Plastics Biodestruction under the Impact of Caves Micromycetes

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Abstract. The article is devoted to the analysis of the plastics biodegradation rate under the impact of micromycetes. Three types of widespread soil micromycetes were selected for the experiments. The strains of thermotolerant micromycetes (optimum growth at a temperature of 12-25°C) isolated from karst caves were used. Penicillium chrysogenum, was highlighted with gypsum sinter deposits of the Novoafonskaya cave named after G. Sh. Smyr (Abkhazia). P. nigrum, was isolated from clay deposits of Divya cave (Russia, Perm region). Sporotrichum sp. was allocated from the clay deposits of the cave Mchishta-Akshasha (Abkhazia). Four types of plastics were biodegraded: high-pressure polyethylene, polypropylene, polyethylene terephthalate, low-pressure polyethylene. The experiments were carried out in the Chapek-Dox nutrient medium in liquid and agar medium. Evaluation of the biodegradation rate was carried out by calculating the mass loss of samples. Qualitative changes in the surface were studied by using scanning electron microscopy. It was found that P. chrysogenum had the highest rate of biodegradation and P. nigrum had the lowest rate. The weight loss was the reatest in polyethylene terephthalate, lower - in polypropylene, and the lowest one – in the low-pressure polyethylene.

1. Introduction

The accumulation of plastic waste in the environment is increasing every year and poses a threat to living beings. Plastics are destroyed very slowly in nature, and therefore the actual problem of search of species capable of their rapid biodegradation. The burning of some plastics leads to the release into the atmosphere of organic pollutants known as furans and dioxins, which can accumulate in living organisms and have toxic and carcinogenic effects [1].

A large amount of plastic waste has accumulated in landfills and in nature [2, 3]. There is information that polymers are destroyed to micro and nanoparticles and in this form accumulate in living organisms [1]. It is assumed that the types of communities developing on them depend on the type of plastic [4, 5], but an alternative opinion is also expressed [5] .

Biodegradation of polymers includes the following stages: adhesion of microorganisms on the polymer surface; growth of microorganisms using the polymer as a carbon source; primary degradation of the polymer; final degradation [6]. Changes in the surface topography, such as the appearance of cracks, are associated with the associated biota [7, 8], suggest that further bio-fragmentation of the material occurs, and the absorption of low molecular weight biota products [9].

Most studies state indirect signs of polymer biodegradation, but some studies have demonstrated the consumption of the polymer by detecting monomers in the medium or by monitoring the fate of the labeled polymer [10, 11].

The rate of biodegradation of plastics is extremely low. The creation of biodegradable plastics using various modifiers introduced into their composition is a priority direction for the creation of new materials [1]. Given the widespread use of plastics in industry and medicine, as well as in extreme conditions, there is a problem of long-term stability of materials in biological media, which, in particular, is solved by chemical or physical modification of plastics [6].

The role of various enzymes and microorganisms in the destruction of plastics is considered in detail in the literature. It is noted that the consortia of microorganisms have the greatest destructive effect, substrate preferences of a number of species of micromycetes and bacteria are revealed [1, 12, 13, 14].

A number of studies are connected with the transformation of plastics while the process of biodegradation, as well as the influence of environmental factors which can accelerate the disintegration of the material, including pollutants of anthropogenic nature [1, 15, 16]. Taking into account the problem of plastics disposal at the landfills, studies on plastics' biodestruction under the influence of microbiota isolated from landfills are of particular importance. Assessment of the susceptibility of polymers to degradation in soil conditions with admixture of municipal waste also requires special attention [17]. Another direction is the study of biodegradation of plastics in various natural environments under the influence of microbiota isolated from the studied environment, for example, marine microorganisms [1, 18, 19], soils [20], etc.

The aim of this work was to evaluate the biodegradation of plastics with thermotolerant strains of widespread soil micromycetes.

