

EWA ŚLIWKA*, BARBARA KOŁWZAN**,
KAZIMIERZ GRABAS**, ELENA KARPENKO***,
PIOTR RUTKOWSKI****

INFLUENCE OF RHAMNOLIPIDS FROM *PSEUDOMONAS* PS-17 ON COAL TAR AND PETROLEUM RESIDUE BIODEGRADATION

The efficiency of biological processes utilized for eliminating hydrocarbons from soil and water environment depends on many factors, among others on the type and level of contaminants and their bioavailability. Pollutants comprising highly hydrophobic and toxic hydrocarbons are characterized by low susceptibility to biodegradation. Among sources of such pollutants are coal tars and heavy petroleum fractions. This paper shows how rhamnolipids isolated from *Pseudomonas* PS-17 stimulate biodegradation of coal tar waste obtained from former gas work and petroleum residue obtained from atmospheric distillation of light petroleum. The study demonstrates low susceptibility of coal tar component to biodegradation. Addition of rhamnolipids initiated biodegradation process by increasing bioavailability of coal tar components. The highest efficiency of the coal tar biodegradation (28%) was observed at the rhamnolipids concentration of 125 mg in 1 dm³ of the cultivation medium. For the petroleum residue the maximum efficiency was 55% at the rhamnolipids concentration of 250 mg/dm³.

1. INTRODUCTION

The use of fuels and other petroleum and coal products results in the contamination of soil and water environment with crude oil, its final products and by-products. Taking account of the physicochemical characteristics of those substances, they are mainly multi-component mixtures of hydrocarbons (paraffinic, cycloparaffinic, mono- and polycyclic aromatic hydrocarbons) and heteroorganic compounds. Unlike petroleum products, liquid

* Faculty of Chemistry, Wrocław University of Technology, ul. Gdańska 7/9, 50-344 Wrocław, Poland. E-mail: ewa.sliwka@pwr.wroc.pl

** Faculty of Environmental Engineering, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland. E-mail: barbara.kolwzan@pwr.wroc.pl

*** Lviv Department of Physical-Organic Chemistry, National Academy of Science of Ukraine, Lviv, Ukraine.

**** Faculty of Chemistry, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland.

products obtained from coal contain greater amounts of PAH and significant amounts of phenols and heterocyclic compounds [1]–[4]. Serious contamination of water and soil environments with hydrocarbons results mainly from damages during exploitation, transportation and processing of crude oil as well as during distribution, storage and transportation of liquid fuels, when multi-tone spills of pollutants take place [5]–[7]. Immediate spill-response operations on the spill site are conducted to limit its environmental impacts. The other source of serious pollution is a long and improper storage or uncontrolled dumping of oil wastes (e.g. from drilling and refining or spent motor oils) or liquid coal products (e.g. creosotes, coke-oven and gas-works tars) [8]–[12]. They contain more higher molecular weight hydrocarbons, particularly polynuclear aromatic hydrocarbons. Those compounds are highly toxic, durable and capable of bio-accumulating [13], [14]. In the environment, they undergo very slow physical, chemical and biological changes. Such contaminants can be removed from water and soil by means of proper, specific methods. Biological methods proved to be quite promising. Biodegradation processes are more and more frequently being applied to technologies for removing different environmental pollutants [10], [15]–[19]. They are based on natural mechanisms of organic compounds degradation by microorganisms utilizing hydrocarbons as a source of carbon and energy. A proper process of bioremediation takes place when hydrocarbons are transformed into safe, nontoxic substances, preferably CO₂ and H₂O. Microbiological methods of water and soil decontamination are limited by low solubility of hydrocarbons, which can reduce their availability to the microorganisms being used in the process. In the bioremediation processes of aquiferous layer contaminated with PAH compounds the main problem stems from the migration of bacteria. They are quite easily adsorbed onto the solid particles of the aquiferous layer. The biosurfactants and synthetic surfactants – because of their surface activity – reduce that adhesion and increase the solubility of the hydrocarbons, thus contributing to their bioavailability for microorganisms [20]–[24].

The aim of the investigation was to determine the influence of rhamnolipids from *Pseudomonas* P-17 on coal tar and petroleum residue biodegradation.

2. MATERIALS AND METHODS

2.1. SAMPLE

Coal tar (gas-works tar) was collected from the surface of underground water contaminated with wastes from gas-works which before its closing produced gas based on dry coal distillation process.

Atmospheric residuum (petroleum residue) is an oil fraction remaining after distillation of fractions boiling below 345 °C.

2.2. RHAMNOLIPIDS AND MICROORGANISMS

Rhamnolipids are biosurfactants extracted with a solvent from *Pseudomonas* P-17 culture.

