EFFECTIVENESS OF INTRINSIC BIODEGRADATION ENHANCEMENT IN OIL HYDROCARBONS CONTAMINATED SOIL

IWONA ZAWIERUCHA¹, GRZEGORZ MALINA², WOJCIECH CIESIELSKI¹*, PIOTR RYCHTER¹

¹Institute of Chemistry, Environmental Protection and Biotechnology, Jan Dlugosz University of Czestochowa, Armii Krajowej 13/15, 42-200 Czestochowa, Poland
²Department of Hydrogeology and Engineering Geology, AGH University of Science and Technology, Mickiewicza 30, 30-059 Cracow
*Corresponding authors e-mail: w.ciesielski@interia.pl

Keywords: Intrinsic and enhanced biodegradation, biostimulation, bioaugmentation, combined enhancement.

Abstract: Studies were conducted using a 10-chamber Micro-Oxymax (Columbus, OH, USA) respirometer to determine the effect of bioaugmentation, biostimulation and combination of them on enhancing intrinsic biodegradation of oil hydrocarbons in soil. Contaminated soil was collected from a former military airport in Kluczewo, Poland. Bioaugmentation was realized by addition of indigenous or exogenous bacteria to soil. Biostimulation was done by aerated water supply and surfactant addition. Bioaugmentation + addition of a surfactant was applied as the combined treatment. The intrinsic and enhanced hydrocarbons biodegradation rates were estimated from the slopes of linear regressions of cumulative curves of O2 uptake. Pertinent biodegradation rates were recalculated on the basis of the stoichiometric reaction (mass balance equation) and conversion equation. The results showed that combined treatment (indigenous bacteria bioaugmentation + addition of a surfactant) was the most effective method of biodegradation enhancement as the 20-fold increase of biodegradation rate was observed.

INTRODUCTION

Petroleum hydrocarbons and their derivatives are the most widely used chemicals in modern societies today [9]. They have become one of the most important energy sources in the world [15]. However, contamination of water and soil by petroleum hydrocarbons as a result of exploration, production, maintenance, transportation, storage and accidental releases, presents substantial hazards to humans and the environment [15, 26].

Current technologies for cleaning up hydrocarbons contaminated soil include soil washing, solvent extraction, thermal treatment, composting, chemical oxidation and bioremediation [12, 17]. Physical and chemical approaches are expensive and byproducts may cause secondary contamination of soil and water resulting in the need for additional post-treatments [15]. Moreover, most of these techniques require continuous monitoring and control for optimum performance. In addition, they do not usually result in a complete destruction of the contaminants [9].
As such, there is a widespread interest in bioremediation – a treatment method that uses biological systems to catalyze the destruction or transformation of various organic compounds to less harmful forms [10]. Bioremediation is relatively simple practical approach for the complete mineralization of hydrocarbons to carbon dioxide and water under aerobic conditions. Moreover, it is a cost-effective method and provides in situ remediation without disturbing native ecosystems [24, 32].

Nowadays the cost-effective strategies for bioremediation of soil contaminated with oil hydrocarbons include natural attenuation (NA) [28]. NA is an approach that relies on natural processes to dissipate contaminants through physical, chemical and biological transformations. Biochemical processes, especially intrinsic biodegradation with participation of indigenous microorganisms, play the most important role in these natural self-purification processes [35]. Intrinsic biodegradation of oil hydrocarbons in soil can be enhanced by bioaugmentation, in which microorganisms able to degrade the contaminant are provided, and by biostimulation, when the proper agents are applied to stimulate indigenous microbial activities [18].

Bioaugmentation is a promising and low-cost bioremediation method, in which effective bacterial isolates or microbial consortia capable of degrading oil hydrocarbons are introduced to contaminated soil. Multiplied indigenous microflora is generally applied in this technique, however, inoculating of soil with exogenous or laboratory modified bacterial cultures still arouses many reservations [7–8, 35]. This is because the survival and degrading ability of microorganisms introduced to a contaminated site are highly dependent on environmental conditions [33]. Thus, in many cases, potentially degrading strains isolated from site A are not necessarily applicable to site B, and isolates, including genetically engineered microorganisms, that are efficient hydrocarbons degraders under laboratory conditions, are not necessarily effective in situ [25]. Moreover, it is not easy to get public acceptance for introducing alien species to soil [11].