2. Objects and methods

The rate of biodegradation was estimated for four types of plastics: polypropylene (PP), polyethylene terephthalate (PET), high-pressure polyethylene (PELD) and low-pressure polyethylene (HDPE).

The samples were washed, crushed and dried plastics. For the experiment, the strains of thermotolerant micromycetes with growth optimum at a temperature of 12-25°C were used, isolated from soil samples of karst caves. Used three strains: *Penicillium chrysogenum* Thom, highlighted with gypsum sinter deposits of the Novoafonskaya cave named after G. Sh. Smyr (Abkhazia); *Penicillium nigrum* (Sopp) Biourge, isolated from clay deposits of Divya cave (Ural); *Sporotrichum sp.* allocated from the clay deposits of the cave Akshasha-Mchishta (Abkhazia). Micromycetes were cultivated on Chapek-Dox medium. For the experiment agar and liquid medium were used. Pieces of plastics were washed with 96% alcohol, samples were weighed in an air-dry state. On Petri dishes with agarose medium, micromycetes were applied to obtain a uniform lawn and pieces of plastics were laid out. In the second experiment the liquid medium of Chapek-Dox was used, which was poured into sterile flasks with added micromycetes (0.01 g of mycelium/150 ml), into which plastic particles were placed. The suspension was stirred and left at a temperature of 24°C, stirring was carried out every 2-3 days for an hour on an automatic stirrer.

The duration of the experiment was three weeks. At the end of the exposure, the plastics were dried to a stable mass at a temperature of 50 ° C and weighed to an accuracy of 0.0001 g.the changes in the surface of the plastics were Analyzed by scanning electron microscopy. Plastic samples were washed from micromycetes under running water, rinsed with 96% alcohol, mounted on a slide table, sprayed with carbon (layer thickness 20 nm) in an ion-spraying unit IB-3 (EIKO). The material was examined in a SAM-scan microscope (Hitachi) at an accelerating voltage of 15 kV and an operating increase of 10000 to 100000.

3. Results and discussion

Mass loss in plastic samples after incubation with various microorganisms was estimated (Table 1), the largest change in mass was noted under the action of the species *Penicillium chrysogenum*.

The greatest biodegradation was found for samples of polyethylene terephthalate and polypropylene, the smallest - for low-pressure polyethylene. This can be due to the high density of polyethylene and, as a result, the complexity of contact between micromycetes and the surface of plastics.

Table 1. The change in mass with respect to the initial mass.

Type of plastic	The change in mass with respect to the initial mass, %					
	Penicillium chrysogenum		*Penicillium nigrum*		*Sporotrichum sp.*	
	for 21 days	in a year	for 21 days	in a year	for 21 days	in a year
PELD	0,592±0,0012	10,29	0,614±0,0012	10,67	0,624±0,0013	10,85
HDPE	0,543±0,001	9,44	0,545±0,0011	9,47	0,496±0,001	8,62
PP	1,124±0,0023	19,54	1,092±0,0022	18,98	1,081±0,0022	18,79
PET	1,962±0,0039	34,10	1,743±0,0035	30,30	1,823±0,0036	31,69

Figures 1-16 show the change in the surface of plastics after the action of micromycetes. Despite the short duration of the experiment, in all experiments, the surface of the plastics changed, which indicates biodegradation of the plastics.

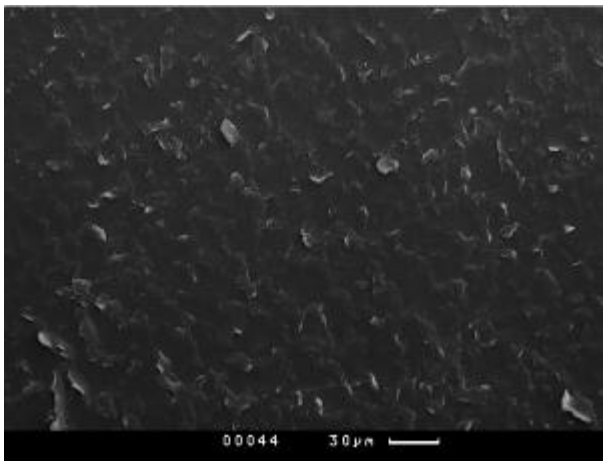

Figure 1. PELD, control sample.

