***Paper 3C-09***, in: A.R. Gavaskar and A.S.C. Chen (Eds.), *Remediation of Chlorinated and Recalcitrant Compounds—2004.* Proceedings of the Fourth International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2004). ISBN 1-57477-145-0, published by Battelle Press, Columbus, OH, www.battelle.org/bookstore.

# BIOLOGICAL FEASIBILITY AND OPTIMIZATION OF BIOSPARGING AT A HYDROCARBON-CONTAMINATED SITE

***Jason G. Waudby*** and Yarrow M. Nelson, Ph.D. (ynelson@calpoly.edu)
(California Polytechnic State University, San Luis Obispo, California)

**ABSTRACT:** The purpose of this study was to identify any biological/chemical factors which may be limiting the biodegradation of total petroleum hydrocarbon (TPH) contaminants at a biosparge site located at a former oil field near Guadalupe, California. Laboratory experiments using a combination of respirometry and TPH analyses were conducted to determine if biodegradation of TPH at the site is limited by a lack of hydrocarbon-degrading microorganisms, depleted inorganic nutrient concentrations, insufficient dissolved oxygen supply, or the chemical composition of the partially biodegraded petroleum constituents in the groundwater. No increase in total $CO_2$ production was observed in samples with added nutrients, inoculum, or both, over the 28-day experiment. No significant TPH biodegradation benefit could be attributed to the addition of nutrients or inoculum indicating both were sufficiently available at the site. Decreasing dissolved oxygen (DO) concentration decreased short-term $CO_2$ production, but considerable $CO_2$ production was observed even in samples with DO concentrations as low as 0.5 mg/L. In a long-term experiment, TPH degradation rates decreased significantly after initial observed biodegradation.

## INTRODUCTION

Biosparging is an *in-situ* remediation technology used to biodegrade organic constituents dissolved in groundwater and adsorbed to soil within the saturated zone and capillary fringe. In biosparging, aerobic biodegradation of contaminants by indigenous microorganisms is stimulated by the injection of air (or oxygen) into the saturated zone. A horizontal biosparge system was installed as part of the Guadalupe Restoration Project (GRP) to stimulate the bioremediation of petroleum hydrocarbon-contaminated groundwater. Groundwater contamination at this site is a complex mixture of hydrocarbons that originated from accidental leakage of diesel-fuel-like mixtures used to dilute crude oil for transport (Haddard, 1996). After three years of biosparge operation, the concentrations of TPH in the groundwater had remained relatively unaffected by the biosparging. The current study was undertaken to identify any potential limitations of biodegradation due to nutrient availability, suitability of the microbial community, oxygen requirement, or chemical constituents.

Two primary factors may limit the effectiveness of biosparing: (1) the permeability of the soil at the contaminated site, and (2) the biodegradability of the contaminants (USEPA, 1994). The rate of oxygen transfer to the groundwater depends upon the soil permeability. Recent studies at the Guadalupe site indicated that oxygen transfer is sufficient (Coffey, 2003). Relatively small concentrations of DO can significantly stimulate hydrocarbon biodegradation. For instance, a study of *in-situ* biodegradation of MTBE in groundwater reported an 83% reduction of MTBE concentrations when groundwater DO levels were increased from < 0.2 to 2 mg/L (Landmeyer et al., 2001).

A greater concern, therefore, is the long-term biodegradability of the hydrocarbon contaminants under site conditions. The weathering or "aging" of hydrocarbons can reduce their availability to microorganisms and extend their life in the environment (Siddiqui and Adams, 2001). Inorganic nutrients [nitrogen (N), phosphorus (P) and sulfur (S)] are needed to support microbial cell growth and for the production of metabolic enzymes required for biodegradation (Suthersan, 1997).

The experiments described herein were performed to address the following questions: (1) Are sufficient hydrocarbon-degrading organisms present in groundwater at the site? (2) Is TPH degradation in this groundwater nutrient limited? (3) Are DO concentrations present in the groundwater sufficient for TPH biodegradation? (4) Does TPH biodegradation stop at a threshold TPH concentration? (5) What are the kinetics of TPH biodegradation under experimental conditions? These questions were addressed by performing a series of laboratory experiments using respirometry and TPH analyses of groundwater from the biosparge site. In Experiment 1 (EXP1), short-term respirometry and TPH measurements were used to examine nutrient and inoculum requirements for biodegradation at the site by measuring $CO_2$ production and TPH degradation. In respirometry experiments 2-4 the production of $CO_2$ was measured and compared for samples containing varied concentrations of DO. Finally, a long-term laboratory biosparge experiment was conducted to investigate the kinetics and long-term biodegradability of the petroleum hydrocarbons present in the site groundwater.

