Inżynieria i Ochrona Środowiska 2018, 21(4), 407-418
Engineering and Protection of Environment
https://ios.is.pcz.pl/

p-ISSN 1505-3695
e-ISSN 2391-7253
DOI: 10.17512/ios.2018.4.7

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Testing of Biopreparations for Bioremediation of Soils Contaminated with Petroleum Substances

Testowanie biopreparatów do bioremediacji gleb zanieczyszczonych substancjami ropopochodnymi

At present, the natural environment is heavily polluted by xenobiotics, which is a global problem. Among the wide range of pollutants present in the surrounding environment, particular attention should be paid to crude oil and its derivatives. Petroleum compounds include lubricants, fuels, bitumens etc. where the demand is enormous; therefore the contamination of the soil environment with oil-derived substances is inevitable. It is therefore necessary to take all actions aimed at environmental protection by reducing this type of pollution. One of the methods leading to lowering the concentration of oil products in the soil environment is bioremediation - a method that uses specific properties of microorganisms to break down petroleum into compounds that are less toxic to the environment. The aim of the experiment was to examine the degree of degradability of petroleum compounds present in soil by biopreparations constructed on the basis of bacteria and to assess the impact of Fenton's reactions on bioremediation processes. Biopreparations were applied into contaminated soil in immobilized and non-immobilized form. In the conducted study biopreparations on the basis of *P. putida* and *P. Fluorescens* were tested for petroleum hydrocarbons contaminated soil. The CO_2 amount was measured as an indicator of effective bioremediation process using Micro-Oxymax respirometer. Moreover, the kinetics of the degradation of petroleum hydrocarbons has also been investigated using Fenton's reaction in the experiment and a modified reaction in the presence of siderophores and citric acid. It was found that the higher bioremediation potential properties have been demonstrated for biopreparation - alginate capsules with *P. putida*, *P. fluorescens* with modified Fenton reaction (citric acid + Fenton reagent). For these treatments the bioremediation kinetics of total petroleum hydrocarbons had the highest values.

Keywords: **soil, biopreparations, total petroleum hydrocarbons, bioremediation**

Introduction

The devastation and contamination of the natural environment represents the progressive process, resulting from human industrial activity. In order to mitigate the adverse impacts of anthropogenic substances produced and released into the environment (e.g. petroleum derivatives), specific actions have been taken to restore the original condition of the natural environment (soil, water, air). One of the methods used is bioremediation, recommended for combating the growing problem of environmental pollution with petroleum derivatives [1]. Crude oil and its derivatives are of key importance for the global economy. These compounds are very common, consequently leading to progressing contamination of soils and water. A particular threat to the environment is posed by all kinds of industrial accidents occurring in technological or storage facilities where petroleum derivatives are stored. Release of these substances leads to contamination of soil, water and air and may have a negative impact on human life [2].

1. Soil contamination by petroleum derivatives

Soil contamination by petroleum and its derivatives can occur in various ways. It may be caused by deliberate discharge of such compounds into the soil environment (components of pesticides or petrochemical wastewater) or as a result of losses which occurred during production, e.g. tank failures. Another possibility of the release of petroleum compounds into the soil environment is transport (diesel oils, lubricants leaking from various means of transport, oil spills) and the use of chemical raw materials. Among the places where petroleum is present in the soil are, among others, areas of ports and fuel stations [3].

The presence of oil on the soil surface for a longer period of time contributes to the initiation of the phenomenon of petroleum sorption on soil particles. Environmental conditions have a very significant impact on the migration of components contained in oils. These include in particular rainfall, because with water, the components of oils can penetrate into deeper parts of the soil and even into the groundwater. If petroleum derivatives become components of groundwater, there is a risk of contamination of potable water. With migration of petroleum to deeper parts of the soil, the spaces through which water and air should go through become clogged. This leads to clumping of the soil and, consequently, changes in its biological, physical and chemical properties. The soil contaminated with petroleum is characterized by low sorption capacity. The disturbance of the chemical-physical configuration of the soil generates many modifications of the biological structure (the extinction of microorganisms). Another effect is the synthesis of very large amounts of nitrogenous organic substances, leading to a substantial remodelling of the quantitative and qualitative composition of soil microflora (Table 1).

