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EFFECT OF *PSEUDOMONAS* BACTERIA ON BIODEGRADATION OF POLYMER STRUCTURES

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Abstract

The growing accumulation of polymer waste has become a global environmental challenge, necessitating the development of sustainable mitigation strategies. Bioremediation, based on the use of microorganisms to degrade pollutants in soil and water, is considered a promising approach. This study provides a theoretical analysis of current research on the ability of soil microbiota to decompose synthetic polymers and proposes a method for polymer waste degradation using a combined biological system. The approach involves the joint application of Eisenia foetida culture and bacterial strains of the genus Pseudomonas. Polymer–polymer blends based on corn starch were selected as model materials, specifically ethylene–vinyl acetate copolymer (EVA) and chlorinated polyethylene (CPE). Experimental substrate variants were inoculated with Pseudomonas chlororaphis subsp. aurantiaca UKM-9 and Pseudomonas chlororaphis subsp. aureofaciens UKM B-109 at a concentration of 1.1×10^{7} CFU/g of dry substrate. The results demonstrated that E. foetida significantly enhances microbial development, increasing the total microbial count from 10^{7} to 10^{9} CFU/g. This indicates a high adaptive potential of the microbiota to substrates obtained via vermiculture. The biochemical characteristics and taxonomic affiliation of the bacterial strains were confirmed using the oxidase test. It was established that physicochemical parameters of the substrate, including temperature, moisture, electrical conductivity, and pH, play a key role in regulating microbial activity and growth. Significant changes in electrical conductivity (up to 3999 µS/cm in CPE samples) suggest polymer bond cleavage, ion release, and the formation of soluble degradation products. Optical analysis confirmed substantial biodegradation levels, reaching 72.1 % for CPE and 67 % for EVA. The findings demonstrate the high efficiency of combined biological systems in polymer degradation and underline their potential for developing environmentally safe technologies for the management of synthetic polymer waste.

Keywords: biodestruction; polymer composite materials; biodegradable polymers; soil; biomass; bacteria; technogenic pollution; soil protection technologies, vermiculture.

ВПЛИВ БАКТЕРІЙ РОДУ *PSEUDOMONAS* НА БІОДЕСТРУКЦІЮ ПОЛІМЕРНИХ СТРУКТУР

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Анотація

Загроза забруднення полімерними відходами стала глобальною проблемою для навколишнього середовища. Одним з методів її рішення є біоремедіація з використанням мікроорганізмів для розкладання забруднювачів ґрунту та води. Проаналізовані сучасні дослідження щодо здатності мікробіоти ґрунтів до біологічного розкладу синтетичних полімерів. Стаття присвячена розробці способу деградації полімерних відходів з використанням комбінованого впливу культури *E. foetida* і штамів бактерій роду *Pseudomonas*. В якості досліджуваного матеріалу обрано полімер-полімерні суміші на основі кукурудзяного крохмалю: сополімер етилена з вінілацетатом (EVA) та хлорований поліетилен (CPE). Для визначення загальної кількості мікроорганізмів у дослідні варіанти субстрату вносили штами бактерій *Pseudomonas.chlororaphis subsp. aurantiaca* УКМ-9, *Pseudomonas.chlororaphis subsp. aureofaciens* УКМ В-109 у кількості 1.1×10^{7} КУО/1 г абсолютно сухого субстрату. Експериментально підтверджено, що культура *E. foetida* підсилює розвиток мікробіоти в субстраті від 10^{7} до 10^{9} КУО/1 г. Приріст мікробіоти свідчить про високу адаптаційну здатність популяції до умов субстрату. Оцінка біохімічних властивостей досліджуваних штамів *P. chlororaphis subsp. aurantiaca УКМ-9*, *P. chlororaphis subsp. aureofaciens* УКМ В-109 та їх належність до роду *Pseudomonas* підтверджено оксидазним тестом. Доведено, що для регуляції мікробіологічної активності, росту та розмноження бактерій значну роль відіграють фізико-хімічні характеристики субстратуНайбільші зміни значень електропровідності в зразках 3,4-CPE (2464 мкСм/см, 3999 мкСм/см) вказують на розщеплення зв'язків полімерних структур, вивільнення іонів та утворення розчинних продуктів. Оптичні дослідження показали, що присутність об'єктів біологічного комплексу сприяє біодеструкції CPE на 72.1 %, EVA на 67 %. Ефективність біологічних комплексів у біодеструкції полімерних матеріалів показують їх перспективність для створення екологічно безпечних технологій утилізації полімерних відходів.

