BIOREMEDIAN TREATABILITY AND FEASIBILITY STUDIES AT A POLISH PETROLEUM REFINERY

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Abstract

Bioremediation is one of the most attractive technologies for remediation of environments contaminated with petroleum products. Treatability and feasibility studies are essential to the successful implementation of bioremediation, either *in situ* or *ex situ*. As part of the bioremediation demonstration at a petroleum refinery in Poland, a treatability and feasibility study was performed under laboratory and field conditions. The laboratory studies examined different soil matrices, and different amendments with appropriate controls. Samples were monitored for changes or differences in contaminant, nutrient, microorganisms, and end product concentrations. Biodegradation rates were measured by biomass increases, carbon dioxide production, and oxygen consumption.

Introduction

The contamination of soils and aquifers with crude petroleum and its products is an important environmental problem. Enhanced bioremediation has been shown to be an efficient technology for the cleanup of petroleum contaminated soil and ground water [1-3]. The contaminated environments can be remediated by using different techniques, including physical containment, *in situ* or in place treatment with the addition of chemicals and/or microorganisms, and *ex situ* treatment by using physical, chemical or biological processes. The degradation capacities of indigenous microorganisms can often be stimulated with simple environmental injections. For a successful bioremediation, the hydrology, chemistry and microbiology of the soil and aquifer has to be examined. The basic steps of an *in situ* bioremediation process are: (1) site investigation, (2) microbial degradation study (treatability and feasibility study), (3) system design, (4) operation, and (5) monitoring.

Combined chemical and microbiological investigations are a prerequisite for hazard assessment and the prediction of success of *in situ* bioremediation. Although microbial transformation of many hydrocarbons has been shown in laboratory experiments, the degradation of various organic compounds in the environment may be limited by many environmental factors, including nutrient concentrations and decreased bioavailability caused by strong adsorption of hydrophobic compounds to the soil matrix. In general, it is difficult to stimulate the complex environmental media in laboratory experiments and, therefore, extrapolations from the laboratory to the environment must be regarded carefully.

The present study is a part of the bioremediation demonstration at the Czechowice-Dziedzice Refinery (Upper Silesia Region of Poland). Enhanced on-site treatment of soil is planned for the emptied oil waste basin of the Refinery. This laboratory pre-investigation is to examine the biodegradation rates of soil and water petroleum contaminants, including recalcitrant compounds (asphaltenes, resins), and to determine the optimal conditions for microbial growth. The study will also demonstrate the presence of microorganisms capable of degrading these contaminants. The extreme conditions of the environment at this place (low pH, high clay) could present difficulties for biostimulation. The data obtained will be extrapolated to the on-site situation. The presentation includes the preliminary results of the treatability study.

Material and methods

Water and three kinds of petroleum contaminated soil (litter, organic soil and clay) intended for on-site bioremediation were taken for experimentation from the petroleum waste basins in the refinery. Water samples were tested in 1 and 2 L Virtis bioreactors whereas soil samples were assayed in 100 and 500 mL Erlenmeyer flasks. The following water experiments were performed: aerated and unaerated water, aerated water amended with 10, 25, 50 and 100% of minimal salts medium (MSM), aerated water amended with dolomite (11.1%; w/v), and aerated water with the addition of formaldehyde (1.5%; v/v). The composition of MSM added to the water was that of Woroszylova & Dianova [4]. The following soil experiments were carried out: soil, dolomite and compost alone; soil, dolomite and compost with the addition of formaldehyde (14%; v/w), soil amended with compost and dolomite (7:2:1), and amended soil with formaldehyde (14%; v/w). Formaldehyde was added to the samples in order to eliminate the activity of microorganisms.

The incubation of water and soil samples was carried out at room temperature for 3 weeks. Changes in CO_2 production, redox potential, pH, temperature and microbial density were controlled during the experiments. The CO_2 production was measured by means of the titration method. The pH and redox changes were controlled potentiometrically with an Orion pH/mV-meter (model 250A). In the water experiments, the oxygen concentration and uptake were measured using an Orion O_2-meter (model 830).