The biodegradation process was carried out using a mixed culture of three strains, i.e. *Acinetobacter calcoaceticus* H29, *Rhodococcus erythropolis* H45 and *Brevibacterium brevis* B1, donated to us by the Group of Biology and Ecology. The bacteria were isolated from a soil contaminated with petroleum products.

2.3. ISOLATION AND OBTAINING BIOSURFACTANTS

Biosurfactants were obtained from the strain of *Pseudomonas* PS-17. The bacteria were grown in 250 cm³ flasks containing 50 cm³ of mineral medium of pH 7.0, and glycerol (30 g/dm³) [22] was used as the source of carbon. The culture incubation, while placed on a rotary shaker (140 rpm), was run at 30 °C for 96–144 hrs. At the end, the cells were separated by centrifuging. The supernatant was acidified with HCl to pH 2.0 and extracted with ethyl acetate. The solvent was evaporated and the residue was chromatographically separated by TLC on silica gel (Silica gel No. 5745, E. Merck AG, Darmstadt, Germany) using a mixture of chloroform, acetone and acetic acid (90:10:6:1 v/v).

2.4. PROCEDURE OF BIODEGRADATION

The microorganisms were grown in 100 cm³ Erlenmeyer flasks containing 30 cm³ of mineral medium according to Siskinea-Trocenko ((KH₂PO₄ – 1.56 g, Na₂HPO₄ – 2.13 g, (NH₄)₂SO₄ – 0.5 g, Mg SO₄·7H₂O – 0.2 g, CaCl₂·2H₂O – 0.02 g, 1000 cm³ of redistilled water, microelements) and coal tar or petroleum residue at 0.1% w/v. In the surfactant-stimulated cultures, the concentration of rhamnolipid ranged from 15.6 to 500 mg/dm³ of the culture. The microorganisms were introduced into the medium as a suspension in a phosphate buffer. The cultures were incubated at 20±2 °C for 14 days and shaken at 140 rpm. Then the residual coal tar or petroleum residue was extracted with dichloromethane from the culture and its loss during the biodegradation process was determined.

2.5. CHEMICAL ANALYSIS

A chemical analysis comprised group components determination and main individual compounds identification in the substrates and products of biodegradation. The content of such group components as aliphatic hydrocarbons, aromatic hydrocarbons and polar compounds was determined by liquid chromatography. The fractions separation was performed in the column filled with silica gel, using consecutively the following eluents: hexane, mixture of hexane and dichloromethane (1:1), methanol and

as a result the respective aliphatic, aromatic and polar fractions were obtained. After evaporation of the solvents the fractions were determined gravimetrically. In the coal tar sample, asphaltenes (components insoluble in hexane) were determined as well.

The qualitative analysis was carried out by GC-MS, using Hewlett-Packard ser. II GC 5890, MS 5971 apparatus equipped with capillary column HP-17MS, in programmed temperature regime. The following temperature programs were used: an initial temperature of 40 °C for 4 min, rising the temperature by 5 °C/min to 250 °C, then this temperature was maintained for 20 min for coal-derived samples; an initial temperature of 40 °C for 3 min, rising the temperature by 10 °C/min to 250 °C, then this temperature was maintained for 20 min for petroleum-derived samples. Electron ionization (EI) at 70 eV with scanning parameters from 15 to 450 amu was used. The following chromatograms were recorded: TIC and selected fragmentation ions.

3. RESULTS AND DISCUSSION

3.1. CHEMICAL COMPOSITION OF SUBSTRATES

Two samples obtained from different raw materials (coal and petroleum) were investigated. The coal tar is a mixture of compounds eluted in the range of n -C₁₁– n -C₂₈ (figure 1), while the petroleum residue (from light petroleum) contains high-boiling compounds with carbons numbers C₁₆–C₃₂ (as n -alkanes) (figure 2). In coal tar, there are also present higher molecular weight compounds, i.e. asphaltenes, although at relatively low concentration (4.4%) (table 1). Coal tar contains about 26% of aliphatic hydrocarbons (paraffinic and naphthenic), 41% of aromatic hydrocarbons and 25% of polar compounds (table 1). The content of aromatic hydrocarbons compared to that of coke-oven tar and coal tars obtained from closed gas-works is lower [4], [26]. The group composition of the petroleum residue is as follows: 47% of paraffin-naphthenes, 50% of aromatic hydrocarbons and small amount of polar compounds (3%). The level of aromatic hydrocarbons higher than that in crude oil and petroleum products (fuels, oils and lubricants) is characteristic of heavy oil fractions [1], [2], [23].

