Effects of oxygen supply on the biodegradation rate in oil hydrocarbons contaminated soil

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Abstract. Respirometry studies using the 10-chamber Micro-Oxymax respirometer (Columbus, Ohio) were conducted to determine the effect of biostimulation (by diverse ways of O₂ supply) on enhancing biodegradation in soils contaminated with oil hydrocarbons. Soil was collected from a former military airport in Kluczewo, Poland. Oxygen was supplied by means of aerated water, aqueous solutions of H₂O₂ and KMnO₄. The biodegradation was evaluated on the basis of O₂ uptake and CO₂ production. The O₂ consumption and CO₂ production rates during hydrocarbons biodegradation were estimated from the slopes of cumulative curve linear regressions. The pertinent intrinsic and enhanced biodegradation rates were calculated on the basis of mass balance equation and O₂ uptake and CO₂ production rates. The biodegradation rates of 5-7 times higher as compared to a control were observed when the aqueous solution of KMnO₄ in concentration of 20 g L⁻¹ was applied. Permanganate is known to readily oxidize alkene carbon – carbon double bonds; so it can be successfully applied in remediation technology for soils contaminated with oil hydrocarbons. While hydrocarbons are not completely mineralized by permanganate oxidation reactions, their structure is altered by polar functional groups providing vast improvements in aqueous solubility and availability for biodegradation. The 3% aqueous solution of H₂O₂ caused significant improvement of the biodegradation rates as compared to a control (on average about 260%). Aerobic biodegradation of hydrocarbons can benefit from the presence of oxygen released during H₂O₂ decomposition. Adding of aerated water resulted in an increase of biodegradation rates (about 114 - 229%) as compared to a control. The aerated water can both be the source of oxygen for microorganisms and determine the transport of substrate to bacteria cells.

1. Introduction
Dynamic development of transportation causes that more of petroleum substances may penetrate natural environment, resulting in degradation of soils, surface water and groundwater [1]. Nearly 2.5 million tons of oil products is estimated to enter the environment yearly [2]. Since late 70-ties intensive studies have been carried out in Poland to estimate the degree of soil and groundwater contamination. A number of locations have been identified and documented with very high contamination of oil hydrocarbons [3]. Oil-hydrocarbon contaminants may appear locally and periodically as a consequence of leaks of crude oil from mining shafts, damage pipelines, cisterns, tankers, and storage and distribution stations of petroleum products. They can also appear permanently on definite areas and derive from refineries or engine industry [4]. Moreover, floods are a serious factor of contamination spreading in the environment [5]. Oil products in soils affect vegetation,
threaten quality of surface water and groundwater, and are also potentially hazardous for humans and the environment [2].

Under natural conditions soils contaminated with oil hydrocarbons may undergo self purification, mainly due to biodegradation with the participation of indigenous microorganisms.

The activity of native microflora decides about efficiency of biodegradation, and it can be enhanced by providing optimal conditions for the microbial growth [6,7].

In the case when O\textsubscript{2} is considered as a limiting factor, biostimulation by oxygen supply is applied to stimulate microbial activity and enhance aerobic biodegradation rates. Commonly used oxygen supply techniques may include tilling, forced aeration and chemical methods [8,9,10]. Tilling has been recommended as a physical method to accelerate biodegradation in landfarming. This technique is effective only for topsoils. Forced aeration techniques, including injection of aerated water, air and pure oxygen, are commonly used for enhancing biodegradation in subsurface soils and groundwater contaminated with petroleum hydrocarbons [9,10]. Chemical methods involve addition of alternative oxygen sources, such as oxygen releasing compounds ORC\textsuperscript{®}, or agents (e.g. KMnO\textsubscript{4}). Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and ozone (O\textsubscript{3}) are also applied, but they can have toxic effects to microorganisms [10].

In this paper, the respirometry studies were conducted to determine the effects of biostimulation (by diverse ways of oxygen supply) on enhancing intrinsic biodegradation of oil hydrocarbon, both in case of fresh leakage and aged contamination. Oxygen was supplied by means of aerated water, aqueous solutions of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) or potassium permanganate (KMnO\textsubscript{4}). The intrinsic and enhanced biodegradation rates were evaluated by the mean O\textsubscript{2} uptake and CO\textsubscript{2} production rates obtained using a linear regression of the cumulative curves of O\textsubscript{2} uptake and CO\textsubscript{2} production.

2. Materials and methods

2.1. Soil samples
Soil samples contaminated with oil hydrocarbons (“historical” - aged contamination) originated from the former military airport in Kluczewo, Poland. Contaminated soil samples were collected at the depths of 1.5 m (G1) and 2.0 m (G2). Uncontaminated soil samples were also collected at the depths of 1.5 m (G1R) and 2.0 m (G2R).

