ISOLATION OF MICROORGANISMS FROM LANDFILL SOIL CONTAMINATED WITH POLYETHYLENE

IZOLAREA MICROORGANISMELOR DIN SOLUL DE GUNOIȘTE CONTAMINAT CU POLIETILENĂ

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Abstract. *The aim of the research was the isolation and characterization of microbial consortia formed on the surface of LDPE extracted from the soil. The soil was amended with mineral salts under aerobic and anaerobic conditions. The duration of LDPE incubation in the soil was six months. Isolation of microorganisms on solid media revealed the existence of 12 species of microorganisms. Most of the consortia were formed by micromycetes from genera Trichoderma, Penicillium, Ascomycota. The isolated consortia demonstrated the ability to populate the LDPE surface and produce mechanical changes in the polyethylene.*
Key words: LDPE, microbial consortia, micromycetes

Rezumat. *Scopul cercetărilor a fost izolarea și caracterizarea consorțiilor de microorganisme formate pe suprafața LDPE din sol. Solul a fost amendat cu săruri minerale în condiții aerobe și anaerobe. Durata incubării LDPE in sol a fost de șase luni. Izolarea microorganismelor pe medii solide a relevat existența a 12 specii de microorganisme. Majoritatea consorțiilor au fost formate de micromicete din genurile Trichoderma, Penicillium, Ascomycota. Consorțiile izolate au demonstrat capacitate de a popula suprafața LDPE și de a produce schimbări mecanice ale polietilenei.*
Cuvinte cheie: LDPE, consorții microbiene, micromicete

INTRODUCTION

The problem of environmental pollution with plastic materials will remain current for a long time, because the degree of pollution is exorbitant, not all types of plastic are recycled, and plastic remains indispensable for the modern economy (Plastic Europe, 2022). One of the most used plastic materials in the world economy is polyethylene. Due to the structure of the hydrophobic backbone of carbon atoms, polyethylene is durable and recalcitrant to the action of external factors. The negative impact of non-recyclable plastic waste accumulated in landfills and

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oceans continues to grow, despite ongoing efforts to process and recycle (Montazer *et. al*., 2020; Rose *et. al*., 2020).

Along the decades of the existence of this problem, enough materials have been accumulated to demonstrate that a way to solve it is biodegradation (Montazer *et. al*., 2018; Mohanan *et. al*., 2020). Sources for the isolation of microorganisms capable of destroying the polymeric polyethylene chain were diverse, from polluted and unpolluted soils, to solid plastic waste or wastewater (Usha *et. al*., 2011; Harshvardhan and Jha, 2013; Divyalakshmi *et. al*., 2016; Bergman *et. al*., 2019; Montazer *et. al*., 2020).

Over time, a wide range of variables have appeared that allow the measurement of plastic biodegradation, such as weight loss, changes in mechanical and physical properties (tensile strength, crystallinity, and water absorption), changes in the chemical structure of the polymer, and the emission percentage of carbon dioxide (Mohanan *et. al*., 2020, Bhatia *et. al*., 2014).

The aim of our research was the isolation and characterization of consortia of microorganisms that populate the surface of low-density polyethylene films extracted from the soil contaminated with polyethylene.

MATERIAL AND METHOD

Soil samples polluted with plastic and other contaminants, collected from the dump near the village Slobozia-Duşca, Criuleni district, Republic of Moldova, served as a research material. Soil samples was cleaned of roots and other impurities, homogenized, sieved (mesh No. 2) and air-dried at 22-23˚C.

Low-density polyethylene (LDPE) films produced by Kraus Folie Sp.J., with a thickness of 35 µm, were also used as material. The films were cut into longitudinal and transversal strips.

The incubation experiments were carried out under laboratory conditions in pots with polluted soil (150 g/pot) in which the LDPE strips were added. The experiment protocol included 6 experimental variants: 3 variants were incubated in oxic conditions and 3 in anoxic conditions. Under oxic conditions the soil humidity was kept constant at 60%. Anaerobic conditions were created by saturating the contaminated soil with water, 80% of the water holding capacity. To stimulate the indigenous microflora, mineral salts were added in different concentrations (tab. 1). The duration of polyethylene incubation in the soil was 180 days (6 months).

Table 1

The content of mineral salts in the experimental variants, g/150 g soil

Experimental variants		K_2HPO_4	KH_2PO_4	$(NH_4)_2HPO_4$
Oxic conditions	**1**	Soil without amendments		
	2	0.075	-	0.075
	3	-	0.075	0.075
Anoxic conditions	**4**	Soil without amendments		
	5	0.75	-	+
	6	-	0.75	0.75

Consortia of microorganisms were isolated from LDPE surface, through enrichment techniques. Enrichment cultures were prepared by adding 10 mL aliquot to

90 mL mineral salt medium (MSM). The composition of the mineral media was as follows, g/L: K_2HPO_4 – 1.0, KH_2PO_4 – 1.0, NH_4NO_3 – 1.0, $MgSO_4 \cdot 7H_2O$ – 0.2, $FeCl_3$ – 0.05, $CaCl_2$ – 0.02, pH=6.0 (Postolachi et. al., 2021). At the consortia creation stage, LDPE strips were added to the culture medium. As a growth inducer in the media was added glucose, in a concentration of 0.1%. The culture period was for 100 days, on a shaker, at 28°C.

