

**MICROBIOLOGICAL CHANGES IN PETROLEUM-CONTAMINATED
SOIL DURING BIOREMEDIATION
AT A POLISH PETROLEUM REFINERY**

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Abstract

The changes in bacterial and fungal numbers and in dehydrogenase activity during the bioremediation of clayey soil heavily contaminated with petroleum hydrocarbons in the column experiment and in the biopile at one of the Polish petroleum refineries are presented. Bacterial populations played a crucial role in the biodegradation of petroleum contaminants in the subsurface soil while fungal populations were much more active in the surface soil. Soil aeration was a critical factor determining the rate of microbial activity and the contribution of microbial groups (bacteria, microscopic fungi) to the bioremediation process. Among the microbiological parameters measured, dehydrogenase activity was the best indicator of microbiological changes in the soil under bioremediation.

Introduction

The contamination of the environment with petroleum hydrocarbons provides serious problems to many countries, especially to those from the Eastern Europe, including Poland. The Institute for the Ecology of Industrial Areas (IETU) in Katowice joined solving the problems by signing, in August 1995, the

agreement with the U.S. Department of Energy. Within this agreement, IETU commenced the project on the bioremediation of clayey soil heavily contaminated with petroleum hydrocarbons at one of the Polish petroleum refineries. The project technology was presented in a separate paper (Worsztynowicz *et al.*, 1997). The microbiological studies within the project yields important information on the compositions and activities of indigenous microorganisms in the biodegradation of petroleum contaminants in the soil. The microbiological studies are divided into three main parts:

1. treatability and feasibility study
2. monitoring of microbiological changes in the column experiment (under laboratory conditions)
3. monitoring of microbiological changes in the biopile at the refinery (under field conditions).

The results of the treatability and feasibility study were presented in a previous paper (Ulfig *et al.*, 1996). The present paper is to show the data on microbiological changes in the columns and the biopile.

Material and methods

Within the column experiment, four columns were set up (A, B, C and D). Each column had six layers:

Layer 1 – topsoil (garden compost) covered by the grass

Layer 2 – layer intermediary between layers 1 and 3

Layer 3 – clay soil from the refinery heavily contaminated with petroleum hydrocarbons and mixed with wood chips (10% v/v)

Layer 4 – layer intermediary between layers 3 and 5

Layer 5 – dolomite

Layer 6 – leachate

Columns A, B and C were actively aerated with an air pumping system while column D was aerated passively with Baro-ballsTM. Commercially available

complex fertilizer was added each week to columns A and D. Column B was fertilized with NH_4NO_3 and TEP (PhoStarTM system) whereas NH_4NO_3 and $(\text{NH}_4)_3\text{PO}_4$ were added to column C. The leachates were turned back to the columns. At two-week intervals, samples from each layer of the columns were collected for microbiological and physico-chemical examination. The following microbiological analyses were performed in each sample:

- total number of bacterial cells with the epifluorescence DAPI method;
- total number of fungal propagules with the epifluorescence Calcafluor white (CFW) method;
- dehydrogenase activity with the TTC method;
- number of naphthalene- and petroleum-degrading microorganisms.

The DAPI method was that of Kepner & Pratt (1994) whereas the CFW method was adapted from medical mycology (Sparkes *et al.*, 1994). The TTC method was that of Alef (1996). The most probable number (MPN) and selective enrichment techniques were used in “Biolog” boxes for enumeration of naphthalene- and petroleum-degrading microorganisms in soil and leachate at 20 and 37°C. The column experiment lasted for 14 months.

Twenty-three locations were set up on the biopile at the refinery. The biopile is divided into the actively and passively aerated zones and treated with commercially available fertilizer. At each location, samples are taken from the shallow (30 cm of depth) and deep (100 cm of depth) soil layers. The biopile experiment has lasted for 11 months. During this time period, four soil samplings have been performed. The scope of microbiological analyses is the same as in the column experiment.

Total petroleum hydrocarbons (TPH) and total petroleum hydrocarbons + polar compounds (TPOC) were measured by means of ultra red spectrophotometric method (IR). Statistical analysis of the results obtained was performed with the STATISTICA for Windows program.

