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Cold-Climate, In Situ Biodegradation of Petroleum Fuel
in Ground Water, Moose Factory, Ontario, Canada
By: Dale R. Van Stempvoort, Greg Bickerton, Suzanne
Lesage and Kelly Millar
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Cold-Climate, In Situ Biodegradation of Petroleum Fuel in Ground Water, Moose Factory, Ontario, Canada

Dale R. Van Stempvoort, Greg Bickerton, Suzanne Lesage and Kelly Millar

Abstract

Fuel spillage from a tank farm at a Health Canada site in Moose Factory, Ontario has resulted in a "smear zone" of residual LNAPL petroleum in the underlying sand unit, adjacent to the fluctuating water table. Monitoring of groundwater at the site suggests that in situ biodegradation of hydrocarbons is being mediated by cold-adapted microorganisms, under both aerobic and anaerobic conditions. Sulfate reduction and iron reduction appear to be important terminal electron accepting processes for anaerobic biodegradation. Bench scale laboratory tests with contaminated sand from the site were used to further examine the inferred biodegradation processes, and to provide information on achievable rates of enhanced aerobic and anaerobic biodegradation, at $\sim 5^{\circ}\text{C}$ and at $\sim 23^{\circ}\text{C}$. These tests confirmed the importance of Fe^{3+} and sulfate as electron acceptors, and indicated potential for enhanced in situ bioremediation at $\sim 5^{\circ}\text{C}$. DNA is being extracted from sediment samples to identify the dominant microorganisms in contaminated zones that play a role in intrinsic bioremediation. The laboratory test results will be helpful to guide decisions for remediation of this cold climate site.

Biodégradation sur place, sous un climat froid, de combustibles pétroliers dans l'eau souterraine, à Moose Factory (Ontario) Canada

Dale R. Van Stempvoort, Greg Bickerton, Suzanne Lesage et Kelly Millar

Résumé

Le déversement accidentel de combustibles à un parc de stockage de Santé Canada à Moose Factory (Ontario) a formé une « zone visqueuse » constituée de fractions pétrolières immiscibles dans la couche sous-jacente de sable, voisine de la nappe phréatique fluctuante. Le suivi des caractéristiques de l'eau souterraine à ce site semble montrer que la biodégradation sur place des hydrocarbures se fait par l'intermédiaire de microorganismes adaptés au froid, dans des conditions aussi bien aérobies qu'anaérobies. La réduction du sulfate et du fer apparaissent comme étant d'importants processus d'acceptation d'électron terminal dans la biodégradation anaérobie. Des essais à l'échelle du banc de laboratoire avec du sable contaminé provenant du site ont servi à examiner de plus près les processus de biodégradation présumés et à obtenir l'information sur le taux de biodégradation aérobie et anaérobie pouvant être atteint à ~ 5 °C et à ~ 23 °C. Ces essais ont confirmé l'importance du Fe^{3+} et du sulfate comme accepteurs d'électrons et ont montré que les possibilités de biorestauration sur place étaient meilleures à ~ 5 °C. L'ADN est extrait des échantillons de sédiments afin de caractériser les microorganismes dominants qui jouent un certain rôle au niveau de la biorestauration intrinsèque dans les zones contaminées. Les résultats des essais en laboratoire aideront à orienter les décisions en matière de restauration de ce site de région froide.

NWRI RESEARCH SUMMARY

Plain language title

Degradation of petroleum fuel in cold ground by bacteria, Moose Factory, Ontario, Canada.

What is the problem and what do scientists already know about it?

The cold climate in Canada's north may prevent or retard the natural recovery of sites affected by petroleum spills. Little is known about the presence of bacteria that break down hydrocarbons in the ground under such conditions, especially in the subsurface when there is a lack of oxygen.

Why did NWRI do this study?

NWRI was asked to assist Health Canada in finding a suitable approach to remediate a petroleum spill at a hospital site in Moose Factory, northern Ontario.

What were the results?

This laboratory study provides strong evidence that cold-adapted bacteria in the contaminated ground at the site are able to degrade hydrocarbons, under low oxygen conditions. Other tests suggest that injection of air to the subsurface may stimulate hydrocarbon-degrading bacteria. The results suggest that the bacteria already present in the subsurface can be utilized to clean up the site.

How will these results be used?

Together with ongoing field investigations, these findings are useful for selecting an appropriate remediation approach to clean up the site.

Who were our main partners in the study?

