O33

REAL-SCALE BIOPILE REMEDIATION OF A KEROSENE-CONTAMINATED SOIL: MICROBIAL DIVERSITY AND GEOCHEMICAL CHARACTERIZATION

José R. GALLEGO[1], Isabel MORI[1], Demelza MENÉNDEZ-VEGA[1], Ana I. PELÁEZ[1] and Jesús SÁNCHEZ[1]

1. Grupo de Biotecnología Ambiental, Instituto Univ. de Biotecnología de Asturias (IUBA), Universidad de Oviedo, C/Julián Clavería S/N, 33006- Oviedo (Spain). jgallego@uniovi.es

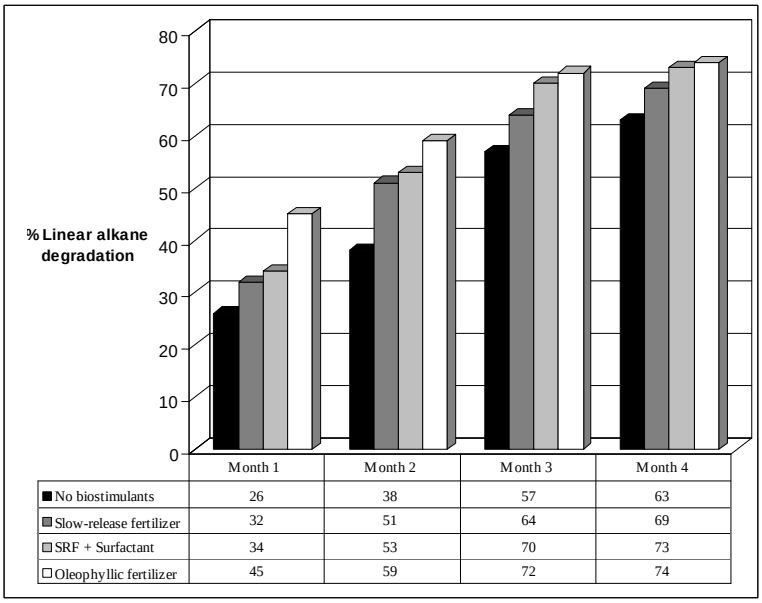
Biopiles technology involves heaping contaminated soils and stimulating aerobic microbial activity through the aeration and/or addition of nutrients, surfactants, moisture and other amendments. Biopiles, like landfarming treatments, have been proven effective in reducing concentrations of the constituents of petroleum products though volatilization and biodegradation. However, general knowledge about biopiles design and efficiency can be increased by detailed studies of microbial processes taking place within the treatment (Chaîneau et al., 2004). Therefore, in this work we selected a soil affected by historical kerosene spills within the last 20 years that had generated an average TPH (Total Petroleum Hydrocarbons) content of 4.000 mg/kg. After a biotreatability study, several biopiles (see below) were on-site disposed for four months to remediate pollution; microbial and geochemical effects of the biodegradation extents were monitored.

Initially, agronomic and geochemical analyses of the soil were performed in order to determine feasibility of the treatment. Results revealed moderate weathering of the hydrocarbons, low initial concentrations of nitrogen and phosphorus, and the presence of appropriate microbial populations. Additional specific microbiological studies were carried out by cultivation of representative soil samples in a synthetic medium for selective enrichment, using kerosene as sole carbon source. The occurrence of several degrading microorganisms, able to attack both aliphatic and aromatic compounds in kerosene, was detected; subsequently, the most abundant of them were isolated, and identified after sequence analysis of 16S bacterial ribosomal DNA. Moreover, confocal laser scanning microscopy of these bacteria, stained with fluorescent indicators, revealed emulsion effects of the hydrocarbons and, interestingly, one of them (*Pseudomonas sp.)* showed the capability of producing a biosurfactant.

Given that the biotreatability study was successful, four different real-scale biopiles (80 m long x 5 m width x 80 cm depth) were implemented on-site. In short, all the treatments had in common frequency of tilling and watering in order to hold moisture content and oxygenation, while they were differenced in the dosages and types of biostimulants: i) no biostimulant , ii) slow-release fertilizer (SRF) to achieve a C:N:P ratio of 100:10:1 surfactant,

iii) SRF + surfactant, iv) oleophilic fertilizer. Monitoring of microbial diversity and activity were monthly done by direct plate counts and isolation of the predominant degrading microorganisms. These strains are now being compared with those isolated in the previous biotreatability study, with the final objective of understanding the evolution of microbial populations under different biostimulation conditions.

Chemical determinations were performed trying to distinguish between volatilization and biodegradation. In this sense, usually C_{17}/Pristane and C_{18}/Phytane ratios are used to determine the advances of the biodegradation, given the differential biodegradability of linear alkanes with respect to the isoprenoids (Gallego et al., 2006). However, kerosene has negligible contents of C_{16}^{+} compounds and, therefore, the use of other isoprenoids such as Farnesane (2,6,10-trimethyl-dodecane) is a feasible alternative. As a result, we have defined a Biotransformation Index for Kerosene (BIK) using n-C_{13} and n-C_{14} abundances, with respect to Farnesane abundance. The use of this index to monitor biodegradation in the biopiles is shown in Figure 1.



	Month 1	Month 2	Month 3	Month 4
No biostimulants	26	38	57	63
Slow-release fertilizer	32	51	64	69
SRF + Surfactant	34	53	70	73
Oleophyllic fertilizer	45	59	72	74

Figure 1. Biotransformation indices of linear alkanes (GC-MS) obtained in monthly sampling after the beginning of the biopile treatments (errors are within 5%). All along the treatment, linear alkane degradation was greater in the biopile where the oleophilic fertilizer was applied, although results in the four biopiles were convergent after four months.

REFERENCES

Chaîneau C.H, Yepremian C., Vidalie J.F., Ducreux J. and Ballerini D. (2003) Bioremediation of a crude oil-polluted soil: biodegradation, leaching and toxicity assessments. Water & Air & Soil Pollution 144, 419-440.

Gallego J.R., García-Martínez M.J., Llamas J.F., Belloch C., Peláez A.I. and Sánchez, J. (2006) Biodegradation of oil tank bottom sludge using microbial consortia. Biodegradation (in press).