Ключові слова: біодеструкція; полімерні композиційні матеріали; біодеградабельні полімери; ґрунт; біомаса; бактерії; техногенне забруднення; технології захисту ґрунтів; вермікультивування.

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Introduction

The threat of pollution caused by polymer waste has become a global environmental problem, prompting the search for sustainable approaches to mitigate its negative impact. One such approach is bioremediation, which involves the use of microorganisms to degrade contaminants in soil and water. This method is considered cost-effective, environmentally friendly, and renewable [1; 2]. Researchers [3] have focused on the use of bacteria of the genus *Pseudomonas* for the biodegradation of polyethylene. A bacterial strain isolated from soil and identified as *Pseudomonas aeruginosa* was studied. It was found that the *P. aeruginosa* strain was capable of degrading polyethylene by 11.5 % after 16 days of incubation. Another study [4] investigated the use of fungi of the genus *Aspergillus* for the efficient degradation of low-density polyethylene and polyurethane, achieving degradation rates of up to 55 % within 30 days. Analysis of previous studies [5; 6] indicates that *P. aeruginosa* and related bacterial species are capable of effectively degrading low-density polyethylene due to their enzymatic activity. These findings were confirmed by the authors using morphological, spectroscopic, and chromatographic analyses. In study [7], the authors examined the biodegradation of several polymers, including polyethylene, polyurethane, polyethylene terephthalate, polystyrene, polypropylene, polyvinyl chloride, and polyvinyl alcohol. A wide range of microorganisms capable of catalyzing polymer depolymerization were described, including bacteria (*Pseudomonas, Bacillus, Ideonella*) and fungi (*Aspergillus, Penicillium, Fusarium,* among others).

In another study [8], it was demonstrated that the isolate *P. aeruginosa* VITARK5 is capable of actively colonizing the surface of polystyrene and utilizing it as the sole carbon source. Due to biofilm formation and metabolic activity, the microorganisms caused a reduction in sample mass by 14.53 %, as well as the formation of cracks and surface erosion. Furthermore, a number of organic compounds with potential industrial value were identified, indicating not only the environmental but also the economic potential of applying the *P. aeruginosa* VITARK5 strain for polystyrene bioremediation.

The biodegradation of polyvinyl alcohol (PVA) involving the bacterium *Pseudomonas alcaligenes* was investigated in studies [9; 10]. The authors found that the degradation rate depends on the initial polymer concentration, pH of the medium, inoculum size, and process conditions. The researchers noted that *P. alcaligenes* exhibits significant potential for the bioremediation of PVA-based materials, and that the use of different technological approaches can improve degradation efficiency. Studies [11; 12] explored the possibility of biodegrading disposable masks made of polypropylene using *Pseudomonas fluorescens*. The experiments were conducted over a 12-month period under both controlled and natural conditions using three strategies: bioaugmentation of soil-like substrates with P. fluorescens, biostimulation through nutrient supplementation, and a combination of both approaches. The results demonstrated that the application of *P. fluorescens* under soil conditions can significantly accelerate the degradation of polypropylene waste, particularly disposable masks, opening prospects for environmentally safe disposal of this type of polymer. Another study [13] demonstrated that strains of *Pseudomonas taiwanensis* and *Pseudomonas alloputida*, isolated from anthropogenically degraded environments, exhibit high hydrolytic activity and are capable of effectively degrading polylactide (PLA) under mesophilic conditions. The authors highlighted that the ability of these bacteria to form biofilms plays a key role, as it ensures direct contact with the polymer surface and accelerates the degradation process. The findings suggest the potential application of *P. taiwanensis* and *P. alloputida* strains in composting processes and soil remediation from biopolymer waste. At the same time, it is emphasized that a major limitation remains the presence of non-biodegradable polymers, particularly polypropylene (PP) and polyethylene (PE), for which alternative disposal methods are still required. Overall, the analysis of recent studies indicates that the biodegradation of synthetic polymers by microorganisms is considered one of the most environmentally safe approaches for reducing polymer pollution.

