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Sem of *in Situ* Microbial degradation of Biopol®-based plastic materials

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Summary. Biodegradability of BIOPOL®-based plastic have been investigated *in situ* by scanning electron microscopy (SEM). It has been shown that the best conditions provided for expression of microbial degradative potential are those in bank mud of the River Danube where the annual weight-loss of the plastic samples of about 63% has been recorded. Judging by quantitative composition of microbial population it may be concluded that the principal role in biodegradation-process occurring in Danube water play bacteria. On the other hand, degradation in terrestrial environments was found to be result of a joint activity of both bacteria and fungi.

Introduction. Studies motivated by replacement of biologically undegradable materials with degradable ones have been intensified recently. Biodegradable plastics based on copolymer of poly-β -hydroxybutyrate (PHB) and poly-β-hydroxyvalerate (PHV), under the trade name BIOPOL® [1] has been designed and commercialized as packaging material. Some of them are designed as completly biodegradable; others are designed by blending and mixing more or less compatible hemosynthetic and biosynthetic materials, becoming partially degradable. These consisting mainly of nondegradable with only small percentage of biodegradable components have been designated as biodestructible ones [2]. Being aware of the potential pollution problems caused by introducing this new materials into our environment, a research has been focused on biodegradability of those plastics under conditions in which, used and discarded, they probably would be degraded by microbial enzymes. Part of this research was published earlier [3,4]. In this paper the focus is on microorganisms potentially responsible for biodegradation of these materials in water and bank mud of the river Danube, as well as in the Danube bank meadow and forest soils.

Material and methods. Preweighted samples of BIOPOL® have been exposed to the microbial enzymes in 30-40 cm depth and investigated at five-weeks intervals. The investigation had started in April, and ended in March next year. The weight loss was followed gravimetrically and qualitative changes of material were determined by scanning electron microscopy. The qualitative and quantitative composition of samples from surrounding microbial population were investigated by counting aerobic saprotrophic bacteria and fungal particles [2]. In order to obtain comparable results on microbial density/activity from four different aquatic, terrestrial and intermediary environments all nonaquatic original natural samples were brought to 100% water saturation and therefore brought to the comparable parameters ($1cm^3$ = 1g). The soil samples were passed through the fine sieve; saturated by sterile tap water (until the first surplus drop of water appeared set apart of soil sample) and then homogenized under the aseptic conditions. 1g of each pretreated sample was then diluted in 9ml of sterile tap water and sonicated 5x1 min with intermittent stirring and cooling for 1 min each. Serial dilution of the resulting suspension were plated onto appropriate media and incubated at 22^oC. The obtained results allowed the comparison of data for different: water, river bank mud, and bank meadow- and forest soils of the complex River Danube ecosystem [3, 4].

Results and discussion. After one-year-period of exposing BIOPOL® to *in situ* microbial action, the most intensive biodegradation was recorded in bank mud of Danube (about 63% of sample weight-loss), than in Danube water (about 30%), while soil samples offered less favourable conditions: meadow soil about 17% and forest soil about 12% (Fig.1) The results of statistical analysis of accompanied bacteria potentially responsible for BIOPOL® degradation are shown in Fig. 2. The highest annual ratio of saprotrophic bacteria was recorded in meadow soil while the lowest values were found to be the characteristics of the Danube water. The number and the seasonal dynamics of mycopopulation is shown in Fig. 3. Differences in quantitative composition of this part of microbial population in different environments were found to be more clear than in the case of bacteria, although in this case the highest number of the fungal particles and active fungi were also recorded in meadow soil, while the lowest annual rates were found to be the characteristics of Danube water.

Conclusion. On the basis of characteristics of microbial population in the investigated environments, it could be concluded that the principal role in degradation of the plastic samples in Danube water play bacteria (Fig 5), and that the most intensive BIOPOL® biodegradation in bank mud of the river is a result of synergistic activity of both bacterial and fungal enzymes. Important role of fungi in biodegradation of this material in investigated natural conditions was confirmed by SEM analysis of the plastic samples (Fig 6). The lack of correlation between the intensity of BIOPOL® degradation and the number of investigated groups of microorganisms shows the importance of other, first of all, abiotic ecological factors (humidity, temperature, pH), providing conditions for the expression of microbial degradable potentials.

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4. Matavulj M., Molitoris H.P. (2000): Biodegradation of polyhydroxy-alkanoate-based plastic (BIOPOL) under different environmental conditions – I. weight loss of substrate. Hoppea, 61, 735-749.

