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MTBE BIOREMEDIATION WITH BIONETS™
CONTAINING ISOLITE®,
PM1, SOLID OXYGEN SOURCE (SOS) OR AIR

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ABSTRACT

MTBE, a gasoline additive, is a persistent and foul tasting contaminant that is more mobile in groundwater than BTEX (benzene, toluene, ethylbenzene, xylenes). It is turning up at many American crossroads. The objective of this well controlled study was to determine if biologically active in situ BioNets could bioremediate MTBE contaminated groundwater. Seven BioNets, most containing 3 fractures each, were placed in a site on the Flathead Indian Reservation in Montana. The MTBE and BTEX plume from a retail gasoline station was contaminating farmland and threatening Native American owned surface waters.

The BioNets contained:

1) Sand or Isolite® as a fracture material, which created bioremediation zones by facilitating inoculation, allowing attachment of the bacteria, presenting a zone for addition of oxygen by way of aeration or addition of Solid Oxygen Source (SOS) and enhancing the porosity/permeability of the subsurface;

2) PM1, an aerobic bacteria known to degrade MTBE, which can be monitored with a genetic probe;
3) Nutrients; and
4) Oxygen as air or SOS.

Results indicate that 12 months after inoculation the reductions of MTBE in the groundwater samples were as high as 85 percent where optimum conditions existed for biodegradation, which included PM1 inoculated Isolite with SOS or air. The use of SOS stimulates more or as much reduction as the use of oxygen as supplied air at various flow rates. After 12 months, DNA of PM1 was isolated from soils from the inoculated BioNets, but not the uninoculated BioNet. PM1 and naturally occurring MTBE degraders were consistently identified on subsurface soil samples using Taqman geneprobe and standard microbial techniques.

INTRODUCTION

One of the goals of the United States Environmental Protection Agency (USEPA) Underground Storage Tank Program is to encourage the demonstration of innovative remediation technologies at petroleum release sites in order to: (1) educate regulators and responsible parties on the effectiveness of these alternative technologies on MTBE and other compounds; and (2) provide cost and performance data for comparison. One of the goals of the USEPA Office of Research and Development, National Risk Management Research Laboratory (USEPA-NRMRL) is to develop and study innovative bioremediation technologies for contaminated sediments, soils, and groundwater. One of the goals of Foremost Solutions Inc. is to develop and prove its patented BioLuxing™ technology of in situ bioremediation of MTBE with BioNets. These three entities, along with their contractors, came together through a Cooperative Research and Development Agreement (CRADA) to develop and study this innovative in situ bioremediation technology at a site with MTBE contaminated soils and groundwater from a leaking underground storage tank.

- **Site Description.**

  George's British Petroleum is located on Highway 93 South in Ronan, Montana, within the boundaries of the Flathead Indian Reservation. In April 1994, a leaking 16,000gallon underground storage tank (UST) was removed. It is estimated that at least 10,000 gallons of product was lost from this tank. Site investigations show that a free product plume is present on groundwater directly west of the tank removal area and has migrated south and west under Highway 93. A 1,500 foot long dissolved phase methyl-tertiary butyl ether (MTBE) plume extends south and west from the free product plume under a grain field to a creek. Our study area is in the grain field, approximately 300 feet south and west of the source, in the dissolved phase MTBE plume. Free product recovery is occurring on site and levels have been reduced from over five feet to less than two feet in five years. An air sparge interceptor trench is in place immediately upgradient of our study area.

