CHARACTERIZATION OF MICROBIAL CONSORTIA ISOLATED FROM THE SURFACE OF LOW DENSITY POLYETHYLENE FILMS

CARACTERIZAREA CONSORTIILOR MICROBIENE IZOLATE DE PE SUPRAFAȚA FILMELOR DE POLIETILENA DE JOASĂ DENSITATE

RASTIMESINA Inna[1]*, POSTOLACHI Olga[1], VORONA Valentina[1], MAMALIGA Vera[1], VOINESCU Alina[1], GUTUL Tatiana[2], STATI D.[2]
*Corresponding author e-mail: inna.rastimesina@imb.utm.md

Abstract. *The ability of microorganisms to transform or detoxify organic compounds is a well-known fact that promotes the development of environmental decontamination biotechnologies. The aim of this work is the characterization of the microbial consortium isolated from the surface of low density polyethylene films. LDPE films extracted from the soil were cultivated in liquid mineral salt media (MSM) with various composition and acidity. The microbial consortia isolated from MSM pH 5.5 and MSM pH 6.5 had a mixed composition. The analysis of the LDPE films by the FTIR method showed that the microorganisms, which populated the surface of the LDPE samples, cause chemical changes, observed on the absorption spectra of the films, such as the appearance of some absorption bands, or their splitting.*
Key words: microbial consortia, polyethylene, LDPE, mineral salt media

Rezumat. *Capacitatea microorganismelor de a transforma sau detoxifica compușii organici este un fapt bine-cunoscut, care contribuie la dezvoltarea biotehnologiilor de decontaminare a mediului. Scopul acestei lucrări este caracterizarea consorțiului microbian izolat de pe suprafața filmelor de polietilenă de joasă densitate. Filmele LDPE extrase din sol au fost cultivate în medii minerale lichide (MSM) cu diverse compoziții și aciditate. Consorțiile microbiene izolate din MSM pH 5,5 și MSM pH 6,5 au avut o compoziție mixtă. Analiza filmelor LDPE prin metoda FTIR a arătat că microorganismele, care au populat suprafața probelor de LDPE, provoacă modificări chimice, observate pe spectrele de absorbție ale filmelor, precum apariția unor benzi de absorbție, sau scindarea acestora.*
Cuvinte cheie: consorții microbiene, polietilenă, LDPE, medii minerale

[1]Institute of Microbiology and Biotechnology, Technical University of Moldova, Republic of Moldova
[2]Institute of Electronic Engineering and Nanotechnologies 'D.Ghitu', Technical University of Moldova, Republic of Moldova

INTRODUCTION

Nowadays, polyethylene-based plastic materials are widely used for packaging products, in construction, transport, medicine. Low-density polyethylene (LDPE, PE-LD), the main material for the production of disposable bags, the most widespread type of plastic packaging, accumulates and pollutes the environment. According to legislative changes, in the Republic of Moldova, polyethylene bags will be gradually withdrawn from circulation (Lege…, 2016, 2017), but still LDPE will remain for a long time the main cause of persistent environmental pollution. In the year 2022, almost 100 thousand tons of plastic materials were imported. The analysis of the last 10 years showed that the amount of plastic materials has registered a constant increase, with an average of 4% annually, but the recycling rate of plastic waste in the Republic of Moldova is only 3% (Studiu…, 2023). Evaluating the ability of microorganisms to transform or detoxify organic compounds is a recognized trend in the development of environmental decontamination biotechnologies. Microorganisms participate both in the degradation of synthetic and natural polymers. Thus, polymeric materials represent a potential source of carbon and energy for heterotrophic microorganisms, bacteria and fungi.

The aim of this work is the characterization of the microbial consortium isolated from landfill soil polluted with low density polyethylene.

MATERIAL AND METHOD

The soil was collected from the landfill, located near the village of Slobozia-Duşca, the Criuleni district, the Republic of Moldova. Kraus Folie Lamilen LDPE films (35 μm) were placed in the soil amended with mono- and dipotassium and diammonium phosphates, in concentration of 0.05% each, according to the protocol represented on table 1.