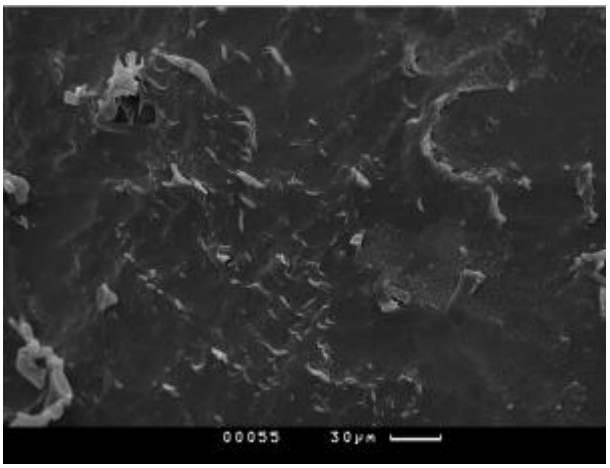

Figure 2. PELD, impact *P. chrysogenum.*

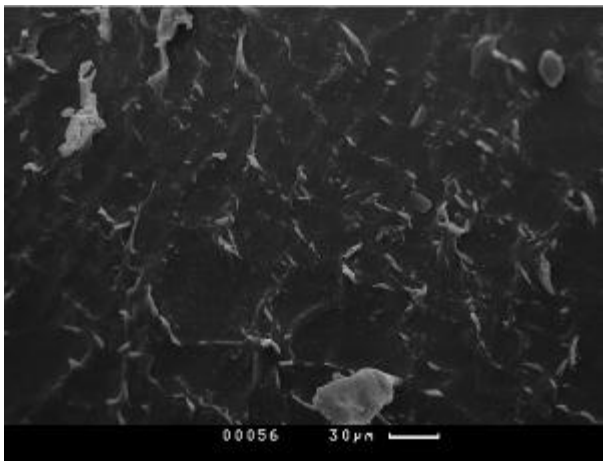

Figure 3. PELD, impact *P. nigrum.*

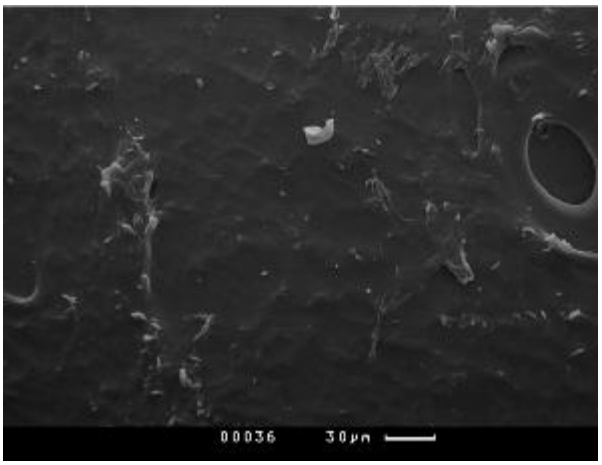

Figure 4. PELD, impact *Sporotrichum sp.*

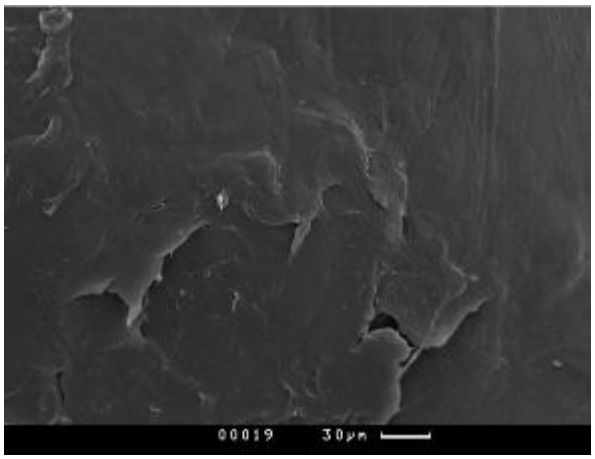

Figure 5. HDPE, control sample.

