

Use of a Bioreactor with an Indigenous Microbial Population to Remediate Hydrocarbons – Pilot Scale Test Conducted at Former Oilfield Landfill

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ABSTRACT

Residual hydrocarbons adsorbed to the soil present challenges for remediation due to the difficulty in physically removing the residual product and the associated time and cost requirements to do so. In addition, site conditions of fractured bedrock limit both the technical and the cost effectiveness of traditional technologies, such as pump and treat, to address the issue due to difficulties in defining specific flow pathways and potentially significant volume of waters to be treated.

The pilot-scale project tests the assumption that enhanced microbially mediated hydrocarbon degradation will be effective in remediating the Site. Ex-situ growth of a super-concentrated population of microbes indigenous to the pilot location was followed by injection into the subsurface. The effectiveness of the microbes in remediating the hydrocarbons was monitored. Once the microbial/nutrient plume has moved past downgradient monitoring points, groundwater from the nutrient/microbial plume will be collected and placed in a tank to be used as inoculum for growth of a higher concentration of microbes. Since the population has already been enhanced in numbers and acclimatized to the in-situ conditions, it is anticipated that the inoculum will result in a larger and more rapid growth of microbes to be re-injected into the ground. The effectiveness of the microbes in remediating the hydrocarbons and the type of microbial response will be monitored throughout the pilot scale.

INTRODUCTION

The oil and gas industry is faced with the challenge of dealing with numerous sites impacted by past practices that reflect the state of the industry at the time of impact. In particular, the oil and gas industry is presently grappling with methods of cleaning up residual weathered hydrocarbons, which are frequently sorbed to the geological matrix. Often traditional remedial methods are either cost-ineffective or physically unable to obtain a level of remediation sufficient to obtain site closure.

The purpose of the pilot-scale project is to address residual hydrocarbon impacts at the Site. The pilot-scale project is being conducted to evaluate the ability of a bioreactor to increase rates of remediation at the site. The bioreactor uses enhanced natural attenuation

through nutrient injection, coupled with growth of an elevated population of indigenous microbes, to attenuate and remediate the hydrocarbons at the pilot location.

The scope of the pilot-scale test includes the following three phases:

- 1) Injection of nitrate containing fertilizer at the pilot location. Nutrient injection was undertaken for three reasons; first, it allowed evaluation of the efficiency and success of the local consortia of microbes to remediate the existing hydrocarbons; secondly, it minimized the potential for nutrient availability to be the limiting factor in microbial biodegradation; and finally, it allowed for the evaluation of the degree to which nitrate is the limiting factor.
- 2) Ex-situ growth of a concentrated population of microbes indigenous to the pilot location, followed by injection into the subsurface. The effectiveness of the microbial consortia in remediating the hydrocarbon-impacted groundwater was monitored.
- 3) Following the first injection of microbes and associated monitoring, a second consortia of microbes was cultured and injected at the pilot location. The consortia was collected in a well downgradient of the injection well at the pilot location. It is expected that the initial microbial population will grow and thrive within the substrate and reinjection of this microbial population, following a period of enhanced ex-situ microbial growth, allowed for a greater population of acclimatized microbes. Following this ex-situ growth, the second injection took place at the pilot location and the effectiveness of the second-growth microbial consortia was and is being monitored.

Groundwater was and will continue to be monitored for changes in hydrocarbon concentrations and indicator parameters for biodegradation, including dissolved oxygen (DO), oxygen reducing potential (ORP), pH, oxidative/reductive pairs, and biological oxygen demand (BOD) during the course of the pilot test.

BACKGROUND

The Site is a former oilfield landfill used to support oilfield operations from the 1950s and 1960s up until its use was discontinued in 1985. The landfill was used for the disposal of inert material, produced water (300,000 to 400,000 L) and hydrocarbon-impacted soil. Landfill pits (unlined) were dug and filled as necessary. The site also had storage tanks, a wellhead and a boneyard. The Site is located on a topographic high relative to the surrounding landscape. Remedial activities in 2000 altered the surface topography of the site by creating a depression in the center of the landfill.

