

Low Temperature Ex Situ Bioremediation of Hydrocarbon Contaminated Soils

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ABSTRACT

Many oil degrading bacteria thrive within the mesophilic temperature range, between 20°C and 40°C; bioremediation practitioners view this as the optimal temperature range for microbially driven hydrocarbon remediation. Environmental managers generally halt active bioremediation projects during the winter months as microbial activity is thought to be insignificant and not cost effective. Recent research has cast some doubt on this assumption. In temperate climates there is a sizeable proportion of a soil's biomass that consists of cold adapted bacteria, that can efficiently degrade hydrocarbon at temperatures around 5°C. When properly stimulated these psychrophiles and psychrotrophs can continue to remove contaminants from soil at temperatures just above the freezing point of water. Considering Southern Alberta's regular Chinook cycles, low temperature bioremediation holds great promise.

In this paper, recent results are presented from the cold climate bioremediation research being carried out in the University of Calgary's Engineering for the Environment program. Approximately 300 m³ of clay-sand subsoil, moderately contaminated with diesel and crude oil (1395 - 3130 ppm), was transferred from an industrial area northeast of Calgary to an engineered treatment pad at the Shepard Landfill. A treatment pile was constructed for the soil and arranged into four treatment cells (one control and three test). The control cell was left untouched to provide a baseline against which the test cells could be compared. The first test cell was mechanically aerated on a monthly basis to release entrapped volatiles and oxygenate the soil. The second test cell was provided with optimal nutrient, moisture and oxygenation regimes. The final test cell was treated the same as cell two, but was not aerated. Total petroleum hydrocarbon (TPH) concentrations at the site were monitored for six months from January to June. Hydrocarbon concentrations were determined gravimetrically and the concentration of specific hydrocarbon fractions by gas chromatography (flame ionization detection). Microbial enumeration was carried out using most probable number (MPN) methodology.

Results showed that under optimized conditions alkane based hydrocarbon contaminants can be significantly reduced (>60% TPH reduction) during cold temperature periods (January – May).

INTRODUCTION

Using indigenous bacteria to remediate hydrocarbon contaminated soils has become a reliable and commonly used treatment strategy. In moderate temperatures most saturated aliphatics and lower molecular weight aromatic contaminants can be rapidly mineralized using simple ex situ treatments. Properly designed biopiles and land farms can substantially reduce the TPH (total petroleum hydrocarbon) concentrations in soil, even in situations of extreme contamination (> 25,000 ppm crude) (Hoffman and Chaw, 1999). TPH removal can also be done relatively quickly. Land treatment systems have shown greater than 80% reductions in both saturated alkanes and 2 to 3-ring PAHs within one year (Chino et al 1999). Land farming is also the cheapest of the available ex situ treatments (Bergerson et al, 1999), and in many cases equally or more effective than composting and biopiles at remediating crude oil contaminated soils (van Zyl and Lorenzen, 1999), (Hoffman and Chaw, 1999).

Much has been written about optimizing land treatment systems using active aeration, nutrient amendments, moisture monitoring, compost addition and other treatments. This research is carried out almost exclusively under favourable temperature conditions (20 - 40 °C) and in many cases is discontinued if the average temperature falls below this ideal range (Troy et al, 1994). This has created a knowledge gap regarding low temperature bioremediation. This is unfortunate for countries such as Canada where much of the year is spent at temperatures below 20°C. Canada has many contaminated sites in remote locations where on-site biological remediation would be the best clean-up option. The small body of work currently available regarding low temperature bioremediation has demonstrated that in most North American and European soils a large component of the bacterial biomass is active at 5°C. A sizeable proportion of these can efficiently degrade crude oil (Sexstone and Atlas 1977, Kerry 1992, Sadowsky and Turco, 1999, Wilson 1999, Gibb 1999, Eriksson et al, 2001). Thus there is both good reason to develop effective low temperature land treatment strategies and good evidential support that they can be successful.