Table 1. **Effects of soil contamination with petroleum: accumulation of nitrogenous organic substances [3]**

Soil contaminated with petroleum: effect of nitrogenous organic substances on soil microflora
• Reduction in the number of microorganisms, mainly aerobic bacteria
• Reduction in nitrogen-assimilating bacteria
• Reduction in fiber-oxidizing bacteria
• Reduction in the number of fungi
• Accumulation of hydrocarbon-binding microorganisms
• Large number of ammonification bacteria
• Presence of large amounts of ammonia (small number of nitrifying bacteria)

Maintaining biological balance is extremely important. However, if it is disturbed due to the effect of unwanted factors, the problems arise with oxygen availability and available forms of P and N for plants. All these consequences are caused by the presence of significant carbon concentrations, which has a very adverse effect on the activity of microorganisms present in the soil and capable of oxidising hydrocarbons that are components of petroleum. Consequently, microbiological decomposition in the soil environment is quite limited and slow [3].

2. Methods of removing petroleum pollutants

Contamination of the environment by petroleum derivatives is enormous and this problem inspires human activities aimed at reducing this type of pollution, because the presence and impact of these substances on the environment is very undesirable. However, before an appropriate method of elimination of petroleum-derivative pollutants is used, it is very important to consider and analyse many parameters, and use the results of the analysis to choose the remediation method [4, 5].

The parameters determining the choice of the method of treatment of soil contaminated with petroleum derivatives are the type of soil (organic soils, non-cohesive and cohesive soils, layered or homogeneous soils), the type of pollutant, its concentration and physical and chemical properties, the time for which the given pollutant is present, the area covered by the pollution (depth and surface), vegetation, climatic conditions, economic and technical limitations [4, 5].

The methods used to treat the environment are usually based on the immobilisation of pollutants through application of insulation or its complete removal [6]. The methods used for soil treatment from petroleum derivatives include physico-chemical methods (blowing the soil with air with simultaneous suction, steam blowing, extraction, combustion) and biological methods (bioremediation) [7].

3. Biological elimination of pollutants: bioremediation

Bioremediation is a concept that was first introduced into scientific literature in 1987 [8]. It means a biological method allowing for elimination of various organic pollutants from both soil and water. It should be added that it is a very effective and economical process. This method is based on the activity of microorganisms (bacteria, fungi, yeasts). Bioremediation allows for elimination of such organic compounds as: petroleum derivatives, PAHs (polycyclic aromatic hydrocarbons) and halogen derivatives. The effect of microorganisms against petroleum-based compounds leads to their mineralization to compounds less harmful to the environment (CO_2 and H_2O) or transformation or neutralization of pollutants into natural and safe compounds. However, it should be emphasized that during the soil remediation process, intermediate products may also be formed. The most common group of microorganisms used in bioremediation are bacteria. This choice is not accidental and it is very much related to the potential of these microorganisms for decomposing contaminants, because during the metabolic transformation of bacteria, products causing the decomposition of contaminants are generated, and the bacteria have the potential for relatively rapid growth. Biological elimination of pollutants (bioremediation) is a technology that does not interfere in any harmful way with the ecosystem, thus being environmentally friendly [9, 10]. The only disadvantage of bioremediation is long time, but this method also has many advantages, which include: low financial outlays (economical method) on the process, elimination of pollution can be carried out in situ, the possibility of using soil directly after the end of the process, lack of synthesis of harmful compounds (bacteria convert the components of pollutants to CO_2 and water, which are environmentally neutral), no need to use expensive equipment [2]. The above mentioned advantages make bioremediation an unrivalled technology compared to traditional techniques of soil environment treatment. Its unrivalled quality is particularly evident when the contamination covers a very large area, where the use of techniques of soil translocation is infeasible due to the wide area of contamination (extensive contamination).

Two groups of factors affecting the bioremediation process can be distinguished. The first category includes parameters that have a positive effect on bioremediation processes, while the second category includes parameters that have a limiting effect.

