TASK 1.15 – ENHANCED BIOREMEDIATION OF COAL - TAR-CONTAMINATED SOIL

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TASK 1.15 – ENHANCED BIOREMEDIATION OF COAL TAR-CONTAMINATED SOIL

1.0 BACKGROUND

The remediation of sites where soils have been contaminated with hydrophobic organic compounds is a major problem. This is especially true for manufactured gas plants and similar industries. Gasification of fossil fuels has results in the production of tars that contain polycyclic aromatic hydrocarbons (PAHs). PAHs are of concern because they are persistent in the environment and because many of them are carcinogenic. When soils contain very high concentrations of PAH and other tar components, it is generally most feasible to use a chemical/physical remediation technique such as incineration. However, when the contaminant concentrations are medium to low, the most inexpensive technology is generally biological treatment. Biological treatment is an environmentally acceptable method of remediation that is relatively low cost for many organic contaminants. PAH and similar hydrophobic compounds, however, present major challenges to the microbial methods. These problems can be broken down into two related areas: mass transfer and bioavailability.

The water solubility of most PAHs is very low, and they have very low vapor pressures. As a result, the diffusion of sorbed PAHs to a location where a biodegrading microorganism can encounter them is often limiting. Complicating this is that, especially in aged samples, the PAHs are bound so strongly to soil components, they are not available for the microorganisms. These two phenomena can be better understood by examining of a typical biodegradation curve such as might be observed for mixed PAHs. In Figure 1, there are three phases of the biodegradation process with respect to time. In the first phase, concentrations are changing only slowly as the microbes adapt to the conditions, grow to a larger population, and begin to biodegrade the PAH. The second phase is the phase of rapid, often logarithmic, biodegradation. The third and last phase is that period when available concentrations of PAHs are dropping so that the microorganisms cannot degrade them. The rate observed during the second phase is dependent on the size of the microbial population that can degrade the contaminants, the availability of the contaminants, and suitable nutrients (e.g., phosphorus) and electron acceptors (e.g., oxygen). When readily biodegradable contaminants are present, this phase is often very rapid. However, when any one of the above factors is limiting, a reduced rate is observed.

Studies where conditions have been optimized for mass transfer of hydrophobic compounds, such as by rapid mixing or by the addition of surfactants, have shown that these rates are generally much less than would be possible with higher mass transfer rates (mass transfer limited). Techniques to improve mass transfer would allow this phase to end more quickly, resulting in reduced costs. In the third phase, the availability of contaminants has dropped to near zero, and even with prolonged incubation, concentrations will not drop significantly. If the lower limit achievable in a particular soil is above the regulatory limit, the biological technology will not be suitable and a more expensive and disruptive technique (e.g., incineration) must be considered.

Figure 1. Total PAHs versus time for three phases of the biodegradation process.

Studies have shown that the concentration of contaminants in that third phase is on the soil and is removable by strong solvents. In fact, these contaminants can be removed by a strong solvent, and when these contaminants are added back to a soil containing a suitable biodegrading microbial population, they will be rapidly and nearly completely degraded. This indicates that although the strong solvent can solubilize the contaminants, the microbes are not able to encounter them. Other investigators have suggested that the contaminants are present in a physical or kinetic trap within the soil matrix. The Gas Research Institute (GRI) has been active in a program to investigate these bioavailability phenomena because of the great impact they have on these and related industries.

Techniques are needed to mobilize contaminants to improve their availability for biodegradation to lower the achievable remediation endpoint (Phase 3) and to improve mass transfer so that remediation goals can be achieved more quickly (Phase 2). One technique that has potential for both of these problems is the use of acoustic energy. The addition of energy is apparently necessary to overcome the binding energy of the contaminants and mobilize them. Acoustic energy has a history of use in the laboratory and in industry to make emulsions and solutions and to remove scale and fouling, among others. Weiss Associates of Emeryville, California, has conducted tests using lower-frequency acoustics to mobilize nonaqueous-phase liquids with good success. The use of lower frequency is important because, although ultrasound is more energetic, it is also dampened quickly in soils, greatly reducing the radius of influence and applicability. Lower-frequency acoustics have the potential to supply the required energy in a more cost-effective manner. In addition, research by Weiss Associates has shown that it is possible to steer the beam of sonic energy to the zones that contain the most contamination.

2.0 OBJECTIVE

Determine the ability of acoustic energy to improve bioavailability or improve mass transfer of PAHs from soil to water phases where microorganisms can access them.

3.0 STATEMENT OF WORK

To meet the objective, an experiment was designed whereby the biological remediation of a PAH-contaminated soil was compared in a series of bottles, some of which were treated with acoustic energy, and others which were not.