In order to study the effectiveness of a land treatment system at low temperatures a modified landfarm was constructed on an engineered treatment pad at the Shepard Landfill in Calgary, Alberta. In order to minimize space requirements approximately 300 m³ of crude contaminated soil was built into a levelled pile approximately 1.5 m deep, 32 m long and 6 m wide. The pile was divided into four (8m _ 6m) treatment cells which were sampled monthly. They included one control and three test cells of which two were mechanically aerated and two amended with 20% v/v mature compost, N.P.K. fertilizer and water (Figure 1). Time zero was January 5th and treatment continued until June 24th. This timeframe was chosen because it included a cold temperature period January to May and a subsequent moderate temperature period.

TPH concentrations were determined gravimetrically on a monthly basis during the 170 day treatment period in order to compare the effectiveness of the different treatments. The combined concentrations of seven *n*-alkanes common to crude oil were also monitored during this period using GC-FID. Microbial enumeration (which included

total heterotrophs, 20°C oil degraders and 4°C oil degraders) was performed using MPN analysis in April and July.

OBJECTIVES

- 1) To determine whether landfarm bioremediation is viable at low temperatures.
- 2) To determine how seasonal changes affect the populations of warm and cold adapted oil degrading bacteria and the total heterotrophic population.
- 3) To determine which treatments, if any, best stimulate biological TPH removal at low temperatures.

MATERIALS AND METHODS

Contaminant source. Approximately 300 m³ of soil was excavated from the area between a well head and a diesel storage sump at an abandoned lease site near Balzac, Alberta. The soil texture was clayey with some sand content (roughly 60% clay, 40% sand). Gas chromatographic analysis identified the contaminant as an F3 fraction (CCME, 2001) crude oil mix (Figure 2.) containing no asphaltenes. Due to site subsurface heterogeneity, clay texture and large excavation area, the soil TPH concentrations ranged from 1300 to 3300 ppm.

Site construction. A modified landfarm was constructed on a pre-built treatment pad at the Shepard Landfill in Calgary, Alberta. The pad has a 1m impermeable clay base with an internal leachate collection system and a fully bermed perimeter. The frozen soil was brought in by dump truck and formed into a large treatment pile (length 32m, width 6m, depth 1.5m), which was squared off and flattened by bulldozer. Compaction relief and some homogenization was done by trackhoe using a cleanup bucket. Any large rocks encountered were removed. The pile was divided into four treatment cells of roughly 8m by 6m (see Figure 1). The two middle cells (2 & 3) were amended with approximately 20% mature leaf compost (v/v) which was mixed in evenly by trackhoe.

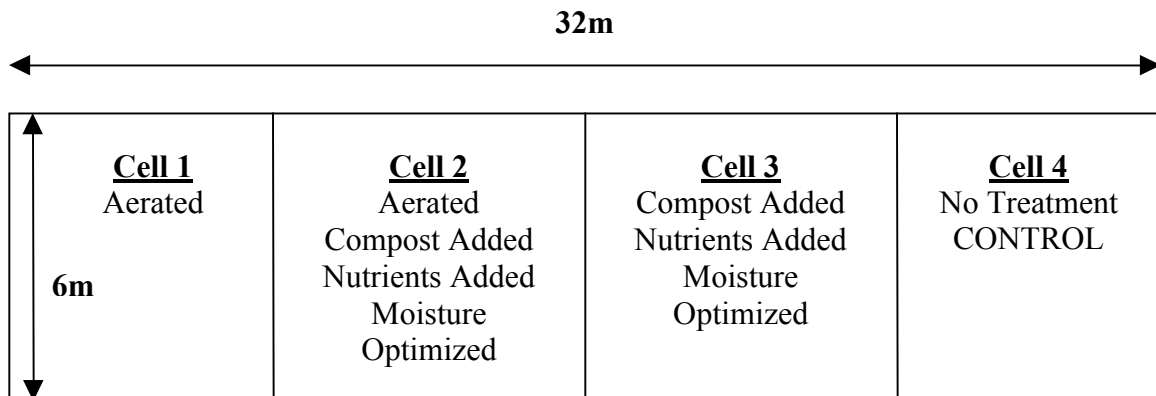


Figure 1. Configuration of the modified landfarm.