Characterization of density and diversity of obtained consortia was performed by cultivation on solid media MSM, Nutrient agar (Oxoid, England) and Czapek-Dox agar. The identification of all isolates was performed according to the colonial aspects and microscopic morphology (Garibova and Lekomtseva, 2005). The morphological characters observation of isolates was performed with optical microscope Optica® Microscopes B-510 PH (Italy).

The modification of the LDPE films was evaluated under the optical microscope and through the tensile test. The tensile testing of polyethylene was performed using the tensile testing machine CQ-508B (COMETECH Testing Machines Co., LTD). Such parameters as elongation at break (%) and tensile strength at break (MPa) were determined.

RESULTS AND DISCUSSIONS

The creation and isolation of microbial consortia, which populate the surface of the LDPE film, is a long process, which required long incubation in soil, and then long cultivation in submerged conditions. In the end, 6 microbial consortia were obtained, with different density, and on this index, as seen in figure 1, the mineral salts added to stimulate the indigenous microflora had an influence.

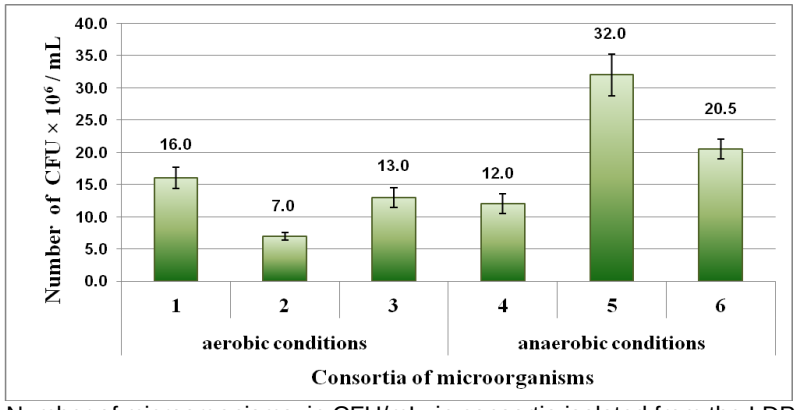


Fig. 1. Number of microorganisms, in CFU/mL, in consortia isolated from the LDPE surface extracted from the soil treated under aerobic and anaerobic conditions

The obtained data show that after 100 days of cultivation the microorganisms in the consortia retain their viability, the titer being from 7.00 × 10^6 CFU/mL, up to 32.00 × 10^6 CFU/mL. If we compare the consortia obtained in oxic conditions (1, 2, and 3) with those in anoxic conditions (4, 5, and 6), then we can say that in the anoxic variants the viability, in general, was higher. It should

be noted that in the oxic variants, Consortium 1 (Aerobic control) had the highest viability. In anoxic variants, Consortium 5 had the highest population density of microorganisms compared to all obtained consortia.

Isolation of microorganisms on solid media revealed the existence of 12 species of microorganisms. Most of the consortia were formed by micromycetes. The isolated micromycetes belonged to different genera: 3 species of the genus *Trichoderma*, 4 species of the genus *Penicillium*, 1 species of the genus *Fusarium*, and 3 representatives of the phylum *Ascomycota*. Isolated bacteria were part of the phylum *Actinobacteria*, genus *Streptomyces*.

The diversity of microorganisms in the consortia also varied depending on the previous incubation conditions in the soil (Figure 2): **Consortium 1** consisted of *Trichoderma* sp.2, *Trichoderma* sp.3, and *Penicillium* sp.1; **Consortium 2** consisted of *Trichoderma* sp.2, *Penicillium* sp.1, and *Penicillium* sp.2; **Consortium 3** consisted of *Trichoderma* sp.1, *Trichoderma* sp.3, *Penicillium* sp.1, and *Fusarium* sp.1; **Consortium 4** consisted of *Trichoderma* sp.2, *Penicillium* sp.1, and *Ascomycota* var.1; **Consortium 5** consisted of *Ascomycota* var.1, *Trichoderma* sp.1, *Trichoderma* sp. 2, and *Penicillium* sp.1; **Consortium 6** consisted of *Ascomycota* var.1, *Ascomycota* var.2, *Ascomycota* var.3, *Penicillium* sp.1, *Penicillium* sp.4, *Trichoderma* sp. 2, and *Streptomyces* sp.1.

It was observed that in each of the consortia, 1 or 2 strains of microorganisms prevailed. The most frequently encountered microorganisms were: *Penicillium* sp. 1 (in 5 consortia), *Trichoderma* sp. 2 (in 4 consortia) and *Ascomycota* var. 1 (in 3 consortia).