Table

Group composition of coal tar and petroleum residue

Sample	Group composition (%wt)			
	Aliphatic hydrocarbons	Aromatic hydrocarbons	Polar compounds	Asphaltenes/losses
Coal tar	26.3	41.3	24.7	4.4/3.3
Petroleum residue	47.0	50.0	3.0	–

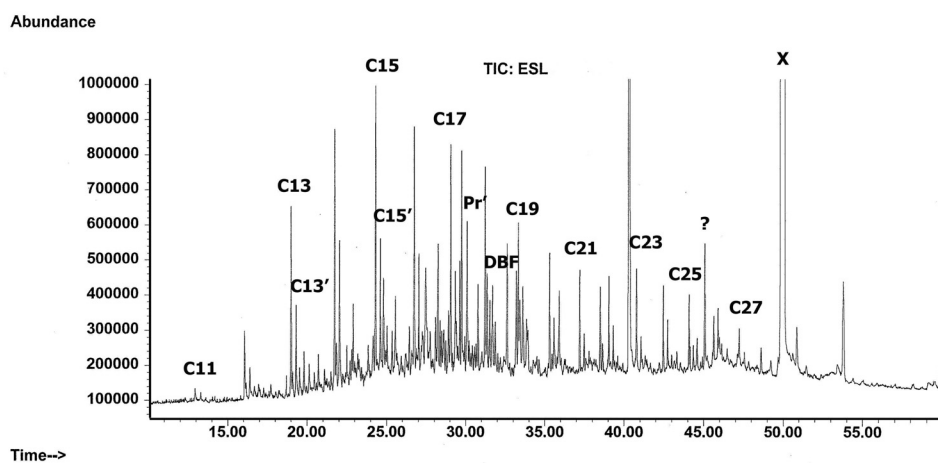


Fig. 1. GC-MS chromatogram of coal tar (C11–C27 – *n*-alkanes; C13', C15' – *n*-alk-1-enes, Pr' – prist-1-ene, DBF – dibenzofuran, X – artefact, ? – unidentified isoprenoid hydrocarbon)

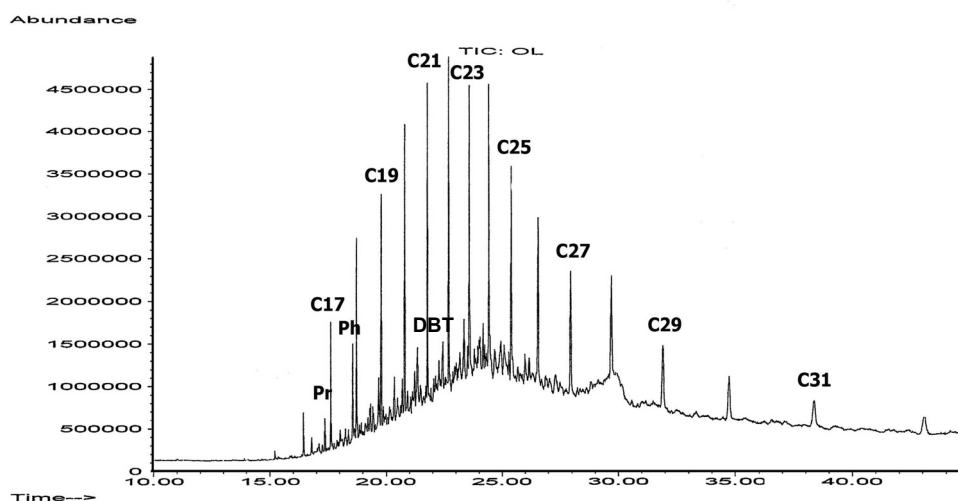


Fig. 2. GC-MS chromatogram of petroleum residue (C17–C31 – *n*-alkanes, Pr – pristane, Ph – phytane, DBT – dibenzothiophene)

Based on GC-MS analysis (figures 1 and 2) it was found that the main compounds in both samples are *n*-alkanes. In the coal tar, *n*-pentadecane prevails and docosane and tricosane are the main components of petroleum residue. The coal tar, obtained at higher temperature than petroleum residue, contains the products of coal pyrolysis: *n*-alkenes (*n*-alk-1-enes) and unsaturated isoparaffinic hydrocarbons (prist-1-ene, prist-2-

ene and unidentified compound, labelled on chromatogram as „?”). Among the aromatic hydrocarbons in coal-derived sample there were identified: *n*-alkylbenzenes and PAHs (mostly unalkylated parent PAH – phenanthrene, anthracene, fluorene, acenaphthene, naphthalene, and fluoranthene as well as methyl naphthalenes and cadalene), while in the petroleum-derived sample, trimethylnaphthalenes, alkylazulenes and methylphenanthrene were predominant. Both in coal tar and heavy petroleum fractions there are found heterocyclic compounds with N, S, O atoms. In the coal tar sample tested, dibenzofuran and methyl dibenzofuran were identified, while in the sample of petroleum residue, dibenzothiophene and its methyl derivatives were found.