The quantification of the total microbial population and that capable of decomposing crude petroleum in water and soils was carried out by1186Xeiables (plate and MPN) and direct count epifluorescence methods (AODC, FITC, DAPI, Calcafluor White). In the plate method, different media such as Standard Methods Agar (SMA), 1%SMA, Sabouraud glucose agar (SGA), diluted SGA (1:10) + salts [5], mineral agar by Woroszylova & Dianova [4] saturated with petroleum vapours were used. In the MPN method, nutrient broth and mineral medium with two drops of filtered crude petroleum were applied. Water and soil samples were diluted with phosphate buffer. In order to compare the results of different experiments, microbial generation time was calculated. The bacteria and fungi growing on the mineral medium amended with petroleum were isolated and examined for petroleum degradation in pure culture. The microbiological methods were those of the US EPA Microbiological Methods for Monitoring the Environment [6] and Westinghouse Savannah River Technology Center [2].

At the starting and ending points of the experiments, concentrations of total petroleum hydrocarbons (TPH), total petroleum organic carbon (TPOC), BTEX, naphta ether extracts (NEE) and salts (NO_3, NO_2, NH_4, PO_4) were measured. TPH and TPOC analyses were performed with the IR spectrophotometric method using carbon tetrachloride for extraction. BTEXs were measured by the GC/MS technique. NEE and salts concentrations were determined using the methods described in the Polish Standards. PAHs were also quantified by the HPLC method with fluorescence detection but the results of PAH analyses will be presented later. On the basis of the results obtained, substrate percent mass losses were calculated.

Results

The highest TPH, TPOC and NEE contamination was observed in the litter while the highest total BTEX concentration was noted in the clay (Tab. 1). In the organic soil and water, the contamination was much smaller. The water and clay were extremely acid whereas the organic soil showed the neutral reaction.

Tab. 1. Selected physico-chemical characteristics of water, soils, dolomite and compost used in the treatibility study

Material	Water	Litter	Organic soil	Clay	Dolomite	Compost
TPH[1]	5.93	245.2	1.34	42.0	0.04	0.12
TPOC[1]	12.73	272.8	2.9	53.2	0.04	0.12
BTEX[2]	2.7	292.12	91.71	1098.47	16.77	12.89
NEE[3]	ND	28.11	0.71	9.98	0.51	0.01
pH	2.69	4.7	6.9	3.0	8.93	6.75

[1] - mg/L or g/kg   ND - not done
[2] - µg/L or µg/kg
[3] - %

In the water experiments, the CO_2 production (2.71-7.6 $\mu LCO_2/L/h$) and oxygen uptake (0.024-0.104 $mgO_2/L/h$) were too low to be good indicators of hydrocarbon biodegradation processes. In the aerated water, the dissolved oxygen concentration ranged between 6.4-8.4 mgO_2/L whereas in the unaerated the range was 4.5-6.9 mgO_2/L. Three paths of microbiological and physico-chemical changes were observed in the bioreactors. In the aerated and unamended water, pH increased from 2.69 to over 4 during 21 days of the experiment and the abundant growth of filamentous iron bacteria (*Clonothrix sp.*) and algae appeared. In the aerated and amended water, no significant pH changes were noted but the abundant growth of mobile rod bacteria and yeasts was noticed. These microorganisms were also found to be good degraders of petroleum in pure culture. The dolomite caused the pH increase (up to 7.95) and eliminated the yeasts from the water.

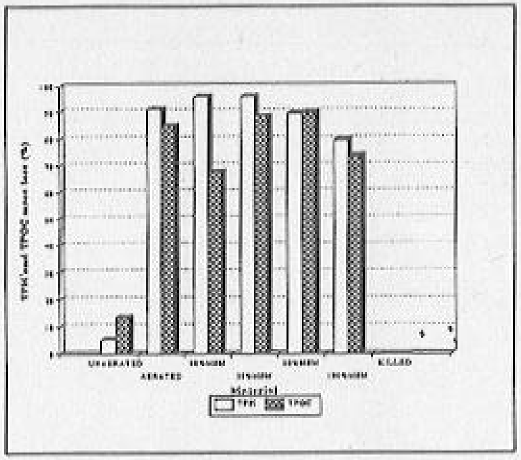

Figure 1. TPH and TPOC mass losses in water experiments

Except for the unaerated and unamended water, the TPH and TPOC percent mass losses were high (Fig. 1). The BTEX mass losses were also high (up to 78.2%). However, the low initial water TPH, TPOC and total BTEX concentrations must be emphasized. Since formaldehyde efficiently killed the aqueous micro-organisms and, since the BTEX loss in the killed water was 57.2%, it can be supposed that, volatilization was a significant BTEX reduction factor. The amendment of water with high quantities of salts or dolomite markedly shortened the microbial generation time (up to 100x).

