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The influence of bioaugmentation and biosurfactant addition on bioremediation efficiency of diesel-oil contaminated soil: Feasibility during field studies

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ABSTRACT

The study focused on assessing the influence of bioaugmentation and addition of rhamnolipids on diesel oil biodegradation efficiency during field studies. Initial laboratory studies (measurement of emitted CO_2 and dehydrogenase activity) were carried out in order to select the consortium for bioaugmentation as well as to evaluate the most appropriate concentration of rhamnolipids. The selected consortium consisted of following bacterial taxa: *Aeromonas hydrophila*, *Alcaligenes xylosoxidans*, *Gordonia* sp., *Pseudomonas fluorescens*, *Pseudomonas putida*, *Rhodococcus equi*, *Stenotrophomonas maltophilia*, *Xanthomonas* sp. It was established that the application of rhamnolipids at 150 mg/kg of soil was most appropriate in terms of dehydrogenase activity. Based on the obtained results, four treatment methods were designed and tested during 365 days of field studies: I) natural attenuation; II) addition of rhamnolipids; III) bioaugmentation; IV) bioaugmentation and addition of rhamnolipids. It was observed that bioaugmentation contributed to the highest diesel oil biodegradation efficiency, whereas the addition of rhamnolipids did not notably influence the treatment process.

1. Introduction

The use of biological methods for remediation of polluted areas has been recognized as a cost-effective and promising strategy. Several biotic and abiotic factors which play a crucial role during such processes have been identified in the framework of numerous studies (Fernández-Luqueño et al., 2011). Although biological treatment processes have received much scientific attention, the development of efficient *in situ* bioremediation processes for decontamination of petroleum-contaminated soils still remains a challenging task. The progress of such processes is directly associated with the catabolic potential of microorganisms present in the polluted area and the bioavailability of the contaminants, hence these two factors are common causes of limitation (Antizar-Ladislao et al., 2006).

Generally, it has been established that microorganisms possessing relevant catabolic genes (such as the *alkB* alkane hydroxylaze gene) are commonly present even in uncontaminated environments (Kloos et al., 2006). However, the relative abundance of hydrocarbon degraders in a given microbial population may be marginal under normal circumstances (Vomberg and Klinner, 2000). This corresponds to a low initial biodegradation potential and slow biodegradation rate. One of the strategies to overcome this problem involves the introduction of selected microorganisms into the polluted area. The concept of inoculating the soil with fast degrading microbes in order to increase the efficiency of the process is commonly known as bioaugmentation (Thompson et al., 2005). Several studies confirm that increasing the initial biomass level may enhance the biodegradation rate and improve the treatment process by reducing the time needed for adaptation (Mishra et al., 2001; Mukherjee and Bordoloi, 2011). On the other hand, it was also reported on numerous occasions that employing this strategy did not contribute to the desired effect (Bouchez et al., 2000; Wagner-Döbler, 2003). In most cases, failure was related to poor survivability and adaptability of the introduced microorganisms due to improper strain selection. Hence, although bioaugmentation seems promising, it requires pretreatment preparations in order to be valid.

While obtaining and maintaining high microbial activity is one priority, another major factor is associated with the bioavailability of

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pollutants for the microorganisms (Angelova and Schmauder, 1999). The limited bioavailability of hydrocarbons in a terrestrial environment due to low water solubility or interactions with the soil matrix often corresponds to inhibition of the degradation rate (Harms and Bosma, 1997). It is believed that surface-active compounds may be used to increase the bioavailability of otherwise poorly accessible carbon sources, thus helping to overcome the diffusion-related mass transfer limitations (Makkar and Rockne, 2003; Singh et al., 2007). Therefore, the application of surfactants to enhance the biodegradation efficiency of petroleum hydrocarbons has been an object of numerous studies (Pantsyrnaya et al., 2011; Wyrwas et al. 2011; Yu et al., 2007). Recently, surface active compounds of microbial origin have gained much scientific attention, since these compounds offer superior biodegradability and environmental-friendliness compared to their synthetic counterparts (Bordoloi and Konwar, 2009; Whang et al., 2009a,b). Rhamnolipids are a perfect example, since the congeners which constitute this bioemulsifier are among the most investigated and well-described group of compounds (Chrzanowski et al., 2012b). Additionally, this biosurfactant proved to be efficient during flushing of contaminated soil and mobilization of recalcitrant pollutants (Mulligan and Eftekhari, 2003; Wang and Mulligan, 2004). Both of these qualities make rhamnolipids potential agents for enhancing the efficiency of *in situ* bioremediation attempts in polluted terrestrial environments.