Chemical analysis of both samples has shown that they contain mainly aromatic hydrocarbons which are not readily biodegraded [15]. A considerably lower concentration of aliphatic hydrocarbons, among them *n*-alkanes (most readily biodegradable group of hydrocarbons), and higher hydrophobicity and toxicity of parent, unalkylated aromatic hydrocarbons [3] present in the coal-derived products suggest that the coal tar will be biodegraded slower than petroleum residue aromatic hydrocarbons.

3.2. EFFECT OF RHAMNOLIPIDS ON EXTRACTION EFFICIENCY

The biodegradation process has been evaluated through determination of coal tar and petroleum residue biodegradation products obtained as a result of their extraction from water solution containing mineral medium, microorganisms and different amounts of biosurfactant. Variable composition of the analytes and matrix affects the properties of the analyte–matrix system and the separation of analytes from water phase into the organic phase. Taking the above into account, the effect of the biosurfactant added (in the range of 0–500 mg/dm³) on the extraction efficiency has been evaluated in a series of experiments (figure 3).

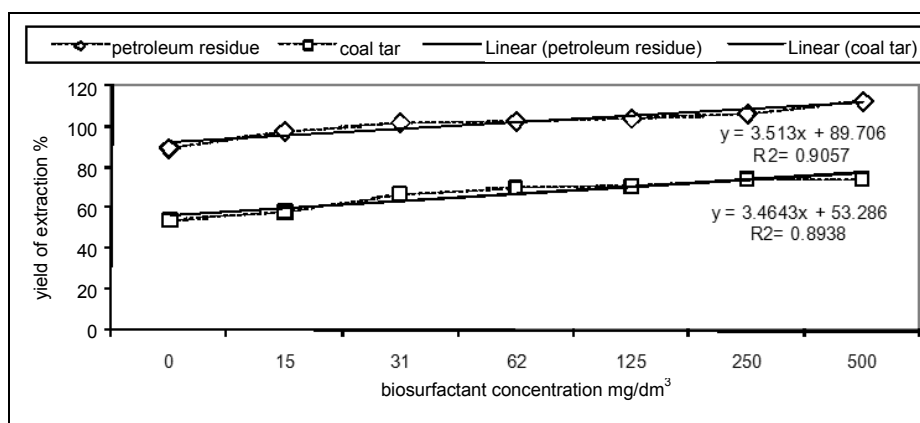


Fig. 3. Effect of rhamnolipid added on yield of extraction

Substantial differences in the efficiency of gas-works tar and petroleum residue extraction have been observed. Extraction efficiency of the petroleum residue (sample without bacteria and surfactant) was high (above 90%). Addition of biosurfactant has caused an extraction efficiency increase and at significant amounts of the biosurfactant the efficiency exceeded 100%. Comparing the recovery of the petroleum residue with that of coal tar (sample without bacteria and surfactant) it was revealed that the recovery efficiency of the latter was almost twice lower (a slightly above 50%), but the biosurfactant added increased the extraction efficiency. These results testify to an important effect of biosurfactant concentration on the analyte recovery. Thus in all quantitative analyses, the control of each concentration of surfactant without bacteria is taken into account.

3.3. EFFECT OF CHEMICAL COMPOSITION AND BIOSURFACTANT ADDITION ON BIODEGRADATION EFFICIENCY

The results, expressed as the efficiency of biodegradation process (loss of the sample mass vs. substrates, in %) as a function of the amount of the biosurfactant in the culture, are presented in figure 4.