Table 2. **Factors limiting and stimulating bioremediation processes [11]**

Limiting factors	Stimulating factors
• Environmental (e.g. absence of biogenic elements) • Microbiological (e.g. microorganisms growth rate) • Substrate (e.g. very high concentration of pollutants) • Difficulties in transmission of factors (e.g. O_2 diffusion and solubility) • Physicochemical availability of the pollutant (e.g. irreversible sorption)	• Oxygen (anaerobic metabolism - oxygen concentration lower than 1%, aerobic metabolism: oxygen concentration over 0.2 mg O_2) • pH (5.5÷8.5) • Temperature (4÷45°C) • Access of H_2O (25÷85%) • Presence of biogenic elements (C:N:P of 100:10:1)

4. Microorganisms involved in the decomposition of petroleum-derived compounds

The potential of microorganisms for transformation, i.e. transformation of petroleum derivatives has been formed for centuries as a result of the release of oil from deep layers of the soil environment to the surface. As a result of the long-lasting process described above, some of the bacteria have developed processes in their metabolisms that enable the use of this type of compounds. The selection of bacteria showing the characteristics of the decomposition of petroleum derivatives should be made in areas rich in crude oil or natural gas [12].

The digestive processes in microorganisms are closely connected with the synthesis of enzymes and this is quite a complicated process since most of them are produced only when they respond to certain chemical compounds that are components of the nutrients [12]. Bacterial enzymes such as dehydrogenases, hydrolases, oxygenases are required for the biodegradation of hydrocarbons being the component of petroleum. Microorganisms use hydrocarbons for basic life processes (growth, reproduction), which is related to the genetic information of a particular microorganism because it encodes the synthesis of enzymes involved in the transformation of hydrocarbons to acetyl-CoA. Genetic information is usually recorded on plasmids or in the bacterial genome [13].

Biodegradation of petroleum derivatives can occur at two levels, under aerobic (Fig. 1) and anaerobic conditions (Fig. 2) [14]. A significant number of microorganisms present in the environment are involved in bioremediation processes under aerobic conditions.

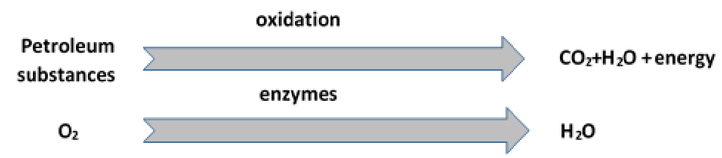

Fig. 1. Degradation of petroleum compounds under aerobic conditions [14]

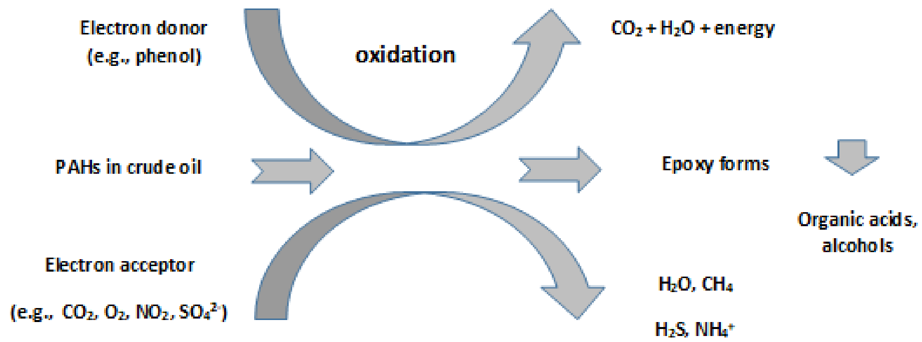

Fig. 2. Degradation of petroleum compounds under anaerobic conditions [14]

Microorganisms, with their specific abilities to bioremediate petroleum derivatives, are an important tool in all bioremediation processes. It is very important to select an appropriate consortium of microorganisms, because on its basis it is possible to create appropriate bacterial or fungal or mixed biopreparations, which, after application to soil contaminated by oil derivatives, intensify the bioremediation process. During preparation of an appropriate biopreparation, many factors are important for the effective action of the biopreparation. Special attention should be paid to the precise choice of microorganisms, often isolated from areas rich in specific contaminants. Biopreparations represent inocula, i.e. biological vaccines, which, after application to the soil environment can perform two basic tasks:

they intensify the process of biological treatment and facilitate the efficient growth of microflora [14].

