FINAL REPORT

Cryogenic Collection of Complete Subsurface Samples for Molecular Biological Analysis

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14. ABSTRACT

The objective of this project has been to demonstrate the efficacy of cryogenic core technology as an integrated approach to sample collection and handling for use as a tool for monitoring subsurface remediation. This has included the development of a in situ cryogenic coring system, the evaluation of molecular biological tools (MBTs) analysis in conjunction with that system and the demonstration that the cryogenic sampling protocols are robust and ready for application by the remediation user community. In addition, cryogenic sample handling has allowed development of a new approach to directly determine local degradation activity using the ratio of labile mRNA to stable DNA for target genes.

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LIST OF ACRONYMS, ABBREVIATIONS AND SYMBOLS

BP	boiling point
cDNA	complementary DNA
cis-DCE	*cis*-1,2-dichlororethene
CT	carbon tetrachloride
DART-PCR	Data Analysis for Real-Time PCR
DNAPL	dense non-aqueous phase liquid
DO	dissolved oxygen
DoD	Department of Defense
ESTCP	Environmental Security Technology Certification Program
kg	kilogram
LN	liquid nitrogen
MBT	molecular biological tools
mg	milligrams
mL	milliliter
mm	millimeter
mRNA	messenger RNA
O.D.	outside diameter
^{o}C	degrees Celsius
PCE	tetrachloroethene
PCR	polymerase chain reaction
PCR-SSCP	PCR single-strand conformation polymorphism
ppb	parts per billion
ppm	parts per million
ppmV	parts per million, volume basis
qPCR	quantitative polymerase chain reaction
r	correlation coefficients
rRNA	ribosomal RNA
SERDP	Strategic Environmental Research Development Program
SOP	standard operating procedure
TCA	trichloroethane
TCE	trichloroethene
tmoA	Toluene monooxygenase gene
trans-DCE	*trans*-1,2-dichloroethene
VC	vinyl chloride

KEYWORDS

Activity ratio
Cryogenic coring
DNA
Molecular biological tools
mRNA
qPCR

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ABSTRACT

OBJECTIVES

The objective of this project has been to demonstrate the efficacy of cryogenic core technology as an integrated approach to sample collection and handling as a tool for monitoring subsurface remediation. This has included the development of a *in situ* cryogenic coring system, the evaluation of molecular biological tools (MBT) analysis in conjunction with that system and the demonstration that the cryogenic sampling protocols are robust and ready for application by the remediation user community.

TECHNICAL APPROACH

The performance of the cryogenic coring system was evaluated for both sample collection and sample preparation. The effects of sample collection and handling were evaluated using "benchmark" samples consisting of both laboratory-generated frozen cores samples and cryogenically-sampled cores taken from a well-controlled physical model environment. These samples were used to systematically assess the effects of whole-sample freezing on the integrity of biomolecules relevant to bioremediations. Impacts of freezing on DNA and RNA were assessed using quantitative polymerase chain reaction (PCR) as well as the community fingerprinting method, PCR single-strand conformation polymorphism (PCR-SSCP).

RESULTS

The cryogenic coring system described allows samples to be frozen *in situ*. Once brought to the surface, the frozen cores can be packed in dry ice and shipped to the laboratory for further processing and analysis. The approach prevents redistribution of fluids during sample recovery and shipping, and because the cores are frozen *in situ* there is little loss of solid material during retrieval to ground surface. The data indicate that the vertical distribution of DNA within the core can be measured at the centimeters scale, providing unprecedented characterization of subsurface biogeochemical interfaces.

Additionally, we did not observe any significant degradation due to freezing or storage for a suite of genes and gene transcripts, including short-lived messenger RNA (mRNA) transcripts, from *P. putida* F1 or from *B. subtilis* JH642 in single-species samples, or from archaea in enrichment culture samples that also contained members of diverse bacterial phyla. Similarly, freezing did not change the relative abundance of dominant phylotypes in enrichment culture samples as measured by PCR-SSCP of bacterial 16S rDNA. Furthermore, freezing, and storage for 5 months at –80ºC did not affect the microbial community composition of samples from the model aquifer. Of even greater significance is that freezing and storage did not affect the relative abundance of 16S ribosomal RNA (rRNA) phylotypes, since in-vivo rRNA content is often correlated with cellular growth rate. Thus we conclude that cryogenic preservation and storage of intact sediment samples can be used for accurate molecular characterization of microbial populations.

Following our characterization of the effects of whole-sample freezing, quantitative PCR was used to profile the abundance of genes associated with Toluene degradation across a contaminant plume in a model aquifer. This approach is important because profiles generated from sediment samples better reflect in situ contaminant degradation. Experiments using Nitrate to stimulate anaerobic Toluene biodegradation revealed that bacteria containing the gene for benzylsuccinate synthase (*bssA)*, although present, were not actively degrading Toluene in groundwater lacking Nitrate. Results were consistent with the hypothesis that organisms, and their genes, detected in groundwater may be artifacts of microbial transport from upstream where chemical conditions favored their growth. The transcription of *bssA* genes from denitrifying Toluene degraders was induced by Toluene, but only in the presence of Nitrate. Transcript abundance dropped rapidly following the removal of either Toluene or Nitrate. The drop in *bssA* transcripts pursuant to the removal of Toluene could be described by an exponential decay function with a rate constant of 0.44 hr^{-1} and a half-life of 1.6 hrs.

Interestingly, *bssA* transcripts never disappeared completely, but rather appeared to be constitutively transcribed in the absence of inducers. A significant implication of this is that the detection of transcripts may not be sufficient as evidence of contaminant degradation. Instead, an integrated approach combining functional gene abundance and gene transcript analysis is recommended. This approach circumvents the complications associated with interpreting pore water gene abundance data which arise from microbial transport, thus making reliable assessments possible from water samples, as opposed to sediment samples. It also avoids the possibility of mistakenly associating basal-level gene expression with actively degrading microbial populations.

BENEFITS

One of the primary benefits of this project was the demonstration that labile biomolecules can be preserved in aquifer solids if those solids are collected cryogenically. This result will make it possible to analyze aquifer materials for biomolecules that serve as indicators of activity (e.g., mRNA of targeted genes). This represents a significant advancement over the current approach that relies on DNA of functional genes which, while providing direct confirmation of the presence of specific types of organisms, does not provide direct evidence of their activities.

As with most research projects, this one yielded unexpected benefits. In this case, our improved ability to collect labile molecules led directly to improvements in diagnosis of in situ activity. Specifically, the physical model studies conducted as part of this work showed that ratios of mRNA to DNA for functional genes could be used to identify areas where bacterial populations were and were not actively degrading contaminants. Of perhaps equal importance, the approach also allowed us to identify where degradation activities were not taking place, even though DNA analyses indicated that those organisms were present (i.e., under conditions where DNA-only analysis would have given a false positive result.)

The cryogenic sampling technique developed during this project builds on current practice for core sampling, and was designed to be easily integrated into routine field sampling.

As a consequence, it is now possible to collect intact aquifer samples containing both solids and pore water. This approach couples well with quantitative polymerase chain reaction (qPCR) methods, which are the basis for molecular-tool based approaches for groundwater remediation.

INTRODUCTION

1.1 SERDP RELEVANCE

Molecular biological tools (MBT) have tremendous potential to improve the design, field performance, and monitoring of subsurface remediation. However, MBT use in the operational cleanup community is limited at present. In order to reduce the time and cost of environmental restoration activities, it is important to integrate MBT into widely-used protocols applicable to all phases of those remediation activities. To accomplish this, a robust, accurate sampling methodology was developed and tested here.

1.2 PROJECT OBJECTIVES

1) Develop and evaluate a sampling methodology for cryogenic collection of "complete" subsurface core samples (i.e., water plus aquifer solids) for MBT analysis

2) Evaluate the applicability of cryogenic cores for the collection and preservation of a suite of biomolecules important for subsurface remediation.

BACKGROUND

1.3 CRYOGENIC CORE BACKGROUND

Cryogenic collection of environmental solids samples has been a standard approach in a number of disciplines for decades. The technique has been used to examine physical characteristics of soils and sediments, including soil accretion (Cahoon & Reed, 1995), bulk density (Knaus & Cahoon, 1990), soil compaction and water content (Cahoon et al., 2000). It has also been used to examine stream ecology (e.g., siltation of gravel beds, Everest et al., 1980; Petts et al., 1989) and to measure the distribution of non-aqueous-phase liquids near the water table in the subsurface (Durnford et al., 1991),

In addition to the physical and chemical applications discussed above, cryogenic coring can be applied to microbial characterization. In particular, characterization of biogeochemical interfaces can be critical to understanding many processes in the subsurface. An increasingly important aspect of this characterization is the use of MBT to understand microbial communities and physiology. Both DNA and RNA can been used in this context and, when used in combination, they can provide evidence of both the presence and activity of a broad range of microbial species.

As an example of the application of cryogenic coring, restoration of groundwater contaminated with chlorinated solvents and petroleum hydrocarbons often relies heavily on a biological component for success. However, assessing biological activity in the subsurface is challenging. Historically, a number of indirect means have been used as characterization tools, with varying degrees of success. Geochemical measurements (e.g., oxidation/reduction potential) have been used to indicate whether conditions are appropriate for bioremediation (e.g., US EPA, 1998). Alternately, the appearance of daughter products of the original contaminants provides direct evidence that transformations have occurred, but little insight into the mechanisms by which those transformations occurred or whether they continue to occur (e.g., US EPA, 2000). Measurement of shifts in the ratios of stable isotopes has also been proposed as a measure of transformation (US EPA, 2008); however, once again, this approach does not provide insight into how and when transformations occurred.

More recently, MBT are increasingly being used to assess subsurface restoration. Currently, most applications are based on DNA measured in water samples (e.g., Environmental Security Technology Certification Program [ESTCP], 2011). While water/DNA samples can provide direct evidence that specific organisms are present, it is difficult to differentiate between cells which are part of the active, local microbial community and those that are inactive and being transported in groundwater. (Recent work utilizing ratios of labile mRNA to DNA may significantly improve our ability to directly measure activity of water samples (Brow, 2011; Lee et al., 2008); however, this approach is still being developed.)

For many subsurface systems, including most groundwater systems, collection of intact subsurface solid samples represents an desirable alternate approach to water-only samples because the numbers of surface-attached organisms typically exceed those of un-attached ones (Doong et al.1997; Haglund et al., 2002; Holm et al., 1992). Furthermore, a mounting body of

evidence suggests that biodegradation "hotspots" in the subsurface may exist on spatial scales too fine to be adequately captured by conventional groundwater sampling (e.g., along horizontal interfaces, Wilson et al., 2004; Winderl et al., 2008). However, field processing of solid samples is more difficult than for water samples and, as a result, if solid samples are to be used, there are significant advantages to postponing sample preparation until the samples are in the laboratory. However, in this case biomolecule stability is a concern, and thus there is a need for new methods to collect and preserve core samples for MBT analyses. We have recently demonstrated that biomolecules can be quantitatively recovered from frozen solid samples (Brow et al., 2010), and as a result, if core samples are frozen on site, they can be returned frozen to the laboratory for subsequent processing. To facilitate this, and to enhance the collection and recovery of the cores (e.g., minimize fluids redistribution and loss of cohesionless materials) we have developed a new *in situ* cryogenic coring system. The capabilities of this new system are examined here by comparing cryogenically-collected core samples collected across a biogeochemical interface with high-resolution water and solids samples from adjacent locations in a very large physical model.

1.4 CRYOGENIC PRESERVATION BACKGROUND

Subsurface bioremediation strategies should be based on knowledge of indigenous microbial organisms, including their metabolic capabilities and the ways in which they respond to changing environmental conditions (Lovley, 2003; Watanabe, 2001). Because most microorganisms are not easily cultured in the laboratory, the use of MBT for gene detection and quantification, community fingerprinting, and gene expression has tremendous potential to improve the design, monitoring, and field performance of subsurface remediation.

Many studies suggest that 90% to 99% of bacterial populations, including those that degrade a variety of contaminants, are attached to solid phase materials in both laboratory-scale column experiments and in the field (Doong et al., 1997; Haglund et al., 2002; Holm et al., 1992; Lehman et al., 2001; Lehman & O'Connell, 2002). There are additionally a number of reports documenting the enhancement, by particle-attached bacteria, of the rates of dechlorination of chlorinated hydrocarbons (Doong et al., 1997), biodegradation of aromatic hydrocarbons (Holm et al., 1992) and other cellular activities (Haglund et al., 2002; Lehman et al., 2001; Lehman & O'Connell, 2002), compared to rates observed for unattached bacteria. Collection of intact core samples is, therefore, an optimal approach for accurately characterizing subsurface microbial populations.

Furthermore, the degradation process may largely be confined to the fringes of contaminant plumes, where overlapping "counter-gradients" of electron donors and acceptors exist. This "plume fringe theory" has been repeatedly validated in both laboratory (Bauer et al., 2008; Rees et al., 2007) and field studies (Mayer et al, 2001; Tuxen et al., 2006; Wilson et al., 2004; Winderl et al., 2008), further demonstrating the need for fine-resolution sampling such as that provided by sediment coring.

However, the collection and storage of core samples for molecular analyses can be problematic. Whereas water samples can be filtered on site and stored in extraction buffer or preservation media (RNAlater®, etc.) to prevent the degradation of nucleic acid-based biomolecules, it is

difficult to separate cells from soil in the field. For this reason, soil samples for molecular analyses are often stored in buffers as well, but doing so with core samples is not practical. Disturbing or destroying the core would obscure biogeochemical interfaces that may be relevant to bioremediation. Consequently, the preservation of biomolecules such as DNA and RNA within intact core samples is an important issue. This is particularly true due to the rapidly increasing interest in the use of gene expression mRNA as a diagnostic tool. Gene expression, rather than gene presence, is a better indicator of physiological activity because mRNA molecules are relatively short-lived compared to DNA molecules. For example, reported *in vivo* mRNA half-lives for the reductive dehalogenase genes *vcrA* and *tceA* are 4.8 and 6.1 hours, respectively (Lee et al.,, 2006), underscoring the importance of sample preservation.

Cryogenic preservation is commonly used for environmental samples, but the effects of whole-core freezing on the integrity of biomolecules such as DNA and RNA has not been systematically examined. Additionally, cryogenic coring (freezing the core in-situ prior to extraction to the surface) has been used to preserve the in-situ macrobiological and physical characteristics of core samples for at least 30 years (Everest et al., 1980; Moser et al., 2003; Petts et al., 1989; Petts et al., 1991), though its use for molecular biological analysis has been limited.

DNA and RNA become increasingly fragmented as samples degrade. This can lead to reductions in PCR amplification efficiencies, increases in detection limits, and even amplification failure. These effects become increasingly pronounced for longer target sequences as the yield of complete target fragments is greatly reduced (Chung et al., 2004; Piyamongkol et al., 2003; Timken et al., 2005). Additionally, sample degradation can lead to the preferential amplification of undamaged targets (Piyamongkol et al., 2003), thus affecting assessments of microbial community structure. In the work reported here, we examine the impacts of whole-sample freezing on the integrity of DNA and RNA through the detection and quantification of a suite of genes and gene transcripts from Bacteria and Archaea. Single-strain experiments using laboratory-generated samples were performed with both *Pseudomonas putida* F1 and *Bacillus subtilis* JH642, two bacterial strains with very different cellular properties (e.g., cell wall composition and structure) potentially resulting in different responses to the freezing and thawing processes. PCR single-strand conformation polymorphism (PCR-SSCP) was used to assess the effects of freezing on relative phylogenetic type (phylotype) abundance within more complex laboratory samples, as well as in sediment samples taken from a physical-model aquifer. A subset of frozen samples was also stored at –80ºC before DNA and RNA were extracted in order to determine the effects of frozen storage on intact samples.

1.5 ACTIVITY RATIOS BACKGROUND

As has been repeatedly demonstrated in both column and field studies, functional gene abundance is not always predictive of contaminate degradation, nor does it consistently correlate with gene expression or contaminant concentration (Behrens et al., 2008). Implicitly, this is understood because the presence of a gene does not necessarily guarantee its expression or activity (Lovley, 2003). For example, it has been demonstrated that *Dehalococcocies* strain 195 can decouple growth from dechlorination (Maymo-Gatell, 1997). Additionally, even under optimal laboratory conditions, organisms such as *Dehalococcoides* have doubling times that are disproportionately longer the time required to either up or down regulate reductive dehalogenase

(RDase) gene expression. Reported doubling times are 19.2 hours for strain 195 (Maymo-Gatell, 1997), and 2.2 days for BAV1 (He et al., 2003). Conversely, up-regulation of RDase genes has been shown to occur in as little as 3 hours following exposure to chlorinated solvents (Johnson et al., 2005), and RDase mRNA half lives are on the order of 4 to 6 hours (Lee et al., 2006).

It has also been suggested that genes recovered from groundwater samples may only represent conditions up-gradient of where they were sampled because planktonic microorganisms are subject to transport. This "false positive" effect can be circumvented by sediment sampling (Brow et al., 2012b) however, the relative simplicity of groundwater sampling makes it more attractive from a practical perspective.

As part of this project we used sediment and water samples collected from a large-scale model aquifer to evaluate the efficacy of using a combination of functional genes and gene transcripts in water samples to assess the *in-situ* microbial activity. In the process, we demonstrated the usefulness of a combined mRNA and DNA approach to characterize degradation activity in water samples. Unlike the functional gene-only approach, this method was successful in providing results that were consistent with biogeochemistry, and observed Toluene degradation. Of perhaps even greater significance is the possibility that, using this approach, the spatial extent of active degradation can be accurately assessed using groundwater samples as opposed to more labor-intensive sediment samples.

MATERIALS AND METHODS

1.6 CRYOGENIC CORE MATERIALS AND METHODS

1.6.1 Cryogenic core development overview

Direct-push sampling for aquifer materials (e.g., Geoprobe MacroCore™ or DualCore™) is a well-developed technology, and is in wide use by the operational cleanup community. Since it may be desirable/necessary to collect aquifer materials for accurate MBT analyses, direct push techniques are potentially well-suited to this task. However, as with all core sampling, there are a number of contamination and sample collection issues that may limit the quality of samples. As discussed above, cryogenic sampling is one means to maximize sample quality. However, to our knowledge, direct-push-type samplers have not been used in conjunction with cryogenic sampling (and specifically for cryogenic sampling for MBT).

To be most useful, the protocol for cryogenic core collection should be as close as possible to conventional core sampling, with the addition of a cryogenic cooling step once the sample is inside the core barrel. The additional cryogenic step can be accomplished by delivery of liquid nitrogen from a pressurized dewar to the core barrel outside of the sleeve containing the core. The coolant will then be vented back to the atmosphere via a return tube (see Section 3.1.3 for a detailed procedure and Figure 3.1 for a picture of the setup in action). As with the (Durnford et al., 1991) design, with this approach it will be possible to freeze the sample without introducing any coolant vapor into the sample or the subsurface. The cores can be collected using either in aluminum or stainless steel sleeves (metal rather than plastic sleeves are used because of better heat transfer characteristics, aluminum sleeves were used here). Once frozen cores are removed from the subsurface, it is a relatively standard approach to pack them in dry ice and ship them by an overnight express service to the laboratory. Because the samples will be solid, it is possible to delay decontamination of the cores until they arrive at the laboratory. This represents a significant simplification of field operations. Decontamination of the soil cores in a sterile laboratory environment has been evaluated using both chemical treatment (Rogers et al., 2004) and by sub-coring using a sterile diamond-tipped core drill. We have previously used cryogenic core sampling with a core barrel similar to the (Durnford et al., 1991) design.

Figure 3.1. Direct-push Cryogenic Coring System Venting N_2 gas During Freezing Phase of Core Collection.

1.6.2 Design of the cryogenic core "CryoCore" sampler

Figure 3.2 shows schematic drawings of the principal components of the cryogenic core sampler. (Photographs of the individual components can be found in Appendix A.1: Cryogenic Core Supporting Data [Figure A.1]). The sampler is designed to be compatible with Geoprobe® direct-push coring equipment and, as a result, a number of the components are commercially available. The standard operating procedure (SOP) for the sample collection process was modified from the approach developed for the Geoprobe DT325 "Dual Tube" procedure (Geoprobe, 2011), as discussed below. In brief, probe rods are advanced to just above the sample collection depth. The drive tip is then removed and a sample sheath containing the sample sleeve is lowered into the probe rod. The probe rod and sample sheath/sleeve assembly are then advanced together to fill the sample sleeve.

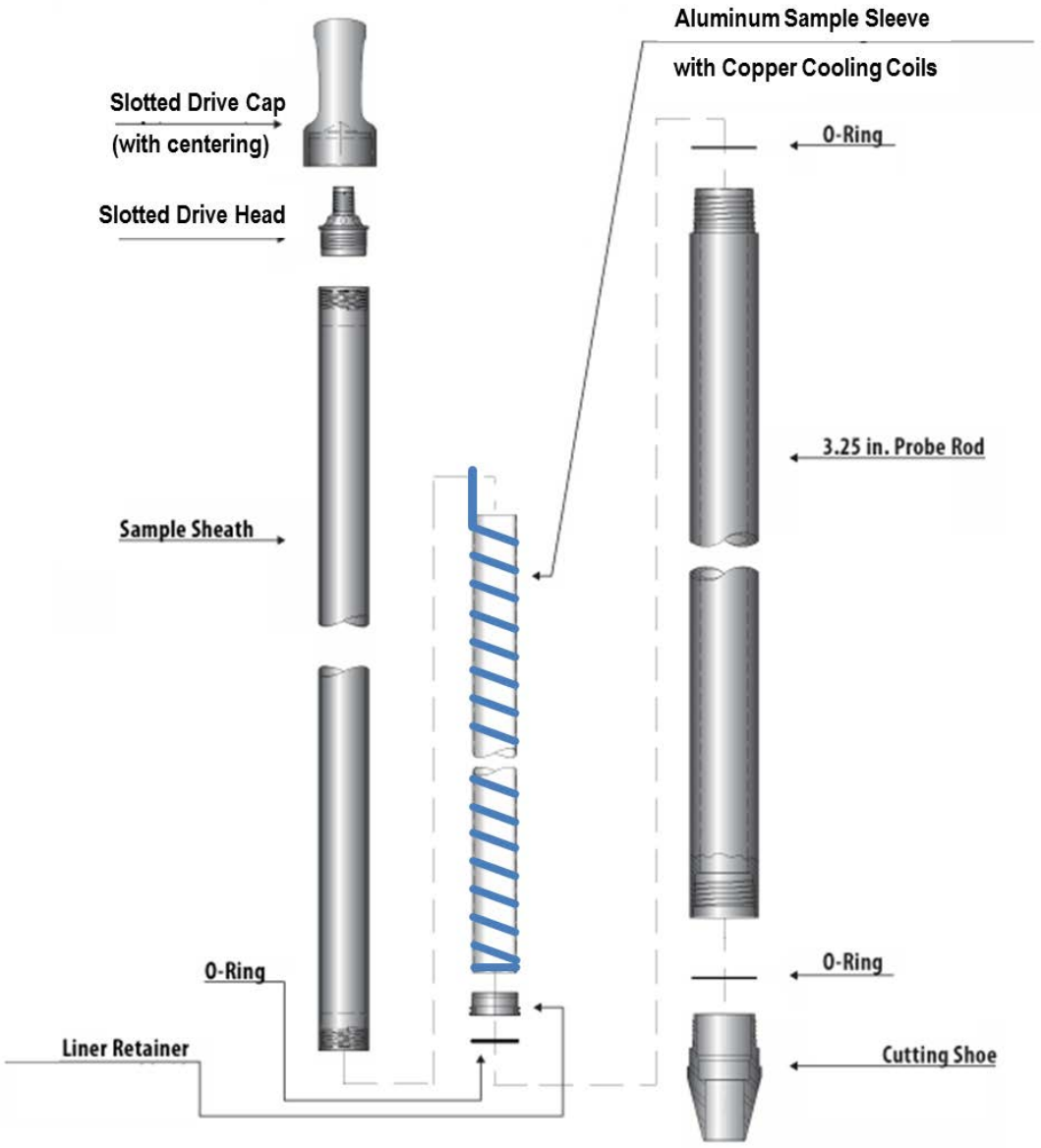

Figure 3.2. Schematic Drawing of the Tooling used for Cryogenic Sample Collection. Not Shown are 1) 1.25 –inch diameter drill rod is used to connect the slotted drive cap and the slotted drive head; 2) insulated copper tube extending from the top of the sample sleeve to ground surface.

For non-cryogenically-collected samples, the sheath containing the sleeve and sample would then be brought to ground surface. For cryogenic cores, an insulated copper tube, which is attached to the coils surrounding the sleeve (Figure 3.2), is connected to a pressurized liquid nitrogen (LN) tank and LN is injected into the tube for a period of ~5 minutes to freeze the core. Following retrieval of the frozen sample sheath/sleeve/core to ground surface, the sleeve can be removed from the sheath, or the entire assembly can be packaged in dry ice and shipped via overnight courier.

The sleeve containing the sample can be sectioned while the core remains frozen. To accomplish this, the aluminum tube is cut using a tubing cutter, and then the frozen core section is cleaved using a sterile chisel. (It should be noted here that plastic liners are not advised for cryogenic cores for two reasons. First, as mentioned above, heat transfer through the plastic is much lower than for aluminum and, as a consequence, it takes longer to initially freeze the cores. Second, at temperatures of -20^{o}C and below, the plastic is very brittle and tends to shatter during sectioning of the core.) Our standard procedure is to remove the aluminum ring (created when the section is cleaved) from around the core by cutting the aluminum on a small vertical bandsaw. The core sections are then wrapped in sterilized aluminum foil and stored at -80^{o}C until needed. As shown in Figure 3.3, all of the core sectioning operations are carried out in a -20^{o}C chest freezer that can be transported to the field.

Figure 3.3. Chest Freezer Containing Bandsaw, Tubing Cutter, Aluminum Core Sleeve and Core Sections Wrapped in Tinfoil.

1.6.3 Cryogenic core sampling procedure

The sequence on the left in Figure 3.4 shows the process by which a typical core is collected using a Geoprobe dual core samples. Briefly:

1. The drill rod and temporary drive point are driven into the ground to the desired core depth.
2. The temporary drive point is removed.

3. The core liner and sheath are placed in the drill rod and an additional rod section is added
4. The drill rod and coring system are advanced to fill the core liner.
5. The core liner and sheath are removed, and the sample can be repeated as desired.

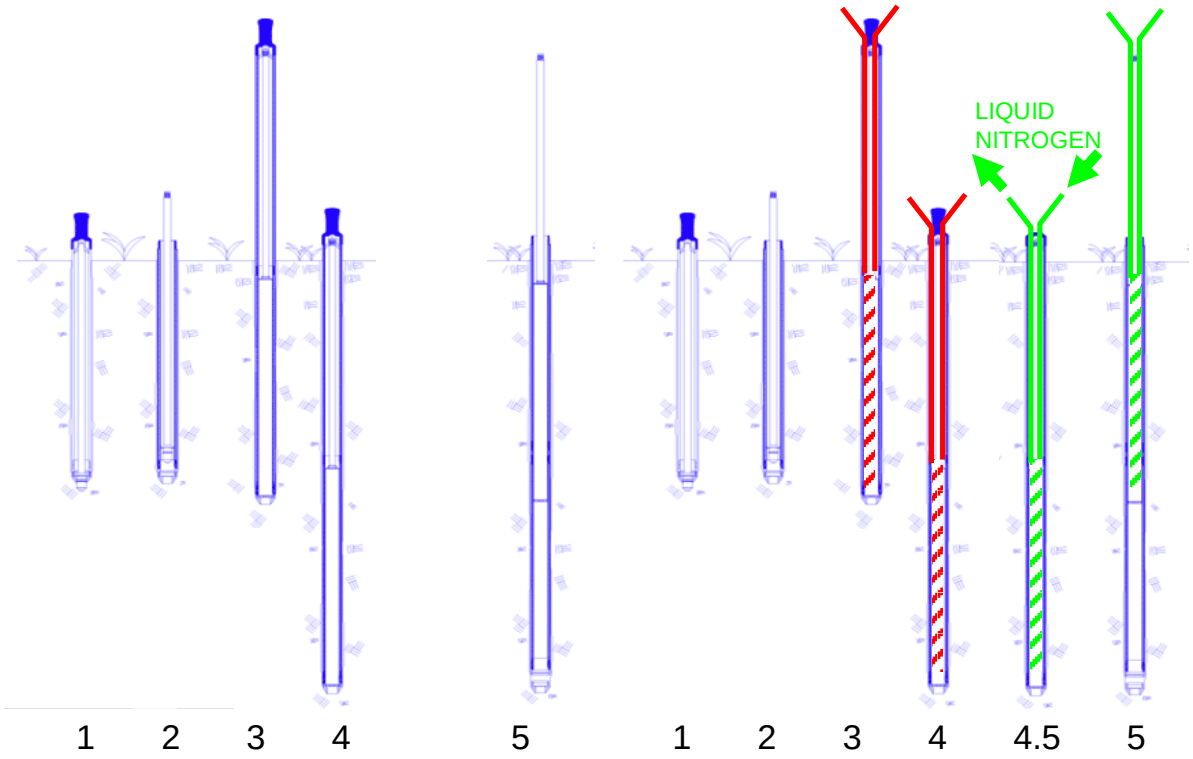

Figure 3.4. Steps in collecting DualCore and CryoCore Subsurface Samples.

As discussed above, we have replaced the plastic liner and sheath with a metal liner and cooling coils. We have modified the process by adding one additional step (4.5) in which liquid nitrogen is circulated through the copper cooling coils for 3-5 minutes prior to recovery of the sample.

The core barrel is then brought to the surface. Because of the possibility that the core barrel may become frozen within the drive casing (which has not proved to be the case in our work), it may be important to immobilize the drive casing during core barrel removal. Most core collection rigs are equipped with a hydraulic system that can be used for this purpose. In our case, an additional short section of casing equipped with "chain attachment wings" is used (Figure 3.5).

Figure 3.5. "Chain attachment wings" Attached to Cryogenic Core Sampler.