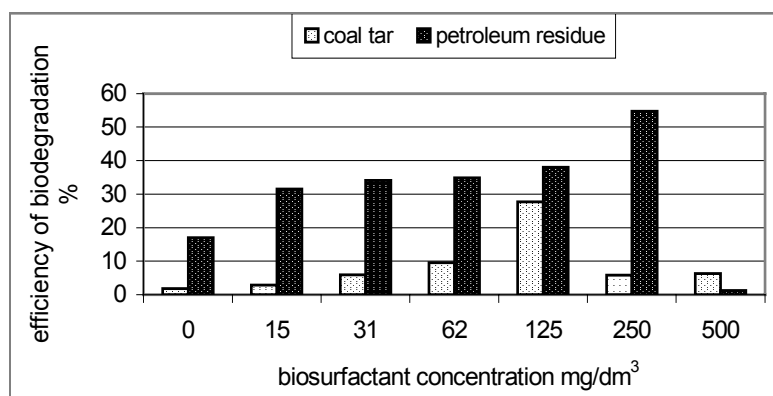


Fig. 4. Effect of rhamnolipid added on efficiency of biodegradation

The coal tar proved to be not susceptible to microbiological decomposition. Despite the fact that for this purpose hydrocarbon degrading strains were used, no signs of a biological decomposition of tar were observed unless a surfactant was added. The introduction of the rhamnolipid initiated the biodegradation process, but at low concentration of the rhamnolipids the loss of the substrates was insignificant. At the dose of the rhamnolipids as high as 125 mg/dm³, its stimulating effect on biodegradation was confirmed. The loss of the gas-work tar in this case reached 27.7% wt. After 14 days of incubation. Unlike the coal tar, the petroleum residue was readily biode-

gradated (17.2% for the sample without surfactant) and the process efficiency doubled in the presence of rhamnolipids at its minimal amount (15.6 mg/dm^3). A further increase in the biosurfactant concentration had a positive effect on the process efficiency, which at 250 mg/dm^3 reached the maximum of 54.7%.

The changes in a chemical composition of the samples during their biodegradation were evaluated based on the group analysis and identification of the main compounds. The group analysis (figures 5 and 6) shows the increased amounts of polar compounds in the biodegradation products of both “petroleum” and “coal” samples. A higher content of polar compounds can result from their lower susceptibility to biological decomposition and from the presence of metabolites produced in the process.

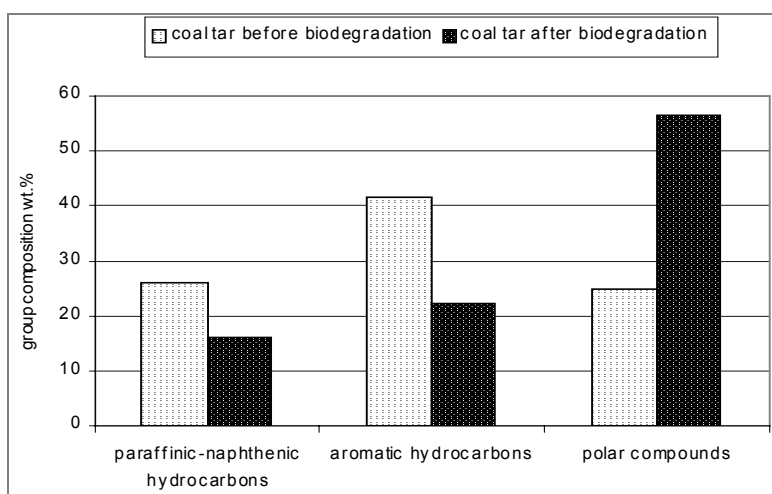


Fig. 5. Group composition of coal tar substrates and biodegradation products

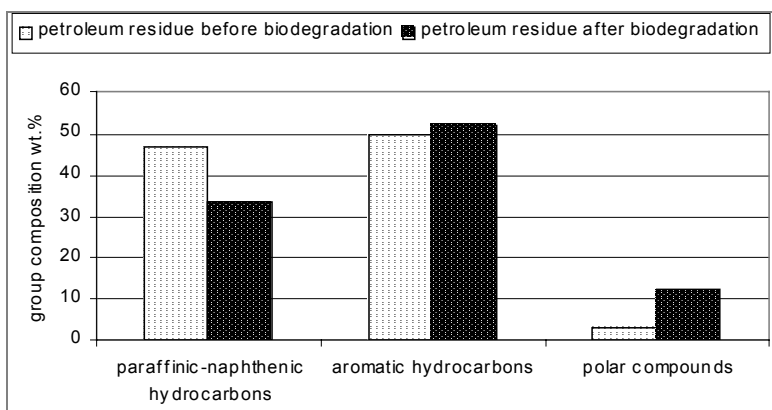


Fig. 6. Group composition of petroleum residue substrates and biodegradation products

