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Monitoring of Bioventing Process for Diesel-Contaminated Soil by Dehydrogenase Activity, Microbial Counts and the Ratio of *n*-Alkane/Isoprenoid

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Abstract–Monitoring parameters were evaluated for a bioventing process that was designed to treat soils contaminated with diesel fuel. Statistical analyses were conducted to evaluate correlations between total petroleum hydrocarbon concentrations in the contaminated soil and physico-chemical parameters of soil such as microbial counts, dehydrogenase activity, and *n*-alkane/isoprenoid ratio. The correlation coefficients (r^2) obtained showed that TPH concentrations in the bioventing system were strongly correlated with dehydrogenase activity (DHA), total heterotrophic bacterial count, and hydrocarbon utilizing bacterial count. Thus, it was concluded that these parameters could useful monitoring parameters for soils contaminated with diesel fuel.

Key words: Bioventing, Monitoring, Total Petroleum Hydrocarbons (TPH), Dehydrogenase Activity, Microbial Counts, *n*-Alkane/Isoprenoid, Correlation

INTRODUCTION

Petroleum hydrocarbons are major contaminants at sites contaminated with diesel fuel, and certain microorganisms have activities in degrading petroleum hydrocarbons in soil environments. Hydrocarbon-degrading bacteria are generally distributed in most soil environments and they are rapidly enriched once exposed to diesel fuel. Bioventing is an *in situ* remedial technology that utilizes microorganisms *in situ* and aeration. It became a valuable alternative to physical and chemical treatment methods for contaminated soils due to its cost efficiency and the advantage of not producing secondary pollutants [Dupont, 1993; US EPA, 1995; Allard and Neilson, 1997]. The efficiency of the bioventing process relies on the type of contaminant, indigenous microbial populations, pH, water content, nutrient and oxygen. To accelerate biodegradation rate in the bioventing process by indigenous microorganisms, it is needed to optimize aeration rate, nutrients addition, pH, and temperature [Norris, 1994; Atlas and Bartha, 1992].

Effective monitoring is crucial in successful operation of the bioventing process. Several kinds of soil biological parameters such as microbial community, microbial counts, dehydrogenase activity (DHA), and oxygen consumption rate are known to provide information on microbial activities in bioventing processes [van Beelene and Doelman, 1997; Nocentitni et al., 2000; Hur and Park, 2003]. Dehyrogenase activities are not consistently correlated with microbial numbers and hydrocarbon degradation, but are widely used to reflect a broad range of soil microbial oxidative activities. The ratio of *n*-alkane/isoprenoid is also a useful parameter in monitoring of bioventing processes for petroleum-contaminated soils [Seklemova et al., 2001].

The goal of this research was to test the effectiveness of monitoring techniques for bioventing by investigating correlations between the concentration of total petroleum hydrocarbons (TPH) in soils and biological/chemical parameters such as microbial counts, dehydrogenase activity, and the ratio of *n*-alkane/isoprenoid. An optimum condition for nutrients and venting rate was also investigated for the bioventing process to treat a soil contaminated with a diesel fuel.

MATERIALS AND METHODS

1. Soil Characteristics

The soil used was obtained from a site in Geumjeong Mountain in Busan, Korea. The characteristics of the soil sample are shown in Table 1. The soil sample was artificially contaminated with diesel fuel to yield a concentration of approximately 10,000 mg TPH/kg soil. The contaminated sample was left outdoors for two weeks to allow substantial fraction of volatile organics to be removed.

2. Bioventing System

A schematic of the bioventing system with three borosilicate glass column reactors is presented in Fig. 1. A humidifier was used to maintain an appropriate level of moisture content in soil column reactors. The activated carbon trap was used to entrap volatilized hydrocarbons and to account for abiotic loss of the TPH. Each column reactor with 4.7-L working volume (H=600 mm, D=100 mm) was sealed to avoid environmental effects and was maintained at 25±2.5 °C in a thermostatic room.

