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# Bench Scale Studies of the Soil Aeration Process for Bioremediation of Petroleum Hydrocarbons

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## ABSTRACT

An alternative to traditional hydrocarbon bioremediation is to pump air through unsaturated soils to create aerobic conditions and induce biodegradation. This study examines the effects of moisture and nutrient augmentation on biodegradation of petroleum hydrocarbons in aerated soils. Findings indicate that forced aeration, coupled with additions of nutrients and moisture, stimulate hydrocarbon-degrading microorganisms and present a feasible approach to bioremediation management.

**Index Entries:** Biodegradation; bioreclamation; bioremediation; petroleum hydrocarbons; soil aeration.

## INTRODUCTION

Traditional approaches to enhancing bioremediation of petroleum hydrocarbons involve injecting nutrients and an oxygen source or an alternative electron acceptor, such as nitrate, into contaminated soil. These systems are typically oxygen limited, and the rate of bioreclamation is dependent on delivering sufficient electron acceptor (*1,2*).

To fully mineralize a hydrocarbon, such as benzene or hexane, a mass ratio of oxygen to hydrocarbon of 3.1 or 3.5, respectively, is required. Assuming that soil contaminated with 10,000 mg/kg of benzene is to be treated, the mass of water required to deliver sufficient electron acceptor for complete biodegradation may be estimated as follows. At a density of

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1600 kg/m$^3$, 1.6 kg of hydrocarbon would be present in a cubic meter of soil. At a porosity of 0.30, under saturated conditions, approx 300 L of water would be present. Using the following oxygen concentrations: 8 mg/L for air-saturated water, 40 mg/L for pure oxygen-saturated water, and 250 mg/L for hydrogen peroxide at 500 mg/L, approx 2000, 400, or 70 pore volumes of water would be required to deliver sufficient oxygen to fully mineralize benzene.

A potentially promising alternative to traditional approaches is to pump air through unsaturated soils to create aerobic conditions and induce biodegradation. Ely and Heffner (3) observed that $CO_2$ was generated in such a soil venting system, indicating that fuel hydrocarbons do biodegrade in unsaturated soil. In addition, Woo and Coleman (4) have observed depressed oxygen concentrations in soil gas at fuel hydrocarbon contaminated sites, possibly indicating biological activity.

In a study of the effect of soil venting on *in situ* biodegradation at a jet fuel contaminated site at Hill Air Force Base, Utah, Hinchee et al. (5) found that by venting alone, with no nutrient or moisture regulation, approx 15% of the hydrocarbons (2200 kg) removed from unsaturated soil over a 70-d period was the result of biodegradation. The objective of the present study was to determine the effect of moisture and nutrient augmentation on biodegradation in aerated soil.

## METHODS

Soil samples were collected from two locations 210 m apart at the Hill Air Force Base, Utah. One location was uncontaminated and one was the site contaminated with JP-4 jet fuel described by Hinchee et al. (5). Soil samples were collected prior to any remedial efforts. The samples were collected using a hammer-driven split spoon sampler from depths up to 20 m below the land surface. Immediately after being brought to the surface, representative soil samples from specific depths were placed in sterile polyethylene bottles, sealed, and placed on ice. The samples were stored at 4°C and maintained at field-moist conditions prior to microbial enumeration.

Microbial characterization included an enumeration of total platable organisms on both nutrient agar (Difco) and a mineral salts agar with JP-4 as the sole carbon source. The mineral salts agar contained (g/L): 0.05 $KH_2PO_4$, 0.50 $NaNO_3$, 0.15 $MgSO_4 \cdot 7H_2O$, 0.05 $CaCl_2 \cdot 6H_2O$, 0.05 NaCl, and 0.01 $FeC_{13} \cdot H_2O$.

The enumeration was carried out in triplicate using a 10-fold sterile distilled water dilution method. Appropriate soil dilutions were pour-plated in the case of the nutrient agar (for total colony counts) and spread-plated in the case of the mineral salts agar plus JP-4 (for hydrocarbon degrader enumeration). After several days of dark incubation at 25°C, colonies were counted with a Quebec-lighted colony counter. The results are expressed as the mean number of colony-forming units per gram soil on a dry weight basis.