## MATERIALS AND METHODS

**Respirometer Experiments for Nutrients and Inoculum.** A respirometer experiment (EXP1) was initiated to evaluate the biosparge-mediated biodegradability of the contaminated groundwater at the biosparge site. The purpose of this experiment was to determine the respiration rate of unamended site groundwater and to see if nutrient (N, P, S) addition or inoculum addition would increase the respiration rate and/or TPH degradation rate. The respirometry experiments were conducted using a Micro Oxymax respirometer manufactured by Columbus Instruments, Inc., Columbus, Ohio. The Micro Oxymax system is a closed-circuit respirometer used to detect low levels of carbon dioxide evolution. Carbon dioxide production was measured on three-hour intervals over an experimental term of 28 days. TPH was analyzed only at the beginning and end of the experiment for each sample because of the sample volume required for TPH analysis. Initial unamended groundwater samples were also analyzed for inorganic nutrients. The respirometry experiment was performed using nine (9) sample bottles with contents as described in Table 1. Duplicate bottles were run for unamended, nutrient amended inoculated, and both. All bottles initially contained a total of 1.5 L of groundwater (D.I. water for control). Diluent-contaminated Guadalupe groundwater, used in a previous experiment, was added as inoculum at 10% of the total liquid volume (bottles 6-9). Ambient air (20.9% $O_2$) was used to refresh the headspace in the sample bottles for this respirometry experiment. The bottles were contained in a temperature controlled water bath at 19°C. All sample bottles were stirred with magnetic stirrers. The final concentration of DO was measured for each bottle at approximately 14°C.

**TABLE 1. A description of respirometry bottle contents for Experiment 1 (EXP1).**

| Bottle Number | Bottle Description and Contents |
|---|---|
| 1 | Control - D.I. Water |
| 2 | Unamended groundwater |
| 3 | Unamended groundwater |
| 4 | Groundwater plus added nutrients (6.5: 1.7: 1.7: 1.7 mg/L, N: P: K: S) |
| 5 | Groundwater plus added nutrients (6.5: 1.7: 1.7: 1.7 mg/L, N: P: K: S) |
| 6 | Groundwater plus added inoculum (10% inoculum by volume) |
| 7 | Groundwater plus added inoculum (10% inoculum by volume) |
| 8 | Groundwater plus added nutrients and inoculum (as above) |
| 9 | Groundwater plus added nutrients and inoculum (as above) |

Initial and final TPH concentrations were measured using GC/MS analysis by Zymax Envirotechnology, Inc. Aqueous samples were extracted using methylene chloride according to EPA Method 3510. The methylene chloride extracts were concentrated and analyzed by GC/MS according to modified EPA Method 8015. The TPH was quantified against diluent standards, over an analytical range of C8-C40 with a Practical Quantitation Limit (PQL) of 50 µg/L. Inorganic nutrients analyses were performed to quantify the initial content of macronutrients in the TB8 groundwater. The initial samples were analyzed for ammonia-N (EPA Method 350.3); nitrate-N (EPA Method 300.0); nitrite-N (EPA Method 300.0); ortho-phosphate (EPA Method 300.0); and sulfate (EPA Method 300.0).

**Description of Respirometer Experiments for DO Effect.** A series of three, 48-hour respirometer experiments were performed to investigate the effects of DO concentration on the rate of $CO_2$ production for site groundwater. Sample DO concentrations were manipulated using $O_2/N_2$ gas mixtures of varying concentrations for the refresh gas in the respirometer. Since the samples were stirred continuously, equilibrium was maintained resulting in controlled DO concentrations. The $O_2/N_2$ gas mixtures were prepared by filling two 85.7 L Tedlar® bags with $O_2/N_2$ mixtures from a Dasibi® gas calibrator Respirometry experiments were performed using 8.02, 1.00, and 0.25 percent $O_2$ in $N_2$ gas, for the refresh air supply. At the end of each run, the DO concentration in the sample waters was measured and the $CO_2$ production data was compiled. The average 48-hour $CO_2$ production rates for all four respirometer experiments (EXP1-EXP4) were then compared (including the unamended run described in Section 2 for the highest DO concentration).

