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VIA FEDERAL EXPRESS

November 9, 2011
In reply refer to: SHEA-111597

Mr. Mark Malinowski
Project Manager, Brownfields and Environmental Restoration Program
California Environmental Protection Agency
Department of Toxic Substances Control
8800 Cal Center Drive
Sacramento, CA 95826-3200

Subject: Treatability Study Work Plans for the Santa Susana Field Laboratory

Dear Mr. Malinowski:

Please find attached responses to DTSC's conditional approval of the SSFL treatability studies for bedrock and groundwater. Treatability study work plans were originally submitted to DTSC on June 1, 2009. DTSC conditionally approved the treatability study work plans in their letter dated April 29, 2011. Attachments to the responses to DTSC's comments and conditions include the following:

Table 1 – General Outline for SSFL Treatability Study Reports

Table 2 – List of Testing and Sampling Procedures – SSFL Treatability Studies

Figure 1 – Schematic Cross-section B-B' of BVE Well and Monitoring Probes, BVE Field Experiment

Attachment A - Permanganate In Situ Chemical Oxidation (ISCO) Laboratory Treatability Study Work Plan

Attachment B - Characterization of the Microbial Populations from Fractured Rock Cores (Revised Work Plan, with Addendum)

Attachment C – Laboratory Evaluation of Biostimulation to Treat Chlorinated Ethenes in the Chatsworth Formation (Revised Work Plan, with Addendum)

We continue developing the Bedrock Vapor Extraction (BVE) and ISCO Field Experiment work plan addenda, and anticipate delivery of these documents to DTSC before December 15, 2011, respectively. Additionally, we do not believe a work plan addendum is needed for the thermal laboratory experiment as it appears that the

clarifications provided in the response to comments/conditions adequately address those items.

We are requesting DTSC's approval of the following laboratory experiment work plans due to the greater length of time needed to complete them, and because they are separate stand alone projects:

- Work Plan, Rock Core Thermal Testing, Santa Susana Field Laboratory (Appendix D of the Treatability Study Work Plans, MWH, June 2009, with clarifications provided in the attached response to comments/conditions)
- Permanganate ISCO Laboratory Treatability Study Work Plan (attached),
- Characterization of the Microbial Populations from Fractured Rock Cores (Revised Work Plan, with Addendum, attached), and
- Laboratory Evaluation of Biostimulation to Treat Chlorinated Ethenes in the Chatsworth Formation (Revised Work Plan, with Addendum, attached).

We look forward to commencing the studies outlined in the work plans upon receiving your approval. Thanks in advance for your consideration of this matter.

Regards,

Michael O. Bower, P.E.
The Boeing Company
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Enclosures
cc: Roger Paulson, DTSC w/enclosure
Richard Andrachek, MWH without enclosure

DTSC Comments: Overall Conditions	SSFL Response
Supplemental experiments may be needed to further study the currently proposed tests in the CFOU (i.e., a thermal treatment field test to build on the results from the laboratory study.)	Comment noted. It is recognized that the State reserves the right to require further experiments or tests.
Supplemental experiments may be needed to study Surficial OU treatment technologies.	Comment noted.
Data evaluation methods and how the results will be reported are not presented in the TS Work Plan, and need to be developed and submitted in the treatability study-specific work plan addendum.	In general, the results from each treatability study will be used to evaluate its effectiveness, implementability and cost in the Feasibility Study (FS). A typical outline for a treatability study data presentation and evaluation report is presented in the attached **Table 1**. A proposed table of contents for the relevant test report may be presented in specific work plan addenda to tailor the typical outline provided in Table 1 to a particular treatability study.
Testing and sampling procedures and methods need to be presented in the treatability-study-specific work plan addendum. If the testing and sampling procedures exist in an approved site-wide document (Quality Assurance Project Plan, Standard Operating Procedures, etc.), the work plan addendum may reference the document and method.	Testing and sampling procedures and methods will be provided with work plan addenda for the items identified below in the specific comments to each work plan. Some testing and sampling procedures will be submitted by the contractor(s) selected to implement the Bedrock Vapor Extraction (BVE) and In Situ Chemical Oxidation (ISCO) field experiments.
Submit a project schedule for each treatability study.	Additional information regarding activities and estimated durations for each treatability study are provided in the treatability study addenda. Detailed project schedules for the BVE and ISCO field experiments will be generated by the contractor selected to implement the work.
General Comments: Bedrock Vapor Extraction Field Experiment Work Plan	
1. Detailed design of the wellfield and treatment system needs to be fully developed in order to permit and construct the test system(s). Similarly, schedules and methods for sampling and analysis and operations and maintenance need to be developed prior to beginning the test.	Some additional detail on the well field and system designs will be presented in the BVE Work Plan Addendum. The goal is to provide enough information to the prospective contractors so they can provide bids that are comparable. The final detailed well field and treatment system design will be fully developed by the contractor selected to implement the field experiment and will be transmitted to DTSC after contractor selection and engagement. This contractor will also develop operation and maintenance schedules and requirements.
2. Data evaluation methods and how the results will be reported are not presented in the Work plan, and need to be developed and submitted in the work plan addendum.	Outlines of the data collection and data analysis reports are included in the BVE Work Plan Addendum.
• Testing and sampling procedures and methods need to be presented in the work plan or by reference to an existing document created and approved for site-wide use (QAPP, Health & Safety Plan (HASP), Standard operating Procedures (SOP), etc.) Additionally, a project schedule should be submitted.	Some testing and sampling procedures will be submitted with the work plan addendum. A listing of these procedures is provided in **Table 2**. Additional testing and sampling procedures, as well as a project schedule, will be developed by the contractor selected to implement the field experiment and will be transmitted to DTSC after contractor selection and engagement.
Specific Comments: Bedrock Vapor Extraction Field Experiment Work Plan	
2.1 Chatsworth Formation, page2-1 A reference to the data source should be included for the physical properties presented (porosities, organic content). A table presenting ranges of values for the site and layers would be helpful.	Specific information on porosities and organic content is presented in the Draft Site-wide Groundwater Remedial Investigation (RI) Report (MWH, 2009, see specifically figure 6-4 for porosity values) and the Site Conceptual Model (SCM) for the Migration and Fate

