SUBTASK 1.16 – SLOW-RELEASE BIOREMEDIATION ACCELERATORS

Final Report

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SUBTASK 1.16 – SLOW-RELEASE BIOREMEDIATION ACCELERATORS

ABSTRACT

Low-cost methods are needed to enhance various bioremediation technologies, from natural attenuation to heavily engineered remediation of subsurface hydrocarbon contamination. Many subsurface sites have insufficient quantities of nitrogen and phosphorus, resulting in poor bioactivity and increased remediation time and costs. The addition of conventional fertilizers can improve bioactivity, but often the nutrients dissolve quickly and migrate away from the contaminant zone before being utilized by the microbes. Through this project, conducted by the Energy & Environmental Research Center, polymers were developed that slowly release nitrogen and phosphorus into the subsurface. Conceptually, these polymers are designed to adhere to soil particles in the subsurface contamination zone where they slowly degrade and release nutrients over longer periods of time compared to conventional fertilizer applications. Tests conducted during this study indicate that some of the developed polymers have excellent potential to satisfy the microbial requirements for enhanced bioremediation.

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EXECUTIVE SUMMARY

The Energy & Environmental Research Center (EERC) has developed and tested several compounds that will provide a slow release of nutrients for the remediation of subsurface contaminants. Nutrients such as nitrogen (N) and phosphorus (P) are often limiting in the biological remediation, either engineered or natural attenuation, of subsurface contamination. When salts of N and P are injected into the groundwater, the flow of water will often move them out of the contamination zone more quickly than microbial uptake occurs. Therefore, a slow-release form of nutrients is desired to provide a continuous supply of N and P to decrease the time required for remediation. Through this research, simple polymers containing an organic structure with an attached phosphate ester have been synthesized. The polymers are water-soluble, but will stick to soil particles and hydrophobic contaminants in the subsurface near the point of injection. These polymers will then degrade, through biotic or abiotic means, releasing N and P for uptake within the zone of contamination. Compared to conventional subsurface nutrient additions, the polymers developed through this project were more effective in providing nutrients at a consistent rate that is more desirable for microbiological degradation of contaminants. The sorptive behavior and slow-release characteristics of the polymers make them ideal candidates for use as a less-invasive, low-cost approach to subsurface remediation.

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INTRODUCTION

The most common type of contamination in subsurface soils and groundwater is from petroleum hydrocarbons such as diesel and gasoline, and leaking underground storage tanks (LUSTs) are the most common source of these petroleum contaminants (EPA, 2003). According to the most recent U.S. Environmental Protection Agency (EPA) corrective action report, as of the fall of 2002 there were over 427,000 confirmed releases of petroleum hydrocarbons from underground storage tanks within the United States, over 150,000 of which remain to be remediated (EPA, 2003). Remediation approaches to these contaminants vary from extensively engineered methods to monitored natural attenuation. In many cases, some form of biological treatment is the most cost-effective approach. Typical biological treatment involves supplying oxygen to the contaminant zone, as this is generally the limiting factor that prevents rapid biodegradation (Alexander, 1994). However, in the biological treatment of contaminants, once oxygen has been supplied, up to three additional factors may limit bioactivity: mass transfer due to poor mixing; low temperatures, and the absence of nutrients. It is difficult to address the mixing and temperature limitations, but the nutrient limitations can be addressed.

Nutrients are generally required in ratios matching that of their occurrence in cell biomass (Alexander, 1994). Table 1 shows the typical composition of a microbial cell for six major elements. The elements carbon, oxygen, and hydrogen come from water, organic substrates, and atmospheric components and, while sometimes limiting, are adequate in an aerated hydrocarbon spill scenario. Sulfur is usually abundant in soil; however, nitrogen and phosphorus are required in fairly large amounts and are generally not abundant in soils or groundwater (Roberts et al., 1993). Therefore, in many cases, addition of nutrients, especially nitrogen and phosphorus, are desirable to enhance the microbial decomposition of contaminants.

One of the largest obstacles to the utilization of nutrients for enhanced microbial degradation is how to best supply the nutrients to the subsurface zones in which they are needed. Currently, the most common approach is to inject a common agricultural fertilizer (i.e., diammonium phosphate or ammonium nitrate) into the subsurface through groundwater wells located within the contaminant source zone. However, the nutrient uptake rates are slow,

Table 1. Typical Composition of a Microbial Cell (Stanier et al., 1986)

Element	% Dry Weight
Carbon	50
Oxygen	20
Nitrogen	14
Hydrogen	8
Phosphorus	3
Sulfur	1

often resulting in the migration of the solution through the area of bioactivity before utilization. This can be overcome by repeated additions, but results in increased labor and amendment costs. An attractive, cost-effective alternative would be to add a nutrient formulation that sticks to soil particles to eliminate off-site migration and is slowly released over a period of time, ideally at a rate that equals microbial uptake.

Through this project, simple polymers containing an organic structure with an attached phosphate ester have been developed. These polymers are high-molecular-weight compounds that are derived from the combination of smaller molecules through chemical reactions and were developed to contain the desired nutrients appropriate for biological remediation applications. The polymers are water-soluble for ease of injection and distribution but will stick to soil particles and hydrophobic contaminants in the subsurface near the point of injection. Once in the subsurface, these polymers will degrade through biotic or abiotic means, release N and P for uptake within the zone of contamination and, ideally, result in an enhanced rate of bioremediation.

A product with a similar concept, an oxygen releasing compound (ORC), has been developed and is commercially available by Regenesis Bioremediation Products of San Clemente, California. This product provides a slow-release source of oxygen available for microbes within subsurface contaminant zones and has been utilized at thousands of sites in the United States and the world. A goal of this project was to develop a similar nutrient-releasing compound (NRC) that could be used alone or in combination with ORC to address nutrient limitations at sites contaminated with petroleum hydrocarbons.

OBJECTIVE

The objective of this project was to develop and evaluate polymers for their ability to supply nitrogen and/or phosphorus in a steady and continuous manner to soil microbes, facilitating microbial growth and remediation of petroleum-related contaminants. The goals of this research were accomplished by completion of the following tasks:

- Synthesis of water-soluble, readily biodegradable polymers containing nitrogen and phosphorus.
- Evaluation of potential microbial inhibitory impacts from the polymers.
- Assessment of polymer nutrient availability to microbes.
- Determination of polymer sorptive behavior.
- Demonstration of polymer suitability as a nutrient source in the biodegradation of petroleum contamination.
- Assessment of potential field trial demonstrations with select polymers.

EXPERIMENTAL

Polymer Synthesis

Two general types of polymers containing phosphate were synthesized in this project: 1) polymers with phosphate in the backbone copolymer chain (backbone phosphates) and 2) polymers with phosphate groups attached to a chain composed of combinations of carbon, oxygen, and nitrogen atoms (appended phosphates).

Nitrogen was also incorporated into some polymers, mainly in the backbone chain as peptide or urea functionality groups. Several polymers of each type were synthesized in this project, so that a variety of solubilities and biodegradabilities could be evaluated.

Several different techniques were utilized to create a variety of polymers. The developed polymer abbreviations and their associated phosphorus content by weight percent are listed in Table 2. A commercially available compound, lecithin, was also included as a potential polymer. The polymers with the greatest potential as bioremediation accelerators were then selected for use in respirometric analyses. The selection was based on a variety of polymer characteristics such as, solubility in water, amount of available phosphorus, and relative cost of production.

Polymer Purification

Purification of the polymers was necessary because, in most cases, phosphoric acid was used to add phosphate to the polymers, resulting in excess, unbound phosphate. Removal of the unbound phosphate is necessary to eliminate excess nutrient sources that could alter the

Table 2. Synthesized Polymers and Associated Phosphorus Content

Polymer/Compound	Phosphorus, wt%
PPGDP	2.2
PPGDP	2.6
BDP	2.3
PTGP	2.7
PTUP	7.4
PTUP-2	8.2
PGG	2.3
PGGP	7.1
PEC	5.0
PPA	5.7
PEC-2	3.4
PPA-2	4.2
PVAP	4.4
GCP	0.2
GP	1.5
PUP	4.5
Lecithin	1.7

experimental results. To accomplish this, two approaches were implemented. For non-water-soluble polymers, the solids were washed with distilled water. For water-soluble polymers, the polymer was dissolved in a small volume of water and dialyzed. Initially, dialysis tubing with a molecular weight cutoff (MWCO) of 12,000 was used and dialyzed against distilled water. Two unfortunate events resulted: 1) the molecular weight of most polymers was apparently less than 12,000, resulting in a loss of the polymer to the dialysis water and 2) the osmotic effect of some polymers is high, resulting in a large influx of water to the dialysis bag followed by rupture and loss of the polymer. To remedy this, dialyzers with a MWCO of 500 (Spectra/Por Float-a-lyzer, Spectrum Labs, Rancho Dominguez, California) were used and dialyzed against polyethylene glycol 1000. Following dialysis, the polymer was dried at ca. 65°C, weighed, and total phosphate determined by persulfate digestion and colorimetry via the molybdate method. Once the purification process was complete, the polymers were ready for utilization in the biological uptake analyses.

Respirometric Analysis

Respirometric analyses were used to determine the rate of polymer phosphorus availability to the microbes in soil. A total of thirteen of the seventeen developed polymers were selected for evaluation in the respirometric analyses. The selection was based on a variety of polymer characteristics such as solubility in water, amount of available phosphorus, and relative cost of production. A clean sand was selected as the test material because it contained little or no organic matter or nutrients. Hexadecane was selected as the test organic compound because it is representative of typical petroleum hydrocarbons, has low volatility, and can be obtained in pure form. Low volatility is important in this type of respirometry test as the headspace of the test chamber containing the sand, hexadecane, and polymer is swept with air twice a day and could result in significant losses of high-volatility hydrocarbons.

