przeprowadzono eksperymenty dotyczące degradacji folii polietylenowych przez wyizolowane szczepy bakteryjne. Dla przyspieszenia degradacji plastiku, bakterie były hodowane także w obecności biosurfaktantów (ramnolipidy, 0,1 g/l). Eksperyment trwał 28 dni. W hodowlach bez biosurfaktantów zaobserwowano tylko niewielkie wahania zmętnienia czyli nie uzyskano przyrostu biomasy. Natomiast w hodowlach zawierających ramnolipidy, między czwartym a szóstym dniem zaobserwowano gwałtowny wzrost zmętnienia podłoża. Wskazuje to na adaptację metabolizmu bakterii do biosurfaktantów i wykorzystanie ramnolipidów jako źródła węgla. Po dwudziestym dniu wzrostu obserwujemy gwałtowny spadek zmętnienia podłoża, będący skutkiem tworzenia się grudek biomasy.

Analizując widma uzyskane metodą ATR-FTIR, zauważono zmniejszenie wysokości pików dla liczb falowych 2850 i 2920 (odpowiadających wiązaniom C-H) oraz pojawienie się niewielkiego zaburzenia dla liczby 2350. Pokrywające się widma folii LDPE degradowanych przez bakterie w obecności lub przy braku biosurfaktantu, sugerują brak wpływu ramnolipidu na biodegradację tego tworzywa.

Po raz pierwszy zbadano i porównano toksyczność i fitotoksyczność produktów biodegradacji polietylenu i bioplastiku, obecnych w płynie pohodowlanym. Wykazano bezpieczeństwo produktów biodegradacji polietylenu i biotworzyw dla wybranych roślin jedno- i dwuliściennych (pszenica zwyczajna (*Triticum aestivum* L. i rzeżucha siewna *Lepidium sativum*). Stopień ich toksyczności nie przekraczał 10%. Jednak dla środowiska wodnego produkty biodegradacji polietylenu są niebezpieczne i wykazują toksyczność ostrą dla skorupiaków *Daphnia magna* (stopień toksyczności 51% dla polietylenu i 55% dla polietylenu z ramnolipidami w porównaniu z 7% dla biotworzyw). Takie rezultaty wskazują na szkodliwość dla środowiska wodnego nie tylko tworzyw sztucznych, ale także produktów ich biodegradacji. Wykazano, że w przeciwieństwie do polietylenu produkty biodegradacji biotworzyw (worki na odpady organiczne, produkowane ze skrobi, 7P0595) są bezpieczne zarówno dla roślin, jak i dla skorupiaków *Daphnia magna*.

Spadek kąta zwilżania po 28 dniach hodowli obserwowany był we wszystkich układach, co oznacza, że folie polietylenu stały się bardziej hydrofilowe i podatne na biodegradację. Odnotowano także nieznaczny spadek masy folii. W celu weryfikacji skuteczności wyizolowanych bakterii, eksperymenty należy powtórzyć zdecydowanie wydłużając czas prowadzenia hodowli.

*Słowa kluczowe: biodegradacja, polietylen, ramnolipidy*

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