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Biostimulation-based bioremediation of diesel fuel: field demonstration

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Abstract

Ex-situ bioremediation of leached cynamonic forest soil at initial diesel oil contamination of 6000 mg kg^{-1}, 4000 mg kg^{-1} and 2000 mg kg^{-1} was investigated after biostimulation with inorganic fertilizers. It was found that the added nutrients had no effect on the decontamination of polluted soils. A precise and reliable approach for evaluation of the biodegradation process is proposed. It comprises application of sensitive and easily accessible diagnostic parameters and relations, calculated on the basis of n-alkanes and isoprenoids – pristane (2.6.10.14-tetramethylpentadecane, i-$C_{19}H_{40}$) and phytane (2.6.10.14-tetramethylhexadecane, i-$C_{20}H_{42}$) distribution.

Introduction

The natural ability of microorganisms to remove or transform organic chemicals is used for purposes of bioremediation of a wide range of soil pollutants. Upon hydrocarbon pollution the first stages of microbiological destruction of crude oil/petroleum products include degradation of n-alkanes and isoprenoids, as well as some aromatic compounds, while aromatic substances with higher molecular weight, resins and asphaltenes are considered as unsusceptible to it (Atlas 1981).

Diesel fuel bioremediation has been demonstrated both in laboratory and field trials (Braun et al. 1992; Geerdink et al. 1996a, 1996b; Margesin & Schinner 1997a; Biggar et al. 1997; Margesin & Schinner 1998). A few studies have been carried out for determination the effect of biostimulation on the biodegradation of hydrocarbons (Owens et al. 1987; Lindstrom et al. 1991; Pritchard & Costa 1991; Kerry 1993; Bragg et al. 1994; Wardell 1995; Braddock & McCarthy 1996; Braddock et al. 1997). All of them reported a favourable influence of fertilizers addition.

In all cases when bioremediation techniques are applied, application of precise and reliable methods for evaluation of pollutants biodegradation process are necessary. Some of the currently used methods include: enumeration, isolation and identification of strains with potential for hydrocarbons degradation (Chaineau et al. 1995); determination of dehydrogenase activity and basal soil respiration (Margesin & Schinner 1997b; Braddock et al. 1997; Smith et al. 1998). Along with the above mentioned, the literature sources outline compounds effective for evaluation of biodegradation, aiming source identification and differentiation of oils, evaluation and monitoring of oil weathering: n-alkanes and isoprenoids, biomarkers (hopanes, norhopanes, methylhopanes), naphthalenes, phenanthrenes and dibenzothiophenes (Alexander 1991; Oudot 1994; Prince et al. 1994; Wang et al. 1994; Wang et al. 1995; Douglas et al. 1996; de Jonge et al. 1997; Wang et al. 1998).

The proposed research aims determination of degradation activities of the indigenous microorganisms at conditions of nutrient biostimulation along ex-situ bioremediation of leached cynamonic forest soil, polluted with diesel fuel under field experiment. Evaluation of the effect of microbial biodegradation on the loss of hydrocarbons by means of application of sensitive and easily accessible diagnostic parameters and relations, calculated on the basis of n-alkanes and isoprenoids (pristane and phytane) distribution was performed.

Description of the experimental procedures

The selected for investigation soil is located within the region of Nova Zagora (Bulgaria). The main soil characteristic features (A horizon) comprise: pH_{H_2O} 6.6 ± 0.6, pH_{KCl} 6.6 ± 0.0, humus 2.3 ± 0.7%, $CaCO_3$ 0.0 g kg^{-1}, $H^+ + Al^{3-}$ 0.0 mg eq kg^{-1}, total N and P 0.14 ± 0.04% and 0.15 ± 0.0% respectively (USDA 1996).

A cynamonic forest soil, polluted with diesel fuel, spilled after an emergency failure at the product pipeline of LUKoil Neftochim Bourgas Bulgaria, has been excavated and shaped in hills with the following dimensions: 2.0 × 1.0 × 0.3 m. The excavated soil was taken from A horizon. The initial hydrocarbon concentrations were approximately 6000 mg kg^{-1}, 4000 mg kg^{-1} and 2000 mg kg^{-1} soil. Nutritious components were added to the polluted soil. The mineral nitrogen was ensured via addition of NH_4NO_3 (34–35%N). Phosphorus and potassium were added as $Ca(H_2PO_4)_2.H_2O$ (35–37%P_2O_5) and KCl (48% K_2O). The C:N and N:P:K ratios were 32:1 and 1.0:0.8:0.6 respectively. Control soils received the same amount of hydrocarbons without adding any nutrients. All experiments were performed in three replicates.