Health Canada, Public Works and Government Services Canada, Cree First Nation

Sommaire des recherches de l'INRE

Titre en langage clair

Dégradation de combustibles pétroliers par les bactéries dans le sol froid, à Moose Factory (Ontario), Canada.

Quel est le problème et que savent les chercheurs à ce sujet?

Le climat froid du nord du Canada peut empêcher ou retarder la restauration naturelle des sites touchés par des déversements de produits pétroliers. On connaît peu de choses sur la présence de bactéries qui décomposent les hydrocarbures dans le sol sous ces conditions, particulièrement sous la surface où il y a absence d'oxygène.

Pourquoi l'INRE a-t-il effectué cette étude?

On a fait appel à l'INRE pour aider Santé Canada à trouver une méthode appropriée pour restaurer un site d'hôpital à Moose Factory, au nord de l'Ontario, suite à un déversement de produits pétroliers.

Quels sont les résultats?

Cette étude en laboratoire montre avec beaucoup de certitude que les bactéries adaptées au froid dans le sol contaminé du site peuvent dégrader les hydrocarbures dans des conditions de présence très faible d'oxygène. D'autres essais semblent montrer que l'injection d'air sous la surface du sol peut stimuler les bactéries dégradant les hydrocarbures. Les résultats laissent supposer que les bactéries déjà présentes sous la surface du sol peuvent être employées pour nettoyer le site.

Comment ces résultats seront-ils utilisés?

De concert avec les recherches permanentes sur le terrain, ces résultats sont utiles pour le choix d'une méthode de restauration appropriée visant à nettoyer le site.

Quels étaient nos principaux partenaires dans cette étude?

Santé Canada, Travaux publics et Services gouvernementaux Canada, Première nation des Cris.

Cold-Climate, In Situ Biodegradation of Petroleum Fuel in Ground Water, Moose Factory, Ontario, Canada

Dale R. Van Stempvoort, Greg Bickerton, Suzanne Lesage and Kelly Millar
National Water Research Institute, Burlington, Ontario, Canada

Abstract

Fuel spillage from a tank farm at a Health Canada site in Moose Factory, Ontario has resulted in a "smear zone" of residual LNAPL petroleum in the underlying sand unit, adjacent to the fluctuating water table. Monitoring of groundwater at the site suggests that in situ biodegradation of hydrocarbons is being mediated by cold-adapted microorganisms, under both aerobic and anaerobic conditions. Sulfate reduction and iron reduction appear to be important terminal electron accepting processes for anaerobic biodegradation. Bench scale laboratory tests with contaminated sand from the site were used to further examine the inferred biodegradation processes, and to provide information on achievable rates of enhanced aerobic and anaerobic biodegradation, at $\sim 5^{\circ}\text{C}$ and at $\sim 23^{\circ}\text{C}$. These tests confirmed the importance of Fe^{3+} and sulfate as electron acceptors, and indicated potential for enhanced in situ bioremediation at $\sim 5^{\circ}\text{C}$. DNA is being extracted from sediment samples to identify the dominant microorganisms in contaminated zones that play a role in intrinsic bioremediation. The laboratory test results will be helpful to guide decisions for remediation of this cold climate site.

Introduction

Beneath an aboveground storage tank farm (ASTF) at Moose Factory Ontario, a surficial sand aquifer (MF sand) has been contaminated by fuel leakage. This included a major fuel spill associated with spring flooding in 1976. Residual LNAPL occurs predominantly in a "smear zone", adjacent to the water table which fluctuates seasonally between 0.3 and 2.3 meters below ground surface. In 2002-2003, groundwater temperatures in monitoring wells near the water table fluctuated seasonally between 0.7 and 10.2°C . Average air temperature at this northern site ranges from -20.5 in January to 15.1 in July.

Our investigation at this site indicates that anaerobic microorganisms play a role in the in situ biodegradation of hydrocarbons in cold ($\sim 5^{\circ}\text{C}$) ground water. Little previous information is available for such conditions. Groundwater monitoring of the plume, down gradient of the zone of residual phase contamination suggests that in situ biodegradation of hydrocarbons is being mediated by cold-adapted microorganisms. Patterns of dissolved concentrations of iron, sulfate, (Figure 1), manganese and oxygen (not shown) in the groundwater show that, in addition to aerobic processes, sulfate reduction, iron reduction and manganese reduction appear to be important electron accepting processes linked to hydrocarbon degradation.