	of Contaminants in Groundwater at SSFL (Groundwater Advisory Panel, 2009, see specifically element 2-2 for porosity and element 3-2 for organic carbon). It should be noted that the work plan contained a typographical error regarding the organic carbon content. The value should have been reported as 0.021% instead of 0.21%.
2.3 VOC Release and Transport Mechanisms in the Bedrock Vadose Zone, page 2-2 Regarding the statement: "Currently, the vast majority of VOC mass is present in the rock matrix blocks of the vadose zone, with very little being present in the fracture network." the text should be revised to clarify that difference in masses is related to the difference in volumes as well as concentration.	The following statement is intended to clarify the quoted sentence: "The occurrence of the VOC mass in the rock matrix blocks is attributable to both the fact that the vast majority of groundwater is stored in the rock matrix and diffusion has transferred the dissolved VOC mass from water flowing in the fractures to the water stored in the rock matrix (i.e., matrix diffusion).
2.4 Conditions Controlling Mass Removal/ Transformation, page 2-4: Consider using the term 'semi-pervious" instead of 'low to moderate". Verify and cite data source of bulk intrinsic permeability.	Consideration has been given to modifying the description, however, the SSFL groundwater team believes the "low-to-moderate" description reasonably conveys our understanding. The bulk intrinsic permeability values are presented in the Draft Site-wide Groundwater RI Report (MWH, 2009, see specifically figure 6-8, noting that these are hydraulic conductivity values). Intrinsic permeability values were calculated by converting the hydraulic conductivity values by considering the fluids of interest (i.e., water and air).
2.4 Conditions Controlling Mass Removal/ Transformation, page 2-4: Verify and cite data source of rock matrix air permeability. Use standard descriptor of permeability.	The rock matrix permeability values are presented in the Draft Site-wide Groundwater RI Report (MWH, 2009, see specifically figure 6-7, noting that these are hydraulic conductivity values). Intrinsic permeability was calculated by converting the hydraulic conductivity values and considering the fluid of interest (i.e., water). Intrinsic permeability (k) is related to hydraulic conductivity (K) by the following: $K=\rho*g*k/\mu$, where ρ=fluid density, g=force of gravity and μ=absolute viscosity (see Equation 3-19 in McWhorter & Sunada or Eq 2.28 in Freeze & Cherry and corresponding Tables 2.2 and 2.3).
2.5 Implications of Vadose Zone Pneumatic and Containment on Extraction Field Experiment Design, pages 2-4, 2-5: In the bulleted items there are numerous uses of "will" to describe the untested conditions. Revise the text for clarity, i.e.: "is expected to be".	Comment noted.
4.0 Extraction Well and Monitoring System Design, page 4-1: It would improve the work plan to present another section along Array 2 and to describe the expected conditions at test area well locations south of the Bowl Structure.	**Figure 1** is attached and presents the section (identified as B-B') along Array 2. A description of the conditions south of the Bowl Structure was not provided in the work plan because they are unknown. Faults and fault features display varied influence on the flow of fluids at SSFL. Because there have been no tests to assess fluid movement across the Bowl Structure at this location, we believe it premature to provide a description.
4.0 Extraction Well and Monitoring System Design, page 4-2: The test should be designed to collect sufficient data to evaluate the influence of the bedrock vapor extraction test on groundwater chemistry below the test area. This could be accomplished by extending the deep well into saturated bedrock or by installing a dedicated monitoring well.	There is respectful disagreement on this recommendation. We do not believe that groundwater chemistry data are required to achieve the objectives of the work plan that have been identified. However, the work plan addendum will incorporate groundwater sampling of corehole C-4 both before and after the bedrock vapor extraction test(s).
4.0 Extraction Well and Monitoring System Design, page 4-3: Note that all surface facilities have been removed from the site, including the asphalt cover.	It is so noted.

4.1 Discussion of Conceptual Well Layout: See comments on GW well and Bowl Structure (Array 2) above.	Comment noted.
4.2 Operations, page 4-5: Verify that operations and sampling will be further developed and presented in an implementation plan.	Additional descriptions on operations and sampling will be provided by the contractor selected to conduct the field experiment.
General Comments: In situ Chemical Oxidation Field Experiment Work Plan	
1. Detailed design of the well field and test equipment will need to be fully developed in order to permit and construct the test system(s). Similarly, schedules and methods for sampling and analysis and operations and maintenance need to be developed prior to beginning the test.	Some additional detail on the well field design test equipment will be presented in the ISCO Work Plan Addendum. The goal is to provide enough information to the prospective contractors so they can provide bids that are comparable. The final detailed well field design and test equipment specification will be fully developed by the contractor selected to implement the field experiment and will be transmitted to DTSC after contractor selection and engagement. This contractor will also develop operations and maintenance schedules and requirements.
2. Testing and sampling procedures and methods need to be presented in the work plan or by reference to an existing document created and approved for site-wide use (QAPP, HASP, SOP, etc.) Additionally, a project schedule should be submitted.	Some testing and sampling procedures will be submitted with the work plan addendum. A listing of these procedures is provided in **Table 2**. Additional testing and sampling procedures, as well as a project schedule, will be developed by the contractor selected to implement the field experiment and will be transmitted to DTSC after contractor selection and engagement.
Specific Comments: In situ Chemical Oxidation Field Experiment Work Plan	
1.2 Work Plan Objective, page 1-2: The description of the six performance criteria is difficult to follow. Consider using bullets to clarify the criteria.	We do not plan to re-submit the work plan. We plan on submitting work plan addenda that address the technical issues and provide additional detail where requested.
2.0 ISCO Field Experiment Location, page 2-1: Corehole C-1 is going to be utilized as an interim remedial measure extraction well. Discuss how this will affect the test and describe the coordination between the two activities.	It is proposed that groundwater extraction from the northeast area (corehole C-1, and wells RD-72 and RD-84) be deferred until the ISCO field experiment is completed.
2.4 Injection location, page 2-4: Present the rationale for selecting a relatively shallow well for injection and the expected movement of injected fluid.	Primary rationale are listed in the work plan and include the following: RD-35A contains some of the highest concentrations and equivalent TCE mass measured at SSFL, and is centrally located within an extensive array of monitoring intervals that will support monitoring during the field experiment. Additionally, it is believed that injecting in the upper portions of the aquifer will allow the permanganate to follow the natural flow field (post injection). Very local to the injection well the flow field is unknown. However, site contaminant data indicate flow northeastward, parallel to strike of the Shear Zone.
3.2 TCE-Permanganate Oxidation Reactions, page 3-3: Address the mass(es) of reactants here and in Section 3.5.	The following sentence is provided in response to this comment: "Using these stoichiometric equations and considering the molar weights of KMnO4 (316) and TCE (130), it theoretically requires 2.4 grams of $KMnO_4$ to oxidize a gram of TCE."
3.4 Chemical Oxidation Bench Test, page 3-5: A work plan is described for this activity and needs to be submitted.	The work plan is attached to this response to comments (**Attachment A**).
3.5 Injection Parameters, page 3-6: Discuss the dose rate in terms of the mass of TCE. The discussion should include: the mass that would be oxidized with a 1:1 stoichiometric ratio; the planned dose ratio; the target mass of TCE; and the expected performance based on similar projects.	Dosage calculations can be made prior to the field test to help assess the scale of the chemical injection operation relative to the possible extent of contamination. As noted in section 3.2 of the ISCO Field Test Work Plan, it requires 2.4 grams of $KMnO_4$ to oxidize a gram of TCE. Under theoretically ideal conditions with negligible NOD, the planned 9,000 lb of $KMnO_4$ injection called out in the Work Plan could oxidize 3,700 lb (about 1,700 kg) of TCE. If a 10 mg/L TCE concentration in groundwater (consistent