Biostimulation relies on increasing the activity of indigenous bacteria by providing nutrients, oxygen or water to contaminated soil [4], or modifying the environmental conditions (e.g. temperature, pH, redox potential). It seems to be effective because indigenous bacteria are best adapted to the environment of the site that is being treated [22]. However, biostimulation does not always work well because of the scarcity of indigenous hydrocarbons degraders or high concentrations of contaminants [30].

This study addresses two specific issues: (i) the effect of bioaugmentation, biostimulation and combined treatment on the biodegradation rate in oil hydrocarbons contaminated soil; and (ii) the comparison of methods of biodegradation enhancement to select the best treatment option for optimal soil remediation.

MATERIALS AND METHODS

Soil samples
The intrinsic and enhanced biodegradation rates were evaluated for soil I samples. Soil I samples contaminated with oil hydrocarbons (“historical” – aged contamination) originated from the former military airport in Kluczewo, Poland. Soil II, collected from another site contaminated with similar compounds, was used only to isolate exogenous bacteria (that are not indigenous to the investigated area) capable of hydrocarbons...
biodegradation. Contaminated soil I samples were collected at the depths of 1.5 m (G1) and 2.0 m (G2), and soil II at 2.0 to 2.5 and 3.0 to 3.5 m. Uncontaminated (reference) soil I samples were also collected at the depths of 1.5 m (G1R) and 2.0 m (G2R).

**Soil characteristics**

pH and Eh of soil suspension were determined according to PN-ISO 10390:1997, soils moisture – PN-ISO 11465:1999, soil organic matter (SOM) content – PN-78/C-04541, content of nitrogen – PN-ISO 11261:2002, content of phosphorus – PN-ISO 11263:2002.

**Contents of hydrocarbons in soil**

Contents of hydrocarbons in soil were determined using a headspace method and the capillary gas chromatography with the mass detector (GC-MS). The Shimadzu GC-14A gas chromatograph was used coupled with the Shimadzu QP 5000 mass spectrometer. The analyses were done according to PN-89/C-04641/03. Total aliphatic hydrocarbons (TAH) were also determined according to PN-C-04643:1994.

**Microbial tests**

**Initial number of bacteria**

Microbial enumeration of bacteria was measured as the number of colony – forming units (CFU) per gram, using the standard agar-plate technique. This method relies on executing dilution series of soil suspension, and inoculating stable volumes of the dilutions on the agar medium. The agar medium was composed of agar – 20.0 g, broth bouillon – 15.0 g, and distilled water – 1.0 L. The dilution series of soil suspension were made with sterile distilled water. Bacteria were incubated at 28°C for 48 hours.

**Isolating bacteria capable of hydrocarbons degradation**

Bacterial strains isolated from contaminated soil samples were grown on the solid medium composed of: yeast extract – 2.5 g, agar – 15.0 g, trypton – 5.0 g, K₃HPO₄ – 1.0 g, MgSO₄ – 0.5 g, mixture of toluene and decane – 4.5 mL, distilled water – 1.0 L, surfactant Tween 80 – 0.5 mL. Toluene, decane and Tween 80 were added to the medium before autoclaving. Isolated bacterial strains were transferred and stored on agar slants.

**Cultivation of bacteria**

Isolated bacterial strains were transferred from agar cants to a liquid medium composed of: yeast extract – 4.0 g, K₃HPO₄ – 1.0 g, MgSO₄ – 0.5 g, mixture of toluene and decane – 4.5 mL, distilled water – 1.0 L. The aerobic bacteria were grown in flasks of 0.5 L, aerated by mechanical mixing. Bacteria were grown as a consortium (i.e. several strains in one liquid medium) without identifying the strains. The separation of bacterial suspension from the liquid medium was achieved by centrifuging (at 2000 rpm for 5 minutes). Concentrations of a bacterial consortium (numbers of cells in 1 mL of a suspension) were checked using the Thom’s chamber. The high density of microbial cells was achieved by the repeated multiplication; the cells were grown in a liquid medium, then centrifuged and suspended again in a new medium.
The respirometry test

Biodegradation of oil hydrocarbons under aerobic conditions was analyzed using a 10-chamber Micro-Oxymax respirometer (Columbus Instruments, OH, USA), in conjunction with an Ultra IBM-compatible computer to collect and record all data. This is a closed-circuit respirometer capable to measure on-line the O₂ consumed and CO₂ produced during hydrocarbons biodegradation (Fig. 1).