The surficial geology at the site consists of clay till, with minor amounts shale and sandstone fragments. The till ranges in thickness from 1.6 to 1.8 m in the area of the pilot study, but is up to 2.8 m thick at the site. The bedrock at the site consists of interbedded sandstone, shale and mudstone. The sandstone is tan to light brown in color and medium to coarse-grained, with shale interbeds 1-5 mm thick. The sandstone ranges from

competent to highly fractured. The sandstone is interbedded with fine-grained dark grey mudstone and shale. The average bedding orientation of the bedrock is $165^{\circ}/45^{\circ}$, with a main set of fractures parallel to the bedding and a secondary set of fractures oriented $046^{\circ}/78^{\circ}$ (EBA, 1998). The site is dominated by a main fracture system running through the middle. The fracture system is associated with the sandstone bedding planes (Figure 1).

Groundwater at the site occurs in the bedrock, at a depth of 7 to 8 m below grade, in the vicinity of the pilot study. The pit excavation appears to act as a recharge area in the spring and impacts localized groundwater flow patterns. This pattern is not reflected in the near-surface wells and water table contour map in the fall. Contouring of the groundwater elevations at the site yields a groundwater flow direction toward the west and southwest. Based on difference in the groundwater elevations in deep and intermediate wells on the site during spring and fall sampling events, it appears that the main fracture acts as a recharge area in the spring and has a greater effect on localized groundwater flow patterns in the spring than in the fall.

Borehole logs displayed clean bedrock interrupted by discoloration in the fractures. Previous remedial activities failed to remove these sorbed hydrocarbons in the fractures.

METHODOLOGY

Pilot –Scale Set-Up

A series of 6 wells are considered part of the pilot-scale set-up (Figure 2). The wells are installed in two lines located perpendicular to one other. The first row of wells is located along the main fracture plane and consists of BH00-06, BH04-22, BH04-21 and BH04-20; screens of these wells are located approximately a 3 m distance from each other. The second set of wells are located perpendicular to the fracture and hydraulically downgradient of BH00-06, based on groundwater contour maps created using water level measurement data. Boreholes BH00-06, BH04-23 and BH00-13 have screens located approximately 3 m from one another along the groundwater flow path.

All six boreholes were logged during installation. The monitoring wells were constructed of 50 mm ID Schedule 40 PVC pipe. The screens are 6 m in length and machine-slotted, with the bottom of the screen located approximately 10 m (i.e. 9.3 m to 10.4 m) below grade. The annulus between the borehole wall and the well standpipe was backfilled with Sil 9 sand to an elevation of approximately 0.3 m above top of screen. The remainder of the annulus between the borehole wall and the well standpipe was filled with bentonite chips and hydrated. All of the monitoring wells were completed above grade, with a stick up of approximately 1 m (0.91 m to 1.09 m).

The infrastructure for the pilot-scale test was set up as follows. A vacuum pump was attached to BH04-23 using 4-inch diameter Schedule 41 PVC pipe. The vacuum pump was used to withdraw water from BH04-23 and place it in a 300 L tank for use in the