Laboratory biodegradation experiments

Laboratory biodegradation experiments with MF sand were designed to simulate in situ bioremediation technologies under both "oxygen depleted" and "air injection" conditions. The objective of the oxygen depleted experiments was to simulate enhanced in situ bioremediation with various electron acceptors in a low-temperature and oxygen depleted environment that is representative of existing field conditions. Chemicals were added to provide alternative electron acceptors that might be used by microorganisms to degrade hydrocarbons. The air injection tests were intended to simulate enhanced in situ bioremediation under aerobic conditions, such as bioventing or biosparging techniques.

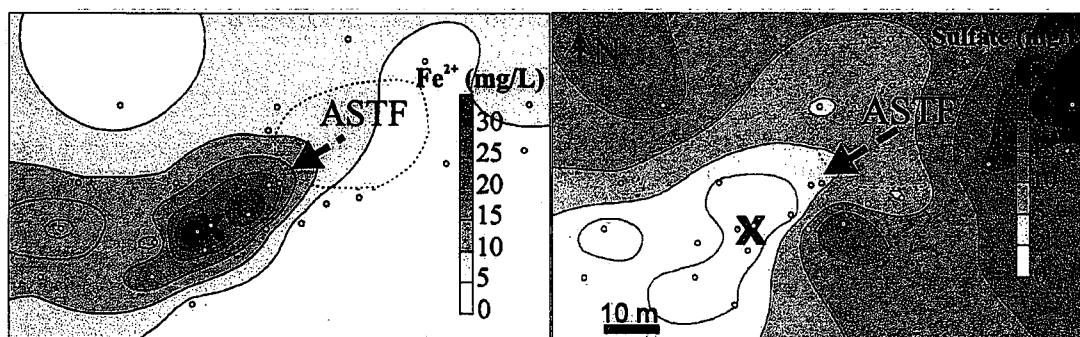


Figure 1. Two plan views of the site showing distribution of dissolved iron and sulfate in groundwater, October 2003. Groundwater sampling locations are indicated by gray circles; general groundwater flow direction from ASTF area is indicated by dashed arrow; sediment sampling location is indicated by "X."

Preparation of samples of MF sand

A sample (~6 kg) of hydrocarbon-contaminated MF sand (see Figure 1 for sample location) was subdivided into 64 sections, or "splits" in a glovebox under nitrogen. These splits were transferred to 125 mL Mason jars, sealed and stored at $5 \pm 2^\circ\text{C}$. "Start-up" concentrations of the splits indicated consistent levels of total petroleum hydrocarbons (TPH - average 4,080 mg/kg), the $\text{C}_6\text{-C}_{10}$ hydrocarbon fraction (average 228 mg/kg), and moisture content (average 14%).

Oxygen-Depleted Batch Tests

Five weeks later, 36 of the splits were placed in a glove box under nitrogen. Either Fe^{3+} (as FeOOH) or SO_4 (as $\text{Fe}^{2+}\text{SO}_4$) was added in triplicated "oxygen-depleted" tests to provide potential electron acceptors for biodegradation of hydrocarbons (Table 1). A colloidal ferric iron oxide precipitate, prepared using a modification of a procedure by Sugimoto *et al.* (1993), provided an alternative mineral form of Fe^{3+} with a larger surface area than the FeOOH . In several tests $(\text{NH}_4)_2\text{HPO}_4$ was added as a nutrient source, and/or a commercial humic substance (BlackEarth Dry Soluble 80, manufactured by Luscar Ltd., Edmonton, Alberta, Canada) was added (colloidal phase) as a potential agent or catalyst of biodegradation (cf. Lovley *et al.*, 1996; 1998; Cervantes *et al.*, 2001). All oxygen-depleted batch tests were conducted at $5 \pm 2^\circ\text{C}$, in the dark, except a "frozen control", which was placed at -20°C . In another control test, HgCl_2 was added to poison the microorganisms (Table 1). At 110 days, the remaining TPH and $\text{C}_6\text{-C}_{10}$ fractions were analyzed.

Table 1. Details of the oxygen-depleted batch tests, which were prepared in 125 mL Mason jars in a glove box, under nitrogen.