	with some of the recent data for well RD-35A shown on Figure 2-6 in the Work Plan) were uniformly distributed in a cylindrical volume of homogeneous sand 50 feet thick with 13 percent porosity (comparable to that of Chatsworth sandstone), it would cover an area more than 300 meters in diameter. However, the actual conditions (concentrations, flow paths, porosity, etc.) at SSFL are heterogeneous and non-ideal, and the NOD has not yet been measured. Therefore, adjustments are expected to be needed during the testing, and the results may vary significantly.
Figure 2-2: The font of the posted well locations is quite small as presented, and hard to read. Consider revising for clarity.	Comment noted. The figure could be revised by increasing the size of the font by a factor of 2.
Figure 2-3: Add alluvium wells, and alluvium/weathered bedrock and weathered bedrock/bedrock contacts to the sections if the vertical scale allows for the elements to be clearly featured.	The scale of the figure is such that these contacts cannot be displayed with reasonable resolution. Additionally, collection of water level measurements over the history of the facility has not shown perched groundwater to occur at either of the alluvium/weathered bedrock or weathered/unweathered bedrock contacts. Furthermore, there have been no mappable finer-grained units identified between ground surface and groundwater in this area.
General Comments: Characterization of the Microbial Populations from Fractured Rock Cores	
1. Data evaluation methods and how the results will be reported are not presented in the Work plan, and need to be developed and submitted in the work plan addendum.	Two new sections entitled "Data Analysis" and "Reporting and Publication" were added to the updated addendum of the work proposal, which is provided in **Attachment B**.
2. The text varies between past and future tense, making it difficult for the reader to clearly understand what work has been done versus what will be performed. i.e.: has the DNA extraction already been performed?	The reason for the varying between past and future tenses is because DNA from C12, C13 and C14 have already been extracted and are currently stored at -80 degrees awaiting for analyses. C15 samples have been crushed, placed in sterile plastic bags and stored at -20 degrees Celsius waiting for DNA to be extracted and further analyses. A table was added to the work proposal clarifying what has been done and when and what will be done in 2011.
Specific Comments: Characterization of the Microbial Populations from Fractured Rock Cores	
Introduction, page 2: The basis for the statement that "complete degradation is likely occurring in many areas and that it may be an important influence on TCE attenuation" is not fully supported in the Work Plan.	The cited references (Pierce 2005; Hurley 2003; Darlington 2008) all indicate that complete dechlorination of TCE is ongoing at the site. Later work by Zimmerman (2010) demonstrated the presence of final product of reductive dechlorination, i.e. ethene, throughout the site. This latest reference is included in the updated addendum of the work proposal.
Rock Core Microbial Sample Collection Procedure., pages 2-4: Describe how have core samples been preserved. Clarify whether testing is to be conducted on DNA that was previously extracted or if DNA extraction will be part of the proposed test.	The preservation method is indicated in the standard operation procedure presented in the section entitled "Rock Core Microbial Sample Collection Procedures". Clarification of tests on DNA already extracted is added to the updated addendum of the work proposal as well as it has been clarified which samples have not had their DNA extracted.
General Comments: Biostimulation to Treat Chlorinated Ethenes in the Chatsworth Formation	
1. Data evaluation methods and how the results will be reported are not presented in the work plan, and need to be developed and submitted in the work plan addendum.	A plan for data evaluation and reporting is presented in the biostimulation work plan addendum, which is provided in **Attachment C**.
Specific Comments: Biostimulation to Treat Chlorinated Ethenes in the Chatsworth Formation	

1.0 Background, page 1: Verify the redox potential of reductive dechlorination. Revise and expand text if redox levels vary with succeeding dechlorinating steps.	This is addressed in the treatability study addenda in two ways: 1) A reference to the SSFL groundwater redox conditions (Table 8-7 of Draft SSFL Groundwater RI Report), which shows that the mean Eh value collected from well samples across the site is 387 mV, ranging from 51 to 629 mV.; and 2) The following reference is added in support of assessing complete reductive dechlorination and the requirement for increasingly lower Eh levels: Parsons Corporation, 2004. Principles and Practices, Enhanced Anaerobic Bioremediation. Quoting from the relevant part of their report: "More reduced conditions are required as the oxidation state of the compound is lowered (i.e., from PCE and TCE to DCE and VC). For example, anaerobic dechlorination of PCE and TCE to DCE may readily occur under iron-reducing conditions, but this redox condition may not be optimal for further degradation of DCE to VC and ethene. As another example, dechlorinating microorganisms may preferentially degrade PCE and TCE to the exclusion of DCE because they gain more energy from dechlorination of the more highly chlorinated CAHs. Thus, dechlorination of DCE may not proceed until PCE and TCE are depleted. As a result, it is common for incomplete dechlorination to occur due when insufficient substrate loading leads to insufficiently reducing conditions." Note that nearly all such information comes from granular aquifer environments and little is known about fractured sedimentary rock environments. This is particularly true when interpreting parameter measurements collected from monitoring wells, which yield samples of water from the most transmissive fracture(s). There exists a range of conditions along any monitored interval such that hetereogeneity can provide ample opportunity for strongly reducing conditions at a local scale.
3.1 Microcosm Evaluation of Biostimulation and Product Formation, page 4: Explain the concentration of the [^{14}C] TCE dosage (µCi/bottle).	This topic is addressed in the addendum to this work plan (**Attachment C**). In summary, addition of 0.50 µCi/ bottle is sufficient to allow for tracking of daughter products. Lower amounts make this process increasingly difficult. Higher amounts are unnecessary for the objective and begin to raise concerns with disposal of the microcosm contents. Because the labeled material has a high specific activity (i.e., approximately 1-5 mCi/mmole), adding 0.50 µCi/bottle will not appreciably increase the total concentration of TCE or cDCE in the microcosms.
General Comments: Work Plan Rock Core Thermal Testing	
1. Include a discussion of steam and/or steam/air heating and cycling technologies, and whether or not those technologies are applicable to the proposed testing.	All thermal technologies may be applicable in the fractured sandstones and shales of the Chatsworth formation. Thermal technologies (ERH, RFH, conductive, and steam injection) function by heating the subsurface causing the chemicals within the formation to boil and be removed via vapor extraction. For steam and/or steam/air heating with cycling heat, steam/air is injected into the subsurface and will move preferentially through the fractures and then heat the rock matrix via conduction. However, the bench testing is not being conducted to validate a thermal remediation method, but rather to show the rate of diffusion of VOCs from the secondary porosity of the rock matrix after the fractures within the formation have been heated. This information can be used to evaluate the applicability of any of the thermal remedial methods to enhance recovery of