Figure 1. Geology of the Study Area

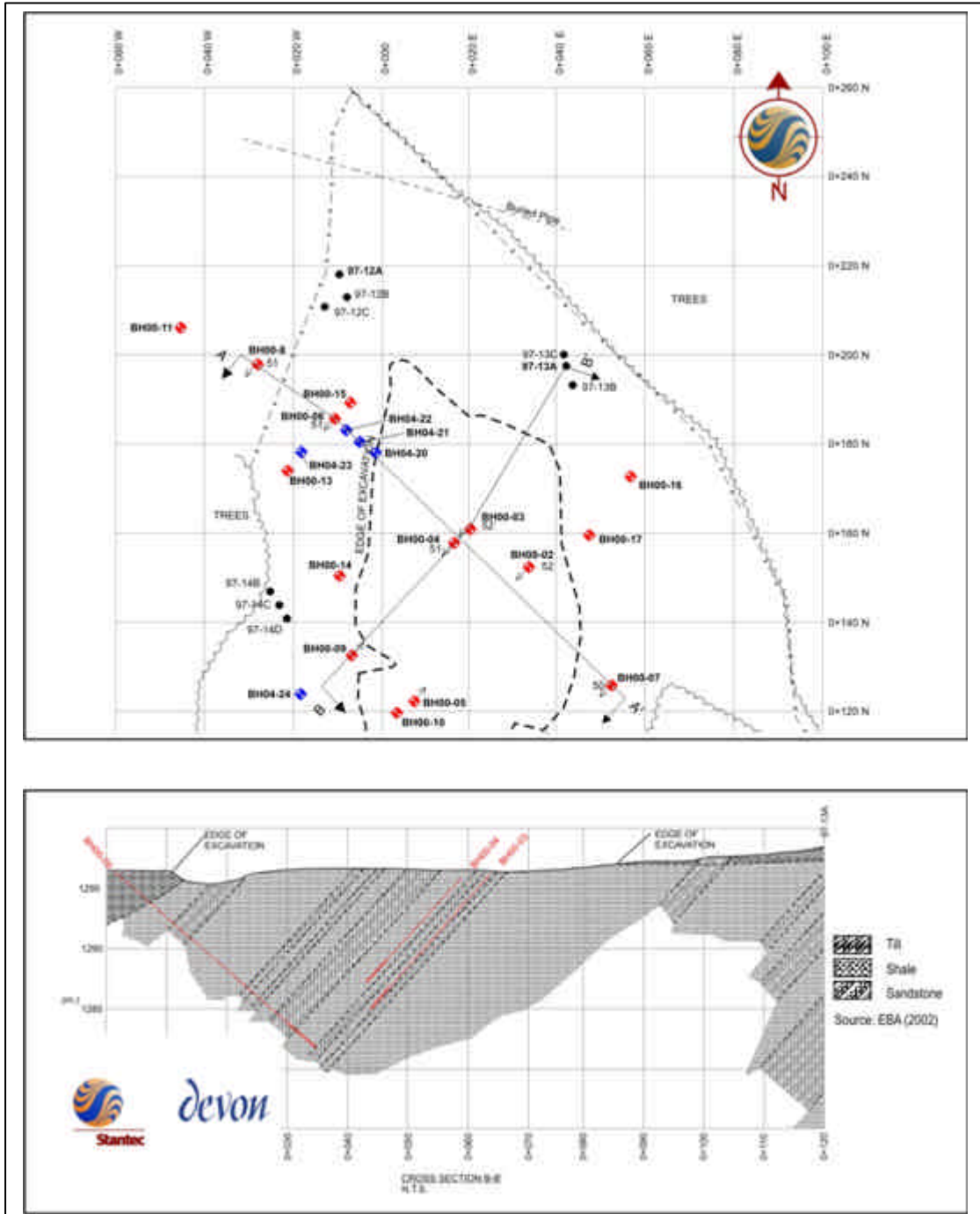
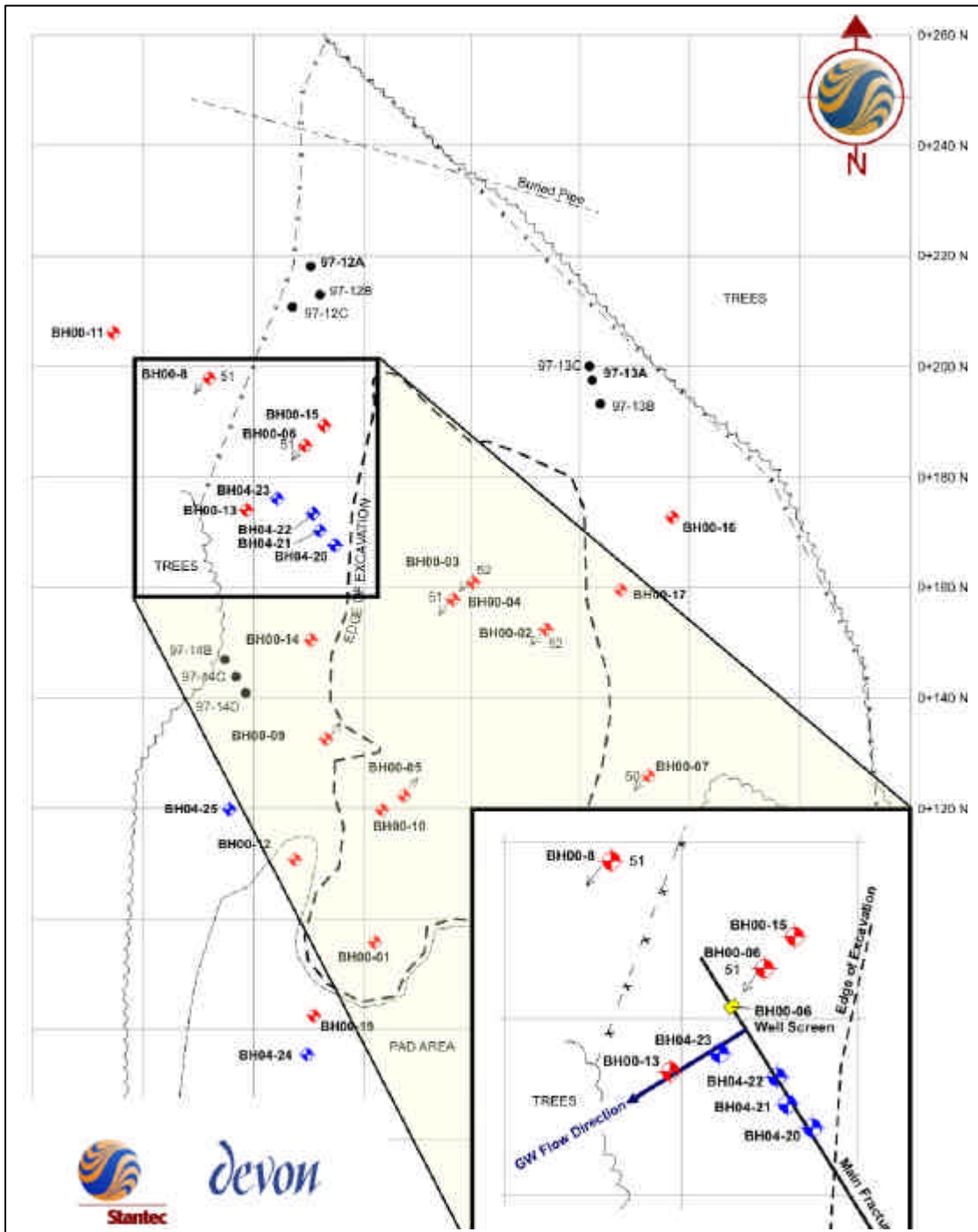