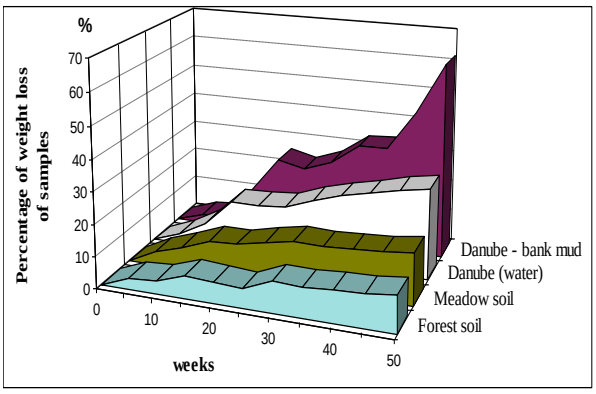


Figure 1. In situ biodegradation of biosynthetic plastic BIOPOL

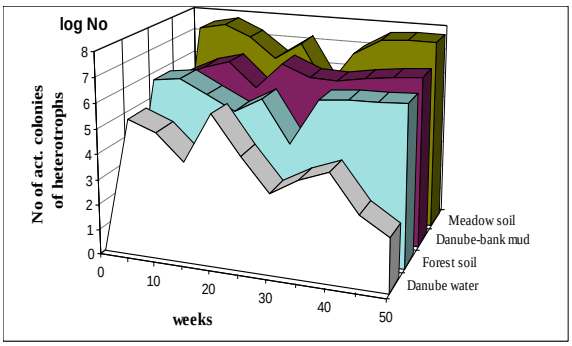


Figure 2. Number of saprotrophic bacteria potentially responsible for degradation of BIOPOL in different environmental conditions

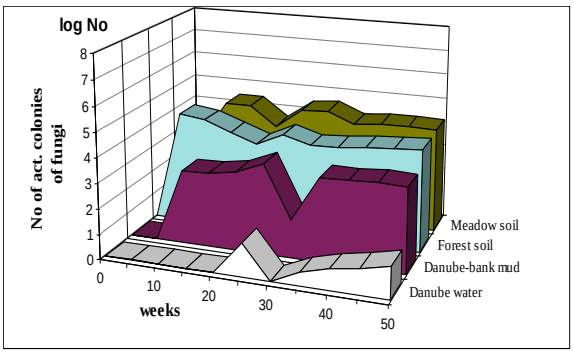
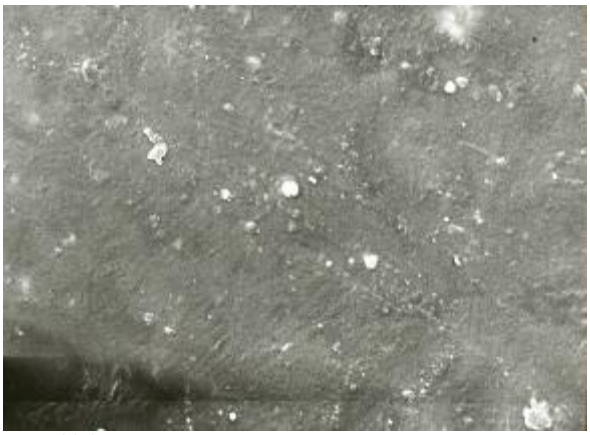
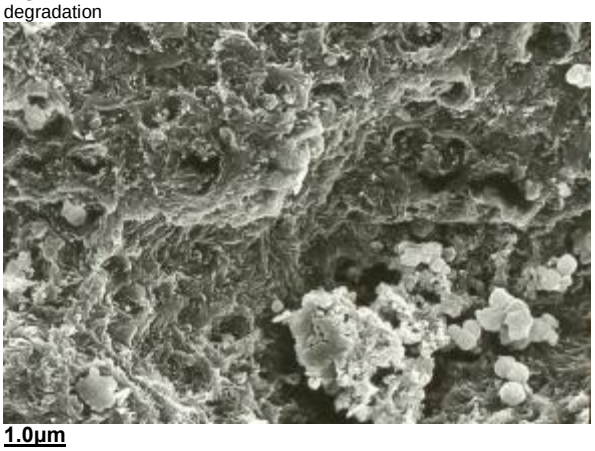


Figure 3. Number of particles of mycelial fungal potentially responsible for degradation of BIOPOL in different environmental conditions



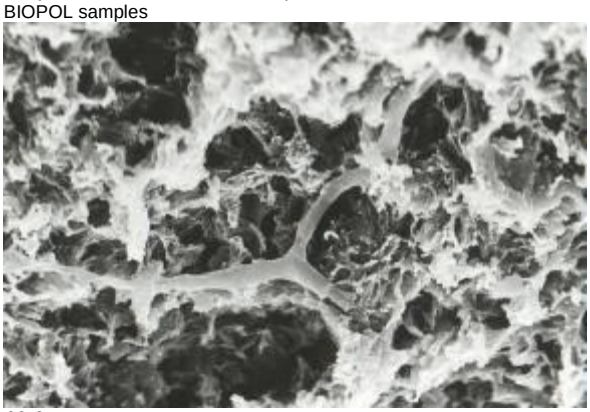
100.0 µm

Figure 4. Surface of BIOPOL plastic not exposed to microbial degradation



1.0µm

Figure 5. Dominating bacteria in the Danube water, by their enzyme action, caused superficial crater-like erosion of BIOPOL samples



10.0 µm

Figure 6. Dominating mycelial fungi in soil samples, by their enzyme action, caused deeper degradaton of the core of BIOPOL samples