	VOC from the sandstone bedrock.
Specific Comments: Work Plan Rock Core Thermal Testing	
2.1 Sample Preparation and Handling, page 3: The core collection procedure does not agree with the TSWP text section 5.0. The core will come from corehole C-15. Describe the preservation procedures used on C-15 core.	Our technical team has revisited the core selection process and believe that fresh core should be selected from upcoming source zone characterization work that is described in the Groundwater Data Gap Sampling and Analysis Plan (MWH, 2010) consistent with the conditions described in DTSC's approval letter (DTSC, 2011).
2.1 Sample Preparation and Handling, page 3: Describe how complete matrix diffusion would be accomplished if the core is spiked.	Long periods of time (years) are required for VOC saturation *in situ* because of the size of the rock matrix blocks. However, diffusion across 4-inch diameter cores can be accomplished within a period of weeks. If spiking the core samples in storage is required, they will be immersed in heated TCE solutions for durations calculated to result in equilibrium concentrations across the core diameter to within 10 percent of the solution concentration. Successful spiking will be confirmed by including extra cores that can be sacrificed for confirmatory analysis after the spiking operation.
2.2 Experimental Setup, page 5: It is unclear if the core testing is targeting saturated or unsaturated core, and why air was selected instead of a prepared gas with the expected soil gas chemistry. Describe how gas chemistry and flow rate was determined.	The purpose of the bench test is to understand the diffusion of VOCs from the rock matrix during heating relative to ambient conditions. Air was selected for simplicity of the experiments to collect the mass that diffuses from the core during testing. As the heating is conducted, the vapors will be removed from the testing apparatus and analyzed for VOC concentrations. The flow rate of 0.1 milliliters per minute (ml/min) was chosen to ensure that the vapors would be pulled through the testing apparatus, but at a slow rate to minimize the amount of stripping that could occur.
2.2 Experimental Setup, page 6: The upper core thermocouple (item 3) is mislabeled on Figure 2.	Comment noted and figure has been revised.

References:

Cherry, J.A., D.B. McWhorter and B.L. Parker (SSFL Groundwater Advisory Panel), 2009. Site Conceptual Model for the Migration and Fate of Contaminants in Groundwater at the Santa Susana Field Laboratory, Simi California. Volumes I through IV, December.

Darlington, R. L. 2008. Laboratory Evaluation of Chlorinated Ethene Transformation Processes in Fractured Sandstone. Clemson University, Clemson, SC, USA.

Freeze, R. Allan and John A. Cherry, 1979. Groundwater.

Hurley, J. C. 2003. Rock Core Investigation of DNAPL Penetration and Persistence in Fractured Sandstone. Waterloo, Ontario, Canada, University of Waterloo.
MWH, 2009. Draft Site-Wide Groundwater Remedial Investigation Report, Santa Susana Field Laboratory, Ventura County, CA. Volumes I through VII, December.

McWhorter, David B. and Daniel K. Sunada, 1977. Ground-water Hydrology and Hydraulics. 6th Printing (1993).

Pierce, A. A. 2005. Isotopic and Hydrogeochemical Investigation of Major Ion Origin and Trichloroethene Degradation in Fractured Sandstone. University of Waterloo, Waterloo, Ontario, Canada.

Zimmerman, L. K. 2010. New Opportunities for Groundwater Hydrochemistry and Degradation Investigations Provided by the Snap Sampler®. University of Guelph, Guelph, Ontario, Canada.

Attachments:

Table 1 – General Outline for SSFL Treatability Study Reports
Table 2 – List of Testing and Sampling Procedures – SSFL Treatability Studies
Figure 1 – Schematic Cross-section B-B' of BVE Well and Monitoring Probes, BVE Field Experiment
Attachment A - Permanganate ISCO Laboratory Treatability Study Work Plan
Attachment B - Characterization of the Microbial Populations from Fractured Rock Cores (Revised Work Plan, with Addendum)
Attachment C – Laboratory Evaluation of Biostimulation to Treat Chlorinated Ethenes in the Chatsworth Formation (Revised Work Plan, with Addendum)

Table 1 – General Outline for SSFL Treatability Study Reports

1. Executive Summary (Abstract)
2. Introduction and Background
   2.1. Statement of Objectives
3. Test Facility Setup or Installation Description
4. Test Procedure Chronology
   4.1. Deviations from Plan
5. Data Summary Tables and Charts
   (Detailed comprehensive data tables included in appendices)
6. Data Quality Assessment
   (May be included as an appendix)
7. Performance Assessment
   7.1. Effectiveness in Achieving Objectives
   7.2. Implementability Considerations
   7.3. Cost Considerations
      7.3.1. Energy Use, Treatment Duration, and Facility Size
8. Conclusions and Recommendations
9. Appendices
   9.1. Data Tables
   9.2. Laboratory Data Validation Summaries
   9.3. Photos & Field Reports

The data tables included in the appendices for each report will be sufficiently detailed to list each analytical result (including "non-detect") for each laboratory sample and each individual field test reading, along with sampling date, time, and location. The data summary tables presented in the body of the report will select (e.g., by omitting pH and temperature) and consolidate (e.g., by listing statistical characteristics) the data to facilitate evaluation. Standard statistical measures will be used to judge the significance of correlated data. Workbook (e.g., Excel) calculations will be prepared to support estimates of energy use, treatment duration, facility size, and other cost drivers.