The following treatments were applied: bioaugmentation, biostimulation by aerated water or surfactant supply and combined treatment: bioaugmentation + addition of a surfactant. Bioaugmentation was done using indigenous or exogenous bacterial consortium containing 2.4 or 4.8×10⁹ cells mL⁻¹. For biostimulation tests, aerated distilled water with the O₂ saturation of 98% and surfactant Tween 80, that is commonly applied for remediation of petroleum contaminated soils [13], were used. In the case of test with combined treatment, bioaugmentation was done using an indigenous bacterial consortium containing 4.8×10¹⁵ cells mL⁻¹ and the surfactant dose was 1% (v/v). Biostimulation by nutrients amendment was not carried out because the presence of these components was maintained in soil samples.

Experiments were carried out using batch tests at a temperature of 20±2°C. Soil samples of 30 g were placed into 500 mL measuring chambers of the respirometer, to which were added: (i) 8.0 mL of the bacterial consortium (bioaugmentation tests), (ii) 8.0 mL of aerated distilled water, (iii) Tween 80 in concentration of 1.0% (v/v), (iv) 7.85 mL of the bacterial consortium + 0.15 mL of Tween 80 (combined treatment). As a control, uncontaminated soil samples G1R and G2R (control 1), as well as contaminated soil samples G1 and G2 without any enhancement (control 2) were used. Control 1 determined the basal respiration, which is the result of soil organic matter (SOM) decomposition, whereas control 2 – the overall respiration, due to organic matter and hydrocarbons biodegradation.
Biodegradation of oil hydrocarbons was evaluated based on the O$_2$ consumption. It is more reliable than using the CO$_2$ production to determine hydrocarbon biodegradation rates because the abiotic CO$_2$ production could be also the result of dissolution of soil carbonates. Cumulative curves of O$_2$ consumption were plotted as O$_2$ content values in the headspace versus time. Rates of O$_2$ uptake were estimated from the slopes of linear regressions of cumulative curves. The coefficients (a) of the linear regression equations ($y=ax$) represented the mean rates of O$_2$ consumption during hydrocarbons biodegradation. The linear regression model was applied because it was found to fit with enough accuracy the experimental data plotted as accumulative curves. It was indicated by the regression coefficients ($R^2 \geq 0.99$).

Having mean O$_2$ uptake rates and assuming that the only products of hydrocarbons biodegradation are biomass, carbon dioxide and water, pertinent biodegradation rates were calculated. Mass balance was done based on the stoichiometric reaction of hydrocarbon decomposition:

$$C_nH_m + aO_2 = YCH_{2}O + bCO_2 + cH_2O$$

where: $m$ – number of hydrogen atoms, $n$ – number of carbon atoms, $a$, $b$, $c$ – stoichiometric coefficients of reaction, $Y$ – microbial yield

Assuming hydrocarbons formula as CH$_{1.5}$ [16, 21] and the microbial yield $Y=0.5$, the biodegradation rates ($k_{NA/ENA}$) were calculated according to the formula [21]:

$$k_{NA/ENA} = 2.144 \frac{4\left(12 + \frac{m}{n}\right)}{4(1 - Y) + \frac{m}{n}} \frac{k_{O_2}}{k_0} \left[ \frac{mg \ of \ hydrocarbons}{kg \ of \ soil \ day} \right]$$

where: $k_{NA/ENA}$ – intrinsic/enhanced biodegradation rates calculated on the basis of O$_2$ consumption rates [mg of hydrocarbons kg of soil$^{-1}$ day$^{-1}$], $k_0$ – O$_2$ consumption rate [μL min$^{-1}$], 2.144 – a conversion coefficient for the O$_2$ consumption rates (converts the Micro-Oxymax respirometer readings to [mmol of O$_2$ kg of soil$^{-1}$ day$^{-1}$])

The “net” values of O$_2$ consumption (excluding basal respiration due to SOM decomposition) obtained for contaminated soil samples (without and with enhancement) were used in formula (2) to calculate the actual hydrocarbons biodegradation rates.

RESULTS AND DISCUSSION

The characteristics of soil, and the initial number of bacteria are presented in Table 1. The contents of aromatic hydrocarbons and TAH in soil samples are listed in Table 2.