The respirometry experiments were conducted using a Model CA-1 CO_2 Analyzer developed by Sable Systems, Inc. The tests were conducted by adding 200 g dry weight of clean, fine-grained Oklahoma quartz sand to 500-mL test chambers. The Oklahoma quartz sand was selected for use to ensure that no natural sources of phosphorus were available for the microbes during the test. Each chamber was wetted to 60% of its moisture-holding capacity before hydrocarbon addition. Two chambers did not receive any amendments to determine the background level of carbon dioxide production. The remaining chambers were all dosed with hexadecane at 1000 mg/kg. All of these chambers except the controls were dosed with ammonium chloride so that nitrogen was not limiting. Pairs of the hexadecane-dosed sand were supplemented with no phosphate as a control, potassium phosphate as a positive control, and selected polymers. The ammonium chloride and the phosphate additives were added to achieve a carbon:nitrogen:phosphate ratio of 100:7:1. Finally, all chambers were inoculated with a suite of microbes that had been extracted from a natural soil.

Respirometric analysis was performed on the chambers by incubating them statically in the dark at room temperature. Every 12 hours, the headspace of each chamber was flushed with carbon dioxide-free air and analyzed for carbon dioxide, by infrared spectroscopy, and for oxygen, using a fuel cell analyzer. Drierite® was used to scrub the headspace gas of moisture prior to infrared analysis of carbon dioxide. After passing through the infrared analyzer, the

headspace gas was scrubbed of carbon dioxide using a molecular sieve before oxygen analysis in the fuel cell. Computer integration then provided data on the production of carbon dioxide and the consumption of oxygen throughout the 30-day incubation period.

Microbial Inhibition Analysis

A simple microbe growth analysis was performed on two selected polymers, PTUP and PTUP-2, to determine potential microbial toxicity effects. The test polymers were selected based on the steady rates of hexadecane biodegradation as indicated by the respirometry results. This analysis was performed by adding the polymer to a standard nutrient broth growth media within test tubes under aerobic conditions. The growth media was distributed to five sets of test tubes in triplicate (one control set and four polymer-amended sets) and was then autoclaved for complete sterilization. An inoculation of microbes from a known contaminated subsurface soil sample was added to each test tube, followed by addition of the selected polymers. One set of the test tubes did not receive any polymer amendment (control set), and the other four sets received the two polymer amendments at concentrations of 50% and 100%, respectively. The test tubes were allowed to incubate for a period of 5 days, after which they were visually inspected for microbial growth.

Sorption Analysis

Sorption of the polymer is believed to be an important parameter for the appropriate and efficacious use of the polymer additives in the field. Sorption was tested with a representative polymer (PGG) by combining it with selected soils in an aqueous media and mixing for 1.5 hours at room temperature. After incubation, an aliquot of the aqueous supernatant was removed and centrifuged at 12,000× g for 1 minute. The clarified supernatant was analyzed for total phosphorus by the persulfate digestion and molybdate colorimetric method. The absorbed phosphorus content was then calculated and plotted in relation to the amount of sediment in each container, creating an absorption isotherm from the resulting data. Two soils were used for these studies: the clean, pure Oklahoma quartz sand used in respirometric studies and a soil from New Mexico that has a moderate clay content of 26%. The Oklahoma sand was selected based on its predicted low absorption properties, and the New Mexico soil was selected because of an expected higher absorption capacity based on clay content.

Polymer and ORC Batch Tests

A bench-scale analysis was performed to evaluate the remediation enhancement potential of two selected polymers (PTUP and PTUP-2). The tests were also performed in combination with a commercially available ORC, developed by Regenesis Bioremediation Products of San Clemente, California, to determine remediation enhancement potential. The polymers and the ORC were evaluated individually and in combination. The tests were completed by adding the EERC-developed polymer and/or ORC to a wide-mouth glass vessel containing 250 grams of sediment collected from a gasoline-contaminated site in Butte, Montana. The sediment was selected to represent a “real-world” contamination scenario. The sediment was then spiked with 10 mg/kg of toluene to serve as the hydrocarbon source. Toluene was selected to represent a component of the contaminant complex BTEX (benzene, toluene, ethylbenzene, and xylene) that

commonly occurs at underground gasoline-contaminated sites. Groundwater, previously collected from the same site in Montana, was added to each sample to attain complete sediment saturation and to ensure proper mass transfer potential. The samples were kept under anaerobic conditions to ensure that the oxygen in the ORC was the primary electron acceptor for the microbes. The samples were allowed to incubate at 25°C for 60 days and then submitted to a commercial analytical laboratory for analysis of toluene concentration.

RESULTS AND DISCUSSION

Respirometric Analysis

The respirometric analysis allows for quantification of cumulative carbon dioxide production or oxygen depletion over time, which is a function of the biodegradation rate occurring in each sample. Biodegradation patterns exhibited during respirometric analysis are bounded by two extremes, including 1) very rapid degradation with essentially no lag period and 2) no biodegradation. Case 1 is undesirable as it indicates that nutrients are almost immediately bioavailable and were, therefore, released very rapidly. While this is not problematic in an enclosed vessel, if utilized in the field to treat groundwater or saturated sediment, the nutrients would likely dissolve quickly and migrate off-site before widespread microbial utilization. Alternatively, Case 2 suggests that nutrients are not being released at all. The ideal nutrient polymer will rest between the two cases. Because phosphorus was the limiting nutrient in these experiments, Case 1 is represented by the sample with a readily available phosphorus source (K_2HPO_4), commonly used in nutrient amendment remedial approaches, while Case 2 is represented by the negative control (no phosphorus).

Figure 1 shows an example of both Cases 1 and 2. The biodegradation of hexadecane with the addition of potassium phosphate exemplifies Case 1, and the blank represents Case 2. Two polymers, PEC-2 and PPA, performed poorly and are similar to the blank samples that exhibited very little CO_2 production. The polymer PEC-2 exemplifies the in-between case, where phosphorus is slowly released. In fact, the data for PEC-2 suggest that the metabolism of hexadecane is very much controlled by the rate of phosphorus release.

Figure 2 shows the results of the respirometry experiment performed with four other phosphate polymers. The data show that the lecithin was the most quickly released, followed by PGG; much slower in release was BEP. Finally, GCP performed about equally to the blank, suggesting little or no phosphorus release.

Figure 3 shows the effect of phosphorus release on hexadecane biodegradation for three additional phosphorus polymers. In this experiment, a newly synthesized PTUP was tested. This polymer was synthesized in an attempt to add even more phosphate to the polymer. In this experiment, the PTUP performed the best at phosphorus release, followed by PPGDP and PVAP.

Figure 4 shows the respirometric data for the final three polymers tested. In this experiment, another polymer (PTUP-2), which was prepared utilizing a slightly different methodology than that of PTUP, performed the best. PTGP and PGGP did not perform as well as

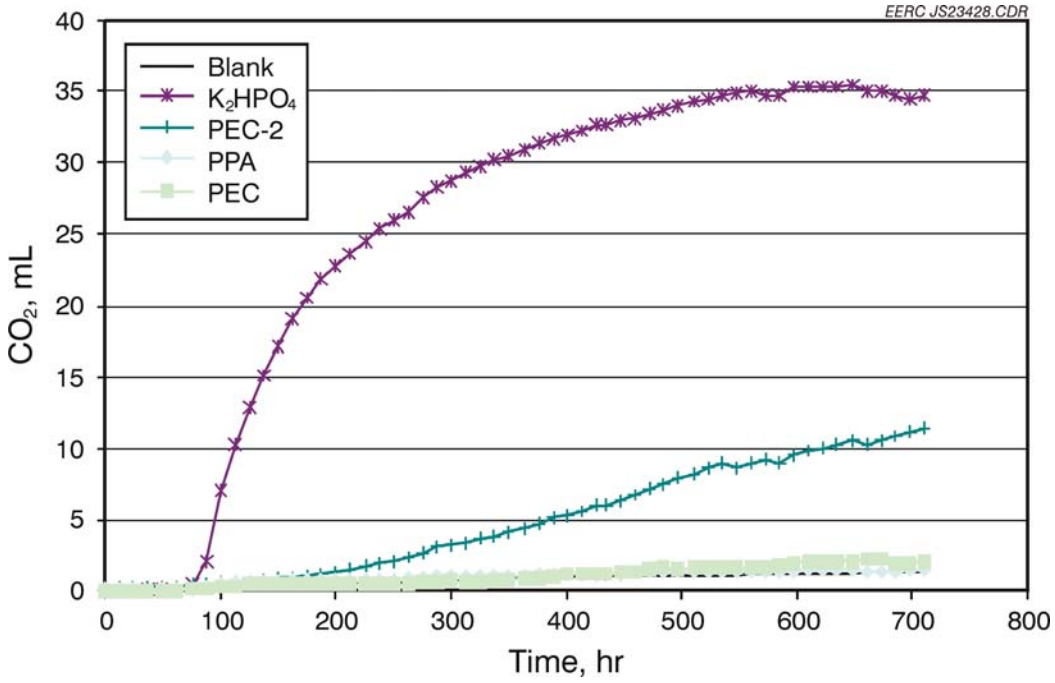

Figure 1. Respirometry results for potassium phosphate and nutrient-releasing polymers.

