BTEX, MTBE BIOREMEDIATION : BIONETSTM CONTAINING ISOLITE, PM1, SOS OR AIR

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ABSTRACT: BTEX (benzene, toluene, ethylbenzene, xylenes) and MTBE are turning up at many American crossroads. The objective of this controlled study was to determine if biologically active in situ BioNets could bioremediate MTBE and BTEX 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 22 months after inoculation the reductions of BTEX in the groundwater samples were as high as 99.7 percent where optimum conditions existed for biodegradation, which included MTBE degrading 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.

Results also 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 un-inoculated 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 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 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 BTEX and MTBE contaminated soils and groundwater from a leaking underground storage tank.

Site Description. As described in Stavnes et al. (2002).

OBJECTIVES OF STUDY

USEPA through this CRADA is investigating the effectiveness of in situ bioremediation through hydraulic fracturing and emplacement of slow releasing Solid Oxygen Source (SOS) (patent pending) (Davis-Hoover, et al. 1991) (Vesper et al. 1994) (Heitkamp, M.A. 1997), Isolite® (diatomaceous earth) inoculated with PM1 (an aerobic bacteria that degrades MTBE) (Hanson, et al., 1999) in BTEX (and MTBE) contaminated soil and groundwater. Air or 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 BTEX 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 (2) feet apart. Hydraulic fracturing was utilized as a delivery mechanism to establish favorable in situ BioLuxing – the process of creating favorable bioremediation conditions in the subsurface environment as described in USEPA (1994) and Stavnes et al. (2002).

MATERIALS AND METHODS

Ground water samples were collected in BioNet monitoring wells 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. USEPA Region 8 Laboratory personnel performed analyses. Standard USEPA quality assurance/quality control procedures were followed, according to USEPA SW846 protocols. The BTEX and MTBE quantification analyses were done using USEPA method 8021B.

Fracture Characteristics. As described in Stavnes et al. (2002) and in Table 1 below.

RESULTS AND DISCUSSION

Benzene, Toluene, Ethyl benzene and Xylene Degradation

BioNets 1-4 all showed reductions in BTEX over the study period except for ethyl benzene in BN-1. Initial concentrations of benzene up gradient of BioNets 1-4 exceeded 29,400 ppb (Figure 1). After 22 months of treatment, benzene concentrations were reduced to less than 8 ppb in Bionets 1-3 (Figure 2). The degree of reduction seen in the BioNets is

TABLE 1. BioNet Components.

BioNet	Fractures		PM1	SOS	Estimated Air Flow Rate (ft^3/day) 10/00-Present					
	material	amount (ft^3)	Microbe injected (liters)	(ft^3)	10/00 -1/01	1/01-6/01	6/01-8/01	8/01-11/01	11/01 -5/02	5/02-7/02
BN-1	sand	8	8.6	0	84	204	0	957	0	957
	sand	2	8.6	0	84	204	0	957	0	957
	sand	13.3	8.6	0	84	204	0	957	0	957
BN-2	Isolite	7.5	5.4	1.67	0	0	0	957	0	957
	Isolite	7	5.0	1.7	0	0	0	957	0	957
	Isolite	2.75	2.8	0.75	0	0	0	957	0	957
BN-3	Isolite	16.8	8.6	0	84	204	0	957	0	957
	Isolite	17.4	8.7	0	84	204	0	957	0	957
	Isolite	5	2.9	0	84	204	0	957	0	957
BN-4	Isolite	10	0	0	84	204	0	957	0	957
	Isolite	10	0	0	84	204	0	957	0	957
	Isolite	3.5	0	0	84	204	0	957	0	957

After 7/02 the air supply was retrofitted to include a telemetry system for continuous monitoring of flow rates.

influenced by a combination of confounding site conditions (continued source and free product) and BioNet contents (Table 1). Bionet 4 was overwhelmed with free product during the first 12 months of the study.

Figures 3a-d show concentrations of BTEX over time in the BioNets. Different treatment conditions were compared by normalizing the BTEX concentrations in each BioNet to the initial concentration on December 2000 (month 2). See equation 1.