The obtained soil physical-chemicals parameters are considered optimal for biodegradation of oil hydrocarbons under aerobic conditions [36]. The initial number of microorganisms ($10^5$ CFU g$^{-1}$) in contaminated soils was sufficient for intrinsic biodegradation [29].
The exemplary cumulative curves of O\textsubscript{2} consumption during intrinsic and enhanced biodegradation of oil hydrocarbons in soil are presented in Fig. 2.

The effectiveness of the intrinsic biodegradation enhancement by bioaugmentation is presented in Fig. 3.

Tab. 1. Soil characteristics and numbers of bacteria

| Soil sample | pH  | Eh (mV) | Moisture (% w/w) | SOM (% w/w) | Number of bacteria (CFU/g) | N (mg kg\textsubscript{-1} d.w.) | P (mg kg\textsubscript{-1} d.w.) |
|-------------|-----|---------|------------------|-------------|---------------------------|----------------|----------------|
| G1          | 7.4 | 451     | 18.18            | 2.76        | 2\times10\textsuperscript{4} | 452            | 314            |
| G1R         | 6.7 | 447     | 26.71            | 3.92        | 1\times10\textsuperscript{5} | 462            | 345            |
| G2          | 7.6 | 457     | 19.23            | 3.20        | 10\times10\textsuperscript{4} | 303            | 305            |
| G2R         | 8.1 | 449     | 13.07            | 1.99        | 3\times10\textsuperscript{5} | 330            | 332            |

Tab. 2. Contents of monoaromatics and total aliphatic hydrocarbons (TAH) in soil

| Contamination | G1 (mg kg\textsuperscript{-1} d.w.) | G2 (mg kg\textsuperscript{-1} d.w.) |
|---------------|-----------------------------------|-----------------------------------|
| Benzene       | < 0.02                            | < 0.02                            |
| Toluene       | < 0.02                            | < 0.02                            |
| Ethylbenzene  | < 0.02                            | 0.02                              |
| m+p Xylene    | 0.07                              | 0.14                              |
| o-Xylene      | < 0.02                            | < 0.02                            |
| TAH           | 357                               | 189                               |

The exemplary cumulative curves of O\textsubscript{2} consumption during intrinsic and enhanced biodegradation of oil hydrocarbons in soil are presented in Fig. 2.

The effectiveness of the intrinsic biodegradation enhancement by bioaugmentation is presented in Fig. 3.

Fig. 2. The exemplary cumulative curves of O\textsubscript{2} uptake during intrinsic and enhanced biodegradation of oil hydrocarbons
Generally, enhanced biodegradation rates of oil hydrocarbons due to bioaugmentation were considerably higher than the rates of intrinsic biodegradation. The highest biodegradation rates were observed in samples with bacterial consortia containing $4.8 \times 10^9$ cells mL$^{-1}$. Generally, in all tests, the rates of enhanced biodegradation were about 7 times higher than intrinsic biodegradation rates. The selection of microorganisms directly from the site of concern seems to give the best results [6]. Capelli et al. [3] reported a 70% decrease of TPH in laboratory studies with soils inoculated with pre-selected bacteria. Bento et al. [2] noted that the addition of a bacterial consortium previously isolated from the Long Beach soil degraded 73–75% of the light ($C_{12}-C_{23}$) and heavy ($C_{23}-C_{40}$) fractions of TPH present in the soil, but had no effect in the case of soil from Hong Kong. Thus, successful bioaugmentation depends on the use of inocula consisting of single microbial strains or microbial consortia that have been well adapted to the site to be treated [11]. Exogenous microorganisms (those in inocula) are usually unable to avoid competition with indigenous bacteria, predators and various abiotic factors. Factors affecting proliferation of microorganism used for bioaugmentation include the chemical structure, concentration and availability of a contaminant to microorganisms, as well as the size and nature of the microbial population and the physical environment [34]. Therefore, it will be more practical to apply bioaugmentation with bacteria isolated from the soil that is to be cleaned-up [11]. However, Shkidchenko et al. [27] showed that the hydrocarbon-oxidative activity of isolated strains to black oil was of 13.1–17.3% (incubation for 10 days at 24°C), while the activity of a mixture of three aboriginal strains was of 17.8%. At the same time, the hydrocarbon-oxidative activity of associations of the strains isolated from other regions was of 24.0–30.0% (in 10 days). The bioaugmentation with exogenous bacteria is recommended in case of more recalcitrant chemicals or when the local microbial population is insufficient or inadequate [19].