Figure 2. Pilot Scale Test Layout



initial nutrient injection. A hose was run from the spigot at the base of the tank to BH00-06. BH00-06 was used as an injection well during the course of the experiments.

Water withdrawn from BH04-23 was also used in the microbial consortia growth and injection. A hose was run from BH04-23 to a 18,000 L holding tank, located on a topographic high to the northeast of the pilot location. The tank was equipped with an aeration line run off of an air compressor. A hose was run from the spigot at the base of the tank to BH00-06.

Initial Site Characterization

The initial site characterization consisted of documentation of borehole logs, slug tests in the newly completed wells and an initial groundwater-sampling program to benchmark groundwater quality prior to initiation of the pilot-scale test. Wells located in the pilot-scale location were field sampled for pH, conductivity, temperature, dissolved oxygen and total dissolved solids (TDS). The samples were submitted for laboratory analysis for benzene, toluene, ethylbenzene and xylenes (BTEX), Canadian Council of Ministers of the Environment Fraction 1 (C6 to C10) and 2 (C11 to C16) petroleum hydrocarbon (CCME F1 and F2 PHC) analyses, nitrate/nitrite, dissolved ferric/ferrous iron, sulphate/sulphite (redox pair) analyses, and BOD. In addition, groundwater samples were collected from the downgradient monitoring wells (BH04-20, BH04-21, BH04-22 and BH04-23) prior to the pilot initiation, and were analyzed for microbial concentrations. The samples were tested for heterotrophic plate counts and the most probable number (MPN) analyses for nitrate, iron and sulphate reducing bacteria.

Addition of Nitrate to BH00-06

Water was removed from BH04-23 and placed in a 300 L tank on site. Approximately 15 mL of CIL Green-Up liquid fertilizer, containing 21% nitrogen (approximately 3 g of nitrogen in total) was added to the tank to make a solution of 11 mg/L nitrate. The water in the tank was then mixed and gravity drained into BH00-06 on 28 June 2004 (Day 1 of the pilot test). Groundwater was monitored regularly (Day 1, Day 15 and Day 22 of the pilot scale test) for changes in hydrocarbon concentrations and indicator parameters for biodegradation, including DO, ORP, pH, redox pairs and BOD. In addition, BTEX and CCME F1 and F2 PHC concentrations were also analyzed.

Primary Growth and Injection of Microbial Consortia

The initialization of the bioreactor required the collection of impacted groundwater from the BH04-23 following Day 1 nitrate additions at BH00-06. Approximately 17,000 L of groundwater were placed in the 18,000 L tank to provide microbial consortia to act as a seed population. The tank was provided with a hydrocarbon food source, a nitrate containing fertilizer to act as a nutrient source, and a continuous air source bubbled through the tank, in an attempt to optimize conditions for microbial growth.

