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# Laboratory-Scale Bioremediation Experiments on Diesel and Polycyclic Aromatic Hydrocarbons Contaminated Soils

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**Abstract** - Laboratory investigations were performed to estimate the potential of bioslurry for bioremediation of PAHs and TPH-contaminated soil of Azimabad region in the south of Tehran refinery plant. The soil slurry-Sequencing Batch Reactor (SS-SBR) consisted of covered 8L Plexiglas vessel with a working volume of 6L. The reactor was equipped with a 400 rpm speed mixer. Oxygen was supplied through a fine-bubble diffuser at the bottom. A granular activated carbon trap as used to collect volatile organics in effluent air, fill period was relatively instantaneous, and Draw period lasted approximately 1 minute so the recent period comprised essentially the entire cycle time. In draw period a fraction of slurry (10%) was removed from period a fraction of slurry (10%) was removed from the SS-SBR weekly and it replaced with untreated slurry. This volumetric replacement volume of slurry (the recycle fraction) remained in the reactor to provide acclimated microorganism for the next batch of untreated slurry.

The soil used in slurry studies was poorly graded sand with clay (SP-SC) which contaminated with approximately 67500 mg/kg TPH and 500mg/kg PAHS and passed through a sieve with an opening diameter of 0.5mm tap water was added to produce solids concentration of 10% (0.1kg dry soil/l slurry). Ammonias nitrate and phosphate were added to provide a C: N: P ratio of approximately 60:2:1 process performance .The TPH concentrations were determined by Gas chromatography with Flame Ionization Detector (GC-FID) after ultrasonic extraction the PAHS concentrations were determined with both of High performance liquid chromatography (HPLC) an GC-FID after silica-gel clean up. The results have shown high overall removal efficiency for TPH close to 96%. whereas PAHs were not detected at the end of each cycles.

**Keywords** : *Bioremediation, PAHs, TPH, SS-SBR.*

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# Laboratory-Scale Bioremediation Experiments on Diesel and Polycyclic Aromatic Hydrocarbons Contaminated Soils

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## I. INTRODUCTION

Bioremediation of contaminated soils offers a number of advantages over conventional treatments on the basis of its environmental friendliness and low costs. The interest in this technology has increased over the last few years (USEPA 2001).

Slurry-phase bioremediation technology is applicable for treating soils and sludge contaminated with hazardous wastes and it is a cost-effective

alternative to cumbersome and often less effective treatment methods such as solid phase remediation (VenkataMohan et al., 2004, in press; Boopathy, 2000; Rahman et al., 2003; Bento et al., 2005; Sarma et al., 2006; Ramakrishna et al., 2006). The bioslurry treatment method has several advantages in that an optimal environment for degradation of the organic contaminants can be maintained with a high degree of reliability. Biological reactions can proceed at accelerated rates in a slurry system because of contact between contaminants and microorganisms, effective mixing and maintenance of high bacterial population, which enhance the degradation rate. (USEPA, 1993). Bioslurry reactors can be operated under aerobic or anaerobic or anoxic conditions (Zappi et al., 1996). Reduction in bioslurry reactors is initially postulated to be a consequence of the (potentially) limiting mass transfer rate i.e., from the soil to the aqueous phase, as it is assumed that microflora could only utilize substrates dissolved in aqueous phase.

Petroleum products such as diesel fuel, heavy oil, gasoline, fuel residues, and mineral oil are common soil contaminants. remediation of oil-contaminated soils to eliminate oily odors and oil. Im on runo. water and in groundwater is required.

The biodegradation of oils in a slurry-phase bioreactor (SPB) has a higher degradation rate than other biological treatment methods (US EPA, 1990; Puskas et al., 1995). Various modes of SPB operation have been tested in laboratories and pilot-scale plants, and one of the most common and best performing modes involves a soil slurry-sequencing batch reactor (SS-SBR).

The fate of polycyclic aromatic hydrocarbons (PAHs) in nature is of great environmental concern due to their toxic, mutagenic and carcinogenic properties (Haeseler et al., 1999). Their environmental importance led the US Environmental Protection Agency (US-EPA) to identify 16 unsubstituted PAHs as priority pollutants, 8 of which are possible human carcinogens (Menzi et al., 1992). The potential use of microorganisms to clean up contaminated soil, sediments and water can provide efficient, inexpensive and environmentally safe cleanup of the waste material (Bishop, 1998).

In this study, we adopted a different method for enhancing the biodegradation of oil: we transferred the oils from the fine soil and increased contact between the

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microorganisms and the oil. The microorganisms must be separated from the soil so that both can be reused.

The use of a support medium bearing the microorganisms also permitted the separation of the microorganisms from the soil after purification.

This paper describes the treatment of a soil with TPH and PAH contamination in laboratory bio-slurry reactor and describe the ability of that to remove oil and polycyclic aromatic hydrocarbons.