4.0 METHODS

4.1 Soil

The soil used in these experiments was obtained from a manufactured gas plant site in Des Moines, Iowa. This soil is also being used in an extensive investigation supported by GRI in the endpoints program. As such, the soil was well mixed and had been characterized by gas chromatography–mass spectrometry (GC–MS) prior to receipt. This soil had not been subjected to engineered biological or other type of treatment. Prior to its use in these experiments, the soil was stored in polyethylene zippered bags at 4°C.

4.2 Preparation of the Soil Slurries

Twenty-g aliquots of the Des Moines PAH-contaminated soil was added to 100-mL borosilicate serum bottles. Twenty mL of Organization for Economic Cooperation and Development (OECD) media, with the composition shown in Table 1, was added to each bottle. Ten μL of an aerobic culture, initiated with the same media and soil 2 weeks previously and maintained at room temperature on a gyratory shaker, was added as inoculum. The headspace of each bottle was replaced with pure oxygen, and the bottles were covered with Teflon-faced butyl-rubber septae and sealed with aluminum crimp seals. At random, one-half of the bottles were marked with yellow (treated) and the other half marked with blue (untreated) adhesive tape. The sealed bottles were placed into a specially built variable-speed rotator that was used to gently mix the samples in an end-over-end manner at approximately 6 rpm at room temperature. Approximately every other day, a pair of bottles, one of each color, was removed from the rotator, marked with the date and time, and stored at -20°C for analysis. To maintain an adequate supply of oxygen, when a vacuum was detected in samples being stored for analysis, additional oxygen was added to all remaining bottles using a syringe.

TABLE 1

Composition of OECD media (OECD, 1981)	
Component	Concentration, mg/L
KH_2PO_4	34
K_2HPO_4	21.8
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	22.5
CaCl_2	27.5
NH_4Cl	20
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.25
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	0.04
$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$	0.035
H_3BO_3	0.057
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.043
Yeast Extract	0.15
pH	7.0

4.3 Acoustic Treatment

A bench-scale acoustic treatment unit, built and supplied by Weiss Associates of Emeryville, California, was used. The acoustic table in the unit was modified to allow for a press, approximately 25 cm in diameter, to be added. Every day during the incubation period, the yellow-labeled bottles were fitted into the press and treated with acoustic energy at 670 Hz applied via a square wave for 10 minutes. After the daily acoustic treatment, samples were stored for analysis, if due on that day, and the remaining bottles were returned to the rotator for further incubation.

4.4 Extraction and Analysis

Hydrocarbon analysis was performed on the soil by extraction and gas chromatography. The extraction was performed by adding 20 mL of tetrahydrofuran and the internal standard, *o*-dichlorobenzene, directly to the serum bottle containing the soil–water mixture. The bottles were capped with Teflon-faced butyl-rubber septae, sealed with aluminum crimp seals, and mixed in an end-over-end fashion for 30 minutes. The mixture was allowed to settle, then 2 μL of the water–solvent supernatant was used for gas chromatography. The gas chromatograph used was a Shimadzu Model GC-17A with electronic pressure control. Detection was by flame ionization.

The separation was made with a DB-5 column 30-m long, with a 0.25-m inside diameter and a 0.25- μ m phase thickness. The injection mode was split with an 80% ratio at 275°C, with the detector at 300°C. Hydrogen was used as the carrier gas at 78 mL/min, and nitrogen was the makeup gas at 30 mL/min. The program began at 65°C for 1 minute, 25°C/minute to 140°C, then 6°C/minute to 290°C. The temperature was held at 290°C for 15 minutes for a total analysis time of 44 minutes. The concentration of naphthalene, fluorene, phenanthrene, pyrene, and benzo(a)pyrene was estimated from a standard curve prepared at concentrations from 2 to 50 mg/kg. The retention times, R^2 value for the least squares linear regression, are shown in Table 2. The total hydrocarbon amount was estimated by using the total chromatogram area minus the area of the solvents, the area of the knowns in Table 2, and the area of the internal standard. This remaining area was converted to a weight by using the calibration factor for naphthalene. At the termination of the experiment, after 48 days of incubation, three bottles of each treatment were harvested to provide an estimate of the variance at the end of the experiment.

TABLE 2

Analytes Used, Retention Time, and R^2 for GC-FID ¹ Analysis		
Analyte	Retention Time, min	R^2 for Regression
Naphthalene	4.3	0.991
Fluorene	8.4	0.994
Phenanthrene	11.2	0.996
Pyrene	16.3	0.996
Benzo(a)pyrene	26.4	0.986

¹ Flame ionization detection.