**Long-Term Biodegradability Experiment.** A long-term laboratory experiment was conducted to investigate the kinetics of the long-term biosparge mediated biodegradation of contaminated groundwater from the biosparge site when ample DO concentrations are maintained. An additional objective of the experiment was to determine if biodegradation stops or slows down after initial biodegradation (all experiments described previously examined relatively short term degradation), and to determine at what concentration biodegradation slows down. Simulated distillation was determined from GC/MS analysis to examine the chemical composition of the partially biodegraded petroleum constituents in

the groundwater. To answer these questions, experimental chambers capable of containing large volumes of groundwater were required to accommodate regular TPH sampling and analyses. Two five-gallon glass carboy bottles initially containing 12 liters of diluent contaminated site groundwater (TPH concentrations of approximately 7000 µg/L) were subjected to specific environmental conditions. The two bottles were incubated at 19°C for several months. The bottles remained open to the atmosphere, and air was bubbled through the water using aquarium air pumps and four air stones (two each). Dissolved oxygen was monitored to ensure oxygen availability to biodegrading microbial flora present in groundwater from the biosparge site. No nutrient or microbial amendments were added during the majority of this experiment.

## RESULTS AND DISCUSSION

**Respirometer Experiments for Nutrients and Inoculum.** The initial TPH concentration of the two unamended groundwater samples was 7350 ± 71 µg/L, and the initial TPH concentration after adding ten percent inoculum water was 6800 ± 141µg/L. The TPH compounds present in all samples were limited to the C10-C32 hydrocarbon range. Simulated distillation of the TPH compounds contained in the water samples showed similar TPH carbon chain distributions for the initial samples (Table 2). The majority of TPH in the groundwater samples was in the $C_{14}$-$C_{24}$ range for both sample sets.

**TABLE 2. TPH Carbon chain distribution of initial groundwater samples.**

| Lab Number Description | 30408-1 (1) 10% Inoculum | 30408-2 (2) 10% Inoculum | 30408-3 (3) Unamended | 30408-4 (4) Unamended |
|---|---|---|---|---|
| C Range | Result (µg/L) | | | |
| C10-C12 | 91 | 83 | 104 | 109 |
| C12-C14 | 363 | 355 | 409 | 405 |
| C14-C16 | 1061 | 1076 | 1182 | 1138 |
| C16-C18 | 1530 | 1551 | 1633 | 1575 |
| C18-C20 | 1631 | 1666 | 1773 | 1739 |
| C20-C24 | 1544 | 1612 | 1701 | 1712 |
| C24-C28 | 392 | 442 | 468 | 484 |
| C28-C32 | 88 | 115 | 130 | 138 |
| C32-C36 | ND | ND | ND | ND |
| C36-C40 | ND | ND | ND | ND |
| Total | 6700 | 6900 | 7400 | 7300 |

Inorganic nutrient analysis of the groundwater samples showed total nitrogen levels of approximately 1 mg/L with the majority in the form of nitrate (Table 3). The concentration of sulfate in all four samples analyzed was 130 mg/L. Results for the orthophosphate analyses were non-detect (ND) for all four samples.

Hydrocarbon biodegradation was similar for groundwater samples with and without nutrients added and with and without inoculation (Figure 1). This suggests that nutrient ammendments and bioaugmentation are not necessary for hydrocarbon biodegradation at this site.

**TABLE 3. Inorganic nutrient analysis of TB8 groundwater**

| Constituent | Method | PQL (mg/L) | Nutrient Concentrations (mg/L) | | | |
|---|---|---|---|---|---|---|
| | | | (1) 10% Inoculum | (2) 10% Inoculum | (3) Unamended | (4) Unamended |
| Ammonia -N | EPA 350.3 | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 |
| Nitrate as N | EPA 300.0 | 0.5 | 0.7 | 0.9 | 1 | 1 |
| Nitrite as N | EPA 300.0 | 0.5 | ND | ND | ND | ND |
| Ortho-Phosphate | EPA 300.0 | 0.5 | ND | ND | ND | ND |
| Sulfate | EPA 300.0 | 1.0 | 130 | 130 | 130 | 130 |