The optimum condition for nutrients and moisture identified in the preliminary study was adopted in the current study. The mass ratio of TPH : N : P was maintained at 100 : 10 : 1 and the moisture content at 60-80% of the field capacity. Three different air flow

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Table 1. Characteristics of the soil sample

Texture	pH	Field capacity (%)	Porosity	Organic matter (%)	Particle density (g/mL)	Dry bulk density (g/mL)	N (g/kg)	P (g/kg)
sand 97.1%, silt 2.9%	6.8±0.3	16.7±0.5	48.6±1.6	2.27±0.1	2.22	1.20	0.01	0.22

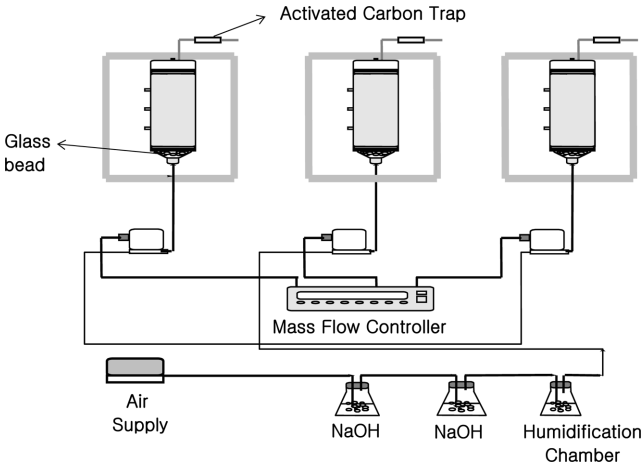


Fig. 1. Experimental set-up of lab-scale bioventing system with three column reactors.

rates evaluated were 5, 10, 20 mL/min.

3. Chemical and Biological Analyses

Soil samples were periodically collected from the column reactors and were analyzed for petroleum hydrocarbons (TPH, *n*-alkane (n-C_8H_{18}~n-$C_{40}H_{82}$), isoprenoid), microbial community and dehydrogenase activity. Petroleum hydrocarbons in soil samples were extracted with a soxhlet extractor and were measured by gas chromatography (HP 5890 Series II equipped with FID and a HP-5 capillary column (50 m×0.32 mm i.d.)).

Dehydrogenase activity (DHA) was determined by a modified spectrophotometric method according to Page [1982]. Dry soil sample of 5 g was treated with a solution of 2,3,5-triphenyltetrazolium chloride (TTC) in 0.1 M tris-buffer solution (pH 7.6) and was incubated for 24 hr at 37 °C. The reduced triphenylformanzan (TPF) formed was extracted with methanol and was quantified spectrophotometrically at 485 nm. All experiments were conducted in triplicate and the activity was expressed as mg TPF/g soil.

Microbial counts of total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) were carried out by using the agar plate microbe-counting method [Carter, 1993]. THB cultivation agar medium was composed of peptone (5 g), yeast extract (2.5 g), glucose (1 g), and agar (14 g) per liter of deionized water. HUB cultivation and counts were performed on a mineral salt medium (MSM) composed of KCl (0.7 g), KH_2PO_4 (2 g), Na_2HPO_4 (3 g), NH_4NO_3 (1 g), $MgSO_4$ (4 mg), $FeSO_4$ (0.2 mg), $MnCl_2$ (0.2 mg), $CaCl_2$ (0.2 mg) per liter of deionized water. All media were adjusted to pH 7.0 and were sterilized by autoclaving at 121 °C for 20 min. 10 ml of a phosphate buffered saline solution was added to 1 g of soil sample and the mixture was vortexed to detach the soil bacteria from soil particles. The buffer solution was then diluted ten times and 100 µl of the solution was spread on the nutrient or mineral agar plate. The plates were incubated at 25 °C for a week and the colonies formed on each plate were counted as CFU.