Table 1

Treatment protocol for soil amendment in laboratory conditions

Amendments	Consortium			
	1	2	3	4
K_2HPO_4	-	+	-	+
KH_2PO_4	-	-	+	+
$(NH_4)_2HPO_4$	-	+	+	-

After six months the films extracted from soil were placed to the flasks with liquid mineral salt medium MSM 2 (pH 6.0) and cultivated under continuous stirring conditions at 28°C. After 90 days of cultivation, cultures were inoculated on MSM 2 (pH 5.5) and MSM 4 (pH 6.5) liquid media, through enrichment techniques. The composition of culture medium MSM 2 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. Culture medium MSM 4 consists of (g/L) NH_4Cl – 4.0, $NaNO_3$ – 2.0, K_2HPO_4 – 1.8, $MgSO_4 \cdot 7H_2O$ – 0.2, NaCl – 0.1, $FeSO_4 \cdot 7H_2O$ – 0.01 (Postolachi *et. al.*, 2021; Postolachi *et. al.*, 2022). Enrichment cultures were prepared by adding 10 mL aliquots to 90 mL MSM. As a

growth inducer in the media was added glucose, in a concentration of 0.1%. LDPE was added to the culture media in the form of films and cultivation was continued for 270 days for microbial consortium formation. At the end of cultivation, catalase activity and pH of culture media was determined.

Samples from the enrichment culture were serially diluted and plated onto culture media: MSM 2 agar, Nutrient agar, and Czapek-Dox agar. The plates were incubating at 28°C for 7-10 days. Growing colonies were selected and streaked successively onto the same media for purification. The isolates were examined for their Gram reaction, endospore formation, and characteristics of a colony, such as color, colony form, margin, surface, and elevation. The morphological peculiarities of the microorganisms and LDPE films after the contact with microbial consortia were studied under the optical microscope Optica® Microscopes B-510 PH, Italy. Parameters of the tensile test, such as stress (tensile strength) at break and elongation at break were studied on a tensile testing machine CQ-508B (COMETECH Testing Machines Co., LTD). The LDPE degradation was determined by Fourier-transform infrared spectroscopy (FTIR).

RESULTS AND DISCUSSIONS

The results of long-term cultivation of microbial consortia in the presence of LDPE are shown on figure 1.

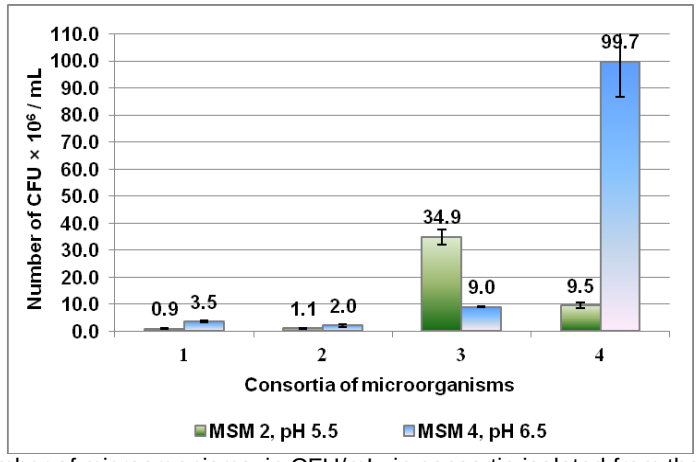

Fig. 1. Number of microorganisms, in CFU/mL, in consortia isolated from the surface of LDPE films, extracted from the soil with different amendments, and cultivated on mineral salt media.

The number of microorganisms in consortia, cultivated on MSM 2 medium (pH 5.5), obtained after LDPE incubation varied from 0.93×10^6 to 34.87×10^6 CFU/ml. The titer of microorganisms in consortia, cultivated on MSM 4 (pH 6.5) medium, obtained after LDPE incubation varied from 2.0×10^6 to 99.73×10^6 CFU/ml, in experimental variant 4, grown on MSM 4 medium (pH 6.5). This was due to the large number of bacteria in this consortium, up to 95% of the

population. All consortia cultivated on MSM 2 and MSM 4 had a mixed composition (tab. 2, tab. 3).