Samples were collected from the tank on a weekly basis to evaluate and document the chemistry of the water and allow adjustment in response to the changing conditions within the tank. The tank water was measured for field parameters including pH, conductivity, temperature, DO and TDS. The samples were submitted for laboratory analysis of BTEX, CCME F1 and F2 PHC, redox pairs and BOD. A sample was also collected from the tank on Day 23 of the pilot test to document the microbial population in the tank prior to injection that same Day. The samples were tested for heterotrophic plate counts and the MPN analyses for nitrate, iron and sulphate-reducing bacteria.

Following weekly measurement of the field-screening parameters and collection of laboratory samples, the nutrient and hydrocarbon levels within the tank were adjusted. From Day 7 to Day 23 of the pilot scale test, a total of approximately 900 g of nitrate and 1.34 L of weathered free-product was introduced into the tank. The tank was allowed to gravity drain into BH00-06 on Day 23 of the pilot-scale test following approximately two weeks of microbial growth within the tank. Groundwater samples were collected from monitoring wells in the pilot study area on a biweekly basis (Day 37 and Day 51). Due to depleted nutrient levels in the tank waters immediately prior to injection and nutrient levels in monitoring wells following injection, additional nitrate (300 g) was added directly to BH00-06 on Day 37 of the pilot test. Additional nitrate was added on Day 51 (300 g) and Day 65 (200 g) of the pilot test to ensure that nutrient levels were not a limiting factor to microbial degradation.

Secondary Growth and Injection of Microbial Consortia

The secondary groundwater collection event was conducted at BH04-23 between 17 August 2004 and 19 August 2004 (Days 51 to 53 of the pilot test) to provide an acclimatized microbial consortia to act as a seed population. Approximately 2200 L of groundwater was placed in the 18,000 L tank. The tank was provided with a hydrocarbon food source, a nitrate containing fertilizer to act as a nutrient source, and a continuous air source bubbled through the tank, in an attempt to maximize the microbial population size.

Samples were collected from the tank on a weekly basis (i.e. Days 53, 58 and 65 of the pilot test) to document the chemistry of the water and optimize the conditions for microbial growth. The tank water was measured for field parameters including pH, conductivity, temperature, dissolved oxygen and total dissolved solids. The samples were submitted for laboratory analysis for BTEX, CCME F1 and F2 PHC, redox pairs and BOD. A sample was also collected from the tank on 31 August 2004 (Day 65 of the pilot test) to document the microbial population in the tank prior to injection. The samples were tested for heterotrophic plate counts and the MPN analyses for nitrate, iron and sulphate-reducing bacteria.

Following weekly measurement of the field-screening parameters and collection of laboratory samples, the nutrient and hydrocarbon levels within the tank were adjusted. From Day 53 to Day 65 of the pilot scale test, a total of approximately 500 g of nitrate and 1.0 L of weathered free-product was introduced into the tank. Due to mechanical problems, air was not bubbled into the tank from Day 58 to Day 65 of the experiment.

The tank was allowed to gravity drain into BH00-06 on Day 67 of the pilot-scale test, following approximately two weeks of microbial growth within the tank. Groundwater samples were and will continue to be collected from monitoring wells in the pilot study area on a biweekly basis (starting Day 65 of the pilot test).

RESULTS AND DISCUSSION

Initial Site Conditions

With the exception of hydrocarbon and microbial distribution, the pilot-scale location showed relatively uniform conditions throughout, prior to conducting the test. Field screening of groundwater conditions indicated the following conditions: EC of 1494 $\mu\text{S}/\text{cm}$ (BH04-22) to 1560 $\mu\text{S}/\text{cm}$ (except for BH04-13 with EC of 2354 $\mu\text{S}/\text{cm}$); TDS of 971 mg/L to 1526 mg/L (except BH04-23 with TDS of 4013 mg/L); DO of 0.53 mg/L to 2.61 mg/L, indicating depressed concentrations; and ORP of 213.1 mV to 282.2 mV. Anomalously high EC and TDS values at the two wells decreased rapidly in subsequent sampling events to the range of concentrations observed at the other locations within the pilot test area, suggesting that the initial high values may have been an artifact of initial drilling and establishment of the wells.