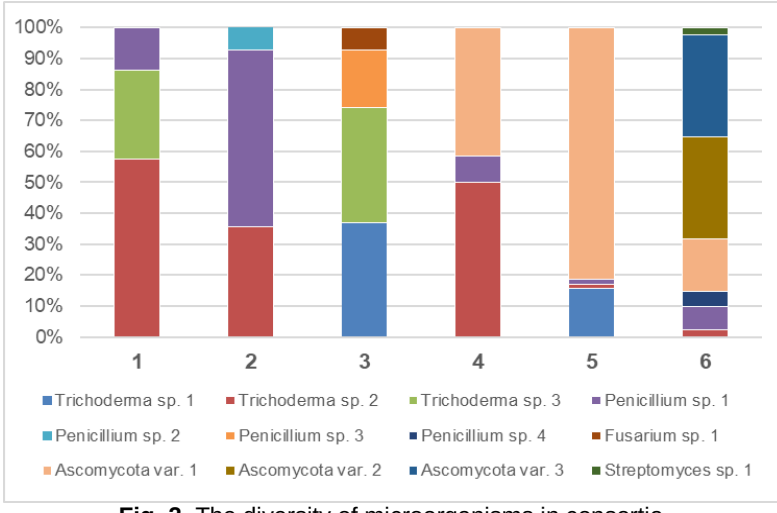
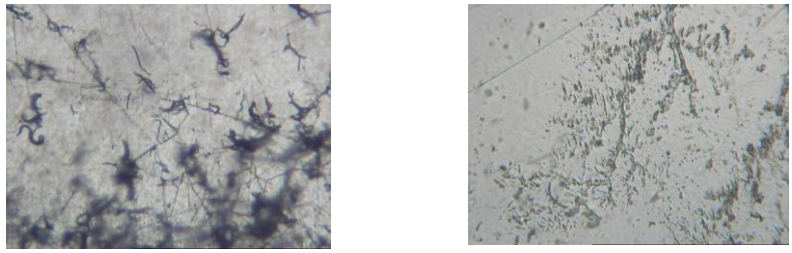


Fig. 2. The diversity of microorganisms in consortia

In the last stage of the research, the changes in the LDPE strips produced during the incubation period were studied. It is known that microorganisms have

the ability to fix and colonize the surface of plastic materials (Rose *et. al*., 2020). In the case of our experiments, it was also observed under the optical microscope that both adhesion of fungal cells and damage to the polyethylene surface occurred.



Adhesion of fungal hyphae on the surface of polyethylene

Surface bioerosion of the polyethylene

Fig. 3. The optical microscope photos of LDPE films

According to data from the literature, as a result of the vital activity of microorganisms on the polyethylene surface, bioerosions are formed, which in turn lead to a decrease in tensile strength, elongation at break and contact angle; in addition, new polar groups such as –OH, C=O, COOH and COO- appear on the main chain of the polymer matrix (Suresh *et. al*., 2019, Montazer *et. al*., 2020).

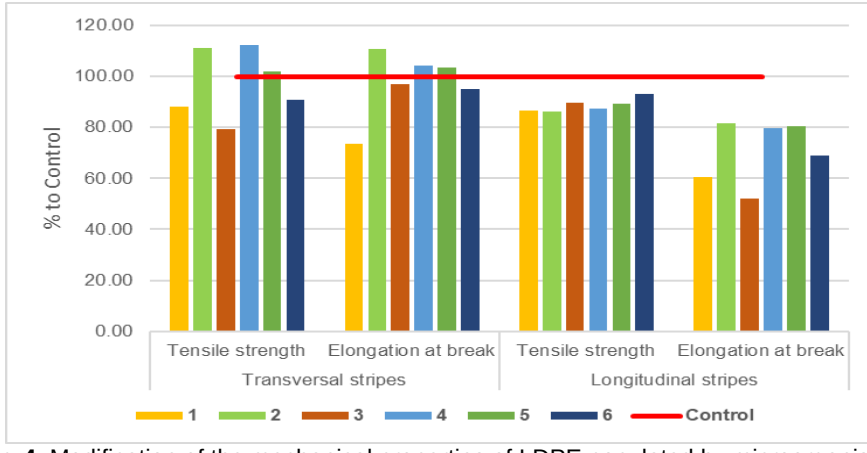


Fig.4. Modification of the mechanical properties of LDPE populated by microorganisms

Performing polyethylene tensile tests showed that in all experimental variants the mechanical properties, such as tensile strength and elongation at break, under influence of microorganisms have changed (Figure 4). It was established that under the influence of Consortia 1, 4 and 5 the mechanical resistance of the transversal strips is higher or practically equal to that of the control film. The mechanical resistance of the longitudinal strips, in all experimental variants, is significantly lower than that of the control film.

CONCLUSIONS

Amending the soil with potassium and ammonium phosphates and creating oxic/anoxic incubation conditions influence both the density and the diversity of the population of microorganisms in the consortia that populate the polyethylene surface.

The isolated consortia demonstrated the ability to populate the LDPE surface and to produce physical changes in the plastic, which allows us to consider these consortia have potential in the biodegradation of non-recyclable plastic.

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