Table 2

Composition of microbial consortia isolated from the LDPE surface and cultivated on culture medium MSM 2, pH, and catalase test

Consortium	Microorganisms	% from population	pH	Catalase
1	**Fungi**	92.86	**3.70**	+
	Bacteria	7.14		
2	**Fungi**	68.75	3.85	+
	Bacteria	31.25		
3	Fungi	14.91	4.98	+
	Bacteria	85.09		
4	Fungi	21.83	**5.31**	+
	Bacteria	78.17		

Of 4 consortia obtained on MSM 2 medium, 2 consortia predominantly contained micromycetes, and 2 consortia contained predominantly bacteria (tab. 2). Of 4 consortia obtained on MSM 4 medium, 2 consortia contained predominantly micromycetes, in 1 consortium bacteria were predominant, and 1 consortium contained bacteria and micromycetes in approximately equal proportions (Table 3). In fungal consortia, the titer of microorganisms was comparative low, from 0.93×10^6 to 9.0×10^6 CFU/ml, with the increase in the proportion of bacteria in the population, the titer also increased.

Table 3

Composition of microbial consortia isolated from the LDPE surface and cultivated on culture medium MSM 4, pH, and catalase test

Consortium	Microorganisms	% from population	pH	Catalase
1	**Fungi**	54.72	5.96	+
	Bacteria	45.28		
2	**Fungi**	76.67	**5.77**	+
	Bacteria	23.33		
3	Fungi	82.22	**6.07**	+
	Bacteria	17.78		
4	Fungi	5.08	5.97	+
	Bacteria	94.92		

Indifferently of experimental media, culture medium became more acidic, especially in variant 1 (MSM 2). Catalase test in both variants of cultural media was positive.

The composition of microbial consortia was studied in detail, and includes mycelial fungi, representatives of the genera *Trichoderma*, *Penicillium*, *Fusarium* and bacteria from the genera *Bacillus*, *Pseudomonas*, *Streptomyces*.

The results of microscopic investigations of LDPE films are presented on figure 2.

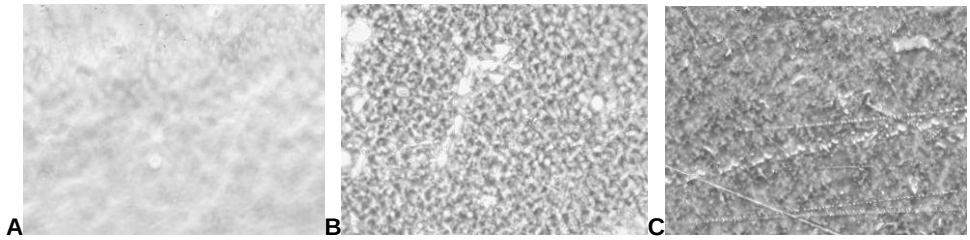

Fig. 2. Microscopic images of LDPE films before and after cultivation with microbial consortia: (A) control, (B) LDPE film, cultivated on MSM 2 with microbial consortia 1, (C) LDPE film, cultivated on MSM 2 with microbial consortia 3.

Compare with untreated LDPE, mechanical parameters of the tensile test, such as stress at break and elongation at break are decreasing, in all variant of microbial consortia, especially for variant 1 (fig. 3).

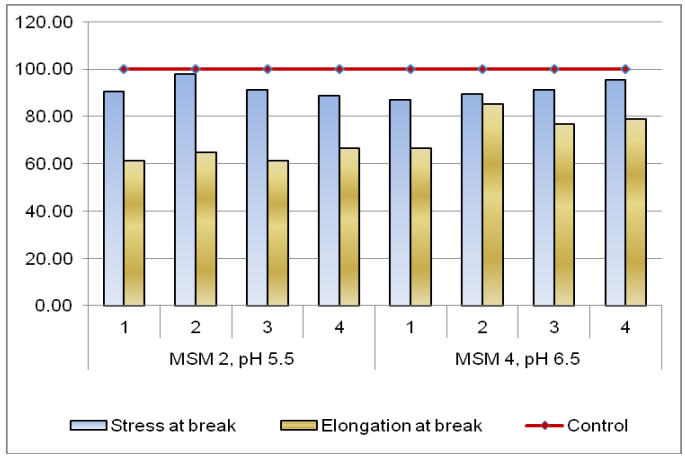

Fig. 3. Tensile test of LDPE films, cultivated with microbial consortia

FTIR is a useful tool to determine the formation of new or disappearing functional groups. Thus, this technique can determine the degradation products, the chemical fragments incorporated in the polymer molecules. The analysis of the LDPE films by the FTIR method showed that the microorganisms, which populated the surface of the LDPE samples, cause physical changes, observed on the absorption spectra of the films, such as the appearance of some absorption bands (891, 879, and 802 cm^{-1}), or their splitting (2988, 2971 cm^{-1}), as presented on figure 4.