5. Bacteria degrading petroleum derivatives

Among microorganisms, bacteria are the highest in the ranking when it comes to bioremediation properties in the soil environment. This is not a coincidence, because they have the possibility of degradation of a wide group of petroleum derivatives, rapid growth rate, large number, susceptibility to genetic manipulation, ease of growing).

Pseudomonas species of bacteria are characterized by a wide spectrum of degrading contaminants, but bacteria such as *Nocardia*, *Mycobacterium* also attract much interest since they have potential for transformation and degradation of hydrocarbons which are components of petroleum (Table 3).

Table 3. **Bacteria capable of decomposing aliphatic and aromatic compounds [14]**

Bacteria that decompose aliphatic compounds in petroleum	Bacteria that decompose aromatic compounds in petroleum
Acinetobacter	*Nocardia sp.*
Methylomonas	*Flavobacterium sp.*
Aeromonas	*Pseudomonas putida*
Micromonospora	*Moraxella sp.*
Bacillus	*P. fluorescenes*
Nocardia	*Achromobacter sp.*
Brevibacterium	*P. mildenbergii*
Pseudomonas	*Beijerinckiasp*
Flavobacterium	*P. aeruginosa*
Spirillum	*Corynebacterium renale*
Methylococus	
Vibrio	

The process of degradation of the above mentioned compounds using bacteria, especially in the first phase of the reaction, requires the presence of molecular oxygen. Its occurrence has a huge impact on the speed of reaction. Degradation of aromatic compounds is a rather complicated process since biochemical decomposition pathways occur in many stages.

Particular attention should be paid to bacterial biosurfactants, as the ability of bacteria to degrade petroleum derivatives is closely related to them. In addition to their positive impact on bioremediation processes (the decomposition process is significantly facilitated), the substances listed above are also safe for the surrounding natural environment [15]. The synthesis of biosurfactants by bacteria depends on certain environmental parameters and also on the availability of nutrients. The effect of bacterial biosurfactants consists primarily in catalysis of dispersion, which results in a process causing dispersion of oil particles. Only molecules in this state of dispersion can be susceptible to specific enzymes and undergo transformations. Bacteria have properties that allow for synthesis of two types of biosurfactants: multimolecular biosurfactants, strongly connecting to the surface, and low-molecular-weight biosurfactants, reducing surface tension [16].

6. Fungi decomposing petroleum-derivatives

Fungi present in the natural environment have potential for accumulating substances with a relatively high degree of toxicity. Fungi belong to the group of microorganisms that have widely colonized the natural environment. Therefore they have acquired many features that make it easier for them to adapt to unfavourable environmental conditions [17].

Fungi that break down aliphatic hydrocarbons include in particular: *Hansenula*, *Candid*, *Saccharomyces Sporobolomyces*, *Trichosporon*, *Pichia*, *Selenotila* [18].

Petroleum derivatives get into the environment as a result of such processes as asphalt production or fuel combustion (anthropogenic activity), and are very harmful and have mutagenic, genotoxic and carcinogenic effects. Filamentous fungi decompose them by means of appropriate intracellular enzymes (monoxygenase produced by cytochrome P450) and extracellular enzymes (ligninolytic enzymes) [19].

7. Experimental part

The experimental part of the study involved several stages. The first stage was screening of microbiological material (bacteria, fungi) from the soil anthropogenic environment contaminated with petroleum derivatives. The isolated microorganisms were used to prepare biopreparations (immobilization of *Pseudomonas fluorescens*, *Pseudomonas putida* in alginate), which were introduced into soil contaminated by oil derivatives. Using 0.5 kg of soil, 10 g of *P. fluorescens* capsules were introduced into the first sample and 10 g of *P. putdia* capsules into the second sample. The above mentioned bacteria were also introduced into the soil samples in a non-immobilized form - 40 ml of medium containing bacteria (OD 600 equal to 1) per 0.5 kg of soil. With the applied biopreparations, these soil samples were put into a respirometer (Respirometer Micro-Oxymax) measuring the level of carbon dioxide produced as a result of the use of petroleum derivatives as a source of carbon and energy by microorganisms.