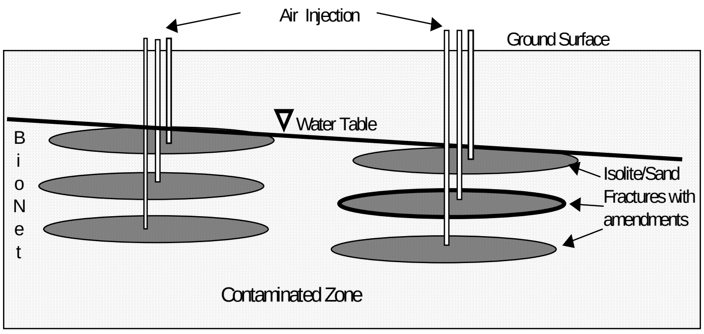
OBJECTIVES OF STUDY

USEPA through this CRADA is investigating the effectiveness of in situ bioremediation through hydraulic fracturing and emplacement of Isolite® (diatomaceous earth) inoculated with PM1 (an aerobic bacteria that degrades MTBE) (Hanson, et al., 1999) on MTBE contaminated soil and groundwater. Air or slow release oxygen supplement (SOS) is supplied to the fractures to enhance aerobic degradation. Six horizontal fracture sets, consisting of three fractures each (BioNets), and a seventh BioNet consisting of one fracture, have been installed by the CRADA team into the dissolved phase portion of the plume, west of Highway 93. The effectiveness of this technology on degradation of MTBE at this site will be determined by monitoring contaminants and microbial activity at fracture locations in the subsurface soil and in groundwater. Data from four of these BioNet treatment zones will be discussed.

PROJECT DESIGN

In October 2000, seven BioNets containing 19 fractures were installed at the study site, at or near the top of the unconfined aquifer at a vertical spacing of approximately two feet apart (Figure 1).

FIGURE 1. Schematic diagram of fractures.



Hydraulic fracturing was utilized as a delivery mechanism to establish favorable in situ bioremediation conditions in the subsurface environment as described in USEPA (1994).
Fracturing procedures included:

1) installing a dedicated steel casing fitted with a drive point;
2) dislodging the drive point downward to expose a short section of open hole;
3) cutting a thin notch in the sidewall at the base of the hole for fracture initiation with a horizontal hydraulic high pressure water;
4) injecting solids-laden slurry downhole, which included the fracture material (sand or Isolite, nutrients, inoculum, and/or SOS) to propagate and form the fracture; and
5) monitoring the injection pressure and surface deformation, to deduce fracture shape (Table 1).

TABLE 1. BioNet components.

BioNet	Fracture Number	Fractures		PM1 Microbes Injected (liters)	Solid Oxygen Source SOS (ft^3)	Air Flow Rate (ft^{3}per day) 10/00-Present			
		material	Amount (ft^3)			10/00-1/01	1/01-6/01	6/01-8/01	8/01-present
BN-1	1	sand	8	8.6	0	84	204	0	957
	2	sand	2	8.6	0	84	204	0	957
	3	sand	13.3	8.6	0	84	204	0	957
BN-2	1	Isolite	7.5	5.4	1.67	0	0	0	957
	2	Isolite	7	5.0	1.7	0	0	0	957
	3	Isolite	2.75	2.8	0.75	0	0	0	957
BN-3	1	Isolite	16.8	8.6	0	84	204	0	957
	2	Isolite	17.4	8.7	0	84	204	0	957
	3	Isolite	5	2.9	0	84	204	0	957
BN-4	1	Isolite	10	0	0	84	204	0	957
	2	Isolite	10	0	0	84	204	0	957
	3	Isolite	3.5	0	0	84	204	0	957

Fracture Characteristics

Wellhead injection pressure was monitored continuously during fracturing. The final upward surface displacement caused by opening the aperture of each fracture, a feature called uplift, was monitored and recorded. The presence of the fractures was confirmed by drilling two-foot offsets from fracture centroids (2 to 8 feet). Isolite (CG-1, 1millimeter) was inoculated prior to injection with microbes and nutrients. Isolite is a porous ceramic material made from diatomaceous earth kiln fired at 1,800 degrees Fahrenheit, with average pore size of 1.4 microns, large surface area (20 m^2/gm) and high porosity (74%). Sand fractures included the use of 10-20 Colorado Silica Sand with an effective size of just over 1.0 millimeter and a uniformity coefficient of 1.55. Fracture diameters are estimated to be as large as 35 feet. These values were calculated using the volumes of solids and liquid injected, the maximum uplift during fracture formation, and with confirmatory sampling.

BioNet performance was evaluated, in part, by reviewing the influence on groundwater as pressurized air flow was applied to a fracture. Several increased pressure tests, or air sparging tests, indicated that the radius of influence of these BioNet treatment zones is in excess of 25 feet from fracture centers. Nutrient supplements were added at installation (October 2000) and in August 2001. Nutrient levels were evaluated as part of the sampling program. Oxygen was provided as SOS or supplied air at various flow rates. PM1 microbes and SOS were provided at installation only.

MATERIALS AND METHODS

Ground water samples were collected in BioNet monitoring wells (BNWs) by USEPA Region 8 UST Program personnel using low flow sampling and purging methodology. BNWs were installed within each BioNet, approximately 10 feet downgradient of the fracture centroids. Analytical work was performed by USEPA Region 8 Laboratory personnel. Standard USEPA quality assurance/quality control procedures were followed, according to USEPA SW846 protocols.