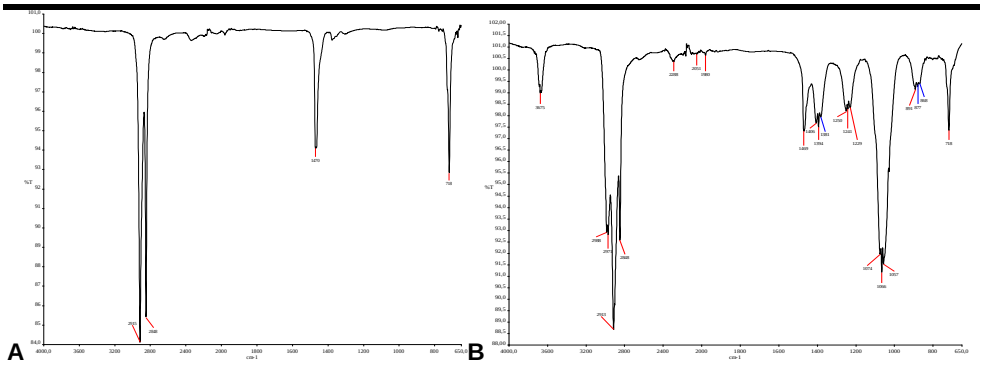

Fig. 4. FT-IR chromatogram of LDPE films: (A) control LDPE, (B) LDPE film, cultivated on MSM 2 with microbial consortia 1

CONCLUSIONS

1. By using accumulation cultures with different pH, with the involvement of microorganisms that colonized the surface of the LDPE films, used in the experiments with polluted soil from the landfill near com. Slobozia-Dușca, 8 microbial consortia with high viability, up to 99.73 × 10^6 CFU/ml, were isolated and analyzed.

2. Practically all consortia obtained by cultivation on MSM 2 and MSM 4 media had a mixed composition, and include mycelial fungi and bacteria.

3. Fourier transform infrared spectroscopy (FTIR) analysis confirmed the degradation of LDPE polyethylene films after the contact with the microorganisms.

Acknowledgments: This work was funded by NARD of the Republic of Moldova within State Program Project 20.80009.7007.03 "Microbial tools for degradation of non-recyclable plastics waste".

REFERENCES

1. **Postolachi O., Rastimesina I., Josan V., Mamaliga V., Cotoman A., Stati D., Gutul T., 2021 -** *Screening of cultivation media for LDPE biodegradation by Penicillium verrucosum CNM-FP-02*. Romanian Journal of Ecology & Environment Chemistry, 3(2), p. 48-53.
2. **Postolachi O., Rastimesina I., Vorona V., Mamaliga V., 2022 -** Screening of cultivation media for LDPE biodegradation by *Pseudomonas fluorescens*. *Scientific Bulletin. Series F. Biotechnologies*., vol. XXVI, nr. 2, p. 151-157.
3. *****, 2016 -** Lege privind deşeurile Nr.209 din 29.07.2016. Publicat: 23.12.2016 în Monitorul Oficial Nr. 459-471, art Nr: 916. http://lex.justice.md/md/368030/
4. *****, 2017 -** Lege pentru completarea Legii nr. 231/2010 cu privire la comerţul interior Nr. 134 din 07.07.2017. Publicat: 28.07.2017 în Monitorul Oficial Nr. 265-273, art Nr: 438.http://lex.justice.md/md/371663/
5. *****, 2023 -** Studiu privind deșeurile din Plastic în Republica Moldova. https://e-circular.org/wp-content/uploads/2023/02/STUDIU-PRIVIND-DESEURILE-DIN-PLASTIC-IN-REPUBLICA-MOLDOVA.pdf