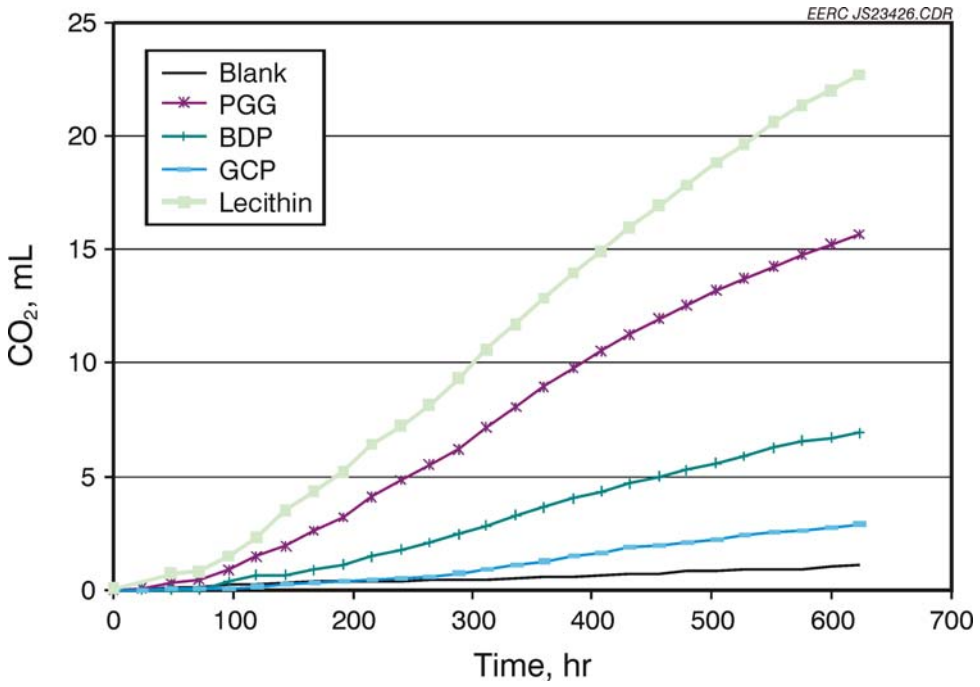

Figure 2. Respirometry results for four nutrient-releasing polymers.

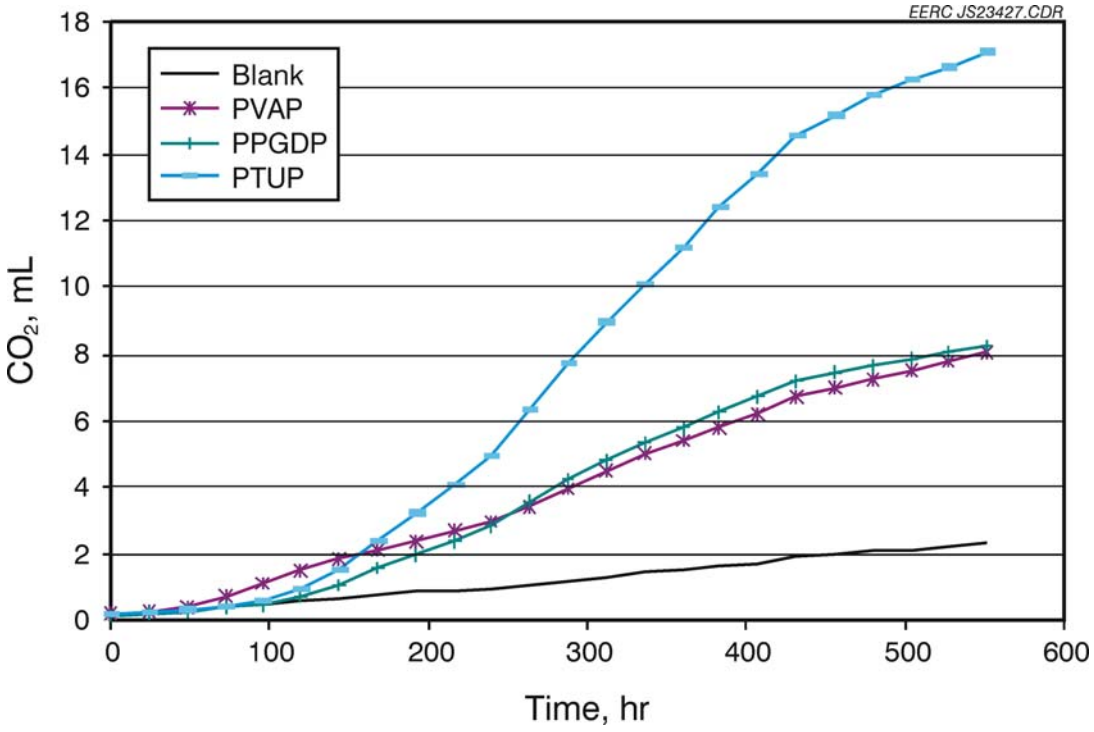

Figure 3. Respirometry results for three nutrient-releasing polymers.

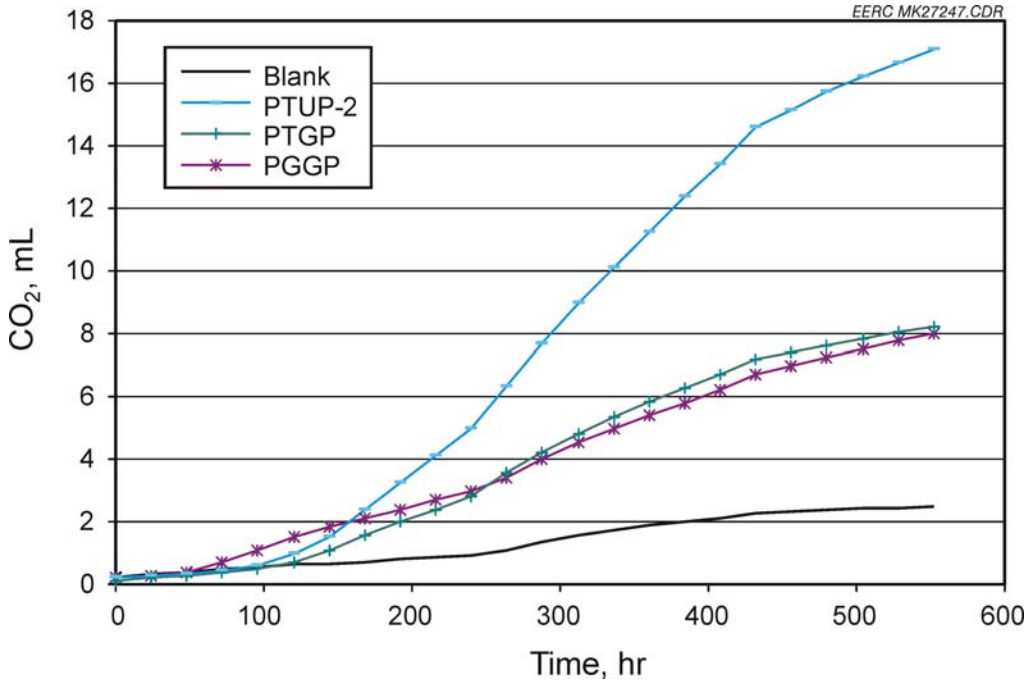

Figure 4. Respirometry results for three nutrient-releasing polymers.

the PTUP-2; however, these polymers demonstrate the slow-release properties that are desirable for nutrient release in the subsurface.

Overall, the respirometry experiments showed that there were essentially two classes of phosphorus polymers: those that released little or no phosphorus and those that released phosphorus at an intermediate level (i.e., between Cases 1 and 2, above). PTUP and PTUP-2 were selected as the best polymers for further evaluation, based on their rate and amount of phosphorus release. Lecithin performed well, however, due to its cost, it was not considered economically feasible for large-scale applications, and therefore no further evaluations were conducted. The polymers (PGG, PTGP, and PGGP) are also potential candidates as they are synthesized from an inexpensive food-grade gelatin that contains a relatively high amount of nitrogen (ca. 18%) and is readily biodegraded.

Microbe Inhibition Analysis

Two polymers, PTUP and PTUP-2, were selected and evaluated for potential microbial inhibition characteristics. As shown in Figure 5, no apparent difference in the growth media clarity could be visually detected between the control sample and the two samples with polymer additions. Each sample appears to have similar microbial growth throughout the test tube. Although this simple evaluation is qualitative in nature, it is sufficient to indicate that there was no microbial inhibition from the addition of the polymer compounds.