The comparison of GC-MS chromatographic profiles of the biodegradation products of coal tar (figure 7), of heavy petroleum oil (figure 8) as well as of substrates (figures 1 and 2) shows the substantial changes of their qualitative composition. These changes occurred during a 14-day process in the presence of biosurfactants. In the products of the coal tar degradation, isoparaffins and aromatic hydrocarbons: pristenes, unidentified isoprenoid hydrocarbon, phenanthrene, anthracene, fluoranthene and pirenene were found most frequently, while in the petroleum oil sample, phytane, pristane, methyl derivatives of naphthalene and phenanthrene prevailed. Dibenzothiophene and its methyl and dimethyl derivatives, present in the initial petroleum sample at considerable concentrations, appear to be susceptible to biodegradation. Straight-chain hydrocarbons and naphthalene were removed most efficiently. Dibenzofuran and methyl dibenzofuran, present in the original coal tar sample, were not detected. Based on the concentration of group components in the degradation products and substrates it was found that paraffin-naphthenes, present in the heavy petroleum oil, undergo the biodegradation most readily. It was also shown that the efficiency of the biodegradation process depends on the qualitative and quantitative composition of hydrocarbons and on the rhamnolipids concentration. Similar conclusions can be found in MULLIGAN's review [22] which deals with biosurfactant-stimulated biodegradation of model hydrocarbons and environmental contaminants.

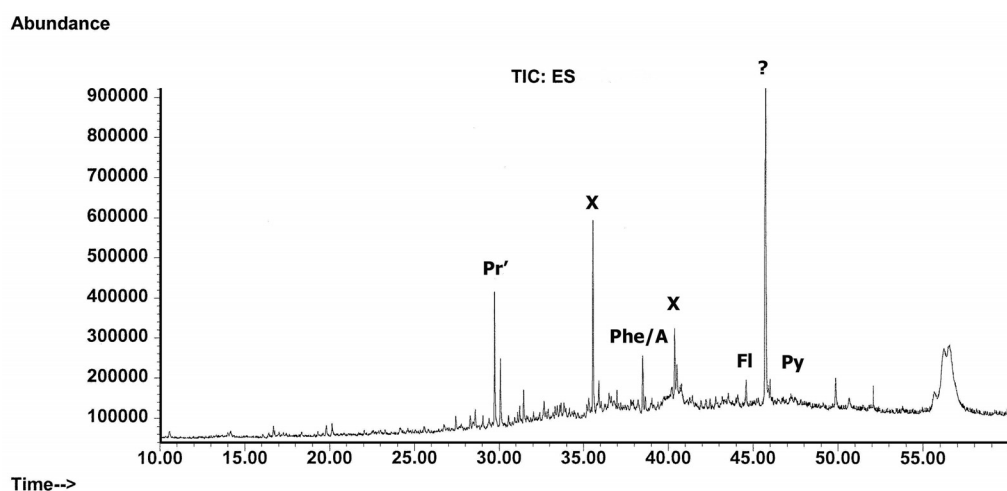


Fig. 7. GC-MS chromatogram of coal tar product biodegradation (Pr' – prist-1-ene, X – artefact, ?, Phe/A – phenanthrene/anthracene, FI – fluoranthene, Py – pirenene)

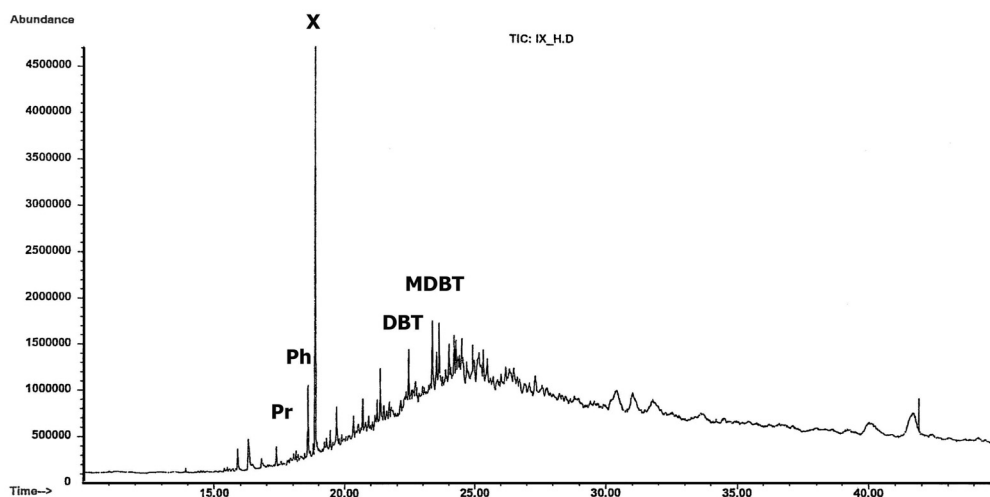


Fig. 8. GC-MS chromatogram of petroleum residue product biodegradation (Pr – pristane, Ph – phytane, X – artefact,?, DBT – dibenzothiophene, MDBT – methyl dibenzothiophenes)