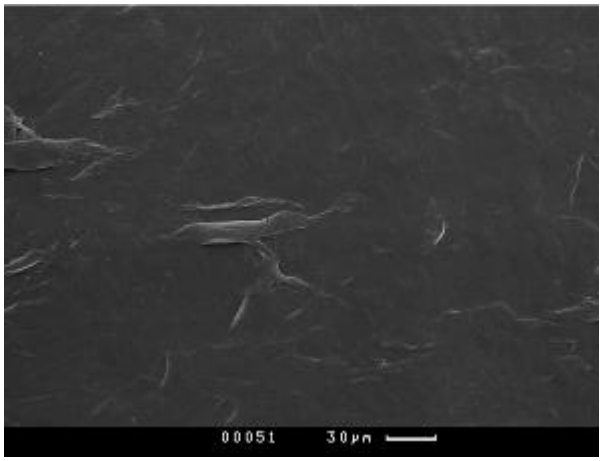

Figure 6. HDPE, impact *P. Chrysogenum.*

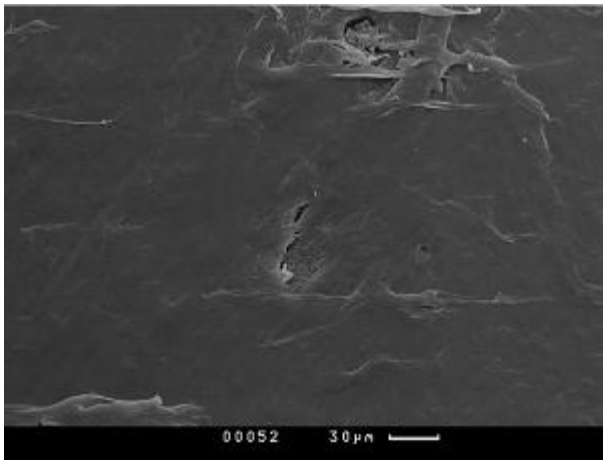

Figure 7. HDPE, impact *P. nigrum.*

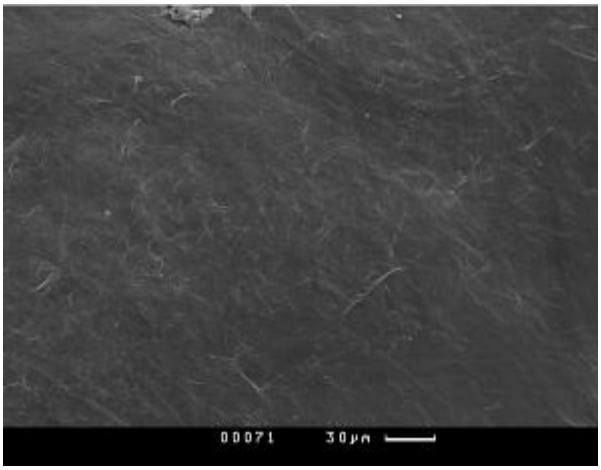

Figure 8. HDPE, impact *Sporotrichum sp.*

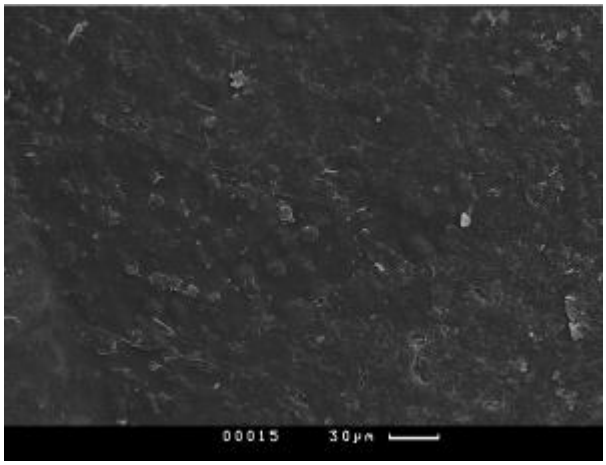

Figure 9. PP, control sample.

