A SYSTEMS' APPROACH TO IN-SITU BIOREMEDIATION: FULL SCALE APPLICATION

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ABSTRACT: An 18-month, in-situ biological program, specifically aimed at accelerating the current attenuation process such that the owner can sell the property began May 5, 2000. The remedial approach focused on bacterial population management, nutrient augmentation, terminal electron acceptor enhancement, and an efficient subsurface delivery of the enhancement liquids. Specifically, a novel delivery system, utilizing direct push advancement equipment coupled with high-pressure air and liquid systems allowed for uniform lateral distribution of the materials to the impacted capillary fringe zone. The treatment zone has extended from 9 to 11 feet below grade across the 20,000 square foot property. Preliminary analytical results from soils and groundwater at the site indicate that the targeted 18-month remediation time-line will be met. Samples from the groundwater and the soils 300 days into the eighteen-month program indicate total BTEX concentrations in the soils have been reduced an average of 76% with a 92% decrease in toluene. Groundwater BTEX levels have also seen a dramatic decrease, approaching targeted treatment levels in several of the wells within ten months.

INTRODUCTION

There are few communities across the country that have been spared the issue of contamination to their community and individual drinking water supplies. Most often this threat comes from leaking underground storage tanks (USTs) at local gas stations.

When faced with reporting and remedial guidelines from regulatory agencies, the station owner must rely on an environmental consultant to represent the owner's best interests while meeting the reporting requirements. As the process moves from reporting the incident to the investigation phase, the owner must rely on the capabilities of the consultant. In the event that an investigation leads to a remedial action plan and eventual site remediation, the environmental consultant must thoroughly evaluate, recommend and implement a wide variety of remedial technologies. Often, the data available to the consultant, for biologically based remediation focuses on individual elements necessary for effective biological processes. As a consequence, when faced with the conflicting field data and the limited pool of qualified remedial contractors, gas station owners and their consultants are hesitant to recommend biological remediation.

By focusing on a systematic and managed approach to bioremediation in which all the known elements of the biological process are addressed, the feasibility of applying in-situ biological solutions becomes more realistic. These elements include bacterial population enumeration, nutrient evaluation, dissolved oxygen concentration monitoring, contaminant concentrations in both the soils and groundwater, effective delivery mechanisms and understandable data interpretation. To date, most field applications of bioremediation have not focused on all the parameters necessary to successfully establish and sustain a robust and productive consortia of bacteria. A full-scale project focused on monitoring and maintaining a supportive environment for the biological mineralization of petroleum hydrocarbons, more specifically benzene, toluene, ethyl benzene and xylenes (BTEX compounds), would go a long way in advancing biological solutions in the general UST market. Results from a managed project are presented including the issues associated with data interpretation and field experiences.

Objective. The objective of the project was to demonstrate the efficacy of in-situ bioremediation when it is applied as a system. The goal for the customer is to remediate the soils and groundwater to a level at which his environmental consultant may petition for a "No Further Action" letter from the state regulatory agency. To meet this goal the objectives listed in Table 1 will need to be met.

TABLE 1. The soil and groundwater cleanup objectives for Georges 66 Station, Princeton, Illinois.

BTEX	Soil Cleanup Objective	GW Cleanup Objective
Benzene	0.17 ppm	0.025 ppm
Toluene	29 ppm	2.5 ppm
Ethyl benzene	19 ppm	1 ppm
Total Xylenes	150 ppm	10 ppm

Site Description. On May 1, 1998 six gasoline USTs and one waste oil UST at a facility commonly known as George's 66 in Princeton, Illinois were removed. The combined capacity of the gasoline USTs was 13,000 gallons and the waste oil tank was 500 gallons. The facility had been in operation for more than 50 years prior to its closing in 1998. During the tank removal activities, it was discovered that three of the six gasoline tanks and the waste oil tank had leaked. Subsequent to the tank removal activities, approximately 500 cubic yards of soil were removed from the source area and replaced with clean backfill material. An early action report was filed with the Illinois Environmental Protection Agency (IEPA) followed by a series of on-site and off-site investigations to better define and delineate the impacted soils and groundwater. These investigations revealed the presence of petroleum hydrocarbons on two adjacent residential properties. A corrective action plan and budget to address the cleanup needs of the site and the adjacent properties was filed with the IEPA on February 1, 2000. The budget was approved on April 3, 2000 and remedial activities were initiated on May 4, 2000.