The aim of the experiment was to examine which of the biopreparations used showed the best properties in terms of decomposition of petroleum derivatives. In order to determine the effect of biopreparations on the distribution of contaminants present in the soil, an experiment was conducted using a Respirometer Micro-Oxymax device. The device recorded the amount of carbon dioxide in consecutive measurement cycles. The amount of CO_2 was measured in µl. The amount of accumulated carbon dioxide suggested the bioremediation potential of a given biopreparation. Soil samples, in which the apparatus recorded high concentrations of carbon dioxide, contained bioreparations with significant bioremediation potential (Table 4).

Table 4. **Amount of accumulated carbon dioxide in the process using bacterial biopreparations (soil contaminated with petroleum substances from external layers of soil); values in the table expressed in µl**

M.c.	Control	*P. fluorescens*	Alginate capsules + *P. fluorescens*	*P. putida*	Alginate capsules + *P. putida*
0	0.00	0.00	0.00	0.00	0.00
1	2157.05	4782.38	2679.35	4484.51	2195.01
2	4506.16	9965.50	8191.88	9372.55	7748.33
3	7062.94	15152.97	13641.52	14204.79	13302.09
4	9486.23	20347.24	19162.59	19099.72	18862.59
5	12054.47	25527.03	24597.39	23915.82	24400.71
6	14446.30	30679.40	30065.69	28759.55	29902.99
7	16968.17	35837.21	35474.82	33548.86	35417.05
8	19305.80	41016.36	40959.93	38421.64	40950.34
9	21783.60	46230.57	46419.14	43267.33	46516.95
10	24055.70	51445.57	51939.77	48173.93	52078.54
11	26472.58	56655.42	57395.07	53017.2	57641.29
12	28722.98	61877.92	62922.85	57941.35	63214.19
13	31107.56	67109.09	68399.27	62816.37	68793.80
14	33304.64	72346.77	73937.66	67757.72	74380.47
15	35630.98	77591.02	79426.58	72644.89	79975.20
16	37804.01	82836.21	84973.48	77573.54	85567.29
17	40109.89	87859.41	90450.67	82419.75	91142.30
18	42268.72	92642.46	95971.33	87251.79	96713.22
19	44576.89	97181.34	101444.2	92047.13	102280.10
20	46745.13	101384.60	106946.00	96793.41	107837.30
21	49063.68	105179.00	112390.10	101521.40	113394.40
22	51203.30	108605.60	117888.10	106279.50	118944.8
23	53525.04	111889.80	123328.00	111005.20	124499.00
24	55696.23	115129.20	128823.70	115720.70	130048.20
25	58062.36	118338.10	134266.10	120428.70	135600.30
26	60274.16	121651.50	139779.80	125157.50	141155.50
27	62661.91	124944.80	145191.80	129882.00	146668.10
28	64856.44	128225.60	150650.40	134531.20	152172.10
29	67300.41	131421.50	156102.20	139138.00	157732.80
30	69542.73	134540.80	161636.80	143670.40	163318.60
31	72047.50	137593.80	167118.30	148166.00	168908.00
32	74313.41	140600.70	172660.00	152599.50	174499.10
33	76907.45	143609.60	178117.20	157034.40	180081.20
34	79235.97	146701.00	183619.50	161552.40	185643.20
35	81918.51	149817.30	189056.00	166034.80	191190.60
36	84266.09	152970.90	194549.30	170513.70	196733.30
37	86998.13	156061.40	200050.20	174912.30	202282.00
38	89612.9	159147.70	205451.90	179335.30	207799.80
39	91926.21	162220.00	210908.10	183730.70	213307.70
40	94647.26	165251.50	216304.10	188061.50	218817.30
M.c. - measurement cycle					

The highest value of accumulated carbon dioxide expressed in µl in the last measurement cycle recorded by the Respirometer Mikro-Oxymax was found for the biopreparation of alginate balls + *P. putida* (it was equal to 218817.3 µl). Not much less (only by 2513.2 µl) of accumulated carbon dioxide was recorded by the device for the biopreparation of alginate balls + *P. fluorescens* (216304.1 µl). Comparison of these concentrations with control leads to the conclusion that the tested biopreparations showed the best properties in terms of decomposition of petroleum derivatives.