To determine the feasibility of engineering an increase in the biodegradation observed in the field study (*5*), a composite of Hill Air Force Base soil was augmented with nutrients and moisture. Fifteen soil columns were operated in the laboratory; 12 were treatment columns and three were sterilized controls. The columns were 30 cm long and 3.8 cm diameter. Each column contained 250 g (dry wt) of soil. The soil in the columns was a composite of the JP-4 contaminated soil samples collected from the Hill Air Force Base site. The concentration of fuel in the composited soil was adjusted to approx 5000 mg/kg by dosing with JP-4.

The experimental design included three soil moisture levels: 25, 50, and 75% of field capacity corresponding to 6.1, 12.2, and 18.3% soil moisture. The average soil moisture measured in the field was less than 6%. Duplicate columns at each moisture level were amended with a 2% (w/w) nutrient mixture. The nutrient mixture consisted of 50% ammonium chloride, 20% sodium phosphate, 17.5% sodium tripolyphosphate, and 12.5% monosodium phosphate. Three method blank columns (no soil) were included in the experimental design. The temperature was maintained at 25.5°C. At least weekly over the 48-d test period, the soil columns were weighed to determine moisture loss. Distilled water was added to maintain the columns at their initial moisture content.

Each soil column was sealed on both ends with a rubber stopper containing a glass tube. The glass tube entering the bottom of each column was fitted with a fritted glass diffuser and was attached by rubber tubing to the air inlet. The glass tube exiting the top of each column was connected by rubber tubing to individual alkali traps to capture evolved $CO_2$. The columns were positioned vertically, and the gas was introduced in an upflow manner. All columns were vented with humidified air, which was passed through alkali scrubbers to remove background $CO_2$. The off-gases from each column were passed through individual traps of 1 *N* NaOH.

## RESULTS AND DISCUSSION

The results of the soil enumerations are shown in Fig. 1. Microbial activity was observed at all test locations, but whereas activity was observed throughout the entire profile in the contaminated area, very little microbial activity was observed below 6 m at the uncontaminated background location. Hydrocarbon degraders were present throughout much of the contaminated site.

The cumulative evolution of $CO_2$ through 48 d for the column studies is shown in Fig. 2. Those columns receiving nutrients showed the greatest evolution of $CO_2$-C. Among the nutrient-treated soils, moisture con-

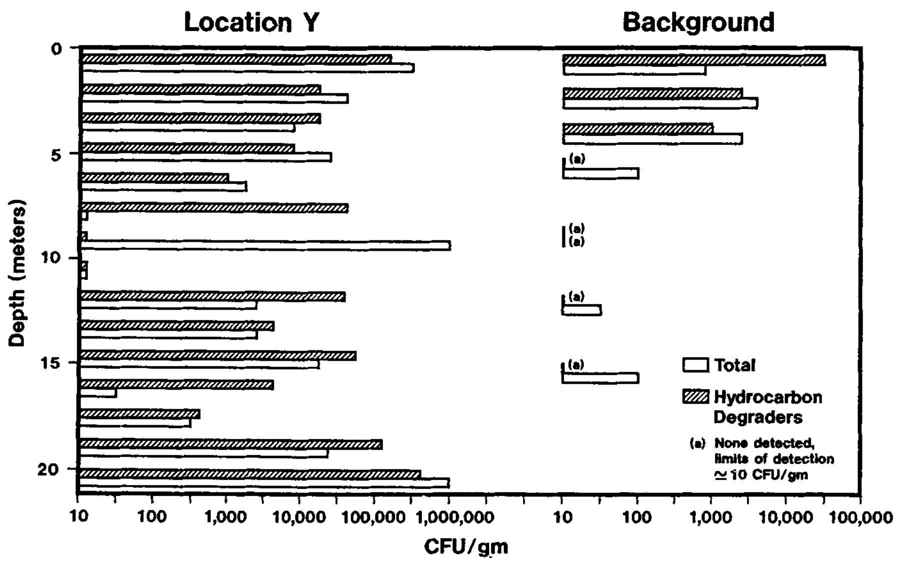


Fig. 1. Vertical distribution of total and hydrocarbon degrading microorganisms. (The lower detection limit was 10 CFU/gm.)