Table 2 – List of Testing and Sampling Procedures – SSFL Treatability Studies

A. Bedrock Vapor Extraction Field Experiment

Procedures for Drilling and Coring Bedrock Vapor Extraction Well

Procedures for Management of Investigation Derived Waste

Procedures for Drilling and Coring Bedrock Vapor Monitoring Wells

Procedures for Borehole Geophysical Logging

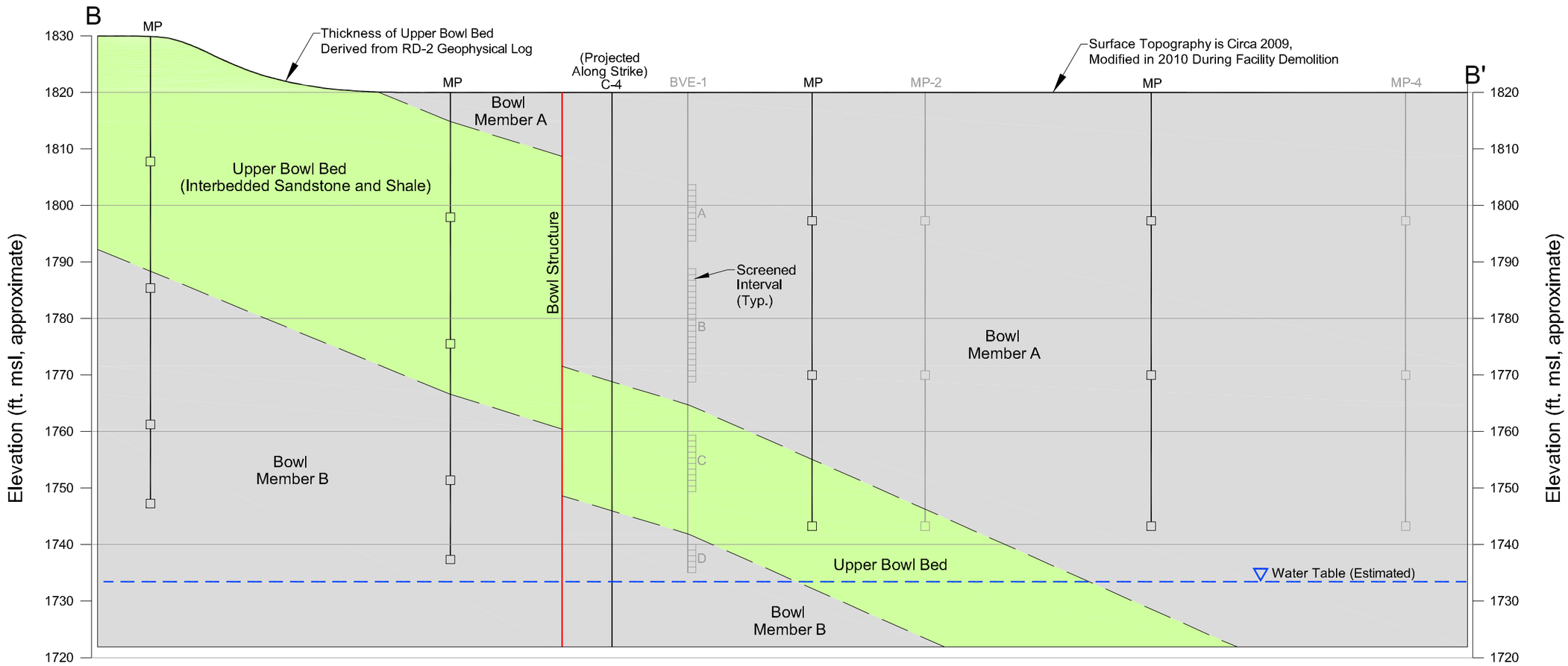
B. In Situ Chemical Oxidation Field Experiment

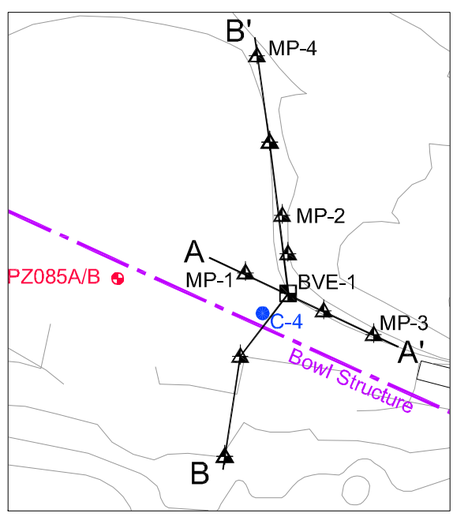
Procedures for Drilling and Coring Wells and Coreholes

Procedures for Management of Investigation Derived Waste

Procedures for Borehole Geophysical Logging

Procedures for Collecting and Analyzing Rock Core Samples for Volatile Organic Compound Analysis





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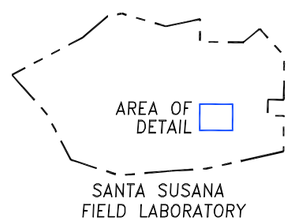

SANTA SUSANA FIELD LABORATORY

MWH

SANTA SUSANA FIELD LABORATORY
VENTURA COUNTY, CALIFORNIA
JUNE 2011

SCHEMATIC CROSS-SECTION B-B' OF BVE WELL AND MONITORING PROBES

FIGURE 1

FILE No. CAD MLUEBKE\BOEING SANTA SUSANA\WORKPLAN 4 09\BVE X SECTION 6 11

Attachment A

Permanganate ISCO Laboratory Treatability Study Work Plan

Permanganate ISCO Treatability Study Work Plan
Santa Susana Field Laboratory, Ventura County, California – USA

Prepared by: ***Dr. Tom Al[1], Amanda Pierce, M.Sc.[2], Steven Chapman[2], M.Sc. and Dr. Beth Parker[2]***
[1] **Department of Geology, University of New Brunswick**
[2] **School of Engineering, University of Guelph**

Prepared for: ***The Boeing Company, NASA and DOE***
Santa Susana Field Laboratory – Treatability Study Plan – Addendum

Date: December 8, 2011

INTRODUCTION

The purpose of this document is to outline the goals, objectives and to provide an overview of proposed methods for laboratory studies involving *Insitu* Chemical Oxidation (ISCO) treatment of TCE-contaminated porous sandstone bedrock. These laboratory studies are designed to complement the proposed field chemical oxidation trial at the SSFL site.

In the field, the ISCO treatment method involves injection of aqueous permanganate solutions into contaminated bedrock through boreholes. The permanganate is expected to spread through the sandstone, initially by advective transport in permeable fractures, followed by diffusive transport into the pore space of the matrix blocks adjacent to the fractures (Figure 1).