7.1. Modified Fenton's reaction

The Fenton's reaction is a radical reaction. The combination of H_2O_2 with Fe^{2+} ions results in a reaction that produces highly reactive hydroxyl radicals that are capable of reacting with several different contaminants. For this reason, the attempts were made to verify the usefulness of the Fenton's reaction in bioremediation processes. Furthermore, a modified Fenton's reaction in the presence of citric acid and siderophores (low molecular weight compounds synthesized by *Pseudomonas fluorescens*) was also performed. The control was also enriched with the addition of citric acid. Three samples were tested. The first sample contained 0.5 kg of soil and 20 ml of H_2O_2 and 20 ml of citric acid, the second - 0.5 kg of soil and 20 ml of H_2O_2 and 20 ml of FMM medium (Fiss Minimal Medium - the medium needed to initiate the production of siderophores by *P. fluorescens* bacteria), and the third - 0.5 kg of soil and 40 ml of H_2O_2 (Table 5).

Table 5. **Amounts of accumulated CO_2 in soils in the modified Fenton's reaction (petroleum-derived soil from external soil layers). Values in the table expressed in µl**

M.c.	Control	Fenton H_2O_2 + citric acid	Fenton + H_2O_2 siderophores	Fenton + H_2O_2	M.c.	Control	Fenton H_2O_2 + citric acid	Fenton + H_2O_2 siderophores	Fenton + H_2O_2
0	0.00	0.00	0.00	0.00	21	91511.34	111796.6	104658.10	103819.20
1	2261.85	2685.70	4470.32	1885.33	22	96044.02	117225.5	109633.80	108902.80
2	6889.08	8156.99	9503.60	7100.32	23	100563.10	122641.00	114598.70	113968.90
3	10375.77	13625.43	14524.39	12064.00	24	105068.70	128052.60	119559.10	119028.90
4	14134.36	19110.38	19552.6	17234.68	25	109591.90	133472.90	124532.00	124103.40
5	18726.01	24546.95	24539.78	22306.42	26	114157.90	138946.20	129549.20	129222.00
6	23257.02	29949.27	29502.04	27349.36	27	118723.30	144417.80	134564.40	134339.60
7	27766.81	35354.9	34472.18	32392.19	28	123286.00	149885.70	139573.40	139448.90
8	32278.21	40762.84	39449.13	37432.49	29	127866.10	155376.50	144602.30	144583.50
9	36826.86	46218.35	44458.45	42517.63	30	132453.00	160870.70	149639.20	149729.80
10	41376.07	51674.25	49469.53	47602.63	31	137054.80	166378.10	154693.30	154885.30
11	45924.6	57129.23	54480.33	52696.54	32	141659.30	171892.40	159746.20	160039.50
12	50473.48	62586.81	59486.10	57789.16	33	146266.10	177405.40	164793.50	165196.30
13	55026.89	68047.17	64495.95	62888.68	34	150871.50	182908.80	169838.00	170352.90
14	59590.99	73522.34	69526.08	68009.31	35	155480.20	188421.60	174886.60	175511.30
15	64158.25	79003.20	74559.30	73133.60	36	160120.00	193973.90	179964.60	180707.30
16	68725.89	84481.96	79589.40	78257.30	37	164749.80	199517.10	185042.70	185891.30
17	73286.96	89951.29	84608.64	83373.99	38	169348.50	205023.40	190089.00	191045.40
18	77842.39	95413.18	89622.30	88487.98	39	173957.90	210531.50	195133.30	196216.30
19	82400.49	100874.5	94638.49	93603.00	40	178586.00	216070.00	200207.20	201398.90
20	86957.81	106341.2	99655.14	98713.71	M.c. - measurement cycle				

The highest value of accumulated carbon dioxide was recorded for the soil in which the modified Fenton's reaction with citric acid was carried out, with the value of accumulated CO_2 in the final cycle for this sample being 216070 μl. Comparison of the results with the control sample reveals that the additives introduced into the soil led to initiation of the bioremediation process.