Hydrocarbons exceeding CCME guidelines (i.e., F2 and/or benzene) were found in all wells except BH00-13. CCME F2 PHC appears highest along the main fracture system (i.e., 91 to 300 mg/L) with a lower concentration at BH04-23 (16 mg/L). Benzene was not found in groundwater collected from BH00-06 and BH00-13, was highest in BH04-23 (0.11 mg/L) and ranged in concentration from 0.013 mg/L to 0.015 mg/L along the main fracture, exempting BH00-06.

The redox pair results indicate that the nutrient concentrations are generally low: no nitrite is found and nitrate was only detected in BH00-06 (0.14 mg/L) and BH00-23 (10.9 mg/L). Dissolved ferric (1.6 mg/L to 27.7 mg/L) and ferrous (0.6 mg/L to 44.2 mg/L) iron varies in both concentration and dominance at each well. Sulphate (10.2 mg/L to 253 mg/L) was found in all wells, whereas sulphite was not present at detectable levels in any of the wells.

The wells located adjacent to BH00-06 (i.e., BH04-22 and BH04-23) had the largest population of microbes and the most varied microbial consortia. In general, aerobic bacteria are dominant at the pilot location, followed respectively, by nitrate reducing bacteria, iron reducing bacteria, and sulphate-reducing bacteria (Table 1).

Table 1. Initial Pilot Site Microbial Populations in Groundwater

Bacteria Class	BH04-20	BH04-21	BH04-22	BH04-23
Heterotrophic (cfu/mL)	3.1×10^5	5.4×10^3	3.2×10^3	4×10^4
Denitrifying (MPN/mL)	70	70	4.9×10^2	2.2×10^4
Iron Reducing (MPN/mL)	0	0	1.3×10^2	4.6×10^3
Sulphate Reducing (MPN/mL)	0	0	0	1.3×10^2

cfu – colony forming units

MPN – most probable number analyses

Addition of Nitrate to BH00-06

After injection, the following observations in groundwater well conditions were made. Field pH appears to have increased in all wells from initial values ranging from 3.3 to 4.58 to Day 23 values ranging from 6.47 to 7.35. Field conductivity and TDS, and laboratory sulphate and dissolved ferrous iron results appear to increase strongly in wells located along the main fracture, but not in the groundwater flow direction perpendicular to the fracture. Field conductivity along the main fracture increased from initial values on Day 1 ranging from 1594 $\mu\text{S}/\text{cm}$ to 1543 $\mu\text{S}/\text{cm}$, to Day 23 values of 1892 $\mu\text{S}/\text{cm}$ to 3910 $\mu\text{S}/\text{cm}$. In a similar manner, TDS values along the main fracture have increased from initial values ranging from 975 mg/L to 1002 mg/L, to Day 23 values of 1229 mg/L to 2541 mg/L. Sulphate values along the main fracture increased from initial values ranging from 105 mg/L to 253 mg/L, to Day 23 values of 304 mg/L to 626 mg/L. Dissolved ferrous iron concentrations (with the exception of BH00-06) have also increased along the main fracture, from initial values ranging from 1.5 mg/L to 19.1 mg/L, to Day 23 values of 21.9 mg/L to 49.3 mg/L.

ORP and F2 CCME PHC levels appear to decrease in all wells except BH00-13, where F2 CCME PHC concentrations are low (0.008 to 0.21 mg/L). Generally no increase or decrease was found in DO (remains below 3.7 mg/L), BTEX, sulphite and nitrite (low to non-detect), ferric and ferrous iron (vary in both dominant form found at a well location and whether concentration increases or decreases were observed) and nitrate.

Based on the preliminary results, based on the increasing pH, decreasing F2, decreasing ORP and no observable increase in nitrate concentrations it appears some biodegradation is occurring. Increases in alkalinity (e.g., reflected in pH) are expected with the biodegradation of hydrocarbons.

Field conductivity, TDS, sulphate and dissolved ferrous iron results appear to increase strongly in wells located along the main fracture. The reason for this may be three-fold. First, just the act of increasing flow of groundwater (through gravity drainage) into the ground may have resulted in stirring up of sediments along large fracture planes, resulting in higher conductivity and TDS. More likely, the oxygenated waters, when allowed to flow into the ground, acted to oxidize ferric sulphide found in formation to elemental sulphur and sulphates. If high levels of both ferrous iron and sulphides were present, insoluble ferrous iron could have formed. Ferrous sulphide imparts a black colour

characteristic of many anaerobic wetlands. It is interesting to note that during the bailing of wells prior to sampling on 20 July 2004 (Day 23), very black groundwaters were observed in water samples collected from BH04-20 and BH04-22. Finally, sulphides may be present in sorbed hydrocarbons that coat the surfaces of the fracture planes, and may be released to some extent during bioremedial activities.