Experimental Section

The objects of investigation were polymer–polymer blends based on plasticized corn starch combined with synthetic polymers, namely ethylene–vinyl acetate copolymer (EVA) and chlorinated polyethylene (CPE) [14]. Biohumus obtained through vermicultivation [15] was used as a substrate to study the biodegradation potential of polymer blends by the bacterial strains *Pseudomonas chlororaphis subsp. aurantiaca* UKM-9 and *Pseudomonas chlororaphis* *subsp. aureofaciens* UKM B-109 in the presence of the earthworm culture *Eisenia fetida*. The duration of the experiment was 60 days. The mass of the samples was measured using torsion balances (WT-500). Temperature, electrical conductivity, and pH were determined using a Hanna HI 991300 measuring device. The morphology of polymer samples (EVA and CPE) before and after biodegradation was examined using a Digital Microscope MM-2288-5X-BN. The degree of biodegradation of the polymer materials was determined according to methodologies regulated by ISO 14855 and ASTM D5338 standards [16; 17]. The bacterial strains *P. chlororaphis subsp. aurantiaca* UKM-9 and *P. chlororaphis subsp. aureofaciens* UKM B-109 were cultivated in meat-peptone broth (MPB) at pH 8.2–8.4. The culture medium was sterilized in an autoclave for 30 minutes at 121 °C. To isolate microorganisms from the substrate and to further analyze colony formation on different media, eleven samples of equal weight were prepared. The composition of these samples is presented in Table 1.

Table 1

Composition of experimental samples for determining the CFU of bacteria of the genus Pseudomonas

Sample No	Polymer	Suspension Volume, mL	Biological Complex	
			Bacterial strain	Eisenia foetida Culture
0	Control	-	Biohumus	+
1	EVA	10	Pseudomonas chlororaphis subsp aureofaciens УКМ В-109	+
2		30		+
3	CPE	10	Pseudomonas chlororaphis subsp aureofaciens УКМ В-109	+
4		30		+
5	EVA	10	Pseudomonas chlororaphis subsp aurantiaca УКМ-91	+
6		30		+
7	CPE	10	Pseudomonas chlororaphis subsp aurantiaca УКМ-91	+
8		30		+
9	EVA	10	Pseudomonas chlororaphis subsp aureofaciens УКМ В-109	-
10	CPE	10	Pseudomonas chlororaphis subsp aurantiaca УКМ-91	-

To determine the total number of microorganisms (excluding micromycetes) in the substrate, the method of inoculating microbial suspensions onto nutrient media was used [18]. The microbial suspension was prepared according to a standard procedure followed by serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, etc.). Morphological and cultural characteristics of the colonies were evaluated, including colony color, pigmentation intensity, cell shape, Gram staining properties, pigment production, as well as the transparency and consistency of colonies grown on the specified media [19; 20]. The morphometric characteristics of microbial cells were analyzed using light microscopy with a Carl Zeiss Primo Star optical microscope. The total number of microorganisms (excluding micromycetes) in the substrate was determined using meat-peptone agar (MPA) prepared on Conda nutrient broth (Fig. 1). The number of bacteria belonging to the genus *Pseudomonas (Pseudomonas chlororaphis subsp. aurantiaca, Pseudomonas chlororaphis subsp. aureofaciens,* and *Pseudomonas fluorescens)* in the substrate was determined by inoculation onto selective nutrient media, namely King A medium (Pseudomonas P agar) Conda and King B medium (Pseudomonas F agar) Conda [21; 22].

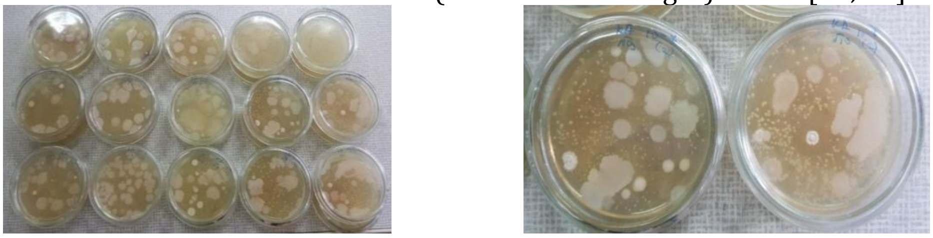


Fig. 1. Determination of the total number of microorganisms and the number of bacteria of the genus Pseudomonas in the composition of experimental samples