Microcosm Name	Chemical Addition per g of soil (wet wt)	$(\text{NH}_4)_2\text{HPO}_4$ per g soil (wet wt)	BlackEarth hydrosol, 10g/L in water per g soil (wet wt)	Water added per g soil (wet wt)
II-CF (-20°C)	None			0.05 mL
II-CHg	500 μg HgCl_2			0.05 mL
II-FS	49 mg $\text{Fe(II)SO}_4 \cdot 7\text{H}_2\text{O}$			0.05 mL
II-FSS0	65 mg $\text{Fe(II)SO}_4 \cdot 7\text{H}_2\text{O}$			0.05 mL
II-FSN	49 mg $\text{Fe(II)SO}_4 \cdot 7\text{H}_2\text{O}$	0.47 mg		0.05 mL
II-FSH	49 mg $\text{Fe(II)SO}_4 \cdot 7\text{H}_2\text{O}$		0.05mL	
II-F	124 mg FeOOH			0.05 mL
II-FN	124 mg FeOOH	0.47 mg		0.05 mL
II-FH	124 mg FeOOH		0.05mL	
II-FNH	124 mg FeOOH	0.47 mg	0.05mL	
II-FCo	195 mg "colloidal" iron*			~0.2mL
II-FCoH	195 mg "colloidal" iron*	0.47 mg	0.1mg (dry)	~0.2mL

*dry weight basis, including some NaCl

Air Injection Experiments

In four triplicated experiments, splits of the hydrocarbon-contaminated MF sand were placed in foil-wrapped, 250 mL filtering flasks. In two tests, colloidal humic product (BlackEarth Dry Soluble 80) was

added as a potential catalyst. Two temperatures were selected, $4 \pm 2^\circ\text{C}$ and $23 \pm 2^\circ\text{C}$. For each 60-day flow-through test, a pump injected humidified air at rate of 20 cc/minute into the flask containing the soil/sand mixture. Volatilization of hydrocarbons was monitored for 6 hour intervals by passing the air exiting the system through Charcoal Tube Samplers (ORBO™-32 Tube).

DNA Analyses of Bacterial Communities

Total DNA was extracted from 1.0 g sediment samples using the Ultraclean™ Soil DNA Kit (MoBio Laboratories) and purified through Sephacryl S400HR spin columns (Amersham Pharmacia Biotech). Bacterial 193-bp fragments were amplified by touchdown PCR using primers 341F-GC and 534R as described by Muyzer *et al.* (1993). Amplified products were separated by DGGE using a denaturing gradient of 30-65%. DGGE was conducted at a constant temperature of 60°C at 65V for 16.5 hours. DGGE bands were excised, reamplified, sequenced, and identified using the Sequence Match program of the Ribosomal Database Project (Michigan State University).

Results

Oxygen-Depleted Batch Tests

There was some evidence for leakage of air into the batches. At 110 days, the uppermost thin layer of batches amended with FeSO_4 had visible evidence of oxidation of Fe^{2+} , and in some batches this layer was partially dried. Unexpectedly, frozen and Hg-poisoned controls indicated losses of ~20-25% of TPH and of the $\text{C}_6\text{-C}_{10}$ fraction at 110 days (Figures 2 and 3). We suggest that these losses occurred during the 5 week storage prior to the batch tests.

Batches amended with FeOOH had the largest losses of hydrocarbons (~40 % TPH; ~50-60% of $\text{C}_6\text{-C}_{10}$ fraction), significantly more than controls (student's t tests, 90-99% confidence). In contrast, there were no significant differences between hydrocarbon losses in most FeSO_4 or colloidal ferric iron amended batches compared to the two controls (Figures 2 and 3). Colloidal iron may have been less effective than FeOOH because of higher ionic strength of the colloidal iron batches, or differences in mineral form(s).

Addition of the humic product had no noticeable effect (Figures 2 and 3). Addition of nutrients enhanced losses of hydrocarbons in the FeSO_4 amendments by ~15-20% (both $\text{C}_6\text{-C}_{10}$ fraction and TPH). The nutrients may have stimulated sulfate reducing hydrocarbon-degraders. In contrast, the addition of nutrients did not stimulate hydrocarbon losses in the FeOOH amended tests.

Air Injection Tests

There were significant losses of TPH and $\text{C}_6\text{-C}_{10}$ hydrocarbons in all air injection tests, averaging 23% to 65% for TPH, and 30% to 86% for the $\text{C}_6\text{-C}_{10}$ fraction (Figure 4). The greatest losses of both TPH and $\text{C}_6\text{-C}_{10}$ were achieved in tests at 23°C without humic product (Figure 4). The smallest declines were observed in tests at 4°C with humic product added. Based on Student's t tests, there were significantly smaller losses of both TPH and $\text{C}_6\text{-C}_{10}$ fractions at 4°C and in the presence of the humic product.