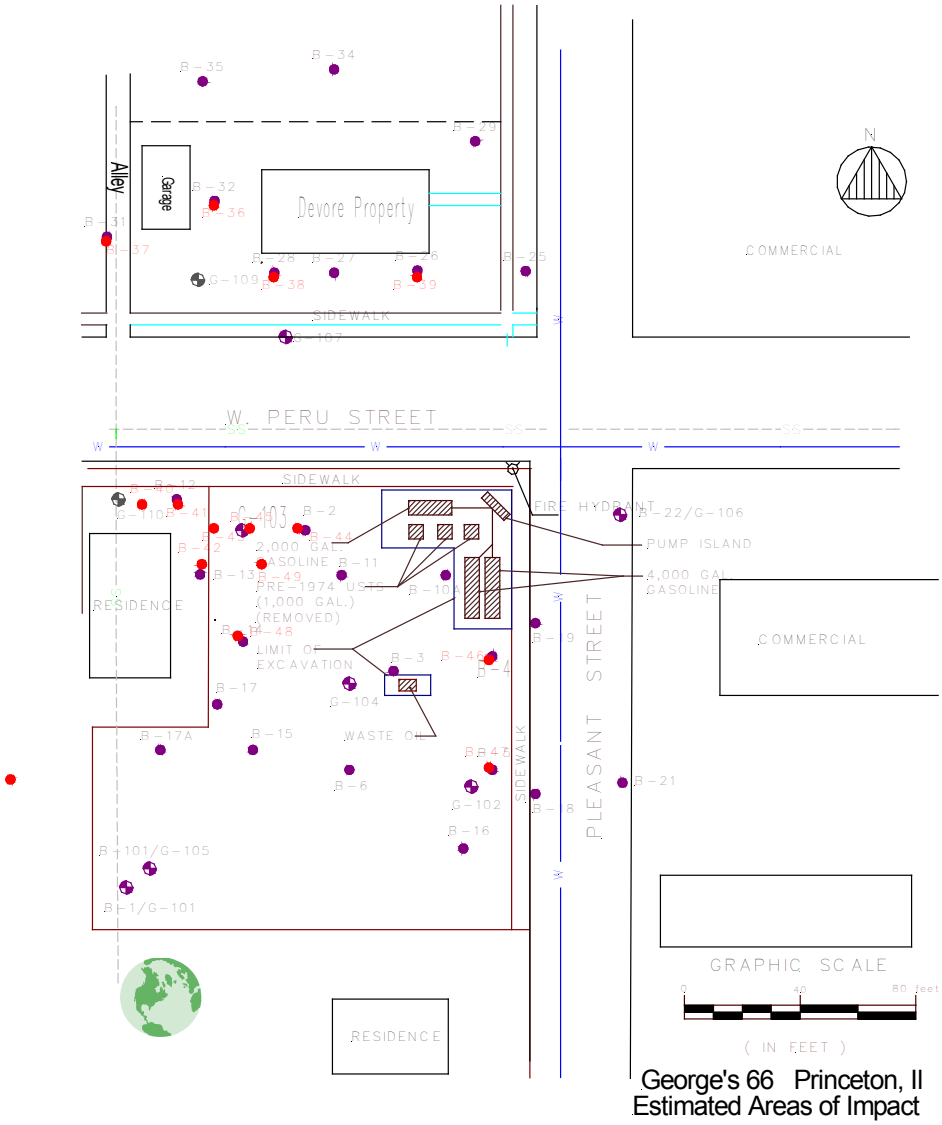

FIGURE 1. George's 66 site, Princeton Illinois

Groundwater depth at the site varies seasonally from between 4 and 10 feet bgs. The soils are generally a low to medium plasticity silty clay with a trace of sand with a calculated hydraulic conductivity of $1.049X10^{5}$ cm/sec. The groundwater gradient trends in a generally northwesterly direction.