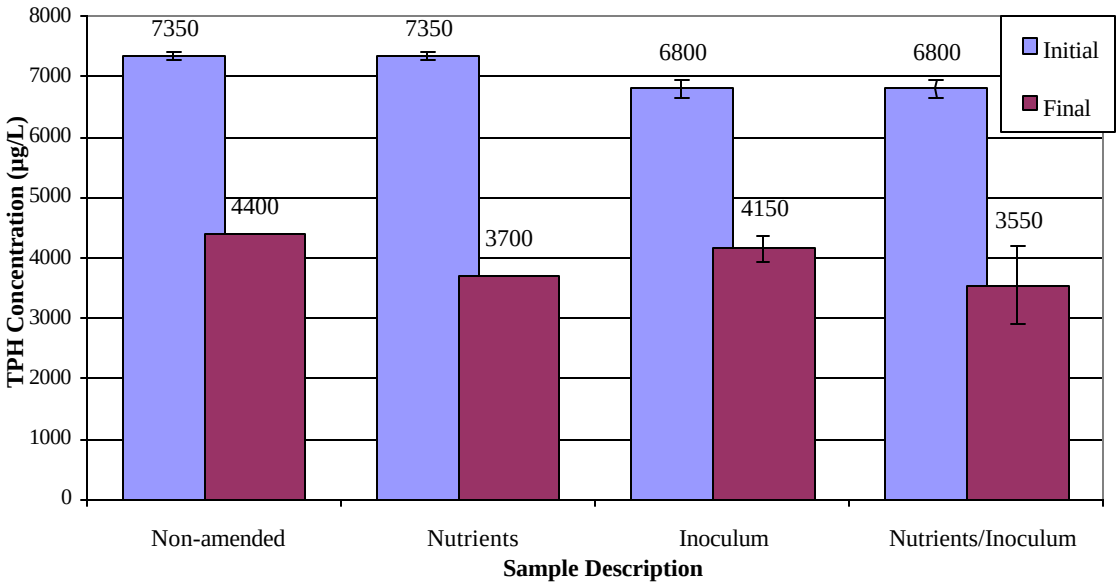


**FIGURE 1. Initial and final average TPH concentrations in Non-amended groundwater and groundwater with added nutrients, inoculum, or both.**

Respiration rates were also unaffected by nutrients or inoculum. The average $CO_2$ production observed for the unamended and the nutrient amended samples was 255.2 ± 3.9 µg/mL and 257.2 ± 6.2 µg/mL, respectively. The samples containing nutrients and inoculum produced an average of 232.2 ± 4.0 µg/mL $CO_2$. The samples containing inoculum only produced 218.8 ± 1.9 µg/mL. In comparison, the total background $CO_2$ produced in the sample control was 1.9 µg/mL.

**Results of the Effects of Dissolved Oxygen Experiments.** Decreasing the oxygen concentrations in the respirometry refresh air reduced the average DO levels in the sample waters as expected (Table 4). Reducing the dissolved oxygen in the sample waters significantly decreased the $CO_2$ production rates. It is interesting to note that significant biodegradation was observed even with only 0.5 mg/L dissolved oxygen (Table 4). Although these samples had only 5% as much DO as the samples purged with air, the $CO_2$ production rate was 50% of that of samples purged with air.

**TABLE 4. Oxygen percentages in respirometer refresh air, average dissolved oxygen concentrations, and corresponding 48-hour average carbon dioxide production values.**

| Experiment | Oxygen in Refresh Air (%) | Avg. Final DO (mg/L) | Standard Deviation (mg/L) | Avg. $CO_2$ Rate (µg/mL-hr) | Standard Deviation (µg/ml-hr) | Avg. 48-hour $CO_2$ (µg/mL) | Standard Deviation (µg/mL) |
|---|---|---|---|---|---|---|---|
| EXP1 | 20.9 | 10.7 | 0.1 | 2.6 | 0.1 | 122.8 | 2.4 |
| EXP2 | 8.02 | 3.9 | 0.2 | 2.0 | 0.1 | 94.4 | 6.5 |
| EXP3 | 1.00 | 1.9 | 0.1 | 1.5 | 0.0 | 74.3 | 0.9 |
| EXP4 | 0.25 | 0.5 | 0.1 | 1.3 | 0.0 | 64.7 | 0.6 |

**Long-Term Degradability Results.** The TPH data for the long-term experiment was somewhat sporadic, showing some initial biodegradation followed by very slow biodegradation (Figure 2). Most of the TPH degradation in Bottle 1 was observed in the first 20 days of the experiment.