The reduced losses of hydrocarbons in tests with humic product added may have been an artifact. These tests had approximately 10% higher moisture content, because the humic product was added as a colloidal phase in water. The higher moisture content may have impeded the flow of air.

During the experiments 6-12% of the TPH were volatilized. There was less volatilization in batches with humic product. The amount of $\text{C}_6\text{-C}_{10}$ volatilized was not measured, but probably substantial.

Comparison of Rates of Hydrocarbon Losses under Oxygen-Depleted and Air Injection Tests

Unexpectedly, the overall rates of the losses of hydrocarbons in the two types of tests, oxygen-depleted and air injection, were similar (Table 2). In the oxygen-depleted tests the overall rate of hydrocarbon loss

decreased with duration of the test, suggesting that a fraction of hydrocarbons was not readily bioavailable. Oxygen-depleted batches amended with FeOOH had faster losses of hydrocarbons than the other batches.

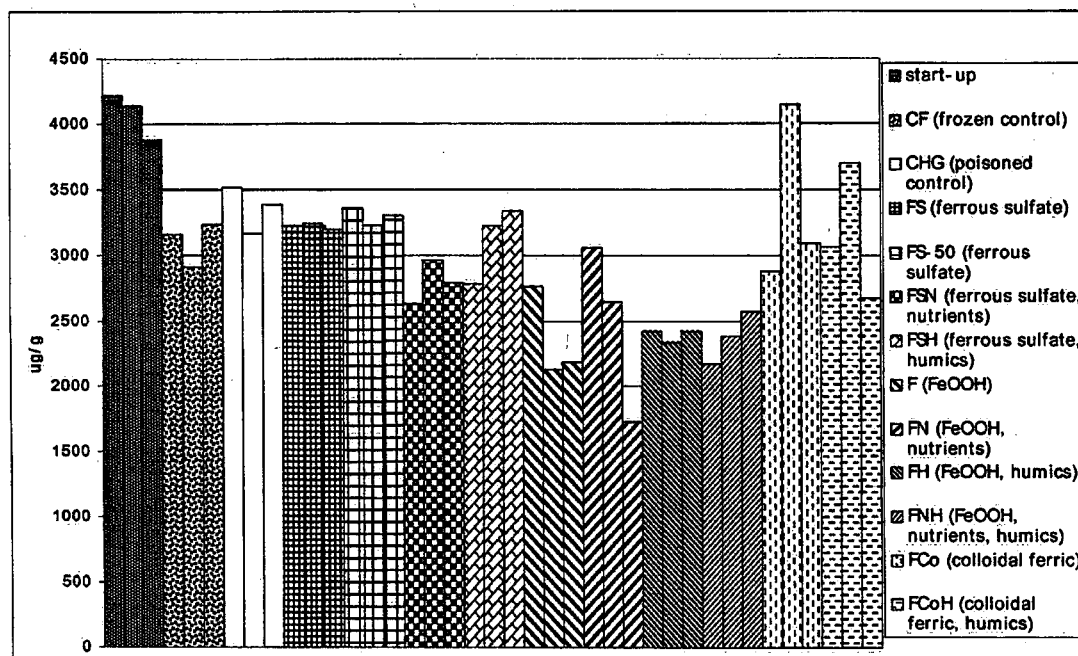


Figure 2. TPH concentrations at start-up and at 110 days in the triplicate oxygen-depleted batches. Results were determined on a dry weight basis, and were corrected for dry weight of chemicals added.