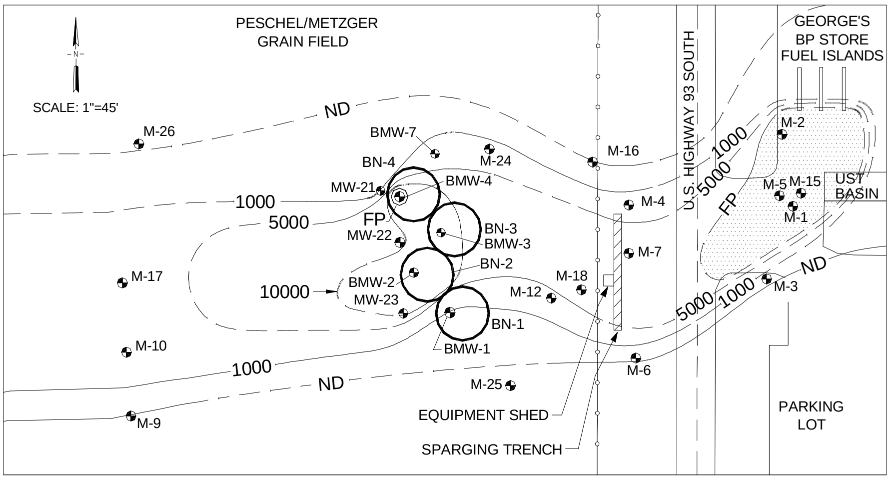


FIGURE 2. Site map before BioNet treatment.

The MTBE quantification analyses were done using USEPA method 8021B and the presence of MTBE was confirmed by mass spectrometry using USEPA method 8260. The presence and quantification of PM1 was done using the method referenced in Hristova, et al., 2001.

The quantification of the naturally occurring MTBE degrading bacteria was done as follows: samples were stored at 4°C until processing; then mixed, split and subsampled; the sample was serially diluted, plated in triplicate, utilizing the Spiral Biotech Autoplate 4000 plater; plates were incubated for 3 weeks in Coleman Ice Chests at 8°C with 2 x 250 ml Erlenmeyer flasks with 1000 mg/l MTBE solution.

RESULTS AND DISCUSSION

BioNets 1-4 all showed reductions in MTBE over the study period. Initial concentrations of MTBE in BioNets 2-4 exceeded 10 mg/l (Figure 2). After 10 months of treatment, MTBE concentrations were reduced to less than four mg/l (Figure 3). The degree of degradation seen in the BioNets is related to a combination of confounding site conditions (continued source and free product) and BioNet contents (Table 1).

Figure 4 shows that the concentrations of MTBE over time were reduced in BioNets. Bio-Net-1 (sand, air, PM1) showed a marked decrease in MTBE concentrations in the beginning of the study. MTBE concentrations rose from May to August 2001, when air was lacking. MTBE concentrations continued to rise after the addition of air in August 2001. This continued rise in MTBE could be due to the activation of the bacteria by the supplied air, causing the area to be enhanced with bacterial surfactant leading to a desorbing of MTBE from the sand, a fingerprint for biodegradation. Alternatively, iron oxide may be forming on the sand that could clog the system and reduce its effectiveness.

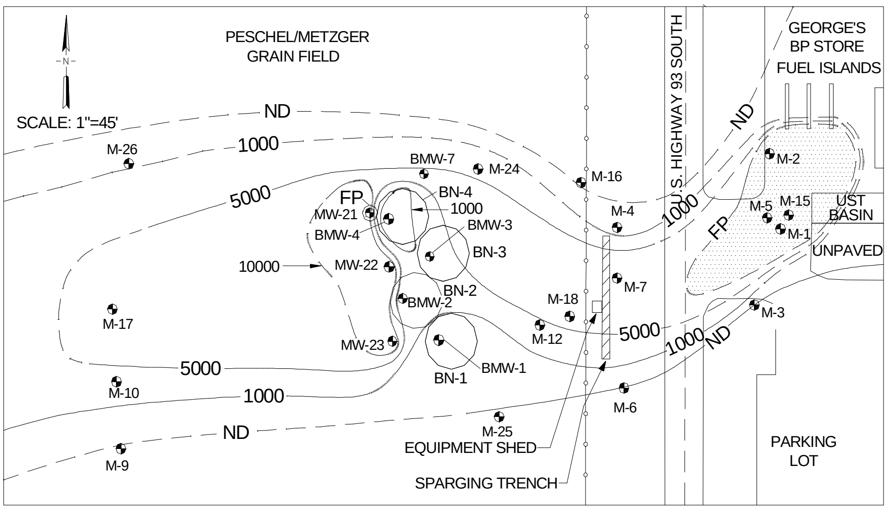


FIGURE 3. Site map after BioNet treatment.