![Fig. 3. The effect of bioaugmentation on biodegradation rates of oil hydrocarbons](image-url)

Legend: G1, G2 – contaminated soil without enhancement – depths of 1.5 and 2.0 m; 1A/2A – addition of exogenous/indigenous bacterial consortium ($2.4 \times 10^9$ cells mL$^{-1}$); 1B/2B – addition of exogenous/indigenous bacterial consortium ($4.8 \times 10^9$ cells mL$^{-1}$)
In our study, application of indigenous bacteria was more efficient in comparison to exogenous consortia as indicated by the increase of biodegradation rate of about 14–23%. This could be explained by the adaptation that allows autochthonous microorganisms to be physiologically compatible with their habitat, as compared to transient allochthonous organisms that do not occupy a functional niche [1]. Ueno et al. [30] also noticed that bioaugmentation capacity of isolated bacterial species in soil microcosms contaminated with diesel oil was much higher than that of exogenous *P. aeruginosa* strain WatG. According to D’Annibale et al. [5], several isolated indigenous fungal species from an aged and heavily contaminated soil were inoculated to the same oil-contaminated soil, and a remarkable removal of naphthalene, dichloroaniline, *o*-hydroxybiphenyl, and 1,1’-binaphthalene was achieved. Thus, the best bioaugmentation performance for the case of aged contamination can be approached by the use of multiplied indigenous microorganisms to increase their abundance in the soil. Taking into account the fact that further doubling of microorganisms resulted in a subsequent increase of biodegradation rates, it may suggest that bioavailable contaminants still remained in the studied soil samples. Therefore, for designing optimum bioaugmentation, it is necessary to evaluate the fractions of bioavailable contaminants to determine the required concentration of a degrading inoculum to be added [35].

The effectiveness of the intrinsic biodegradation enhancement by biostimulation and combined treatment is presented in Fig. 4.

The addition of aerated water resulted in the 3-fold increase of biodegradation rate. Aerated water can be an effective O₂ carrier for biodegradation of oil hydrocarbons in soil. It is the source of oxygen for microorganisms and facilitates the transport of substrates to bacterial cells. Moreover, its application is safe for people and ecosystems [16]. In the case of application of Tween 80 enhanced biodegradation rates were on average 4 times higher than intrinsic biodegradation rates. Surfactants can improve biodegradation effectiveness in soil contaminated with oil hydrocarbons [2, 23]. They contain both hydrophobic
and hydrophilic fractions, and are useful in reducing the interfacial tension between hydrocarbons and soil water, thereby improving the water solubility of hydrophobic substrates [31]. Consequently, surfactants increase bioavailability of hydrocarbons to microorganisms and hence their biodegradation [14]. On the other hand, generation of chemical surfactant byproducts and the potential for the further spread of contaminants must be considered. Also, the efficiency of surfactants is limited in the case of low permeable soils [15].

Combined treatment resulted in the 20-fold increase of biodegradation rate. These results suggest that bioavailability of contaminants could be the limiting factor of oil hydrocarbons biodegradation in studied soils. Therefore, the combination of bioaugmentation with various amendments such as surfactants, fertilizers or O$_2$-releasing compounds can enhance intrinsic aerobic biodegradation of oil hydrocarbons in long-term weathered, historically contaminated soils. Menendez-Vega et al. [20] noted that after the sequential application of hydrogen peroxide, an oleophilic fertilizer and a surfactant, contamination levels clearly declined over a short period of time.

The comparison of enhancement methods of oil hydrocarbon biodegradation was based on the ratio of enhanced – to – intrinsic biodegradation rate (k$_{ENA}$/k$_{NA}$) (Fig. 5). An increase of the biodegradation rate for each of enhancement methods was presented as a mean of values obtained for soil samples G1 and G2.