Groundwater flowing perpendicular to the fracture did not exhibit an immediate impact. However, based on our understanding of groundwater flow at the pilot location, the effects would be much slower since flow along the main fracture is appears to be greater than along the direction of the groundwater gradient.

The microbial consortia appear to have increased in microbial diversity since initial groundwater conditions prior to injection (Table 2). In addition, with the exception of BH00-06, after three weeks following the nitrate injection, the microbial population along the fracture had shifted from a primarily aerobic bacterial population to one that also has a higher nitrate reducing and sulphate reducing bacterial population. Lack of oxygen has been found to limit abiotic activity below levels of approximately 1 mg/L (Lyman, 1982), and oxygen concentrations in wells BH04-21 and BH04-22 dipping below this concentration during the sampling events.

Table 2. Microbial Populations in Groundwater for Day 23 Following Nitrate Injection

Bacteria Class	BH00-06	BH04-21	BH04-22	BH04-23
Heterotrophic (cfu/mL)	1.3×10^4	2.5×10^2	9.0×10^2	2.2×10^3
Denitrifying (MPN/mL)	$>1.6 \times 10^4$	6.4×10^2	2.4×10^3	9.2×10^3
Iron Reducing (MPN/mL)	1.5×10^2	2.3×10^3	2.3×10^3	2.3×10^3
Sulphate Reducing (MPN/mL)	60	20	20	1.4×10^2

cfu – colony forming units

MPN – most probable number analyses

It appears that the microbial population in BH04-23 has reduced in size, however, the reason for this is largely an artifact of the test protocol. The initial Day 1 microbial plates were incubated at 25°C for 10 Days, whereas, due to laboratory error, the Day 23 microbial samples were incubated at 35°C for 3 to 5 Days. Since the initial Day 1 microbial samples were incubated at a longer time and at temperatures that more closely reflect summer groundwater temperatures, they are seen to more accurately reflect microbial conditions in the subsurface. It is expected the Day 23 results would actually be higher if Day 1 laboratory analysis procedures had been followed.

In summary, the injection of the nutrients and oxygenated waters appears to have increased the diversity of the microbial populations in groundwaters within the pilot test location. This implies that nutrients are a limiting factor to the biodegradation process.

Primary Growth and Injection of Microbial Consortia

The waters present in the tank immediately prior to injection were oxygenated (13.26 mg/L DO) and with a nitrate concentration (11.9 mg/L) below CCME Drinking Water criteria. The only hydrocarbons detected were low level CCME F2 PHC (0.84 mg/L). Aerobic bacteria (6.8×10^5) and nitrate reducing bacteria ($>1.6 \times 10^4$) dominated. Sulphate reducing bacteria were found at concentrations of 4.3 bacteria/mL and iron reducing bacteria were evaluated to be present at <10 MPN/mL. Note that while dissolved ferric and ferrous iron were both at concentrations of 0.02 mg/L, sulphate levels were 66 mg/L (greater than initial background concentrations in the wells).

Nutrients were provided throughout the course of the experiment to minimize the potential that they would be the limiting factor for microbial remediation. The nitrate increased at BH00-06 due to the spot nitrate injection but this is not seen in any of the other wells at the pilot location.

Based on the preliminary results, the increasing pH (ranges from 4.98 to 8.67 in sampling events), decreasing F2 (except at BH04-21 where concentrations increased from 19 mg/L to 53 mg/L), ORP values once again in the negative ranges, and no significant increase in nitrate concentrations except at BH00-06, it appears that some biodegradation is occurring. Increases in alkalinity (e.g. reflected in pH) are expected with the biodegradation of hydrocarbons. Microbial count and MPN analyses results are pending.

Secondary Growth and Injection of Microbial Consortia

Laboratory results have not yet been received to assess the progress of this stage in the pilot test. Monitoring is presently ongoing.

CONCLUSIONS

Initial results appear to indicate that injection of the nutrients and oxygenated waters have increased the diversity of the microbial population and the number of microbes in groundwaters within the pilot test location. The nutrients appear to be a limiting factor to the biodegradation process. The efficacy of the enhanced biodegradation for removing the residual hydrocarbons has not yet been assessed.

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