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

2.1.2. 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 of dilution series of soil suspension, and inoculating stable volumes of the dilutions on the agar medium. The dilution series of soil suspension were made with sterile distilled water. Bacteria were incubated at 28°c for 48 hours.

2.1.3. Contents of hydrocarbons in soil
Soil samples were extracted with mixture of methanol:dichloromethane (4:1) by ultrasounds. Contents of aliphatic and aromatic hydrocarbons were determined using a gas chromatograph with mass detector - Varian Star 3400 CX.

2.2. Respirometer 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 O\textsubscript{2} consumed and CO\textsubscript{2} produced during hydrocarbons biodegradation. Experiments were carried out using batch tests at a temperature of 25±5°c. Soil samples of 30 g were placed into 500 mL measuring chambers of the respirometer, to which were added: 8 mL of aqueous
solutions of KMnO₄ (concentrations of 5, 10, 20 g L⁻¹), H₂O₂ (in dilution of 1:10), and aerated distilled water with the O₂ concentrations of 6.67, 7.36, 7.83 mg L⁻¹, which corresponded to the O₂ saturation (under existing conditions i.e. t = 27°C and barometric pressure = 1013 hPa) of 83, 92 and 98%, respectively. The initial concentration of H₂O₂ was of 300 g kg⁻¹. All tests were done in triplicate. 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 rate, which is the result of soil organic matter degradation, whereas control 2 determined an overall respiration, including basal respiration and hydrocarbons biodegradation rates.

Biodegradation of oil hydrocarbons was evaluated based on the cumulative curves of O₂ consumption and CO₂ production. Cumulative curves were plotted as O₂/CO₂ content values in the headspace versus time. Rates of O₂ uptake and CO₂ production were estimated from the slopes of linear regressions of cumulative curves. The coefficients of the linear regression equations represented the mean rates of O₂ consumption and CO₂ production 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 (R²) within the range of 0.94-0.99.

Having data on mean O₂ uptake and CO₂ production rates, and assuming that the only products of hydrocarbons biodegradation are biomass, carbon dioxide and water, pertinent biodegradation rates were calculated.

Mass balance equation is as following:

\[ C_nH_m + a \text{O}_2 = Y \text{CH}_2\text{O} + b \text{CO}_2 + c \text{H}_2\text{O} \]  \hspace{1cm} (1)

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

Assuming that hydrocarbons formula is CH₁.₅ [11,12], hence m/n =1.₅; whereas microbial yield Y=0.₅.

Hydrocarbons biodegradation rates \( k'_\text{NA/ENA}, k''_\text{NA/ENA} \) were calculated according to formulas [12,13]:

\[ k'_{\text{NA/ENA}} = 2.144 \left( \frac{12 + \frac{m}{n}}{4(1-Y) + \frac{m}{n}} \right) k_{\text{O}_2} \left[ \frac{\text{mg of hydrocarbons}}{\text{kg of soil} \cdot \text{day}} \right] \]  \hspace{1cm} (2)

\[ k''_{\text{NA/ENA}} = 58,090 \ k_{\text{CO}_2} \left[ \frac{\text{mg of hydrocarbons}}{\text{kg of soil} \cdot \text{day}} \right] \]  \hspace{1cm} (3)

where: \( k'_\text{NA/ENA} \) – natural/enhanced biodegradation rate calculated on the basis of O₂ consumption rate, \( k''_\text{NA/ENA} \) – natural/enhanced biodegradation rate calculated on the basis of CO₂ production rate, \( k_{\text{O}_2} \) – O₂ consumption rate [μl/min], \( k_{\text{CO}_2} \) – CO₂ production rate [μl/min], 2.144 – a conversion coefficient for O₂ consumption rate to [mmol of O₂/kg of soil*day]; 58,090 – conversion coefficient for CO₂ production rate to [mg of hydrocarbons/kg of soil*day]

The values of O₂ consumption and CO₂ production rates obtained for contaminated soil samples (without and with enhancement by O₂ supply) minus the values obtained for uncontaminated soil samples were placed to formulas (2) and (3). It provided to calculate the “net” hydrocarbons biodegradation rates.
3. Results and discussion

The characteristics of soils are presented in Table 1. The obtained soil physical-chemical parameters are considered optimal for biodegradation of oil hydrocarbons under aerobic conditions [11,14,15]. Sufficient number of microorganisms (10^5 CFU g^-1) [16] for biodegradation was also present in impacted soils.