1.6.4 Model aquifer description

Samples from a large physical model aquifer were used to assess cryogenic core collection under environmentally realistic transport distances, time frames and biogeochemical conditions. The physical model was in continuous operation for more than 4 years and has dimensions of ~6.1 m long × 2.4 m high × 0.5 m thick (Figure 3.6) and is filled with a sand (median grain size ~0.3 mm) (see Section 3.4 for details). A dissolved Toluene source was introduced near the up-gradient end of the model and results in an anoxic zone within an otherwise oxic aquifer. Groundwater flow in the model was approximately 30 cm/d and the model had been in continuous operation for more than a year at the time the samples were collected. Samples for the MBT analyses discussed here were collected across the oxic/anoxic interface. The target

sampling zone was initially identified using chemical analyses from high-resolution groundwater samples collected from horizontal ports on the side of the physical model (Figure 3.6).

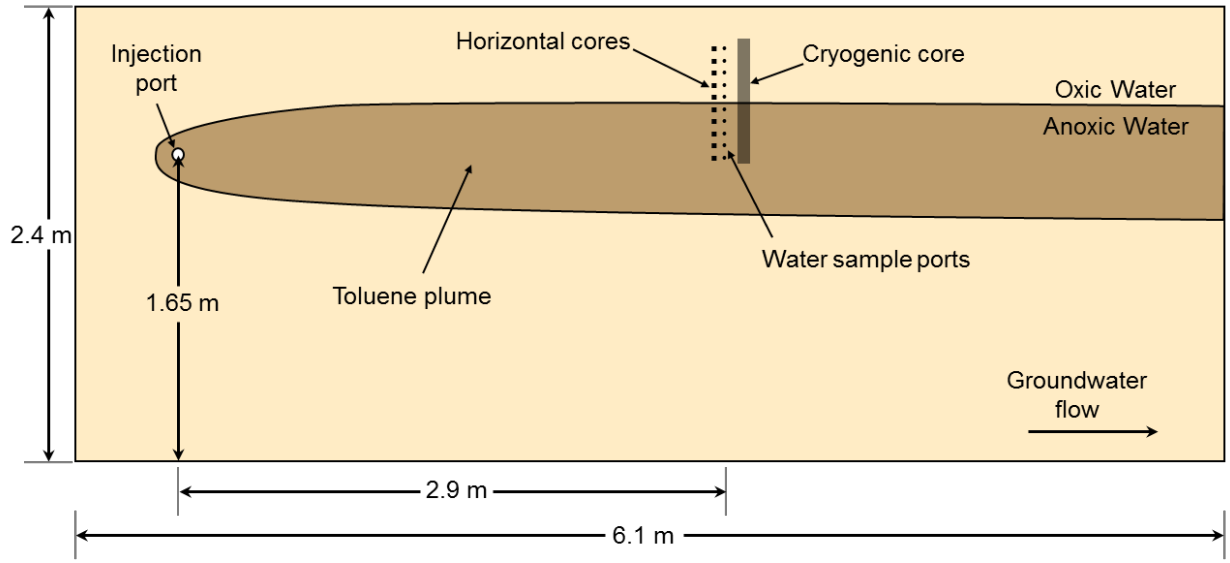

Figure 3.6. Schematic Drawing of the Large Physical Model Aquifer Used for this Study Showing the Locations of the Water and Soil Collection Points.

1.6.5 Water geochemical analyses

Water samples collected from ports on the side of the physical model (Figure 3.6) were analyzed for a suite of geochemical indicators, including dissolved oxygen, Nitrate, Toluene and fluorescein (the latter used here as a conservative tracer). Approximately 15 mL of water was collected from each sample port to perform chemical analyses. All water samples were collected with gas-tight syringes. Samples for Toluene analyses were injected into sample vials sealed with Teflon coated silica septum. Toluene concentrations were measured by headspace gas chromatography (HP 7694 Headspace Sampler attached to an HP 5890 GC with an FID detector). The GC method had a detection limit of 50 µg/L Toluene and an associated error of ±5% (n=15). Dissolved Oxygen (DO) was measured using a flow-thru oxygen electrode (Model 8-730 by Microelectrodes, Inc.) which had a detection limit of ~0.1 mg/L. Approximately 5 mL of water were collected for Nitrate and fluorescein analyses. Nitrate was analyzed by ion chromatography (Dionex IC25) and had a detection limit of 0.01 mg/L. Fluorescein was measured using a flow-through fluorometer (Gilson Model 121) and had a detection limit of 0.005 mg/L.

1.6.6 Horizontal aquifer solids samples for MBT analysis

Aquifer solids samples were collected from 9 locations approximately 10 cm hydraulically up-gradient of the water sampling ports (Figure 3.6) These samples were collected by drilling through the plexiglass wall of the physical model and inserting a sterile 1.27 cm (0.5 inch) Outside Diameter (OD) stainless steel tube. The tube was capped and sealed in place using a Swagelok fitting, which allowed the tube to be sequentially advanced to collect multiple samples. During each sampling event approximately 30 mL of solids and water were collected. For most experiments the samples were immediately processed for MBT analysis as discussed

below. For one experiment, samples were split and one half was frozen to examine the effects of freezing on tmoA gene recovery.

1.6.7 Cryogenic core samples for MBT analysis

Cryogenic core collection was accomplished using a core system similar to the one described above. In this case the core barrel was advanced into the aquifer using vibration rather than direct hammering (see Appendix A.1: Cryogenic Core Supporting Data Figure A.2). As discussed above, once the core barrel was in place, liquid nitrogen was circulated through the cooling coils for 5 minutes and then the sampling sleeve was removed from the probe rod. The sleeve was immediately sectioned into 2.54 cm (1 inch) sections, removed from their aluminum rings, wrapped in sterile aluminum foil and stored in a -20^{o}C freezer.

1.6.8 Water samples for MBT analysis

The same sampling ports used to collect water samples for chemical analyses were also used to collect samples for MBT analyses. In this case, 25 mL of water was filtered through 0.2 μm filters and the filters were taken directly to the laboratory to initiate sample preparation.

1.6.9 MBT sample preparation and qPCR analyses

Detailed descriptions of the water and solids preparation methods have been presented elsewhere (Brow, 2011). For the current work, only quantitative polymerase chain reaction (qPCR) analysis of the Toluene monooxygenase gene (*tmo*A) will be discussed. *tmo*A was chosen because it was known to be present in the aquifer and was expected to be at its highest concentration at the interface between the oxic an anoxic zones (i.e., where both Toluene and oxygen are present due to counter-diffusion) providing an excellent test of the vertical resolution of the cryogenic core sampler. DNA extractions were performed on filtered water samples, and 1 g fractions of sediment using the FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH). Independent DNA extractions were performed on duplicate sediment sub-fractions from each depth. (Duplicate water samples were not possible without reducing sample resolution.) qPCR was performed in triplicate reactions in a MyiQ real-time qPCR detection system using iQ SYBR Green Supermix. Primers targeting several genes were used but only tmoA results are discussed here. qPCR data were analyzed using LinReg PCR and gene copy numbers were determined by comparison to standard curves.

1.6.10 Preliminary test of cryogenic cooling

A laboratory experiment was conducted to test cryogenic cooling of the core within the core sleeve under realistic conditions. To accomplish this, a section of probe rod was placed in a large drum filled with water-saturated aquifer material. A sample sheath/sleeve assembly filled with water-saturated aquifer material was then placed in the probe rod section. The sleeve also contained thermocouples to measure temperature in the core as it cooled. The plot in Figure 3.7 shows the temperature at the center of the core as a function of time after liquid nitrogen flow was initiated. The data indicate that only ~2 minutes are required to fully freeze the sample. Our current approach is to apply liquid nitrogen for 5 minutes prior to sample recovery to ensure complete freezing.

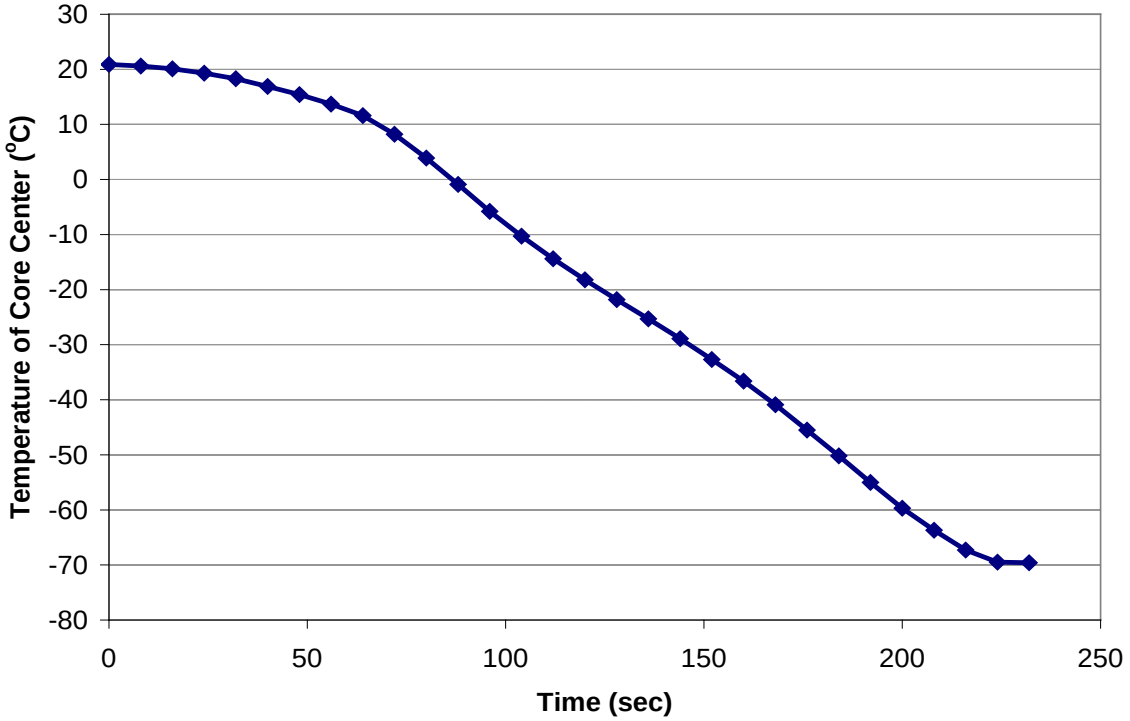

Figure 3.7. Plot of Core Temperature versus Time for During the Preliminary Cryogenic Cooling Experiment.

1.7 CRYOGENIC PRESERVATION MATERIALS AND METHODS

1.7.1 Laboratory-generated samples

Pseudomonas putida F1 and *Bacillus subtilis* JH642 strains were obtained from Dr. Dan Arp, and Dr. Michiko Nakano, respectively. Cultures of *P. putida* F1 were grown at room temperature, overnight, in 2xYT medium (Sambrook & Russell, 2001) with shaking at 120 rpm. *B. subtilis* JH642 was grown at 37ºC, overnight, in 2xYT (Sambrook & Russell, 2001) medium with shaking at 150 rpm. An archaeal enrichment culture also containing members of diverse bacterial phyla was grown as described by Simon et al. (2005). Each culture was used to create a set of three samples (unfrozen, frozen, and frozen with granular media) consisting of 300 µL of the culture in 2 mL microcentrifuge tubes. One of each set of samples contained 0.5 g of sterile 0.1 mm zirconia/silica beads (Biospec Products, Inc. Bartlesville, OK) to simulate soil. This "simulated core sample" and one of the non-bead-containing samples were frozen by immersion in liquid nitrogen for 10 seconds.

1.7.2 Sediment samples from a large physical model aquifer

Sediment from a large laboratory physical model was used to assess the effects of whole-sample freezing on DNA and RNA integrity under complex, environmentally realistic conditions (see Section 3.4. for description of physical model). Sediment (approximately 50 g) was collected through a port installed through the side of the model aquifer from within the anaerobic Toluene-containing zone. The sample was homogenized, and divided into 0.5 gram fractions for MBT

analysis with unfrozen samples processed immediately for DNA and RNA, and remaining samples frozen by immersion for 10 seconds in liquid nitrogen and stored at -80ºC for 5 and 10 months for DNA and RNA analysis, respectively.

1.7.3 DNA and RNA extraction

DNA from laboratory-generated samples was extracted via bead beating for 30 s at a speed of 5.5 m/s using a Bio101 FastPrep instrument (Thermo Fisher Scientific, Waltham, MA), and purified using the Wizard SV96 genomic DNA purification kit (Promega Corporation, Madison, WI). DNA from model aquifer samples was extracted and purified using a FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH). Total RNA (which includes rRNA and mRNA) was isolated as described in Smit et al. (Smith et al., 2010), except that each extraction was performed on 0.5 g sediment as opposed to filters. Total DNA and RNA concentrations were determined fluorometrically using PicoGreen and RiboGreen reagents, respectively (Invitogen Corporation, Carlsbad, CA) and a NanoDrop fluorometer (Thermo Scientific, Wilmington, DE). DNA from select laboratory-generated samples was electrophoresized and visualized in a 1% agarose gel. Additionally, the quality of RNA extracted from the laboratory-generated samples was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA). All nucleic acid extracts were stored at –80ºC until use.

1.7.4 Effects of freezing on quantification of genes and gene transcripts

The effects of freezing on the quantification of genes and gene transcripts were assessed by quantitative PCR (qPCR) of DNA and RNA recovered from the laboratory-generated samples. Equal amounts of either DNA or RNA were used from each set of extracts. Total RNA (including ribosomal RNA (rRNA) and messenger RNA (mRNA)) was converted to complementary DNA (cDNA) using SuperScript III First-Strand Synthesis Supermix (Invitogen Corporation, Carlsbad, CA). qPCR was performed in 25 µL reactions in a MyiQ real-time qPCR detection system using iQ SYBR Green Supermix (BioRad Laboratories Inc., Hercules, CA). Several independent master mixes were prepared for each set of samples (n=10 and 5 for DNA and RNA, respectively) and were tested in triplicate within a single microtiter plate.

Dilution series spanning six orders of magnitude were tested in duplicate for each experiment to ensure that product concentrations were within the instrument's linear dynamic range. Experiments performed with each set of extracts were replicated using primers targeting the following genes/transcripts: *todE* (Hendrickx et al., 2006), *todC1* (Kabir et al., 2003), and 16S rRNA (Johnsen et al., 1999) for *P. putida* F1; *thrB*, *pheA*, and *trpC* (Jun et al., 2006) for *B. subtilis* JH642; and archaeal 16S rRNA (Francis et al., 2005) and *amoA* for the enrichment culture. Primer sequences and cycling conditions can be found in Appendix A.2: Cryogenic Preservation Supporting Data (Table A.1). All primers were added at a final concentration of 200 nM. qPCR data were analyzed using Data Analysis for Real-Time PCR (DART-PCR) software (Peirson et al., 2003). DART-PCR was used to calculate fold differences relative to the unfrozen sample, and to assess differences in amplification efficiencies between frozen (both frozen and frozen with granular media) and unfrozen samples based on observation of individual-sample reaction kinetics.

1.7.5 Effects of storage on quantification of genes and gene transcripts

Storage experiments were conducted using replicates of the single-strain simulated core samples described above (i.e., with granular media). One unfrozen sample was processed immediately and served as the t = 0 reference sample. Additional samples were stored at –80ºC for 1 week, 2 weeks, or 1 month. At each time point, samples were removed from storage, and the DNA and RNA were extracted and purified as described previously. Purified DNA and RNA extracts from each time point were subsequently stored at –80ºC until use in qPCR experiments. After 1 month, equal amounts of either DNA or RNA (n=5) from all 4 time points were evaluated by qPCR on the same microtiter plate, ensuring that the results were directly comparable.

1.7.6 Effects of freezing and storage on relative phylotype abundance

PCR-SSCP was used to assess the effects of freezing on the relative abundance of bacterial 16S ribosomal DNA (rDNA) and RNA (rRNA) phylotypes recovered from the enrichment culture samples (frozen, unfrozen, and frozen with granular media). Additionally, PCR-SSCP was used to assess both the effects of whole-sample freezing and storage at –80ºC on the relative abundance of bacterial 16S rDNA and rRNA phylotypes recovered from model aquifer samples. RNA was converted to cDNA as described. DNA and cDNA from the enrichment culture samples were PCR-amplified using universal bacterial 16S rRNA gene primers 357F (5´ - CCT ACG GGA GGC AGC AG -3´) and 5´-phosphorylated 519R (5´- phosphorylation- ACC GCG GCT GCT GGC AC -3´) (Lane, 1991). Cycling parameters consisted of 4 min initial denaturation at 95ºC and 20 touchdown cycles of 30 s denaturation at 95ºC, 1 min annealing starting at 66ºC, and 1.5 min extension at 72ºC. The annealing temperature was decreased 0.5ºC with every cycle until a final annealing temperature of 56ºC was reached. The touchdown step was followed by 20 cycles of 30 seconds denaturation at 95ºC, 1 min annealing at 56ºC, and 1.5 min extension at 72ºC, with a final extension of 7 min at 72ºC.

After amplification, PCR products were purified using the Wizard SV gel and PCR clean-up system (Promega Corporation, Madison, WI). Purified phosphorylated PCR products were digested (to form single-stranded products), desalted, combined with SSCP stop solution (Lonza, Basel, Switzerland), denatured at 95ºC for three minutes, and placed on ice as described in Sliwinski and Goodman (2004). Products were run on a 0.75 mm 1×MDE polyacrylamide gel (Lonza, Basel, Switzerland) at 300 V for 25 hours at 17ºC. After electrophoresis, the gel was imaged directly on a Typhoon variable mode imager (GE Healthcare Bio-Sciences Corporation, Piscataway, NJ) following staining with GelRed (Biotum, Inc., Hayward, CA). Alternatively, it was subsequently found that the use of FAM-labeled 357F with nonphosphorylated 519R eliminated the need for the digestion to single-stranded DNA and staining steps, thus this approach was used in PCR-SSCP experiments with DNA and RNA from the model aquifer sediment samples.

Gel fingerprint patterns were analyzed using GelCompar II software (Applied Maths, Austin, TX), which was used to detects bands, create densitometric profile curves, and calculate the area under each peak. This information was transformed into a relative area under each peak (relative to the sum of the areas under all peaks), thus making possible the comparison of independent samples. A similarity matrix of the densitometric curves was calculated based on pair-wise Pearson's correlations.

1.8 ACTIVITY RATIOS MATERIALS AND METHODS

1.8.1 Model aquifer

Initial investigations in the model aquifer involved water samples collected from horizontal ports on the side of the physical model oriented vertically and spanning the upper interface of the anoxic plume. A sediment core was collected cryogenically at the same downstream distance (2.9 m) using the method described by Johnson et al. (2012). Subsequent experiments incorporated the addition, and subsequent removal of the electron acceptor, Nitrate (80 mg/l), from the injection source, as well as water samples collected from ports installed along the centerline of the plume between the injection port and 2.9 m.

1.8.2 DNA and RNA Extraction.

DNA extractions were performed on filtered water samples, and 1 g fractions of sediment using the FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH). Independent DNA extractions were performed on duplicate sediment sub-fractions from each depth. (Duplicate water samples were not possible without reducing sample resolution.) RNA was extracted from filters as described by (Smith et al., 2010). A similar procedure was used for sediment with the exception that 4 independent extractions were performed on replicate 1 g sub-fractions, and the RNA was pooled during elution. RNA was treated with Turbo DNA-free (Applied Biosystems, Carlsbad, CA) and was confirmed to be free of DNA contamination by a RT-minus qPCR. Total RNA was converted to complementary DNA (cDNA) using SuperScript III and random primers (Invitogen Corporation, Carlsbad, CA) at a concentration of 300 ng per 20 ul reaction.

1.8.3 Quantification of *bssA* genes and gene transcripts

Quantitative PCR was performed in a MyiQ real-time qPCR detection system using iQ SYBR Green Supermix (BioRad Laboratories Inc., Hercules, CA). Primers targeting *bssA* genes from denitrifying organisms (Brow et al., 2012b) were added at a final concentration of 200 nM. Cycling conditions consisted of 5 min initial denaturation at 95ºC followed by 40 cycles of 45 s denaturation at 95ºC, 1 min annealing at 61.5ºC, and 1.5 min extension at 72ºC. qPCR data were analyzed using LinReg PCR (Ruijter et al., 2009) and gene copy numbers were determined by comparison to standard curves.

1.8.4 Lifetime of *bssA* transcripts following removal of Toluene

To test the lifetime of *bssA* transcripts, 800 ml of pore water was collected from a port along the plume centerline located 0.5 m downgradient of the injection port (i.e., a location where active anaerobic degradation of the Toluene was known to be occurring). The initial concentration of Toluene in the sample was 11.2 mg/L and the initial Nitrate concentration was 15.5 mg/L. Toluene in the sample was removed via sparging with helium gas, which was also used to keep the sample anoxic. Subsamples (100 ml) for RNA analysis were collected at the beginning of the experiment (t = 0), and at 2, 4, 8, 12, 20, and 30 hours. Additionally, 15 ml samples were collected periodically in gas-tight syringes for analysis of Toluene and Nitrate concentrations. Samples for DNA analysis (25 ml) were also collected at the beginning, and end of the experiment.

1.8.5 Geochemical analyses of water samples

Water samples collected from the model aquifer ports, and from the *bssA* lifetime experiment were analyzed for fluorescein, Toluene, dissolved oxygen, and Nitrate using methods described by Johnson et al. (2012). Briefly, 15 ml water samples were collected in gas-tight syringes. Toluene concentrations were measured by headspace gas chromatography, dissolved oxygen (DO) with a flow through electrode, Nitrate by ion chromatography, and fluorescein with a flow through fluorometer.

1.9 LARGE PHYSICAL MODEL AQUIFER MATERIALS AND METHODS

Several experiments were carried out in a large physical model aquifer pictured below (Figure 3.8). The model aquifer is filled with sand and water and is approximately 6.1m long × 2.4m high × 0.5m wide. Water flows laterally down the tank at a rate of approximately 30 cm (1 foot) per day. There are two injection ports and more than 150 sampling ports over the length of the tank. The system has been in continuous operation for over two year. Water samples were collected directly from sample ports with gas tight syringes. About a dozen soil samples ports were also installed throughout this study which allowed for a comparison of soil samples collected directly from the model aquifer to samples collected by cryogenic core extraction.

Figure 3.8. Picture of Large Physical Model Aquifer Used in this Study. Dimensions are Approximately 6.1×2.4×0.5 M.

We use a series of pumps to inject the model aquifer with a combination of Toluene, TCE, Nitrate, and fluorescein dye (Figure 3.9). Injection concentrations used throughout this study were as follows: 25 mg/L Toluene, 1 mg/L TCE, 1 ppm Fluorescein, and either 0 or 52 mg/L

Nitrate. The injection setup allows for easy sampling and adjustment of the injection mixture as well as monitoring of the system.

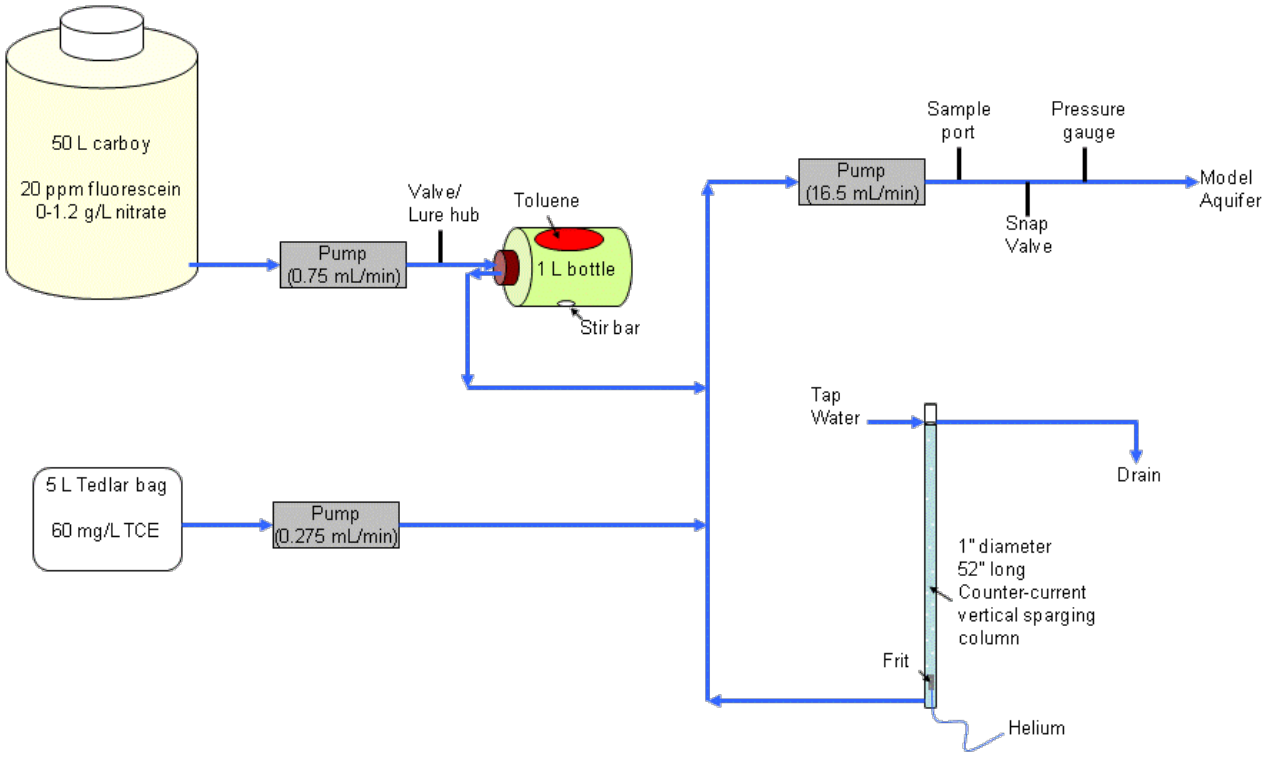

Figure 3.9. Schematic of Model Aquifer Injection Setup. All Connections are Made with Swagelok® Fittings, all Tubing is Silica or Stainless Steel. Injection Concentrations were as follows: 25 mg/L Toluene, 1 mg/L TCE, 1 ppm Fluorescein, and either 0 or 52 mg/L Nitrate.

RESULTS AND DISCUSSION

1.10 CRYOGENIC CORE RESULTS AND DISCUSSION

1.10.1 Characterization of geochemical conditions at the interface using high-resolution water samples

The water chemistry data in Figure 4.1.A. show that the oxic/anoxic interface within the aquifer is on the order of 20 cm thick. Simple diffusion calculations suggest that the shape and extent of the interface are primarily controlled by molecular diffusion in the vertical direction. The dissolved oxygen concentration decreases from near equilibrium atmospheric values to below detection level over a ~5 cm interval, and Nitrate is removed over roughly that same interval. The Toluene concentration gradient extends over a somewhat larger distance, which is due in part to flow conditions that exist around the injection location (~2.9 m up-gradient). Fluorescein is used here as a conservative tracer and shows a distribution that extends slightly higher (i.e., farther into the interface) than the Toluene. This is likely because aerobic degradation of the Toluene continuously removes the Toluene wherever Nitrate and/or oxygen are present, and this "sharpens" its concentration gradient at the interface. It is worth noting here that the samples show very little overlap of Toluene with the primary electron acceptors (oxygen, Nitrate), and as a consequence a very narrow active microbial Toluene oxidation zone might be expected.

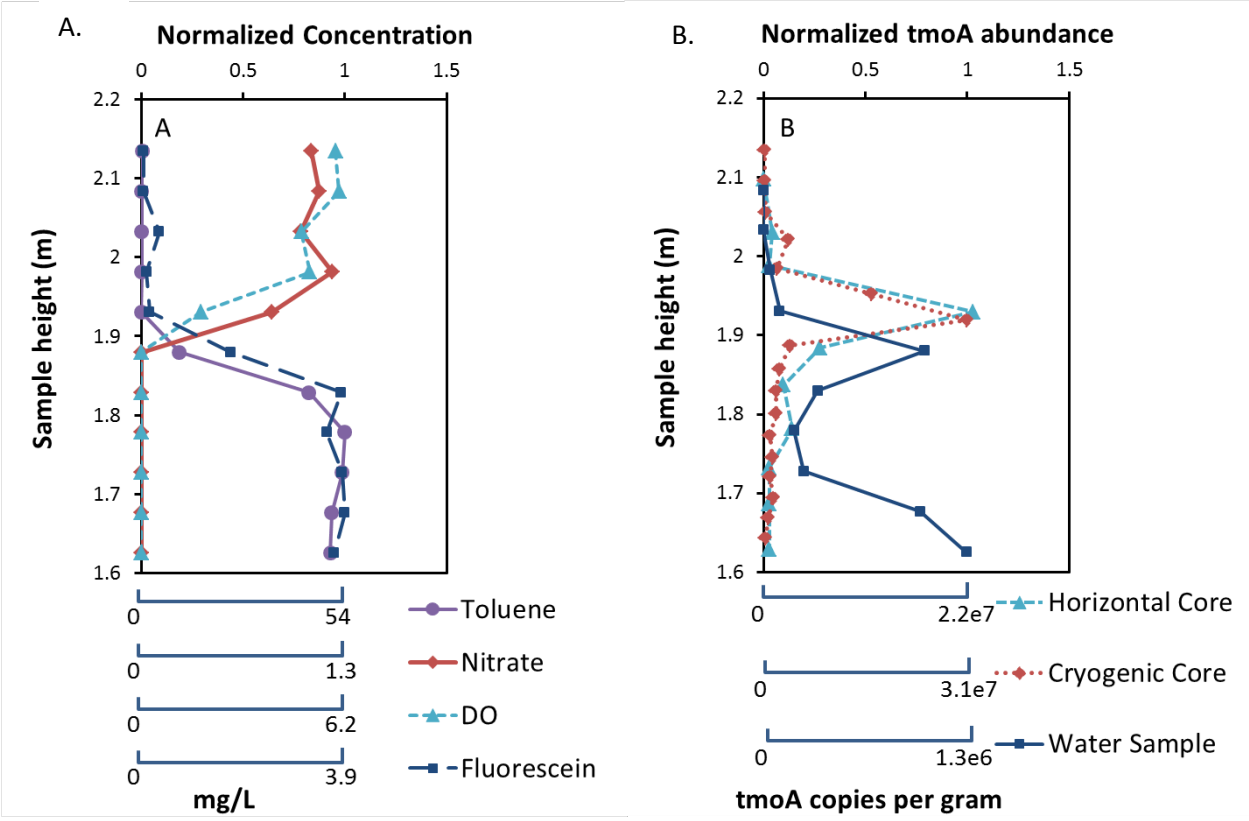

Figure 4.1. A) Geochemical Conditions at the Interface Between the Oxic and Anoxic Aquifer Zones. B) Abundance of tmoA in Water, Core and Cryogenic Core Samples Collected at the Interface.