The soils were stirred once per month for provision of sufficient air rate and availability of terminal electron acceptor oxygen. Control soils were treated in the same way.

The experiment began in September 1997. The duration of the active experimental period was 373 days and then the stirring was suspended. The selected parameters have been a subject of monitoring until the 686th day. Following the methods described below the residual hydrocarbons concentration, n-alkanes and isoprenoids – pristane (2,6,10,14-tetramethylpentadecane, i-$C_{19}H_{40}$) and phytane (2,6,10,14-tetramethylhexadecane, i-$C_{20}H_{42}$) were determined.

The residual hydrocarbon concentration was determined by means of Fourier transform infrared spectroscopy (FTIR) (Bruker, Germany) after extraction of soil samples with tetrachlormethane (CCl_4) and applying a clean up procedure for polar compounds on Al_2O_3 following the method of Kovacheva & Belcheva (1994).

Capillary GC (Hewlett Packard, USA) with flame ionization detection (GC-FID) was applied for determination of n-alkanes and isoprenoids – pristane and phytane, using a capillary column ULTRA – 2 (25 m × 0.32 mm × 0.52 μm). The concentrations of individual n-alkanes and isoprenoids were calculated by the method of external standard (Milina et al. 1999).

For deducing significant differences among means, the t-test was used. Significance was accepted at α = 0.05 (95% confidence).

Results and discussion

The reduction of hydrocarbons in the studied range of pollution is shown in Figure 1. Statistically significant losses of hydrocarbon concentrations were found within the whole range of pollution, despite nutrient stimulation of diesel oil degrading bacteria. There were no statistically significant differences in the residual hydrocarbon concentrations at the end of the 373 days period, differentiated by the level of initial pollution with or without added fertilizers. The potential for pollutant reduction in the investigated soil type was not influenced by stimulation with nutrients added in the above mentioned concentrations. The result is not in compliance with what was reported. Possible reason for our results is that in great extent the constructive and energy requirements of the specific microflora, responsible for carbon destruction are provided by the available native concentrations of nutrients. Probably, the decrease of the introduced nutrients (data not shown) is due to abiotic and biotic processes, which are not related with the activities of aerobic hydrocarbon utilizing microorganisms, some of which comprise: immobilization onto soil matrices, leaching, nitrification, assimilation by microflora.

Table 1 shows the change of three biodegradation indicators within the whole process: ratios Pr/Ph, n-C_{17}/pristane and n-C_{18}/phytane. These indicators were selected as appropriate for estimation of the effect of biodegradation on hydrocarbon destruction in polluted soils (Fayad et al. 1992; Wang et al. 1999; Papazova & Pavlova 1999). Additionally the changes in the following parameters Σ(n-C_{10} + n-C_{11} + n-C_{12} + n-C_{13} + n-C_{14}) (Σn-C_{10}-n-C_{14})(%), Σ(n-C_{15} + n-C_{16} + n-C_{17} + n-C_{18} + n-C_{19} + n-C_{20}) (Σn-C_{15}-n-C_{20})(%), Σ(n-C_{21} + n-C_{22} + n-C_{23} + n-C_{24} + n-C_{25} + n-C_{26} + n-C_{27}) (Σn-C_{21}-n-C_{27}) (%) was monitored. Tendencies, valid for the whole studied range of pollution, with and without nutrient stimulation were found.

It is obvious that the amount of n-alkanes with length up to 14 carbon atoms were statistically significantly reduced within the first couple of weeks as a result of abiotic losses comprising evaporation

Table 1. Dynamics of biodegradation indicators and parameters of Investigated treatments