8. Discussion and conclusions

Bacteria have the capability to break down petroleum derivatives and are active until the nutrients are exhausted [20]. The analysis of available literature data confirms that a wide range of studies conducted worldwide have focused in particular on the processes carried out by selected species of microorganisms and on the study of mechanisms in terms of the effect of bacterial hydrolytic enzymes. Currently, studies also explore the problems related to expression of genes which are responsible for the synthesis of specific enzymes [21]. Two strains of bacteria were selected for the study: *Pseudomonas fluorescens*, *Pseudomonas putida*, which have been isolated from soils contaminated with petroleum derivatives. Tests performed using the Micro-Oxymax device demonstrated that strains of bacteria are capable of bioremediation of petroleum derivatives. Biopreparations based on the above-mentioned strains of bacteria and introduced into soils with high content of petroleum derivatives during the testing that lasted 3 weeks caused the decomposition of petroleum derivatives into carbon dioxide. This leads to the conclusion that these microorganisms are effective in degradation of petroleum-based compounds.

The aim of this experiment was to verify the opportunities for transformation of petroleum compounds into carbon dioxide by means of biopreparations made based on *P. putida* and *P. fluorescens* bacteria. The amount of released carbon dioxide was measured in soil samples to which biopreparations were introduced by means of a Respriometer Micro-Oxymax device. Based on the amount of gas accumulated in specific soil samples, it is possible to identify the biopreparation which has the best potential for the initiation of bioremediation processes. In general, more carbon dioxide generated in a specific sample of the contaminated soil translates into better bioremediation properties of the biopreparation used in the sample. Kinetics of the decomposition of petroleum derivatives was also studied using the basic Fenton's reaction and the modified Fenton's reaction in the presence of siderophores and citric acid in the experiment.

In conclusion, the best potential bioremediation properties were demonstrated for: biopreparation of alginate capsules + *P. putid*, *P. fluorescens*, modified Fenton's reaction (citric acid + Fenton's reagent).

Acknowledgements

The paper was presented at the conference "Wastewater treatment plants and sewage sludge management: universities for industry". The conference was co-financed by funds of the Ministry of Science and Higher Education for activities to promote science No. 805/P-DUN/2018. Participation of Paulina Kokot, M.Sc., in the conference was financed by BS/MN-401-304/18.

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Streszczenie

Obecnie środowisko naturalne jest w znacznym stopniu zanieczyszczone przez ksenobiotyki, co stanowi problem o charakterze globalnym. Wśród szerokiej gamy zanieczyszczeń obecnych w otaczającym środowisku należy zwrócić szczególną uwagę na ropę naftową i jej pochodne. Do związków ropopochodnych zalicza się smary, paliwa, asfalty itd., na które zapotrzebowanie jest ogromne, w związku z tym skażenie środowiska glebowego substancjami będącymi pochodnymi ropy naftowej jest nieuniknione. Należy więc podejmować wszelkie działania mające na celu ochronę środowiska poprzez redukcję tego typu zanieczyszczeń. Jedną z metod prowadzących do obniżenia stężenia produktów ropy w środowisku glebowym jest bioremediacja - metoda wykorzystująca specyficzne właściwości mikroorganizmów do rozkładu ropy naftowej na związki mniej toksyczne dla środowiska. Celem doświadczenia było zbadanie stopnia zdolności rozkładu związków ropopochodnych obecnych w glebie przez biopreparaty skonstruowane na bazie bakterii oraz ocena wpływu reakcji Fentona na procesy bioremediacyjne. Biopreparaty mikrobiologiczne wprowadzono do zanieczyszczonej gleby w postaci immobilizowanej i nieimmobilizowanej. W przeprowadzonym badaniu testowano biopreparaty opracowane na bazie mikroorganizmów *P. putida* i *P. fluorescens* na glebie zanieczyszczonej węglowodorami ropopochodnymi. Podczas badania mierzono ilość CO_2 jako wskaźnik skutecznego procesu bipremedacji z użyciem respitometru Micro-Oxymax. Ponadto zbadano również kinetykę degradacji węglowodorów ropopochodnych, stosując reakcję Fentona i jej modyfikację w obecności sideroforów i kwasu cytrynowego. Stwierdzono, że wyższe właściwości potencjału bioremediacji wykazano dla biopreparatów - kapsułki alginianowe z *P. putida*, *P. fluorescens* ze zmodyfikowaną reakcją Fentona (kwas cytrynowy + odczynnik Fentona). W przypadku tych zabiegów odnotowano najwyższe wartości parametrów kinetyki rozkładu węglowodorów ropopochodnych.

Słowa kluczowe: gleba, biopreparaty, bioremediacjia, węglowodory ropopochodne