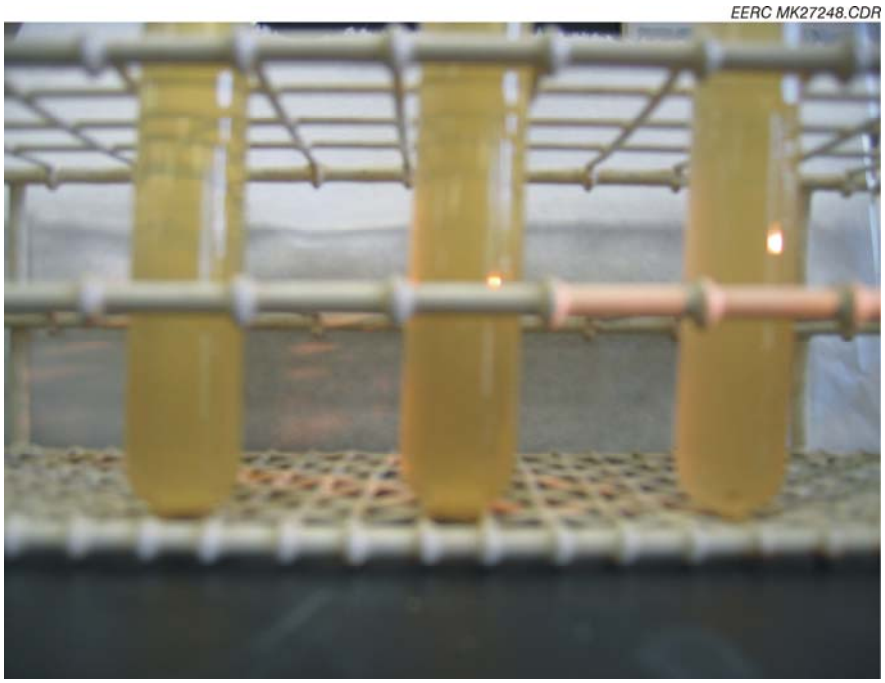

Figure 5. Microbe inhibition test samples from left to right: control; PTUP; and PTUP-2.

Sorption Analysis

One simple test of polymer sorption was performed using a representative polymer, PGG, on the Oklahoma sand (predicted to have low absorption) and the New Mexico soil (significant clay content). This test revealed that the sand did not absorb at all (data not shown), but that the New Mexico soil sorbed quite strongly, quickly, and in a simple linear fashion (Figure 6). The data suggest that for the desired soil/polymer sorption to occur, there must be sufficient clay content present in the sediments.

Polymer and ORC Batch Tests

The two polymers selected for further evaluation, PTUP and PTUP-2, were used in batch microcosm evaluations to determine their hydrocarbon remediation enhancement potential alone and in combination with ORC. The results, as summarized in Table 3, indicate that the nutrient-releasing polymer additions resulted in total degradation of the toluene (detailed analytical reports are contained in Appendix A). However, the control sample, which had no ORC or nutrient amendments, also illustrated a similar reduction (99%) in toluene content. This may suggest that adequate nitrogen and phosphorus is naturally present in the soils utilized in this experiment. The soil used was selected to more accurately represent potential field site conditions; however, a more sterile soil (e.g., Oklahoma white sand) should have been used to reduce natural nitrogen and phosphorus interferences.

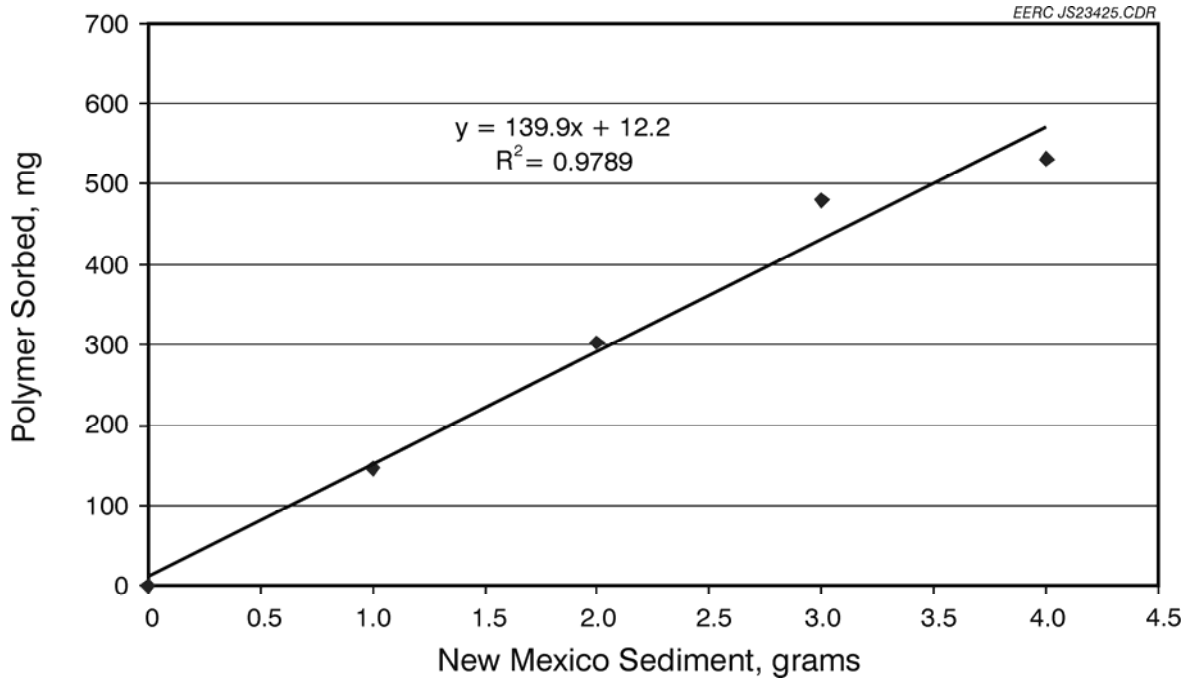

Figure 6. Sorption of the polymer (PGG) to a New Mexico soil.

Table 3. Results of Toluene Degradation Analysis

Sample Description	Initial Toluene Concentration, mg/kg	Toluene Concentration (after 60-day incubation), mg/kg	% Reduction
Control (no amendments)	10	0.1	99
ORC Only	10	5.8	42
PTUP Polymer Only	10	ND	100
PTUP-2 Polymer Only	10	ND	100
PTUP Polymer + ORC	10	2.9	71
PTUP-2 Polymer + ORC	10	6.4	36

The samples that received ORC additions, alone and in combination with the nutrient-releasing polymer, illustrated limited reduction of toluene. A discussion with a Regenesis staff member revealed that the ORC could cause an increase in the pH of the microcosm, which can then be inhibitory to microbial activity. However, this pH increase is typically not an issue in the field because of sufficient natural buffering capacity in groundwater and soils (Von Arb, 2005).

Field-Trial Assessment

A basic assessment of the potential for a field trial evaluation of an EERC-developed polymer was completed. The Montana Department of Environmental Quality (MDEQ) has expressed interest in cooperating on a field trial by providing an appropriate demonstration site with hydrocarbon-contaminated soils. The site is instrumented with an appropriate configuration of monitoring wells for proper evaluation of the polymer remediation potential. A specific demonstration methodology (according to specific site characteristics) and an associated proposal will be prepared upon identification of a potential funding source. The most promising polymers, as determined by the results of this project, will be selected for use in the field trial.

CONCLUSIONS

Through this research, several nutrient-rich polymers have been developed and evaluated for enhanced bioremediation potential. Based on the project results, five of the polymers, PTUP, PTUP-2, PGG, PTGP, and PGGP, have revealed the potential to be an adequate slow-release source of nutrients for enhanced bioremediation of petroleum hydrocarbons. The results also indicate that the polymer chemical structures possess sorptive characteristics that are desirable for source zone distribution and for resistance to off-site migration, therefore, providing a long-term source of nutrients for microbial utilization.

This research involved developing and modifying a variety of chemical reaction methods for creating the desired polymers. These chemical reactions, although safely manageable at the bench-scale level, may provide a unique challenge at the larger, mass-production scale, due to the excessive heat of reaction. However, it is believed that these same chemical reactions can be safely conducted on a larger scale with the addition of a heat transfer agent.

The next step in this research will be to evaluate the polymers at the field scale to evaluate their bioaccelerating potential in real-world subsurface contamination sites. In order to assess this potential, sufficient amounts (approximately 100 pounds) of polymer will be needed. As such, the EERC research team will develop an appropriate methodology to mass-produce these polymers in a safe and effective manner and identify the issues crucial to the commercialization potential of the product.

The overall goal of the field trial demonstration will be to evaluate the microbiological enhancement potential of the developed polymers. A secondary objective of the demonstration may be to evaluate the effectiveness of the combined use of the nutrient-releasing polymer and the already commercially available ORC developed by Regenesis.

ACKNOWLEDGMENTS

We would like to acknowledge the late John Gallagher, an EERC Microbiologist and friend, for his contributions to this project. Prior to his passing in December 2004, John designed the experimental testing procedures for the microbiological components of the project and provided technical guidance for achieving the project objectives. John's unique insight and enthusiasm for this and other projects will be dearly missed.

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APPENDIX A

ANALYTICAL REPORTS

ENERGY LABORATORIES, INC. • P.O. Box 30916 • 1120 South 27th Street • Billings, MT 59107-0916
800-735-4489 • 406-252-6325 • 406-252-6069 fax • eli@energylab.com

LABORATORY ANALYTICAL REPORT

Client: Energy and Env Research Center
Project: Bioaccellerator Project
Lab ID: B06030453-001
Client Sample ID: #2

Report Date: 03/16/06
Collection Date: 03/06/06 15:00
Date Received: 03/07/06
Matrix: Soil

Analyses	Result	Units	Qual	RL	MCL/ QCL	Method	Analysis Date / By
PHYSICAL CHARACTERISTICS							
Moisture	26	wt%		0.01		SW3550A	03/14/06 08:27 / elf
VOLATILE ORGANIC COMPOUNDS							
Toluene	0.095	mg/kg		0.050		SW8021B	03/14/06 20:00 / bw
Surr: Trifluorotoluene	85.2	%REC			80-120	SW8021B	03/14/06 20:00 / bw

Report Definitions:
RL - Analyte reporting limit.
QCL - Quality control limit.
MCL - Maximum contaminant level.
ND - Not detected at the reporting limit.