MATERIALS AND METHODS

The implementation of the remedial action plan (RAP) consisted of first establishing a 10 foot by 10 foot grid across the site. Throughout the grid oxygen release compound (ORC™), vegetative heterotrophic bacteria cultures, nutrients, nitrification inhibitors and low levels of hydrogen peroxide were injected via a patented high pressure injection process into the saturated every 100 days, with

the second and third injections focusing on those areas exhibiting elevated BTEX levels.

Across the site grid, bio-injection points consisting of concentrated cultures and bioslurry were over-laid on an ORC^{TM} injection grid. The bioslurry consisted of 700 ppm hydrogen peroxide, nutrients composed of a proprietary blend of bioavailable slow-release nitrogen, ortho-phosphate and nitrification inhibitors. Liquid heterotrophic cultures were vegetative *Pseudomonas* consortia with a cell count of no less than $3X10^9$ Colony Forming Units//mL.

Groundwater and soil samples were collected periodically and evaluated for BTEX compounds via EPA Method 8260, ortho phosphate via EPA Method 365.2 in acidified and non-acidified samples, ammonia nitrogen via EPA Method 350.3 in acidified and non-acidified samples, total calcium via Method SM3500B in acidified and non-acidified samples and iron via Method SM3111D in acidified and non-acidified samples. Total and selective heterotrophic evaluations were also performed on groundwater samples.

Total heterotrophic enumeration was performed by obtaining samples in sterile bottles. Dilution occurred as quickly as possible after having been received into the laboratory, samples were shaken and the foam allowed to settle, 1mL of sample was transferred from the sample into 99 mL of sterile phosphate dilution water to give a 10^{-2} dilution. Samples were shaken and allowed to settle. Dilution was continued using either 99 mL or 9 mL dilution water bottles until the required dilution level was reached. Samples were then plated (into sterile petri dishes) at three dilution levels in duplicate e.g. 10^{-5}, 10^{-6}, 10^{-7}, in the expected range. Pour plates were prepared with molten PCA agar (temperature approx. 45^{o}C). A control plate was prepared to check for sterility. Plates were swirled gently to mix inoculums and agar. Plates were allowed to set. Plates were inverted, stacked and incubated at 30^{o}C +/- 2^{o}C for 48 hours. Plates were checked after 24 hours for spreading colonies. If present, a quick count was performed and the sample was re-incubated for another 24 hours. Plates with between 30 and 300 colonies were counted and results recorded.

Selective enumeration of the samples utilized both MacCokey Agar and PIA agar. Using the fixed volume 100 μL micro-pipet, 0.1 mL of the undiluted sample was pipetted onto the center of a MacCokey Agar plate. With a sterile glass rod the sample was carefully spread over the surface of the plate by spinning it on a turntable. Plates were incubated at 35^{o}C for 24-48 hrs. The plates were examined for growth and the results recorded.

RESULTS AND DISCUSSION

In November 1999 a series of soil borings were advanced across the site to better delineate the extent of impacted soils. The results of these soils borings, in combination with the groundwater monitoring data, have served as verification of the methods employed to date at the site.

TABLE 2A: Source Area – – Soil Sample Results at $T_{0\ days}$ and $T_{300\ days}$

Boring Number	B-2	B-44	Change
Depth of Sample (ft Bgs)	6	8.5	
Date of Sample	11/12/98	2/28/2001	
BTEX Total (ppb)	328780	134687.4	-59%
Benzene (ppb)	4380	729.4	-83%
Toluene (ppb)	44200	15363	-65%
Ethylbenzene (ppb)	53200	35445	-33%
Xylenes Total (ppb)	227000	83150	-63%

TABLE 2B: Perimeter of Soil Excavation – Soil Sample Results at $T_{0\ days}$ and $T_{300\ days}$

Boring Number	B-4	B-46
Depth of Sample (ft Bgs)	10.5'	11'
Date of Sample	11/12/98	2/28/01
BTEX Total (ppb)	31292	ND
Benzene (ppb)	452	ND
Toluene (ppb)	9940	ND
Ethylbenzene (ppb)	3700	ND
Xylenes Total (ppb)	17200	ND

TABLE 2C: Cross Gradient – Soil Sample Results at $T_{0\ days}$ and $T_{300\ days}$

