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Influence of bacteria on degradation of bioplastics

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Abstract. The degradation rate of bioplastic in soil is closely related to the diversity of soil microbiota. To investigate the effect of soil bacterial on biodegradation, 4 bacterial strains of soil – *Pseudomonas chlororaphis*, *Kocuria rosea*, *Cupriavidus necator* and *Bacillus cereus*, were used to accelerate the decomposition of bioplastics manufactured from Polylactid acid (PLA) by direct action during 250 days. The best results were obtained with bacterial strains *Cupriavidus necator* and *Pseudomonas chlororaphis* that were isolated of lagoons with anthropogenic sediments.

1. Introduction

Bioplastics are products with character of conventional plastics that are made from plant biomass or its parts – organic substances like polysaccharides (starch, cellulose), polyesters produced by organisms and also synthetic biodegradable polymers. Biopolymers are high-molecular organic compounds mostly produced by metabolic reactions of living organisms [1].

All polymers that are subject to change by microorganisms like bacteria, fungi, algae and protozoa, are considered to be biodegradable. These biologically degraded plastics are more environmentally friendly than the products from petroleum because they decompose in shorter time intervals. Not only the process of decomposition but also the final products of bioplastics degradation are significant – only water, carbon dioxide, eventually methane and residual biomass are produced [2]. With regard to the fact that bioplastics serve as a raw material for manufacturing household items, packaging materials and other everyday life needs, they make a big amount of municipal waste and that is the reason why is necessary to focus on faster and more efficient recycling [1].

Degradation capacity of the environment is defined as a maximal level of microbial activity that is possible to expect based on conditions of ecosystem where biodegradation takes place. Resulted activity of microbial consortium depends not only on physical-chemical conditions but also on biological factors like number of microorganisms, their ability to adapt, genetic predispositions and other physiological conditions. Other factors that affect the whole process of biodegradation are the presence of chemical substances, eventually properties of contaminating substances like toxicity, bioavailability and biodegradability [3].

The aim of this study was to compare biodegradation rate and efficiency of conventional and ecological plastics when exposed to bacterial biomass for 250 days. Progress of experiment was continuously monitored visually by taking photos at 15 days interval with binocular microscope. Intermediate result of this experiment was to verify which bacterial species effect the decomposition of bioplastics the most and to use this acquired knowledge for further study of biodegradation processes.

2. Methods and experimental

Plastic product that is often used as an everyday item was monitored in the experiment. It is a plastic drinking cup with volume of 0.2 L manufactured by American company Nature Works LLC (see figure 1). Since 1989 Nature Works LLC deals with production of environmentally friendly plastics and because of their wide field of activity it is now possible to buy these products in Europe and even in Czech Republic. This plastic cup is made from polylactic acid (PLA). This compound is derived from renewable resources such as starch that is easy to decompose. Lactic acid is obtained by fermentation of the glucose that is followed by polymerization to obtain polylactic acid [1].

For experimental purpose the plastic cup was cut to samples of size 2 x 1 cm. During the process of biodegradation every sample of plastic cup was added in bacterial medium to observe rate and efficiency of reaction and also to monitor changes during experiment.

Four strains of soil bacteria were used for the experiment – *Pseudomonas chlororaphis, Kocuria rosea, Cupriavidus necator* and *Bacillus cereus*. Strains were isolated from metal contaminated soils and some of them were recently used for successful biodegradation of chemical substances [2,6,7].
Bacteria were statically cultivated at 25°C on tryptone-soya agar (M290, HiMedie Laboratories, Mumbai, India). Plastic samples of size 2 x 1 cm were leached in the trypton-soya broth (M011, HiMedie Laboratories, Mumbai, India) with bacterial culture added in the exponential phase of growth with total volume of 100 mL. Progress of experiment was continuously monitored visually by taking photos (zoom 40x and 100x) at 15 days interval with binocular microscope Olympus CX4.

Figure 1. Plastic drinking cup 0.2 L (Nature Works LLC, USA) and its parts used in degradation experiments.

3. Results and discussion

Biodegradable rate and efficiency with selected bacterial strains is summarized in table 1.

Table 1. Biodegradation process of plastic cup sample

Bacterial strain	Number of days				
	50 d	100 d	150 d	200 d	250 d
Bacillus cereus ZK-27	+	+	++	++	++
Cupriavidus necator POP-31	++	++	++	+++	+++
Kocuria rosea SL-1	+	+	+	+	++
Pseudomonas chlororaphis ZK-1	++	++	+++	+++	+++

As shown in table 1, bacterial strains *Cupriavidus necator* POP-31 and *Pseudomonas chlororaphis* ZK-1 were the most suitable for biodegradation of the sample. After 250 days biodegradation reached its maximal effect and caused distinctive damage of the sample not only on the surface but also in the structure of material. Progress of biodegradation is shown in figures 2 – 4.