To recalculate the number of microorganisms per 1 g of absolutely dry substrate (a.d.s.), the moisture content of the experimental samples was determined according to the procedure described in [23]. To confirm the affiliation of the bacterial isolates to the genus *Pseudomonas*, oxidase test discs (Himedia, DD018) were used. The absence of a color change on the disc or the development of coloration within 60 s at a temperature of 25–30 °C was interpreted as a negative reaction [24].

Results and Discussion

The moisture content determined in the substrate samples, depending on their composition (CPE and EVA), enabled a reliable comparison of the total number of microorganisms (excluding micromycetes) and the number of bacteria of the genus *Pseudomonas*. Based on the experimental data on substrate moisture content, a diagram was constructed illustrating the changes in EVA and CPE samples (Fig. 2).

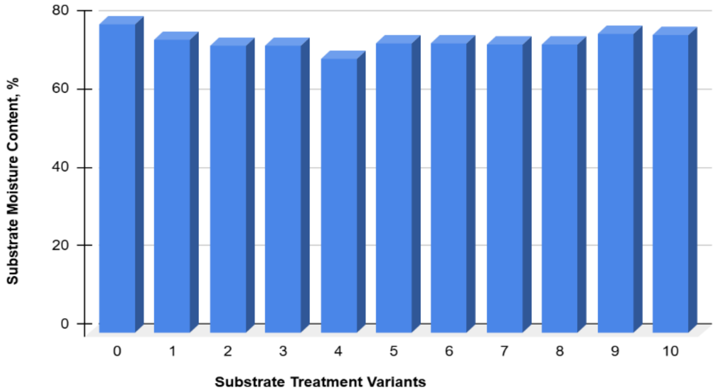


Fig. 2. Moisture content in substrate samples depending on their composition (CPE, EVA)

The obtained results indicate that the slight differences in the water absorption capacity of the samples are due to the varying hygroscopicity of their components (plasticized starch and synthetic polymers). Specifically, the control sample exhibited a moisture content of 78.64 %, while the lowest value of 69.86 % was recorded for the fourth sample. To determine the total number of microorganisms, the experimental substrate variants were inoculated with the bacterial strains *P. chlororaphis subsp. aurantiaca* UKM-9 and *P. chlororaphis subsp. aureofaciens* UKM B-109 at a concentration of 1.1×10^7 CFU per 1 g of absolutely dry substrate (a.d.s.). Subsequent counts demonstrated a positive growth trend (Table 2).

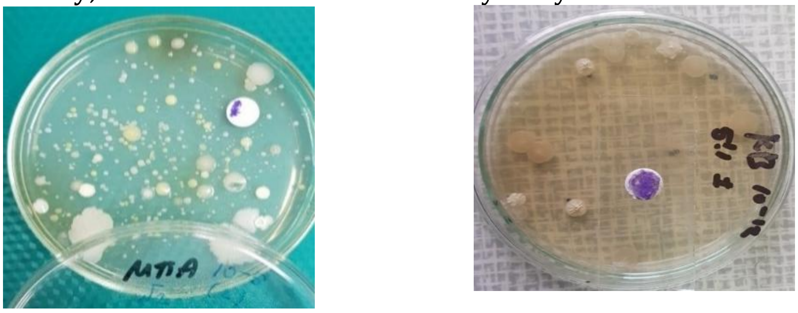
Table 2

Total number of microorganisms (except for microfungi) and number of bacteria of the genus Pseudomonas in substrates with the addition of polymer-polymer mixtures (in 1 g of dry matter)