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LABORATORY ANALYTICAL REPORT

Client: Energy and Env Research Center
Project: Bioaccellerator Project
Lab ID: B06030453-002
Client Sample ID: #3

Report Date: 03/16/06
Collection Date: 03/06/06 15:00
Date Received: 03/07/06
Matrix: Soil

Analyses	Result	Units	Qual	RL	MCL/ QCL	Method	Analysis Date / By
PHYSICAL CHARACTERISTICS							
Moisture	24	wt%		0.01		SW3550A	03/14/06 08:28 / elf
VOLATILE ORGANIC COMPOUNDS							
Toluene	5.8	mg/kg		0.050		SW8021B	03/14/06 21:14 / bw
Surr: Trifluorotoluene	95.1	%REC			80-120	SW8021B	03/14/06 21:14 / bw

Report Definitions:
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MCL - Maximum contaminant level.
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LABORATORY ANALYTICAL REPORT

Client: Energy and Env Research Center
Project: Bioaccellerator Project
Lab ID: B06030453-003
Client Sample ID: #4

Report Date: 03/20/06
Collection Date: 03/06/06 15:00
Date Received: 03/07/06
Matrix: Soil

Analyses	Result	Units	Qual	RL	MCL/ QCL	Method	Analysis Date / By
PHYSICAL CHARACTERISTICS							
Moisture	26	wt%		0.01		SW3550A	03/14/06 08:28 / elf
VOLATILE ORGANIC COMPOUNDS							
Toluene	ND	mg/kg		0.050		SW8021B	03/14/06 22:28 / bw
Surr: Trifluorotoluene	88.2	%REC			80-120	SW8021B	03/14/06 22:28 / bw

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QCL - Quality control limit.
MCL - Maximum contaminant level.
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LABORATORY ANALYTICAL REPORT

Client: Energy and Env Research Center
Project: Bioaccellerator Project
Lab ID: B06030453-004
Client Sample ID: #5

Report Date: 03/20/06
Collection Date: 03/06/06 15:00
Date Received: 03/07/06
Matrix: Soil

Analyses	Result	Units	Qual	RL	MCL/ QCL	Method	Analysis Date / By
PHYSICAL CHARACTERISTICS							
Moisture	27	wt%		0.01		SW3550A	03/14/06 08:28 / elf
VOLATILE ORGANIC COMPOUNDS							
Toluene	ND	mg/kg		0.050		SW8021B	03/15/06 20:27 / bw
Surr: Trifluorotoluene	84.1	%REC			80-120	SW8021B	03/15/06 20:27 / bw

Report Definitions:
RL - Analyte reporting limit.
QCL - Quality control limit.
MCL - Maximum contaminant level.
ND - Not detected at the reporting limit.

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LABORATORY ANALYTICAL REPORT

Client: Energy and Env Research Center
Project: Bioaccellerator Project
Lab ID: B06030453-005
Client Sample ID: #6

Report Date: 03/16/06
Collection Date: 03/06/06 15:00
Date Received: 03/07/06
Matrix: Soil

Analyses	Result	Units	Qual	RL	MCL/ QCL	Method	Analysis Date / By
PHYSICAL CHARACTERISTICS							
Moisture	35	wt%		0.01		SW3550A	03/14/06 08:28 / elf
VOLATILE ORGANIC COMPOUNDS							
Toluene	2.9	mg/kg		0.050		SW8021B	03/15/06 21:04 / bw
Surr: Trifluorotoluene	81.2	%REC			80-120	SW8021B	03/15/06 21:04 / bw

Report Definitions:
RL - Analyte reporting limit.
QCL - Quality control limit.
MCL - Maximum contaminant level.
ND - Not detected at the reporting limit.

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LABORATORY ANALYTICAL REPORT

Client: Energy and Env Research Center
Project: Bioaccellerator Project
Lab ID: B06030453-006
Client Sample ID: #7

Report Date: 03/16/06
Collection Date: 03/06/06 15:00
Date Received: 03/07/06
Matrix: Soil

Analyses	Result	Units	Qual	RL	MCL/ QCL	Method	Analysis Date / By
PHYSICAL CHARACTERISTICS							
Moisture	26	wt%		0.01		SW3550A	03/14/06 08:28 / elf
VOLATILE ORGANIC COMPOUNDS							
Toluene	6.4	mg/kg		0.050		SW8021B	03/15/06 02:09 / bw
Surr: Trifluorotoluene	82.2	%REC			80-120	SW8021B	03/15/06 02:09 / bw

Report Definitions:
RL - Analyte reporting limit.
QCL - Quality control limit.
MCL - Maximum contaminant level.
ND - Not detected at the reporting limit.

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QA/QC Summary Report

Client: Energy and Env Research Center
Project: Bioaccellerator Project

Report Date: 03/16/06
Work Order: B06030453

Analyte	Result	Units	RL	%REC	Low Limit	High Limit	RPD	RPDLimit	Qual
Method: SW8021B									Batch: 20170
Sample ID: LCS-20170	Laboratory Control Spike				Run: VARIAN1_060314A				03/14/06 18:46
Toluene	2.08	mg/kg	0.050	104	70	130			
Surr: Trifluorotoluene			0.10	98	80	120			
Sample ID: MB-20170	Method Blank				Run: VARIAN1_060314A				03/14/06 19:23
Toluene	ND	mg/kg	0.050						
Surr: Trifluorotoluene			0.10	98.7	80	120			
Method: SW8021B									Analytical Run: VARIAN1_060314A
Sample ID: CCV_0314VAR12r-S	Continuing Calibration Verification Standard								03/14/06 18:10
Toluene	6.52	mg/kg	0.050	109	85	115			
Surr: Trifluorotoluene			0.10	103	85	115			
Method: SW8021B									Analytical Run: VARIAN1_060315A
Sample ID: CCV_0315VAR14r-S	Continuing Calibration Verification Standard								03/15/06 18:38
Toluene	6.57	mg/kg	0.050	109	85	115			
Surr: Trifluorotoluene			0.10	105	85	115			

Qualifiers:

RL - Analyte reporting limit.

ND - Not detected at the reporting limit.

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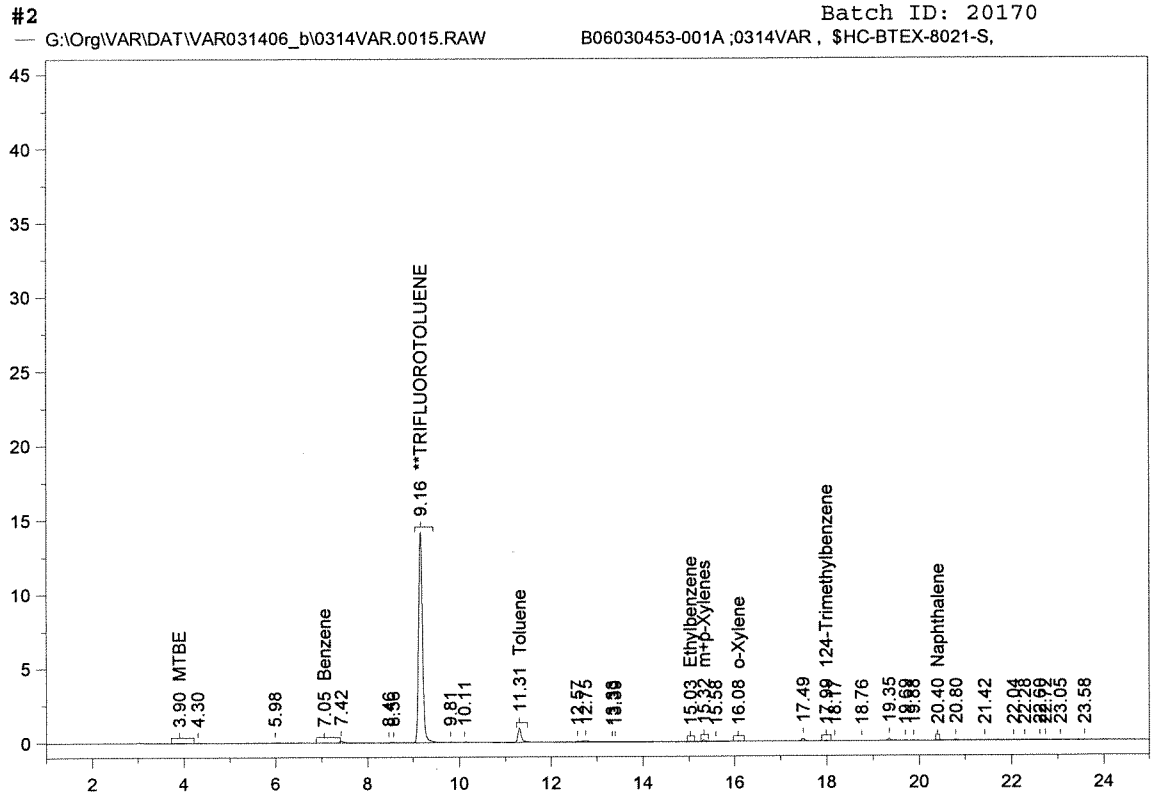

PHOTOIONIZATION DETECTOR TARGET COMPOUND CHROMATOGRAM
Sample Name: B06030453-001A ;0314VAR , $HC-BTEX-8021-S,
Raw File: G:\Org\VAR\DAT\VAR031406_b\0314VAR.0015.RAW
Date & Time Acquired: 3/14/2006 8:00:57 PM
Method File: G:\Org\VAR\Methods\0606VA2.MET
Calibration File: G:\Org\VAR\Cals\0606MAS.CAL
Sample Weight: 50 Dilution: 1 S.A.: 1