Subsequent sampling will help resolve these observations. BioNet-2 (Isolite, SOS, PM1) demonstrated a steady decrease in MTBE concentration with time, probably because it enjoyed a steady supply of oxygen for 10 months due to the SOS. After August 2001, air was added to the SOS to provide additional oxygen and the decrease in MTBE continued.

BioNet-3 (Isolite, air, PM1) behaved similarly to BioNet-1, early on. However, once air was added in August 2001, there was a dramatic decline in MTBE concentrations. A similar decline in DO was also observed, even though the air supply increased dramatically (Table 1). This indicates that the oxygen provided to Bionet-3 since August 2001, is being consumed by the bacteria that in turn are effectively degrading MTBE. BioNet-4 (Isolite, air, no PM1) showed a decrease in MTBE concentration after August 2001. This is remarkable because the BioNet became saturated with free product that had migrated from the source area, for much of the test period.

The addition of air in August 2001 may have helped the achieve this reduction of MTBE, at least in part, but further data will be required to determine if this trend holds, since the free product has also migrated downgradient.

Table 2 shows the presence of PM1, both at and subsequent to inoculation. BioNets that were inoculated during fracture installation showed a presence of PM1 a year later (except for BN-2 which showed presence for ten months). Where PM1 was not inoculated, it was not found. This also indicates that although BioNets 3 and 4 were not designed to intercept the contaminant plume (i.e. fractures did not overlap) and there was no cross mobilization of PM1 from BN-3 to BN-4 which was 35 feet away.

TABLE 2. Presence of PM1 vs. BioNets vs. time.

BioNet	**PM1 Microbes Inoculated 10/00**	**PM1 Microbes Presence (+) Absence (-)**		
		6/01	**8/01**	**10/01**
BN-1	Yes	+	+	+
BN-2	Yes	+	+	**Not Detected**
BN-3	Yes	+	+	+
BN-4	No	**-**	**-**	**-**

Figure 5 compares the different treatment conditions by normalizing the MTBE concentrations in each BioNet to the initial concentration on December 2000.
See equation 1.

$$\text{Percent Reduction } = \frac{MTBE(Dec00) - MTBE(new)}{MTBE(Dec00)} * 100 \quad (1)$$