Substrate Treatment Variant	Microorganism Count, CFU / 1 g a.d.s.	
	Pseudomonas spp. *s*	Total (excluding micromycetes)
0	$\geq8.4 \pm 0.1\times10^6$	$\geq11.2 \pm0.2\times10^{10}$
1	$\geq5.9 \pm 0.2 \times 10^7$	$\geq9.0 \pm 0.2\times10^{10}$
2	$\geq7.4 \pm 0.2\times10^7$	$\geq1.5 \pm 0.3\times10^9$
3	$\geq1.1 \pm 0.2\times10^9$	$\geq1.2 \pm 0.2\times10^{10}$
4	$\geq2.3 \pm 0.4\times10^9$	$\geq1.7 \pm 0.3\times10^{10}$
5	$\geq1.9 \pm 0.3\times10^9$	$\geq1.3 \pm 0.3\times10^{10}$
6	$\geq1.7 \pm 0.2\times10^9$	$\geq5.0 \pm 0.3\times10^{10}$
7	$\geq4.5 \pm 0.1\times10^7$	$\geq2.8 \pm 0.2\times10^{10}$
8	$\geq6.7 \pm 0.1\times10^7$	$\geq6.4 \pm 0.1\times10^{10}$
9	$\geq8.3 \pm 0.1\times10^7$	$\geq22.5 \pm 0.2\times10^{10}$
10	$\geq1.2 \pm 0.2\times10^7$	$\geq13.1 \pm 0.2\times10^{10}$

The results indicate that the inoculation of bacteria at a concentration of 1.1×10^7 CFU/g led to significant changes in the microbial proliferation within the tested substrate samples. In samples 3,4-CPE and 5,6-EVA, the maximum increase in bacterial counts (over a 200-fold rise compared to the initial inoculation) demonstrated a high adaptive capacity of the bacterial populations to the substrate conditions. In contrast, bacterial growth in samples 1,2,9-CPE and 7,8-EVA was moderate; specifically, in sample 2-CPE, counts increased 6.7-fold, in 8-EVA 6.1-fold, and in 9-EVA 7.5-fold. In the control sample, microbial counts remained at the level of the natural background. The intense increase in microbial populations in the experimental substrates indicated that the presence of the Eisenia fetida culture enhances the development of microbiota, with counts rising from 10^7 to 10^9 CFU per 1 g of substrate.

Microscopic examination revealed the cultural and morphological characteristics of *P. chlororaphis subsp. aurantiaca* UKM-9 and *P. chlororaphis subsp. aureofaciens* UKM B-109. The bacteria were Gram-negative, straight to slightly curved rod-shaped cells, averaging 0.5–1.0 µm in size. Colonies on MPA nutrient media were smooth, convex, and shiny; some colonies produced pigments (pyocyanin, pyoverdine), giving them characteristic coloration (Fig. 3a, b). The oxidase test was employed to confirm the affiliation of the isolated cultures to the genus Pseudomonas, as most representatives of this genus possess a cytochrome oxidase system. The test was conducted by transferring a colony onto an oxidase disc at 25 °C, and within 10 s, a violet-blue coloration appeared, indicating the activity of the enzyme cytochrome c oxidase (Fig. 3b).



a) **b)**

Fig. 3. Visualization of the oxidase-positive reaction of *Pseudomonas*

Most bacteria of the genus *Pseudomonas* are oxidase-positive, indicating their ability to utilize the cytochrome system for electron transport during aerobic respiration, which is a characteristic diagnostic feature of the genus. Thus, the oxidase positivity of the isolates confirms their species-specific affiliation and allows differentiation from related genera that lack this enzyme.

In the studied substrate samples (Table 1) of CPE and EVA polymer blends, the dynamics of pH changes during biodegradation (after 15 days) in the presence of the biological complexes are presented in Figure 4.

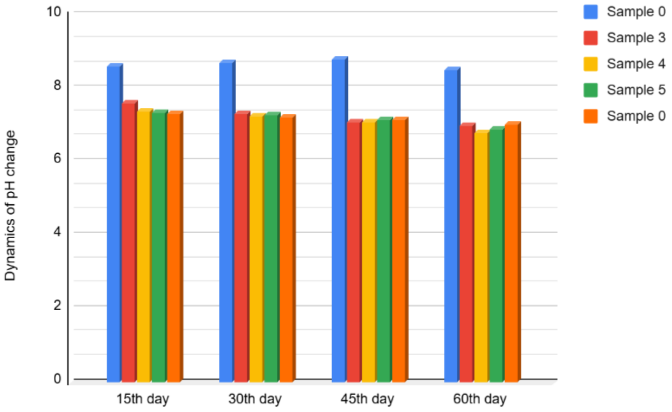


Fig. 4. Dynamics of the pH environment during the biodegradation of polymer mixture samples

In the control sample, the pH remained stable, whereas in the experimental samples, a gradual decrease in pH was observed, indicating the formation of an acidic environment as a result of microbial activity. The most pronounced acidification was observed in sample 4-CPE, suggesting the highest biodegradation activity. The results of electrical conductivity measurements show the dynamics of changes in the parameters during the biodegradation of polymer material samples (Fig. 5), specifically in samples 3,4-CPE and 5,6-EVA.