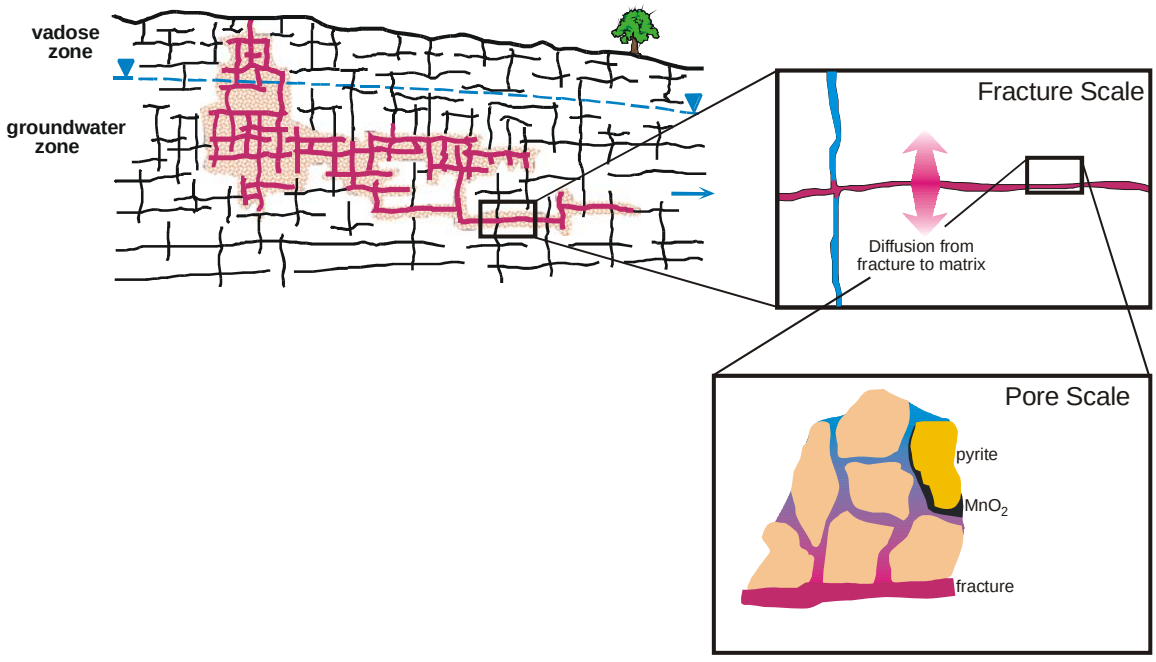

Figure 1. Conceptual model showing advective and diffusive spreading of permanganate in **f**ractured porous sandstone.

Permanganate is a strong oxidant, and in contact with aqueous and sorbed chlorinated ethenes such as trichloroethylene (TCE), it is well known that permanganate will oxidatively degrade the TCE to CO_2 and Cl^- (e.g. Schnarr et al., 1998):

$$2MnO_4^- + C_2HCl_3 \rightarrow 2CO_2 + 2MnO_{2(s)} + H^+ + 3Cl^-$$

However, in the contaminated bedrock system, permanganate will also contact naturally occurring minerals and solid organic carbon. Reactive minerals present in the Chatsworth Formation sandstone include biotite, chlorite, pyrite, magnetite and ilmenite (Loomer and Al, 2009). Reactions with reactive minerals and solid organic carbon cause additional consumption of permanganate, as illustrated, for example, by the following reactions of permanganate with pyrite and organic carbon:

$$FeS_{2(s)} + 5MnO_4^- + H_2O + H^+ \rightarrow Fe(OH)_{3(s)} + 5MnO_{2(s)} + 2SO_4^{2-}$$

$$3CH_2O + 4MnO_4^- + H^+ \rightarrow 3HCO_3^- + 4MnO_{2(s)} + 2H_2O$$

Such reactions consume some fraction of the total oxidant supplied (referred to as natural oxidant demand or NOD). A large NOD can result in significant depletion of the injected oxidant, thus limiting the amount of oxidant available for reaction with the targeted contaminants, and diminish the efficiency of treatment. In addition, production of precipitates (e.g. manganese oxides, iron hydroxides) has potential to clog up fractures and decrease porosity within the rock matrix (which leads to reduced effective diffusion coefficient), which can also have detrimental effects on ISCO treatment.

OBJECTIVES

The objective of the proposed treatability investigations include:

1) measurement of diffusion properties of representative SSFL sandstone samples (porosity and diffusion coefficient) before and after treatment by permanganate;
2) identification of mineral-permanganate reactions that contribute to natural oxidant demand;
3) measurement of permanganate consumption by reaction with naturally occurring minerals and organic carbon; and
4) assess the potential for detrimental effects of such reactions (e.g. fracture clogging, reduced matrix porosity, etc.) on treatment efficiency.

LABORATORY METHODS

Laboratory tests will be conducted via different test methods and procedures ranging from:

1) batch tests on crushed rock samples;
2) 1-D diffusion tests on intact core samples;
3) 1-D diffusion-reaction tests on inteact core samples; and

4) mineralogical investigations to identify important MnO_4^- consuming reactions, and the extent of pore clogging by Mn oxides.

Measurement of natural oxidant demand is commonly conducted by crushing and disaggregating rock samples, then using batch reaction techniques to measure permanganate consumption normalized to the rock mass. However, this approach artificially enhances the amount of mineral surface area available to react with permanganate compared to the surface area exposed to the permanganate-containing pore fluid within intact rocks. The batch reaction technique is therefore expected to overestimate the natural oxidant demand. Batch tests also do not provide any insight into potential effects of precipitation reactions that may cause clogging of fractures and reduced porosity within the rock matrix (with commensurate reductions in diffusion coefficient). Therefore, in order to avoid such artifacts, the main thrust of the proposed laboratory investigations involves measurements performed on intact rock samples. However, measurements of NOD via batch tests will be conducted to provide a benchmark for comparison to measurements made on intact samples.

Batch Tests of Natural Oxidant Demand (NOD)

A series of batch tests will be conducted to evaluate the natural oxidant demand (NOD) of SSFL sandstone. Representative samples from rock cores will be crushed and pulverized, and then placed in vials with different initial permanganate concentrations. Crushed rock mass to solution volume ratios will be selected to provide measurable changes in permanganate concentrations. The samples will be kept well-mixed using a sample rotator, and periodic measurements of MnO_4^- concentrations made using a HACH DR/2010 spectrophotometer (or similar equipment) at selected time intervals (e.g. at 1 day, 3 days, 1 week, 2 weeks, 3 weeks) until MnO_4^- concentrations stabilize. NOD can then be estimated based on the mass of rock sample and amount of MnO_4^- consumed. As discussed above, such tests are expected to overestimate the NOD, such that the results will provide an upper bound on the oxidant loading required to overcome the NOD in the field.

Quantification of the various components contributing to the overall NOD will be assessed via before / after measurements on the crushed rock samples (e.g. decline in organic carbon content, mineralogical changes, etc.) and in the solution (e.g. sulfate produced by pyrite oxidation).