1.10.2 Characterization of vertical tmoA distribution using water samples, horizontal cores and cryogenic core

Normalized concentrations of tmoA in water and solids samples are seen in Figure 4.1.B. The vertical distributions of the horizontal cores and the cryogenic core are nearly identical. In addition, the absolute numbers of tmoA gene copies per gram for the two samples are very similar. In contrast, the maximum number of gene copies for the water samples is only about 5% of the values in the core samples. The vertical distribution of tmoA genes in groundwater samples is also different than in the core samples. In particular, tmoA gene copies in the water samples are elevated within the anoxic zone (1.6-1.7 meter elevation). Based on other data from the aquifer (Brow et al., 2012a), this is likely due to transport of bacteria in the groundwater from a location near the upstream interface of the groundwater plume, where Toluene and electron acceptors will have mixed at that depth. The contrast between core samples and water samples is important in this context. By utilizing core samples (with their higher number of gene copies), it is possible to differentiate between (local) populations of attached organisms containing tmoA (which we believe are actively oxidizing Toluene) and suspended transported organisms (which, in this case at least, are not degrading Toluene).

1.10.3 Comparison of frozen and unfrozen core sub-samples

To confirm that the agreement between horizontal and cryogenic cores in Figure 4.1 actually reflected similar tmoA concentrations in the aquifer materials, each of a set of horizontal core samples was homogenized and split into two fractions. One set of fractions was frozen and stored for two weeks while the other set was immediately processed for MBT. Both sets were analyzed using qPCR and are presented in Figure 4.2. The data show good agreement between the frozen and unfrozen fractions, indicating that both the recovery of biomolecules and their integrity were unchanged by the freezing process. This again support the conclusion that cryogenic collection and storage of aquifer solids represents an excellent approach for characterization of subsurface microbial activity using molecular tools.

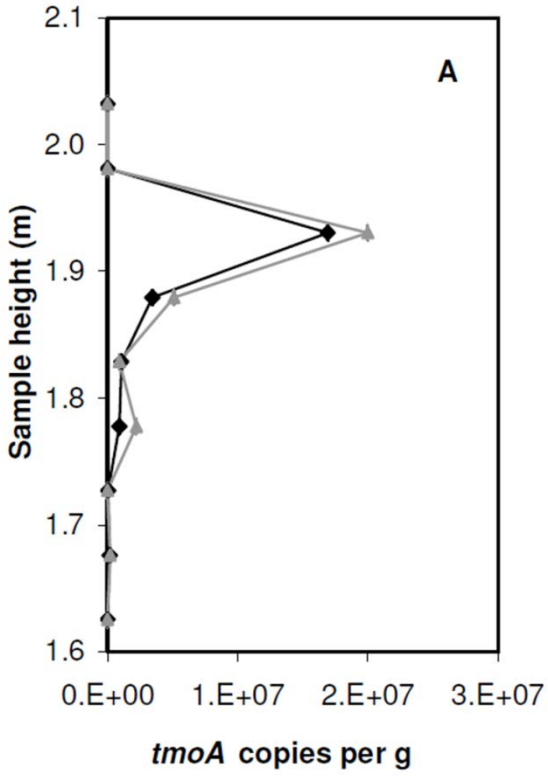

Figure 4.2. Depth Distribution of tmoA Genes Recovered from Unfrozen Fractions of Port-collected Sediment (black diamonds), and Fractions which were Frozen and Stored at -80ºC for Two Weeks Prior to Processing (grey triangles).

1.11 CRYOGENIC PRESERVATION RESULTS AND DISCUSSION

1.11.1 Effects of freezing and storage on quantification of genes and gene transcripts

Quantification of target genes and gene transcripts from laboratory-generated samples (unfrozen, frozen and frozen with granular media) was accomplished by qPCR with equal starting amounts of either DNA, or RNA, respectively. Fold differences for the two frozen samples relative to the unfrozen sample should be equal to 1, barring any effects of DNA or RNA degradation. Our

experiments indicated 10% variability in replicate fluorometric DNA and RNA concentration measurements (data not shown), thus fold differences of less than 10% were not considered to be indicators of sample degradation.

All qPCR dilution series were linear (R^2 >0.98) and confirmed that concentrations examined were within the linear dynamic range of the instrument. Fold differences, relative to the unfrozen sample, for genes and gene transcripts from frozen single-species and enrichment culture samples are shown in Table 4.1. The results of t-tests indicated no statistically significant differences between target genes or gene transcripts from frozen and unfrozen samples (p>0.05, n=10 and 5 for DNA and RNA, respectively) when calculated to allow for a 10% uncertainty in initial concentration. It is particularly noteworthy that freezing did not affect the recovery of mRNA molecules examined here (i.e., *todC*, *todE*, *trpC*, *thrB*, *pheA*, and *amoA* gene transcripts), which are particularly vulnerable to decay, make up only a fraction (≈3%) of the total RNA pool (Lamond, 1985), and yet are crucial to gene expression analysis. Additionally, results obtained targeting archaeal genes and gene transcripts from the enrichment culture samples are significant because the enrichment culture samples represent a more microbially complex, and, therefore, environmentally-relevant system. Furthermore, we evaluated individual sample reaction kinetics with DART-PCR software and observed no trends in amplification efficiency for any target DNA or RNA (e.g., better efficiencies for unfrozen vs. frozen samples). Amplification efficiencies for all targets from unfrozen and frozen samples were comparable (p>0.05, n=10 and 5 for DNA and RNA, respectively) and were greater than 94.8% and 88.2% for all DNA and RNA, respectively.

Table 4.1. Effect of Freezing and Thawing Processes on Quantification of Individual Genes and Gene Transcripts From Laboratory-generated Samples.

a. *P. putida* F1

Target		Frozen	Frozen with granular media
DNA	16S rDNA	0.86 ± 0.09	0.85 ± 0.18
	todC	0.84 ± 0.07	0.88 ± 0.11
	todE	0.83 ± 0.08	0.91 ± 0.06
rRNA	16S rRNA	0.95 ± 0.19	1.12 ± 0.22
mRNA	*todC*	0.95 ± 0.11	1.03 ± 0.07
	todE	0.96 ± 0.06	0.93 ± 0.07

b. *B. subtilis* JH642

Target		Frozen	Frozen with granular media
DNA	*trpC*	0.96 ± 0.21	0.97 ± 0.11
	thrB	0.96 ± 0.08	0.96 ± 0.18
	pheA	0.98 ± 0.11	0.90 ± 0.11
mRNA	*trpC*	1.03 ± 0.17	0.96 ± 0.13
	thrB	1.02 ± 0.15	0.92 ± 0.19
	pheA	1.13 ± 0.09	0.99 ± 0.14

c. Mixed culture (targeting archaea)

Target		Frozen	Frozen with granular media
DNA	16S rDNA	0.87 ± 0.18	1.14 ± 0.15
	amoA	0.84 ± 0.11	1.10 ± 0.11
rRNA	16S rRNA	0.86 ± 0.11	1.06 ± 0.14
mRNA	*amoA*	0.82 ± 0.23	1.05 ± 0.18

For practical reasons, field-collected cores are likely to be stored frozen until further sub-coring and/or processing can be carried out in a laboratory setting. Results from storage qPCR experiments with both single-strain sets of laboratory-generated cores are shown in Table 4.2. Fold differences were calculated with respect to DNA or RNA from a sample that was extracted immediately after freezing (0 day). T-tests were performed and indicated that no storage time for either strain was statistically different from the 0 day sample (p>0.05, n=5) when allowing for a 10% uncertainty. Furthermore, calculated amplification efficiencies were comparable (p>0.05, n=5) among all storage durations for both strains regardless of the primers used, indicating that storage time did not affect the integrity of the DNA or RNA.

Table 4.2. Effect of Cryogenic Storage on Quantification of Individual Genes and Gene Transcripts from Laboratory-generated Samples.

a. *P. putida* F1

Target		1 week	2 weeks	1 month
DNA	16S rDNA	1.12 ± 0.20	1.10 ± 0.14	1.15 ± 0.12
	todC	1.15 ± 0.04	1.04 ± 0.07	0.95 ± 0.11
	todE	1.11 ± 0.07	1.09 ± 0.12	1.15 ± 0.10
rRNA	16S rRNA	1.05 ± 0.06	0.96 ± 0.09	0.94 ± 0.06
mRNA	*todC*	1.04 ± 0.16	0.89 ± 0.16	1.07 ± 0.24
	todE	1.06 ± 0.08	0.89 ± 0.05	0.99 ± 0.06

b. *B. subtilis* JH642

Target		1 week	2 weeks	1 month
DNA	*trpC*	0.97 ± 0.07	1.04 ± 0.11	1.03 ± 0.06
	thrB	0.98 ± 0.10	0.93 ± 0.07	1.16 ± 0.07
	pheA	0.85 ± 0.14	0.91 ± 0.06	1.10 ± 0.10
mRNA	*trpC*	0.96 ± 0.04	0.95 ± 0.03	0.92 ± 0.05
	thrB	1.00 ± 0.06	1.04 ± 0.12	0.92 ± 0.12
	pheA	1.02 ± 0.20	1.00 ± 0.10	0.85 ± 0.19

It is also important to note that freezing and storage did not result in any trends in either DNA or RNA yields (Figure A.3) from laboratory-generated cores, nor was the quality of DNA affected, as visualized by electrophoresis in a 1% agarose gel (Figure A.4). Moreover, measurement of RNA using an Agilent 2100 Bioanalyzer indicated that cryogenic preservation did not result in RNA degradation. Specifically, RNA Integrity Numbers, or RINs, did not change as a function of time at -80ºC (Appendix A.4.).

1.11.2 Effects of freezing and storage on the relative abundance of bacterial phylotypes

To further evaluate the effects of freezing and storage on DNA and RNA from a broad range of bacterial phyla (Table A.2), PCR-SSCP was used to generate profiles of the bacterial community present in the enrichment culture samples. Bacterial 16S rRNA genes (rDNA) and gene transcripts (rRNA) from the suite of enrichment culture samples (unfrozen, frozen, and frozen with granular media) were profiled in triplicate. Additionally, triplicate bacterial 16S rDNA and rRNA profiles from an unfrozen model aquifer sample were compared to profiles from replicate samples stored at –80ºC for 5, and 10 months, respectively.

rDNA PCR-SSCP gel fingerprint patterns and associated densitometric profiles (Figure A.5) from the enrichment culture samples contained 20 distinct bands/peaks, each corresponding to a unique phylotype. This compared reasonably well with the number of individual groups identified by the clone library analysis (16 groups, Table A.2). Pair-wise comparisons of the densitometric profiles were performed and the associated Pearson's correlation coefficients (*r*)

were calculated (Figure A.5). DNA from frozen samples produced densitometric profiles as similar to those of unfrozen profiles ($r > 0.95$) as to replicates from the same sample ($r > 0.95$). The area under each peak in each densitometric profile was converted to a relative peak area by dividing by the area under all peaks in the profile. Peaks comprising >5% of the total peak area were plotted in Figure 4.3.A (top), which also shows the corresponding positions in a representative PCR-SSCP gel lane (middle) and densitometric profile (bottom). PCR-SSCP profiles from all samples, frozen and unfrozen, were comparable in relative abundance of the dominant phylotypes, i.e. freezing did not disproportionately affect any individual phylotype. Additionally, relative phylotype abundance in the model aquifer samples also appeared to be unaffected by freezing and 5 months of storage at –80ºC (Figure 4.3.B**,** Figure A.6).

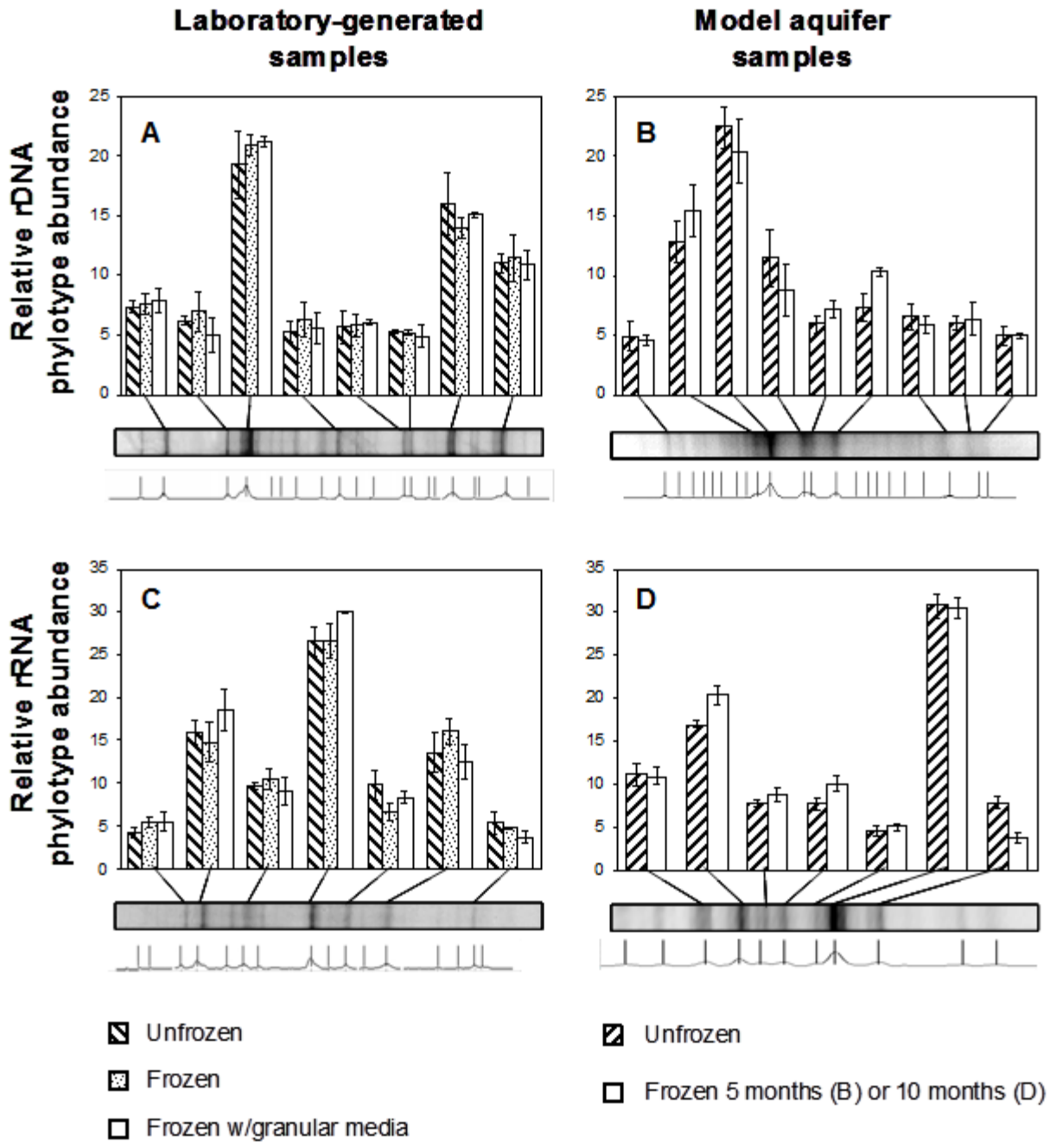

Figure 4.3. Relative Abundance of Bacterial Phylotypes Determined by PCR-SSCP of 16S rDNA (A and B) and rRNA (C and D).

Similarly, results from the rRNA PCR-SSCP analysis indicated that freezing had no effect on the relative abundance of 16S rRNA gene transcripts from the enrichment culture samples (Figure 4.3.C**,** Figure A.7), or the model aquifer samples (Figure 4.3.D**,** Figure A.8). This is important because biodegradation depends upon active bacterial populations, and *in-vivo* rRNA content is often correlated with cellular growth rate (Binder & Liu, 1998; Lamond, 1985; Wagner, 1994).

The dominant rDNA phylotypes did not necessarily correlate with the most active ones in our samples. For example, PCR-SSCP densitometric profiles differed substantially between rDNA and rRNA recovered from the model aquifer samples ($0.18 < r < 0.22$, $n=3$).

Though sediment cores provide an optimal approach for sampling microbial populations, sample preservation for molecular biological analysis can be problematic. This is particularly true with respect to RNA, which is much more susceptible to degradation by nuclease activity than DNA (Burlage et al., 1998). Our results indicated that neither freezing, nor storage for one month at -80ºC affected our ability to detect and quantify individual genes. Especially noteworthy are the qPCR results which demonstrated that short-lived mRNA molecules were not degraded upon freezing, or storage. Additionally, the relative abundance of dominant bacterial 16S rDNA and rRNA phylotypes was unaffected by freezing and storage of sediment at -80ºC. The implications of this are that whole-core freezing is a viable way to preserve the molecular characteristics of microbial populations in sediment in terms of both gene abundance, and more significantly, gene expression. In fact, due to the short half-lives of mRNA molecules, immediate freezing, e.g. with cryogenic coring, may be the only viable way to preserve the in-situ microbial signature of sediment.

1.12 Activity Ratios Results And Discussion

The introduction of Toluene to the model aquifer resulted in the formation of an anoxic Toluene plume within an otherwise oxic aquifer. This can be seen in Figure 4.4.a which profiles the pore water concentrations of Toluene, dissolved oxygen, and Nitrate in a vertical transect extending from above the plume, to the plume center. The abundance of *bssA* genes from organisms that couple denitrification to Toluene degradation was quantified in both water samples, and the core sample collected from this transect (Figure 4.4.b). *bssA* encodes benzylsuccinate synthase, the key enzyme involved in anaerobic Toluene degradation, and has been found in all isolated organisms capable of anaerobic Toluene degradation to date (Winderl et al., 2007).

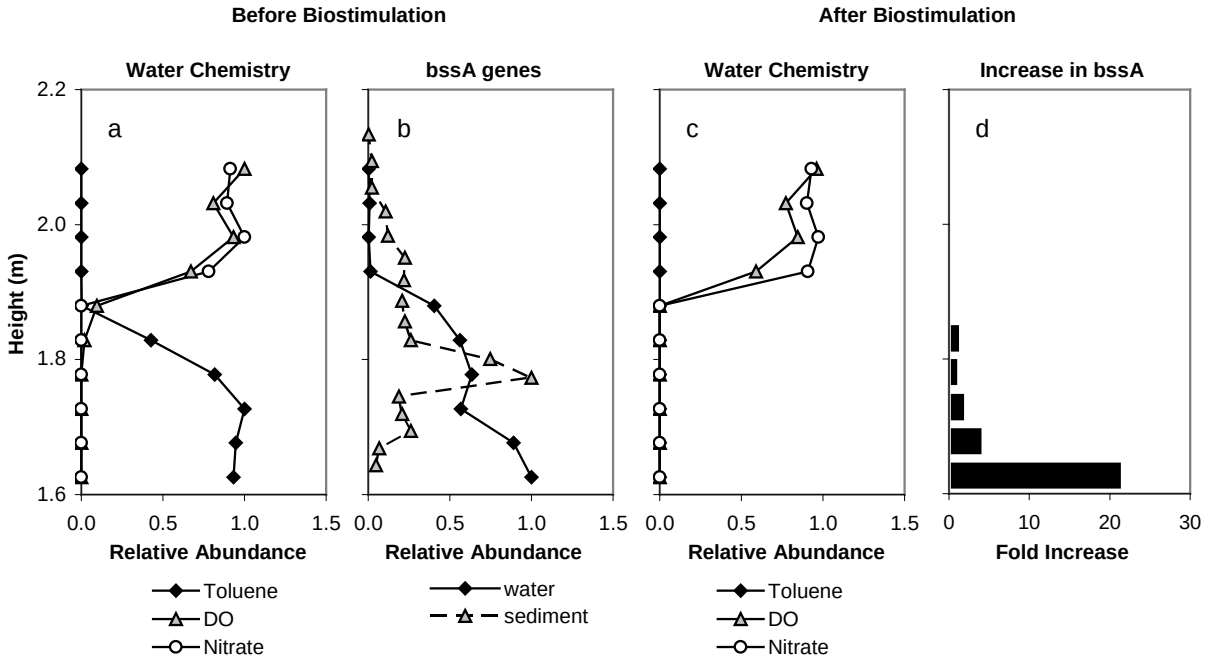

Figure 4.4. Vertical Chemical Profiles (a & c) and *bssA* Gene Abundance (b) in Samples Collected in a Vertical Transect 2.9 m Downgradient of the Injection Port. Data are Displayed as Values Relative to the Sample of Greatest Concentration. (In a., 82% Saturation for Dissolved Oxygen, 1.7 mg/l for Nitrate, and 61 mg/l for Toluene. In b., 9.08E6 Copies/g for Water, and 5.23E7 Copies per g for Sediment. In c., 83% Saturation for Dissolved Oxygen, 2.3 mg/l for Nitrate, and 0 mg/l for Toluene.) Panel d Shows the Fold Increase in *bssA* Gene Abundance Following Biostimulation with Nitrate.

Maximum *bssA* recovery was greater from sediment than from an equivalent volume of water (~600% greater). This same result has been repeatedly shown in both column experiments, and in the field (Brad et al., 2008; Doong et al., 1997; Haglund et al., 2002; Holm et al., 1992; Lehman et al., 2001; Lehman et al., 2001), and was therefore expected. For these data, it is the difference in the pattern of distribution between the water and the sediment that is significant, and it appears that the sediment samples better reflected chemical conditions within the plume. The highest abundance of *bssA* genes in the groundwater was deep within the center of the plume, where the electron acceptor, Nitrate, was not detectable. *bssA* abundance in the sediment, on the other hand, was largely confined to a narrow peak near the plume interface where electron acceptor availability would be more favorable.

Implicit in these results is the idea that gene abundance in pore water samples does not necessarily correlate with gene expression, and, by extension, actively degrading populations. The high abundance of *bssA* genes in Nitrate-depleted water would suggest that these genes are present, but not expressed. These results are consistent with those recently reported involving *tmoA*, a gene involved in the aerobic degradation of Toluene (Brow et al., 2012b). They also support the idea that genes recovered from pore water may belong to organisms that originated upstream of the sampling point in an area where chemical conditions favored their growth and activity. Given the long distances a microorganism can travel in the subsurface (Laskin, 1988),

this limits the likelihood of developing useful correlations between gene abundance and contaminant concentration, gene expression, or degradation activity.

The samples collected from within the Toluene plume contained no detectable Nitrate, thereby precluding any direct assessments of the relationship between gene expression and Nitrate availability. It should also be noted that, running under those conditions, no appreciable degradation of Toluene was observed in the plume. Nitrate was therefore added to the injection solution at a concentration of 80 mg/l to stimulate anaerobic biodegradation under denitrifying conditions. This resulted in the removal of >97% of the Toluene within 0.5 m of the injection port, with no Toluene detectable at any downgradient location. Additionally, Nitrate was also consumed within less than 0.5 m of the source. This is illustrated in Figure 4.4.c which depicts the Toluene, DO, and Nitrate profiles 2.9 m downgradient of the injection port after biostimulation.

bssA gene abundance in pore water at the 2.9 m location increased as much as 21 fold within the plume (Figure 4.4.d). The increased recovery of *bssA* genes from Nitrate and Toluene-depleted pore water further supports the idea that the organisms harboring these genes originated upstream where biostimulation succeeded in degrading the Toluene plume. However, under these conditions it was still not possible to assess the relationship between gene expression and chemical conditions, since no plume samples contained either Nitrate or Toluene. To remedy this, the injection concentration of Nitrate was reduced to 52 mg/l, and water sampling ports were installed along the centerline of the plume between the injection port and the transect at 2.9 m in an attempt to capture the leading edge of the plume.

As shown in Figure 4.5.a, the concentrations of Toluene and Nitrate within the plume decreased to 4 and 0% of their influent concentrations, respectively, over 2.9 m. Over this distance, the abundance of *bssA* genes increased initially, and remained fairly constant along the length of the plume (Figure 4.5.b), showing no apparent relationship to either Toluene or Nitrate concentrations. In contrast, the abundance of *bssA* gene transcripts peaked at 0.5 m where Toluene and Nitrate were present at 40 and 24 % their influent concentrations, respectively, and then rapidly decreased over 300 fold by 2.9 m. This supports the hypothesis that *bssA* genes detected downgradient in the plume were present, but not highly expressed, and likely originated nearer to the source zone of the plume.

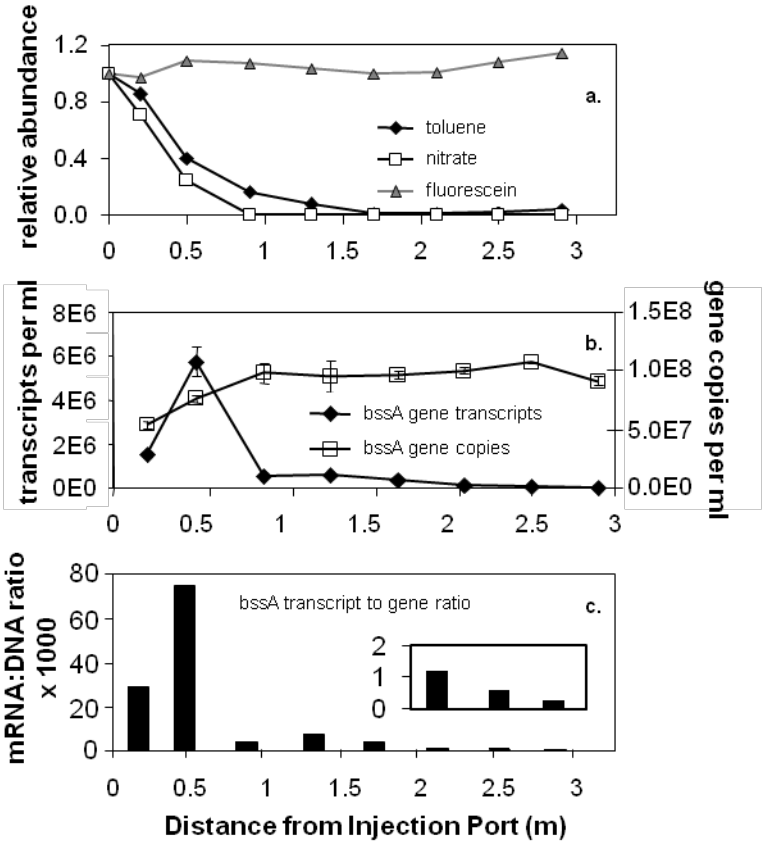

Figure 4.5. (a) Relative Abundance of Toluene, Nitrate, and Fluorescein in Pore Water Collected Along the Centerline of the Plume Downgradient of the Injection Port. (b) the Abundances of *bssA* Genes and Gene Transcripts, and (c) the Ratio of bssA Transcripts per *bssA* Gene. Error Bars in (b) Represent the Standard Deviation of two Independent PCR Reactions. The Insert in (c) Shows the Ratios for the Last Three Locations on an Expanded Scale.

The results indicate that gene expression is a more sensitive indicator of both geochemical conditions within the plume, and actively degrading populations. Given the short-lived nature of bacterial mRNA (Lamond, 1985), this is not necessarily surprising. However, the data do suggest that caution must be exercised in interpreting gene expression data. The detection of functional gene transcripts, such as those involved in biodegradation, is often considered evidence of activity. A closer examination of the data warns against the use of this type of presence/absence approach. Even at 2.9 m, *bssA* transcripts were still detectable on the order of 10^4 to 10^5 per ml. Two possible explanations for this are: 1) slow, and potentially incomplete down-regulation of *bssA* transcription, and 2) that *bssA* genes are continually transcribed at some basal level, even in the absence of Toluene and Nitrate.

To assess the rate at which *bssA* gene expression decreases following the removal of Toluene, an 800 ml sample of pore water was collected from the plume centerline port at 0.5 m which had the peak abundance of *bssA* transcripts. Toluene was removed from the sample via sparging with helium gas, and *bssA* gene expression was monitored over the next 30 hours. The concentration

of Toluene fell to 3 and 1% of its initial value within 1 and 2 hours respectively, after which Toluene was non-detectable. In the same 2 hours, the abundance of *bssA* transcripts fell 92%. Transcript abundance after this point remained fairly constant at ~10^4 per ml (Figure 4.6).

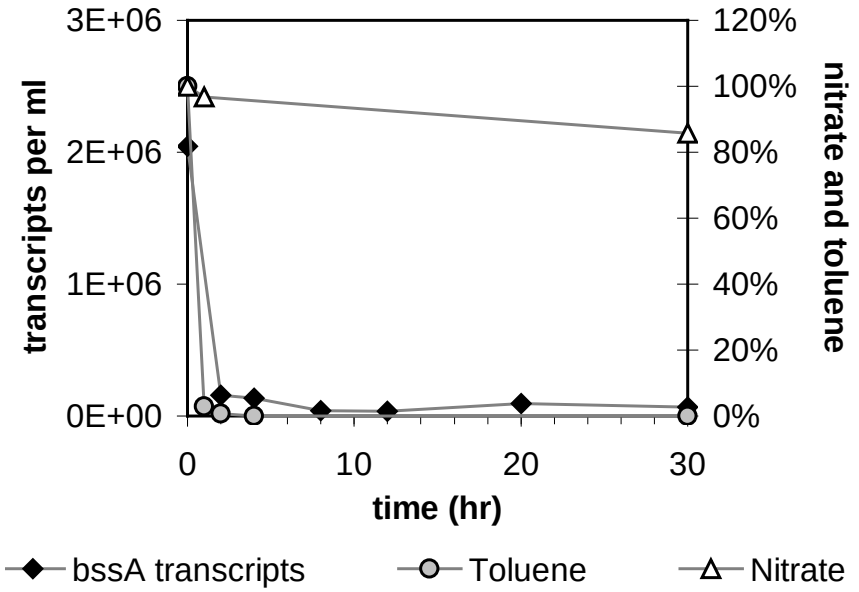

Figure 4.6. *bssA* lifetime. Abundance of *bssA* Transcripts per ml Following Removal of Toluene via Sparging with Helium Gas. Also Shown are the Concentrations of Nitrate and Toluene Relative to their Initial Concentrations.