Treatment: diesel oil 6000 mg kg^{-1}, without fertilizer	Biodegradation indicators and parameters						Treatment: diesel oil 6000 mg kg^{-1}, with fertilizer	Biodegradation indicators and parameters					
Time (days)	Pr/Ph	n-C_{17}/ pristine	n-C_{18}/ phytane	Σ n-C_{10}-n-C14 (%)	Σ n-C_{15}-n-C20 (%)	Σ n-C_{21}-n-C27 (%)	Time (days)	Pr/Ph	n-C_{17}/ pristine	n-C_{18}/ phytane	Σ n-C_{10}-n-C14 (%)	Σ n-C_{15}-n-C20 (%)	Σ n-C_{21}-n-C27 (%)
0	1.29	1.55	1.69	4.1	8.8	4.5	0	1.29	1.55	1.69	4.1	8.8	4.5
13	1.01	1.19	1.11	1.0	9.4	6.9	13	1.04	1.15	1.03	1.0	11.4	6.3
69	1.00	1.14	1.05	0.9	11.2	6.8	69	1.02	1.20	1.06	1.3	10.7	7.1
148	0.92	1.17	1.07	0.9	10.7	7.5	148	0.87	1.14	0.98	0.8	10.1	9.0
220	0.87	1.11	1.04	0.7	12.3	8.4	220	0.82	1.11	0.96	0.6	12.1	8.1
275	0.84	1.13	1.06	0.3	14.1	10.8	275	0.79	1.13	1.12	0.2	8.8	11.0
338	0.87	1.14	1.07	0.7	12.9	12.0	338	0.74	1.14	0.96	0.5	13.1	11.7
554	0.89	1.11	1.03	0.1	4.1	3.2	554	0.69	1.14	0.99	0.1	3.7	2.6
686	0.86	1.16	1.02	0.1	4.7	2.9	686	0.66	1.13	0.91	0.1	5.5	2.9
Treatment: diesel oil 4000 mg kg^{-1}, without fertilizer	Biodegradation indicators and parameters						Treatment: diesel oil 4000 mg kg^{-1}, with fertilizer	Biodegradation indicators and parameters					
Time (days)	Pr/Ph	n-C_{17}/ pristine	n-C_{18}/ phytane	Σ n-C_{10}-n-C14 (%)	Σ n-C_{15}-n-C20 (%)	Σ n-C_{21}-n-C27 (%)	Time (days)	Pr/Ph	n-C_{17}/ pristine	n-C_{18}/ phytane	Σ n-C_{10}-n-C14 (%)	Σ n-C_{15}-n-C20 (%)	Σ n-C_{21}-n-C27 (%)
0	1.29	1.55	1.69	4.1	8.8	4.5	0	1.29	1.55	1.69	4.1	8.8	4.5
13	0.99	1.12	1.01	1.2	12.5	6.8	13	1.02	1.22	1.07	1.3	11.6	6.4
69	0.94	1.18	1.01	0.8	11.7	6.9	69	0.80	1.05	0.89	0.9	10.6	4.6
148	0.94	1.19	1.03	0.9	12.0	7.4	148	0.84	1.11	1.03	0.7	11.3	4.9
220	0.86	1.08	1.03	0.3	8.2	4.7	220	0.90	1.15	1.06	0.4	8.3	5.5
275	0.74	1.18	1.13	0.5	16.0	10.7	275	0.82	1.14	0.98	0.4	13.4	8.6
338	0.69	1.13	0.93	0.4	16.3	11.8	338	0.72	1.12	0.97	0.6	16.8	10.6
554	0.78	1.03	0.91	0.1	5.8	3.4	554	0.66	1.01	0.96	0	5.4	3.4
686	0.61	0.94	0.77	0	4.4	3.0	686	0.69	0.94	0.91	0	5.1	3.5
Treatment: diesel oil 2000 mg kg^{-1}, without fertilizer	Biodegradation indicators and parameters						Treatment: diesel oil 2000 mg kg^{-1}, with fertilizer	Biodegradation indicators and parameters					
Time (days)	Pr/Ph	n-C_{17}/ pristine	n-C_{18}/ phytane	Σ n-C_{10}-n-C14 (%)	Σ n-C_{15}-n-C20 (%)	Σ n-C_{21}-n-C27 (%)	Time (days)	Pr/Ph	n-C_{17}/ pristine	n-C_{18}/ phytane	Σ n-C_{10}-n-C14 (%)	Σ n-C_{15}-n-C20 (%)	Σ n-C_{21}-n-C27 (%)
0	1.29	1.55	1.69	4.1	8.8	4.5	0	1.29	1.55	1.69	4.1	8.8	4.5
13	1.03	1.08	0.98	1.5	11.5	6.3	13	0.91	0.88	0.75	0.9	11.1	5.3
69	1.01	1.07	0.94	1.7	13.1	6.9	69	0.84	0.83	0.75	0.7	9.8	6.1
148	0.93	1.00	0.86	1.0	10.9	7.4	148	0.80	0.90	0.81	0.6	9.2	5.5
220	0.94	1.02	0.87	1.9	11.8	6.5	220	0.81	0.91	0.80	0.1	9.5	4.1
275	0.77	0.95	0.81	0.5	14.0	8.9	275	0.71	0.75	0.67	0.4	12.8	7.7
338	0.71	0.89	0.72	0.2	13.5	9.6	338	0.67	0.76	0.66	0.2	13.0	10.6
554	0.69	0.87	0.77	0	4.5	4.0	554	0.58	0.69	0.57	0	3.6	2.6
686	0.61	0.64	0.53	0	4.7	4.1	686	0.54	0.59	0.49	0	5.0	4.8