TARGET ANALYTES	RT	CAL RRT	RRT	AREA	AMOUNT	FLAG
MTBE	3.903	3.903	3.903	119	.1	U
Benzene	7.051	7.051	7.051	108	.05	U
Toluene	11.307	-2.12	-2.145	5477	.095	
Ethylbenzene	15.031	15.031	15.031	161	.05	U
m+p-Xylenes	15.322	15.322	15.322	605	.05	U
o-Xylene	16.076	16.076	16.076	208	.05	U
124-Trimethylbenzene	17.986	17.986	17.986	182	.2	U
Naphthalene	20.399	20.399	20.399	401	.1	U

SURROGATE COMPOUND	RT	ACTUAL	MEASURED	%REC	QC LIMITS
**TRIFLUOROTOLUENE	9.162	5.	4.258	85.16	80-120

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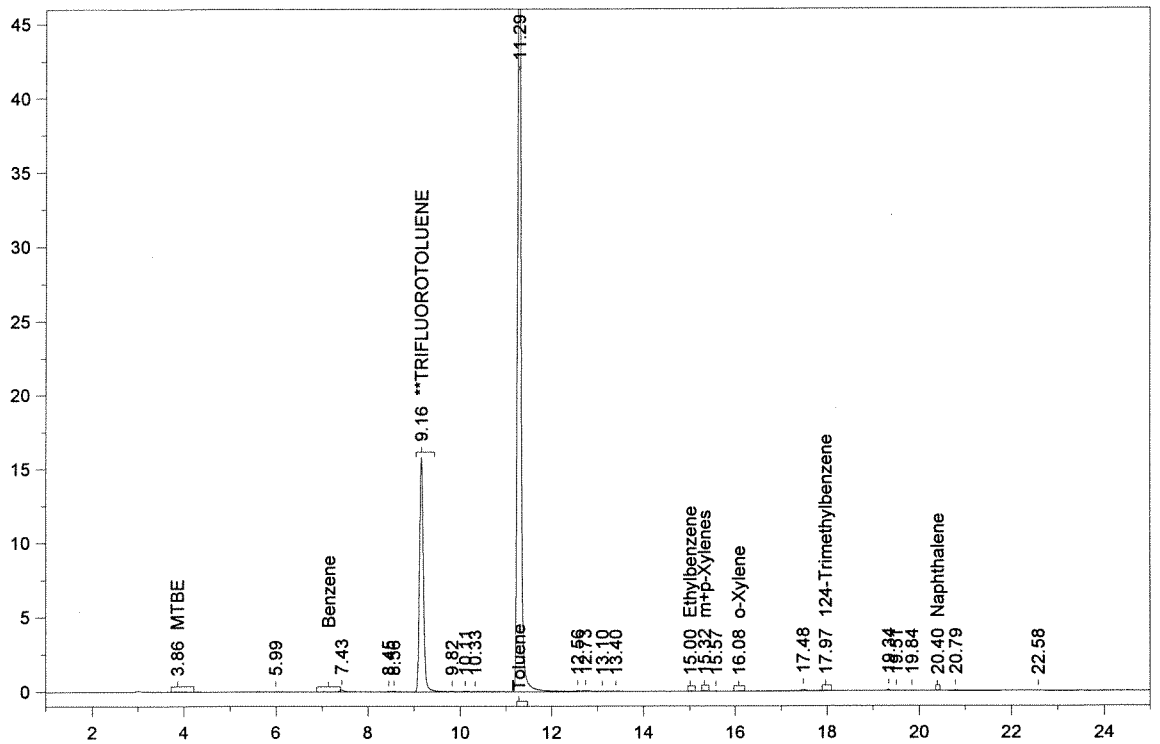
#3 Batch ID: 20170

G:\Org\VAR\DAT\VAR031406_b\0314VAR.0017.RAW B06030453-002A ;0314VAR , $HC-BTEX-8021-S,



PHOTOIONIZATION DETECTOR TARGET COMPOUND CHROMATOGRAM

Sample Name: B06030453-002A ;0314VAR , $HC-BTEX-8021-S,
Raw File: G:\Org\VAR\DAT\VAR031406_b\0314VAR.0017.RAW
Date & Time Acquired: 3/14/2006 9:14:32 PM
Method File: G:\Org\VAR\Methods\0606VA3.MET
Calibration File: G:\Org\VAR\Cals\0606MAS.CAL
Sample Weight: 50 Dilution: 1 S.A.: 1

TARGET ANALYTES	RT	CAL RRT	RRT	AREA	AMOUNT	FLAG
MTBE	3.861	3.861	3.861	125	.1	U
Benzene	.	.	.		.05	U
Toluene	11.29	-2.12	-2.133	334849	5.796	
Ethylbenzene	15.004	15.004	15.004	167	.05	U
m+p-Xylenes	15.321	15.321	15.321	313	.05	U
o-Xylene	16.076	16.076	16.076	120	.05	U
124-Trimethylbenzene	17.969	17.969	17.969	205	.2	U
Naphthalene	20.396	20.396	20.396	203	.1	U

SURROGATE COMPOUND	RT	ACTUAL	MEASURED	%REC	QC LIMITS
**TRIFLUOROTOLUENE	9.157	5.	4.756	95.11	80-120

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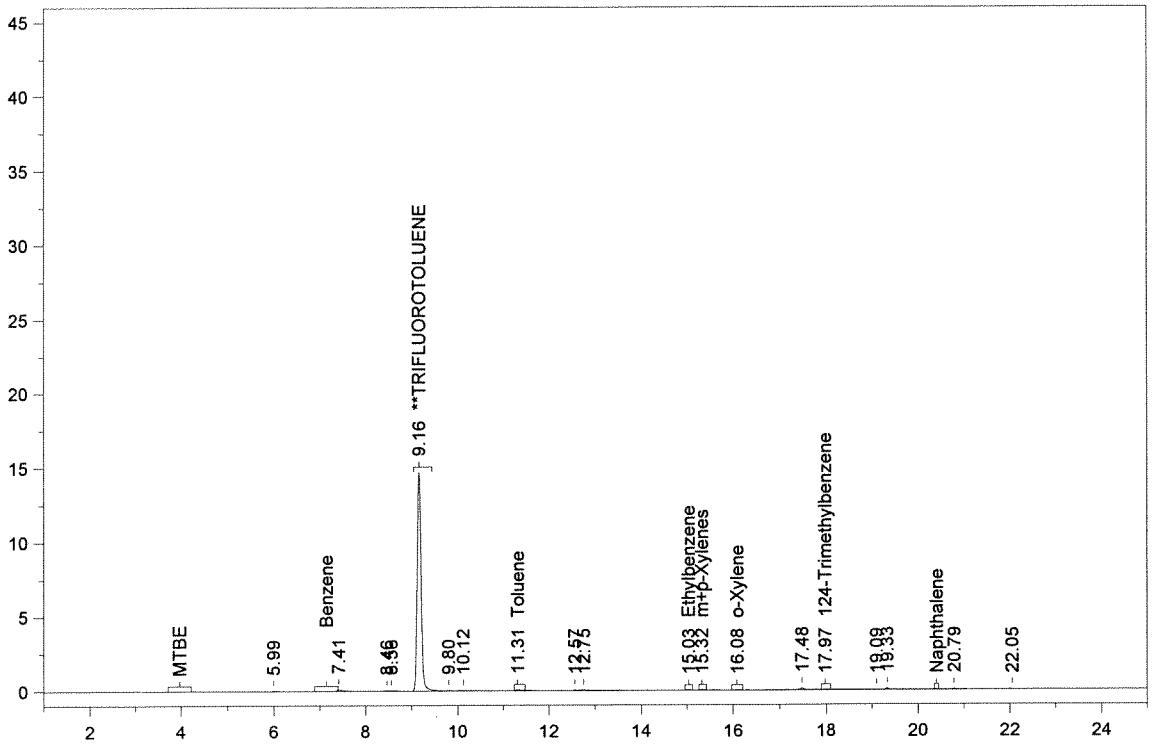
#3 Batch ID: 20170

G:\Org\VAR\DAT\VAR031406_b\0314VAR.0019.RAW B06030453-003A ;0314VAR , $HC-BTEX-8021-S,



PHOTOIONIZATION DETECTOR TARGET COMPOUND CHROMATOGRAM

Sample Name: B06030453-003A ;0314VAR , $HC-BTEX-8021-S,
Raw File: G:\Org\VAR\DAT\VAR031406_b\0314VAR.0019.RAW
Date & Time Acquired: 3/14/2006 10:28:21 PM
Method File: G:\Org\VAR\Methods\0606VA4.MET
Calibration File: G:\Org\VAR\Cals\0606MAS.CAL
Sample Weight: 50 Dilution: 1 S.A.: 1

TARGET ANALYTES	RT	CAL RRT	RRT	AREA	AMOUNT	FLAG
MTBE	.	.	.		.1	U
Benzene	.	.	.		.05	U
Toluene	11.313	11.313	11.313	1180	.05	U
Ethylbenzene	15.034	15.034	15.034	108	.05	U
m+p-Xylenes	15.322	15.322	15.322	347	.05	U
o-Xylene	16.083	16.083	16.083	443	.05	U
124-Trimethylbenzene	17.974	17.974	17.974	615	.2	U
Naphthalene	.	.	.		.1	U