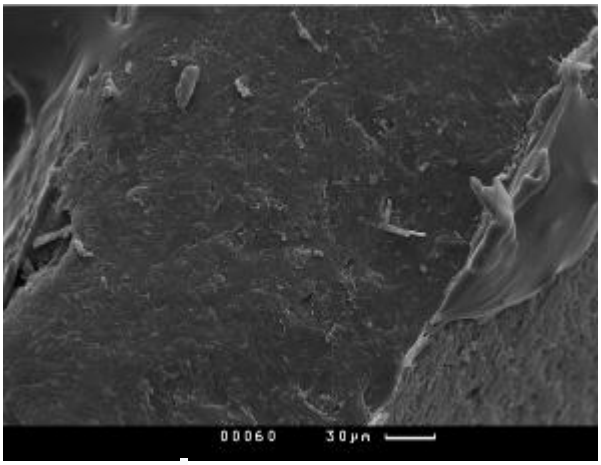

Figure 10. PP, impact *P. chrysogenum.*

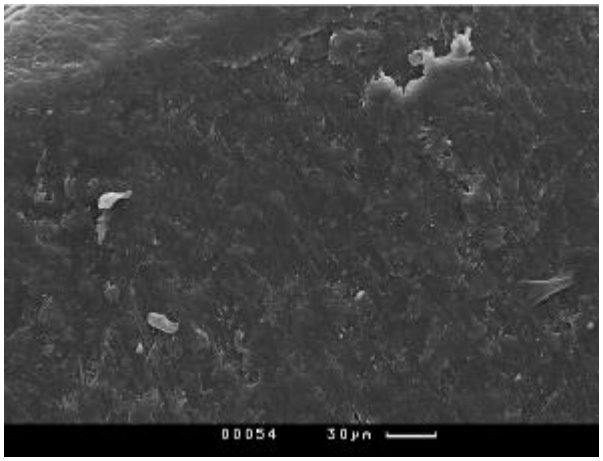

Figure 11. PP, impact *P. nigrum.*

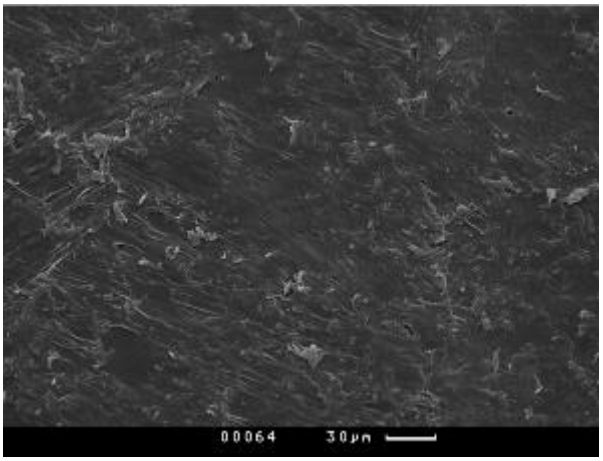

Figure 12. PP, impact *Sporotrichum sp.*

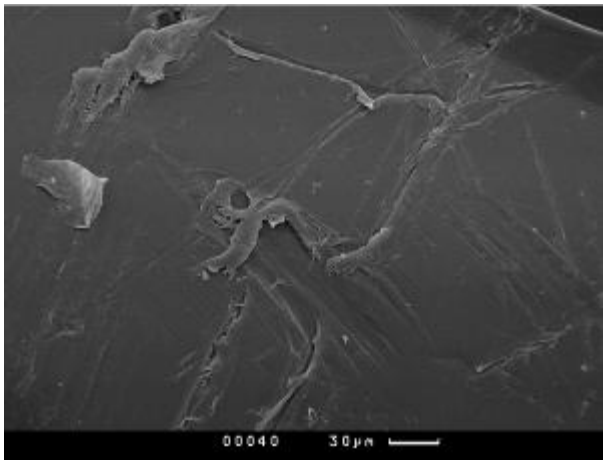

Figure 13. PET, control sample.