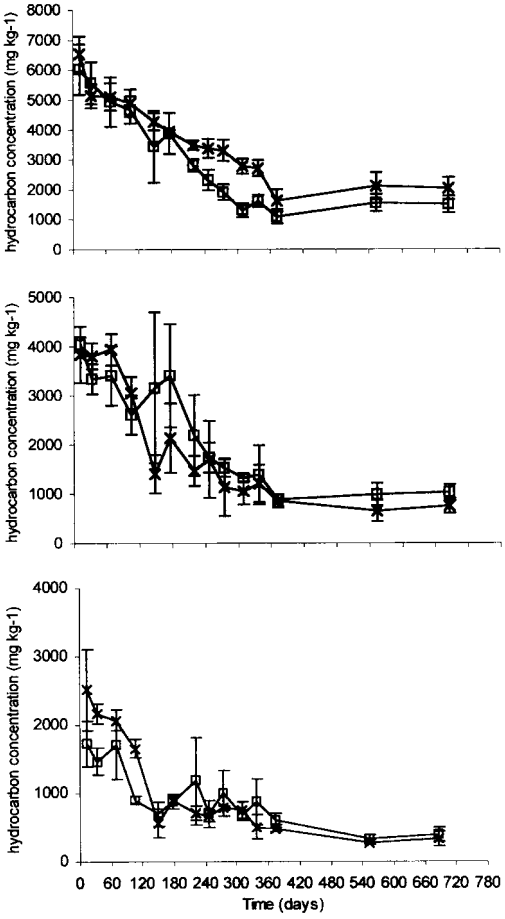

Figure 1. Diesel oil concentration in treated soils. □ with fertilizer, × without fertilizer. Error bars indicate standard error of the mean (n = 3).

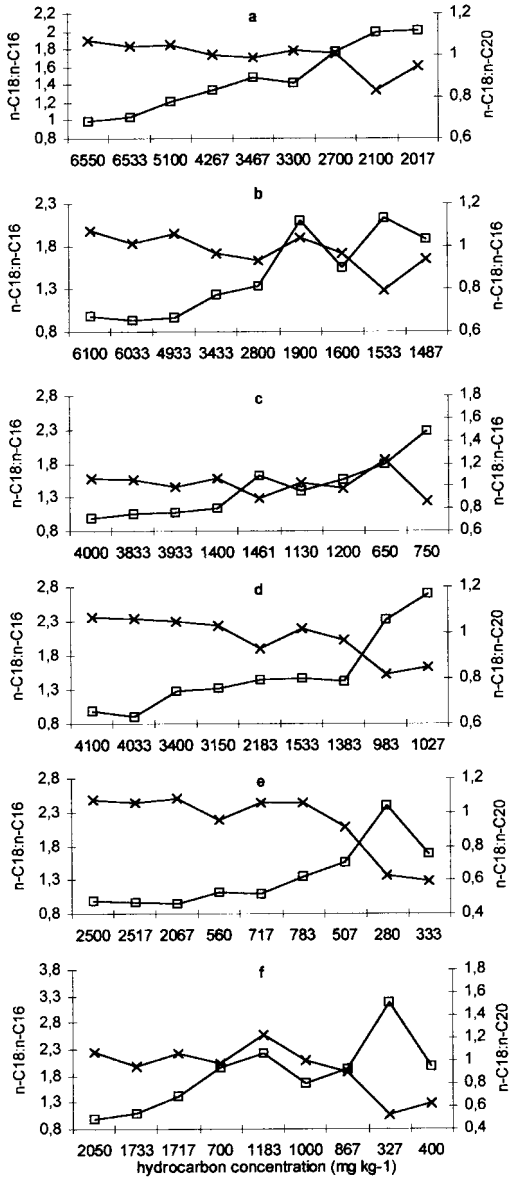

Figure 2. n-C_{18}:n-C_{16} and n-C_{18}:n-C_{20} as a function of hydrocarbon concentration. □ n-C_{18}:n-C_{16} × n-C_{18}:n-C_{20} a, c, e without fertilizer b, d, f with fertilizer.

of lower alkanes. The evaporation is the major process that takes place after spillage and its duration is quite short – from some hours up to several days (Bongiovanni et al. 1989; Oudot 1994).