The drop in *bssA* transcripts during the first 8 hours of the experiment could be fit to an exponential decay function with a rate constant of 0.44 per hour, and a half-life of 1.6 hours. This indicates that *bssA* gene expression is down-regulated quickly in the absence of Toluene. However, the persistence of transcripts extending at least 30 hours after the removal of Toluene signals that *bssA* transcription may occur at a basal level even in the absence of Toluene. Despite the sensitivity of *bssA* gene expression to presence of Toluene, clearly the presence of transcripts alone is inadequate to suggest active Toluene degradation.

Results from both the model aquifer and the *bssA* lifetime experiment indicate that *bssA* gene expression is upregulated in response to Toluene, but only in the presence of the electron acceptor, Nitrate. It is therefore important to also understand how sensitive *bssA* gene expression is to the availability of Nitrate. Unlike Toluene, Nitrate cannot be sparged from a sample. To evaluate how quickly *bssA* gene expression is down-regulated in response to the depletion of Nitrate, Nitrate biostimulation in the model aquifer was ceased, and *bssA* gene abundance and expression were monitored at two locations along the centerline of the plume (0.5 m and 2.9) over the subsequent 3 weeks. Concomitantly, Nitrate and Toluene concentrations were monitored at 11 locations along the length of the plume over the same time period.

Toluene degradation ceased rapidly following the termination of biostimulation, and the Toluene plume evolved down the length of model aquifer (Figure 4.7). Within 3 weeks, the Toluene

plume had reached its pre-biostimulation state. Changes in groundwater chemistry were accompanied by significant changes in the abundance and expression of *bssA* genes at 0.5 m. For example, on the third day following the end of biostimulation, a spike was observed in *bssA* gene abundance (Figure 4.8.a). This coincided with the complete disappearance of Nitrate from the system, as well as the first observed movement of the leading edge of the Toluene plume (Figure 4.7, 3 days). The spike in *bssA* gene abundance was accompanied by a sharp drop in the abundance of *bssA* gene transcripts (Figure 4.8.a). The fact that this drop coincided with the first day in which Nitrate was no longer detectable within the plume is significant, and indicates that *bssA* gene expression is tightly coupled to the availability of Nitrate.

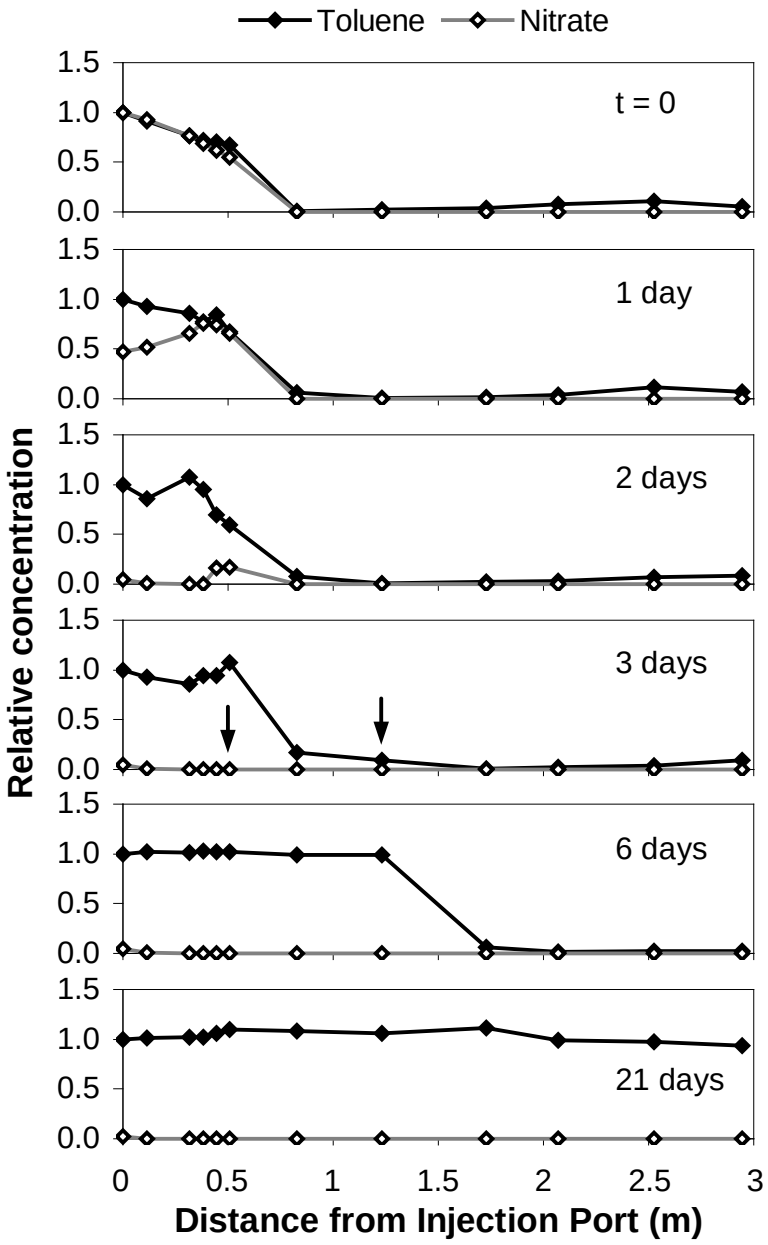

Figure 4.7. Evolution of Toluene and Nitrate in the Model Aquifer following the Termination of Biostimulation with Nitrate. Toluene Data Presented Relative to Injection

Concentration. Nitrate Data Presented Relative to Injection Concentration Prior to Termination. Arrows are Present to Indicate the Points at Which Nitrate is No Longer Detectable and the Leading Edge of the Toluene Plume Begins to Move.

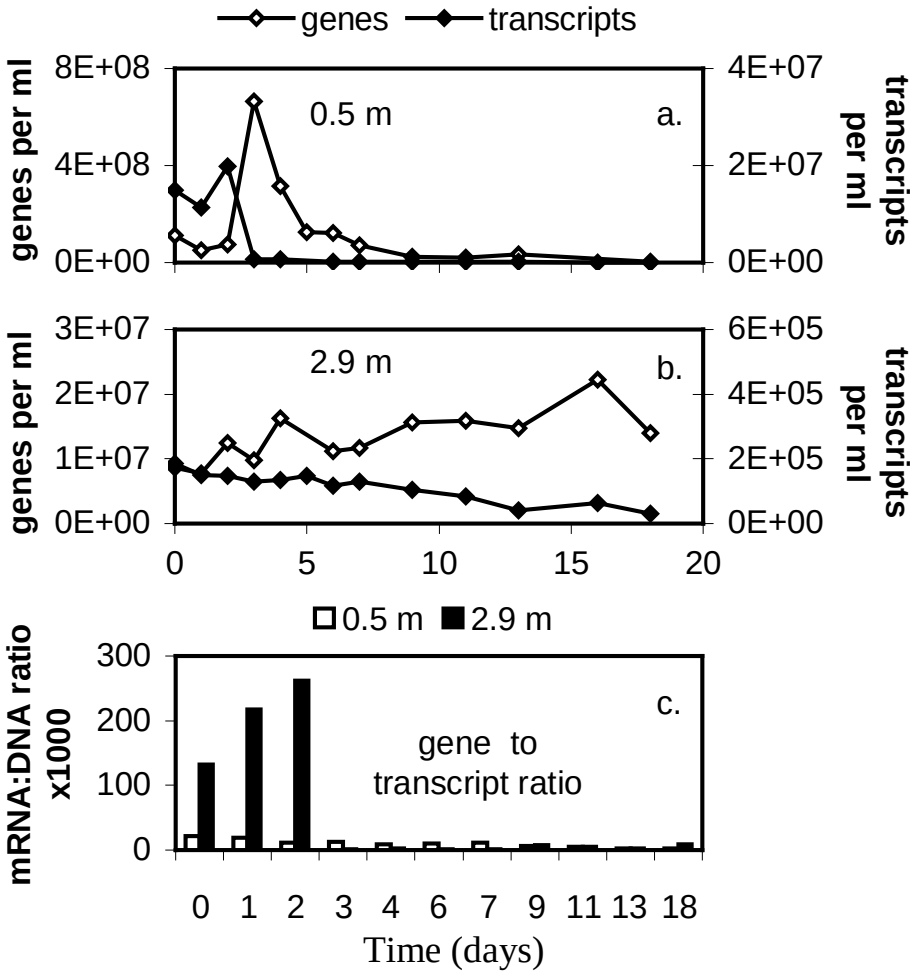

Figure 4.8. Abundance of *bssA* Genes and Gene Transcripts in Pore Water from Along The Centerline of the Plume at 0.5 m (a) and 2.9 m (b) Downgradient of the Injection Port Over the 18 Days Following the Termination of Biostimulation with Nitrate. Also Shown are the bssA Gene Transcript to Gene Ratios at Those Locations.

The spike in *bssA* gene abundance observed at 0.5 m on day three is likely due to re-entrainment of attached cells following the reduction in pore water ionic strength caused by the removal of Nitrate. Re-entrainment upon decreases in ionic strength results from expansion of the electrical double layer surrounding particles and sediment surfaces. As a result, the effective distance of electrostatic repulsion increases, causing the release of particles reversibly-held in secondary energy minima (Tong et al., 2005). This phenomenon has been well documented for both biological (Lee et al, 2010) and non biological colloids (Tong & Johnson, 2007) in saturated porous media.

A slight gradual increase in *bssA* gene abundance over time was observed at 2.9 m (Figure 4.8.b), but nothing to the degree of the spike seen at 0.5 m. The fact that a similar, but delayed pulse of *bssA* genes was not evident in pore water collected at 2.9 m is not necessary surprising. The combined actions of dispersion and additional attachment-detachment events occurring over the intervening distance would likely attenuate the peak, making it difficult to detect.

The abundance of *bssA* gene transcripts at 2.9 m decreased slowly following the end of biostimulation, but transcripts continued to be detected on the order of $>10^4$ per ml even three weeks after Nitrate depletion. These results are consistent with those from upgradient at 0.5 m, where *bssA* gene expression also plateaued at around 10^4 transcripts per ml. From these results, it is evident that a presence/absence approach to interpreting gene expression data is inadequate to assessing the *in-situ* physiological state of the Toluene degraders in this system. In fact, roughly 10^4 *bssA* transcripts per ml could be detected in all plume samples lacking both Nitrate and Toluene, and also those from the *bssA* lifetime experiment. This suggests that *bssA* genes are expressed at a low levels, even in the absence of inducers.

Constitutive expression of catabolic genes is thought to infer a selective advantage for organisms inhabiting oligotrophic environments. In carbon- and energy-limited environments, constitutive expression increases an organism's capacity to react quickly to transiently available nutrients (Ihssen & Egli, 2005). Given the oligotrophic nature of most groundwater systems, it is possible that many of the catabolic genes associated with contaminant degradation also exhibit some degree of constitutive expression, thus making a presence/absence approach to interpreting gene expression data insufficient.

To differentiate between constitutive expression, and gene expression associated with actively degrading populations of organisms, it is necessary to take an integrated approach. For example, model aquifer samples containing both Toluene and Nitrate consistently had higher *bssA* gene transcript to gene copy number ratios than those samples lacking either Nitrate, or both Toluene and Nitrate (Figure 4.5.c and Figure 4.8.c). Ratios on the order of 10^{-1} were consistently associated with samples in which Toluene degradation was actively taking place coupled to Nitrate reduction, while ratios closer to 10^{-3} were observed elsewhere. This relationship also held for samples from the *bssA* lifetime experiment in which the transcript to gene ratio fell from 0.12 to 0.001 following the removal of Toluene.

In the likelihood that many genes involved in contaminant degradation may also be constitutively expressed, an integrated mRNA/DNA approach could provide a valuable line of evidence that degradation is actively taking place. An appropriate "activity threshold" (mRNA:DNA ratio) would need to be determined experimentally for each gene in question, but in doing so, accurate assessments of *in-situ* degradation could be made from water samples, as opposed to sediment samples. Molecular analysis of sediment samples has been shown to better reflect solution chemistry and degradation activity when using a DNA-only approach (Brow et al., 2012b). However, sediment sampling comes with a unique set of challenges which make groundwater sampling more practical, particularly when repeated sampling is necessitated, as is the case for monitored natural attenuation. An integrated mRNA/DNA approach circumvents the complications of interpreting pore water gene abundance data which result from microbial

transport. Additionally, it prevents the pitfalls that could arise from applying a presence/absence approach to the interpretation of gene expression data, thus avoiding the confusion between constitutive levels of gene expression, and levels associated with contaminant degradation.

CONCLUSIONS AND IMPLICATIONS FOR FUTURE RESEARCH/IMPLEMENTATION:

1.13 CONCLUSIONS

The results presented in this report outline two new strategies for determining subsurface microbial activity. The first allows cryogenic collection of aquifer solids and associated water in a manner that preserves aquifer structure as well as labile biomolecules. The second approach provides a local, direct measure of subsurface biodegradation activity by utilizing the ratio between labile mRNA and more-stable DNA of targeted genes.

Collection of solid samples from aquifers is important because, as observed in this project, microbial populations associated with aquifer solid surfaces are typically 10-100 times higher than from corresponding water-only samples. Aquifer solids are also more-likely to contain intact and functioning microbial communities than those in water samples. Since in situ bioremediation activity is typically associated with microbial communities, analysis of solid-sample populations is more likely representative of those populations. Finally, for water-only samples it can be difficult to differentiate between cells that are locally active versus inactive cells being are transported with the groundwater. As a result, MBT analysis of aquifer solids is likely to be more representative of subsurface conditions than are water samples (if the quality of MBT from the aquifer solids can be kept at a high level – e.g., by cryogenic sampling.) During this project we developed an approach for collection of aquifer solids that both immobilizes pore water within core material and preserves biomolecules within those cores. The latter is important because extraction of biomolecules from aquifer solids is difficult to accomplish under field conditions. As a result, cryogenic collection allows extraction of those samples to be postponed until they arrive at the laboratory. An important conclusion from this project is that biomolecules can be quantitatively recovered from frozen core samples without loss of quality, even after a month or more of storage at -80^{o}C.

The second strategy developed in this project allows the analysis of local microbial activity. As part of this project we have demonstrated that cryogenic collection and preservation of samples allows mRNA to be preserved. As a consequence, the ratios of mRNA to DNA can be used to assess local microbial activity. Previous approaches provide either indirect evidence (e.g., presence of DNA, but no indication of activity) or direct evidence that activity is occurring at some location up-gradient from the sampling point (e.g, daughter product formation, shifts in stable isotopes). In contrast, the new approach evaluates activity based on the presence of elevated levels of mRNA relative to levels of the corresponding DNA. For many species of bacteria, mRNA levels remain at a low, baseline level unless actively being used. Upon cessation of activity, mRNA levels typically decrease with half-lives of a few hours. As a result, observation of elevated mRNA/DNA ratios indicates that the bacteria at that location are active (or at least had been with the last few hours)

Large-scale physical model (mesocosm) studies were used in this project to highlight the applicability of the cryocoring technique by allowing cryocore sections to be directly compared to high-resolution water samples and unfrozen soil cores collected from horizontal sampling ports adjacent to where the cryocore sections were collected. To provide a framework for

comparison, a dissolved-phase Toluene plume within an anoxic zone was developed in the physical model. Dissolved Nitrate levels were adjusted to biostimulate existing microbial populations. We then used cryocoring and high-resolution sampling to measure microbial populations across the oxic/anoxic Toluene interface. This approach allowed us to demonstrate that cryocores can provide abundance information at scales of a few centimeters, which is much smaller than one would typically obtain from un-frozen cores. This can be very important, as observed here, because boundaries between active and inactive remediation zones can be quite thin in the vertical direction.

Significant levels of DNA from Toluene-degrading organisms were observed in water samples from areas in which Toluene degradation was not occurring. We were able to demonstrate that the likely source of these bacteria was an area hydraulically up-gradient, in which Toluene degradation was occurring, and that these organisms were transported in the groundwater. If only DNA measurements had been made, they might have been incorrectly interpreted as degradation activity in the down-gradient water samples. Utilization of mRNA/DNA ratios, however, allowed the correct interpretation to be made. It is interesting in this context that some of the highest DNA levels we observed in groundwater samples during these experiments occurred when important metabolic constituents (e.g., Nitrate) were reduced. This resulted in significant "die-off" and detachment of organisms, and the observed increase in numbers. However, as expected, there was no elevation of the mRNA/DNA ratio, so we were able to correctly show those increases in number were not contributing to increased degradation. Based on our results, we believe that interpretation of degradation activity can be much more robust using mRNA/DNA ratios, rather than simply using DNA concentrations.

1.14 IMPLICATIONS FOR FUTURE RESEARCH

From our perspective, the primary limitations to implementation of the techniques developed here are the need to: 1) identify specific genes within field samples which are relevant from a remediation perspective; 2) determine the mRNA/DNA activity ratios characteristic of those genes; and 3) determine quantitative relationships between those activity ratios and degradation of the contaminants of concern. Each of these is discussed briefly below.

Current research utilizing microarrays, including current projects funded by SERDP, is providing an increased understanding of microbial communities associated with specific contaminants and remediation activities. We believe that this will greatly facilities application of qPCR-based mRNA (and DNA) analysis to assess local microbial activity because it will help to quickly identify the specific genes that should be tested by qPCR.

In the work reported here, detailed characterization of geochemical conditions measured as part of our physical model studies was used to relate microbial activity to mRNA/DNA ratios. While some work looking at mRNA/DNA ratios in field systems has been done (Lee et al., 2008), there is a need for additional research activities to assess the site-to-site variability of those ratios and their relationship to contaminant degradation. This will also need to include the possibility that ratios can be elevated due to processes other than the reactions of interest. It is likely that, to be sufficiently robust, activity ratios of other genes (e.g., hydrogenases) may need to be measured in order to ensure that the suite of ratios is a robust indicator of contaminant degradation.

Finally, the extent to which degradation rates can be correlated with mRNA/DNA ratios is yet to be determined. This may not be necessary for useful implementation of the ratio approach (i.e., at many sites it may be sufficient to demonstrate that the reactions are occurring locally and the overall rate can be evaluated using other tools). However, if correlations between activity ratios and degradation rates can be established, they would certainly prove useful, and we therefore believe further research in this area is warranted.

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APPENDIX A: SUPPORTING DATA

Appendix A.1: CRYOGENIC CORE SUPPORTING DATA

Throughout this project, we developed several prototype cryogenic core designs involving only minor modifications to the Geoprobe systems. One example of a modified DualCore sampler is shown in "cut-away" view in Figure A.1 (left).

Figure A.1. Prototype Cryogenic Sampler "cut-away" View (left)**, Adaptor** (center)**, and Slotted Slip Cap** (right)**.**

Other prototypes included a vibrated rather than driven core barrel (Figure A.2) as well as a design where freezing occurs on the outside of the tube. In both cases we had limited success. With the core barrel design, we were limited in our ability to collect deep samples at specific depth intervals. With the external freezing design, at depths greater than a few meters the frozen sample was stripped from the inner rod by the overlying aquifer materials. In contrast, we have used MacroCores extensively and successfully to collect depth-specific samples at field sites. (The primary difficulty we have had with the MacroCore approach is retaining noncohesive aquifer materials in the core barrel during removal from the subsurface, and this should be eliminated if the samples are frozen once they are in the core barrel.) Based on our experience, we are confident that the modified MacroCore will be effective for cryogenic core sampling (and at the same time will go a long way towards addressing the fluids redistribution and contamination issues.)

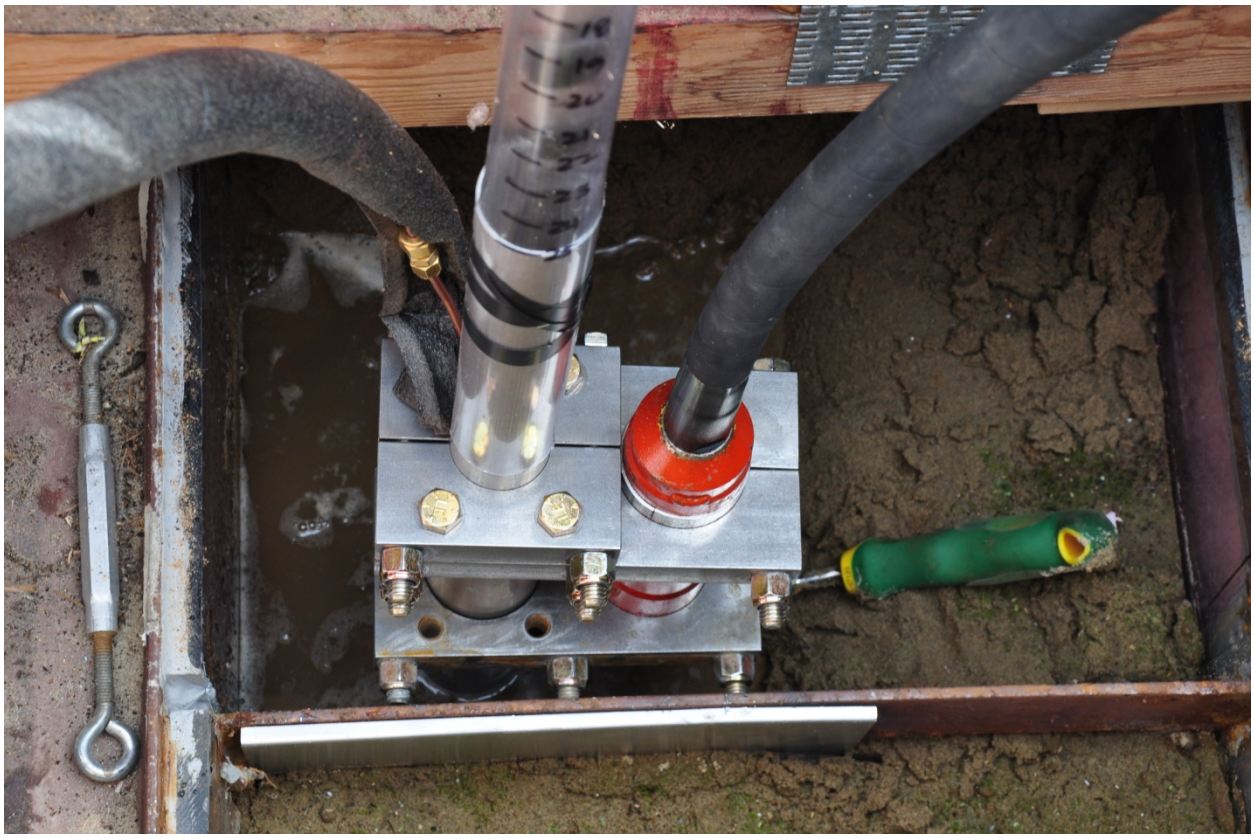


Figure A.2. Vibration Core Setup (viewed from above)**. A Concrete Vibrator** (right of center) **was Bolted to Cryogenic Core Barrel** (left of center)**.**

Appendix A.2: CRYOGENIC PRESERVATION SUPPORTING DATA

Table A.1. PCR Primer Sequences and Cycling Conditions.

Gene (organism)	Primer sequence	Denaturation		Annealing		Extension		Reference
		sec	ºC	sec	ºC	sec	ºC	
todC1 *(P.putida F1)*	Fwd: 5'-ATCCTGCGAGGCCACAAG-3' Rev: 5'-TTCCTCGCTGTAGACGTTGTTG-3'	30	92	60	57	60	72	Kabir et al. (2003)
todE *(P.putida F1)*	Fwd: 5'-GGATTTCAAACTGGAGACCAG-3' Rev: 5'-GCCATTAGCTTGCAGCATGAA-3'	60	94	60	58	120	72	Hendrickx et al. (2006)
16S rRNA (*P.putida* F1)	Fwd: 5'- GAGTTTGATCCTGGCTCAG-3' Rev: 5'- CCTTCCTCCCAACTT-3'	30	95	60	60	60	72	Johnsen et al. (1999)
trpC *(* B.subtilis *JH642)*	Fwd: 5'-TTCTCAGCGTAAAGCAATCCA-3' Rev: 5'-GCAAATCCTTTAGTGACCGAATACC-3'	30	95	60	60	60	72	Kanno et al. (2006)
pheA *(B.subtilis JH642)*	Fwd: 5'-GCCAATGATATGGCAGCTTCTAC-3' Rev: 5'-TGCGGCAGCATGACCATTA-3'	30	95	60	60	60	72	Kanno et al. (2006)
thrB *(B.subtilis JH642)*	Fwd: 5'-CCTGCATGAGGATGACGAGA-3' Rev: 5'-GGCATCGGCATATGGAAAC-3'	30	95	60	60	60	72	Kanno et al. (2006)
amoA *(enrichment culture)*	Fwd: 5'-TCGTGGTGTTCTCCGTATC-3' Rev: 5'-GATTGTGGCGTAGTATGTGG-3'	60	95	60	60	60	72	This study
16S rRNA (Archaeal)	Fwd: 5'-GTAGCCGGTTCTACAAGTC-3' Rev: 5'-ACTGGTGGTCTTCAATGGATC-3'	30	95	45	61	30	72	Simon et al. (2005)
bssA *(Bacterial)*	Fwd: 5'-CTGCTGTGGCCSTAYTACAAGGC-3' Rev: 5'-GATGGCGTCGGTCATGTCGKT-3'	45	95	60	61	60	72	This study
tmoA *(Bacterial)*	Fwd: 5'-GGCTTYACCAACATGCAGTTYC-3' Rev: 5'-CATRAACTCCTTGAABGACT-3'	45	95	60	55	60	72	This study

Primers and cycling conditions used in qPCR. All reactions included 4 min initial denaturation at 95ºC, 4 minutes final extension at 72ºC, and generation of a melt curve.

Characterization of bacteria in the archaeal enrichment culture.

DNA from the enrichment culture was extracted and purified as described in section 3.2 Cryogenic Preservation Materials and Methods. Bacterial 16S rRNA gene sequences were amplified by PCR using primers 27F (bacteria biased, 5'- AGA-GTT-TGA-TCM-TGG-CTC-AG - 3') and 1492R (universal, 5' – GGY-TAC-CTT-GTT-ACG-ACT-T - 3') (Lane, 1991). PCR was performed in a MyiQ real-time PCR detection system (BioRad Laboratories Inc., Hercules, CA) in 25 µL reactions containing 15 µL iQ Supermix (BioRad Laboratories Inc., Hercules, CA), 1 µL of template DNA, and primers at a final concentration of 800 nM. The PCR cycling conditions were as follows: 1 min initial denaturation at 94°C followed by 30 cycles of 30 s denaturation at 94°C, 1.5 min annealing at 55°C, and 2.5 min extension at 72°C, and a final extension of 5 min at 72°C. After amplification, the PCR products were purified with the Wizard SV gel and PCR clean-up system (Promega Corporation, Madison, WI) and cloned with pCR®2.1 vector system using the TOPO TA cloning kit (Invitrogen Corporation, Carlsbad, CA) according to the manufactures' instructions. Randomly-selected clones were analyzed by PCR and gel electrophoresis to ensure the presence of insert, after which sequencing was performed in

an ABI 3730XL high-throughput capillary DNA Analyzer (Washington University - Genome Sequencing Center, St. Louis, MO). For complete coverage, each clone was sequenced with 4 primers; 2 targeting vector sequences (M13F 5'-GTA-AAA-CGA-CGG-CCA-G-3' and M13R, 5'-CAG-GAA-ACA-GCT-ATG-AC3-'), (Yanisch-Perron, Vieira, & Messing, 1985) and 2 targeting internal sequences (870F, 5' –CCT-GGG-GAG-TAC-GGT-CGC-AAG-3' (Sy et al., 2001) and U926Rmod, 5'-CCG-TCW-ATT-CCT-TTR-AGT-TT-3' (Baker, 2003)). Sequences were assembled and aligned using the CLC Genomics Workbench 1.1.1 (http://www.clcbio.com) and refined manually to remove or correct regions of ambiguity. Sequences from 90 clones were analyzed using Basic Local Alignment Search Tool (BLAST) (Stephen F. Altschul, Warren Gish, Webb Miller, Eugene W. Myers, 1990) against the GenBank database to determine closest phylogenetic affiliations.

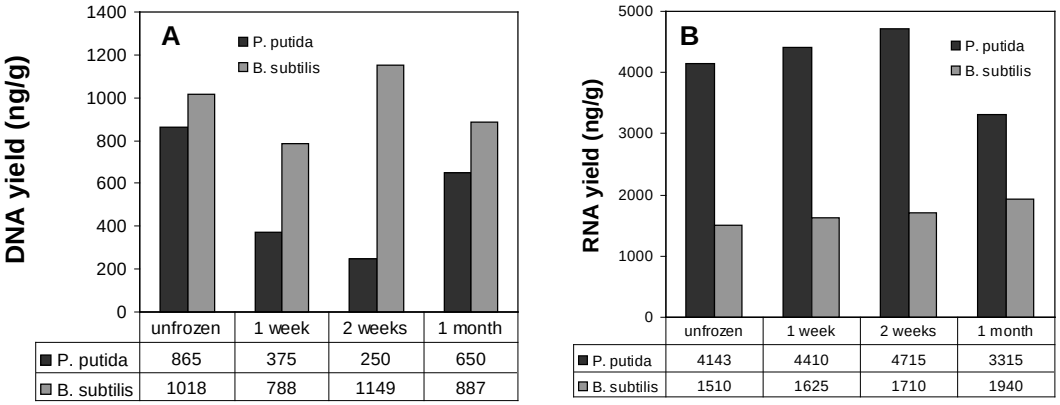


Figure A.3. DNA and RNA Yields from Laboratory-generated Samples. DNA (A) and RNA (B) Yields from Single-species Laboratory-generated Samples Containing Zirconia/Silica Beads. Data Shown for Unfrozen Samples, and Intact Samples Stored at -80ºC for one Week, two Weeks, and one Month.

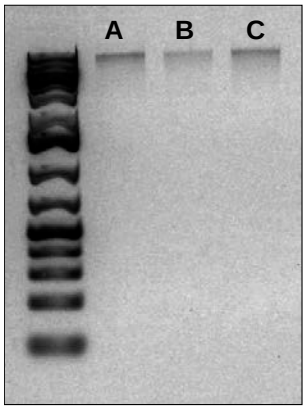


Figure A.4. Agarose Gel Image of DNA Extracted from Laboratory-generated Samples, Agarose Gel Image of DNA Extracted from Laboratory-generated Pseudomonas Putida F1 Samples (unfrozen: A, frozen: B, and frozen with Granular Media at -80ºC for one month: C)

Table A.2. Bacterial Clone Library Analysis of an Archaeal Enrichment Culture.