![Fig. 5. The effect of enhancement methods on the biodegradation rate of oil hydrocarbons in soil](image)

The most effective method of biodegradation enhancement in oil hydrocarbons contaminated soil was combined treatment. Combination of bioaugmentation (with using of indigenous bacterial consortium containing 4.8 × 10$^9$ cells mL$^{-1}$) and addition of the surfactant (Tween 80 in dose of 1% v/v) resulted in the 20-fold increase of intrinsic biodegradation rate. Combined treatment was significantly more effective than bioaugmentation or addition of surfactant separately (Tab. 3). When this remediation method was applied, the 62–65% decrease of TAH in soil samples was noted. These results, together with the fact that surfactant can improve the dissolution of residual
substrates, may indicate that major limiting factors of oil hydrocarbons biodegradation in aged soil are: number and activity of indigenous microflora and bioavailability of substrate for microorganisms.

Tab. 3. The soil hydrocarbons concentration after remediation

| Remediation method                                      | Content of TAH in treated contaminated samples (after 8 days of incubation) (mg kg⁻¹ d.w.) |
|--------------------------------------------------------|------------------------------------------------------------------------------------------|
| Intrinsic biodegradation (without enhancement)         | G1    345  G2    183                                                                 |
| Bioaugmentation – addition of exogenous/indigenous bacterial consortium (4.8×10⁹ cells mL⁻¹) | G1    269  G2    144                                                                 |
| Biostimulation – addition of aerated distilled water    | G1    319  G2    175                                                                 |
| Biostimulation – addition of surfactant                 | G1    302  G2    168                                                                 |
| Combined treatment – bioaugmentation + addition of a surfactant | G1    124  G2    72                                                                 |

CONCLUSIONS

Each of the studied enhancement methods resulted in an increase of the biodegradation rate in oil hydrocarbons contaminated soil.

The highest biodegradation rate due to bioaugmentation was achieved when the bacterial consortium containing 4.8×10⁹ cells mL⁻¹ was applied. Moreover, application of indigenous bacterial consortia was more efficient in comparison to the exogenous bacteria, as the 8-fold increase of the biodegradation rate was achieved. Native microorganisms are well adapted to their environment, and a rapid growth of population guarantees better biodegradation.

In the case of biostimulation by aerated water supply or surfactant addition the 3–4-fold increase of biodegradation rate was observed, respectively.

Both bioaugmentation and biostimulation appeared to be effective in enhancing intrinsic biodegradation of oil hydrocarbons in soil; however, the combined enhancement increased the biodegradation rate more efficiently.

In the case of aged contamination, the best enhancement performance was achieved by the use of bioaugmentation + addition of a surfactant. When such treatment was applied, the enhanced biodegradation rate was 20 times higher than the intrinsic biodegradation rate. Inoculating of soil with indigenous microorganisms seems to be the most effective option, while applying a surfactant makes a substrate more available for microorganisms.

ACKNOWLEDGMENTS

This work was financially supported by MNiSW grant no. 4 T12B 019 30.
REFERENCES

[1] Atlas, R.M. & Bartha, R. (1998). Microbial Ecology: Fundamentals and Applications. Benjamin/Cummings Publishing, Menlo Park, CA 1998.

[2] Bento, F.M., Camargo, F.A.O., Okeke, B.C. & Frankenberger, W.T. (2005). Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation, Bioscience Technology, 96, 1049–1055.

[3] Capelli, S.M., Busalmen, J.P. & Sanchez, S.R. (2001). Hydrocarbon bioremediation of a mineral-base contaminated waste from crude oil extraction by indigenous bacteria, International Biodeterioration and Biodegradation, 47, 233–238.

[4] Coulon, F. & Delille, D. (2003). Effects of Biostimulation on Growth of Indigenous Bacteria in Sub-Antarctic Soil Contaminated with Oil Hydrocarbons, Oil and Gas Science and Technology, 58, 469–479.

[5] D’Annibale, A., Rosetto, F., Leonardi, V., Federici, F. & Petrucciolli, M. (2006). Role of autochthonous filamentous fungi in bioremediation of a soil historically contaminated with aromatic hydrocarbons, Applied and Environmental Microbiology, 72, 28–36.

[6] Devlinny, J. & Chang, S.H. (2000). Bioaugmentation for soil bioremediation. In Wise, D.L., Trantolo, D. (Eds.), Bioremediation of Contaminated Soils, 465–488, Marcel Dekker, New York 2000.

[7] Fantroussi, S.E. & Agathos, S.N. (2005). Is bioaugmentation a feasible strategy for pollutant removal and site remediation? Current Opinion in Microbiology, 8, 268–275.