4. Data Analysis

To evaluate the effectiveness of the monitoring parameters, correlations of TPH concentrations with each parameter were investigated by a statistical program (SPSS, version 12.0). The following equation was used to obtain correlation coefficients:

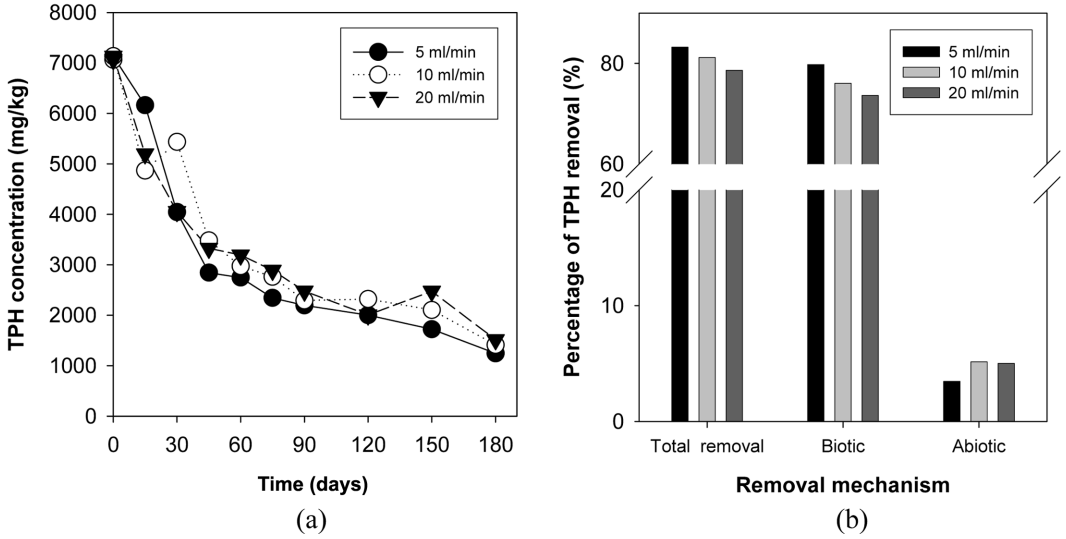


Fig. 2. TPH removal at different air flow rate conditions: (a) Changes in TPH concentration in the soil during the incubation period. (b) Evaluation of the biotic and abiotic portion of the TPH degradation during the incubation period.

$$r = \frac{S_{xy}}{S_x \cdot S_y} = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum(x_i - \bar{y})^2}\sqrt{\sum(y_i - \bar{y})^2}} \quad (1)$$

Where, r=sample correlation coefficient; S_{xy}=covariance; S_x and S_y= standard deviation, x_i and y_i=i^{th} value; $\bar{x}$ and $\bar{y}$ =mean value.

RESULTS AND DISCUSSION

1. Effect of Air Flow Rate on TPH Degradation

Fig. 2 shows time profiles of TPH concentration and the cumulative TPH removal due to biotic or abiotic processes at three different air flow rate conditions during 180 days. The decrease in TPH concentrations was initially rapid around day 45 and slowed down thereafter at three different experiments. This is probably due to rapid degradation of easily biodegradable *n*-alkanes of low molecular weights and subsequent retardation of the biodegradation reactions because of the remaining *n*-alkanes with higher carbon numbers (>C_{20}) and isoprenoids that are not readily biodegradable. This is supported by the separate analyses on petroleum hydrocarbons with lower carbon numbers (C_{10} to C_{25}) (data now shown) and is consistent with the observation by Margesin and Schinner [1997] and Balba [1998].

As seen in Fig. 2(a), the rates of TPH degradation were not substantially different at three different air flow rate conditions. First-order rate constants were 8.8×10^{-3} day^{-1} (r^2=0.91), 8.1×10^{-3} day^{-1} (r^2=0.92) and 7.3×10^{-3} day^{-1} (r^2=0.89) at 5, 10 and 20 mL/min air flow rate condition, respectively. Abiotic portion of the TPH removal accounted for 3.5%, 5.1%, 5.0% for 5, 10, 20 mL air flow rate condition, repectively, confirming that most TPH degration was attributable to biological reactions (Fig. 2(b)). Based on these results, the optimum air flow rate for the bioventing process was selected as 5 mL/min.