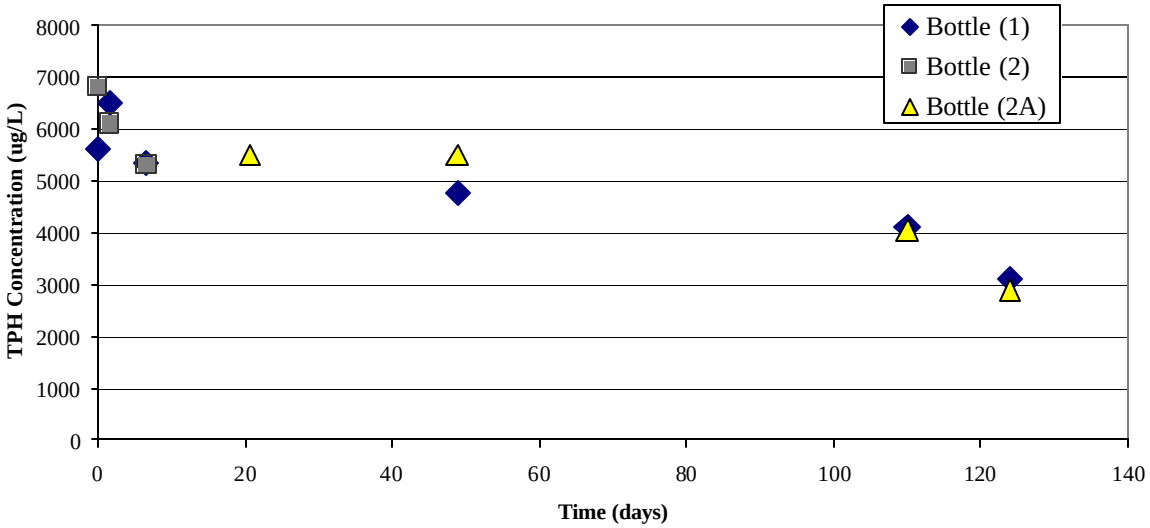


**FIGURE 2. TPH concentration vs. time for long-term degradability experiment.**

Inorganic nutrient analyses for N and P were non-detectable after approximately 117 days of incubation, suggesting a possible long-term nutrient limitation. However, nutrient addition after 124 days did not result in an increase in TPH biodegradation at the end of the long-term experiment.

TPH was biodegraded for all carbon chain lengths in the long-term experiment (Figure 3). Simulated distillation of TPH samples for Bottle 1 at 2, 49, and 124 days show hydrocarbons in the C10-C12 range were nearly reduced to zero by 124 days.