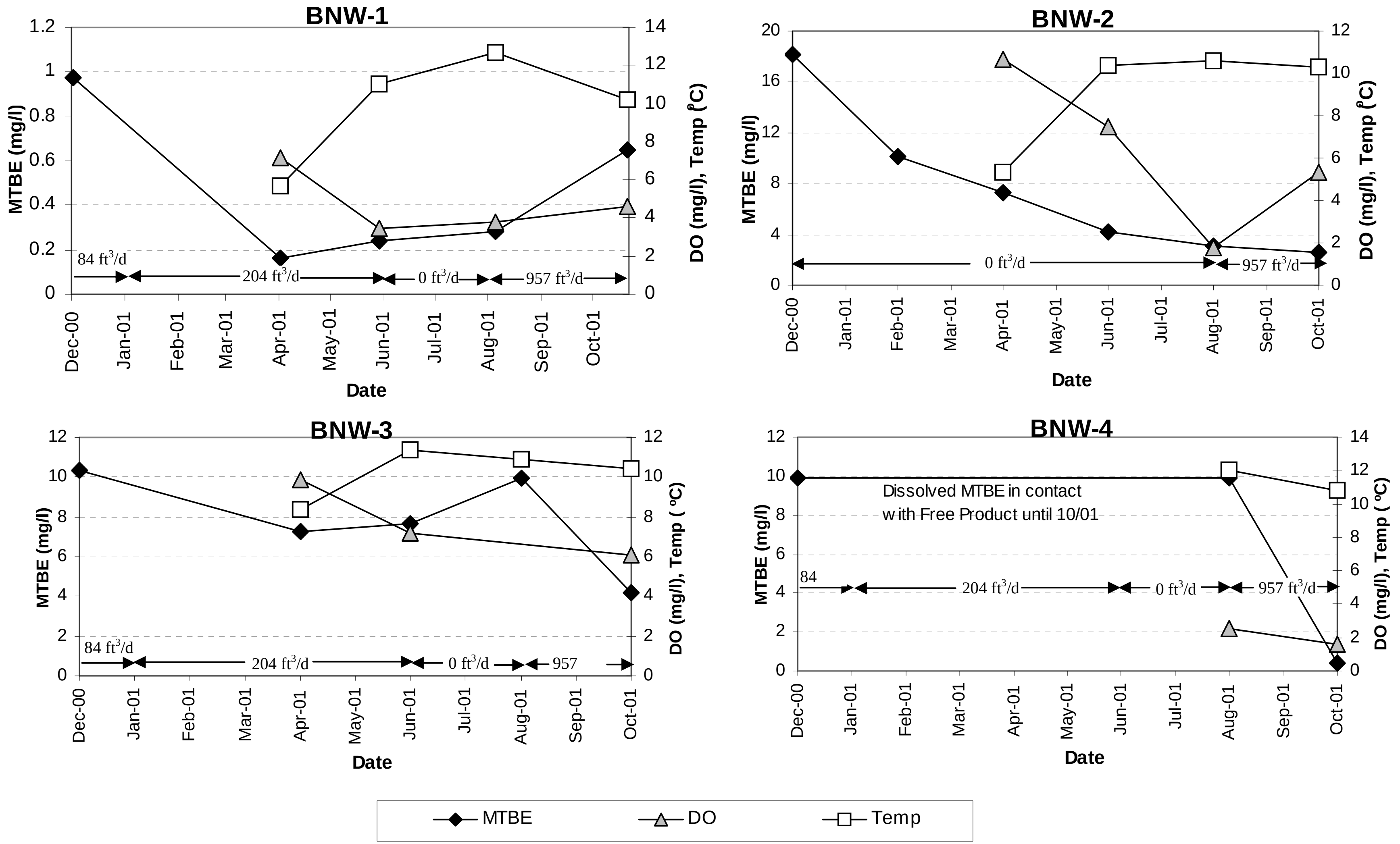


FIGURE 4. BioNets 1,2,3 and 4: MTBE, dissolved oxygen, temperature, supplied air and SOS vs. time

The percent reduction of MTBE is higher by almost 30 percent with the SOS in BioNet-2 (BNW-2) than with the air in BioNet-3 (BNW-3). This indicates that the SOS provides a less drastic (and thus less toxic) concentration of oxygen that is very steady. As we saw in the laboratory (Davis-Hoover, et al., 1991). The bacteria thrive better in this environment.

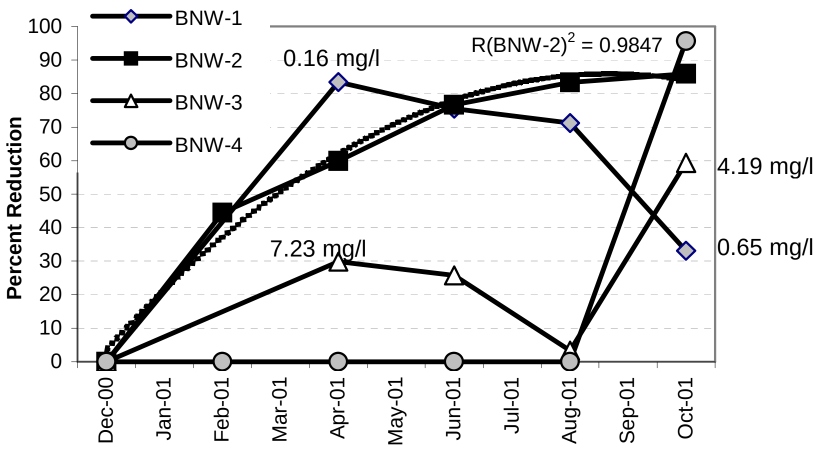


FIGURE 5. Percent reduction of MTBE in BioNets.