Sampling. During the six month treatment period composite sampling was performed on a monthly basis. Care was taken at all times to sample at least 1m from the transition zone between cells. In each cell four 0.3m deep sample pits were dug at least 2m from one another in a diamond pattern. From each pit three grab samples were taken at various depths. The collected soil was then combined into a one litre glass sample jar and stored at 5°C until being analyzed (within 48 hours).

Hydrocarbon extraction and analysis. Petroleum hydrocarbons were extracted using a modification of the CCME methodology (CCME, 2001). A minimum of 10 g (d.w.) soil was placed in a tared cellulose extraction thimble (Whatman International, Maidstone, England) for use in the soxhlet apparatus. A 1:1 v/v hexane : acetone extraction mixture was used; extraction proceeded for 24 hours (cycling rate: 6 cycles/hour). The recovered solvent-hydrocarbon mixture was transferred to a clean 500 ml beaker and left for 24 hrs to evaporate in a fume hood. Polar non-petroleum derived organics were removed from the mixture by re-solubilization in a 40 ml mixture of hexane and dichloromethane (1:1 v/v) to which 5 g of activated silica gel was added. The silica gel was thoroughly mixed for one minute and left to settle for four minutes. The solvent was then recovered (passed through 6mm glass wool) into a tared storage vial. A second solvent rinse of silica residue was done to ensure maximum TPH recovery. The solvent was evaporated off and the vial was accurately weighed. TPH concentration was determined gravimetrically after the silica gel clean-up. The weight of the extracted hydrocarbons was determined and divided by the dry weight of the sample soil. Units were converted from TPH(g)/gram of dry soil to µg/g. The sample was then prepared for gas chromatographic analysis by the addition of exactly 10 ml toluene.

Gas Chromatograph Flame Ionization Detection (GC FID) was used for analysis of all soxhlet-extracted samples. The concentrations of seven major *n*-alkane molecules (C₁₈, C₂₀, C₂₁, C₂₂, C₂₄, C₂₆ & C₂₈) were determined and summed for analysis over the treatment period. They were identified by retention time comparisons to laboratory standards and quantified by area under their peaks. Calibration curves were used to convert the area responses to concentrations.

GC-FID analysis was carried out on a Hewlett-Packard GC 6890 Series gas chromatograph equipped with a flame ionization detector. Column was HP-5 with length 30 m, inside diameter 0.25 mm and film thickness 0.17 µm. Helium was used as the carrier gas at a flow rate of 9.96 ml/min and a pressure of 18.4 p.s.i.. Oven temperature program was as follows: 40°C held for 1 min; programmed ramping rate of 15°C/min to 325°C which was held for 7 min. The injector was set to 300°C, and the detector to 340°C. One micro litre samples were injected in splitless mode using an autosampler.

Enumeration of heterotrophs and oil degraders. Microbial enumerations were carried out twice during the treatment period, once on April 15th to quantify the cold adapted microbial population, and again on July 16th to quantify the warm adapted population. MPN analysis, as described by Haines et al (1996), was slightly modified for enumerating total heterotrophs and crude oil degraders at 20°C and 4°C. Well mixed 10 g samples were diluted with 90 ml of autoclaved tap water. Ninety six-well microtiter

plates (Corning Inc., New York City) were used to prepare eight replicates of the treatments. Two hundred micro litres of sample slurry was transferred via multi-channel pipetter to the first row of the assay plate. Two hundred micro litres of sterile Bushnell-Haas medium (Difco Products, Detroit, MI) was placed into each well of the remaining 11 rows. The serial dilution was carried out by transferring 20 µl of sample from the first to second row where it was thoroughly mixed and then 20 µl transferred to the third row. The procedure of mixing and transferring was continued for the next ten rows (row twelve acted as a negative control). Three plates for each treatment cell were prepared in this manner. The total heterotroph enumeration had 2 µl of sterile 1/10 strength Tryptic Soy Broth (Sigma Chemical Co., St. Louis, MO) added to each well. The 20°C and 4°C plates had 2 µl of diesel oil added to each well. The total heterotroph and 20°C oil degrader plates were placed in plastic bags to minimize evaporative loss and incubated for 13 days at room temperature. The 4°C oil degrader plates were bagged and refrigerated at 4°C for the same time period.