Boring Number	B-14	B-48	Change
Depth of Sample (ft Bgs)	11'	10'	
Date of Sample	11/10/99	2/28/01	
BTEX Total (ppb)	472690	2048.5	-99.57%
Benzene (ppb)	4400	67.5	-98.47%
Toluene (ppb)	3690	1	-99.97%
Ethylbenzene (ppb)	108000	1980	-98.17%
Xylenes Total (ppb)	356600	0	-100.00%

Overall, where comparisons are available of soil borings from similar locations and depths, an average decrease in BTEX compounds of 76% is observed. Further, in those areas where soils have been exposed to the petroleum hydrocarbons for greater lengths of time, as in Table 2A, longer remediation times area seen. For those area up-gradient or cross gradient, such as the results seen in Tables 2B and 2C complete remediation of the soils is accomplished within 300 days. Observed remediation times are a direct function of the absorptive characteristics of the soil and the robustness of the established biofilm. Across the site similar sample locations have seen and average soil benzene concentration decrease of 92.7%.

As with the soil boring data, the groundwater monitoring data is also trending downward across the site. In all wells benzene and toluene values are

trending towards closure limits within the targeted eighteen-month time-line. Variations in the rate at which the targeted compounds are mineralized within the groundwater is directly related to the sorbed mass of contamination in and immediately upgradient from the individual wells.

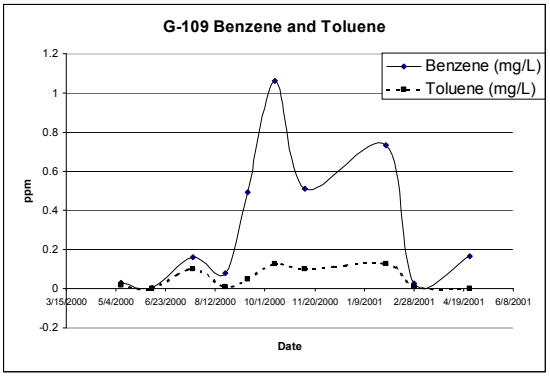

Figure 2. G-109 Benzene and Toluene (down gradient monitoring well)

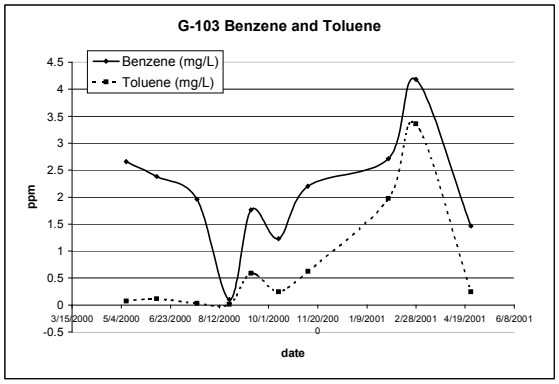

Figure 3. G-103 Benzene and Toluene (source area monitoring well)

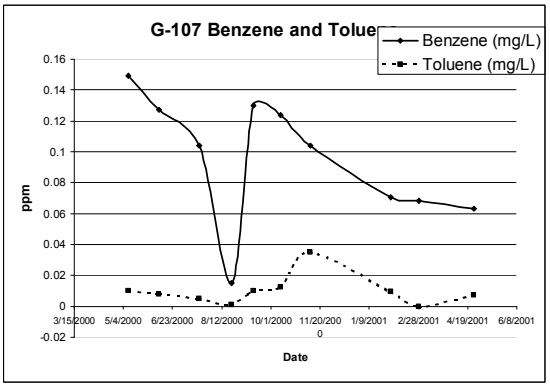

Figure 4. G-107 Benzene and Toluene (cross gradient monitoring well)

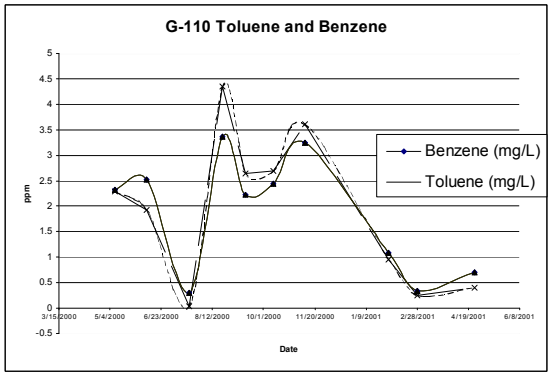

Figure 5. G-110 Benzene and Toluene (down gradient monitoring well)