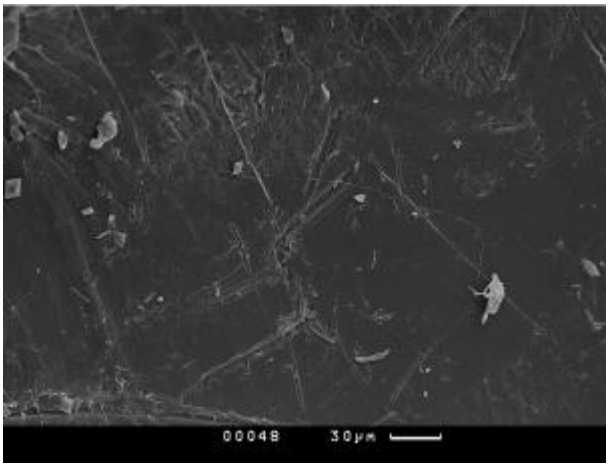

Figure 14. PET, impact *P. chrysogenum.*

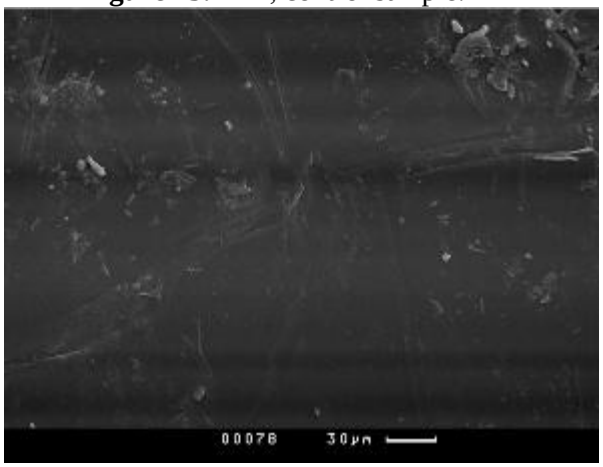

Figure 15. PET, impact *P. nigrum*.

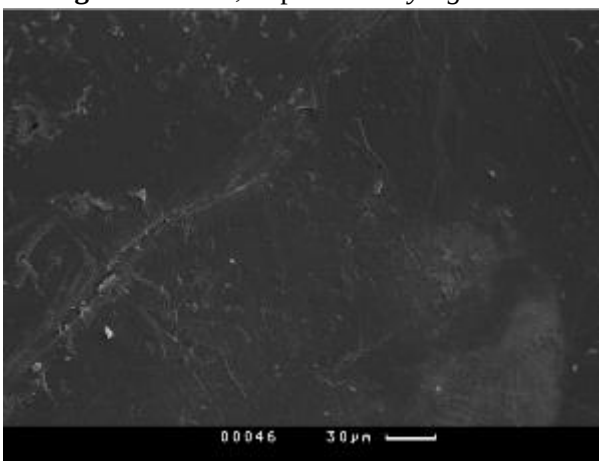

Figure 16. PET, impact *Sporotrichum sp.*

As a result of the study, it was revealed that all three types of micromycetes studied had an effect on the plastics' surface structure. When incubating plastics in a liquid medium with micromycetes, a decrease in the mass of polymers occurred, which allows one to conclude that they are biodegradable. The most effective decomposition of polyethylene terephthalate and polypropylene, with the use of *Penicillium chrysogenum*. The rate of biodegradation of polyethylene terephthalate under the action of micromycetes is above 30% per year.

4. References

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