[8] Gentry, T.J., Rensing, C. & Pepper, I.L. (2004). New approaches for bioaugmentation as a remediation technology, Critical Reviews in Environmental Science and Technology, 34, 447–494.

[9] Gouda, M.K., Omar, S.H., Nour Eldin, H.M. & Checkroud, Z.A. (2008). Bioremediation of kerosene II: a case study in contaminated clay (Laboratory and field: scale microcosms), World Journal of Microbiology and Biotechnology, 24, 1451–1460.

[10] Hamman, S. (2004). Bioremediation capabilities of white rot fungi, Biodegradation, 52, 1–6.

[11] Hosokawa, R., Nagai, M., Morikawa, M. & Okuyama, H. (2009). Autochthonous bioaugmentation and its possible application to oil spills, World Journal of Microbiology and Biotechnology, 25, 1519–1528.

[12] Khan, F.I., Husain, T. & Hejazi, R. (2004). An overview and analysis of site remediation technologies, Journal of Environmental Management, 71, 95–122.

[13] Lai, C.C., Huang, Y.C., Wei, Y.H. & Chang, J.S. (2009). Biosurfactant-enhanced removal of total petroleum hydrocarbons from contaminated soil, Journal of Hazardous Materials, 167, 609–614.

[14] Lee, M., Kim, M.K., Kwon, M., Park, B.D., Kim, M.H., Goodfellow, M. & Lee, S. (2005). Effect of the Synthesized Mycolic Acid on the Biodegradation of Diesel Oil by Gordonia nitida Strain LE31, Journal of Bioscience and Bioengineering, 100, 429–436.

[15] Liang, Y., Zhang, X., Dai, D. & Li, G. (2009). Porous biocarrier-enhanced biodegradation of crude oil contaminated soil. International Biodeterioration & Biodegradation., 63, 80–87.

[16] Malina, G. (1999). The bioventing of unsaturated zone contaminated with oil compounds. Monograph 66, Wydawnictwo Politechniki Czestochowskiej, Czestochowa 1999.

[17] Malina, G. (2007). Risk reduction of soil and groundwater at contaminated areas. Monograph 132, Wydawnictwo Politechniki Czestochowskiej, Czestochowa 2007.

[18] Malina, G. & Zawierucha, I. (2007). Potential of Bioaugmentation and Biostimulation for Enhancing Intrinsic Biodegradation in Oil Hydrocarbon-Contaminated Soil, Bioremediation Journal, 11, 141–147.

[19] Mariano, A.P., Kataoka, A.P., Angelis, D. & Bonotto, D.M. (2007). Laboratory study on the bioremediation of diesel oil contaminated soil from a petrol station, Brazilian Journal of Microbiology, 38, 346–353.

[20] Menendez-Vega, D., Gallego, J.L.R., Pelaez, A.I., de Cordoba, G.F., Moreno, J., Munoz, D. & Sanchez, J. (2007). Engineered in situ bioremediation of soil and groundwater polluted with weathered hydrocarbons, European Journal of Soil Biology, 43, 310–321.

[21] Plaza, G., Ulfig, K., Worsztynowicz, A., Malina, G., Krzeminska, B. & Brigmon, L. (2005). Respirometry for assessing the biodegradation of petroleum hydrocarbons, Environmental Technology, 26, 161–169.

[22] Rahman, K.S.M., Rahman, T.J., Kourkoutas, Y., Petsas, I., Marchant, R. & Banat, I.M. (2003). Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients, Bioresourc Technology, 90, 159–168.

[23] Rous, J.D., Sabatini, D.A., Sulfita, J.M. & Harwell, J.H. (1994). Influence of surfactants on microbial degradation of organic compounds, Critical Reviews in Environmental Science and Technology, 24, 325–370.
[24] Sarkar, D., Ferguson, M., Datta, R. & Birnbaum, S. (2005). Bioremediation of petroleum hydrocarbons in contaminated soils: Comparison of biosolids addition, carbon supplementation and monitored natural attenuation, *Environmental Pollution*, 136, 187–195.

[25] Sayler, G.S. & Ripp, S. (2000). Field application of genetically engineered microorganisms for bioremediation processes, *Current Opinion in Biotechnology*, 11, 286–289.

[26] Scherr, K., Aichberger, H., Braun, R. & Loibner, A.P. (2007). Influence of soil fractions on microbial degradation behaviour of mineral hydrocarbons, *European Journal of Soil Biology*, 43, 341–350.