Statistically significant decrease of the n-C_{17}/Pr and n-C_{18}/Ph ratios was determined within the first couple of weeks after the beginning of the experiment, proving that the indicated n-alkanes were preferentially degraded compared with their respective isomers. By the end of the active experimental period the above mentioned ratios remained constant – obviously the rates of n-alkanes, pristane and phytane degradation were close. The evaluation of the dynamics of the above mentioned parameters could be applied for measurement of the biodegradation process, proving the observations of other authors (Pritchard & Costa 1991), due to the fact that pristane and phytane are relatively persistent isoprenoids. The established trend outlines that the pollutant reduction is a result of the microbial metabolism, showing the impact of biotic factors. The results obtained are of significant importance, having in mind the low temperatures at which the experiment was performed during the winter season (the temperature was approximately 0 °C). The capability of microorganisms to degrade petroleum hydrocarbons at temperatures below 0 °C is reported (Zo Bell 1973).

The content ratio of Σn-C_{15}-nC_{20} and Σn-C_{21}-nC_{27} (%) increased statistically significantly during the active one-year period of the experiment. Statistically significant reduction of the content of these n-alkanes was determined after suspending the stirring procedures. Figure 1 shows that the residual concentrations of petrol products remain constant within the period from 373th to 686th day. One reason for this could be the presence of n-alkanes with branched chains, which due to their molecular structure are more stable to microbial destruction compared with n-alkanes (Atlas 1984). The residual hydrocarbon concentration remained statistically significantly unchanged between the 9th and 10th month of the field experiment, while the content of heavy n-alkanes (Σn-C_{21}-nC_{27}) increased. Obviously, there are other factors, determining the established trend. The season dynamics representing consequent alternation of wet and cold periods (October–April) with dry and warm ones (April–August), especially after cancellation of soil stirring, seems to be important for oxygen diffusion in the soil, thus influencing the passive degradation of pollutants. Besides this, the seasons change, and the related alternation of anaerobic and aerobic conditions causes the most efficient humus accumulation in untilled soils (Turin 1937; Mischustin 1954). Thus, when high concentrations of organic matter are available in soils, the destruction of these substances is directed towards humus formation, on contrary to the mineralization process taking place under strongly aerated conditions. The presence of humus – like ingredients, detected as non-polar hydrocarbons, most probably forms the measured residual hydrocarbon concentrations.

The increase of the ratio n-C_{18}:n-C_{16} (Figure 2) upon reduction of the petroleum hydrocarbon concentration, indicates higher level of degradation of n-C_{16} compared with n-C_{18}. The decrease of the ratio n-C_{18}:n-C_{20} (Figure 2) is an evidence that the destruction of n-C_{18} is preferential to n-C_{20}. Basing on the results obtained, could be assumed that chain length of the n-alkanes is of great importance and degree of degradation of n-alkanes with shorter chains was higher than that of ones with longer chains, independently on the initial rate of pollution in the range investigated. De Jonge et al. (1997) reported that at higher concentrations (12000–4000 mg kg^{-1}) due to the different mechanism of oil distribution in the soil matrix, all n-alkanes were degraded at the same rate, regardless the chain length of n-alkanes. Our results prove that the scheme of microbial hydrocarbon utilization is not universal.

Conclusions

The results of this study in the investigated soil type clearly indicate that the added nutrients had no effect on the decontamination of polluted soils. The result obtained is of significant importance from point of view the physiology of microflora, specific for the studied soil. On the other hand, bioremediation without introduction nutrients in soil results in avoidance of risks of soil and ground water contamination with fertilizers. Obviously the effectiveness of indigenous microbial activity is not enhanced, in respect the parameters of ex-situ bioremediation. Further investigations, applying surfactants could be performed for increase of the bioavailability of the contaminant, having in mind that this is a significant regulatory factor of the biodegradation (Oberbremer et al. 1990; Zhang & Miller 1992; Lee et al. 1995; Churchill et al. 1995). By means of n-alkanes and isoprenoids determination and application of some diagnostic parameters and relations, the diesel oil reduction resulting from the combined occurrence of abiotic and biotic processes was determined. Applying such approach is suitable for the purposes of measurement of bioremediation activities in field pollution. The proved biodegradation capabilities of indigenous microorganisms within the investigated range of contamination during cold environmental conditions demonstrated the opportunity to carry out effective treatment of polluted soils throughout the whole year. When the bioremediation operations commence during the warm season, the biodegradation process will be faster.

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