



Demonstration Bulletin

Augmented In Situ Subsurface Bioremediation Process™

BIO-REM, Inc.

Technology Description: BIO-REM's technology is an in-situ subsurface bioremediation process for the treatment of hydrocarbon contaminated soils. BIO-REM uses a proprietary product, "H-10", of microaerophilic microorganisms and micronutrients, in conjunction with minor components of surface tension depressants and penetrants used to enhance introduction into the pore spaces of the soil. The H-10 slurry does not contain hydrogen peroxide or any other oxygenating compounds. The BIO-REM process was demonstrated on JP-4 (jet fuel hydrocarbons) contaminated soils at Williams Air Force Base, Arizona between May 1992 and June 1993.

BIO-REM claims that the microaerophilic bacteria require minimal oxygen to survive and utilize hydrocarbons as electron acceptors. Therefore, subsurface oxygen concentration is not a limiting factor since the microorganisms can exist and propagate under very low oxygen tensions. They state that no additional sources of oxygen nor additional nutrient treatments are necessary during the course of the remediation.

The H-10 slurry is prepared on-site and above-ground and placed directly into borings and/or wells previously drilled into the contaminant plume.

According to BIO-REM, implementation of the technology consists of the following steps:

1. Definition and characterization of the contaminant plume including the geology, hydrogeology, and gradient of the site, and the dimensions of the contaminant plume.
2. Determination of application methodology. In permeable soils, borings and wells drilled into the contaminant plume are sufficient. Soils containing fine sands and clays may also require lancing.
3. Initialization and propagation of H-10, and placement (inoculation) of the batch solution into the contaminant plume. The batch solution is placed into the contaminant plume by the application methods described in Step 2. The quantity of culture and batch solution placed into the contaminant plume is determined by a proprietary formula, and is based on the type and level of contamination.

Waste Applicability: According to BIO-REM, a wide range of hydrocarbon contaminants can be treated by their bioremediation process. Typical contaminants include gasoline, diesel, JP series

aviation fuels, and other wastes containing polyaromatic hydrocarbons, pentachlorophenols, trichloroethene, and dichloroethene. BIO-REM claims that over two hundred hydrocarbon compounds have been identified as being amenable to their treatment process.

Demonstration Activities: The BIO-REM in situ process was tested at the former Liquid Fuels Storage Area on Williams Air Force Base near Phoenix, Arizona. Soils at the 0.9 acre site are contaminated with hydrocarbons derived from JP-4 jet fuel as a result of leaking underground storage tanks and pipelines. The tanks and pipelines were removed, however, significant hydrocarbon contamination exists in the soil from approximately four feet below land surface (BLS) to the groundwater table at approximately 225 feet below surface.

BIO-REM, through a sub-contract arrangement, was retained by the U.S. Army Corps of Engineers to remediate soil at the site from four feet to twenty-five feet below land surface. The EPA SITE Demonstration Program was invited to participate in the demonstration of BIO-REM's bioremediation process.

BIO-REM claimed that their in situ process would reduce organic contaminant levels in soils (4 to 25 feet BLS) from the Liquid Fuels Storage Area to less than the following project-specific clean-up levels:

CONTAMINANT	CLEAN-UP LEVELS
TRPH *	100 mg/Kg
Benzene	0.130 mg/Kg
Toluene	200 mg/Kg
Ethylbenzene	68 mg/Kg
Xylenes	44 mg/Kg

* Total Recoverable Petroleum Hydrocarbons

In order to deliver the H-10 slurry (microaerophilic bacteria and micronutrients) to the sub-surface soils, 315 borings were drilled to a depth of thirteen feet BLS across the site. The borings were placed on ten foot centers in areas of low to moderate contamination, and on five foot centers in areas of high sub-surface contamination. The H-10 slurry was prepared above-ground in tanks using groundwater from the site. Approximately 100,000 gallons of the H-10 slurry were placed into the sub-surface through the network of borings. BIO-REM claimed that the process would require approximately six months to achieve the site-specific remedial goals and that no additional amendments or oxygen sources would be necessary. As a means of monitoring

the progress of the remediation, BIO-REM utilized periodic vapor analysis for hydrocarbons from several open boreholes throughout the site.

The progress and effectiveness of the bioremediation technology was evaluated by the SITE Program by comparing baseline (time 0) soil contamination levels to contamination levels at one month, three months, and six months after inoculation. During each sampling event, twenty borings, within a highly contaminated portion of the site, were used to evaluate the process. These borings were divided into four depth intervals from four feet BLS to twenty-five feet BLS. All samples were analyzed for benzene, toluene, ethylbenzene, and xylenes (Method SW846 5030/8021) and Total Recoverable Petroleum Hydrocarbons (TRPH) (Method SW846 3550/EPA 418.1). In addition, selected samples were analyzed for Total Petroleum Hydrocarbons (TPH), as per the California Leaking Underground Fuel Tanks Manual Method for diesel contaminated soils.

Demonstration Results: Results from the Demonstration indicate that the BIO-REM process was unsuccessful in reducing target contaminants in the soil to the project clean-up levels.

Baseline sampling indicated that a majority of the soil samples were significantly higher than the cleanup levels of 130 ppb for benzene and 100 ppm for TRPH. Furthermore, soil samples analyzed one and three months after inoculation did not show significant reductions in benzene or TRPH contamination (Table 1). The lack of progress in the remediation prompted concerns regarding the effectiveness of the technology. It was jointly decided between the SITE Program and BIO-REM to collect sixteen samples (four boreholes) at six months to determine the progress of the remediation at the predicted end of the project. Results

from the six month sampling event also indicated a lack of significant reduction in contaminant concentrations.

Based on these results, BIO-REM submitted a request to the Air Force to re-inoculate the site based on their assessment that sub-surface lithological conditions inhibited the remedial process. In March of 1993 BIO-REM re-inoculated the site by injecting approximately 35,000 gallons of H-10 slurry into 104 boreholes deepened to a depth of 23 feet below land surface. The inoculation to deeper depths was implemented to overcome the sub-surface lithological conditions identified by BIO-REM. In June of 1993 a confirmatory sampling event initiated by the Air Force, in conjunction with the SITE Program, indicated that significant contamination existed at the site, and that the re-inoculation was unsuccessful in reducing the target contaminants to the project specific clean-up levels. Based on these results, these site activities were concluded.

A final report will be available in the summer of 1994

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Table 1. Frequency Distribution of Benzene and TRPH for Demonstration Sampling Events

Number of Samples	Benzene (ppb)				
	<50	50-130	130-1,000	1,000-10,000	10,000-100,000
Baseline	33	4	13	26	4
One Month	27	3	20	25	5
Three Months	37	3	12	22	6

Number of Samples	Total Recoverable Petroleum Hydrocarbons (ppm)		
	<100	100-1,000	1,000-10,000
Baseline	27	33	20
One Month	29	34	17
Three Months	22	30	28

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