[27] Shkidchenko, A.N., Boronin, A.M., Kobzev, E.N., Petrikevich, S.B., Chugunov, V.A. & Kholodenko, V.P. (2004). Biodegradation of black oil by microflora of the Bay of Biscay and biopreparations, *Process Biochemistry*, 39, 1671–1676.

[28] Tongarun, R., Luepromchai, E. & Vangnai, A.S. (2008). Natural Attenuation, Biostimulation and Bioaugmentation in 4-Chloroaniline-Contaminated Soil, *Current Microbiology*, 56, 182–188.

[29] Turco, R.F. & Sadowski, M.J. (1995). The Microflora of Bioremediation. In Skipper, H.D. and Turco, R.F. (Eds.), Bioremediation, Science and Applications (pp. 87–103). Special Publication 43. Soil Science Society of America. Washington, DC 1995.

[30] Ueno, A., Ito, Y., Yumoto, I. & Okuyama, H. (2007). Isolation and characterization of bacteria from soil contaminated with diesel oil and the possible use of these in autochthonous bioaugmentation, *World Journal of Microbiology and Biotechnology*, 23, 1739–1745.

[31] Urum, K., Grigson, S., Pekdemir, T. & McMenamy, S. (2006). A comparison of the efficiency of different surfactants for removal of crude oil from contaminated soils, *Chemosphere*, 62, 1403–1410.

[32] Vidali, M. (2001). Bioremediation: an overview, *Pure and Applied Chemistry*, 73, 1163–1172.

[33] Vogel, T.M. (1996). Bioaugmentation as a soil bioremediation approach. *Current Opinion in Biotechnology*, 7, 311–316.

[34] Vogel, T.M. & Walter, M.V. (2001). Bioaugmentation. In Hurst, C.J., Crawford, R.L., Garland, J.L., Lipson, D.A., Mills, A.L. (Eds.), Manual of environmental microbiology (pp. 952–959). American Society for Microbiology Press, Washington DC 2001.

[35] Zawierucha, I. & Malina, G. (2006). Bioaugmentation as a method of biodegradation enhancement in oil hydrocarbons contaminated soil, *Ecohydrology & Hydrobiology*, 6, 163–169.

[36] Zawierucha, I., Szewczyk, A., Malina, G. (2007). Effect of temperature on the biodegradation rate in oil hydrocarbons contaminated soil, *Polish Journal of Environmental Studies*, 16(3B), 520–524.

---

**EFEKTYWNOŚĆ WSPOMAGANIA BIODEGRADACJI W GRUNCIE ZANIECZYSZCZONYM WĘGLOWODORAMI ROPOPOCHODNYMI**

Przeprowadzono badania mające na celu określenie efektywności wspomagania biodegradacji węglowodorów ropopochodnych w gruncie w wyniku zastosowania bioaugmentacji, biostymulacji lub metody kombinowanej. Próbki gruntu użyte do badań zostały pobrane z terenu Centralnej Stacji Tankowania (CST) lotniska Kluczewo niedaleko Stargardu Szczecińskiego. Bioaugmentację przeprowadzono z użyciem autochtonicznych i allochtonicznych mikroorganizmów zdolnych do rozkładu węglowodorów ropopochodnych. Z kolei biostymulacja obejmowała wprowadzenie napowietrzonej wody lub substancji powierzchniowo czynnej (SPC) do zanieczyszczonego gruntu. Biodegradację węglowodorów ropopochodnych szacowano na podstawie konsumpcji O₂ przy...
użyciu respirometru Micro-Oxymax V6.0 COLUMBUS INSTRUMENTS. Średnie szybkości konsumpcji O₂ podczas biodegradacji węglowodorów wyznaczono z równań aproksymacji liniowej krzywych kumulacyjnych. Na podstawie równania bilansu masy i wyznaczonych szybkości konsumpcji O₂ obliczono szybkość biodegradacji węglowodorów, tj. szybkość utraty substratu w czasie. Z przeprowadzonych badań wynika, że metoda kombinowana (kombinacja bioaugmentacji z dodatkiem SPC) była najbardziej efektywną metodą wspomagania biodegradacji węglowodorów ropopochodnych w gruncie – odnotowano wtedy 20-krotny wzrost szybkości biodegradacji.