Organism (phylum/ class/ genus)		Maximum Score	Sequence Length	Expect Value (E)	Maximum Identity	Number of Clones
Proteobacteria						**44**
α- *Proteobacteria*						
	Sphingomonas	2597	1431	0	99%	8
	Nitrobacter	2416	1462	0	96%	2
	Rhodopseudomonas	2385	1483	0	96%	2
	Labrys	1349	759	0	98%	2
β- *Proteobacteria*						
	Burkholderia	2726	1494	0	99%	7
	Ralstonia	2745	1503	0	99%	7
	Pandorae	2599	1455	0	98%	6
	Variovorax	2697	1498	0	99%	5
γ- *Proteobacteria*						
	Rhodanobacter	2605	1499	0	99%	5
Acidobacteria						**22**
Acidobacteria						
	Unclassified *acidobacteriaceae*	2591	1459	0	98%	22
Uncultured *Bacteria* division OP11		1905	1346	0	92%	**11**
Planctomycetes						**7**
Planctomycetacia						
	Environmental samples	1186	896	0	91%	7
Bacteroidetes						**4**
Sphingobacteria						
	Chitinophaga	1247	752	0	96%	2
	Pedobacter	1277	862	0	93%	1
	Sphingobacterium	1428	856	0	96%	1
Gemmatimonadetes						
Environmental samples		1399	838	0	96%	**1**

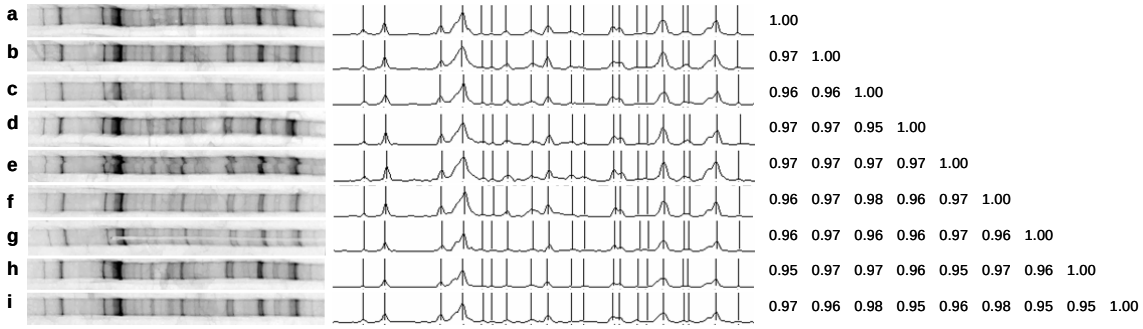


Figure A.5. Bacterial rDNA PCR-SSCP Gel Fingerprints (left) **and Associated Densitometric Profiles** (center)**, and Pearson's Correlation Coefficients** (right) **of Enrichment Culture Samples (unfrozen: A, B & C; frozen: D, E, & F; frozen with Granular Media: G, H, & I)**

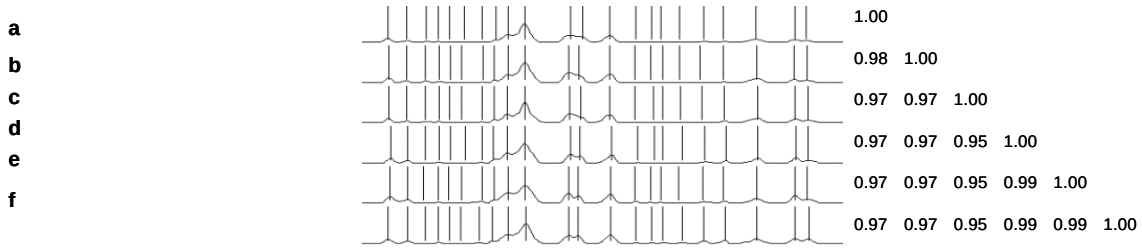


Figure A.6. Bacterial rDNA PCR-SSCP Gel Fingerprints (left) **and Associated Densitometric Profiles** (center)**, and Pearson's Correlation Coefficients** (right) **of Model Aquifer Samples (unfrozen: A, B, & C; frozen five months: D, E, & F)**

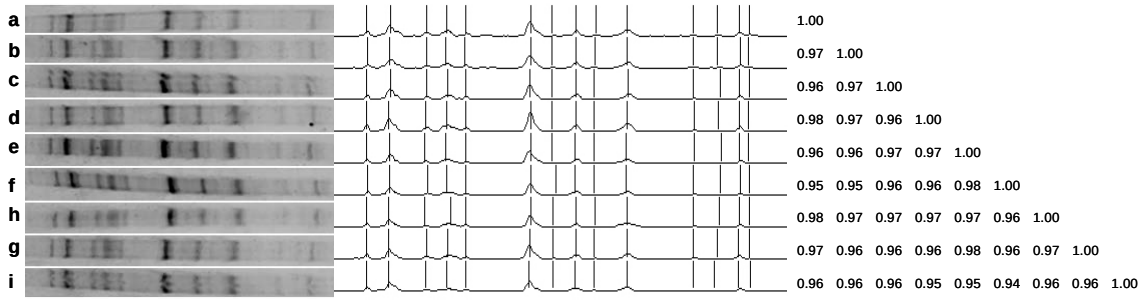


Figure A.7. Bacterial rRNA PCR-SSCP Gel Fingerprints (left) **and Associated Densitometric Profiles** (center)**, and Pearson's Correlation Coefficients** (right) **of Enrichment Culture Samples (unfrozen: A, B & C; frozen: D, E, & F; frozen with Granular Media: G, H, & I)**

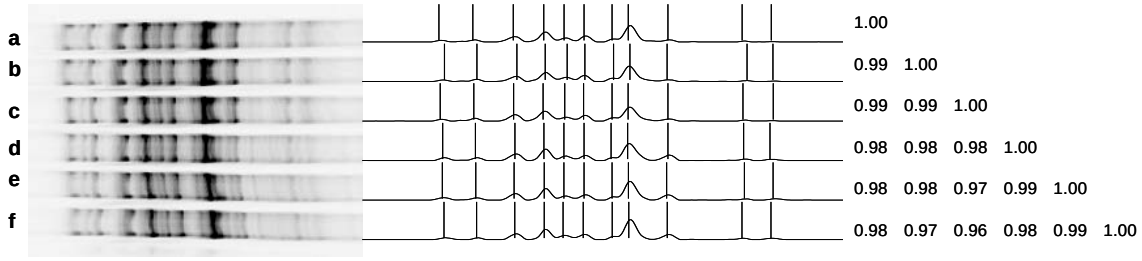
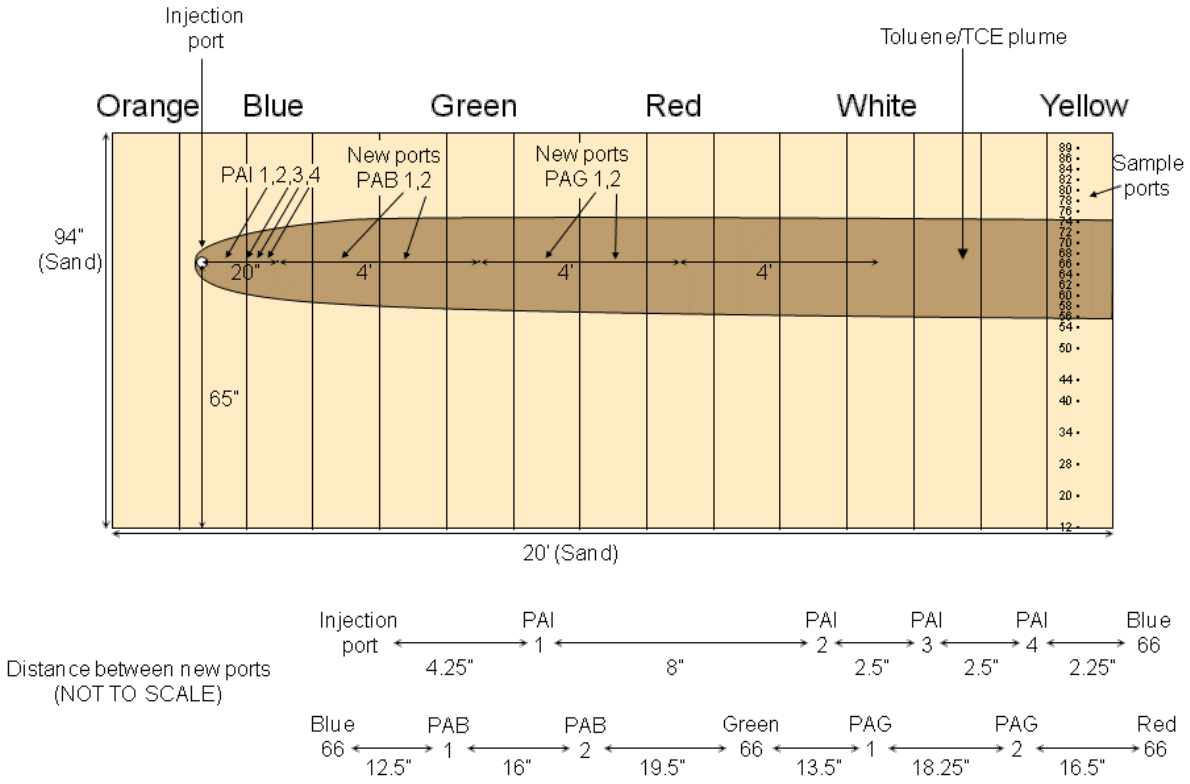


Figure A.8. Bacterial rRNA PCR-SSCP Gel Fingerprints (left) **and Associated Densitometric Profiles** (center)**, and Pearson's Correlation Coefficients** (right) **of Model Aquifer Samples (unfrozen: A, B, & C; frozen 10 months: D, E, & F)**

APPENDIX A.3. MODEL AQUIFER AQUEOUS CHEMISTRY DATA

As described in Section 3.4, a large physical model aquifer was employed throughout this study. The figure below shows the layout of the water sample ports. Each port is labeled by a section color (orange, blue, green, red, white, and yellow) and a height from the bottom of the tank (ranging from 12 to 89 inches). Sample ports are referred to either by section and height or by the first letter of the section and height (i.e. Red 74 is the same as R74). There are addition ports install horizontally downstream of the injection port as detailed in the figure below. The terms injection port and influent port are used interchangeably.



On 9/26/10 we started injecting 80 mg/L Nitrate into the model aquifer along with the 25 mg/L Toluene already being injected. This was based on the stoichiometry $C_7H_8 + 4.8NO_3 + 4.8H + 0.6NH_3 = 4CO_2 + 0.6C_5H_7O_2N + 2.4N_2 + 5.2H_2O$

On 10/22/10 the Nitrate concentration was reduced by 15% in an attempt to leave some Toluene in the middle of the plume

On 10/29/10 the Nitrate concentration was reduced by another 15% (to 57.8 mg/L) in an attempt to leave some Toluene in the middle of the plume

On 11/5/10 the Nitrate concentration was reduced by another 10% (to 52 mg/L) in an attempt to leave some Toluene in the middle of the plume

On 2/25/11 we stopped adding Nitrate to the tank (continued to add Toluene/TCE/fluorescein)

On 4/1/11 we stopped adding TCE to the tank

On 4/4/11 we started adding Nitrate back into the tank at one half of the 11/5/10 concentration (~0.42 mM)

On 4/18/11 we increased the Nitrate concentration to 2 times the 11/5/10 concentraion

Table 1: Toluene Concentrations in Model Aquifer from 9/23/2010 to 11/29/2010.

Date	Section	Port	Toluene (mg/L)
9/23/2010		Influent	20.62210262
9/23/2010	Blue	68	19.6942926
9/23/2010	Blue	64	20.08842587
9/23/2010	Green	68	17.58249952
9/23/2010	Green	64	18.3685487
9/23/2010	Red	89	0
9/23/2010	Red	86	0
9/23/2010	Red	84	0
9/23/2010	Red	82	0
9/23/2010	Red	80	0
9/23/2010	Red	78	0
9/23/2010	Red	76	0
9/23/2010	Red	74	0
9/23/2010	Red	72	1.410330154
9/23/2010	Red	70	9.640572231
9/23/2010	Red	68	16.71522753
9/23/2010	Red	66	20.62911702
9/23/2010	Red	64	19.04825953
9/23/2010	Red	62	20.82644162
9/23/2010	Red	60	20.72284443
9/23/2010	Red	58	19.48445946
9/23/2010	Red	56	15.72979787
9/23/2010	Red	54	5.470722386
9/23/2010	Red	50	0
9/23/2010	Red	44	0
9/23/2010	Red	40	0
9/23/2010	Red	34	0
9/23/2010	Red	28	0
9/23/2010	Red	20	0
9/23/2010	Red	12	0
10/7/2010		Influent	16.55097022
10/7/2010		PAI	11.9896692

Date	Section	Port	Toluene (mg/L)
10/7/2010	Blue	68	14.12937413
10/7/2010	Blue	64	14.6056006
10/7/2010	Green	68	0.18591489
10/7/2010	Green	64	0.049608812
10/7/2010	Red	89	0
10/7/2010	Red	86	0
10/7/2010	Red	84	0
10/7/2010	Red	82	0
10/7/2010	Red	80	0
10/7/2010	Red	78	0
10/7/2010	Red	76	0
10/7/2010	Red	74	0
10/7/2010	Red	72	0
10/7/2010	Red	70	0
10/7/2010	Red	68	0
10/7/2010	Red	66	0
10/7/2010	Red	64	0
10/7/2010	Red	62	0
10/7/2010	Red	60	0
10/7/2010	Red	58	0
10/7/2010	Red	56	0
10/7/2010	Red	54	0
10/7/2010	Red	50	0
10/7/2010	Red	44	0
10/7/2010	Red	40	0
10/7/2010	Red	34	0
10/7/2010	Red	28	0
10/7/2010	Red	20	0
10/7/2010	Red	12	0
10/12/2010		Influent	27.76969422
10/12/2010		PAI	22.81023483
10/12/2010	Blue	72	0.380860664
10/12/2010	Blue	68	0
10/12/2010	Blue	64	0
10/12/2010	Blue	60	0
10/12/2010	Green	72	0
10/12/2010	Green	68	0
10/12/2010	Green	64	0
10/12/2010	Green	60	0
10/12/2010	Red	72	0
10/12/2010	Red	68	0
10/12/2010	Red	64	0

Date	Section	Port	Toluene (mg/L)
10/12/2010	Red	60	0
10/15/2010		Influent	29.09681658
10/15/2010		PAI	26.32969566
10/15/2010	Blue	68	0
10/15/2010	Blue	64	0
10/15/2010	Green	68	0
10/15/2010	Green	64	0
10/15/2010	Red	68	0
10/15/2010	Red	64	0
10/21/2010		Influent	26.82633517
10/21/2010		PAI	23.31139568
10/21/2010	Blue	74	0
10/21/2010	Blue	72	0
10/21/2010	Blue	68	0
10/21/2010	Blue	64	0
10/21/2010	Blue	60	0
10/21/2010	Green	74	0
10/21/2010	Green	72	0
10/21/2010	Green	68	0
10/21/2010	Green	64	0
10/21/2010	Green	60	0
10/21/2010	Red	72	0
10/21/2010	Red	68	0
10/21/2010	Red	64	0
10/21/2010	Red	60	0
10/25/2010		Influent	21.33967384
10/25/2010		PAI	20.49021156
10/25/2010	Blue	72	0
10/25/2010	Blue	68	0
10/25/2010	Blue	66	0
10/25/2010	Blue	64	0
10/25/2010	Blue	62	0
10/25/2010	Blue	60	0
10/29/2010		Influent	28.21301094
10/29/2010		PAI	23.36124867
10/29/2010	Blue	72	0
10/29/2010	Blue	70	0
10/29/2010	Blue	68	0
10/29/2010	Blue	66	0
10/29/2010	Blue	64	0
10/29/2010	Blue	62	0
10/29/2010	Blue	60	0

Date	Section	Port	Toluene (mg/L)
11/1/2010		Influent	30.49209789
11/1/2010		PAI	28.44605736
11/1/2010	Blue	72	0
11/1/2010	Blue	70	0
11/1/2010	Blue	68	0
11/1/2010	Blue	66	0
11/1/2010	Blue	64	0
11/1/2010	Blue	62	0
11/1/2010	Blue	60	0
11/3/2010		Influent	29.25734417
11/3/2010		PAI	27.51898776
11/3/2010	Blue	72	0
11/3/2010	Blue	70	0.017786074
11/3/2010	Blue	68	0.249954211
11/3/2010	Blue	66	0.012291926
11/3/2010	Blue	64	0
11/3/2010	Blue	62	0
11/3/2010	Blue	60	0
11/5/2010		Influent	24.3837274
11/5/2010		PAI	22.70254952
11/5/2010	Blue	72	0
11/5/2010	Blue	70	0
11/5/2010	Blue	68	0.071530267
11/5/2010	Blue	66	0.0079163
11/5/2010	Blue	64	0
11/5/2010	Blue	62	0
11/5/2010	Blue	60	0
11/8/2010		Influent	31.73416532
11/8/2010		PAI	28.83503518
11/8/2010	Blue	74	0
11/8/2010	Blue	72	0.050089304
11/8/2010	Blue	70	0.873573523
11/8/2010	Blue	68	1.8091108
11/8/2010	Blue	66	1.631127963
11/8/2010	Blue	64	1.288509722
11/8/2010	Blue	62	0.94842391
11/8/2010	Blue	60	0.799877105
11/10/2010		Influent	30.73029974
11/10/2010		PAI	20.03572537
11/10/2010	Blue	72	0
11/10/2010	Blue	70	0.11421527
11/10/2010	Blue	68	0.908936934

Date	Section	Port	Toluene (mg/L)
11/10/2010	Blue	66	0.601311889
11/10/2010	Blue	64	0.294285489
11/10/2010	Blue	62	0.174359456
11/10/2010	Blue	60	0.143407118
11/10/2010	Green	72	0
11/10/2010	Green	70	0
11/10/2010	Green	68	0
11/10/2010	Green	66	0
11/10/2010	Green	64	0
11/10/2010	Green	62	0
11/10/2010	Green	60	0
11/10/2010	Red	72	0
11/10/2010	Red	70	0
11/10/2010	Red	68	0
11/10/2010	Red	66	0
11/10/2010	Red	64	0
11/10/2010	Red	62	0
11/10/2010	Red	60	0
11/12/2010		Influent	24.94315379
11/12/2010		PAI	23.47335293
11/12/2010	Blue	72	0
11/12/2010	Blue	70	0.059683386
11/12/2010	Blue	68	0.774446484
11/12/2010	Blue	66	1.007335358
11/12/2010	Blue	64	0.583643968
11/12/2010	Blue	62	0.273844894
11/12/2010	Blue	60	0.15118951
11/15/2010		Influent	29.76126194
11/15/2010		PAI	27.86685597
11/15/2010	Blue	72	0.033110613
11/15/2010	Blue	70	0.003808488
11/15/2010	Blue	68	0.355433982
11/15/2010	Blue	66	0.739358766
11/15/2010	Blue	64	0.062400922
11/15/2010	Blue	62	0.073916972
11/15/2010	Blue	60	0.009704359
11/15/2010	Green	72	0
11/15/2010	Green	70	0.009326268
11/15/2010	Green	68	0.176691023
11/15/2010	Green	66	0.163871343
11/15/2010	Green	64	0.252226701
11/15/2010	Green	62	0.465446917

Date	Section	Port	Toluene (mg/L)
11/15/2010	Green	60	0.757003056
11/15/2010	Red	68	0
11/15/2010	Red	66	0
11/15/2010	Red	64	0
11/18/2010		Influent	18.40130879
11/18/2010		PAI	17.08166556
11/18/2010	Blue	74	0
11/18/2010	Blue	72	0
11/18/2010	Blue	70	0.040889067
11/18/2010	Blue	68	0.53933632
11/18/2010	Blue	66	1.176184912
11/18/2010	Blue	64	0.848032626
11/18/2010	Blue	62	0.587082241
11/18/2010	Blue	60	0.636482315
11/18/2010	Blue	58	0.507915306
11/18/2010	Green	72	0
11/18/2010	Green	70	0
11/18/2010	Green	68	0.024958006
11/18/2010	Green	66	0.022327116
11/18/2010	Green	64	0.016844783
11/18/2010	Green	62	0.109831767
11/18/2010	Green	60	0.243629048
11/18/2010	Green	58	0.338423783
11/18/2010	Green	56	0
11/18/2010	Red	74	0
11/18/2010	Red	72	0
11/18/2010	Red	70	0
11/18/2010	Red	68	0
11/18/2010	Red	66	0
11/18/2010	Red	64	0
11/18/2010	Red	62	0
11/18/2010	Red	60	0
11/18/2010	Red	58	0
11/18/2010	Red	56	0
11/29/2010		Influent	17.42440983
11/29/2010		PAI	19.33103684
11/29/2010		NPAI	18.21154603
11/29/2010	Blue	74	0.146246746
11/29/2010	Blue	72	0.054252253
11/29/2010	Blue	70	0.02249647
11/29/2010	Blue	68	0.013997278
11/29/2010	Blue	66	0.007034085

Date	Section	Port	Toluene (mg/L)
11/29/2010	Blue	64	0.004816734
11/29/2010	Blue	62	0.011130081
11/29/2010	Blue	60	0.004127504
11/29/2010	Blue	58	0.007026208
11/29/2010	Green	72	0.004450457
11/29/2010	Green	70	0.004115688
11/29/2010	Green	68	0.034260643
11/29/2010	Green	66	0.112502041
11/29/2010	Green	64	0.405208209
11/29/2010	Green	62	0.532790604
11/29/2010	Green	60	0.410147034
11/29/2010	Green	58	0.646698674
11/29/2010	Green	56	0.062578153
11/29/2010	Red	74	0
11/29/2010	Red	72	0
11/29/2010	Red	70	0.002800243
11/29/2010	Red	68	0.018561951
11/29/2010	Red	66	0.008168361
11/29/2010	Red	64	0.00732947
11/29/2010	Red	62	0.007341285
11/29/2010	Red	60	0
11/29/2010	Red	58	0
11/29/2010	Red	56	0

Table 2: Chlorine, Sulfate and Nitrate Concentrations in Model Aquifer from 9/26/2010 to 11/29/2010.

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
9/26/2010		IN	25.30901804	6.472931111	0.273308581
9/26/2010		PAI	27.72344689	13.4963859	0
9/26/2010	Blue	78	27.70741483	9.219255544	0.365442794
9/26/2010	Blue	76	27.24488978	8.152038157	0.352722772
9/26/2010	Blue	74	27.44609218	8.807395388	0.38710121
9/26/2010	Blue	72	27.4509018	7.561980626	0.324188669
9/26/2010	Blue	70	31.83967936	9.449968039	0.080382948
9/26/2010	Blue	68	31.59839679	7.752962581	0
9/26/2010	Blue	66	31.10220441	7.933716871	0
9/26/2010	Blue	64	32.50981964	26.94478045	0
9/26/2010	Blue	62	31.67775551	7.874514432	0

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
9/26/2010	Blue	60	33.25450902	8.448443723	0.148422447
9/26/2010	Blue	58	32.34148297	9.664552294	0.192377168
9/26/2010	Blue	56	32.66372745	8.832177804	0.407333815
9/26/2010	Blue	54	30.35511022	8.485223976	0.298651252
9/26/2010	Blue	50	29.64809619	8.234056154	0.298651252
9/27/2010		IN	28.47693511	5.6528081	63.70591974
9/27/2010		PAI	29.44644253	6.656688244	46.47404399
9/27/2010	Blue	78	34.62329945	8.55881708	0.604219305
9/27/2010	Blue	76	32.57623143	8.367955917	0.446111636
9/27/2010	Blue	74	32.4190774	8.576930777	0.419760358
9/27/2010	Blue	72	31.16731822	8.460049765	0.368297862
9/27/2010	Blue	70	29.95621579	8.465007198	0
9/27/2010	Blue	68	31.06333073	7.650462853	0.137026646
9/27/2010	Blue	66	31.12666145	8.629174492	0.622820207
9/27/2010	Blue	64	31.96559812	8.552143613	0.983987723
9/27/2010	Blue	62	30.32759969	8.505620013	0.034101654
9/27/2010	Blue	60	30.70758405	7.977844089	0.063863097
9/27/2010	Blue	58	31.90070367	8.795439162	0.233131307
9/27/2010	Blue	56	33.80688038	9.124345762	0.498814192
9/27/2010	Blue	54	32.13291634	13.16999228	0.404259607
9/27/2010	Blue	50	33.10711493	9.466217955	0.47649311
9/28/2010		IN	30.36757416	7.372007263	85.16063278
9/28/2010		PAI	29.84519179	7.07100204	74.53065686
9/28/2010	Blue	78	38.63723023	8.830048108	0.522591985
9/28/2010	Blue	76	31.51376497	8.585949346	0.369861597
9/28/2010	Blue	74	35.3824449	12.66486962	0.429240336
9/28/2010	Blue	72	34.46884771	9.058984295	0.745631527
9/28/2010	Blue	70	28.78059941	6.401789558	21.06940813
9/28/2010	Blue	68	27.92419736	6.646262706	52.72832011
9/28/2010	Blue	66	30.1509952	7.582037027	54.82725514
9/28/2010	Blue	64	30.33020667	7.889032403	48.29618469
9/28/2010	Blue	62	30.08388622	6.842814623	47.1795099
9/28/2010	Blue	60	27.98749333	6.319424945	46.73963457
9/28/2010	Blue	58	36.17783879	8.455850695	8.002540583
9/28/2010	Blue	56	34.3140395	9.155013946	0.490391575
9/28/2010	Blue	54	33.14497064	8.971003912	0.386700344
9/28/2010	Blue	50	34.09974834	8.391830928	0.39231326
9/28/2010	Green	78	27.13270232	8.113641228	0.392497144
9/28/2010	Green	76	27.25434295	8.052781939	0.462782189
9/28/2010	Green	74	28.20085909	8.264765822	0.400595832
9/28/2010	Green	72	29.64154027	8.457579959	0.230812617
9/28/2010	Green	70	30.35921998	2.759326639	0.113960114

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
9/28/2010	Green	68	29.65218383	0.48650208	0
9/28/2010	Green	66	30.39723268	3.232428509	0.067103417
9/28/2010	Green	64	30.90888357	7.873181899	0.081565361
9/28/2010	Green	62	30.57133082	7.922316003	0.058137012
9/28/2010	Green	60	30.08552857	7.431347186	0.030080842
9/28/2010	Green	58	30.49682594	7.539851667	0.112224681
9/28/2010	Green	56	29.87341772	7.907054652	0.040493442
9/28/2010	Green	54	30.29992017	8.493313853	0.087928616
9/28/2010	Green	50	28.28676778	8.289332874	0.048389663
9/29/2010		IN	31.94739043	8.140999991	80.98688302
9/29/2010		PAI	30.34553541	8.106568895	83.28951364
9/29/2010	Blue	78	30.98794998	11.20573976	0.49315227
9/29/2010	Blue	76	30.61922682	8.687616903	0.433279824
9/29/2010	Blue	74	31.32930399	9.016666512	0.42923048
9/29/2010	Blue	72	30.99175124	7.753882804	14.00147512
9/29/2010	Blue	70	30.95069753	8.259182401	36.42818922
9/29/2010	Blue	68	31.64785038	8.281516085	38.83263193
9/29/2010	Blue	66	31.35895389	8.074743395	38.65851013
9/29/2010	Blue	64	30.86326833	12.10671779	34.02808509
9/29/2010	Blue	62	30.64963698	9.040489108	31.16838041
9/29/2010	Blue	60	30.38354811	7.556415816	28.32660853
9/29/2010	Blue	58	31.13619949	8.970510232	5.622803592
9/29/2010	Blue	56	31.84171513	9.714408018	0.496912375
9/29/2010	Blue	54	31.43954081	8.804124287	0.410140715
9/29/2010	Blue	50	30.57209108	8.456835503	0.400017354
9/29/2010	Green	78	29.61036986	8.597351597	0.449187962
9/29/2010	Green	76	28.95275022	8.63531886	0.426338091
9/29/2010	Green	74	30.11365796	9.055006002	0.448031006
9/29/2010	Green	72	30.69373171	9.909641637	0.439643079
9/29/2010	Green	70	30.79636599	0.181033119	0.034997903
9/29/2010	Green	68	29.88786255	1.349698961	0.056980057
9/29/2010	Green	66	29.90990991	2.858897647	0.048013652
9/29/2010	Green	64	29.90154712	8.029517685	0.056980057
9/29/2010	Green	62	29.65066332	8.690780841	0.065078745
9/29/2010	Green	60	29.89318432	8.53053666	0.039914964
9/29/2010	Green	58	30.41775953	9.08720373	0.074623628
9/29/2010	Green	56	30.32500855	8.783837857	0.06392179
9/29/2010	Green	54	31.29205155	9.34664669	0.147801062
9/29/2010	Green	50	30.55460524	8.74977899	0.504143347
9/30/2010		IN	31.53507014	7.335988592	91.84850674
9/30/2010		PAI	35.6488978	7.273835866	85.34802505
9/30/2010	Blue	78	31.11262525	7.838127551	0.417870906