BNW-2 reductions demonstrate a good fit to a polynomial equation ($R^2 = 0.9847$), which is characteristic of biological reactions. The final addition of air to the SOS after 10 months, appeared to be utilized by the bacteria as a source of oxygen as the reduction of MTBE continued asymptotically. The presence of PM1 appeared to increase the percent reduction of MTBE (BNW-3 vs. BNW-4) but since BioNet-4 (BNW-4) contained free product for most of the study, additional data needs to be collected. When air was supplied to all treatments in August 2001 and after the naturally occurring MTBE degrading bacteria colonized the Isolite in the fractures (see poster 442 for more data*)*, the reduction of MTBE was significant. This has been documented in various field studies where the initial inoculation of bacteria, such as PM1, seems to account for quicker reductions of contaminant. This reduction can continue as a result of a native degrading bacterial consortium, which is better suited to the site conditions. In addition, BioNet-4 (BNW-4) data indicate that if bacteria are not inoculated in the field, but air and nutrients are supplied to the treatment zone (e.g., Isolite fractures), native degrading bacteria will colonize and begin reducing contaminants,.

Although initial data show greater percent reduction in MTBE in sand fractures (BNW-1) as compared to Isolite (BNW-3), throughout the study MTBE concentrations at these two locations varied by an order of magnitude (Figures 4 & 5). The overall response of these two BioNets is similar until air is reintroduced in August 2001 when a dramatic increase in the percent reduction of MTBE was observed in the Isolite-filled BioNets, indicating that the bacteria are more able to rebound in Isolite than in the sand and possibly that the Isolite is more recalcitrant to surfactant or iron fouling.

CONCLUSION

Four in situ bioremediation treatment conditions were evaluated and compared. The largest and most consistent reductions in MTBE concentrations were seen with Isolite, SOS and PM1 inoculated fractures. The sand fractures produced good reductions initially (BioNet-1), only to rebound with time, as compared to the Isolite fractures.

The Isolite, PM1, and air (BioNet-3) should have shown better performance, but due to equipment problem, a continuous air supply was not provided and therefore conditions were not optimum throughout the pilot study. The SOS was more reliable than the supplied air at this site, due to site location and lack of serviceability on air supply. PM1 inoculation was beneficial for initial reduction of MTBE compared to the naturally occurring and degrading bacteria, however native bacteria were found colonized in the BioNets with time and seemed to perform very well based on observed reductions. The presence of free product in the uninoculated BioNet-4 fractures, limits our ability to unequivocally determine the effectiveness of naturally occurring bacteria at this site and compare their activity to that of PM1. Subsequent data will be collected to look at this issue.

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REFERENCES

Davis-Hoover, W. J., L. C. Murdoch, S.J. Vesper, H. R. Pahren, O. L. Sprockel, C. L. Chang, A. Hussain, and W. A. Ritschel. 1991. " Hydraulic Fracturing to Improve Nutrient and Oxygen Delivery for I*n Situ* Bioreclamation." In R. E. Hichee and R. F. Olfffenbuttel (Eds.*), In Situ Bioreclamation: Applications and Investigations for Hydrocarbon and Contaminated Site Remediation*, pp. 67-82. Butterworth-Heinemann, Stoneham, MA.

Hanson, J. R., C. E. Ackerman, and K. M. Scow. 1999.Biodegradation of methyl *tert*-butyl ether by a bacterial pure culture. Appl. Environ. Microbiol**. 65**:4788-4792.

Heitkamp, M. A. 1997. Effects of Oxygen-Releasing Materials on Aerobic Bacterial Degradation Processes. Bioremediation Journal. **1**(2):105-114.

Hristova, K. R., C. M. Lutenegger, and Kate M. Scow. 2001. Detection and Quantification of methyl *tert*-butyl ether-degrading strain PM1 by real-time TaqMan PCR. Appl. Envn. Microbiol. **67:**5154-5160.

Stavnes, S. A., C. A. Yorke, L. Thompson. 1996. "In Situ Bioremediation of Petroleum in Tight Soils using Hydraulic Fracturing." In E. J. Krause & Assoc., Inc. and Environmental Industry Assoc. (Eds.), *Hazwaste World Superfund XVII Conference Proceedings*, pp. 20-29.
USEPA. 2000. *Sampling and Analysis Plan for George's British Petroleum LUST Site, Ronan Montana.* Region 8 UST Program.

USEPA. 1994. *Alternative Methods for Fluid Delivery and Recovery*. EPA/625/R-94/003.

Vesper, S. J., L. C. Murdoch, S. Hayes, and W. J. Davis-Hoover. 1994. Solid oxygen source for bioremediation in subsurface soils. J. Hazardous Materi. **36**:265-274.