SURROGATE COMPOUND	RT	ACTUAL	MEASURED	%REC	QC LIMITS
**TRIFLUOROTOLUENE	9.159	5.	4.411	88.23	80-120

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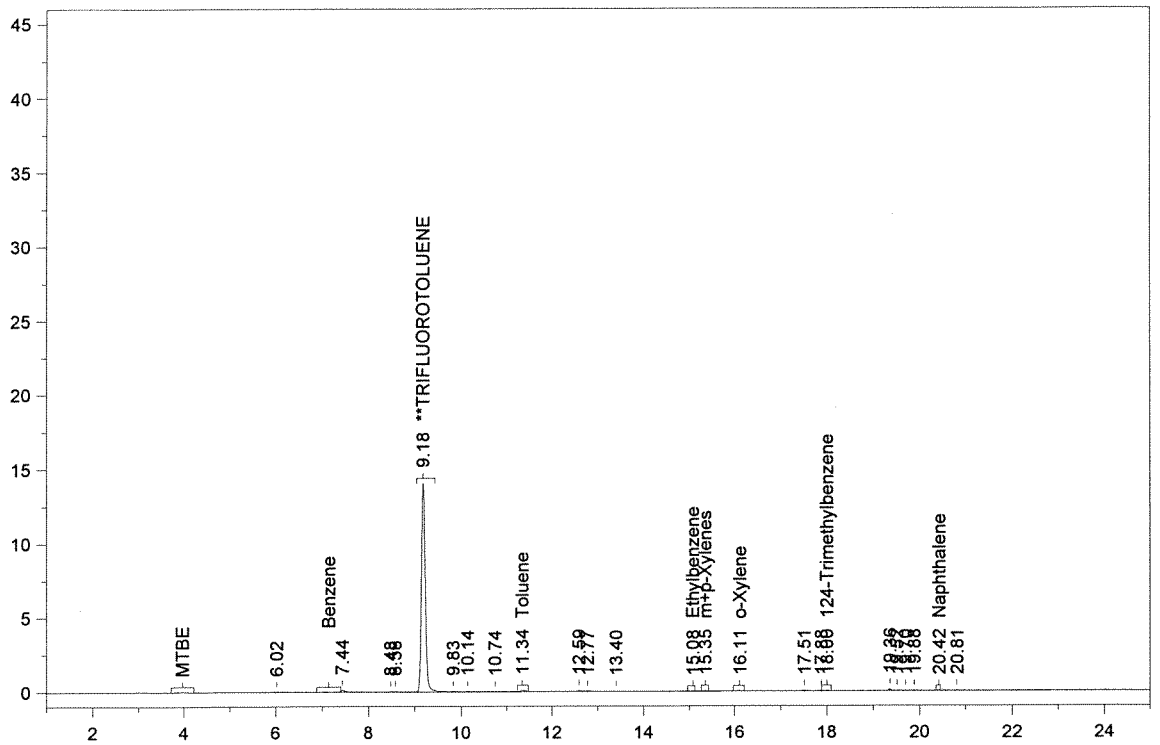
#4 Batch ID: 20170

G:\Org\VAR\DAT\VAR031506_b\0315VAR.0017.RAW B06030453-004A ;0315VAR , $HC-BTEX-8021-S,



PHOTOIONIZATION DETECTOR TARGET COMPOUND CHROMATOGRAM

Sample Name: B06030453-004A ;0315VAR , $HC-BTEX-8021-S,
Raw File: G:\Org\VAR\DAT\VAR031506_b\0315VAR.0017.RAW
Date & Time Acquired: 3/15/2006 8:27:50 PM
Method File: G:\Org\VAR\Methods\0606va8.MET
Calibration File: G:\Org\VAR\Cals\0606MAS.CAL
Sample Weight: 50 Dilution: 1 S.A.: 1

TARGET ANALYTES	RT	CAL RRT	RRT	AREA	AMOUNT	FLAG
MTBE	.	.	.		.1	U
Benzene	.	.	.		.05	U
Toluene	11.345	11.345	11.345	1013	.05	U
Ethylbenzene	15.078	15.078	15.078	142	.05	U
m+p-Xylenes	15.351	15.351	15.351	364	.05	U
o-Xylene	16.112	16.112	16.112	192	.05	U
124-Trimethylbenzene	18.004	18.004	18.004	148	.2	U
Naphthalene	20.42	20.42	20.42	182	.1	U

SURROGATE COMPOUND	RT	ACTUAL	MEASURED	%REC	QC LIMITS
**TRIFLUOROTOLUENE	9.181	5.	4.206	84.12	80-120

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#6 Batch ID: 20170

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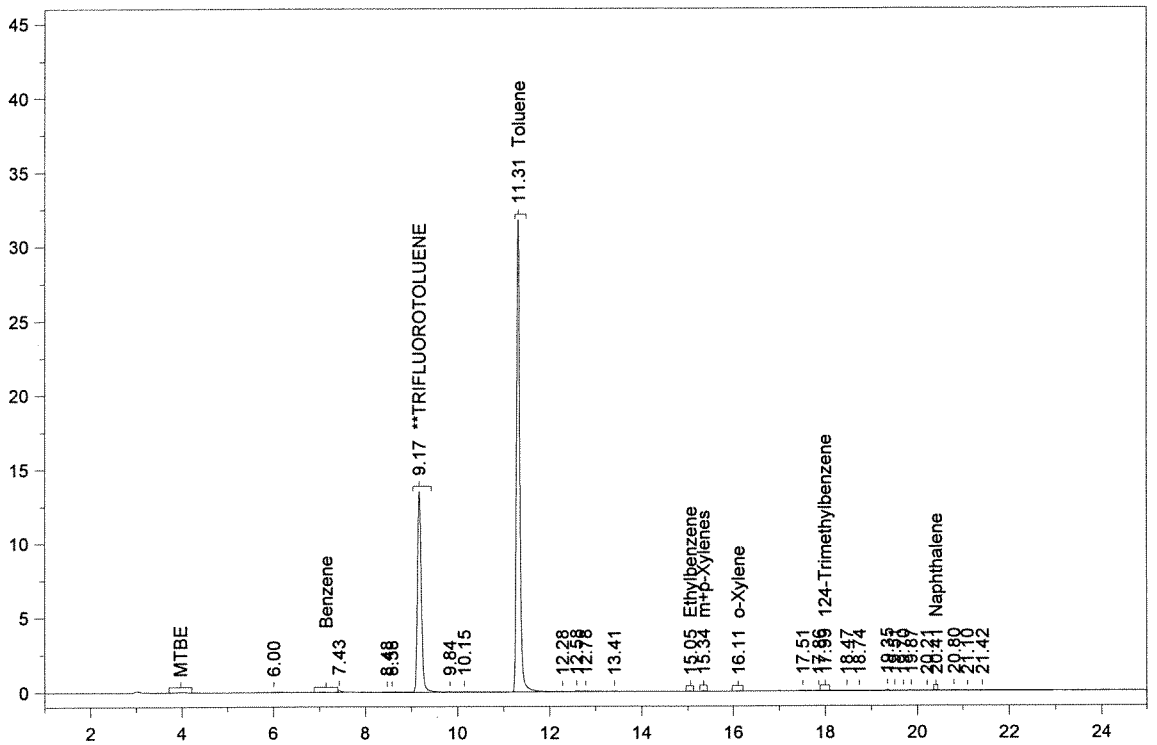

PHOTOIONIZATION DETECTOR TARGET COMPOUND CHROMATOGRAM

Sample Name: B06030453-005A ;0315VAR , $HC-BTEX-8021-S,
Raw File: G:\Org\VAR\DAT\VAR031506_b\0315VAR.0018.RAW
Date & Time Acquired: 3/15/2006 9:04:39 PM
Method File: G:\Org\VAR\Methods\0606var.MET
Calibration File: G:\Org\VAR\Cals\0606MAS.CAL
Sample Weight: 50 Dilution: 1 S.A.: 1

TARGET ANALYTES	RT	CAL RRT	RRT	AREA	AMOUNT	FLAG
MTBE	.	.	.		.1	U
Benzene	.	.	.		.05	U
Toluene	11.313	-2.12	-2.14	169626	2.936	
Ethylbenzene	15.053	15.053	15.053	134	.05	U
m+p-Xylenes	15.343	15.343	15.343	337	.05	U
o-Xylene	16.105	16.105	16.105	141	.05	U
124-Trimethylbenzene	17.992	17.992	17.992	326	.2	U
Naphthalene	20.409	20.409	20.409	245	.1	U