2. Microbial Counts versus TPH Concentrations

Fig. 3 shows changes in TPH concentration and microbial counts of total heterotrophic bacteria and hydrocarbon utilizing bacteria during the bioventing process at 5 mL/min air flow rate. Fig. 3 clearly shows that TPH concentrations are inversely proportional to the counts for total heterotrophic bacteria or hydrocarbon utilizing bacteria. A transition point that differentiates rapid degradation and slow degradation was chosen to be 75th day. The averge removal rate for TPH up to 75th day is 95.3 mg/kg/d, whereas the rate is 11.8 mg/kg/d after 75th day. These results imply that cell counts could be a monitoring parameter for bioventing processes for diesel-contaminated soils [Nocentitni et al., 2000].

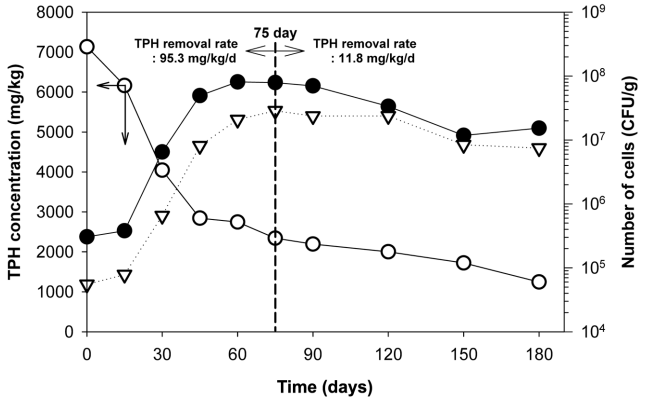


Fig. 3. Time profiles of TPH concentration and microbial counts (total heterotrophic bacteria and hydrocarbon utilizing bacteria) during the bioventing operation at 5 mL air flow rate condition: TPH concentration (○), total heterotrophic bacteria (●), hydrocarbon utilizing bacteria (▽).

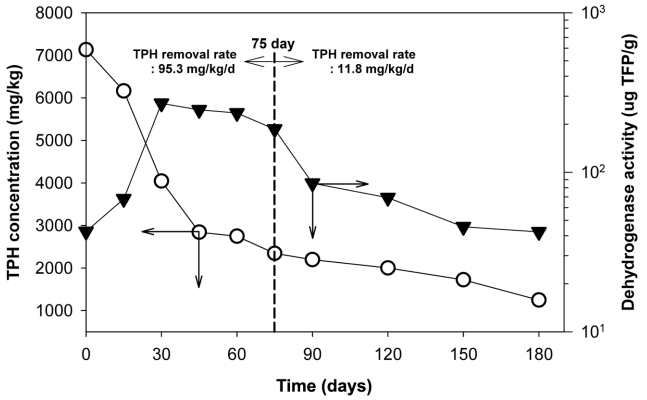


Fig. 4. Time profiles of TPH concentration and dehydrogenase activity during the bioventing operation at 5 mL air flow rate condition: TPH concentration (○), Dehydrogenase activity (▼).

3. Dehydrogenase Activity (DHA) versus TPH Concentration

Dehydrogenase activity was plotted against operating time together with TPH concentration in Fig. 4. The dehydrogenase activitiy was maximized on 30th day as 270 μg TPF/g soil and was maintained at similar values untill 75th day. After the transition point (75th day), the dehydrogenase activity decreased substantially as low as 40 μg TPF/g soil on 180th day. This low activity of dehydrogenase appears to be due to inhibition of intermediates, persistence of long chain *n*-alkanes and isoprenoid, and lacks in propagation factors [Margesin and Schinner, 1997]. The decrement of the dehydrogenase activity matches well with the slow degradation reactions for TPH, suggesting that dehydrogenase activity could be used as a monitoring parameter for the bioventing process [Margesin et al., 2000].