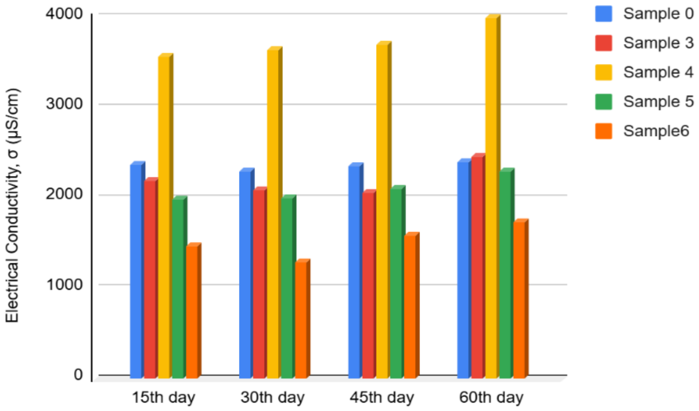
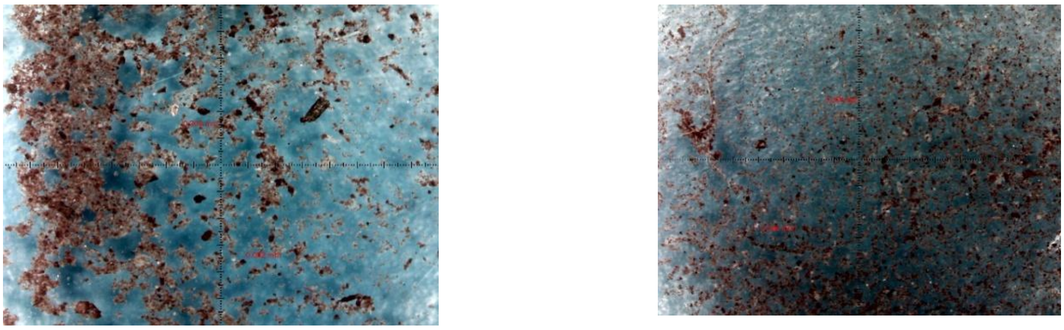


Fig. 5. Dynamics of electrical conductivity during biodegradation of samples 3,4-CPE and 5,6-EVA

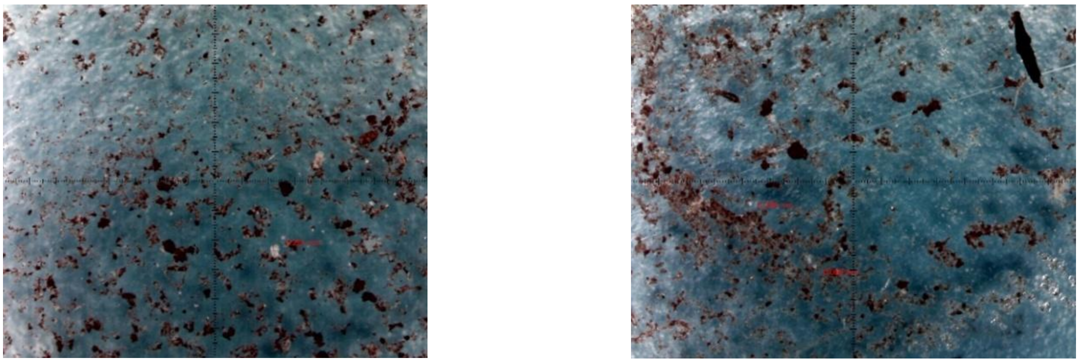
In the control sample, electrical conductivity remained relatively stable over the 60-day period, ranging from 2370 to 2397 µS/cm. An increase in electrical conductivity was observed in sample 3-CPE, from 2109 to 2464 µS/cm, and in sample 4-CPE, from 3565 to 3999 µS/cm. Sample 5-EVA showed an increase from 1985 to 2290 µS/cm, while sample 6-EVA ranged from 1465 to 1738 µS/cm. The most pronounced changes in electrical conductivity were observed in samples 3 and 4-CPE. Therefore, the dynamics of conductivity increase in these samples indicate an intensive progression of polymer matrix degradation processes. Optical examination of sample 4-CPE in vermicompost, in the presence of the biological complex *P. chlororaphis subsp. aurantiaca* UKM-91 and *E. foetida*, is presented in Figure 6.