After the incubation period, 50 µl of *p*-iodonitrotetrazolium violet (3g/L) (Sigma Chemical Co., St. Louis, MO) was added to each well. This chemical oxidant competes with O₂ as a preferential terminal electron acceptor. Once reduced it forms an insoluble pink colored formazan deposit. Pink wells are scored as positive while the others are negative. MPN values were calculated using published tables (Garthright, 2001).

RESULTS

TPH reduction. Hydrocarbon concentrations at time zero ranged from roughly 1400 µg/g in cell 3 to 3130 µg/g in cell 2¹. The TPH chromatographic profile (Figure 2) shows that a large proportion of the identifiable compounds have retention times matching lab standard *n*-alkanes and the contaminant appears as a weathered paraffinic crude.

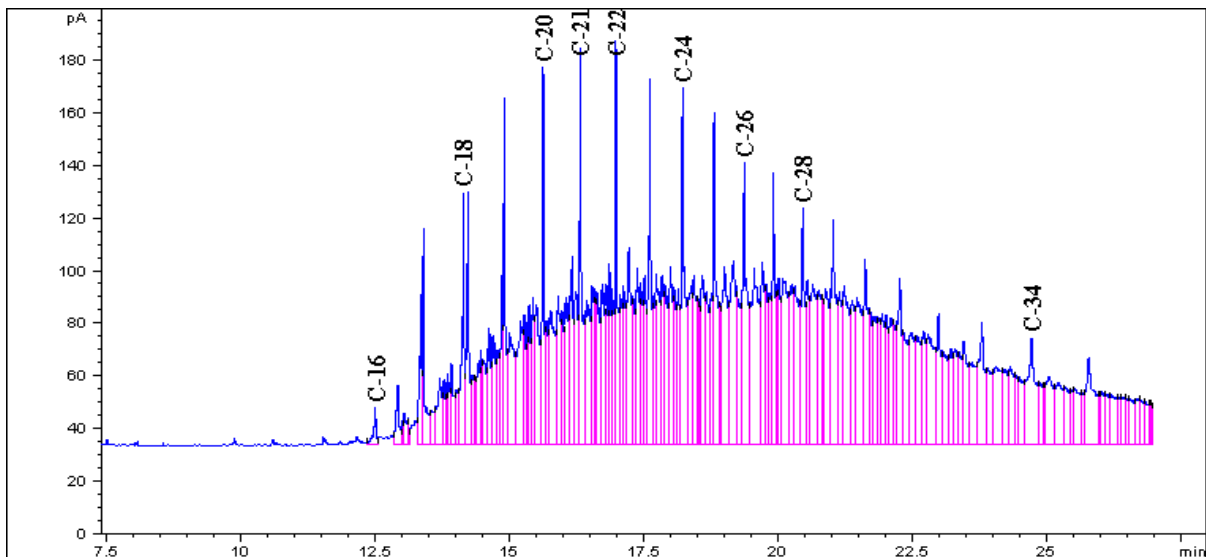


Figure 2. TPH chromatographic profile at time zero (Cell 2, January 5th).

¹ This variability is expected when large volumes of soil are excavated from sites with highly heterogeneous contamination levels and the quantity of material precludes effective homogenization.

Cell 2 showed the greatest reduction in TPH during the 136 day cold weather treatment period (Jan. 5 – May 21), with a 62% decrease. Cell 1 also showed significant reduction (52%) while cell 3, which was not aerated, had the lower TPH decline at only 35% (Fig. 3; Table 1). TPH concentrations in the control cell did not change significantly (<9%).