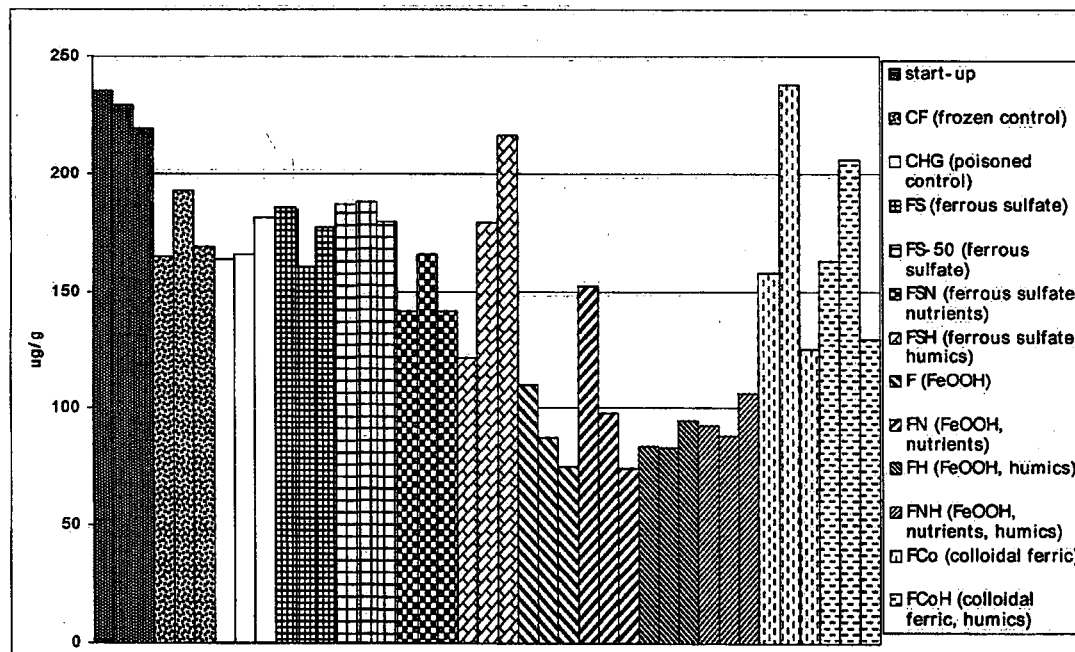


Figure 3. Concentrations of the C₆-C₁₀ fraction at start-up and 110 days, triplicate oxygen-depleted batches.

The air injection tests indicated increased rates of hydrocarbon loss (i.e., inferred biodegradation) with an increase in temperature from 4 to 23°C (Table 2). However, earlier batch tests in our laboratory with another sample of MF sand indicated no such temperature effect on the rates of losses of TPH and the C₆-

Comparison of humic product amendments to similar previous tests

In previous batch experiments, Seibel *et al.* (1996) found that addition of humic substances resulted in either slight increases or decreases in the rate of biodegradation of PAHs, but the overall rate was not noticeably affected. Lesage *et al.* (1997, 1999) found that the addition of Aldrich humic acid (AHA) inhibited mineralization of phenanthrene and pyrene in soil batches, when spiked as pure phases, but this effect was only slight or negligible in the presence of furnace oil, crude oil or weathered hydrocarbons. Addition of AHA enhanced the overall degradation of crude oil. Overall, the results of the batch tests by Seibel *et al.* (1996) and Lesage *et al.* (1997; 1999) are similar to the results of the batch tests of this study.

In contrast to our findings, Lovley *et al.* (1996, 1998) reported that humic acids may serve as chelators of Fe^{3+} , potentially enhancing the microbial biodegradation of hydrocarbons in anaerobic sediments, and possibly acting as electron shuttles to enhance degradation of contaminants. Cervantes *et al.* (2001) found that anaerobic microbial oxidation of toluene to CO_2 was coupled to humus respiration, and inferred that humic substances may contribute to the intrinsic bioremediation by serving as terminal electron acceptors.

Microbial Analyses

DGGE analysis, conducted on samples from the earlier oxygen-depleted microcosm experiment (Table 2) confirmed the presence of an iron-reducing, psychrotolerant species of *Rhodospirillum rubrum* (Fig. 6). Also identified were a number of strains within the family Comamonadaceae (*Acidovorax*, *Hydrogenophaga*, *Rhodospirillum* spp.) with specific hits for isolates found associated with petroleum contamination and biodegradation. Bands 4 and 7 were closely related (83-92%) to *Stenotrophomonas* and *Sphingomonas* species adapted to humic acid-sorbed PAHs.