In all wells a significant "rebound" is seen three to six months following the initial injections. The severity of this rebound, and the recovery period is seen to be related to the starting concentration, the soil type and the period of time the soils in the area of the monitoring well have been in contact with the petroleum hydrocarbon. A comparison of the cross gradient well, G-107 and the source area well, G-103, their rebound and recovery times and the overall rate of disappearence of the targeted compounds in the ground water may be seen in figure 3 and figure 4.

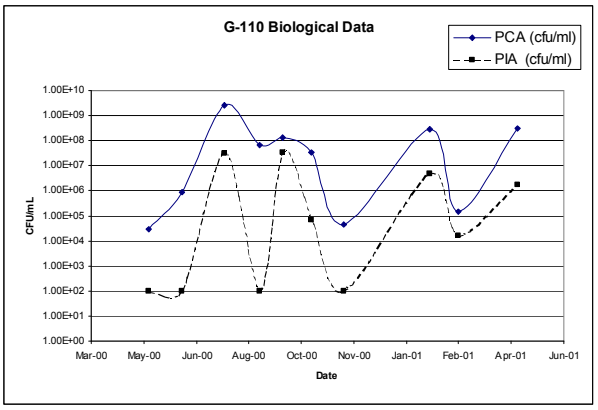

Figure 6. G-110 Biological Analyses

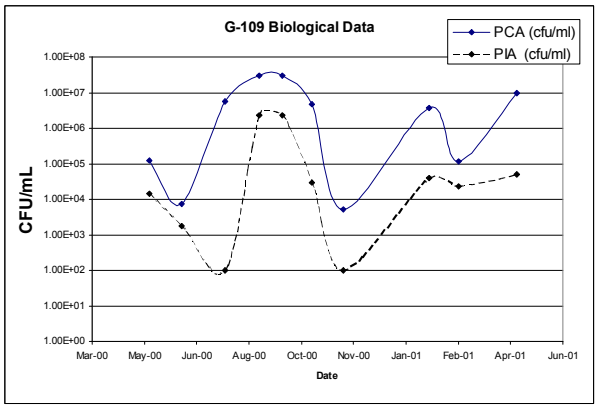

Figure 7. G-109 Biological Analyses

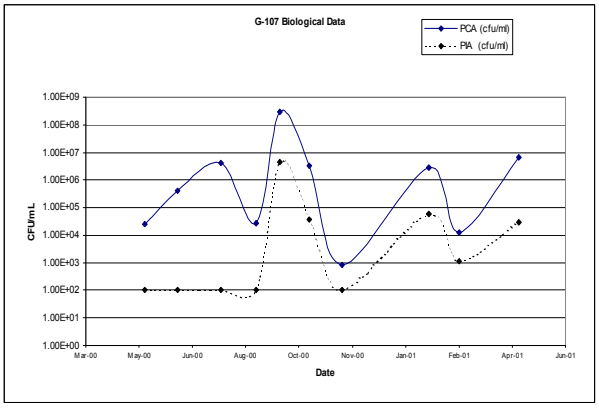

Figure 8. G-107 Biological Analyses

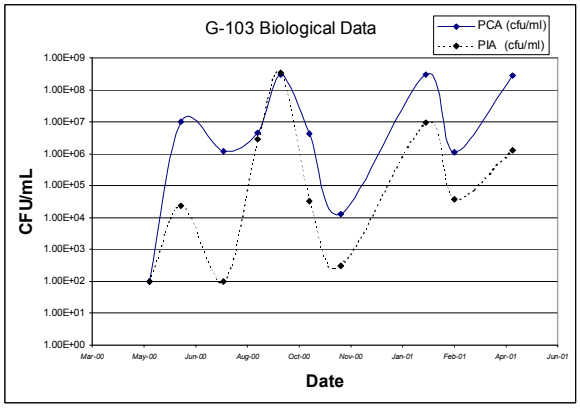

Figure 9. G-103 Biological Analyses

The continued monitoring of the total and selective biological counts throughout the project has allowed for correlations to be drawn between substrate concentration, nutrients and dissolved oxygen. Figure 9, the total and selective heterotrophic enumerations for G-103, a source area monitoring well, has maintained the highest biological counts through the project due to the maintained levels of nutrients, nutrients and growth substrate. In those wells where the substrate is becoming the rate limiting factor, such as G-107 (Figure 8), bacterial counts are several orders of magnitude less than source area or down gradient wells (G-110, Figure 6). Further, the application of vegetative cultures at the beginning of the project has allowed for better population management and a greater proportion of the total heterotrophs to remain as *Pseudomonas*. This population management is best seen as the project progresses in the source area and down gradient wells.

In order to obtain more rapid bacteria counts at a lower analytical cost, plate counts of *Pseudomonas* were used throughout the project in lieu of substrate specific enumerations. To confirm that the reported PIA count represents BTEX degrading cultures, the March 2001 monitoring well samples were further evaluated for substrate specificity. Toluene was chosen as a sole-source growth substrate for this evaluation. The results from this analysis verify

the assumption that the PIA and/MacConkey's agar may be utilized to assess the selective culture counts.

Table 3. Total Viable Count (CFU/mL) vs. Pseudomonas Count (CFU/mL)on MAC vs. Toluene Count (CFU/mL)

Date of Analysis	Well #	Total Viable Count	Pseudomonas Count	Toluene Count
3/5/01	107	1.28×10^4	1.15×10^3	1.00×10^3
3/5/01	103	1.13×10^6	3.66×10^4	7.60×10^5
3/5/01	109	1.15×10^5	2.34×10^4	1.42×10^4
3/5/01	110	1.60×10^5	1.56×10^4	1.00×10^3