Soil samples were mainly contaminated with aliphatic hydrocarbons. Aliphatic hydrocarbons are well susceptible to microbiological decomposition [17,18,19].

| Soil sample | pH   | Eh (mV) | Moisture (% w/w) | OM content (% w/w) | Number of bacteria (CFU/g) | N (mg/kg s.m.) | P (mg/kg s.m.) | Σ aromatic hydrocarbons (mg/kg d.w.) | Σ aliphatic hydrocarbons (mg/kg d.w.) |
|-------------|------|---------|------------------|--------------------|---------------------------|----------------|---------------|-------------------------------------|-------------------------------------|
| G1          | 7.4  | 451     | 18.18            | 2.76               | 2×10^5                    | 452            | 314           | 30                                  | 155                                 |
| G1R         | 6.7  | 447     | 26.71            | 3.92               | 1×10^6                    | 462            | 345           | -                                   | -                                   |
| G2          | 7.6  | 457     | 19.28            | 3.20               | 1×10^5                    | 303            | 305           | -                                   | 82                                  |
| G2R         | 8.1  | 449     | 13.07            | 1.99               | 3×10^5                    | 330            | 332           | -                                   | -                                   |

The exemplary cumulative curves of O_2 consumption and CO_2 production during biostimulation tests are presented in Fig. 1. The mean rates of O_2 uptake and CO_2 production during intrinsic and enhanced biodegradation (obtained from linear regressions of cumulative curves) are presented in Fig. 2.

In our experimental set-up the air in the respirometer is circulated in a closed loop, and soil samples are continuously aerated. The addition of O_2 could cause reading clutter of O_2 contents at the top of measuring chamber, therefore the evaluation of biodegradation enhancement by O_2 supply was based on the biodegradation rate calculated on the basis of CO_2 production rate. Only for contaminated soil samples without enhancement (control), pertinent biodegradation rates were calculated on the basis of O_2 consumption rates and then used to comparative analysis. The effectiveness of biodegradation enhancement (biostimulation) by diverse ways of O_2 supply was presented in Fig. 3.
**Figure 1.** Cumulative curves of $O_2$ uptake (A) and $CO_2$ production (B) during biostimulation tests
Notation: G1R – control 1 (uncontaminated soil - depth of 1.5 m), G1 – control 2 (contaminated soil without enhancement - depth of 1.5 m), D3 – addition of aqueous solution of KMnO$_4$ (concentration of 20 g L$^{-1}$)

**Figure 2.** Mean rates of $O_2$ uptake and $CO_2$ production during intrinsic and enhanced biodegradation (biostimulation tests)
Notation: G1R, G2R – control 1 (uncontaminated soil - depths of 1.5 and 2.0 m); G1, G2 – control 2 (contaminated soil without enhancement - depths of 1.5 and 2.0 m); D1, D2, D3 – addition of aqueous solution of KMnO$_4$ (concentration of 5, 10 and 20 g L$^{-1}$); E – addition of aqueous solution of hydrogen peroxide in dilution of 1:10; F1, F2, F3 – addition of aerated distilled water with oxygen saturation of 83, 92 and 98%, respectively
The biodegradation rates of 5-7 times higher as compared to a control were observed when the aqueous solution of KMnO₄ in concentration of 20 g L⁻¹ was applied. Permanganate is known to readily oxidize alkene carbon – carbon double bonds [20,21], so it can be probably used also for oxidation of aromatic hydrocarbons. Brown et al. [22] observed the reduction of contents in soil for benzo(a)pyrene (of 72.1%), pyrene (of 64.2%), phenanthrene (of 56.2%) and anthracene (of 53.8%) after 30 min oxidation at KMnO₄ concentration of 160 mM. The results suggested that permanganate oxidation can be successfully applied in remediation technology for soils contaminated with oil hydrocarbons. While hydrocarbons are not completely mineralized by permanganate oxidation reactions, their structure is altered by polar functional groups providing vast improvements in aqueous solubility and availability for biodegradation.

The 3% aqueous solution of H₂O₂ caused significant improvement of the biodegradation rates as compared to a control (on average about 260%). Aerobic biodegradation of hydrocarbons can benefit from the presence of oxygen released during H₂O₂ decomposition [23]. However, Goi et al. [24] noted that efficiency of H₂O₂ application was strongly dependent on the soil matrix. Treatment of shale oil and transformer oil adsorbed in peat, used as a model of organic-rich soil, resulted in lower oil removal degree, and required more H₂O₂ than the treatment of oil in sand matrix representing the mineral part of the soil.

Adding of aerated water resulted in an increase of biodegradation rates (about 114 - 229%) as compared to a control. The aerated water can both be the source of oxygen for microorganisms and determine the transport of substrate to bacteria cells. The water facilitated diffusion of substrate for microorganisms and, consequently improved biodegradation rate [25].

5. Conclusions
Biostimulation by oxygen supply can be attractive approach of enhancing biodegradation in soil contaminated with oil hydrocarbons.

The results of biostimulation show that the highest biodegradation rates were achieved when aqueous solution of KMnO₄ in concentration of 20 g L⁻¹ was applied. Then, the biodegradation rates were about 415-585% higher compared to a control.

The adding of aerated water seems to be the most optimal way of O₂ supply. The use of aerated water causes improvement of the biodegradation rate and is safe for natural environment.

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