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

BioNet-1 (sand, air, PM1) showed a marked reduction in benzene concentrations in the first 10 months followed by a series of increase and reductions after month 12. Toluene and xylene show a similar pattern. There was no reduction of ethyl benzene in BN-1. These results could reflect good initial growth of degrading organisms on the sand, but when the air supply was compromised in months 8-10 (see Table 1), the culture could not reestablish itself as it did on the Isolite. It has been shown that Isolite has the optimum surface area for bacterial attachment, air space for aeration and contaminant/nutrient interface (Sumitomo, 1993). Alternatively, iron oxide may be forming on the sand that could clog the system and reduce its effectiveness, although this was not verified here.

In BioNet-2 (Isolite, SOS, PM1) SOS was the only source of oxygen during the first 10 months of treatment. Benzene, toluene, ethyl benzene and xylene were degraded in a very consistent manner. When air was supplied in month 10, contaminant reduction continued but not as predictably, after the air was supplied, the treatment conditions duplicated BioNet 3 and the contaminant reductions in the two behaved similarly.

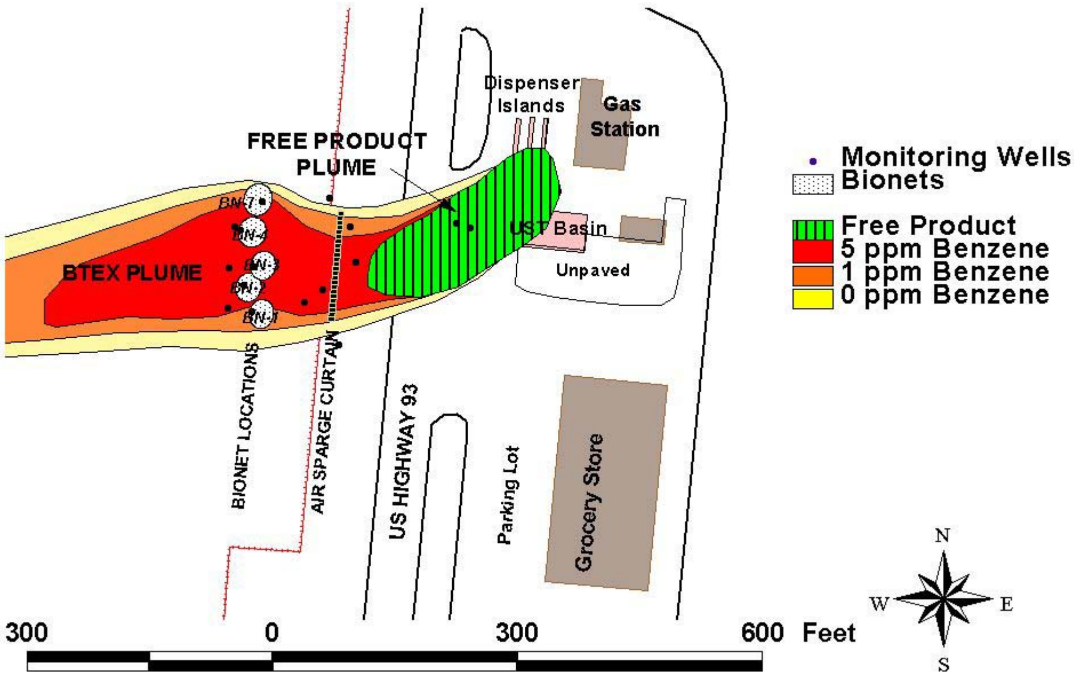


FIGURE 1. Site map at onset of BioNet treatment.