## II. SOIL SAMPLES

The soil used in slurry studies was poorly graded sand with clay (SP-SC) which contaminated with approximately 67500 mg/kg TPH and 500mg/kg PAHS and passed through a sieve with an opening diameter of 0.5mm (Table 1)

## III. BIOREACTOR

The study was carried out in the thermostated chamber (at  $21 \pm 0.3$  °C) using a 8 l pyrex-made reactor with a 17.1 cm diameter and 47.0 cm height (Fig. 1), upper closed with a stainless steel flange to ensure the seal. The reactor operating level was set at 41.0 cm (leaving 6 cm of freeboard); thus the reactor working volume was 6 l. The reactor was equipped with a DO probe (cell OX325, WTW), a pH probe (Mettler Toledo) and a magnetic stirrer with a variable speed impeller, working at 400 rpm and placed in the flange central position. Oxygen was supplied by an air flow of 50 N l h<sup>-1</sup> and distributed through a fine bubble diffuser at the bottom of the reactor. The air flow was forced to pass through a humidifier

Before entering the reactor, in order to minimize evaporation. Off gases firstly were piped to an air-cooled condenser and then collected to an amberlite resin trap (XAD2 Supelco) to catch volatile organic compounds. Peristaltic pumps (Cellai 503U) were used for fill and draw operations.

## IV. THE FEED SLURRY

Before using, the soil was passed through a 0.5mm sieve. The sieved fraction was partially air-dried and was stored at 4°C to maintain biological activity. Prior to using in bioslurry experiments, the samples were spiked with a known concentration of PAHs including Naphthalene, Phenanthrene and Pyrene (750 mg/kg) dissolved in dichloromethane. Tap water was added to produce a solids concentration of 10% (0.1 kg dry soil/L slurry). The Mineral Salt Medium (MSM) provide a C:N:P ratio of approximately 60:2:1 by adding ammonia, nitrate and phosphate as NH<sub>4</sub>Cl, KNO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub>. Sodium sulphate (Na<sub>2</sub>SO<sub>4</sub>) and Magnesium sulphate heptahydrate (MgSO<sub>4</sub>.7H<sub>2</sub>O) were added with 2000 mg/L and 200 mg/L concentrations respectively. Since the samples were collected from an aged contaminated site, the endogenous microorganisms were supposed to degrade contaminants and therefore no adapted

microorganism addition (bioaugmentation) was performed.

## V. EXPERIMENTAL DESIGN

6 L of prepared slurry was fed to reactor through the feed reservoir by gravity. The reactor was operated in sequencing batch mode. Fill period was relatively instantaneous, and Draw period lasted approximately 1 minute so the React period comprised essentially the entire cycle time. In draw period a fraction of slurry (10%) was removed from the SS-SBR weekly and it replaced with untreated slurry. This volumetric replacement strategy provided a 14 days solid retention time (SRT) and 70 days hydraulic retention time (HRT). A certain volume of slurry (the recycle fraction) remained in the reactor to provide acclimated microorganism for the next batch of untreated slurry.

## VI. ANALYSES OF CONTAMINANTS

### a) TPH analysis

At every sampling event, the soil slurry samples were collected from all the reactors for the total petroleum hydrocarbon (TPH) analysis. Diesel fuel components were extracted with dichloromethane and analyzed by gas chromatography as follows. Fifty ml of dichloromethane was mixed with 25 ml of soil slurry sample in an airtight vial. The slurry-solvent mixture was mixed for 10 min with a wrist-action shaker to partition the diesel fuel components to the solvent phase. A 10 ml portion of the solvent was then transferred into a clean glass-barrel syringe. The solvent was filtered through a 0.5 mm TeNon membrane filter (Millipore Millex-SR 0:5 μm /liter unit; Bedford, MA) to remove particulates and through a sodium sulfate cartridge to remove water. Finally, a 2-ml sample of the filtrate was collected for analysis.

A gas chromatograph (GC) (Hewlett-Packard model 5890 T gas chromatograph with autosampler model 7673; Palo Alto, CA) with a capillary column (Supelco PTE-5TM, 30 m, 0.32 mm diameter, 0.25-μm /lm thickness; Bellefont, PA) and a Name ionization detector was used to detect and quantify the TPH. The GC was standardized using standards of refined No. 2 diesel fuel. The GC was operated with the helium carrier gas flow rate at 19.0 cm/s, column inlet pressure at 5.7 psi, inlet temperature at 300°C, and an oven temperature program of 40 °C (held 13 min) to 260°C (held 10 min) at 8°C/min. The total area counts from TPH analyses were used to calculate the percent degradation of TPH under each condition compared to corresponding time zero samples under each condition.

### b) Pah Analysis And Clean-Up Of The Extracts

The extracts in all cases, were concentrated to 2–3 ml using a rotary evaporator and then to 1ml under a stream of nitrogen.

The extracts were passed through a mixed silica column to remove co-extractive substances. The column

was packed at the bottom with cotton wool and then filled with 15 g of activated silica. 1 cm of sodium rinsed with sulfate was added at the top of the column, the column was 30 ml of DCM and 30 ml of n-hexane.