5.0 RESULTS

The change in concentrations of measured analytes and of the estimated total hydrocarbon are shown in Figures 2 through 7. These figures show that naphthalene was about 5 mg/kg and declined rapidly to about 0.3 mg/kg. Other analytes show a much more variable pattern and do not necessarily reflect a decline in concentration throughout the treatment period. Moreover, it is exceedingly difficult, given the high variability, to determine any differences between the control (untreated) and the acoustically treated samples. The initial and final mean concentrations and the standard deviations for them are shown in Table 3. These data show that the only significant difference between acoustically treated and untreated soils is for naphthalene, in which the untreated sample had a mean concentration of 0.27 mg/kg compared to the treated sample with a mean of 0.63 mg/kg. In fact, these differences may not be significant since these concentrations are near the limit of detection by this method. For all other analytes, the differences noted

Figure 2. Changes in naphthalene concentration for acoustically treated and untreated samples of PAH-contaminated soil during biodegradation.

Figure 3. Changes in fluorene concentration for acoustically treated and untreated samples of PAH-contaminated soil during biodegradation.

Figure 4. Changes in phenanthrene concentration for acoustically treated and untreated samples of PAH-contaminated soil during biodegradation.

Figure 5. Changes in pyrene concentration for acoustically treated and untreated samples of PAH-contaminated soil during biodegradation.

Figure 6. Changes in benzo(a)pyrene concentration for acoustically treated and untreated samples of PAH-contaminated soil during biodegradation.

Figure 7. Changes in total hydrocarbon concentration for acoustically treated and untreated samples of PAH-contaminated soil during biodegradation.

TABLE 3

Initial and Final Concentrations in Acoustically Treated and Untreated PAH-Contaminated Soil			
Analyte	Initial, mg/kg	Treated Final, mg/kg	Untreated Final, mg/kg
Naphthalene	5.1 ± 0.5	0.63 ± 0.48	0.27 ± 0.05
Fluorene	4.0 ± 0.2	9.1 ± 3.2	10.2 ± 3.5
Phenanthrene	6.6 ± 0.5	10.6 ± 4.9	16.4 ± 9.2
Pyrene	45.7 ± 6.3	59.2 ± 22.8	62.9 ± 18.2
Benzo(a)pyrene	34.8 ± 30.2	21.3 ± 7.3	21.6 ± 3.8
Total Hydrocarbon	5920 ± 5700	6460 ± 640	6330 ± 1300

between acoustically treated and untreated are explainable by the variance of the samples and analysis. In fact, it is noted that for most analytes, the exceptions being naphthalene and benzo(a)pyrene, the concentrations increased during treatment rather than decreased, as expected. However, during biodegradation, larger molecules are fragmented into smaller molecules. Thus an analyte could increase in concentration, even though the total amount of hydrocarbon, especially the more nonpolar and environmentally hazardous compounds, is decreasing. Alternatively, since the method of analysis used is GC-FID and retention time matching for identification of analytes, it is possible to misidentify. This is especially true when many smaller compounds are being generated during the biodegradation of the complex mixture shown here. In other words, it is likely that the data in Figures 2 through 7 and in Table 3 do not correctly represent what occurred in the samples. However, the data probably are sufficient to show that there appears to be no significant difference between acoustically treated and untreated samples.

The data are not sufficient or appropriate to estimate biodegradation rates. Thus the only comparisons between acoustically treated and untreated must be on the basis of initial versus final concentrations.

A number of factors, if changed, might have increased the ability to detect differences between the treatments or enhanced these potential differences. One of these is improvements in the analytical. Specific ion monitoring, as practiced in mass spectrometry, is likely to have provided better identification and subsequent quantitation of analytes. The use of a soil that had a different spectrum of nonpolar contaminants might have made it easier to detect differences in treatments. Finally, a 10-minute daily exposure to the acoustic energy was selected. It is possible that a longer dose, or even a higher energy dose, would have led to greater differences between treatments. The use of increased energy must be evaluated with caution, since increases in energy result in poorer propagation of the energy through the media.

6.0 CONCLUSIONS

Under the conditions used in these experiments, the use of low-level energy acoustic energy did not result in improvements in the biodegradation of PAHs in a PAH-contaminated soil compared to an untreated control. Expected impacts on biodegradation rates by the acoustic energy could not be evaluated as the data were not conducive to this determination. The acoustic energy was only supplied to the treated samples during 10 minutes per day (0.6944 % of a day). It is possible that using longer treatment times, more exposure to the acoustic energy, and alternate types of contamination might have been able to demonstrate the purported ability of acoustic energy to desorb nonpolar contaminants and improve their biodegradation rate and endpoint.

7.0 REFERENCES

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