Permanganate Diffusion Experiments – 1D

Two types of 1-D diffusion experiments are envisioned for intact sandstone samples: experiments utilizing inward diffusion of permanganate and a conservative tracer with a sufficient sample length and/or short enough duration to provide essentially semi-infinite conditions; and experiments utilizing through-diffusion conditions where shorter length samples are used such that breakthrough occurs and is monitored at the effluent end of the sample (e.g. see Cavé et al, 2009; attached).

For the former, small diameter samples (~11 mm diameter) can be utilized, such that multiple samples can be sub-cored from the same depth interval from a larger diameter core sample, and

test conditions can be varied between these 'replicate' samples (e.g. use different MnO_4^- concentrations and/or different time periods, etc. At the end of the test period and after non-destructive testing (e.g. via radiation transmission methods, described below) the samples can be split longitudinally for visual inspection and thin-sections prepared for optical microscopy and scanning electron microscopy (SEM) imaging to assess MnO_2 distribution, porosity changes, etc.

For the latter involving through-diffusion testing, samples would be disks cut from existing cores (e.g. 63.5 mm diameter HQ-cores) with lengths of 10 to 20 mm. Differences in diffusive transport between the conservative tracer and MnO_4^- will provide insight into the degree of attenuation due to NOD reactions within the intact rock samples.

Porosity and Diffusion Measurements on Intact Samples

The porosity distribution and diffusion coefficient of sandstone samples will be measured using methods described by Cavé et al (2009). The measurements will be conducted on small subsamples cut from cores or rock slabs prior to the oxidant diffusion and injection experiments, and again following the oxidant injection in order to provide quantitative information on the magnitude of change in the diffusion coefficient due to MnO_2 precipitation in the pore spaces.

MnO_2 Distribution in the Rock Matrix

The reaction of permanganate in the rock matrix with natural minerals, organic carbon, and TCE is known to produce insoluble Mn(III/IV) oxide solids. The spatial distribution of these secondary solid products will provide an indication of the extent of penetration of the oxidant into the matrix. The distribution of the secondary oxides in the rock matrix will be monitored periodically throughout the 1-D (semi-infinite case) diffusion experiments using radiation transmission methods that were recently developed at the University of New Brunswick Cavé et al (2009).

Mineral – Oxidant Reactions

The mineralogy of the rock samples used in the oxidant injection experiments will be determined prior to the injections using powder x-ray diffraction (XRD), optical microscopy and scanning electron microscopy (SEM) in a manner similar to that described by Loomer and Al (2009). This will establish an understanding of the initial, or baseline conditions. Mineralogical investigations will also follow the permanganate diffusion experiments in order to identify specific mineral-water reaction processes that contribute significantly to the consumption of permanganate. The post injection mineralogical investigations will involve XRD, SEM and transmission electron microscopy.

PROJECT SCHEDULE

The laboratory studies are proposed for a one year period, ideally beginning in January 2012.

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Attachment B

Characterization of the Microbial Populations from Fractured Rock Cores (Revised Work Plan, with Addendum)

Characterization of the Microbial Populations from Fractured Rock Cores
Santa Susana Field Laboratory, Ventura County, California – USA

Prepared by: ***Gláucia Lima, Ph.D., Amanda Pierce, M.Sc., Beth Parker, Ph.D.***

Prepared for: ***The Boeing Company, NASA and DOE***
Santa Susana Field Laboratory – Treatability Study Plan (Pages 1-8) and Addendum (Pages 9-14)

Date: ***May 29, 2009 – Addendum at the End: July 15, 2011***

INTRODUCTION

The purpose of this document is to outline the goals, objectives and to describe the materials and methods that are being applied for rock core crushing, DNA extraction and molecular biology analyses on rock core samples obtained from boreholes C12, C13, C14 and C15 from SSFL, California. This will provide information on the indigenous microbial community in a level of detail that has yet to be found in the scientific literature. This has relevance to the assessment of cleanup technology options, particularly in-situ microbial technologies, for the SSFL site.

There is abundant evidence from extensive groundwater and rock core sampling that TCE degradation occurs in the Chatsworth Formation. Historical aqueous concentration data from monitoring wells indicate the presence of *cis*-DCE and minor *trans*-DCE, 1,1-DCE, and some vinyl chloride (VC) and ethene in the groundwater (Hurley 2003; Pierce 2005). The main TCE degradation product is *cis*-DCE, which was detected in 42 of 122 wells sampled at the site. CSIA analyses (Compound Specific Isotope Analysis) show that the existence of *cis*-DCE is attributable to microbially mediated processes (Pierce 2005). Redox conditions indicated by measurements on groundwater samples by Pierce (2005) show predominantly Fe(III)-reducing conditions with local occurrences of NO_3^{2-}-, Mn(IV)-, SO_4^{2-}-reduction, and methanogenesis. Rock core samples have also been analyzed showing the existence of TCE daughter products within the rock matrix (Hurley 2003; Hurley *et al.* 2007). The results of Hurley (2003) and Pierce (2005) suggest that microbial reductive dechlorination is a major degradation pathway in groundwater samples. Lab microcosm studies by Darlington et al. (2008) support this conclusion of microbial production of *cis*-DCE but, in contrast to the field study of Pierce (2005), no apparent complete dechlorination due to microbial processes was indicated. Both field and microcosm studies suggest that abiotic processes cause some complete dechlorination and this may account for the minimal occurrences of acetylene measured in the field samples.

The lab microcosm studies by Darlington et al. (2008) were performed as batch tests using crushed rock samples and water from wells, both obtained from SSFL. These experiments were set up as live and autoclaved microcosms. They used samples from the microcosms actively degrading TCE and demonstrated that a *Pseudomonas* sp. was possibly the microorganism responsible for TCE to *cis*-DCE pathway. Darlington et al. (2008) suggested that iron-bearing minerals in the Chatsworth Formation could be responsible for abiotic degradation of TCE and *cis*-DCE, and they estimated the extent of this transformation to be limited to 25%.

The long term sampling of monitoring wells at the site and other evidence, such as lack of TCE or degradation products at seeps, suggests that complete degradation of TCE is likely occurring in many areas and that this may be an important influence on TCE attenuation. The possibilities that biotic

pathways, in addition to abiotic pathways are causing complete dechlorination may be important and the rock core microbial studies described here are intended to elucidate the microbial community so that such possibilities can be further considered. Also, by elucidating the natural microbial community, the effect of enhancement of degradation through remedial amendments can be better assessed.