4. CONCLUSIONS

Two samples from different sources were tested: the sample of coal tar taken from the surface of underground water contaminated with wastes from an old, closed gas-works and a residual fraction from crude oil distillation. It was found that the efficiency of the biodegradation process depends on the chemical composition of the substrate and on the rhamnolipid concentration. The study demonstrated low susceptibility of coal tar component to biodegradation. The rhamnolipids added initiated biodegradation process by increasing the bioavailability of coal tar components. The efficiency of biodegradation increased with an increase of biosurfactant concentration. The highest efficiency of the coal tar biodegradation was observed at the rhamnolipid concentration of 125 mg in 1 dm³ of the culture medium. Compared to the coal tar, petroleum residue – containing greater amounts of higher paraffin-naphthenic hydrocarbons and smaller amounts of unalkylated parent PAH – is characterized by better susceptibility to biodegradation. The maximum process efficiency (55%) has been obtained at the rhamnolipid concentration of 250 mg/dm³.

It has been shown that the biosurfactant has also a substantial effect on the extraction of the biodegradation products which has to be taken into account in a quantitative analysis of the process.

REFERENCES

- [1] *Zanieczyszczenia naftowe w gruncie*, J. Surygala (red.), Ofic. Wyd. Polit. Wroc., Wrocław, 2000.
- [2] SURYGALA J., ŚLIWKA E., *Charakterystyka produktów naftowych w aspekcie oddziaływań środowiskowych*, *Chemia i Inżynieria Ekologiczna*, 1999, 6, 2/3, 131–147.
- [3] NEFF M., STOUT S.A., GUNSTER D.G., *Ecological risk assessment of polycyclic aromatic hydrocarbons in sediments: Identifying sources and ecological hazard*, *Integr. Environ. Assess. Manag.*, 2005, 1, 23–33.
- [4] *Kirk-Othmer Encyclopedia of Chemical Technology*, Copyright© by John Wiley & Sons, Inc, 2002.
- [5] SURYGALA J., ŚLIWKA E., *Wycieki ropy naftowej*, *Przemysł Chemiczny*, 1999, 78, 9, 322–325.
- [6] JACOBS M.A., WALDRON D.M., *Approaches to sheltered-water oil spills*, *Sea Techn.*, 1996, 37, 34–38.
- [7] SURYGALA J., ŚLIWKA E., *Oil spill hazards in Poland on the background of the world's status*, *Fresenius Environmental Bulletin*, 2004, 13, 12b, 1474–1476.
- [8] STECZKO K., PIWOWARCZYK J., *Odpady wiertnicze jako źródło zagrożeń dla środowiska*, *Nafta-Gaz*, 1991, 4–6, 455–465.
- [9] ŚLIWKA E., KOŁWZAN B., GRABAS K., KORZEŃ R., *Skład i właściwości biologiczne odpadów wiertniczych*, *Inżynieria Ekologiczna*, 2003, nr 8, Warszawa, Wydaw. Nauk. Gabriel Borowski, 27–33.
- [10] PŁAZA G., *Bioremediacja gruntów zanieczyszczonych związkami ropopochodnymi z terenu rafinerii metodą bioprzyzmy*, Wrocław, Ofic. Wyd. Polit. Wroc., 2006.
- [11] LANDMEYER J.E., CHAPELLE F.H., PETKEWICH M.D., BRADLEY P.M., *Assessment of natural attenuation of aromatic hydrocarbons in groundwater near a former manufactured-gas plant*, South Carolina, USA, *Environ. Geology*, 1998, 34(4), 279–292.
- [12] BOJAKOWSKA I., IRMIŃSKI W., *Zanieczyszczenie związkami organicznymi gleb na terenie starych gazowni*, *Przeł. Geol.*, 2002, 50, 8, 691–698.
- [13] ATSDR 1999, *Toxicological profile for total petroleum hydrocarbons (TPH)*, Atlanta, GA: Agency for Toxic Substances and Disease Registry.
- [14] ATSDR 2002, *Potential for human exposure*, [in:] *Toxicological Profile for Wood Creosote, Coal Tar Creosote, Coal Tar, Coal Tar Pitch, and Coal Tar Pitch Volatiles*, Atlanta, GA: Agency for Toxic Substances and Disease Registry.
- [15] KOŁWZAN B., *Biodegradacja produktów naftowych*, [w:] *Zanieczyszczenia naftowe w gruncie*, J. Surygala (red.), Ofic. Wyd. Polit. Wroc., Wrocław, 2000, 207–237.
- [16] KOŁWZAN B., *Wykorzystanie mikroorganizmów do oczyszczania gruntów skażonych produktami naftowymi*, *Inżynieria Ekologiczna*, 2002, nr 7, 36–44.
- [17] DURANT N.D., WILSON L.P., BOUWER E.J., *Microcosm studies of subsurface PAH-degrading bacteria from a former manufactured gas plant*, *J. of Contaminant Hydrology*, 1995, 17, 213–237.
- [18] MORASCH B., HUNKELER D., HOHENER P., TEMIME B., *Biodegradation of mono- and polyaromatic hydrocarbons at a former cokery evidenced by isotopes and metabolites*, Gasworks Europe, redevelopment, site management and contaminant issues of former MGP's and other tar oil polluted sites, MGP 2008, Conference in Dresden, Germany, 2008, 59–67.
- [19] FETZNER S., *Bacterial degradation of pyridine, indole, quinoline, and their derivatives under different redox conditions*, *Appl. Microbiol. Biotechnol.*, 1998, 49, 237–250.
- [20] LAHA S., LUTHY, *Inhibition of phenanthrene mineralization by nonionic surfactants in soil–water systems*, *Environ. Sci. Technol.*, 1991, 25 (11), 1920–1930.
- [21] GRABAS K., KOŁWZAN B., ŚLIWKA E., *Effect of surfactants on the biodegradation of petroleum hydrocarbons*, [w:] *Environmental engineering studies. Polish research on the way to the EU*, L. Pawłowski, M. R. Kluwer (eds.), Academic/Plenum Publ., 2003, 389–398.