4. Ratio of *n*-Alkane/Isoprenoid Versus TPH Concentrations

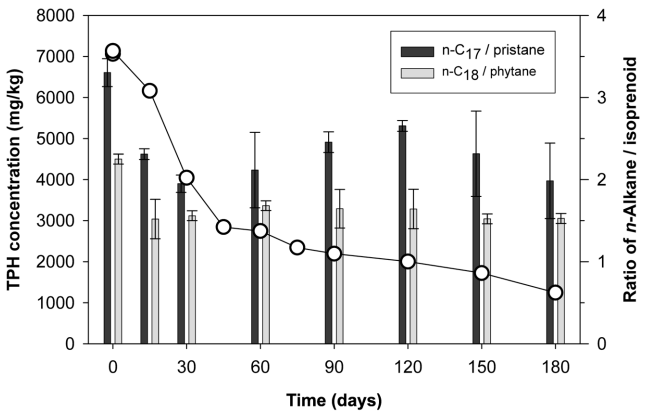


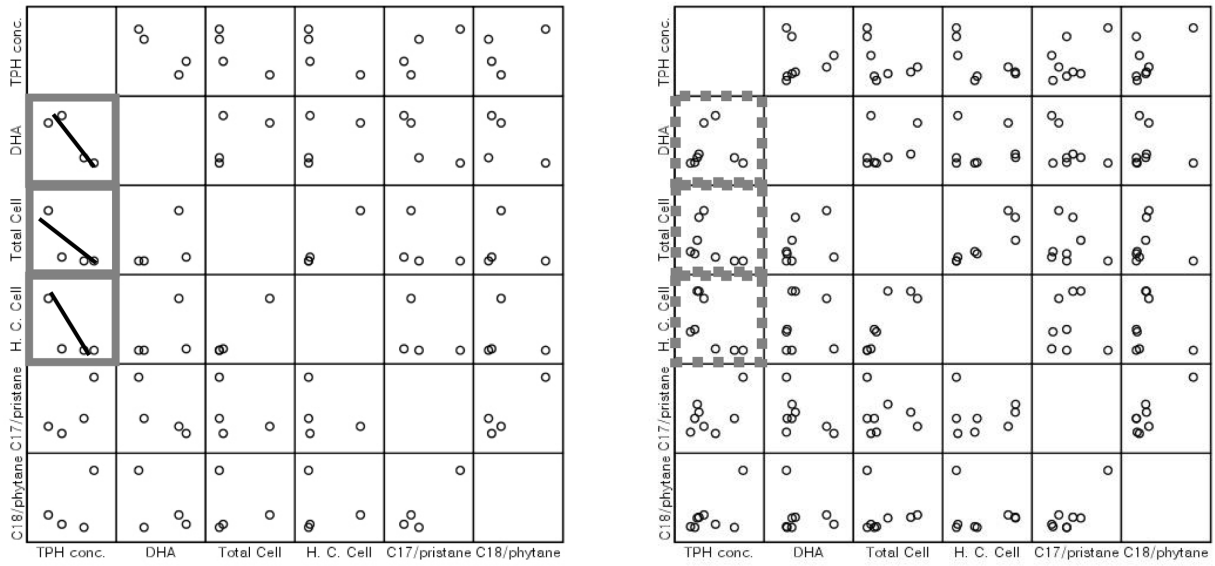
Fig. 5. Time profiles of TPH concentration and the ratio of *n*-alkane/isoprenoid (*n*-C17/pristane and *n*-C18/phytane) during the bioventing operation at 5 mL air flow rate condition. TPH concentration (○).

Fig. 5 shows changes in TPH concentrations and the ratio of *n*-alkane/isoprenoid during the bioventing process at 5 mL/min air flow rate condition. At commencement, the ratios of *n*-C_{17}/pristane and *n*-C_{18}/phytane were 3.3 and 2.5, respectively. The ratios were decreased during the first 30 days of operation and then fluctuated betweem 1.5 to 1.7 for *n*-C_{17}/pristane and between 2.1 to 2.8 for the case of *n*-C_{18}/phytane. Because the isoprenoid is not readily biodegradable, it is generally expected that the ratio of *n*-alkane/isoprenoid would be reduced during the bioventing process. However, the results of this study are not consistent with a previous study [Korda et al., 1997]. Therefore, the ratio of *n*-alkane/isoprenoid could just be useful in the initial period of bioventing processes as a monitoring parameter [Lesson and Hichee, 1995].