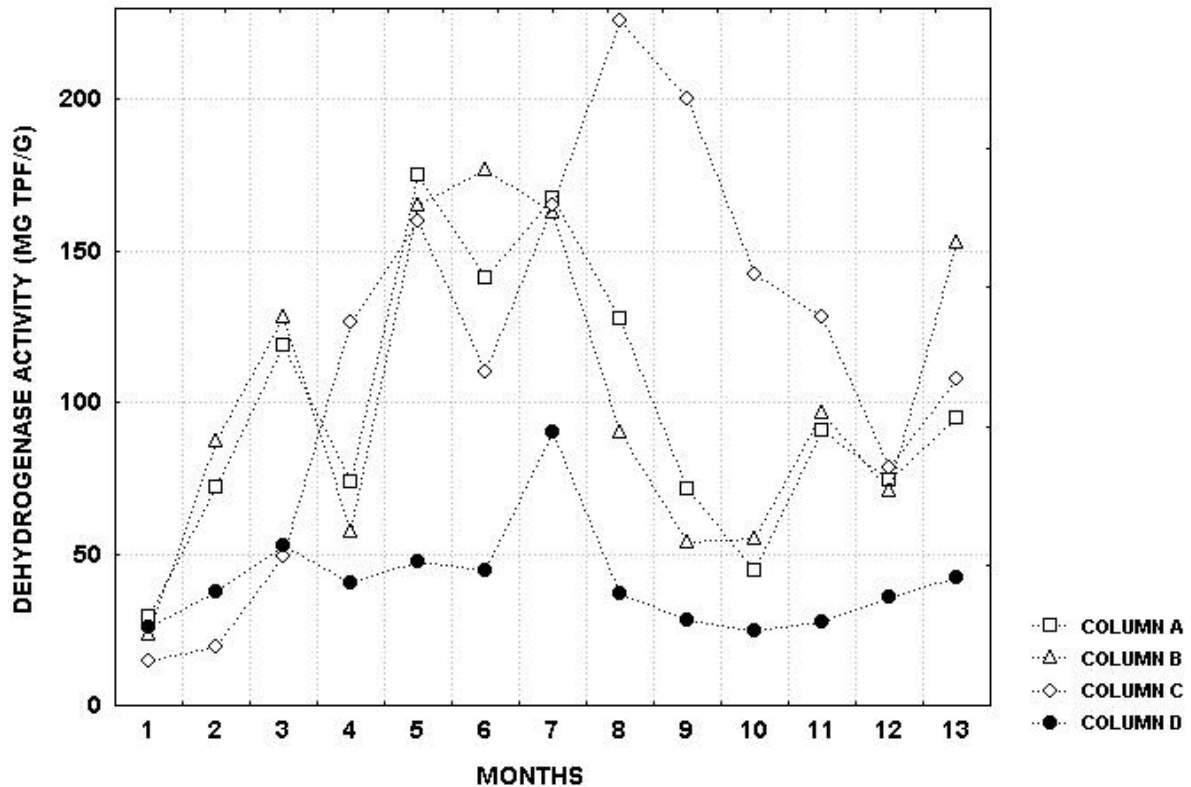


Fig. 1. The seasonal changes of dehydrogenase activity in the columns (layer 3)

Results

Column experiment

Generally, the mean total bacterial and fungal numbers in layer 3 of the columns were extremely high (1.06×10^{11} and 3.78×10^9 bacterial cells or fungal propagules per gram). The majority of microorganisms counted with epifluorescence methods were actively engaged in the biodegradation of petroleum hydrocarbons at 20 and 37°C. The mean total bacterial numbers were significantly higher in the actively aerated columns A, B and C than in the passively aerated column D. The highest mean fungal number was observed in column A while the lowest was counted in column C. Thus, the mean fungal numbers were irrespective of the column aeration systems. However, the clearest differences between the actively and passively aerated columns were observed for the mean dehydrogenase activities. The activities for columns A, B

and C were 97.2, 99.5 and 102.1 mg TPF/g, respectively. The activity in column D was only 38.8 mg TPF/g.