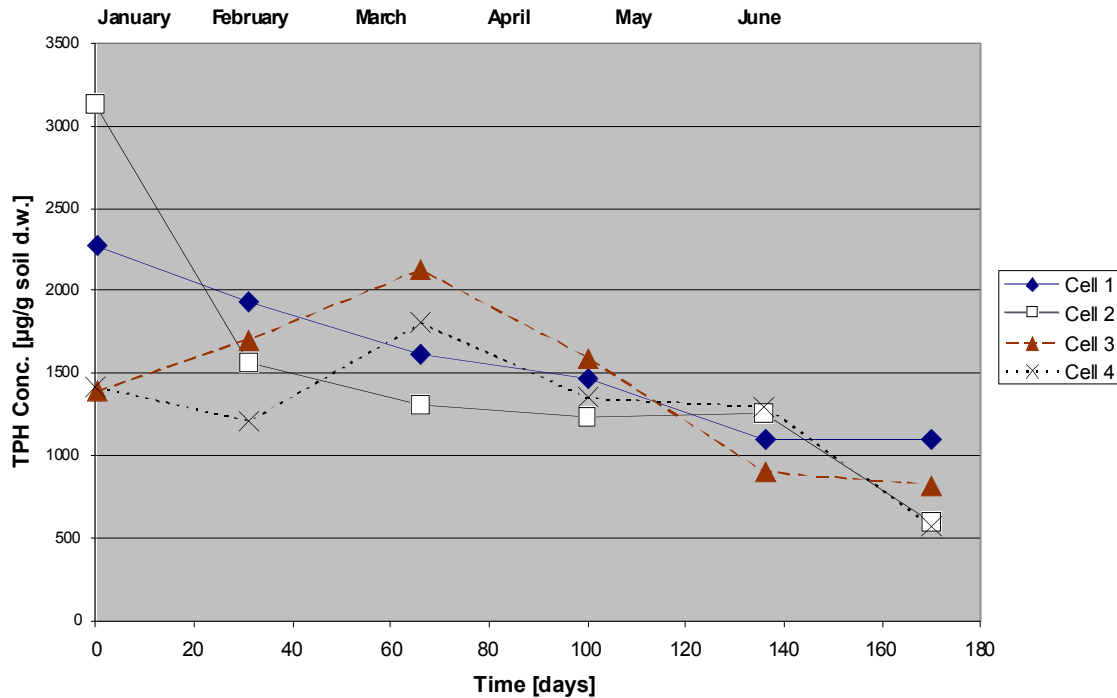


Figure 3. TPH Reduction for each cell over the full treatment period including the May 21 to June 24 moderate temperature period.

Days of Treatment						TPH Concentration (µg/g soil d.w.)
	0	31	66	100	136	
Cell 1	2275	1932	1619	1467	1100	
Cell 2	3130	1566	1305	1229	1258	
Cell 3	1395	1709	2131	1598	904	
Cell 4	1421	1212	1808	1354	1299	

Table 1. Change in TPH concentrations over the cold temperature treatment period (January 5th – May 21st)².

The treatments applied to cell 2 had a clear effect on the initial rate of hydrocarbon biodegradation, with a 50% reduction in TPH concentration seen in the first 31 days of treatment. The degradation rate subsequently slowed, with a decrease of only 19.7%

² Time constraints precluded the possibility of multiple replicates being done for each sample; thus standard deviations could not be calculated. Large variations in TPH concentrations are not uncommon in this type of research due to the heterogeneity of the material being sampled. Some replicates were run to confirm the accuracy of the reported values.

being measured over the next 105 days. Cell 1, the other aerated cell, showed more gradual TPH reduction over time. TPH fluctuations during the treatment period made it difficult to determine a trend for cell 3.

Reduction of *n*-alkanes. All treatments, except for the control, showed significant reduction in alkane concentrations. None of the seven quantified alkanes proved resistant to biological degradation although C₂₁, which was the only odd numbered hydrocarbon analysed, proved most difficult to remove in all treatments (unpublished data). Cell 2 showed the greatest extent of biodegradation with more than 60% of the measured alkanes being removed. Cells 1 and 3 showed roughly equal efficiency at reducing the alkane concentrations, 48.9% and 50% respectively (Table 2).