Throughout the project the limitations of essential nutrients have been seen to affect the biological robustness of the site's soils and groundwater. Increased total and selective counts have been observed following each injection of additional nutrients and peroxide. In that the analyses for biological enumeration were performed some several weeks following an injection, the increase in the biological counts must be attributed primarily to the additional nitrogen and ortho-phosphate made available rather than the increase in dissolved oxygen resulting from the hydrogen peroxide component of the bioslurry.

Throughout the project, dissolved oxygen within all the wells have been generally maintained above 0.7 ppm, with readings as high as 10 ppm noted. This sustained level of oxygen must be attributed to the effective introduction, distribution and disassociation of the ORC™ across the site. At no time has any monitoring well had a measure dissolved oxygen level below 0.65 ppm. As a tertiary confirmation that biological Mineralization of the BTEX compounds is occurring, site monitoring of pH has shown a general rise across all the monitoring wells. After ten months of the in-situ treatment program, pHs generally range from 7.0 to 7.7.

CONCLUSIONS

The notion that biologically based remediation technologies present scale-up issues and frequently do not offer reproducible results may be an outgrowth of the field practioner focusing on only a portion of the biological system. This field project, and others like it demonstrate that employing a "systems" approach to in-situ biological remediation necessitates that all the necessary components critical to in-situ environment need to be addressed. Further, the success of this project demonstrates the efficacy of both the proprietary delivery systems and products employed and capabilities of Oxygen Release Compound to supply sufficient terminal electron acceptor levels throughout the term of an in-situ project. If any of the components below are omitted or poorly managed the less likely the project is to succeed:

1) Uniform subsurface delivery to the effected areas,
2) Maintenance of bioavailable essential nutrients,

3) Maintained levels of terminal electron acceptors,
4) Effective population management and monitoring and
5) Effective interpretation of field data.

Of the five critical elements, four may be addressed by equipment and materials the fifth requires experience and sufficient field analytical data to base field activities. When applying biological solutions to remediate impacted soils and groundwater the interaction of all the in-situ elements must be evaluated. The equalibria between the aqueous phase and the soils cannot be ignored; the role the established biofilm plays in addressing the entrained BTEX compounds on the soils and in the groundwater requires thoughtful consideration. The bioavailablility of essential nutrients must be evaluated continuously and the overall health of the consortia monitored.

When applied as a system, bioremediation of BTEX contamination eliminates the residual source of the contamination, specifically the BTEX compounds entrained within the vadose zone soils, generating reproducible results for the practioner, his client and the property owner.