## VII. RESULTS AND DISCUSSION

Slurry-phase biodegradation experiments were performed to investigate the remediation of PAH and TPH contaminated soil.

The SS-SBR was operated with a 14 days cycle. Hydraulic retention time of 60 days was achieved by replacing 900 mL of the slurry volume every two weeks. TPH concentration at the end of each cycle is shown in Figure 1. TPH concentrations were reduced from 67.5 g/kg in the feed slurry to 2.7g/kg after treatment in the SS-SBR. This represents a removal efficiency of 95% (Fig1). Rates of TPH removal in SS-SBR showed a considerable decrease at first two weeks of operation

Also Fig. 2 shows the decrease in time removal efficiency of TPH in slurry-phase bioreactor treatments with 60 days HRT of the SBR.

But the concentration of PAHs in the external sample could not be detected because it was very low.

## VIII. CONCLUSION

The analyzes show that SS-SBR have a good ability for removing the petroleum and PAHs contaminated Soil.

In this process at the end of 60 days HRT the concentration of TPH is decreased from 67500 mg/kg dry soil to 3003 mg/kg dry soil that have removal efficiency of 96%

Also the analyze of low concentration of PAHs with Clean-up of the extracts can not practicable.

Table 1 : Soil content

Soil constituent	Measured value
Solids concentration (%w/v)	10
Slurry diesel fuel (mg/kg)	67500
Naphthalene concentration (mg/kg)	250
Pyrene concentration (mg/kg)	250
pH	7.6
CFU (107/g soil)	9

## REFERENCES RÉFÉRENCES REFERENCIAS

- Hinchee and Olfenbuttel, 1991 R.E. Hinchee and R.F. Olfenbuttel, On-site bioreclamation: processes for xenobiotic and hydrocarbon treatment
- Freeman and Harris, 1995. H.M. Freeman and E.F. Harris , Composting of contaminated soil ... In: B.C. Alleman and A. Leeson, Editors

- Bioremediation of oil-contaminated desert soil: The Kuwaiti experience M. T. Balba, R. Al-Daher, N. Al-Awadhi H. Chino and H Tsuj
- Cerniglia, C.E., 1992. Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3, 351–368
- Bishop, D.F., 1998. Perspective on remediation and natural recovery of contaminated sediments. In: United States Environmental Protection Agency National Conference on Management and Treatment of Contaminated Sediments, EPA/ 625/R-98/001, 27–36.
- Bispo, A., Jourdain, M.J., Jauzein, M. , 1999. Toxicity and genotoxicity of industrial soils polluted by polycyclic aromatic hydrocarbons (PAHs). Organic Geochemistry 30, 947–952
- Cassidy D. P. and Irvine R. L. (2000b) The effect of operating conditions on the biodegradation of diesel fuel in soil slurry-SBRs. Water Science and Technology (inpress).
- U.S. Environmental Protection Agency (USEPA), EPA's Contaminated Sediment Management Strategy" EPA-823-F-98-004, April, 1998
- S.E. Herbes, Partitioning of polycyclic aromatic hydrocarbons between dissolved and particulate phases in natural waters, Water Res.11 (1977) 493–496.

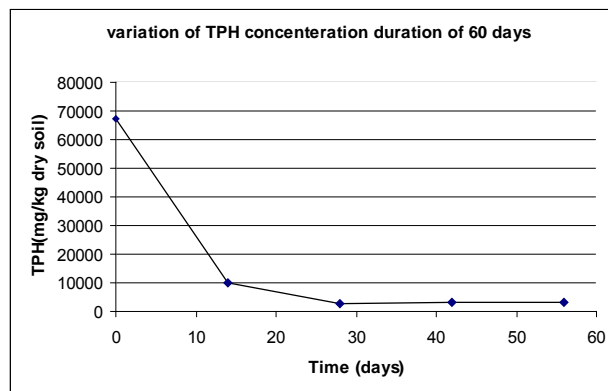


Figure 1 : Variation of TPH concentration duration of 60 days

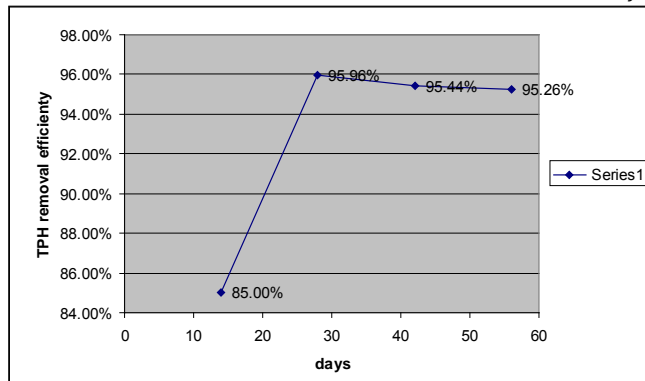


Figure 2 : TPH removal efficiency during 90 days HRT

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