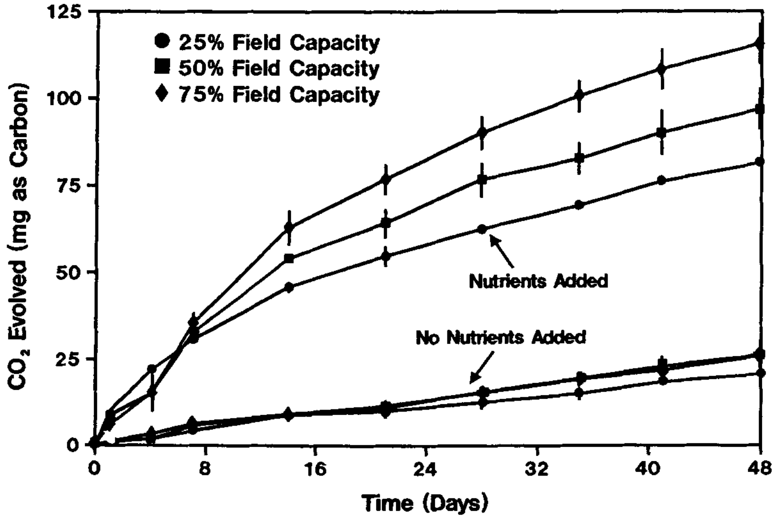


Fig. 2. Results of bench scale treatability studies with Hill AFB, Utah, soils. (The points are the means of two tests with the ends of the bars representing the range of results of each separate test.)

tent appears to be important, with the driest soils having the lowest rate of biodegradation and the wettest soils having the highest rate. Fig. 3 illustrates the results for the sterile control. This control received the 2% nutrient treatment and the moisture level was maintained at 50% of field capacity. As can be seen from Fig. 3, $CO_2$ evolution was substantially higher with the unsterilized biologically active treatment. The $CO_2$ evolved by the sterile control was thought to be of inorganic origin, and released

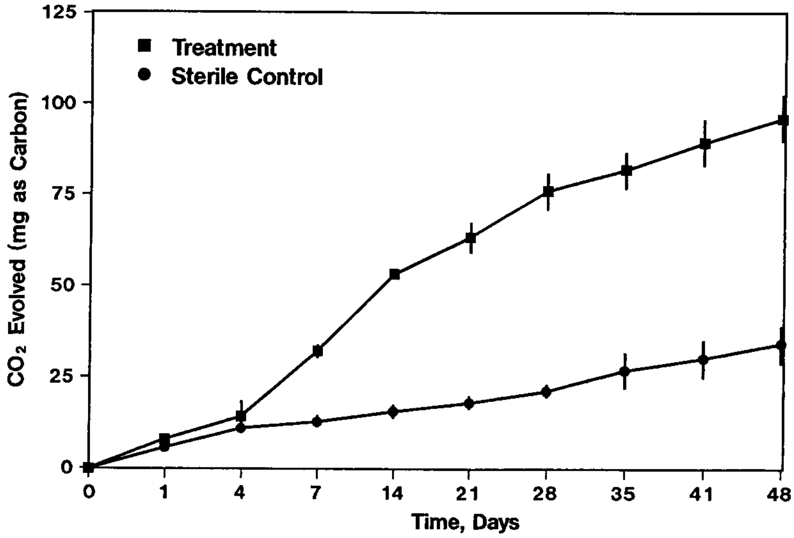


Fig. 3. Results of sterile control for bench scale treatability studies with Hill AFB, Utah, soils. (The points are the means of two tests with the ends of the bars representing the results of each separate test.)

by the soil or soil water during the course of the experiment. Plating of the sterile control soils after incubation revealed no viable organisms.

This study indicates that hydrocarbon-degrading microorganisms are present in unsaturated soil and that forced aeration, coupled with the addition of nutrients and moisture, stimulates degradation of hydrocarbon by those microorganisms. With the widespread application of forced air soil venting (*6*), the technology for aerating unsaturated soil exists. Management of soil and nutrient conditions to increase biodegradation associated with soil venting or management of a soil venting site to optimize biodegradation appears to be feasible.

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