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
9/30/2010	Blue	76	29.86372745	7.07774008	0.338692197
9/30/2010	Blue	74	31.66733467	7.586566357	0.405226397
9/30/2010	Blue	72	32.08256513	7.168215568	1.538415222
9/30/2010	Blue	70	31.26813627	7.328317844	30.33387524
9/30/2010	Blue	68	33.31302605	7.306682402	34.81514933
9/30/2010	Blue	66	34.31583166	7.961056203	36.2036368
9/30/2010	Blue	64	32.11543086	7.40187835	34.4647158
9/30/2010	Blue	62	30.11543086	7.331268132	33.38782514
9/30/2010	Blue	60	30.00320641	7.081280425	31.5841763
9/30/2010	Blue	58	30.77915832	7.479765944	9.869039017
9/30/2010	Blue	56	31.18557114	7.028568619	0.383249037
9/30/2010	Blue	54	29.59679359	6.94143679	0.352842004
9/30/2010	Blue	50	31.28657315	7.449279638	0.360067437
9/30/2010	Green	78	30.98837675	8.274573438	0.394087187
9/30/2010	Green	76	30.24368737	8.151251414	0.349229287
9/30/2010	Green	74	30.4761523	8.112110931	0.348627168
9/30/2010	Green	72	30.54589178	8.104440183	0.516016378
9/30/2010	Green	70	30.26853707	1.524511973	0
9/30/2010	Green	68	29.83006012	0.078280966	0.038234586
9/30/2010	Green	66	29.1238477	1.494812411	0.04425578
9/30/2010	Green	64	29.43246493	7.184933864	0.076469171
9/30/2010	Green	62	29.1006012	6.772286965	0.031912331
9/30/2010	Green	60	29.59038076	8.443526577	0.086103083
9/30/2010	Green	58	28.64048096	7.239022471	0.155647881
9/30/2010	Green	56	30.27815631	7.901853764	0.098446532
9/30/2010	Green	54	29.88216433	7.770270935	0.087307322
9/30/2010	Green	50	30.27334669	8.059989182	0.332972062
9/30/2010	Red	78	25.17675351	6.94714068	0.407634875
9/30/2010	Red	76	26.96112224	7.47189851	0.437138728
9/30/2010	Red	74	29.50781563	4.153611644	0
9/30/2010	Red	72	29.15190381	0.092245661	0.207430154
9/30/2010	Red	70	28.41523046	0.077690908	0.048470617
9/30/2010	Red	68	27.78356713	0.068446674	0.068340559
9/30/2010	Red	66	26.82885772	0.010680041	0
9/30/2010	Red	64	26.41282565	0.047401288	0
9/30/2010	Red	62	26.47775551	0.114274475	0
9/30/2010	Red	60	27.72344689	0.314894036	0.04425578
9/30/2010	Red	58	28.24609218	0.060185868	0.052685453
9/30/2010	Red	56	30.40881764	0.136696661	0.052083333
9/30/2010	Red	54	27.65931864	0.414023701	0.106575145
9/30/2010	Red	50	28.00561122	7.769877563	0.443762042
10/1/2010		IN	28.30883503	7.586397567	94.2947933

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
10/1/2010		PAI	28.94761532	7.25882566	89.07103995
10/1/2010	Blue	78	31.04143862	7.335475199	0.879202641
10/1/2010	Blue	76	30.06645817	7.287807575	0.775037589
10/1/2010	Blue	74	30.85848319	7.300582498	0.592438733
10/1/2010	Blue	72	30.82173573	7.228699723	0.381008479
10/1/2010	Blue	70	31.31118061	7.253105546	15.98685536
10/1/2010	Blue	68	30.80688038	7.331471118	38.00908344
10/1/2010	Blue	66	30.14464425	7.365601136	35.17244586
10/1/2010	Blue	64	30.41047694	7.233275814	33.20850061
10/1/2010	Blue	62	30.31821736	8.432402544	31.91604793
10/1/2010	Blue	60	31.05238468	7.106479937	25.71636724
10/1/2010	Blue	58	30.78967944	7.207725968	1.936973943
10/1/2010	Blue	56	31.35183737	7.28952361	0.452931967
10/1/2010	Blue	54	31.6825645	7.300391828	0.352487096
10/1/2010	Blue	50	31.00469116	7.187705567	0.606079395
10/1/2010	Green	78	30.63096169	8.187009619	0.433711035
10/1/2010	Green	76	30.39640344	8.127520426	0.492613892
10/1/2010	Green	74	30.15089914	8.117986901	0.469982794
10/1/2010	Green	72	29.52071931	7.943714071	0.757366732
10/1/2010	Green	70	29.91868647	7.847997483	0
10/1/2010	Green	68	29.8522283	8.231626515	13.41714073
10/1/2010	Green	66	29.84362783	7.442822686	28.15835568
10/1/2010	Green	64	29.51524629	7.438437265	35.20313735
10/1/2010	Green	62	28.32916341	6.719228166	34.93559438
10/1/2010	Green	60	28.85848319	6.453433499	36.04482817
10/1/2010	Green	58	28.06645817	6.469068479	34.98333669
10/1/2010	Green	56	30.29788898	7.66895789	7.822919412
10/1/2010	Green	54	30.30258014	7.780118788	2.011687567
10/1/2010	Green	50	30.50039093	8.089767668	0.448281742
10/1/2010	Red	78	27.1297889	7.275604664	0.5195852
10/1/2010	Red	76	28.22673964	7.82759574	0.483003426
10/1/2010	Red	74	31.2595778	7.058049632	0.095484631
10/1/2010	Red	72	30.29632525	0.065209309	0
10/1/2010	Red	70	30.31430805	0.036990076	0.109745323
10/1/2010	Red	68	30.67005473	0.090568484	0.129586285
10/1/2010	Red	66	28.92337764	0	0
10/1/2010	Red	64	28.33229085	0.022689789	0.046502255
10/1/2010	Red	62	28.9202502	0.059870535	0.159037713
10/1/2010	Red	60	29.89288507	0.506420829	0.088974315
10/1/2010	Red	58	30.10711493	0.070738753	0.038441864
10/1/2010	Red	56	31.81157154	0.264269303	0.150047277
10/1/2010	Red	54	31.04534793	0.122791797	0.328305923

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
10/1/2010	Red	50	30.31274433	8.146015463	0.43774123
10/4/2010		IN	26.29451689	6.768124895	95.63640123
10/4/2010		PAI	26.45237551	7.217947998	90.17060309
10/4/2010	Blue	78	30.75116297	7.619475487	0.315504941
10/4/2010	Blue	76	30.80683291	7.535238951	0.271783282
10/4/2010	Blue	74	30.58796614	7.500196552	0.307528692
10/4/2010	Blue	72	31.58316175	7.468785684	1.693328016
10/4/2010	Blue	70	28.03706246	7.757623407	30.19600892
10/4/2010	Blue	68	27.44833371	7.591396642	27.53253275
10/4/2010	Blue	66	27.24090597	7.536174912	27.27463405
10/4/2010	Blue	64	27.26378403	7.535426143	27.6648794
10/4/2010	Blue	62	26.89010905	7.308736265	21.92257131
10/4/2010	Blue	60	27.09143598	7.164036615	22.18933251
10/4/2010	Blue	58	29.05589873	7.346361918	7.617317322
10/4/2010	Blue	56	29.77732022	7.521948297	0.462622413
10/4/2010	Blue	54	30.26919851	7.550476404	0.415651172
10/4/2010	Blue	50	30.84496301	7.340746149	0.296302861
10/4/2010	Green	78	30.92884923	7.349918571	0.395562842
10/4/2010	Green	76	30.52619538	7.293386496	0.413878672
10/4/2010	Green	74	31.64722032	7.476834953	0.459668247
10/4/2010	Green	72	31.75017159	7.217573613	0.676799456
10/4/2010	Green	70	31.61137802	9.841073735	0.516683653
10/4/2010	Green	68	31.66170975	11.30154808	11.55935658
10/4/2010	Green	66	31.37115839	8.979053181	27.99367808
10/4/2010	Green	64	30.92732403	7.374253571	27.0406641
10/4/2010	Green	62	31.21177457	7.379682147	31.49702368
10/4/2010	Green	60	31.47563487	7.59776118	29.16352787
10/4/2010	Green	58	31.35666895	7.435091069	29.06072288
10/4/2010	Green	56	31.36429497	7.513337452	26.5978346
10/4/2010	Green	54	32.19629375	7.446884184	1.053751052
10/4/2010	Green	50	31.59460078	7.381928455	0.387882011
10/4/2010	Red	78	31.61519103	8.294303738	0.307233276
10/4/2010	Red	76	31.28574697	8.173003126	0.392904093
10/4/2010	Red	74	30.61389461	8.536343386	0.041653742
10/4/2010	Red	72	31.15839243	2.470751203	0.723770698
10/4/2010	Red	70	30.3431709	0.024147807	0.029541661
10/4/2010	Red	68	29.81163731	0.066715337	0.051993324
10/4/2010	Red	66	30.32944406	0.365960952	0.192611631
10/4/2010	Red	64	30.08541142	0.029389192	0.049334574
10/4/2010	Red	62	30.79844429	0.079369536	0.201178712
10/4/2010	Red	60	29.80248608	1.426966923	0.07237707
10/4/2010	Red	58	30.73133532	7.528874413	0.096305815

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
10/4/2010	Red	56	30.20819035	1.496040883	0.275032865
10/4/2010	Red	54	30.99900862	10.51421726	1.622605279
10/4/2010	Red	50	30.36147335	8.050953745	0.955377321
10/7/2010		IN	25.25137231	7.184155078	80.94186065
10/7/2010		PAI	25.21129215	6.780657396	76.19210165
10/7/2010	Blue	78	26.06953037	7.055415086	0.254428799
10/7/2010	Blue	76	25.67047138	7.733670459	0.24937183
10/7/2010	Blue	74	25.41866341	6.820901812	0.264542739
10/7/2010	Blue	72	25.12241875	6.714917826	1.748131292
10/7/2010	Blue	70	23.27698876	6.091445428	23.06199529
10/7/2010	Blue	68	22.93717871	6.117361989	16.87763713
10/7/2010	Blue	66	23.25346345	7.264011799	19.06920147
10/7/2010	Blue	64	22.96767448	6.345554151	19.72723968
10/7/2010	Blue	62	22.99032848	6.08765276	17.33845352
10/7/2010	Blue	60	21.82974645	5.688369153	16.88364228
10/7/2010	Blue	58	23.05219134	6.060050569	8.420487049
10/7/2010	Blue	56	23.03040864	6.317109145	0.318273045
10/7/2010	Blue	54	22.22270628	5.92246102	0.196589706
10/7/2010	Blue	50	22.25407336	5.881163085	0.413091231
10/7/2010	Green	78	23.73616799	5.527180784	0.312583954
10/7/2010	Green	76	21.45595539	5.384745048	0.271180012
10/7/2010	Green	74	20.3006012	5.133796882	0.251900314
10/7/2010	Green	72	38.64542207	7.669455832	0.382911799
10/7/2010	Green	70	37.30306449	8.74562438	1.943766851
10/7/2010	Green	68	37.31246475	94945.80783	6.648478624
10/7/2010	Green	66	35.93720624	11.23696674	9.601360894
10/7/2010	Green	64	35.5499154	9.621684356	20.48401592
10/7/2010	Green	62	35.44369242	8.289998315	25.98825266
10/7/2010	Green	60	34.56664787	8.116845435	26.83463859
10/7/2010	Green	58	36.04906937	8.376668351	25.91796123
10/7/2010	Green	56	34.18687723	7.9961064	19.67357812
10/7/2010	Green	54	35.61665727	7.922352633	8.895878803
10/7/2010	Green	50	37.27956383	7.708953408	3.230196431
10/7/2010	Red	89	37.96484302	7.435278261	0.359160354
10/7/2010	Red	86	38.78642602	7.32708111	0.413403518
10/7/2010	Red	84	33.41041549	6.796016548	0.337334703
10/7/2010	Red	82	38.37093439	7.594017334	0.474707921
10/7/2010	Red	80	30.71630006	6.252410101	0.284696367
10/7/2010	Red	78	37.32938522	7.353288033	0.529593016
10/7/2010	Red	76	37.96578304	7.349544187	0.488509436
10/7/2010	Red	74	38.65106223	8.032421707	0
10/7/2010	Red	72	38.97913142	9.658935625	0.03819489

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
10/7/2010	Red	70	38.55893965	16.27206529	0.458980614
10/7/2010	Red	68	38.62944162	15.63523708	6.935421749
10/7/2010	Red	66	37.02105659	10.90339005	15.69842085
10/7/2010	Red	64	36.74844896	9.390876247	20.47053537
10/7/2010	Red	62	36.78887009	10.05522173	25.29946078
10/7/2010	Red	60	37.53619101	10.54061137	23.43336757
10/7/2010	Red	58	36.97123519	12.5267217	18.90422391
10/7/2010	Red	56	37.39518707	12.00202168	7.199897291
10/7/2010	Red	54	37.08779846	10.07618727	0.041083579
10/7/2010	Red	50	37.3857868	7.036371464	0.466041854
10/7/2010	Red	44	36.17522091	7.420115685	0.592502247
10/7/2010	Red	40	35.89678511	7.736470676	0.484657851
10/7/2010	Red	34	36.25399511	7.983002939	0.46347413
10/7/2010	Red	28	36.82929122	8.036352745	0.525099499
10/7/2010	Red	20	36.23049445	7.793751521	0.510977019
10/7/2010	Red	12	36.52566272	7.109563655	0.497496469
10/7/2010	White	78	368828.7272	8.211377548	0.368147387
10/7/2010	White	76	36.56984396	9.522098051	0.053601233
10/7/2010	White	74	35.6392179	0.084985305	0.10046219
10/7/2010	White	72	35.20210566	0.123921304	0.125818462
10/7/2010	White	70	34.45666479	0.051852268	0.05520606
10/7/2010	White	68	34.41624365	0.215271148	0.035948132
10/7/2010	White	66	34.82233503	2.191085903	0.114263705
10/7/2010	White	64	34.60800902	0.029763576	0.037231994
10/7/2010	White	62	35.75296108	0.114748882	0.060662473
10/7/2010	White	60	34.5751081	0.067389229	0
10/7/2010	White	58	34.6606505	8.959210797	0.237193478
10/7/2010	White	56	34.40778342	7.461859568	1.643343176
10/7/2010	White	54	39.77721376	8.825555493	1.262999101
10/7/2010	White	50	34.10227486	7.425544262	0.605340865
10/12/2010		IN	25.30363024	7.142715354	76.80141027
10/12/2010		PAI	24.61088702	7.255682177	73.41053631
10/12/2010	Blue	78	24.28375828	6.931398822	0.252762899
10/12/2010	Blue	76	24.05802465	6.740293605	0.223472778
10/12/2010	Blue	74	23.56141065	6.786129984	0.192555427
10/12/2010	Blue	72	23.48369907	6.561820462	17.82900536
10/12/2010	Blue	70	22.17000333	6.21732579	15.74072818
10/12/2010	Blue	68	22.35059024	6.342924686	7.482812394
10/12/2010	Blue	66	22.20034785	6.415829792	4.963048342
10/12/2010	Blue	64	22.14705991	6.300156096	3.701945895
10/12/2010	Blue	62	21.98127521	6.335164984	4.461590616
10/12/2010	Blue	60	21.71187507	6.149653971	15.64309445

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
10/12/2010	Blue	58	22.5926063	6.275433325	23.02420503
10/12/2010	Blue	56	22.81759982	6.455530592	1.887585599
10/12/2010	Blue	54	22.36687266	6.222198161	0.181707234
10/12/2010	Blue	50	22.47566887	6.123307077	0.156213981
10/12/2010	Green	78	22.3550309	6.200182263	0.28368025
10/12/2010	Green	76	22.26103689	6.192061645	0.26740796
10/12/2010	Green	74	21.77330422	6.182858277	0.222116754
10/12/2010	Green	72	21.37734522	5.964323417	1.813546681
10/12/2010	Green	70	21.74147948	6.87690045	9.433859923
10/12/2010	Green	68	20.98286645	6.954677927	9.59495559
10/12/2010	Green	66	20.59652888	6.62660495	7.609736253
10/12/2010	Green	64	20.43296451	5.635889524	9.322123534
10/12/2010	Green	62	19.80683122	5.717095706	9.669808123
10/12/2010	Green	60	19.2939348	5.492425268	9.303681606
10/12/2010	Green	58	19.40273101	5.353833383	7.429927453
10/12/2010	Green	56	18.51681901	5.0521073	6.775510204
10/12/2010	Green	54	18.07793361	5.060588835	5.888399214
10/12/2010	Green	50	28.87514159	8.212810788	0.263910618
10/12/2010	Red	78	31.36272545	7.978508217	0.405189715
10/12/2010	Red	76	29.79088612	8.289717657	0.450070323
10/12/2010	Red	74	29.69852749	10.19047619	0
10/12/2010	Red	72	29.68632918	11.80109566	0
10/12/2010	Red	70	29.54691993	12.40792246	0
10/12/2010	Red	68	28.82373443	13.37252423	0.083756064
10/12/2010	Red	66	29.07467108	13.71007164	6.678044849
10/12/2010	Red	64	28.71917749	13.50589971	11.90189478
10/12/2010	Red	62	28.6538294	12.342815	13.56943062
10/12/2010	Red	60	28.33928727	11.43426043	11.87376539
10/12/2010	Red	58	28.02300253	12.03539823	3.546516222
10/12/2010	Red	56	27.76945195	11.59165613	0.118522733
10/12/2010	Red	54	27.63527054	10.13379688	0
10/12/2010	Red	50	28.20336325	7.622840287	0.43806002
10/12/2010	White	78	30.28143243	8.210493047	0.602411543
10/12/2010	White	76	29.84839244	12.99262537	0.049621517
10/12/2010	White	74	29.98344515	17.60387695	0.112833641
10/12/2010	White	72	30.60294502	16.71007164	6.831650311
10/12/2010	White	70	29.5887427	15.43531395	11.27799112
10/12/2010	White	68	28.94571752	13.20101138	12.35449359
10/12/2010	White	66	28.54665853	12.97787611	11.64525356
10/12/2010	White	64	29.08338416	14.34428993	6.97893456
10/12/2010	White	62	28.72789056	14.39275179	0.139382734
10/12/2010	White	60	28.50483576	12.06257901	0

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
10/12/2010	White	58	28.07266707	9.60619469	0.126740309
10/12/2010	White	56	27.82434434	7.355667931	0.530665782
10/12/2010	White	54	28.75577241	6.701222082	0.410246685
10/12/2010	White	50	29.12956347	6.681415929	0.370106987
10/15/2010		IN	27.28524996	8.519041988	91.26748645
10/15/2010		PAI	29.12397462	9.510898305	86.50598463
10/15/2010	Blue	78	28.13109426	11.38731047	0.348296052
10/15/2010	Blue	76	27.61724191	8.088109454	0.274597044
10/15/2010	Blue	74	27.83934375	7.945042161	1.7485772
10/15/2010	Blue	72	30.21978022	8.861326641	32.21247151
10/15/2010	Blue	70	29.37857917	8.659994039	17.09735093
10/15/2010	Blue	68	29.76319455	9.002278693	10.28619781
10/15/2010	Blue	66	29.6277666	9.354947263	4.855945736
10/15/2010	Blue	64	29.50394676	8.978241849	4.798897244
10/15/2010	Blue	62	29.8630243	9.038045516	6.883214369
10/15/2010	Blue	60	29.69354589	9.10784851	17.89248134
10/15/2010	Blue	58	28.93360161	8.530387378	25.02026723
10/15/2010	Blue	56	27.91673116	7.937542665	10.9601343
10/15/2010	Blue	54	26.96718774	7.787552761	0.209905693
10/15/2010	Blue	50	27.70236805	7.761785264	0.208813855
10/15/2010	Green	78	26.88670484	7.575451652	0.273232247
10/15/2010	Green	76	27.08481659	7.461420866	0.248392952
10/15/2010	Green	74	26.30397771	7.451613834	1.03151315
10/15/2010	Green	72	26.52453181	7.439691559	10.83184343
10/15/2010	Green	70	26.32796781	8.558847001	14.7408934
10/15/2010	Green	68	27.67296084	9.512244368	10.54332546
10/15/2010	Green	66	25.70809472	8.634418837	5.396132167
10/15/2010	Green	64	25.79941186	7.588143106	6.810880157
10/15/2010	Green	62	25.77696951	7.677752459	7.074831789
10/15/2010	Green	60	25.95341278	7.647946773	6.252678413
10/15/2010	Green	58	25.35288655	7.517570933	5.412509724
10/15/2010	Green	56	26.10896146	7.455267434	13.44597453
10/15/2010	Green	54	26.58798948	7.460459392	13.88352827
10/15/2010	Green	50	26.81318681	7.292778371	0.305441443
10/15/2010	Red	78	28.68889465	7.934565863	0.326259407
10/15/2010	Red	76	27.95026459	7.915617753	0.607227609
10/15/2010	Red	74	28.48980498	10.68258308	0
10/15/2010	Red	72	27.89253599	12.27278059	0
10/15/2010	Red	70	27.55578581	12.184717	0
10/15/2010	Red	68	26.87192392	12.58641691	0.036070242
10/15/2010	Red	66	26.10590978	12.42129767	3.783849753
10/15/2010	Red	64	25.8624135	11.79258136	8.743372432

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
10/15/2010	Red	62	25.58487215	10.94641294	10.13600922
10/15/2010	Red	60	24.99500426	10.19588736	7.072208285
10/15/2010	Red	58	24.88694816	11.15827085	1.07641196
10/15/2010	Red	56	25.32509344	10.22530204	0.24435555
10/15/2010	Red	54	25.03941087	10.58820345	0.034714218
10/15/2010	Red	50	25.21333679	6.91172888	0.539155197
10/15/2010	White	78	26.34718573	7.872488248	0.382127602
10/15/2010	White	76	27.50915886	12.32276751	0.082988677
10/15/2010	White	74	25.64038042	14.99138312	0.040138314
10/15/2010	White	72	25.47015505	14.82427884	4.446674351
10/15/2010	White	70	25.45683307	14.47400951	6.911655027
10/15/2010	White	68	25.33767531	14.26467802	8.480846159
10/15/2010	White	66	24.84846242	13.36834222	8.096006509
10/15/2010	White	64	25.21481701	12.72410651	3.619499627
10/15/2010	White	62	24.88472782	12.61258335	0.040680724
10/15/2010	White	60	25.18743293	11.04476265	0
10/15/2010	White	58	24.63012989	9.026337872	0.087599159
10/15/2010	White	56	25.11860267	7.165272627	0.437995796
10/15/2010	White	54	26.61288532	6.805619468	0.488168689
10/15/2010	White	50	26.86230248	6.199821346	0.316224829
10/18/2010		IN	24.44723421	5.429662757	86.33950039
10/18/2010		PAI	25.0513927	5.449816695	83.64025208
10/18/2010	Blue	78	31.57944292	8.708996334	0.193870062
10/18/2010	Blue	76	30.72577481	8.627036987	0.523904735
10/18/2010	Blue	74	28.72342095	7.731626327	17.05499734
10/18/2010	Blue	72	26.07218517	6.78477514	26.02414517
10/18/2010	Blue	70	26.24401726	6.725273038	15.63691124
10/18/2010	Blue	68	26.19537073	7.138716674	9.098225811
10/18/2010	Blue	66	26.0659082	6.750609417	5.605780669
10/18/2010	Blue	64	25.68301295	6.949653544	7.317962087
10/18/2010	Blue	62	26.1867399	7.096873261	6.509073423
10/18/2010	Blue	60	25.35582581	6.880362387	15.45341804
10/18/2010	Blue	58	25.72459788	6.918175013	21.53881198
10/18/2010	Blue	56	28.01333856	6.870957216	27.13725292
10/18/2010	Blue	54	30.67242056	8.573677038	1.0850143
10/18/2010	Blue	50	31.79913692	8.513982994	0.22297588
10/18/2010	Green	78	28.49744998	8.195550778	0.222469692
10/18/2010	Green	76	28.41192625	7.857732394	0.336108932
10/18/2010	Green	74	27.73715182	7.670780629	4.000911139
10/18/2010	Green	72	30.09180071	8.239889441	11.4054314
10/18/2010	Green	70	30.4433111	9.305744832	15.057832
10/18/2010	Green	68	28.36720282	9.797117027	11.06071727

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
10/18/2010	Green	66	26.99097685	8.928002457	3.839184025
10/18/2010	Green	64	27.44291879	7.799190004	4.926982359
10/18/2010	Green	62	27.73872107	8.074243268	5.774594417
10/18/2010	Green	60	28.18281679	8.25505288	4.881172332
10/18/2010	Green	58	29.13456257	8.0176203	4.036344309
10/18/2010	Green	56	29.58650451	7.938348145	9.62111817
10/18/2010	Green	54	30.19458611	9.755273614	16.59006353
10/18/2010	Green	50	28.18124755	7.856388798	0.711194351
10/18/2010	Red	78	28.45921684	8.292903362	0.4907808
10/18/2010	Red	76	27.97322396	7.739863663	2.89118478
10/18/2010	Red	74	28.74400248	11.45769035	0.51370938
10/18/2010	Red	72	28.96687819	14.59305624	0
10/18/2010	Red	70	28.0614456	13.03258435	0
10/18/2010	Red	68	28.45534747	13.26045362	0
10/18/2010	Red	66	26.98576072	13.0233542	0.04230869
10/18/2010	Red	64	27.44853738	12.29551857	3.959820393
10/18/2010	Red	62	27.57390497	11.63594758	5.761897613
10/18/2010	Red	60	27.87416809	11.13213534	3.304445142
10/18/2010	Red	58	27.97554558	11.73017201	0.21864039
10/18/2010	Red	56	28.59077542	11.21578355	1.498819451
10/18/2010	Red	54	27.82464015	12.61972752	1.261071911
10/18/2010	Red	50	28.07847082	7.755439538	1.122408593
10/18/2010	White	78	28.83377186	9.425134847	0.196530687
10/18/2010	White	76	28.22705464	14.58190314	0
10/18/2010	White	74	28.42903575	16.00911477	0.174693944
10/18/2010	White	72	25.86441727	15.90431413	1.331222448
10/18/2010	White	70	27.79987618	16.03699751	4.918453413
10/18/2010	White	68	27.2720941	15.2828175	7.271362476
10/18/2010	White	66	27.30382294	14.40287673	7.23942624
10/18/2010	White	64	27.35644637	13.97290567	2.194592677
10/18/2010	White	62	28.19455193	13.50909073	0.149854649
10/18/2010	White	60	28.43754837	12.2762891	0.094716873
10/18/2010	White	58	27.88964557	10.06932226	0.342290947
10/18/2010	White	56	27.59944281	8.075994885	0.865826862
10/18/2010	White	54	28.93901873	7.815627794	0.586316551
10/18/2010	White	50	29.91100449	7.674675743	0.463757831
10/21/2010		IN	24.92680268	6.397424191	79.21338637
10/21/2010		PAI	25.52717645	6.420683358	84.2327667
10/21/2010	Blue	78	28.51113534	8.311788169	0.371139766
10/21/2010	Blue	76	28.84363806	8.706809554	5.889088744
10/21/2010	Blue	74	25.96636038	5.679465616	22.96126466
10/21/2010	Blue	72	24.14265691	5.25291941	21.36295696

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
10/21/2010	Blue	70	27.81498209	5.286366476	3.477060269
10/21/2010	Blue	68	25.99517209	5.38055649	0.105388493
10/21/2010	Blue	66	24.77885065	5.461098563	0.179109771
10/21/2010	Blue	64	24.64569382	5.501657936	0.378233223
10/21/2010	Blue	62	25.03893475	5.329616993	0.03673397
10/21/2010	Blue	60	24.03052484	5.189485319	4.806069972
10/21/2010	Blue	58	23.4410528	5.117016675	14.42276999
10/21/2010	Blue	56	24.70331724	5.352876159	25.73531275
10/21/2010	Blue	54	28.28531381	8.379643424	7.235832088
10/21/2010	Blue	50	29.7251207	9.60180691	0.263217896
10/21/2010	Green	78	28.42080673	8.110913547	0.22268386
10/21/2010	Green	76	28.1194518	8.199721274	0.514782256
10/21/2010	Green	74	28.08986139	7.983468691	5.194436704
10/21/2010	Green	72	29.48606136	8.694699409	17.84738935
10/21/2010	Green	70	29.42065099	9.918208467	18.51037418
10/21/2010	Green	68	30.29278929	11.0740545	10.40888709
10/21/2010	Green	66	29.98209002	10.27305493	2.558964355
10/21/2010	Green	64	30.58869335	8.951511365	1.662655486
10/21/2010	Green	62	30.49836474	9.309625643	1.139006409
10/21/2010	Green	60	30.04204952	9.637560671	0.049147519
10/21/2010	Green	58	29.79676063	9.155653804	0.034707268
10/21/2010	Green	56	29.24232986	9.243115959	7.535277278
10/21/2010	Green	54	30.30758449	9.245230429	17.93985762
10/21/2010	Green	50	27.66858745	7.691095199	2.234185393
10/21/2010	Red	78	28.9989994	7.946978558	0.723613373
10/21/2010	Red	76	29.83309986	7.962573099	6.218492035
10/21/2010	Red	74	29.87072243	10.17212476	1.959084509
10/21/2010	Red	72	29.31358815	15.68693957	0.054112255
10/21/2010	Red	70	29.15189113	14.46881092	0.075094558
10/21/2010	Red	68	28.72603562	13.31676413	0
10/21/2010	Red	66	28.47388433	13.15068226	0.052731841
10/21/2010	Red	64	28.4914949	13.03040936	0.035614699
10/21/2010	Red	62	28.49629778	12.28323587	0.03478645
10/21/2010	Red	60	28.06163698	11.42923977	0.053007924
10/21/2010	Red	58	28.2113268	12.19785575	0.028988708
10/21/2010	Red	56	27.7726636	11.69181287	0.525109743
10/21/2010	Red	54	28.46828097	12.98479532	3.499627288
10/21/2010	Red	50	28.30258155	8.076413255	4.325115265
10/21/2010	White	78	29.62657595	9.398830409	0.996107231
10/21/2010	White	76	29.66980188	13.47563353	0.28685017
10/21/2010	White	74	28.67480488	15.20662768	0.042516772
10/21/2010	White	72	28.02161297	16.30526316	0.068468568