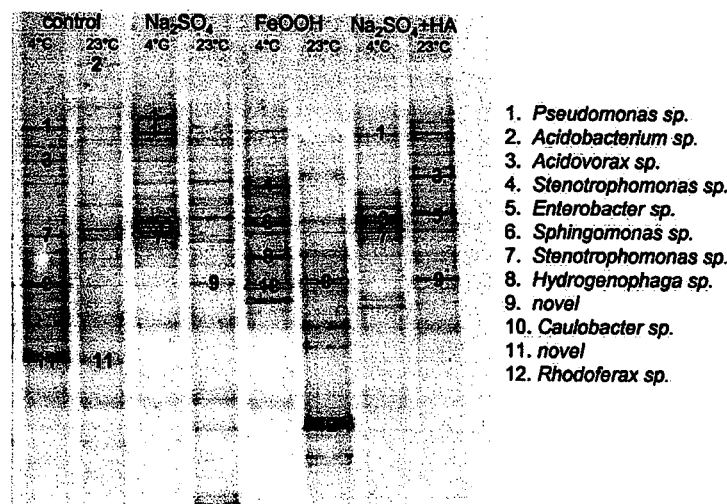


Figure 6. DGGE analysis of bacterial communities in oxygen-depleted microcosms (HA refers to BlackEarth Dry Soluble 80). Numbered bands were excised, purified and identified. Similarity indices exceeded 0.85 for all species reported. Novel sequences had similarity indices below 0.6.

Implications for Remediation of the MF sand

While the rates of hydrocarbon losses observed in these laboratory tests are not directly applicable to field conditions, they indicate that in situ bioremediation techniques, under either air injection or oxygen-depleted conditions, might achieve relatively rapid rates of hydrocarbon degradation. The test results suggest that in situ bioremediation under oxygen-limited conditions might achieve similar rates of

C₁₀ at 5°C and 23°C under oxygen-depleted conditions (Table 2). This key result suggests that the low temperature of the soil and groundwater at the field site will not deter in situ bioremediation of the hydrocarbons under oxygen-limited conditions. We hypothesize that the reason for the lack of a temperature effect in these previous oxygen-depleted tests was that the indigenous hydrocarbon-degrading bacteria in the zone of contamination are adapted to both low temperature and oxygen-limited conditions.

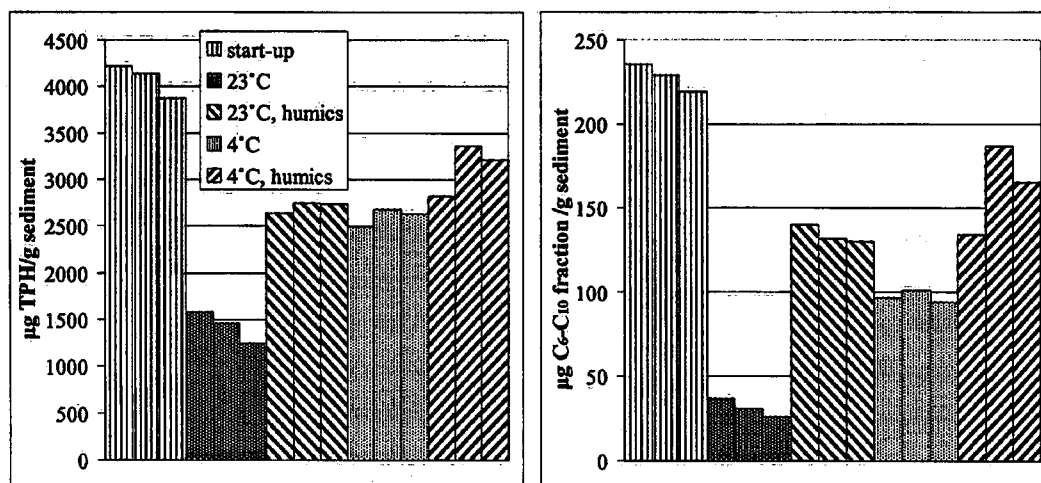


Figure 4. Start-up and final (60 d) concentrations of TPH (left) and of C₆-C₁₀ fraction (right) in triplicated air injection tests.

Table 2. Average rates of losses of hydrocarbons in laboratory tests.

Test(s)	Duration (days)	µg TPH per g soil per day	µg C ₆ -C ₁₀ per g soil per day
Earlier study with MF sand, oxygen-depleted batches, 23± 2°C*			
all tests with FeOOH	40	47.0 ± 7.8	9.2 ± 1.7
all other tests	40	32.2 ± 7.8	6.5 ± 1.9
all tests with FeOOH	194	16.4 ± 4.0	2.7 ± 0.4
all other tests	194	12.3 ± 4.0	2.1 ± 0.6
Earlier study with MF sand, oxygen-depleted batches, 5± 2°C*			
all tests with FeOOH	40	31.8 ± 5.6	9.4 ± 0.9
all other tests	40	34.2 ± 7.8	7.4 ± 1.1
all tests with FeOOH	194	15.6 ± 4.0	3.3 ± 0.3
all other tests	194	11.7 ± 4.7	2.0 ± 1.0
This study, oxygen-depleted batches, 5± 2°C			
all tests with FeOOH	110	15.3 ± 3.1	1.2 ± 0.2
all other tests	110	7.7 ± 3.7	0.5 ± 0.3
This study, air injection tests, 23± 2°C			
without BlackEarth product	60	44.2 ± 2.8	3.3 ± 0.1
with BlackEarth product	60	22.8 ± 1.0	1.6 ± 0.1
This study, air injection tests, 4± 2°C			
without BlackEarth product	60	24.7 ± 1.6	2.2 ± 0.1
with BlackEarth product	60	15.8 ± 4.6	1.1 ± 0.4