SURROGATE COMPOUND	RT	ACTUAL	MEASURED	%REC	QC LIMITS
**TRIFLUOROTOLUENE	9.173	5.	4.061	81.22	80-120

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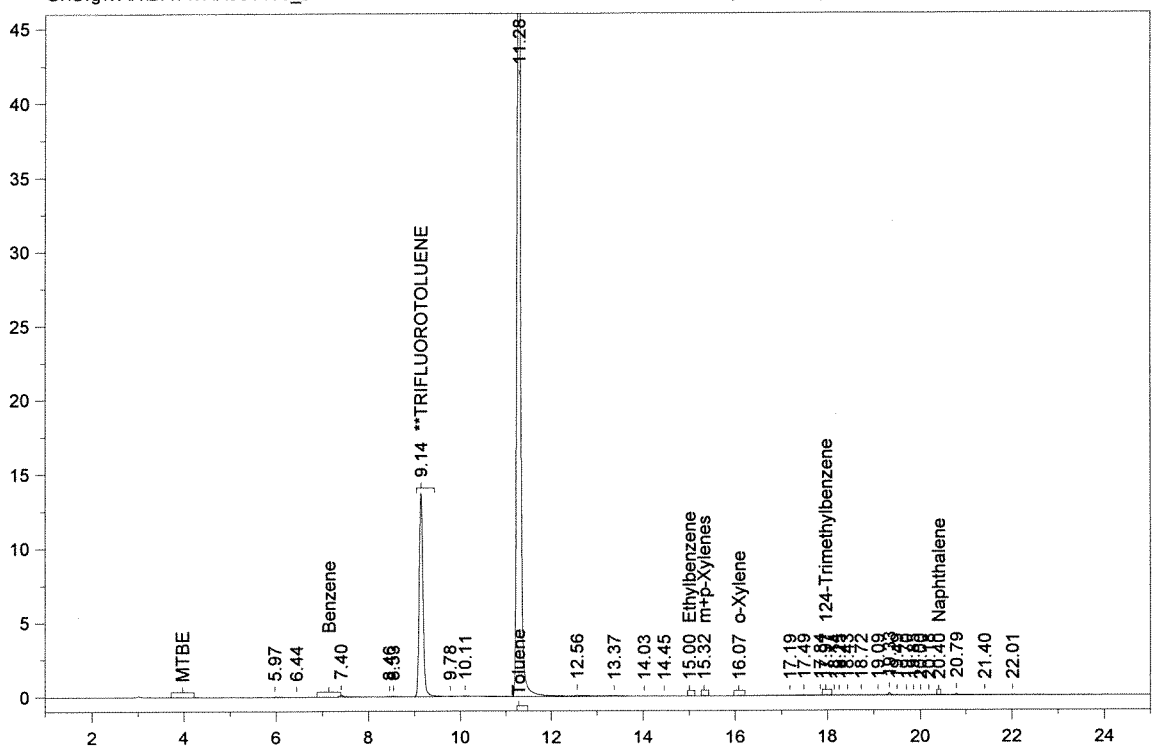
#7 Batch ID: 20170

G:\Org\VAR\DAT\VAR031406_b\0314VAR.0025.RAW B06030453-006A ;0314VAR , $HC-BTEX-8021-S,



PHOTOIONIZATION DETECTOR TARGET COMPOUND CHROMATOGRAM

Sample Name: B06030453-006A ;0314VAR , $HC-BTEX-8021-S,
Raw File: G:\Org\VAR\DAT\VAR031406_b\0314VAR.0025.RAW
Date & Time Acquired: 3/15/2006 2:09:04 AM
Method File: G:\Org\VAR\Methods\0606VA7.MET
Calibration File: G:\Org\VAR\Cals\0606MAS.CAL
Sample Weight: 50 Dilution: 1 S.A.: 1

TARGET ANALYTES	RT	CAL RRT	RRT	AREA	AMOUNT	FLAG
MTBE	.	.	.		.1	U
Benzene	.	.	.		.05	U
Toluene	11.276	-2.12	-2.135	371154	6.424	
Ethylbenzene	15.002	15.002	15.002	152	.05	U
m+p-Xylenes	15.318	15.318	15.318	274	.05	U
o-Xylene	16.074	16.074	16.074	221	.05	U
124-Trimethylbenzene	17.968	17.968	17.968	204	.2	U
Naphthalene	20.399	20.399	20.399	227	.1	U

SURROGATE COMPOUND	RT	ACTUAL	MEASURED	%REC	QC LIMITS
**TRIFLUOROTOLUENE	9.141	5.	4.111	82.21	80-120

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ANALYTICAL SUMMARY REPORT

March 21, 2006

Marc Kurz
Energy and Env Research Center
15 N 23rd St
PO Box 9018
Grand Forks, ND 58203

Workorder No.: B06030453

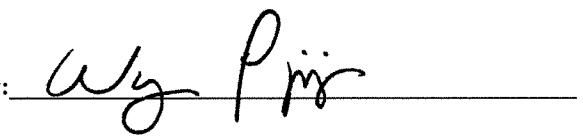
Project Name: Bioaccellerator Project

Energy Laboratories Inc received the following 6 samples from Energy and Env Research Center on 3/7/2006 for analysis.

Sample ID	Client Sample ID	Collect Date	Receive Date	Matrix	Test
B06030453-001	#2	03/06/06 15:00	03/07/06	Soil	Methanol Extraction for Volatiles Purgeable Aromatics Moisture Moisture
B06030453-002	#3	03/06/06 15:00	03/07/06	Soil	Same As Above
B06030453-003	#4	03/06/06 15:00	03/07/06	Soil	Same As Above
B06030453-004	#5	03/06/06 15:00	03/07/06	Soil	Same As Above
B06030453-005	#6	03/06/06 15:00	03/07/06	Soil	Same As Above
B06030453-006	#7	03/06/06 15:00	03/07/06	Soil	Same As Above

There were no problems with the analyses and all data for associated QC met EPA or laboratory specifications except if noted in report comments or the Case Narrative.

If you have any questions regarding these tests results, please call.

Report Approved By: 

Energy Laboratories Inc

Sample Receipt Checklist

Client Name Energy and Env Research Center | Date and Time Received: 3/7/2006

Work Order Number B06030453 | Received by klm

Login completed by: Krystal McDonald (Signature) | 3/7/2006 (Date) | Reviewed by (Initials) | (Date)

Carrier name UPS ARS Ground

Shipping container/cooler in good condition?	Yes ☑	No ☐	Not Present ☐
Custody seals intact on shipping container/cooler?	Yes ☐	No ☐	Not Present ☑
Custody seals intact on sample bottles?	Yes ☐	No ☐	Not Present ☑
Chain of custody present?	Yes ☑	No ☐	
Chain of custody signed when relinquished and received?	Yes ☑	No ☐	
Chain of custody agrees with sample labels?	Yes ☑	No ☐	
Samples in proper container/bottle?	Yes ☑	No ☐	
Sample containers intact?	Yes ☑	No ☐	
Sufficient sample volume for indicated test?	Yes ☑	No ☐	
All samples received within holding time?	Yes ☑	No ☐	
Container/Temp Blank temperature in compliance?	Yes ☐	No ☑	18 °C
Water - VOA vials have zero headspace?	Yes ☐	No ☐	No VOA vials submitted ☑
Water - pH acceptable upon receipt?	Yes ☐	No ☐	Not Applicable ☑

Adjusted? _____ Checked by _____

Any No and/or NA (not applicable) response must be detailed in the comments section below.

Client contacted _____ Date contacted: _____ Person contacted _____

Contacted by: _____ Regarding _____

Comments:

Corrective Action _____

Chain of Custody and Analytical Request Record

Page 1 of 1

PLEASE PRINT, provide as much information as possible. Refer to corresponding notes on reverse side.

Company Name: UND - EERC (Energy & Environmental Research Center)	Project Name, PWS #, Permit #, Etc.: BioAccelerator Project
Report Mail Address: 15N. 23rd ST. GRAND FORKS, ND 58202	Contact Name, Phone, Fax, E-mail: MARC KURZ (701) 777-5278 MKURZ@UNDEERC.ORG Sampler Name if other than Contact:
Invoice Address: SAME	Invoice Contact & Phone #: SAME Purchase Order #: ELI Quote #:

Report Required For: POTW/WWTP ☐ DW ☐ Other _____

Special Report Formats - ELI must be notified prior to sample submittal for the following:

NELAC ☐ A2LA ☐ Level IV ☐

Other _____

EDD/EDT ☐ Format _____

Number of Containers
Sample Type: A Air W Water S Soils/Solids V Vegetation B Bioassay O Other

ANALYSIS REQUESTED

Notify ELI prior to RUSH sample submittal for additional charges and scheduling

Comments: PLEASE DO the extraction on ENTIRE contents of JAR per Bill Brown

Shipped by: ANC NDSU

Cooler ID(s)

Receipt Temp 18 °C

Custody Seal Y (N) Intact Y N Signature Y N Match

	SAMPLE IDENTIFICATION (Name, Location, Interval, etc.)	Collection Date	Collection Time	MATRIX	BTEX	Toluene Only per Bill B.	SEE ATTACHED	Normal Turnaround (TAT)	RUSH Turnaround (TAT)	Lab ID
1	#2 TOLUENE ONLY	3/6	3pm	S	X			X		[illegible]603045341
2	#3 ORC ONLY	↓	↓	↓	X			↓		2
3	#4 NRC-1 ONLY				X					3
4	#5 NRC-2 ONLY				X					4
5	#6 NRC-1 + ORC				X					5
6	#7 NRC-2 + ORC				X					6
7										
8										
9										
10										

LABORATORY USE ONLY

Custody Record MUST be Signed

Relinquished by (print): MARC KURZ	Date/Time: 3/6/06	Signature:	Received by (print):	Date/Time: / Signature:
Relinquished by (print):	Date/Time:	Signature:	Received by (print):	Date/Time: 3/7/06 0900 / Signature:

Sample Disposal: Return to client: _____ Lab Disposal: X

LABORATORY USE ONLY

Sample Type: _____ # of fractions _____

In certain circumstances, samples submitted to Energy Laboratories, Inc. may be subcontracted to other certified laboratories in order to complete the analysis requested. This serves as notice of this possibility. All sub-contract data will be clearly notated on your analytical report.

Visit our web site at *www.energylab.com* for additional information, downloadable fee schedule, forms, & links.