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
10/21/2010	White	70	27.74624775	15.86666667	0.121476492
10/21/2010	White	68	27.451671	15.37076023	3.51011844
10/21/2010	White	66	26.98018811	14.55009747	3.987741918
10/21/2010	White	64	27.48769262	14.41734893	0.285745838
10/21/2010	White	62	27.93356014	13.62222222	0.070401149
10/21/2010	White	60	28.66119672	12.69298246	0.555754949
10/21/2010	White	58	27.96237743	10.39785575	0.182766903
10/21/2010	White	56	28.22813688	8.331384016	0.750117335
10/21/2010	White	54	28.57794677	7.997660819	0.593026145
10/21/2010	White	50	30.31578947	8.310331384	0.484801634
10/25/2010		IN	29.40441102	8.096635754	100.0307566
10/25/2010		PAI	29.4410001	8.165449882	94.42090017
10/25/2010	Blue	78	29.21130196	6.682123125	0.733866209
10/25/2010	Blue	76	28.71023478	6.336572612	4.182894337
10/25/2010	Blue	74	30.36080903	7.05628453	19.97568085
10/25/2010	Blue	72	28.11566216	7.129291634	10.49621801
10/25/2010	Blue	70	28.16038215	6.810132202	0.213865494
10/25/2010	Blue	68	28.14208761	6.764502762	0.046850133
10/25/2010	Blue	66	27.85750584	6.738358327	0.128748458
10/25/2010	Blue	64	27.58105499	6.755870166	0.074387998
10/25/2010	Blue	62	27.32798049	6.593084057	0.048638306
10/25/2010	Blue	60	28.40227665	6.681383189	0.880496397
10/25/2010	Blue	58	26.93871328	6.360497238	2.560306135
10/25/2010	Blue	56	27.95101128	6.810378848	14.38191799
10/25/2010	Blue	54	26.08395162	5.988308998	1.614004971
10/25/2010	Blue	50	25.59304807	5.616860695	0.69667221
10/29/2010		IN	28.79031883	9.090218817	84.15102285
10/29/2010		PAI	29.30882011	9.425692	80.10852136
10/29/2010	Blue	78	25.44100535	6.845677734	0.388085327
10/29/2010	Blue	76	25.0658599	6.720968224	0.37642333
10/29/2010	Blue	74	25.37863626	6.899058926	0.425338927
10/29/2010	Blue	72	25.69606702	7.190124479	0.050535318
10/29/2010	Blue	70	27.07004887	8.405236879	4.734122678
10/29/2010	Blue	68	26.66697696	8.366351442	2.828682032
10/29/2010	Blue	66	26.63625785	8.694001519	2.690681741
10/29/2010	Blue	64	26.10658599	8.340121028	3.220654692
10/29/2010	Blue	62	25.25389807	7.950116196	3.07747129
10/29/2010	Blue	60	24.44403072	7.630289225	6.419929056
10/29/2010	Blue	58	24.42634396	6.834633349	0.319409126
10/29/2010	Blue	56	20.79962765	5.642530084	0.308718962
10/29/2010	Blue	54	19.92366768	5.51022756	0.322972513
10/29/2010	Blue	50	19.0635327	6.645728354	0.372212054

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
11/1/2010		IN	5.083972596	7.613699135	71.11427543
11/1/2010		PAI	5.267123405	8.306711471	67.76252993
11/1/2010	Blue	78	5.350668876	9.224820475	0.625283615
11/1/2010	Blue	76	5.281816473	9.042763764	0.63279453
11/1/2010	Blue	74	5.384838803	9.233106242	0.692255934
11/1/2010	Blue	72	5.144453367	8.17252808	0.098267795
11/1/2010	Blue	70	5.055782406	7.773430307	0
11/1/2010	Blue	68	4.964377851	7.708985454	0
11/1/2010	Blue	66	4.892791854	7.57204014	0.049759807
11/1/2010	Blue	64	4.835044677	7.527158903	0.037241617
11/1/2010	Blue	62	4.727409407	7.300220954	0
11/1/2010	Blue	60	4.655140011	7.298149512	0.036615707
11/1/2010	Blue	58	4.53400762	7.195728227	0
11/1/2010	Blue	56	4.36076609	7.245442828	0.340807736
11/1/2010	Blue	54	4.279270814	7.300911434	0.537030372
11/1/2010	Blue	50	4.010353488	6.639891364	0.486018746
11/3/2010		IN	5.260971943	7.908057731	73.31239001
11/3/2010		PAI	5.259581715	7.810444605	69.3166006
11/3/2010	Blue	78	5.447957668	9.119157738	1.13291337
11/3/2010	Blue	76	5.36471774	8.990633788	1.246731032
11/3/2010	Blue	74	5.39356498	8.833987961	3.068800474
11/3/2010	Blue	72	4.955295467	6.907058359	0
11/3/2010	Blue	70	4.938786504	6.90822042	0
11/3/2010	Blue	68	4.834519372	6.953773212	0
11/3/2010	Blue	66	4.709225035	6.628860948	0
11/3/2010	Blue	64	4.626332664	6.576568201	0
11/3/2010	Blue	62	4.567074177	6.523113394	0
11/3/2010	Blue	60	4.514593054	6.423873382	0.133225875
11/3/2010	Blue	58	4.278601778	6.455016617	0.034211089
11/3/2010	Blue	56	4.13054245	6.375531643	2.022730637
11/3/2010	Blue	54	4.160953697	7.006763195	1.142453001
11/3/2010	Blue	50	3.929480663	6.814093476	0.856921989
11/5/2010		IN	5.773979891	11.27134683	74.81746093
11/5/2010		PAI	5.7500598	11.22353436	69.83293556
11/5/2010	Blue	78	5.609673614	8.953327504	1.517283856
11/5/2010	Blue	76	5.558864043	8.93163481	3.008699669
11/5/2010	Blue	74	5.549790905	8.115281175	8.537993687
11/5/2010	Blue	72	5.553750093	8.229499851	0.634074987
11/5/2010	Blue	70	5.539068106	8.155567607	0
11/5/2010	Blue	68	5.538243276	8.236583179	0.055123566
11/5/2010	Blue	66	5.324612123	8.161986874	0
11/5/2010	Blue	64	5.290959031	7.718836121	0

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
11/5/2010	Blue	62	5.404620702	8.054187465	0.189698976
11/5/2010	Blue	60	5.441243185	8.229057143	0.047116791
11/5/2010	Blue	58	5.402311176	8.36319768	0.112094849
11/5/2010	Blue	56	5.03707614	7.300698372	1.858803603
11/5/2010	Blue	54	5.073038759	8.117052007	2.718608053
11/5/2010	Blue	50	4.934962099	7.948601596	1.367310801
11/8/2010		IN	5.252370756	8.728742714	66.44321374
11/8/2010		PAI	5.385438972	9.227809397	63.3797255
11/8/2010	Blue	78	5.419938139	8.08400994	1.709607513
11/8/2010	Blue	76	5.660752524	9.440811699	10.39955053
11/8/2010	Blue	74	5.206315217	8.303241987	3.108114616
11/8/2010	Blue	72	5.354678631	9.726188977	0
11/8/2010	Blue	70	5.313381598	9.213380209	0
11/8/2010	Blue	68	5.236225825	9.316903908	0
11/8/2010	Blue	66	5.191359913	9.489138028	0
11/8/2010	Blue	64	5.127629924	9.295832713	0
11/8/2010	Blue	62	4.968729819	8.709045727	0.053936913
11/8/2010	Blue	60	4.840250161	8.202878967	0
11/8/2010	Blue	58	4.700723973	8.027438361	0
11/8/2010	Blue	56	4.369158084	7.136264214	0.07255799
11/8/2010	Blue	54	4.463478468	6.90379396	3.348583353
11/8/2010	Blue	50	4.153495802	5.315896156	1.104101453
11/10/2010		IN	5.299967918	7.937958274	67.02040467
11/10/2010		PAI	5.388575706	8.036961028	63.55261091
11/10/2010	Blue	78	5.678537055	8.532387307	2.091014236
11/10/2010	Blue	76	5.653329667	9.358235276	3.100252738
11/10/2010	Blue	74	5.287746154	8.203615663	0.615995316
11/10/2010	Blue	72	5.510335029	8.533006074	0
11/10/2010	Blue	70	5.483599921	8.205884476	0
11/10/2010	Blue	68	5.451365018	8.298287046	0
11/10/2010	Blue	66	5.484363781	8.503305248	0
11/10/2010	Blue	64	5.510335029	8.483092186	0
11/10/2010	Blue	62	5.373756817	8.08522487	0
11/10/2010	Blue	60	5.335105489	7.894438314	0
11/10/2010	Blue	58	5.288815558	7.81337981	0
11/10/2010	Blue	56	5.158195457	8.088731218	0
11/10/2010	Blue	54	5.534625785	8.795775883	1.926234775
11/10/2010	Blue	50	5.600012222	8.229810142	1.38391901
11/10/2010	Green	78	5.663178621	9.439376967	1.352612495
11/10/2010	Green	76	5.62586341	9.301114845	1.561849848
11/10/2010	Green	74	5.682074408	9.081346349	2.979849016
11/10/2010	Green	72	5.580450006	8.208246653	1.771681626

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
11/10/2010	Green	70	5.405941852	7.691257268	0
11/10/2010	Green	68	5.41626308	6.52371046	0
11/10/2010	Green	66	5.295584102	6.677121673	0
11/10/2010	Green	64	5.303999873	6.41489305	0
11/10/2010	Green	62	5.237150071	6.291139916	0
11/10/2010	Green	60	5.300982899	6.531391689	0
11/10/2010	Green	58	5.303841085	6.760975089	0
11/10/2010	Green	56	5.309398669	7.167866859	0
11/10/2010	Green	54	5.339250838	7.535499013	0.057956369
11/10/2010	Green	50	5.273353764	8.601482904	1.680734708
11/10/2010	Red	78	4.52768471	6.46631461	0.44373774
11/10/2010	Red	76	4.793019674	7.204992799	0.466623076
11/10/2010	Red	74	5.105355924	9.179495386	0
11/10/2010	Red	72	5.080267399	10.78337867	0
11/10/2010	Red	70	4.994998174	11.01104177	0
11/10/2010	Red	68	4.895755593	10.81559716	0
11/10/2010	Red	66	4.666465535	8.05739585	0
11/10/2010	Red	64	4.571192658	6.794473782	0.038934792
11/10/2010	Red	62	4.546262921	6.919933856	0
11/10/2010	Red	60	4.646934595	7.851069504	0
11/10/2010	Red	58	4.568652048	7.63535499	0
11/10/2010	Red	56	4.548168379	7.988478157	0.04428461
11/10/2010	Red	54	4.410340283	7.78983304	0
11/10/2010	Red	50	4.102608889	6.448178375	0
11/12/2010		IN	5.511551649	9.005269427	65.90213638
11/12/2010		PAI	5.554823544	8.882719913	62.86207574
11/12/2010	Blue	78	5.723718688	9.103389796	1.721737178
11/12/2010	Blue	76	5.579079761	8.349114696	2.490104152
11/12/2010	Blue	74	5.355084073	7.77210232	1.245052076
11/12/2010	Blue	72	5.632233818	8.445014234	0
11/12/2010	Blue	70	5.638672197	8.128242919	0
11/12/2010	Blue	68	5.606031114	8.222527306	0
11/12/2010	Blue	66	5.629538683	8.493266843	0
11/12/2010	Blue	64	5.631784629	8.461569522	0
11/12/2010	Blue	62	5.563358139	8.005895298	0
11/12/2010	Blue	60	5.46528516	7.617451697	0
11/12/2010	Blue	58	5.429200293	7.393955301	0
11/12/2010	Blue	56	5.234102445	7.200742969	0.042952191
11/12/2010	Blue	54	5.597496519	8.621469383	1.14679543
11/12/2010	Blue	50	5.60258733	9.123175385	1.621796131
11/15/2010		IN	5.104586222	8.110678161	62.03924651
11/15/2010		PAI	5.232455418	8.765015849	58.65837568

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
11/15/2010	Blue	78	5.340710018	8.610163332	3.170040145
11/15/2010	Blue	76	5.270337042	7.718196685	5.700850622
11/15/2010	Blue	74	5.057571084	6.980073086	1.08250751
11/15/2010	Blue	72	5.292646773	8.014778623	0
11/15/2010	Blue	70	5.275128393	7.804405322	0
11/15/2010	Blue	68	5.285310015	7.750297793	0
11/15/2010	Blue	66	5.242187851	7.964305183	0
11/15/2010	Blue	64	5.208049471	7.849629525	0
11/15/2010	Blue	62	5.110725141	7.049928328	0
11/15/2010	Blue	60	5.233503526	7.577678626	0.080008983
11/15/2010	Blue	58	4.995582973	6.677636228	0
11/15/2010	Blue	56	4.840163505	6.262946438	0
11/15/2010	Blue	54	5.19696947	8.478932386	1.450548834
11/15/2010	Blue	50	4.956802971	7.900102966	1.690856517
11/15/2010	Green	78	4.953359187	7.375381074	1.72791331
11/15/2010	Green	76	4.977465674	7.54517373	2.395216305
11/15/2010	Green	74	4.924611077	7.894248047	2.039246512
11/15/2010	Green	72	4.793597556	7.354586017	0.442435642
11/15/2010	Green	70	4.565109976	6.600916598	0
11/15/2010	Green	68	4.594307275	6.049140942	0
11/15/2010	Green	66	4.629044575	6.826431932	0
11/15/2010	Green	64	4.617665114	6.778381216	0.044075124
11/15/2010	Green	62	4.362076452	5.808483576	0
11/15/2010	Green	60	4.374953209	6.174516969	0.06681452
11/15/2010	Green	58	4.270142393	6.400436091	0.033687993
11/15/2010	Green	56	4.041055894	5.813732814	0
11/15/2010	Green	54	3.972479674	5.93103309	0
11/15/2010	Green	50	3.99209427	6.110112859	1.060329581
11/18/2010		IN	5.458397099	8.971916922	66.38903969
11/18/2010		PAI	5.441208621	8.935654406	64.30069431
11/18/2010	Blue	78	5.737031369	8.952767054	8.462241778
11/18/2010	Blue	76	5.395523457	8.039277602	1.285465299
11/18/2010	Blue	74	5.213989008	7.465392725	0
11/18/2010	Blue	72	5.564241935	8.705652267	0
11/18/2010	Blue	70	5.51041486	8.350564819	0
11/18/2010	Blue	68	5.424774024	8.141749768	0
11/18/2010	Blue	66	5.497297338	8.715023479	0
11/18/2010	Blue	64	5.564091159	8.641683559	0
11/18/2010	Blue	62	5.294201904	7.456225235	0
11/18/2010	Blue	60	5.212028919	7.005592169	0
11/18/2010	Blue	58	5.22831274	6.987664633	0
11/18/2010	Blue	56	5.463825039	7.171829322	0

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
11/18/2010	Blue	54	5.811514772	8.943803286	0.855738735
11/18/2010	Blue	50	5.720747548	8.756990211	1.77090774
11/18/2010	Green	78	5.73021527	8.954771559	1.729839961
11/18/2010	Green	76	5.848166791	9.061503174	1.900968912
11/18/2010	Green	74	5.860234689	8.991518643	1.91315461
11/18/2010	Green	72	5.842926783	8.863071425	0.628603697
11/18/2010	Green	70	5.578068184	8.193524297	0
11/18/2010	Green	68	5.679216222	7.209686883	0
11/18/2010	Green	66	5.581402734	7.449511922	0
11/18/2010	Green	64	5.570922718	6.995892676	0
11/18/2010	Green	62	5.511853534	6.560623033	0
11/18/2010	Green	60	5.543769948	6.634661546	0
11/18/2010	Green	58	5.517887483	6.989278285	0
11/18/2010	Green	56	5.4748559	7.52141676	0
11/18/2010	Green	54	5.517093542	7.761455166	0
11/18/2010	Green	50	5.54107055	8.594655145	1.274445699
11/18/2010	Red	78	5.502167458	8.935829733	1.97675801
11/18/2010	Red	76	5.568858472	9.138528831	2.285858646
11/18/2010	Red	74	5.345284787	9.517896197	0.036557094
11/18/2010	Red	72	5.650316782	9.666399957	0
11/18/2010	Red	70	5.274624069	8.158318664	0
11/18/2010	Red	68	5.196976674	7.434362831	0
11/18/2010	Red	66	5.101703797	7.163386142	0
11/18/2010	Red	64	5.073121933	6.825625433	0
11/18/2010	Red	62	5.007701224	6.446471435	0
11/18/2010	Red	60	4.965781158	6.206646397	0
11/18/2010	Red	58	4.936405354	6.433669387	0
11/18/2010	Red	56	4.895914381	7.711100443	0.086191524
11/18/2010	Red	54	4.779999047	7.828239185	0
11/18/2010	Red	50	4.729028058	7.520990025	1.248588242
11/29/2010		IN	7.209058942	6.117548662	86.3294606
11/29/2010		PAI	7.163847957	6.000304136	85.07135238
11/29/2010		NPAI	7.570537508	7.151916058	75.59633292
11/29/2010	Blue	78	7.039099129	5.915754258	19.91898982
11/29/2010	Blue	76	7.016702947	5.631843066	16.78034999
11/29/2010	Blue	74	6.954537843	5.703467153	14.49044311
11/29/2010	Blue	72	6.879186202	5.66879562	11.34401937
11/29/2010	Blue	70	6.976096785	5.771745742	11.95318131
11/29/2010	Blue	68	6.943653717	5.747718978	15.6046934
11/29/2010	Blue	66	6.876046551	5.758515815	13.1360452
11/29/2010	Blue	64	6.972957133	6.058394161	15.87309367
11/29/2010	Blue	62	7.051239116	6.165906326	18.87306484

Sample Date	Section	Port	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
11/29/2010	Blue	60	6.892372739	5.995894161	23.51486148
11/29/2010	Blue	58	6.962700938	6.391423358	35.9708248
11/29/2010	Blue	56	7.42716008	6.45377129	3.06599014
11/29/2010	Blue	54	6.96751507	5.996958637	19.55833598
11/29/2010	Blue	50	6.89425653	6.02113747	35.24403955
11/29/2010	Green	78	7.504814133	5.947840633	2.456251622
11/29/2010	Green	76	7.449765573	5.929288321	1.934154006
11/29/2010	Green	74	7.457300737	6.237530414	10.6754692
11/29/2010	Green	72	7.201105157	5.701490268	29.23025917
11/29/2010	Green	70	6.993678835	6.115723844	4.021679592
11/29/2010	Green	68	7.432602143	5.024482968	0
11/29/2010	Green	66	7.407066309	4.837439173	0
11/29/2010	Green	64	7.340715003	4.27676399	0
11/29/2010	Green	62	7.344901206	4.290298054	0
11/29/2010	Green	60	7.44516075	4.657542579	0
11/29/2010	Green	58	7.386763228	4.934002433	0
11/29/2010	Green	56	7.13224213	51.11344282	0
11/29/2010	Green	54	7.211152043	5.840024331	0
11/29/2010	Green	50	7.252804756	5.86770073	1.140196615
11/29/2010	Red	78	7.601097206	6.48725065	1.503116712
11/29/2010	Red	76	7.368235169	6.389245447	0.225011403
11/29/2010	Red	74	7.168336414	6.513616652	0
11/29/2010	Red	72	7.121716916	5.979011275	0
11/29/2010	Red	70	7.129722284	5.741196878	0
11/29/2010	Red	68	7.025417044	5.504770165	0.288528135
11/29/2010	Red	66	6.797028596	4.822029488	0
11/29/2010	Red	64	6.629857669	4.524197745	0
11/29/2010	Red	62	6.477284767	4.443191674	0
11/29/2010	Red	60	6.385458484	4.496444059	0
11/29/2010	Red	58	6.087376241	4.583694709	0
11/29/2010	Red	56	5.941866899	4.54501301	0.062841022
11/29/2010	Red	54	5.739613623	4.692454467	0
11/29/2010	Red	50	5.402681798	4.733044232	0.962548778

Table 3: Toluene, Chlorine, Sulfate and Nitrate Concentrations in Model Aquifer from 12/9/2010 to 5/1/2010.

Sample Date	Port	Distance from injection (m)	Toluene (mg/L)	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
12/9/2010	IN	0	19.89083739	9.969425223	8.070028116	46.95020085
12/9/2010	AI1	0.1143	20.52454703	10.26961394	8.516138707	44.58564475

Sample Date	Port	Distance from injection (m)	Toluene (mg/L)	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
12/9/2010	AI2	0.3175	15.92331792	10.18476528	8.438163074	33.39242067
12/9/2010	AI3	0.381	13.39642323	10.20875697	8.184292409	24.42517376
12/9/2010	AI4	0.4445	10.43302534	10.20319792	8.275313964	17.87795702
12/9/2010	B64	0.508	8.97688699	10.03993739	8.313102156	8.812727157
12/9/2010	B66	0.508	3.103827531	10.13502641	8.259718838	3.142255946
12/9/2010	G64	1.7272	0.028817694	10.51011601	8.041387067	0
12/9/2010	G66	1.7272	0.064279566	10.37786913	8.568322399	0
12/9/2010	R64	2.9464	0.040542482	9.572684582	7.300618557	0
12/9/2010	R66	2.9464	0.045048078	9.551033544	7.787366448	0
12/20/2010	IN	0	26.10020174	8.946213335	8.334761703	44.43345715
12/20/2010	AI1	0.1143	26.00701783	9.170905037	8.264215284	43.47317291
12/20/2010	AI2	0.3175	24.37078169	8.943078102	8.213696109	33.97344
12/20/2010	AI3	0.381	18.97397628	8.782832857	8.025512183	27.65221064
12/20/2010	AI4	0.4445	17.07893227	8.585313175	7.838952088	20.42610284
12/20/2010	B64	0.508	13.6223686	8.184003344	7.464388492	14.13568208
12/20/2010	B66	0.508	8.804228948	8.443530969	7.68919882	11.24986024
12/20/2010	G64	1.7272	1.649402406	7.907754476	5.163420509	0.05689653
12/20/2010	G66	1.7272	0.701738619	8.237302306	5.449755974	0
12/20/2010	R64	2.9464	0.199329277	7.774681251	3.884202834	0.089692784
12/20/2010	R66	2.9464	0.0304876	8.378736153	4.914613573	0.05341814
12/29/2010	IN	0	27.0403667	9.151161355	8.046784674	45.74816601
12/29/2010	AI1	0.1143	27.51853484	9.376554205	7.811965046	42.96151141
12/29/2010	AI2	0.3175	23.45158508	8.768804783	7.335334976	32.95648679
12/29/2010	AI3	0.381	17.19324994	8.762455689	6.948689222	25.17334941
12/29/2010	AI4	0.4445	13.07419832	8.537768293	7.03006946	16.74354336
12/29/2010	B64	0.508	13.2972765	8.109204423	6.764239301	14.05009547
12/29/2010	B66	0.508	9.799126657	8.251706319	6.881828367	12.80449201
12/29/2010	G64	1.7272	2.384972375	7.817851537	2.11642393	0
12/29/2010	G66	1.7272	1.822749678	8.071462584	3.604750168	0
12/29/2010	R64	2.9464	0.637266068	7.227738488	3.615684517	0.040448196
12/29/2010	R66	2.9464	0.179211636	7.672175094	4.343804616	0.078384082
1/4/2011	IN	0	25.83860545	8.929789385	7.958719791	47.95906863
1/4/2011	AI1	0.1143	26.45314268	8.97400305	7.810145874	46.18161765
1/4/2011	AI2	0.3175	22.40561739	8.818055627	7.665403875	37.46936275
1/4/2011	AI3	0.381	20.26955947	8.602471167	7.588939691	32.13333333
1/4/2011	AI4	0.4445	16.45606914	8.422874574	7.546266057	22.76519608
1/4/2011	B64	0.508	17.45138039	8.178157078	7.541737427	24.77181373
1/4/2011	B66	0.508	14.07091166	8.276523915	7.574657087	21.01348039
1/4/2011	AB1	0.8255	0.324997581	7.971826641	6.653429131	0.031372549
1/4/2011	AB2	1.2319	0.265790749	7.909790414	6.075680383	0.043872549
1/4/2011	G64	1.7272	2.677513128	8.030435453	1.656085347	0.029411765

Sample Date	Port	Distance from injection (m)	Toluene (mg/L)	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
1/4/2011	G66	1.7272	1.762751215	8.153479684	3.361463096	0
1/4/2011	AG1	2.0701	1.162731141	7.445375559	2.49335946	0.043382353
1/4/2011	AG2	2.5273	1.509910115	7.330899868	1.872066188	0.029411765
1/4/2011	R64	2.9464	2.568122468	6.766404469	1.217156543	0.058088235
1/4/2011	R66	2.9464	1.295673776	7.263722516	1.558371435	0.214705882
1/4/2011	IN	0	25.83860545	8.929789385	7.958719791	47.95906863
1/4/2011	AI2	0.3175	22.40561739	8.818055627	7.665403875	37.46936275
1/4/2011	B66	0.508	14.07091166	8.276523915	7.574657087	21.01348039
1/4/2011	AB1	0.8255	0.324997581	7.971826641	6.653429131	0.031372549
1/4/2011	AB2	1.2319	0.265790749	7.909790414	6.075680383	0.043872549
1/4/2011	G66	1.7272	1.762751215	8.153479684	3.361463096	0
1/4/2011	AG1	2.0701	1.162731141	7.445375559	2.49335946	0.043382353
1/4/2011	AG2	2.5273	1.509910115	7.330899868	1.872066188	0.029411765
1/4/2011	R66	2.9464	1.295673776	7.263722516	1.558371435	0.214705882
1/10/2011	IN	0	26.93772656	8.668912623	6.898723837	48.30716959
1/10/2011	AI2	0.3175	23.02249012	8.599383557	6.892007189	34.21987763
1/10/2011	B66	0.508	10.6732555	8.424127303	6.518960916	11.74954336
1/10/2011	AB1	0.8255	4.365067372	8.36069099	4.413382468	0.025801837
1/10/2011	AB2	1.2319	1.924204346	8.741308867	3.149018825	0.032443894
1/10/2011	G66	1.7272	0.167061494	8.610135474	4.731061775	0
1/10/2011	AG1	2.0701	0.237425977	8.135617518	4.597999528	0
1/10/2011	AG2	2.5273	0.532038358	8.791484481	2.947700909	0.04317337
1/10/2011	R66	2.9464	1.027590845	8.503691492	0.958483853	0
1/31/2011	IN	0	27.26119996	9.13784184	7.477005725	37.87268784
1/31/2011	AI1	0.1143	26.64099923	9.250941006	7.103128307	36.8845516
1/31/2011	AI2	0.3175	17.87868729	9.077546069	6.925143121	22.8712846
1/31/2011	AI3	0.381	10.89706229	8.865618924	6.814625896	14.97104222
1/31/2011	AI4	0.4445	8.903580479	8.664751949	6.560128786	6.714887103
1/31/2011	B64	0.508	5.458209906	8.604812958	6.447983649	0.731215716
1/31/2011	B66	0.508	5.604287298	8.591968889	6.346872145	0.05791555
1/31/2011	AB1	0.8255	2.74673152	8.087125604	3.853632508	0
1/31/2011	AB2	1.2319	1.819917927	7.972599319	1.762487452	0.058936089
1/31/2011	G64	1.7272	2.827462024	7.736411153	0.4219913	0.048730705
1/31/2011	G66	1.7272	3.002286217	7.619387409	1.762306572	0.082663605
1/31/2011	AG1	2.0701	2.505351331	7.499152648	0.192093768	0.101033295
1/31/2011	AG2	2.5273	0.052688686	7.310059404	0.393412378	0.06454905
2/11/2011	R64	2.9464	0	7.552312824	0.915429905	0.057150147
2/11/2011	R66	2.9464	0	7.301139911	1.505277152	0.050516648
2/11/2011	IN	0	23.79252553	9.71352832	7.579217839	43.45812369
2/11/2011	AI1	0.1143	22.14408411	10.35539454	7.463861213	41.01853385
2/11/2011	AI2	0.3175	18.78257118	9.970133428	7.373121187	32.83077511

Sample Date	Port	Distance from injection (m)	Toluene (mg/L)	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
2/11/2011	AI3	0.381	18.1482944	10.00265088	7.368449424	27.42424049
2/11/2011	AI4	0.4445	15.79321098	9.834054962	7.347246804	21.25597096
2/11/2011	B66	0.508	20.35607951	10.7717593	7.090659168	25.13980001
2/11/2011	AB1	0.8255	4.823204513	1.092727755	0.298004618	0.045092669
2/11/2011	AB2	1.2319	3.259617159	10.26667845	0.044561438	0.072861601
2/11/2011	G66	1.7272	3.664210968	9.197843952	0.544799518	0
2/11/2011	AG1	2.0701	3.657830667	10.57877529	0.029647731	0.111075728
2/11/2011	AG2	2.5273	1.024014726	8.196165061	0	0.052225973
2/11/2011	R66	2.9464	1.807086432	8.285234603	0	0
2/14/2011	IN	0	26.52358987	9.83784613	7.39721966	43.12679229
2/14/2011	AI1	0.1143	24.79809252	9.862391655	7.302180788	42.08491519
2/14/2011	AI2	0.3175	22.54968617	9.751936795	7.035841349	33.70067222
2/14/2011	AI3	0.381	21.39298087	9.738436757	7.104855712	29.58564081
2/14/2011	AI4	0.4445	14.45970044	9.851959807	6.947720385	24.15357645
2/14/2011	B66	0.508	15.49741706	9.635652374	6.965179866	20.83707754
2/14/2011	AB1	0.8255	1.083465743	9.599447726	5.216925814	0.155805557
2/14/2011	AB2	1.2319	3.752968048	9.285878653	1.10768876	0.26145612
2/14/2011	G66	1.7272	3.362013118	9.039502953	0	0.082662859
2/14/2011	AG1	2.0701	3.484081672	8.660581422	0	0.144660003
2/14/2011	AG2	2.5273	3.975479087	8.83301373	0.075438134	0.214551914
2/14/2011	R66	2.9464	1.75469707	8.229193833	0.097015417	0.079644271
2/17/2011	IN	0	24.98547646	9.692292272	8.182885153	42.76127
2/17/2011	AI1	0.1143	24.7469359	9.838628846	7.958852567	40.98254968
2/17/2011	AI2	0.3175	18.17752169	9.665564131	7.40688322	34.22976248
2/17/2011	AI3	0.381	20.40990247	9.682603321	7.041837827	30.83204072
2/17/2011	AI4	0.4445	17.86316189	9.741739334	6.772757085	27.14081435
2/17/2011	B66	0.508	16.66782819	9.699308409	6.627429321	23.04459525
2/17/2011	AB1	0.8255	3.003798585	9.704988139	4.397631951	0
2/17/2011	AB2	1.2319	2.384125607	9.292372457	0.791363181	0.032719341
2/17/2011	G66	1.7272	2.792169505	9.048478166	1.086506265	0.035385361
2/17/2011	AG1	2.0701	4.745821698	8.586415422	0	0
2/17/2011	AG2	2.5273	2.873604993	8.261000301	0	0.081434804
2/17/2011	R66	2.9464	3.780635713	8.21355785	0	0.095492002
2/25/2011	IN	0	29.67019693	9.562063263	9.781237135	43.75616259
2/25/2011	AI1	0.1143	27.05986207	9.689728134	9.610822299	40.58582352
2/25/2011	AI2	0.3175	22.73136328	9.572020412	9.459938153	33.39194084
2/25/2011	AI3	0.381	21.39140154	9.278284526	9.182870437	30.25606198
2/25/2011	AI4	0.4445	20.83896198	9.111857897	8.955203876	26.94989436
2/25/2011	B66	0.508	20.01352822	9.020821109	8.654201492	23.87740215
2/25/2011	AB1	0.8255	0.213495909	8.908091961	5.190184871	0
2/25/2011	AB2	1.2319	0.606912375	8.630003023	0.393677418	0.057852903