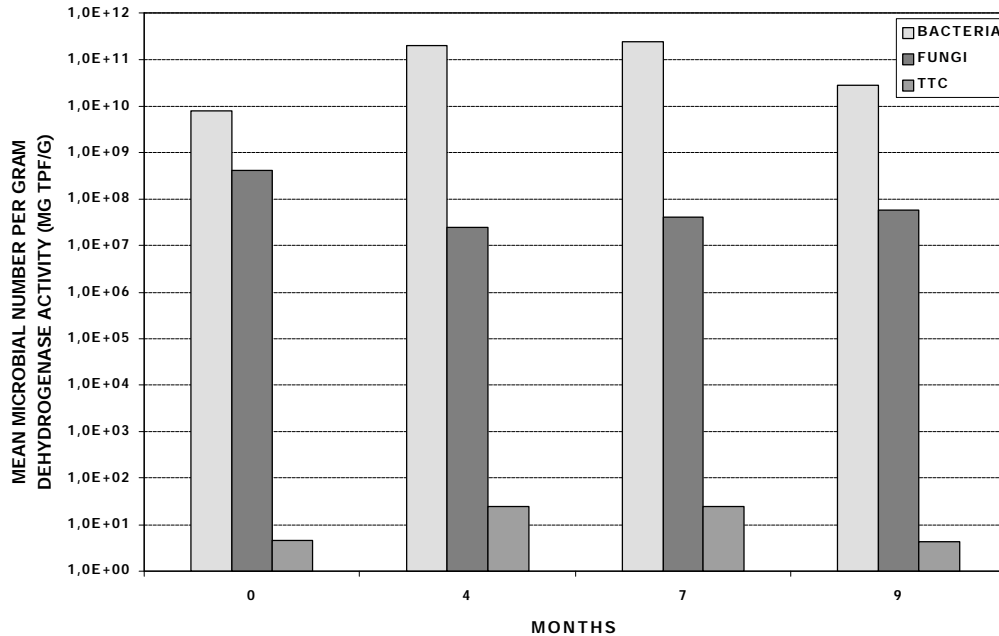


Fig. 2. The seasonal changes of microbiological parameters in the biopile

Bacterial and fungal numbers displayed high fluctuations during the column experiment, with no essential differences observed between the columns. In contrast, the columns considerably differed in the seasonal changes of dehydrogenase activity. The activity increased in all columns to the 5-8th month of the experiment. The highest maximal activity was noticed in column C in the 8th month and the lowest was in column D in the 7th month. The activities decreased during the final stage of the experiment. The seasonal changes in the dehydrogenase activities in columns are illustrated in Fig. 1.

The beginning TPH concentration in the treated soil was 37.98 g/kg. The TPH concentrations in layer 3 increased in all columns up to the 2nd month of the experiment. Slow TPH decrease was observed during the course of the experiment (after the 2nd month). The TPH reductions (calculated from the differences in the TPH concentrations between the 2nd and 13th month) in the

actively aerated columns A, B and C were 74.79, 63.99 and 68.01%, respectively. In the passively aerated column D, the TPH reduction was much lower (35.96%). Similar reductions were observed for TPOC.

Table 1. The mean TPH and TPOC reductions during the 9-month soil bioremediation in the biopile

BIOPILE ZONES	REDUCTION (%)	
	TPH	TPOC
	56,43	49,00
SHALLOW	65,71	56,83
PASSIVE	74,52	41,91
ACTIVE	50,94	68,29
GENERAL	59,50	51,60

Biopile

Generally, the mean total bacterial and fungal numbers in the biopile were high (1.19×10^{11} and 1.21×10^8 bacterial cells or fungal propagules per gram). Most of the microorganisms were able to degrade petroleum hydrocarbons at 20 and 37°C. The deep and shallow soil layers as well as the actively and passively aerated zones of the biopile did not statistically differ in the mean numbers of fungi and bacteria. In contrast, the differences in the mean dehydrogenase activities were statistically important. In the deep layer, the mean dehydrogenase activity was 2.79 times higher than in the shallow layer (21.14 and 7.56 mg TPF/g). In the actively aerated zone, the mean dehydrogenase activity was 1.81 times higher than in the passively aerated zone (18.73 and 10.34 mg TPF/g).

The seasonal changes in microbiological parameters during bioremediation are illustrated in Fig. 2. The total bacterial number and dehydrogenase activity increased up to the 7th month and decreased in the 9th

month of the bioremediation process. The negative correlation between the bacterial and fungal numbers was observed in the biopile.

At the beginning of the experiment, the mean TPH and TPOC concentrations in the biopile were 27.42 and 38.58 g/kg. The values of TPH and TPOC reductions in the biopile during the 9-month bioremediation period are presented in Table 1. In the shallow layer, the reductions were higher than in the deep layer. The passively aerated zone showed higher TPH reduction than the actively aerated zone. In contrast, the TPOC reduction in the passively aerated zone was lower than in the actively aerated zone.

Conclusions

During the microbiological studies, several important observations have been made and conclusions drawn:

- ◆ Bacterial populations played a crucial role in the biodegradation of petroleum contaminants in the subsurface soil of the columns and the biopile at the refinery.
- ◆ Fungal populations were much more actively engaged in the biodegradation process in the surface soil (not presented data).
- ◆ Most of the microorganisms present in the soil under bioremediation (both in shallow and deep layer) were able to degrade petroleum hydrocarbons at 20 and 37°C.
- ◆ The antagonism between petroleum-degrading bacteria and fungi seems to exist in the soil under examination.
- ◆ Soil aeration was a critical factor determining the rate of microbial activity and the contribution of microbial groups (bacteria, fungi) to the bioremediation process.

- ◆ Among the microbiological parameters measured, dehydrogenase activity was the best indicator of microbiological changes in the soil under bioremediation.
- ◆ The mean dehydrogenase activity in the biopile was much lower than the dehydrogenase activity in the columns.

References

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