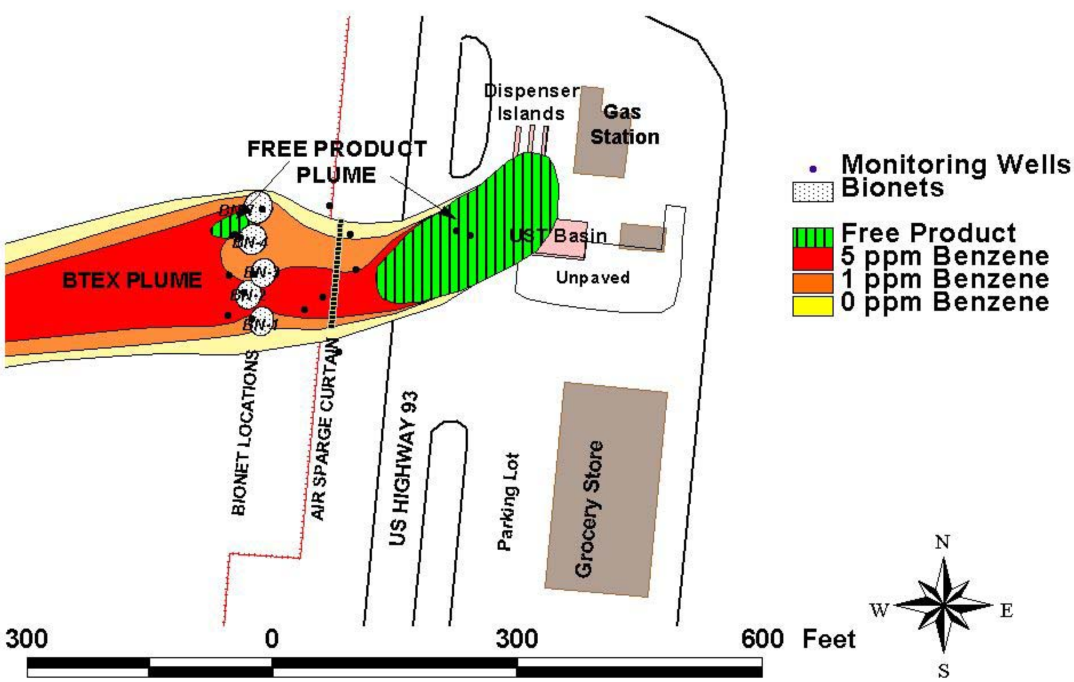


FIGURE 2. Site map after BioNet treatment at 22 Months.

BioNet-3 (Isolite, air, PM1) demonstrated markedly consistent reduction in BTEX over the 22month study.

BioNet-4 (Isolite, air, no PM1) showed no BTEX reduction for the first 10 months as it was overwhelmed with free product during that time. After 10 months, it showed marked reduction in BTEX despite no initial inoculation of the MTBE degrading bacteria.

TPH-G Degradation.

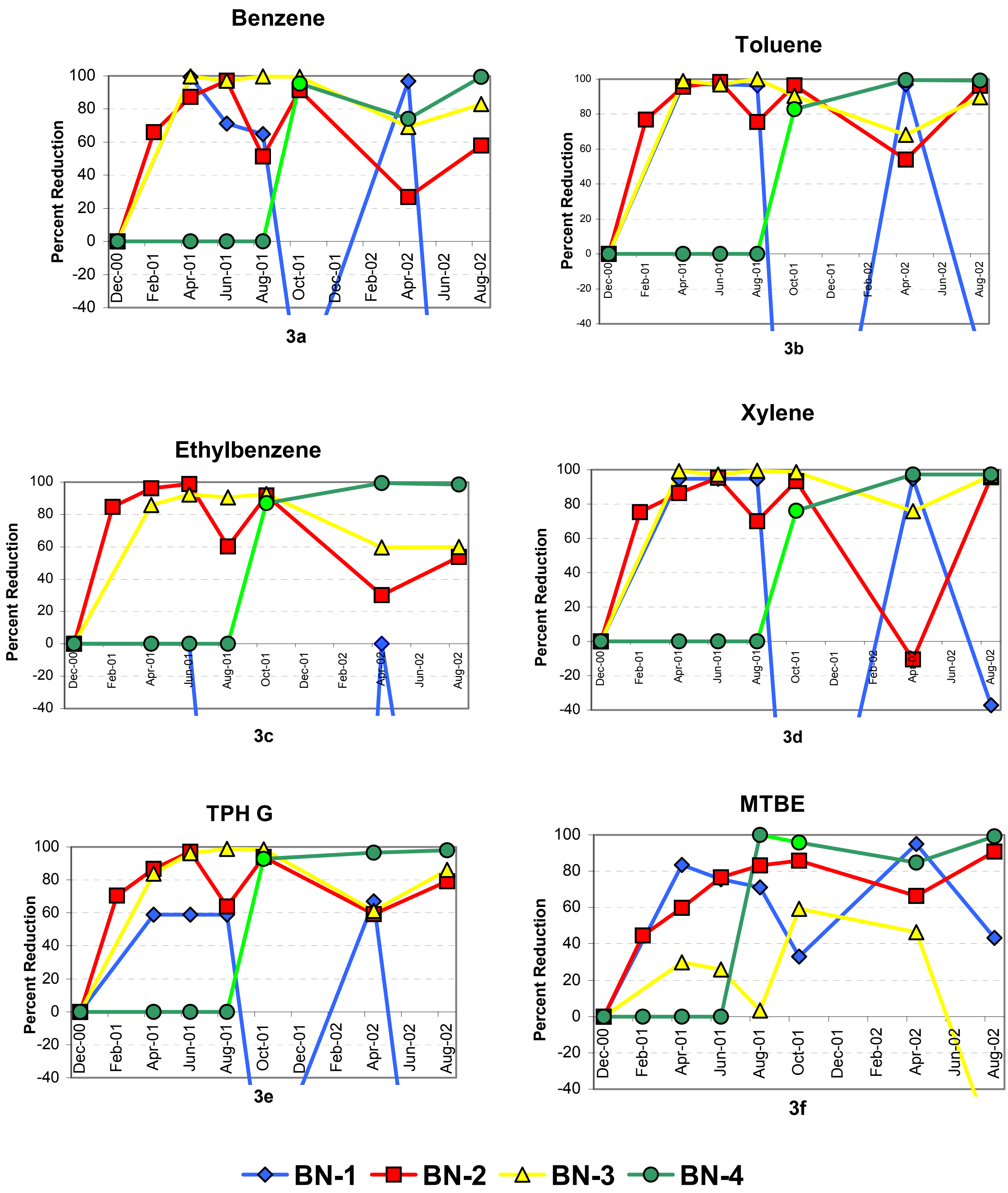
Total petroleum hydrocarbon as gasoline (TPH-G) (Figure 3e) shows a pattern similar to BTEX in BioNet 1, probably due to the loss of air and subsequent problems re-cultivating the sand. BioNet 4 showed no reduction over the first 10 months due to the free product but then responded with close to 100 percent reduction over the next year, similar to BTEX. BioNet 2 showed consistent TPH-G reduction with SOS. (This is interesting as the MTBE degradation showed no lapse with SOS.) When air was supplied to the fractures TPH-G reductions mimicked BioNet 3 as expected.