5. Evaluation of the Monitoring Parameters

The correlations of TPH concentrations with dehydrogenase activity (DHA), total heterotrophic bacteria, hydrocarbon utilizing bacteria, and the ratio of *n*-alkane/isoprenoid are graphically expressed in Fig. 6 and are presented in terms of correlation coefficient in Table 3. Fig. 6(a) depicts the correlations between each parameter for the first 75 days, and Fig. 6(b) shows the correlations during the whole experimental period (180 days). Fig. 6(a) demonstrates that soil biological (total heterotrophic bacteria and hydrocarbon utilizing bacteria) and enzymatic (dehydrogenase activity) parameters corrleate with the profile of TPH concentrations significantly and negatively. However, for the whole period of the bioventing operation, non linear-correlations of each parameter with TPH concentrations are observed (Fig. 6(b)). This is due to the retardation of TPH degradation after 75^{th} day.

Table 2 numerically expresses the correlation matrix of TPH concentration with the soil biological parameters and the ratio of *n*-al-



(a) Correlations up to 75 days of operation (b) Correlations for the whole 180-d period.

Fig. 6. Diagrammatically expressed correlations of monitoring parameters with TPH degradation in bioventing column reactor at 5 mL/min air flow rate.

Table 2. Correlation matrix (correlations by r^2, significance levels) for TPH concentration with, DHA, total heterotrophic bacteria, hydrocarbon utilizing bacteria, and *n*-alkane/isoprenoid ratio during the bioventing operation

Parameter		TPH value	(DHA* value)$^{-1}$	(Total Cell**)$^{-1}$	(H.C. Cell***)$^{-1}$	*n*-C17/pristane	*n*-C18/phytane
TPH value	Correlation by r^2	1					
	Significance level	.					
(DHA value)$^{-1}$	Correlation by r^2	**0.848**	1				
	Significance level	**0.033**	.				
(Total Cell*)$^{-1}$	Correlation by r^2	**0.866**	0.528	1			
	Significance level	**0.026**	0.282	.			
(H.C. Cell**)$^{-1}$	Correlation by r^2	**0.776**	0.372	0.948	1		
	Significance level	**0.022**	0.467	0.004	.		
n-C17/pristane	Correlation by r^2	0.796	0.801	0.379	0.349	1	
	Significance level	0.204	0.199	0.621	0.651	.	
n-C18/phytane	Correlation by r^2	0.563	0.544	0.176	0.156	0.937	1
	Significance level	0.437	0.456	0.824	0.844	0.063	.

DHA*-Dehydrogenase activity; Total Cell**-Total Heterotrophic bacteria; H.C. Cell***-Hydrocarbon utilizing bacteria

kane/isoprenoid during the initial 75 days of operation. Correlation values (r^2) of TPH concentrations with DHA, total heterotrophic bacteria, and hydrocarbon utilizing bacteria were 0.848 ($P<0.05$), 0.866 ($P<0.05$) and 0.776 ($P<0.05$), respectively. These results suggest that the TPH degradation in bioventing processes for diesel-contanminated soils significantly correlates with the soil microbial parameters and that these parameters could be used to monitor the bioventing process. Monitoring of the bioventing process using the soil microbial counts and enzymetic parameter together would be advantageous because the quantification of viable soil microorganims is usually restricted to small parts of soil microorganisms [Schinner et al., 1996; van Beelen and Doelman, 1997].

CONCLUSIONS

Correlations between several biological/chemical parameters and the concentration of total petroleum hydrocarbons (TPH) were evaluated to test the effectiveness of several monitoring techniques for the bioventing process. Statistical analyses using a statitical package (SPSS version 12.0) showed that TPH concentrations in the bioventing process were strongly correlated with dehydrogenase activity (DHA), total heterotrophic bacterial count, and total hydrocarbon utilizing bacterial count with correlation coefficients (r^2) of 0.848, 0.866, 0.776, respectively. The ratio of *n*-alkane/isoprenoid was not significantly related to TPH concentrations. This research revealed that dehyrogenase activity and soil microbial counts could be used as useful monitoring parameters for bioventing processes for soils contaminated with diesel fuel.

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