Figure 2. Green color loss in the sample of plastic

Figure 3. Biodegradation : first holes formation

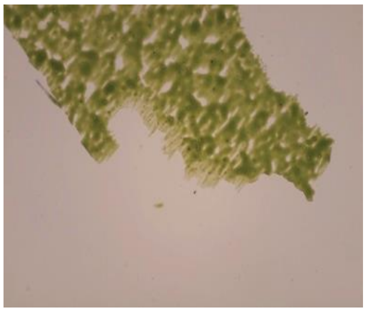

Figure 4. Biodegradation: structure disruption

Bacterium *Pseudomonas chlororaphis* ZK-1 was isolated as an arsenic-tolerant strain from sediments of lagoon in Zemianske Kostoľany [7,8,15]. Bacterium *Cupriavidus necator* POP-31 was identified as a new isolate from lagoon in Poproč and from the soil with high concentrations of antimony and lead [4]. Generally, these bacteria are common soil species that are low nutrient-demanding and due to their adaptability are often used for biodegradation of organic pollutants [7,9,10,11,12,14]. One of the significant factors that determine bacterial behavior in biodegradation process is the characteristics of genome. The fact that bacteria *Cupriavidus* possess wide spectrum of plasmids and megaplasmids has already been proofed by research of the genetic nature of biodegradation [5]. Plasmids and megaplasmids are the reasons why this bacterial strain is resistant to heavy metals and organic toxic substances and is able to degrade them with high rate and efficiency [13,16,17,18]. Biotechnological use of *Pseudomonas chlororaphis*, which contributed on the biodegradation the most, is not fully clarified yet. Bacterial genus *Pseudomonas* is well known for its so called degradable plasmids that are able to metabolized low molecular organic compounds as well as high molecular organic compounds like polymer chain of petroleum substances [14,19].

Study focused on ability of microorganisms to decompose waste plastics on landfill in Bangkok was published in 2015. Lab experiment was based on simulated conditions of landfill environment (lighting conditions with the use of UV-B lamp, presence of indigenous microflora, amount of plastic material in municipal waste). For experimental method were used sample of common plastic products from polyethylene (high density polyethylene HDPE/ low density polyethylene LDPE), polypropylene (PP) and polystyrene (PS), that were cut to pieces of 9 cm^2; from microbial strains were selected bacterial species of *Flavobacterium, Xanthobacter, Methylobacter, Methylococcus, Nitrobacter* and *Nitrosomonas.* Experiment lasted for 210 days and it was concluded that products from HDPE degraded in higher rate with mass loss of 15%, from LDPE of 4, 96%, PP 6, 7% and for products from PS mass loss was 5, 29% [21].

Some studies confirm that ingested polyethylene creates changes in digestive system of fishes, birds and sea animals that cause disruption of function of digestive organs and may cause death. Increasing amount of waste, decrease of landfill capacity and long-term degradation of conventional plastics caused rising interest in biodegradation of PE. Degradation ability of microbial biomass on LDPE was tested in Saudi Arabia [22] and India [20] – especially presence of bacteria able to metabolize LDPE. Bacterial genus *Bacillus, Staphylococcus, Micrococcus* and *Pseudomonas* were tested in this study; the most efficient bacterium was *Brevibacillus borstelensis* isolated from soil. Incubation of polyethylene with bacterium *B. borstelensis* decreased mass weight of 30% and maximal rate of biodegradation was monitored after 90 days of incubation at 50°C [20].

4. Conclusion

The results of experiments show that the biodegradation of PLA materials accelerated the most with effect of gram negative bacteria *Pseudomonas chlororaphis* and *Cupriavidus necator*. These results could be used in processes of controlled biodegradation and in other biotechnological processes that accelerate decomposition of environmentally degradable materials.

Biodegradable materials are becoming a new alternative for plastic manufacturing. Physical-chemical qualities of these substances are improving due to progressive research with the aim of better mechanical properties and resistance to different environmental effects but with preserved easy biodegradation ability.

In these days, biodegradation is considered to be an important part of degradation process when decomposition happens in presence of living microorganisms [23,24,25]. Biological degradation of bioplastics is much more effective and faster than degradation of conventional plastics. Limiting factors of biodegradation process are conditions needed for growth and reproduction of microorganisms but the basic factor that affects the rate of biodegradation is composition on these plastics – from which material are biodegradable plastics made and if this material could be possible source of carbon and energy for their growth and reproduction.

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