- [22] MULLIGAN C.N., *Environmental applications for biosurfactants*, Environ. Pollut., 2005, 133, 183–198.
- [23] GUHA S., JAFFE P.R., *Bioavailability of hydrophobic compounds partitioned into the micellar phase of nonionic surfactants*, Environ. Sci. Technol., 1996, 30, 4, 1382–1391.
- [24] VOLKERING F., BREURE A.M., RULKENS W.H., *Microbiological aspects of surfactant use for biological soil remediation*, Biodegradation, 1998, 6, 401–417.
- [25] SHULGA A., KARPENKO E.V., ELYSSEEV S.A., VILDANOWA-MARTSISHIN R.I., *Strain Pseudomonas sp. PS-17 – producer of extracellular biosurfactant and biopolymer*, Ukr. Pat. 10467A, 1996.
- [26] BROWN D.G., GUPTA L., KIM T.-H., MOO-YOUNG H.K., COLEMAN A.J., *Comparative assessment of coal tars obtained from 10 former manufactured gas plant sites in the Eastern United States*, Chemosphere, 2006, 65, 1562–1569.
- [27] ALTGELT K.H., BODUSZYŃSKI M.M., *Composition and Analysis of Heavy Petroleum Fractions*, Marcel Dekker, Inc, New York, Basel, Hong Kong, 2000.

WPLYW RAMNOLIPIDÓW PRODUKOWANYCH PRZEZ *PSEUDOMONAS* PS-17 NA BIODEGRADACJĘ SMOŁY WĘGLOWEJ I POZOSTAŁOŚCI NAFTOWEJ

Efektywność procesów biologicznych stosowanych do likwidacji skażeń środowiska gruntowo-wodnego węglowodarami zależy od bardzo wielu parametrów, m.in. od rodzaju i poziomu zanieczyszczeń oraz ich biodostępności. Małą podatnością na biodegradację charakteryzują się zanieczyszczenia zawierające silnie hydrofobowe i toksyczne węglowodory. Do źródeł takich węglowodorów należą smoły węglowe i ciężkie frakcje ropy naftowej. W pracy przeanalizowano wpływ ramnolipidów produkowanych przez *Pseudomonas* PS-17 na stymulację procesu biodegradacji odpadów smoły węglowej, pochodzącej z zamkniętej gazowni, i pozostałości naftowej, otrzymanej podczas destylacji atmosferycznej lekkiej ropy naftowej. Biodegradację przeprowadzono, wykorzystując szczepy wyizolowane z gleby skażonej produktami naftowymi. Badania wykazały, że smoła węglowa stanowi substrat bardzo mało podatny na biodegradację. Wprowadzenie ramnolipidów zwiększyło biodostępność składników smoły i zainicjowało proces biodegradacji. Maksymalną efektywność biodegradacji smoły węglowej (28%) uzyskano, stosując stężenie ramnolipidów 125 mg w 1 dm³ podłoża. Dla pozostałości naftowej maksymalna efektywność procesu wynosiła 54%. Uzyskano ją przy stężeniu ramnolipidów 250 mg/dm³.