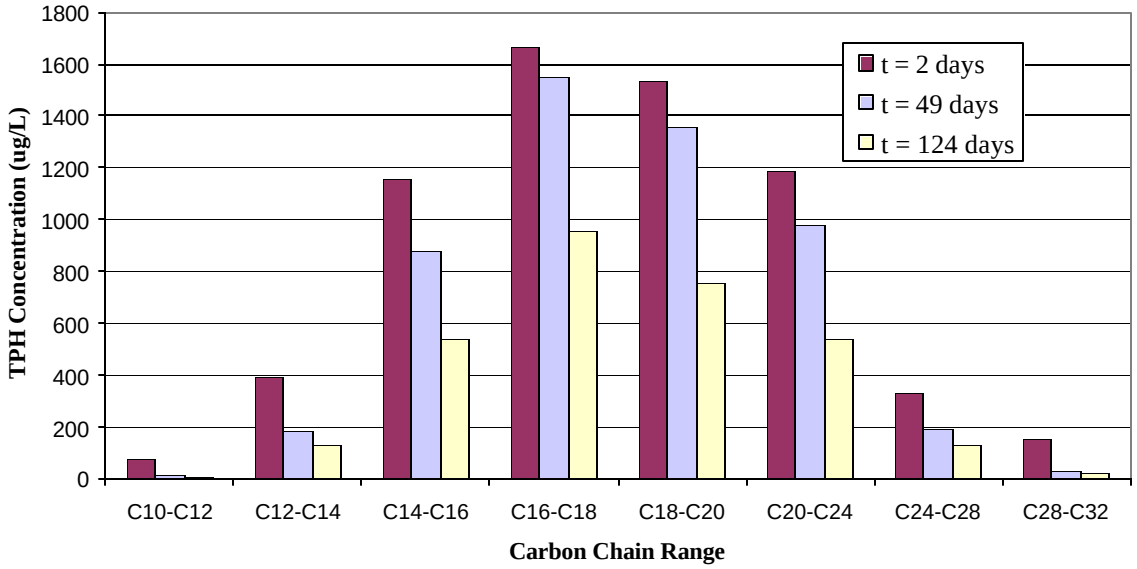


**FIGURE 3. Simulated distillation of TPH concentrations in samples of experimental Bottle 1 groundwater at 2, 49, and 124 days.**

## CONCLUSIONS

Considerable carbon dioxide ($CO_2$) production and concomitant TPH loss was observed in non-amended groundwater samples from the TB8 biosparge site, indicating hydrocarbon-degrading microorganisms were present in the TB8 groundwater. Nutrient addition (N, P, K, & S) increased TPH degradation rates only slightly for both inoculated and non-inoculated groundwater (over 28 days of EXP1), but this increase was not statistically significant. Nutrient analyses suggested that sufficient nitrogen was available in the short-term groundwater experiments, but ortho-phosphate (P) was not detectable in the groundwater samples. This lack of phosphate could limit the biodegradation potential of TPH in this groundwater. Since the phosphate detection limit was 0.5 mg/L, and phosphate concentrations <0.5 mg/L could be sufficient for enhanced hydrocarbon biodegradation, phosphate should be measured with a more sensitive method. At the end of the long-term experiment, inorganic nutrient analysis at 117.4 days resulted in non-detect readings for both nitrogen and ortho-phosphate. Thus, the long-term biodegradation of hydrocarbons in the groundwater could be limited by a lack of inorganic nutrients.

Respirometry experiments showed significant biodegradation at dissolved oxygen concentrations as low as 0.5 mg/L. Respiration rates increased with increasing dissolved oxygen concentration.

During the long-term biodegradability experiment, the initial biodegradation of TPH compounds was relatively rapid, with first order rate constants of 0.0385 $day^{-1}$ and 0.0357 $day^{-1}$ ($R^2$ = 0.9523) over the first 6.6 days of degradation. The TPH degradation beyond 6.6 days was considerably slower. Zero order rate constants of 19.8 µg/L-day ($R^2$ = 0.87) and 24.0 µg/L-day ($R^2$ = 0.88) were determined for the overall non-amended biodegradation of hydrocarbons in long-term experiments (124 and 103 days, respectively).

Although the overall rates were slow, TPH biodegradation was observed throughout the long-term experiment, and no minimum threshold TPH concentration was observed. However, after 124 days, 60% of the initial TPH was still present. Therefore, longer-term experiments need to be conducted in the future to confirm if partially biodegraded diluent constituents can be completely biodegraded.

## ACKNOWLEDGMENTS

Funding for this study was provided by Unocal Corporation through the California Polytechnic State University, Environmental Biotechnology Institute.

## REFERENCES

Coffey, P.A. 2003. "Improving Biosparge Performance for Remediation of Hydrocarbons in Groundwater". M.S. Thesis, California Polytechnic State University, San Luis Obispo, California.

Haddad, R., and S.A. Stout. 1996. *Diluent Geochemistry Report.* Technical Report Prepared for Unocal Corporation, ENTRIX, Inc., Walnut Creek, CA.

Landmeyer, James E., Francis H. Chapelle, Henry H. Herlong, and Paul M. Bradley. 2001. "Methyl tert-butyl ether biodegradation by indigenous aquifer microorganisms under natural and artificial oxic conditions" *Environmental Science and Technology* 35:1118-1126.

Siddiqui, Samina, and W.A. Adams. 2001. "The fate of diesel hydrocarbons and their effect on the germination of perennial ryegrass". *Environmental Toxicology* 17:49-62.

Suthersan, S.S., 1997. *Remediation Engineering Design Concepts.* CRC Press Inc, Boca Raton, FL.

United States Environmental Protection Agency. 1994. How to Evaluate Alternative Clean-Up Technologies for Underground Storage Tank Sites. EPA 510-B-94-003.