The aim of this study was to determine the influence of bioaugmentation, introduction of rhamnolipids and a combined approach (rhamnolipids-mediated bioaugmentation) on the biodegradation efficiency of petroleum hydrocarbons in silty-clay soil freshly spiked with diesel oil in order to evaluate the feasibility of each treatment method. The experiment was divided into two stages: the first stage, carried out under laboratory conditions, focused on the selection of an appropriate microbial consortium for bioaugmentation and an optimal concentration of rhamnolipids, while the second stage allowed for assessment of the chosen treatment methods during 365 days of field studies.

2. Materials and methods

2.1. Chemical reagents

Petroleum diesel oil (EN 590:2004) was purchased from PKN, Orlen, Poland. Rhamnolipids, were obtained from the Jeneil Biosurfactant Company (Saukvulle, WI, USA) as a commercially available product JBR-425 (25% aqueous solution of rhamnolipids). The mixture contains mainly rhamnolipid RL1 (rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate) and RL2 (L-rhamnosyl-L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate).

2.2. Characterization of soil

The soil used throughout the experiments both under laboratory conditions and during field studies originated from a field near Poznań, Poland (N: 52° 10′ 26″ E: 18° 51′ 9″). The soil was characterized as fine grained silty clay type CL (inorganic clays with low to moderate plasticity according to United Soil Classification System). The soil composition (clay, slit and sand content) as well as the content of organic carbon, nitrogen, phosphorus, potassium (expressed as grams per kilogram of dry matter) and the cation exchange capacity were given in Table 1.

Table 1
Characteristics of the experimental soil.

Parameter	Value
Clay [%]	53 ± 3
Silt [%]	25 ± 2
Sand [%]	22 ± 2
Organic carbon [g kg^{-1}]	46.12 ± 2.7
Nitrogen [g kg^{-1}]	4.34 ± 0.6
Phosphorous [g kg^{-1}]	0.7 ± 0.06
Potassium [g kg^{-1}]	1.56 ± 0.1
pH	7.56 ± 0.8
Moisture [%]	20 ± 1
Cation exchange capacity [$cmol_c$ kg^{-1}]	7.4 ± 0.3

± Represent standard deviation ($n = 3$).

2.3. Microorganisms

2.3.1. Microbial consortia for bioaugmentation trials

Ten bacterial consortia (ZomB1, ZomB2, AsU1, AsU2, TotD1, TotD2, DanG1, DanG2, ZaC1 and ZaC2) with a high biodegradation potential towards diesel oil have been selected from the group of 218 consortia isolated in the framework of previous research (Owsianiak et al., 2009a) for potential application in bioaugmentation treatment during field studies. Three distinct responses of the 218 microbial consortia upon introduction of rhamnolipids could be observed during the previous studies: negative (decrease of biodegradation rate), neutral (no effect on the biodegradation rate) or positive (facilitated biodegradation rate). In order to achieve the highest possible biodegradation efficiency and avoid potential issues during rhamnolipids-mediated bioaugmentation, the ten consortia used in the framework of this study were selected from the group which responded to the presence of rhamnolipids in a positive manner. After initial tests concerning the influence of these ten consortia on the microbial activity of autochthonic soil microflora, one consortium was selected for subsequent identification and application in field studies.

2.3.2. Culturing conditions

The microbial consortia have been stored at −80 °C in a 30% (v/v) glycerol stocks. To prepare an inoculum used for bioaugmentation a stock suspension was transferred (1 ml) to a 300-mL Erlenmeyer flask containing 50 ml of mineral medium (composition in Owsianiak et al., 2009b) and diesel fuel (0.5%, v/v), then cultivated for 24 h at 25 °C. Later, 1 ml aliquot of the cell suspension was transferred to a new enrichment flask and the culture was grown for 3 days in the same conditions. This step was repeated three times and cells from the last enrichment were centrifuged at 10 000× *g* and washed twice with 40 ml of mineral medium. Aerobic conditions were provided during all the steps. Polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) revealed that the community in the glycerol stock was the same as the communities in the enrichment cultures (data not shown).

2.4. Laboratory-scale studies

A set of initial experiments was carried out under laboratory conditions in order to establish the most adequate factors for each treatment method during field studies. These experimental steps were focused on: I) the selection and primary characterization of the most appropriate hydrocarbon-degrading bacterial consortium for bioaugmentation treatment; II) the determination of the most appropriate rhamnolipids concentration and assessment of their biodegradability for rhamnolipids and combined treatment (rhamnolipids-mediated bioaugmentation).

For each experiment, an airtight 1 L glass bottle filled with 100 g of soil was used. Following experimental set-ups were tested: non-augmented soil without rhamnolipids (I), non-augmented soil with rhamnolipids (II), augmented soil without rhamnolipids (III),