Sample Date	Port	Distance from injection (m)	Toluene (mg/L)	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
2/25/2011	G66	1.7272	1.092161858	7.956828648	0.429675159	0.053576819
2/25/2011	AG1	2.0701	2.194827582	8.59053004	0	0.049300734
2/25/2011	AG2	2.5273	3.264150324	8.425525862	0	0.046533857
2/25/2011	R66	2.9464	1.663982576	8.167351221	0.314788753	0
2/26/2011	IN	0	24.07217178	9.829617908	10.12656544	18.14666813
2/26/2011	AI1	0.1143	22.35655602	10.03088079	9.989023234	19.81556702
2/26/2011	AI2	0.3175	20.63214963	10.05454071	10.08731518	25.00274454
2/26/2011	AI3	0.381	18.5280011	9.907972161	9.939794102	28.88681524
2/26/2011	AI4	0.4445	20.33590279	9.708860176	9.659886573	28.24217807
2/26/2011	B66	0.508	16.16235867	9.699334757	9.545961049	25.16456252
2/26/2011	AB1	0.8255	1.414201657	9.774309023	5.010394665	0
2/26/2011	AB2	1.2319	0.256933157	9.387761373	0.386847839	0.031177956
2/26/2011	G66	1.7272	0.386732904	8.552289941	0.2454804	0.025688879
2/26/2011	AG1	2.0701	0.901867402	9.372090522	0	0.050719069
2/26/2011	AG2	2.5273	2.728913938	9.994622747	0	0.06938193
2/26/2011	R66	2.9464	1.670162016	9.740509149	0.174131422	0
2/27/2011	IN	0	27.43696545	9.860317554	10.69414575	1.573665789
2/27/2011	AI1	0.1143	23.59749705	10.09679362	10.4181381	0.407956096
2/27/2011	AI2	0.3175	29.54946745	10.14203252	10.343224	0.034260699
2/27/2011	AI3	0.381	26.11729465	10.18110248	10.48114847	0
2/27/2011	AI4	0.4445	19.06602197	9.959314366	10.02960059	6.101999598
2/27/2011	B66	0.508	16.36402345	10.08944964	9.866122799	6.342458945
2/27/2011	AB1	0.8255	2.204193234	9.929350939	6.52212902	0.022417494
2/27/2011	AB2	1.2319	0.158688333	9.751039173	0.277594813	0.033837727
2/27/2011	G66	1.7272	0.616967258	8.981096603	0.123957432	0.04272013
2/27/2011	AG1	2.0701	0.820325577	9.050129988	0.813738483	0.043354588
2/27/2011	AG2	2.5273	1.953707331	10.18345255	0.835006468	0.097706437
2/27/2011	R66	2.9464	2.332327013	10.0897434	0.056979153	0
2/28/2011	IN	0	26.34626869	10.06887552	11.45805823	1.6469314
2/28/2011	AI1	0.1143	24.48432349	10.08540564	11.22649815	0.328053238
2/28/2011	AI2	0.3175	22.57486473	10.02392518	10.85298371	0
2/28/2011	AI3	0.381	24.80027837	10.11382585	10.68571968	0.022286791
2/28/2011	AI4	0.4445	24.78188577	10.10860581	10.42381941	0
2/28/2011	B66	0.508	28.24836254	10.2538969	10.33814375	0
2/28/2011	AB1	0.8255	4.468522776	10.18052635	6.440765893	0
2/28/2011	AB2	1.2319	2.352038993	9.861233959	0.364239452	0
2/28/2011	G66	1.7272	0.286428266	9.317189879	0.055021065	0.024577957
2/28/2011	AG1	2.0701	0.606813914	8.883346625	0	0.021870216
2/28/2011	AG2	2.5273	0.95370932	9.863263974	0	0
2/28/2011	R66	2.9464	2.455069043	10.33190749	0	0
3/1/2011	IN	0	26.47952247	10.03758328	10.15149881	1.60266634

Sample Date	Port	Distance from injection (m)	Toluene (mg/L)	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
3/1/2011	AI1	0.1143	25.50594363	10.16798588	10.42675503	0.277675541
3/1/2011	AI2	0.3175	25.91216008	10.32002733	11.60237343	0.067680857
3/1/2011	AI3	0.381	25.16429819	10.19930528	11.4457887	0
3/1/2011	AI4	0.4445	25.8561434	10.17168726	11.191955	0
3/1/2011	B66	0.508	24.36796176	10.34992313	11.11397087	0.045393203
3/1/2011	AB1	0.8255	19.81569556	10.32287455	4.774755336	0
3/1/2011	AB2	1.2319	1.967452548	9.925402881	0.425676196	0
3/1/2011	G66	1.7272	0.22636285	9.681111554	0.054249827	0
3/1/2011	AG1	2.0701	0.671940249	8.949376459	0	0.023923445
3/1/2011	AG2	2.5273	0.833464271	9.604521383	0.032056716	0
3/1/2011	R66	2.9464	1.844608118	10.22065942	0	0.023105549
3/2/2011	IN	0	26.06412941	9.919601245	9.827415834	1.404860639
3/2/2011	AI1	0.1143	25.96865726	10.22739753	9.774100885	0.196899492
3/2/2011	AI2	0.3175	25.2373999	10.1767083	9.462790609	0
3/2/2011	AI3	0.381	23.81375382	10.27921319	9.793557777	0.021494692
3/2/2011	AI4	0.4445	24.40638929	10.23978823	10.29009154	0.023116933
3/2/2011	B66	0.508	24.68070286	10.37467791	10.67739094	0
3/2/2011	AB1	0.8255	24.39531828	10.17051295	5.691830403	0
3/2/2011	AB2	1.2319	9.147938141	10.01478436	0.102493393	0
3/2/2011	G66	1.7272	0.170606106	9.754016418	0	0
3/2/2011	AG1	2.0701	0.373153102	9.178412019	0	0.033458719
3/2/2011	AG2	2.5273	0.630090198	9.011700765	0	0.026827809
3/2/2011	R66	2.9464	0.905927952	9.85483167	0	0
3/3/2011	IN	0	24.39048186	9.205908734	9.718561385	1.526087565
3/3/2011	AI1	0.1143	24.98749295	9.333165153	9.621659094	0.242191715
3/3/2011	AI2	0.3175	24.69961533	9.880031394	9.924874351	0.040165458
3/3/2011	AI3	0.381	25.12690253	10.02046194	9.82887628	0
3/3/2011	AI4	0.4445	24.80971885	10.22536159	9.687667187	0
3/3/2011	B66	0.508	24.86498723	10.68393318	9.710423401	0.085326619
3/3/2011	AB1	0.8255	24.21541742	10.36439063	6.905682272	0.021381612
3/3/2011	AB2	1.2319	24.08326641	10.20433905	0	0
3/3/2011	G66	1.7272	1.563189569	10.12613522	0.065254576	0.089722838
3/3/2011	AG1	2.0701	0.32063377	9.548155623	0	0
3/3/2011	AG2	2.5273	0.525350857	8.934577867	0	0
3/3/2011	R66	2.9464	0.578078927	9.395672161	0	0.080131087
3/4/2011	IN	0	25.99595471	8.689629983	9.570126648	1.791608111
3/4/2011	AI1	0.1143	23.91835138	9.111623072	9.438091408	0.311383256
3/4/2011	AI2	0.3175	24.32191725	8.973674599	9.535676074	0
3/4/2011	AI3	0.381	25.20160326	9.092518192	9.672719547	0.030917486
3/4/2011	AI4	0.4445	21.75188069	9.358862698	9.720373645	0
3/4/2011	B66	0.508	21.43918291	9.513387464	9.649196027	0

Sample Date	Port	Distance from injection (m)	Toluene (mg/L)	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
3/4/2011	AB1	0.8255	23.61510984	10.41890259	5.944772846	0
3/4/2011	AB2	1.2319	22.87623156	10.23600146	0	0
3/4/2011	G66	1.7272	2.358667417	10.13907229	0	0
3/4/2011	AG1	2.0701	0.507233952	9.837889473	0	0
3/4/2011	AG2	2.5273	0.485596066	9.241142921	0	0
3/4/2011	R66	2.9464	0.479322104	8.903717023	0	0
3/6/2011	IN	0	26.25445143	8.552003742	8.676613579	1.64304074
3/6/2011	AI1	0.1143	26.99080122	8.701387806	8.528751048	0.083177972
3/6/2011	AI2	0.3175	26.35675681	8.707625136	8.436043588	0
3/6/2011	AI3	0.381	25.70623389	8.610634648	8.529253982	0
3/6/2011	AI4	0.4445	25.64402595	8.585997193	8.764626991	0.023828172
3/6/2011	B66	0.508	25.84645086	8.710743802	8.730930427	0
3/6/2011	AB1	0.8255	25.20672719	8.879151723	4.699245599	0
3/6/2011	AB2	1.2319	24.1232772	9.473569312	0	0
3/6/2011	G66	1.7272	21.71787999	12.46530485	0	0
3/6/2011	AG1	2.0701	2.750847208	9.041946047	0	0
3/6/2011	AG2	2.5273	0.187576919	8.486823639	0	0
3/6/2011	R66	2.9464	0.463036581	7.988149072	0	0.028240797
3/8/2011	IN	0	24.51952148	8.235761511	8.583255784	1.384639881
3/8/2011	AI1	0.1143	27.70780923	8.470752172	8.454096204	0.075155247
3/8/2011	AI2	0.3175	27.50139467	8.429244863	8.226101466	0
3/8/2011	AI3	0.381	26.9888438	8.564440096	8.227705933	0.028873202
3/8/2011	AI4	0.4445	23.35685336	8.495063595	8.337772358	0
3/8/2011	B66	0.508	25.93717717	8.708529752	8.35895132	0.053712648
3/8/2011	AB1	0.8255	20.91566738	8.796288061	3.650803838	0
3/8/2011	AB2	1.2319	21.91876495	9.384505915	0.021660302	0.053712648
3/8/2011	G66	1.7272	22.59011837	9.809659344	0.018932709	0
3/8/2011	AG1	2.0701	15.68123669	9.634735687	0	0
3/8/2011	AG2	2.5273	1.606268419	10.36052062	0.323620961	0.120800382
3/8/2011	R66	2.9464	0.164871711	9.127160604	0.034014697	0
3/10/2011	IN	0	27.38716365	8.423314531	7.483367338	1.263395135
3/10/2011	AI1	0.1143	22.14853457	8.682364879	7.511592461	0.080583433
3/10/2011	AI2	0.3175	27.11990779	8.456740383	7.662395265	0.021687362
3/10/2011	AI3	0.381	23.67297365	8.332587221	7.729329129	0
3/10/2011	AI4	0.4445	27.88249952	8.32960277	7.918195528	0
3/10/2011	B66	0.508	26.45689603	8.477034649	8.105771635	0
3/10/2011	AB1	0.8255	26.04314531	8.529262542	4.743594913	0
3/10/2011	AB2	1.2319	23.08934544	8.696988689	0	0.04103589
3/10/2011	G66	1.7272	23.10416192	9.45295013	0	0.02849124
3/10/2011	AG1	2.0701	22.14270959	9.731100964	0	0.039760163
3/10/2011	AG2	2.5273	5.484759049	9.512937595	0	0.029766967

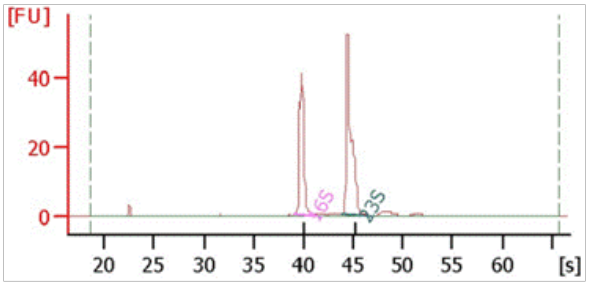
Sample Date	Port	Distance from injection (m)	Toluene (mg/L)	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
3/10/2011	R66	2.9464	0.945954497	9.258363924	0	0.056769859
3/13/2011	IN	0	27.34112308	8.02349176	10.09501295	0.970087053
3/13/2011	AI1	0.1143	25.83475364	8.246201561	9.148492696	0
3/13/2011	AI2	0.3175	21.36380477	8.467261659	8.253043031	0
3/13/2011	AI3	0.381	25.28200293	8.788293713	7.818672345	0.028940545
3/13/2011	AI4	0.4445	25.90539381	8.248181203	7.27530295	0
3/13/2011	B66	0.508	25.67323749	8.362010657	7.117760113	0
3/13/2011	AB1	0.8255	23.70523356	8.321098042	1.692186922	0.087053158
3/13/2011	AB2	1.2319	26.15311492	8.068693601	0	0
3/13/2011	G66	1.7272	23.22435183	7.992477358	0.029956599	0.087284682
3/13/2011	AG1	2.0701	21.7422039	8.880347097	0	0.023847009
3/13/2011	AG2	2.5273	21.36332428	8.791263177	0.022918603	0
3/13/2011	R66	2.9464	9.658622182	8.406222677	0	0
3/15/2011	IN	0	20.7785421	7.550222065	7.364179342	0.8494662
3/15/2011	AI1	0.1143	20.32551708	7.718168181	7.223381381	0.039012522
3/15/2011	AI2	0.3175	24.80037289	7.651989764	7.833718245	0
3/15/2011	AI3	0.381	24.2660266	7.698755846	8.906745242	0
3/15/2011	AI4	0.4445	24.84221507	7.741404159	8.658915346	0
3/15/2011	B66	0.508	21.6681609	7.860231183	9.107111571	0
3/15/2011	AB1	0.8255	23.27319264	8.552016236	2.061161105	0
3/15/2011	AB2	1.2319	25.82416706	8.361716521	0	0
3/15/2011	G66	1.7272	22.50618196	8.520250596	0	0
3/15/2011	AG1	2.0701	22.17314599	9.153798641	0	0
3/15/2011	AG2	2.5273	21.14014764	9.830877379	0	0
3/15/2011	R66	2.9464	19.43642209	9.680872967	0	0
3/18/2011	IN	0	22.60411565	7.771367797	7.401369473	0.964606096
3/18/2011	AI1	0.1143	22.88122946	7.693696017	6.958751759	0.028196878
3/18/2011	AI2	0.3175	23.03096174	7.697885781	7.278065123	0.02774209
3/18/2011	AI3	0.381	23.12243636	7.54221993	7.093108599	0.02615033
3/18/2011	AI4	0.4445	23.93128527	7.394933608	6.845118339	0
3/18/2011	B66	0.508	24.8338104	7.406858322	6.44239321	0
3/18/2011	AB1	0.8255	24.44665214	7.609900735	2.177167972	0
3/18/2011	AB2	1.2319	23.93694484	7.922843883	0	0.040248769
3/18/2011	G66	1.7272	25.09372103	7.775235271	0	0.035246097
3/18/2011	AG1	2.0701	22.45820761	7.93186799	0	0
3/18/2011	AG2	2.5273	22.07084062	8.655085729	0.059752528	0.131888622
3/18/2011	R66	2.9464	21.07592715	8.55517597	0	0
3/21/2011	IN	0	25.31850062	4.206295443	9.669936243	1.453717604
3/21/2011	AI1	0.1143	23.45036022	4.204850291	9.381069152	0
3/21/2011	AI2	0.3175	23.07572625	4.104050942	9.418097106	0
3/21/2011	AI3	0.381	21.12860796	4.058348011	9.262383521	0

Sample Date	Port	Distance from injection (m)	Toluene (mg/L)	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
3/21/2011	AI4	0.4445	24.40041859	3.955561577	8.910985777	0
3/21/2011	B66	0.508	21.40495378	3.925755318	8.831289848	0
3/21/2011	AB1	0.8255	24.30687628	3.822607596	4.302354095	0.073576719
3/21/2011	AB2	1.2319	24.80546138	3.866865375	0	0.045201841
3/21/2011	G66	1.7272	27.31265197	3.888181367	0	0.055100054
3/21/2011	AG1	2.0701	26.15388686	3.878245947	0	0
3/21/2011	AG2	2.5273	23.54974719	3.935148805	0	0.058069518
3/21/2011	R66	2.9464	24.78442608	3.83723976	0	0.193015161
4/4/2011	IN	0	23.22562001	4.301447567	10.52504361	0.968535864
4/4/2011	AI1	0.1143	16.38453494	4.279899812	10.18988288	0
4/4/2011	AI2	0.3175	17.00382589	4.235330951	9.922252679	0.036475025
4/4/2011	AI3	0.381	23.70024353	4.163873439	10.09818091	0.049931831
4/4/2011	AI4	0.4445	17.15100605	4.05687134	9.881883877	0.045682313
4/4/2011	B66	0.508	23.11648929	4.035139416	9.813107401	0.136338687
4/4/2011	AB1	0.8255	23.75874932	3.911746289	5.369548966	0.163606424
4/4/2011	AB2	1.2319	24.42280478	3.787616487	2.970595564	0
4/4/2011	G66	1.7272	21.52230406	3.814505138	6.119112883	0.088177488
4/4/2011	AG1	2.0701	21.39023576	3.73126082	2.637179168	0.049223578
4/4/2011	AG2	2.5273	15.91229812	3.661644996	1.335908298	0
4/4/2011	R66	2.9464	16.21665425	3.308040812	3.409917767	0
4/11/2011	IN	0	26.06173089	3.558012681	6.433019479	25.06032231
4/11/2011	AI1	0.1143	19.65391553	3.828624697	8.124346137	18.71537464
4/11/2011	AI2	0.3175	17.6470981	3.780446159	8.038160713	5.093726124
4/11/2011	AI3	0.381	11.52016567	3.567057694	6.679121208	0
4/11/2011	AI4	0.4445	11.74269246	3.46110183	6.152294126	0.055436115
4/11/2011	B66	0.508	13.8405946	3.428429029	6.165745031	0.279312734
4/11/2011	AB1	0.8255	15.95577869	3.697564307	4.437303841	0.052237878
4/11/2011	AB2	1.2319	14.8479339	3.612651942	4.440292931	0.113715108
4/11/2011	G66	1.7272	11.95346689	3.293307614	3.362975141	0.61903662
4/11/2011	AG1	2.0701	16.87469962	3.529770091	4.029542171	0.052593238
4/11/2011	AG2	2.5273	24.63257884	3.407939306	4.525731082	0.093104245
4/11/2011	R66	2.9464	26.89678236	3.325426638	4.447516565	0.043709245
4/18/2011	IN	0	35.85666702	3.649173076	6.540719829	26.3593236
4/18/2011	AI1	0.1143	31.77280159	3.752607508	6.181127296	22.55919258
4/18/2011	AI2	0.3175	35.76975708	3.713563765	6.291903264	16.81162759
4/18/2011	AI3	0.381	31.46102541	3.712619158	6.114146478	9.718452822
4/18/2011	AI4	0.4445	35.23791171	3.643820304	5.782677301	2.853913657
4/18/2011	B66	0.508	35.63868127	3.716397585	5.75713013	6.449021316
4/18/2011	AB1	0.8255	22.23930814	3.983248975	5.319178626	0.042905169
4/18/2011	AB2	1.2319	16.23580697	3.641773656	2.877598995	0
4/18/2011	G66	1.7272	22.08127359	3.36689311	1.018666609	0

Sample Date	Port	Distance from injection (m)	Toluene (mg/L)	Cl- (ppm)	SO4-- (ppm)	NO3- (ppm)
4/18/2011	AG1	2.0701	21.97172933	3.831167298	2.272839493	0
4/18/2011	AG2	2.5273	16.33429597	3.940584239	3.646804994	0
4/18/2011	R66	2.9464	14.12590828	3.651534592	1.789804747	0.051543794
4/26/2011	IN	0	8.237394316	4.677197165	10.98014731	133.9163213
4/26/2011	AI1	0.1143	9.501595878	4.35999743	9.646553827	130.3814724
4/26/2011	AI2	0.3175	6.370648129	4.173560503	8.568752744	109.2774615
4/26/2011	AI3	0.381	4.861139736	4.067779673	8.262426223	108.2710455
4/26/2011	AI4	0.4445	4.618054195	4.127736419	8.417150383	109.9017595
4/26/2011	B66	0.508	4.944000945	4.185694606	8.527193795	115.4318836
4/26/2011	AB1	0.8255	1.061894811	4.079057252	7.399053705	70.76849593
4/26/2011	AB2	1.2319	1.242981152	3.849223061	5.752304766	43.3339788
4/26/2011	G66	1.7272	0.015336354	3.867352838	10.27325496	71.13563895
4/26/2011	AG1	2.0701	2.385693113	3.809965668	3.754158334	19.38783677
4/26/2011	AG2	2.5273	0.568642383	3.819244688	9.26198722	27.40740741
4/26/2011	R66	2.9464	2.968254005	3.933733521	4.47061119	0.053703058
5/1/2011	IN	0	18.83452731	3.815508044	7.352947838	131.5400946
5/1/2011	AI1	0.1143	18.33794295	3.947668467	8.599805214	126.7696834
5/1/2011	AI2	0.3175	18.92740796	3.571493962	6.144368191	117.3993085
5/1/2011	AI3	0.381	15.58982115	3.530381085	6.302009015	109.2678636
5/1/2011	AI4	0.4445	0	3.508742728	6.456931893	105.0761252
5/1/2011	B66	0.508	0	3.477783234	6.287966297	107.8199685
5/1/2011	AB1	0.8255	0	3.458308713	7.658490181	49.91962878
5/1/2011	AB2	1.2319	0	3.519561907	8.799121198	54.23510858
5/1/2011	G66	1.7272	0	3.258570038	7.531199746	53.7856363
5/1/2011	AG1	2.0701	0	3.431177543	9.974859007	45.79067087
5/1/2011	AG2	2.5273	0	3.182669341	10.30169192	49.99150795
5/1/2011	R66	2.9464	0	2.990753764	9.80000453	48.28066238

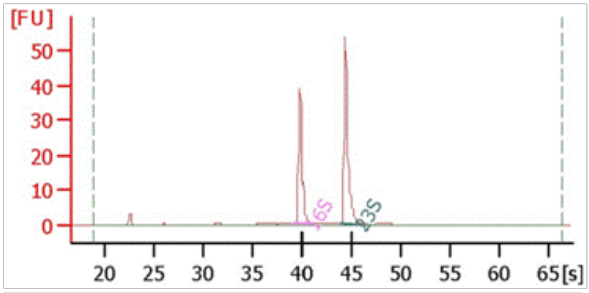
APPENDIX A.4. BIOANALYZER RNA INTEGRITY DATA

Data referred to in Section 4.2.1.



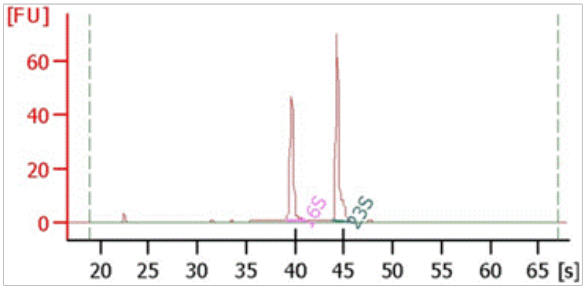
***P. putida* unfrozen**

RNA Concentration: 164 ng/µl
rRNA Ratio [23s / 16s]: 1.6
RNA Integrity Number (RIN): 10.0



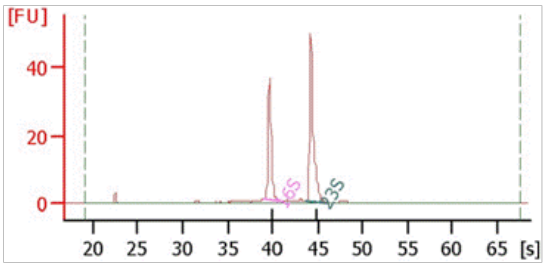
***P. putida* frozen**

RNA Concentration: 140 ng/µl
rRNA Ratio [23s / 16s]: 1.2
RNA Integrity Number (RIN): 10.0



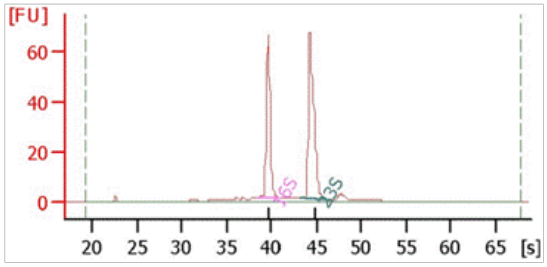
***P. putida* frozen w/ sand**

RNA Concentration: 169 ng/µl
rRNA Ratio [23s / 16s]: 1.3
RNA Integrity Number (RIN): 10.0



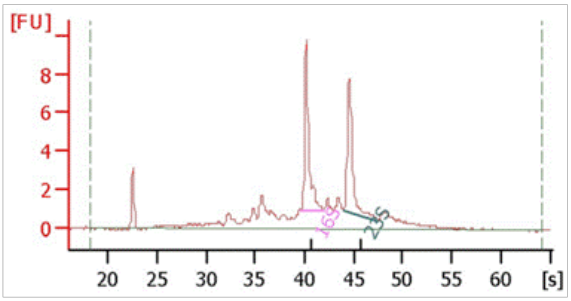
***P. putida* storage sample time 0**

RNA Concentration: 146 ng/µl
rRNA Ratio [23s / 16s]: 1.3
RNA Integrity Number (RIN): 9.9



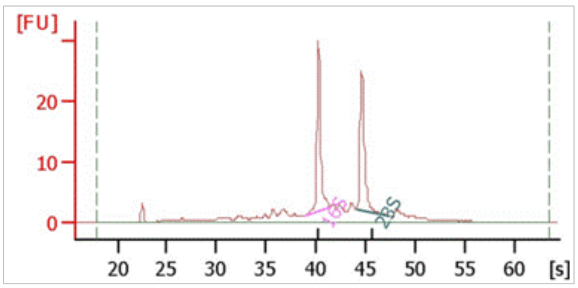
***P. putida* storage sample 1 week**

RNA Concentration: 317 ng/µl
rRNA Ratio [23s / 16s]: 1.4
RNA Integrity Number (RIN): 9.7



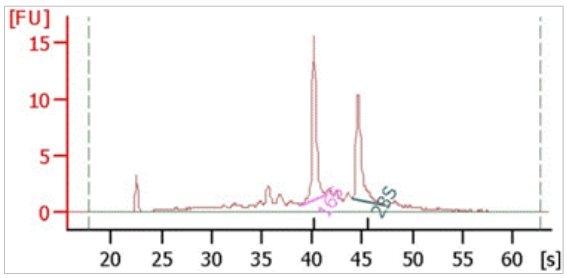
B. subtilis unfrozen

RNA Concentration: 75 ng/µl
rRNA Ratio [23s / 16s]: 0.9
RNA Integrity Number (RIN): 7.3



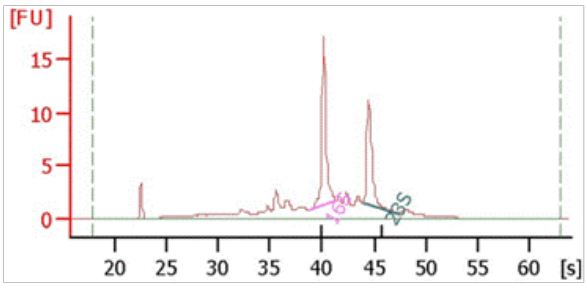
B. subtilis frozen

RNA Concentration: 166 ng/µl
rRNA Ratio [23s / 16s]: 0.9
RNA Integrity Number (RIN): 8.4



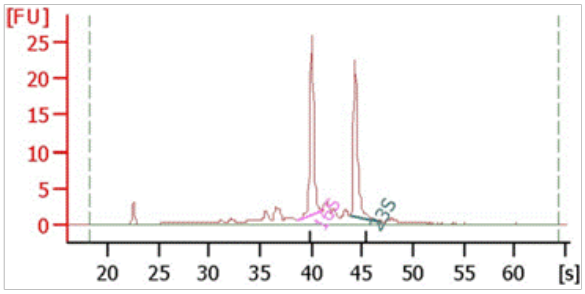
B. subtilis frozen w/ sand

RNA Concentration: 98 ng/µl
rRNA Ratio [23s / 16s]: 0.8
RNA Integrity Number (RIN): 7.8



B. subtilis storage sample time 0

RNA Concentration: 108 ng/µl
rRNA Ratio [23s / 16s]: 0.7
RNA Integrity Number (RIN): 7.7



B. subtilis storage sample 1 week

RNA Concentration: 123 ng/µl
rRNA Ratio [23s / 16s]: 0.9
RNA Integrity Number (RIN): 8.6

APPENDIX B: PUBLICATIONS FROM THIS WORK

Brow, C., R. O'Brien Johnson , R.L. Johnson, & H.S. Simon, 2012, "Comparison of Sediment-Attached and Suspended Microbial Populations by High-resolution Sampling of Sediments and Pore Water Across a Toluene Contaminant Plume in a Well-controlled Model Aquifer", Applied and Environmental Microbiology, In Preparation.

Brow, C.N., R. O'Brien Johnson, H.S. Simon, & R.L. Johnson, 2012, "Integrated mRNA:DNA Ratio Approach for Assessing Localized Activity in Subsurface Contaminant Biodegradation" Environmental Science and Technology, In Preparation.

Brow, C.N., R. O'Brien Johnson, M. Xu, R.L. Johnson, & H.M. Simon, 2010, Effects of Cryogenic Preservation and Storage on the Molecular Characteristics of Microorganisms in Sediments", Environmental Science and Technology, 44(21), 8243-7.

Johnson, R.L., R. O'Brien Johnson, C.N. Brow, & H.S. Simon, 2012. "Cryogenic Core Collection and Preservation of Subsurface Samples for Biomolecular Analyses", Environmental Science and Technology, In Preparation.