a)

b)

Fig. 6. Changes in the surface of sample 4-CPE in the presence of a biological complex: a) before the experiment; b) after 60 days of biodegradation

For sample 4-CPE, it was determined that within the biological complex composed of P. chlororaphis subsp. aureofaciens UKM B-109 and E. foetida, the degree of biodegradation was 72.1%The results of the surface analysis of sample 5-EVA, before and after biodegradation in the presence of the components of *the biological complex (P. chlororaphis subsp. aureofaciens UKM B-109 and E. fetida*), are presented in Figure 7.


a)

b)

Fig. 7. Changes in the surface of the 5-EVA sample in the presence of biological complex objects a) before the experiment; b) after 60 days of biodegradation

For sample 5-EVA, it was determined that in the presence of the biological complex *P. chlororaphis subsp. aurantiaca* UKM B-91, the degree of biodegradation reached 67 %. The interaction mechanism between the microbiota of the biological complex and the CPE and EVA polymer samples occurs in several sequential stages. In the initial stage, *Pseudomonas chlororaphis subsp. aurantiaca* UKM-9 and *Pseudomonas chlororaphis subsp. aureofaciens* UKM B-109 adsorb onto the surface of the polymer matrix and form a biofilm, establishing close contact between the cells and the polymer substrate. Subsequent biodegradation is associated with the enzymatic activity of the microorganisms, resulting in oxidative depolymerization of polymer chains through the cleavage of C–C and C–H bonds. This process leads to the formation of low-molecular-weight products, including monomers, oligomers, and organic acids. These compounds are then utilized by the microorganisms as a source of carbon and energy, promoting further degradation of the polymer matrix.

A comparative analysis of literature data and the results of the present study indicates that the efficiency of polymer blend biodegradation depends on the type of polymer, the composition of the microbial consortium, and the conditions of the biodegradation process (Table 2).

Table 2

Comparison of the effectiveness of polymer biodegradation in different studies

Microorganism	Polymer	Experiment Duration	Degree of Biodegradation
Pseudomonas aeruginosa [3]	Polyethylene (PE)	16th day	11,5 %
Aspergillus sp [4]	Low-Density Polyethylene (LDPE)	30th day	до 55 %
Pseudomonas aeruginosa VITARK5 [8]	Polystyrene (PS)	30th day	14,53 %
Pseudomonas chlororaphis + Eisenia foetida	CPE	60th day	72,1 %
Pseudomonas chlororaphis + Eisenia foetida	EVA	60th day	67 %

Thus, the results of the study indicate that the use of biological complexes enhances the biodegradation processes of polymer materials.

Conclusions

It was determined that the slight differences in the water absorption capacity of CPE and EVA samples are due to the varying hygroscopicity of their components (plasticized starch and synthetic polymers). The highest electrical conductivity values (3565–3999 µS/cm) observed in sample 4-CPE indicate an intensive progression of polymer matrix degradation.

The biological complex *P. chlororaphis subsp. aureofaciens* UKM B-109 in combination with E. fetida contributed to the biodegradation of 4-CPE by 72.1 % and 5-EVA by 67%. The most pronounced increase in the population of bacterial strains *P. chlororaphis subsp. aurantiaca* UKM-9 and *P. chlororaphis subsp. aureofaciens* UKM B-109 in the experimental substrates confirmed that the presence of *E. fetida* promotes the development of microbiota, increasing counts from 10^7 to 10^9 CFU per 1 g of absolutely dry substrate. The study of the efficiency of biological complexes in polymer biodegradation demonstrates their potential for the development of environmentally safe technologies for the disposal of synthetic polymer waste.

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