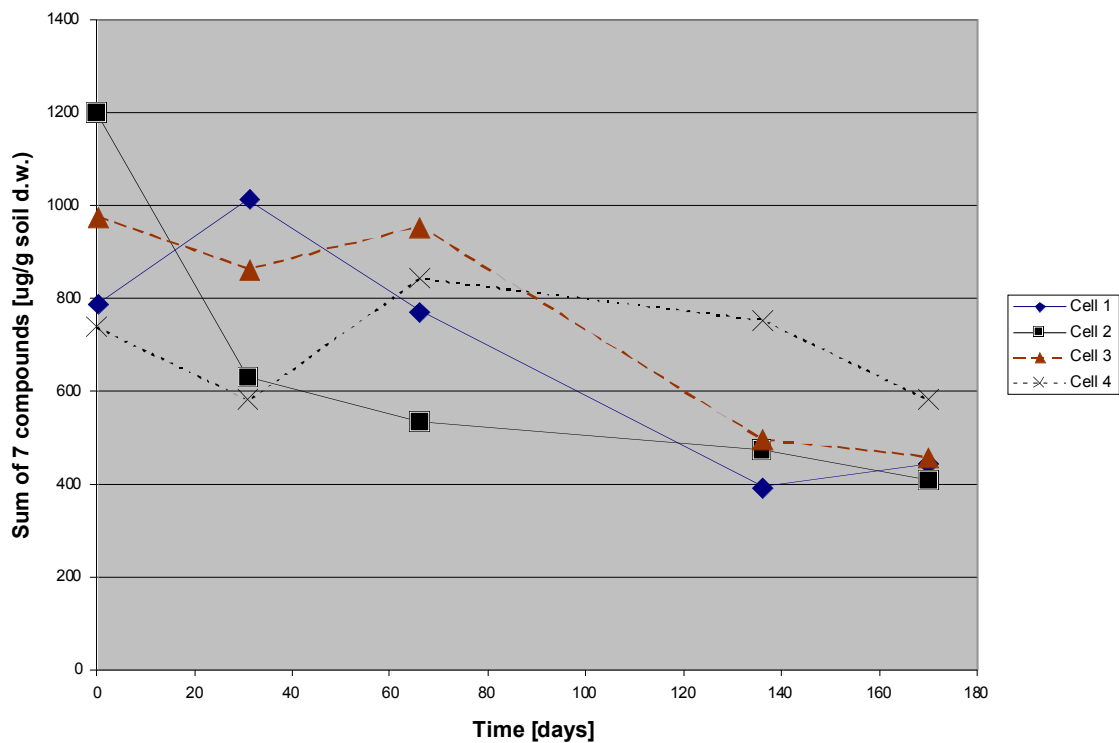


Figure 4. Summed concentration of seven alkanes for each cell over the treatment period.

Consistent with the TPH data, cell 2 showed extensive hydrocarbon reduction in the first 31 days of treatment (47.7%) and gradual reductions after that.

	Days of Treatment				% Reduction
	0	31	66	136	
Cell 1	788	1013	771	394	50.0
Cell 2	1200	628	535	473	60.6
Cell 3	976	864	955	496	48.9
Cell 4	737	581	843	753	0

Table 2. Concentration ($\mu\text{g/g}$) of summed alkanes over the cold temperature treatment period. The Percent reduction from time zero to day 136 is listed in the right hand column³.

Microbial enumeration. At the end of the April 15 sample incubations the total populations of heterotrophs ranged from 2.3×10^7 to 1.6×10^{11} cells per g of soil and populations of 4°C oil degraders ranged from 2.3×10^2 to 7.9×10^3 cells per g of soil. The warm adapted population sampled on July 16 showed consistently higher results with total heterotrophs ranging from 1.8×10^{10} to 1.6×10^{11} cells per g soil and the 4°C oil degraders from 1.3×10^4 to 2.2×10^5 cells per g soil (Table 3). The cell 1 and 3 results are not sufficiently different from the control to assume that the treatments had meaningful affects on the soil biomass.