The amendment of soils with dolomite and compost caused the pH increase up to 6.9, 7.4 and 6.7 in the litter, organic soil and clay, respectively. It also stimulated the CO_2 production during the first 7-10 days of the experiments. The highest CO_2 production was observed in the amended clay (61.2 $\mu LCO_2/100g/h$). From 51.7 to 73.2% of the CO_2 originated from the dolomite as a product of the reaction with acid soil. The remaining CO_2 was the product of microbial activity. The intensive growth of microorganisms was found to occur in both the soil alone and the soil amended with dolomite and compost. The humidity of the soils and amended soils ranged between 22-34%. The majority of microorganisms were killed in the soils and mixtures treated with formaldehyde.

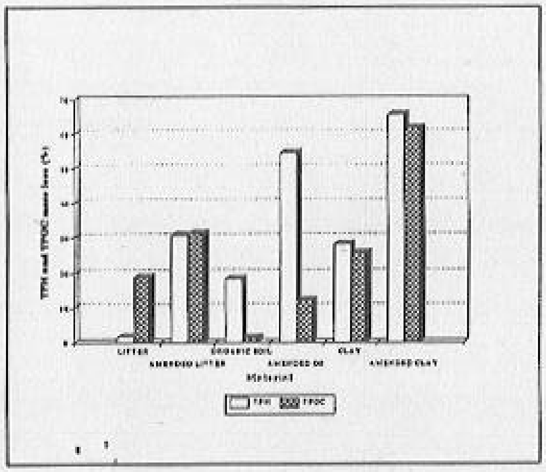

Figure 2. TPH and TPOC percent mass losses in soil experiments

The soils and soil mixtures with dolomite and compost differred in the TPH and TPOC percent mass losses (Fig. 2). The highest hydrocarbon mass losses were noticed in the clay and amended clay. The addition of dolomite and compost to the litter caused the high increase of the TPH mass loss but a low TPOC mass loss increase was obtained. In the organic soil and amended organic soil, the TPOC mass losses were low but the addition of dolomite and compost to the soil markedly increased the TPH mass loss. The total BTEX mass losses ranged between 37-93% and were caused chiefly by the volatilization process. The NEE mass losses were 53.5 and 77.7% in the litter and clay, respectively. No NEE mass loss was detected in the organic soil. The amendment of the soils with dolomite and compost caused no or small increase of the NEE mass losses. The mass losses were 0, 63 and 95.6% in the amended organic soil, litter and clay, respectively. In the soils and mixtures treated with formaldehyde, the NEE mass losses ranged between 0-4.6%.

Conclusions

1. In general, the high percent mass losses of petroleum hydrocarbons were noted in the water, soils and mixtures examined. The greatest contribution of petroleum degraders to the hydrocarbon mass losses was observed in the naphta ether extract fraction (oils) while the lowest occurred in the total BTEX fraction. The BTEX mass losses were caused principally by the volatilization process.
2. The petroleum-hydrocarbon mass losses depended on the specified material and the treatment method. In the water, the reduction of the hydrocarbons was highest. In the

litter, due to the high concentration of recalcitrant compounds (asphaltenes, resins), the reduction was lowest. The aeration and the addition of salts, dolomite and compost to the water and soils intensified the microbial activity and the degradation of hydrocarbons.

3. Petroleum hydrocarbons and their products were degraded by the microbial communities of bacteria, yeasts and filamentous fungi. Numerous yeasts were the active degraders of hydrocarbons at low pH.
4. These studies have demonstrated that, nutrient and pH amendments of the water and soil will stimulate indigenous microorganisms to degrade the contaminants present at the Czechowice-Dziedzice refinery.

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