MTBE Degradation.

As discussed in Stavnes et al, 2002: The percent reduction of MTBE is higher by almost 30 percent with the SOS in BioNet-2 than with the air in BioNet-3 (Figure 3f). This indicates that the SOS provides a less drastic (and thus less toxic) concentration of oxygen that is very steady and the bacteria thrive better in this environment (as confirmed in the laboratory (Davis-Hoover, et al., 1991)). BioNet 2 reductions demonstrate a good fit to a polynomial equation ($R^2 = 0.9847$), which is characteristic of biological reactions (Stavnes et al. (2002). 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 reduction of MTBE (BN-3 vs. BN-4) but since BioNet-4 contained free product for most of the study, additional data needs to be collected before conclusions regarding treatment can be drawn. When air was supplied to all treatment areas in August 2001 and after the naturally occurring MTBE degrading bacteria colonized the Isolite in the fractures (see Slomczynski, and Davis-Hoover. 2002***)***, 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 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 eventually colonize and begin reducing contaminants. Although initial data show greater reduction in MTBE in sand fractures (BN-1) as compared to Isolite (BN-3), this trend did not hold throughout the study. Also, MTBE concentrations at these two locations varied by an order of magnitude

Update on MTBE degradation: After October 01, (see Figure 3f): Excellent consistent reductions in MTBE concentrations continued for BN-2 and BN-4. BN-1 reduction is good but not as consistent, perhaps due to instability of the bacteria on the sand



FIGURES 3a-f. Contaminant percent reductions in BioNets

matrix. BN-3 shows good, then less reduction of MTBE, due to a large increase in upgradient concentration measured during the last sampling event.

CONCLUSION

Four in situ bioremediation treatment conditions were evaluated and compared. The largest and most consistent reductions in BTEX concentrations were seen with Isolite, SOS and air supplied BioLuxing fractures. PM1 (which was designed to degrade MTBE) inoculation of fractures is not as important for BTEX degradation. The sand fractures produced good reductions initially (BioNet-1) at low concentrations, only to rebound with time, as compared to the Isolite fractures. The Isolite, PM1, and air (BN-3) showed better BTEX reduction than MTBE, even with varying air supply conditions. 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 on MTBE and compare their activity to that of PM1, but for BTEX it is clear that the PM1 inoculation was not necessary for significant reductions. Twenty-two months after inoculation the reductions of BTEX in the groundwater samples were as high as 99.7 percent where optimum conditions existed for biodegradation. Twelve months after inoculation the reductions of MTBE in the groundwater samples were as high as 85 percent under optimum conditions.

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