Results are expressed in millions of cells/g	Cell 1		Cell 2		Cell 3		Cell 4	
	April 15	July 16	April 15	July 16	April 15	July 16	April 15	July 16
Total Heterotrophs (20°C)	23	92000	160000	160000	92000	92000	18000	18000
Oil Degraders (20°C)	0.023	0.13	1.3	2.2	0.035	0.11	0.094	0.33
Oil Degraders (4°C)	0.00023	0.049	0.0079	0.22	0.0049	0.013	0.0023	0.023

Table 3. Results of microbial enumeration [by MPN] at two sampling dates representing the cold adapted and the warm adapted populations.

Discussion

The microbial enumeration data demonstrated that all treatment cells had healthy heterotrophic population on April 15th. The high numbers of 4°C oil degraders expected in the April samples did not materialize⁴. However, the results confirm that there is an active low temperature adapted oil degrading population in all the soils. The principal objective of low temperature bioremediation is to simulate the growth of these specialized oil degraders.

³ Data for day 100 (April 15) showed extremely low concentrations for all cells which was deemed the result of laboratory error. Therefore its results were excluded from Fig. 4 and Table 2.

⁴ This may have been an artifact of the relatively short incubation times. The merit of doubling the incubation time is currently being assessed.

MPN results for Cell 2 demonstrate that it is possible using simple treatments to substantially increase the growth rate of these organisms (Table 3). The biomass in cells 1 and 3 did not appear to respond significantly to the treatments when compared against the control. There are a number of possible explanations why proven treatments would not stimulate bacterial growth in these cells. Mechanical aeration can drive moisture out of the soil matrix. Thus, as in the case of cell 1 where moisture is not being added, soil desiccation can occur and lead to a population die-back. Alternatively the aeration may have initiated a period of rapid microbial growth which accelerated the consumption of available nutrients again causing population shrinkage. An available nutrient analysis done in May would seem to support this theory since it showed cell 1 as having only 2 mg/kg of available nitrate. Nitrogen stress might also explain why from May 21st to June 24th, when the temperature was well suited to bioremediation and sufficient natural moisture was available, no TPH removal occurred in cell 1 (Figure 3). Nutrient and moisture stress would not be a factors limiting microbial growth for cell 3 but oxygen availability may be. Oxygen can passively diffuse up to one metre into the surface of most treatments soils, but the depth it penetrates and final concentration is dependent on soil type, degree of compaction and moisture content (Koning et al, 1999). It may be that the oxygen concentration in the surface profile of cell 3 can sustain only a limited population even in the presence of ideal moisture conditions and excess nutrients. This could explain why both non-aerated cells have similar bacterial concentrations.

The reductions in TPH seen over the treatment period correlated well with the decreases seen in *n*-alkane concentration. Cell 1 had a 52% TPH reduction and a 50% decrease in combined alkane concentration; cell 2 showed 62% TPH reduction and 61 % alkane reduction and cell 3 showed 35% and 49% respectively (control had 9% TPH loss and 0% alkane loss). This demonstrates that all treatments positively influenced the rate and extent of biodegradation relative to the control. This was further confirmed by comparing the time zero chromatograms for all treatments with the day 136 chromatograms which show area reductions in all peaks from C₁₆ – C₃₄ (excluding Phytane) (unpublished data).

Data from Environment Canada for the Calgary area shows that during the cold treatment period (January 5th – May 21st) there were 92 days where the daily maximum temperature rose above 0°C and 61 days where the daily mean temperature was above 0°C. This is a substantial amount of time where bioremediation could occur during a period that is traditionally considered non-productive for landfarming. In areas which do not experience regular Chinook cycling, such as Northern Alberta, or areas where prolonged periods of sub-zero temperature are the norm, ex situ bioremediation may not be the best option in the winter. However, such areas could see benefits from well designed land treatment systems in early spring where rapid TPH reductions are achievable as soon as daytime temperatures begin rising above zero.

This study confirms that the bioremediation of alkane based hydrocarbons by ex situ treatment is feasible at low temperatures. The rate and extent of hydrocarbon removal can be significantly improved by using a treatment regime which combines mechanical aeration, nutrient amendments and moisture optimization.

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