*data from earlier, unpublished tests in our laboratory

hydrocarbon degradation to those obtained with active air injection techniques (e.g. bioventing), as long as there are suitable electron acceptors available.

The greatest enhancements of hydrocarbon losses in the oxygen-depleted tests were in the batches amended with FeOOH (Fe^{3+}OOH), suggesting that the dominant hydrocarbon degrading microorganisms present in the MF sand include iron-reducing bacteria. This is consistent with groundwater monitoring results, which indicate elevated Fe^{2+} in the plume down gradient of the ASTF fuel spill zone (Figure 1).

Observation of enhanced hydrocarbon losses in some of the $\text{Fe}^{2+}\text{SO}_4$ -amended, oxygen-depleted tests suggests that sulfate reducing bacteria may also be important hydrocarbon degraders in the MF sand. This is consistent with the lower concentrations of sulfate in the plume of hydrocarbon contamination that extends down gradient of the ASTF fuel spill area (Figure 1). Lower sulfate concentrations appear to indicate that bacterial sulfate reduction is occurring in the plume, linked to biodegradation of the hydrocarbons.

Some remediation methods that might be considered for the site include 1) monitored natural attenuation, 2) enhanced in situ biodegradation, or 3) enhanced ex situ biodegradation. Enhanced in situ or ex situ bioremediation under oxygen-limited conditions would potentially include the addition of an electron acceptor. The results of the air injection experiments suggest that bioventing and/or biosparging could also be used effectively at the site to reduce the petroleum hydrocarbons in the MF sand to acceptable levels.

Acknowledgements

Prabhjeet Grewal and Colin Stewart assisted with the laboratory experiments; Nicole Tran assisted with an earlier phase of batch tests, as reported in Table 2.

References

- Cervantes, F.J., Dijkstra, W., Duong-Dac, T., Ivanova, A., Lettinga, G. and Field, J.A. 2001. Anaerobic mineralization of toluene by enriched sediments with quinones and humus as terminal electron acceptors. *Applied Environ. Microbiol.* 67(10), 4471-4478.
- Lesage, S., Li, W.-C., Millar, K., and Liu, D. 1999. Effect of humic acids on the bioavailability of PAHs from weathered soils. *Proceedings, The Fifth International In Situ and On-Site Bioremediation Symposium*, San Diego, California, April 19-22, 1999, pp. 161-166.
- Lesage, S., Li, W.-C., Millar, K., Brown, S. and Liu, D. 1997a. Effect of humic acids on the bioremediation of polycyclic aromatic hydrocarbons from aquifers contaminated with petroleum. *Proceedings, 6th Annual Symposium on Groundwater and Soil Remediation*, March 18-21, Montréal, Québec, Canada, pp. 325-338.
- Lovley, D.R., Fraga, J.L., Blunt-Harris, E.L., Hayes, L.A., Phillips, E.J.P. and Coates, J.D. 1998. Humic substances as a mediator for microbially catalyzed metal reduction. *Acta hydrochim. hydrobiol.* 26(3), 152-157.
- Lovley, D. R., Woodward, J. C. and Chapelle, F. H. 1996. Rapid anaerobic benzene oxidation with a variety of chelated Fe(III) forms. *Appl. Environ. Microbiol.* 62(1), 288-291.
- Muyzer, G., De Waal, E.C., and Uitterlinden, A.G. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* 59(3), 695-700.
- Seibel, F., S. Heidenreich and F.H. Frimmel, 1996. Interaction of Humic Substances and Polycyclic Aromatic Hydrocarbons (PAHs) during the Biodegradation of PAHs. *Acta hydrochim. hydrobiol.* 24(6), 260-266.
- Sugimoto, T., Muramatsu, A., Sakata, K. and Shindo, D. 1993. Characterization of Hematite Particles of Different Shapes. *J. Colloid Interface Sci.* 158(2), 420-428.

Biographical Sketches

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