OBJECTIVES

The objective of the present work is the characterization of the indigenous microbial communities through molecular biology tools (MBTs), utilizing the DFN approach in order to determine the spatial distribution of microorganisms in fractures and/or in the matrix pore spaces and their role in contaminant attenuation at the Santa Susana Field Laboratory (SSFL), Ventura County, California. Investigations regarding community structures will consider the presence of dechlorinating microorganisms (i.e. *Dehalococcoides* strains and others), the microbial ecology of the communities' samples and enumeration of important players through the use of molecular biology tools.

Specifically, the following questions should be addressed:

1) Is it likely, based on the identified microbial community that TCE degradation in the Chatsworth Formation can include some complete dechlorination by microbial processes?
2) Do the microbes occur only close to fractured or are they distributed throughout the rock matrix?
3) If microbes exist in the rock matrix, what are the community ecologies and how do they vary in distance away from fractures and between lithologies?
4) Is there a correlation or pattern of microbial population community and density, pore sizes, mineralogy (including organic carbon content) and contaminant concentration?

ROCK CORE SAMPLING

Rock samples were obtained at various depths ranging from 50 to 1400 feet below ground surface (ft bgs). Sample frequency was determined according to expected contaminant concentrations and observed changes in lithology and fracture characteristics visually logged in the field. For the rock core procedure (Parker et al., 2008), boreholes were cored using an air rotary drilling technique using an HQ3 coring system, producing a 2.4-in diameter core and a nominal 3.8-in diameter borehole. The core barrels were 5 ft long. The triple tube coring systems utilize a stainless steel core barrel, an inner core barrel, and an outer core barrel all of which minimize contact of the drilling fluid or borehole water with the core. Once the inner core tube is brought to the surface, the stainless steel core sleeve is extruded using water pressure applied to one end of the core sleeve sealed with a plug. The stainless steel sleeve is split along its length to allow easy access to the core for VOC and microbial sampling, physical property sampling, and geologic logging for both structural and lithologic features.

After the rock core is retrieved and extracted from the inner core barrel, it is placed on an aluminum foil-lined PVC tray. The lithology and fractures are then logged by a geologist and the positions where the samples will be taken are determined.

Rock Core Microbial Sample Collection Procedures

C-12, C-13 and C-14 Rock Core Microbial Sample Collection Procedure (Fall 2008)

1) rock core VOC samples were chosen from the core
    a) samples adjacent to the fracture plane, at varying distances away from a fracture plane, into the unfractured matrix are selected
2) a hammer and **CLEAN** chisel were used to break each sample, a puck 2-3 inches in length, out of the core
    a) the chisel was rinsed with purge and trap grade methanol and then wiped dry with a fresh kim wipe between each break
3) selected rock core VOC samples/pucks were then chosen to split for microbial analyses
    a) the selected samples/pucks were split along their vertical axis using a hammer and CLEAN chisel
    b) the chisel was rinsed with purge and trap grade methanol and then wiped dry with a fresh kim wipe between each break
4) once split, each rock core microbial sample was wrapped in packaging used to prevent exposure to oxygen
    a) each split sample was removed from the core using a **NEW** pair of nitrile gloves and a **NEW** (torn directly from the roll still contained in the box) piece of aluminum foil
    b) the sample was wrapped in the clean sheet of aluminum foil (preferably, with the shiny side on the outside)
    c) then the sample was vacuum packed using a food grade plastic bag and a vacuum sealer
5) each sample was labeled and placed on ice or in a refrigerator until packed for shipment to the University of Guelph
6) the samples were shipped to the University of Guelph on ice
    a) the sample ID for each sample was scanned into the shipping form in the field database
    b) the bottom of a cooler was lined with a layer of ice packs, followed by a layer of bubble wrap, followed by a layer of samples
    c) the layering was repeated until the cooler is full

any extra space in the cooler is packed with bubble wrap, the chain of custody is added, and the cooler is taped shut for shipping

C-15 Rock Core Microbial Sample Collection Procedures (March 2009)

The following provides a summary of the rock core microbial sample collection procedures modified in March 2009 prior to C15 coring.

1) rock core microbial samples were chosen from the core
    a) samples adjacent to the fracture plane, at varying distances away from a fracture plane, and in the unfractured matrix are selected
    b) a hammer and CLEAN chisel were used to break each sample, a puck 2-3 inches in length, out of the core; the chisel was rinsed with purge and trap grade methanol and then wiped dry with a fresh kim wipe between each break
    c) information (corehole, run number distance to top of sample, distance to bottom of sample, begin sample time, end sample time, sample ID, sample type, sample position, lithologic description/comments) were recorded on the applicable field sheet
2) once the samples/pucks were broken out of the core they are packaged using the following procedure to maintain in situ REDOX conditions (presumably anoxic at depths below the watertable)

a) the samples were removed from the core using a NEW pair of nitrile gloves and a NEW (torn directly from the roll still contained in the box) piece of aluminum foil
b) the sample was wrapped in the clean sheet of aluminum foil (with the shiny side of the foil on the outside)
c) a sample ID label was applied to the foil and labels indicating which end of the sample represented a fracture plane and which end is up/top are also applied to the foil
d) the foil wrapped sample was then placed in a plastic vacuum sealable bag
e) the bag was then placed in the vacuum sealer for nitrogen purging, evacuation, and sealing
i) the vacuum sealer were set to run 2 nitrogen purge/evacuation cycles
f) the sample vacuum packed in the plastic bag was then be placed in a metalized bag (oxygen barrier) and placed in the vacuum sealer
i) the vacuum sealer was set to run 2 nitrogen purge/evacuation cycles
ii) once evacuated and sealed, a second (duplicate) sample ID label should be applied to the outside of the metalized bag for easy scanning during packaging

3) the metalized bags were stored on ice or in a refrigerator until they are packaged for shipment to the University of Guelph
   a) each sample ID label on each metalized bag was scanned into the shipping form in the field database
   b) each metalized bag was then placed in a large plastic ziptop bag to help protect the metalized bags (the metalized bags are somewhat easily punctured)
   c) the bottom of a cooler was lined with a layer of ice packs, followed by a layer of bubble wrap, followed by a layer of sample bags
   d) the layering was repeated until the cooler is full
   e) any extra space in the cooler was packed with bubble wrap, the chain of custody is added, and the cooler was taped shut for shipping
   f) overnight shipment on ice to the University of Guelph laboratory occurs typically within 1-3 days of sample collection and preservation in the field.

By anaerobically preserving the rock samples as soon as they are lifted from the borehole, the exposure to oxygen will be minimized and recovery of microbial DNA of anaerobic microorganisms will be possible. The extracted DNA will then represent the indigenous conditions of the microbial community and it will be amplified by using sensitive molecular techniques.

DNA EXTRACTION

After samples arrive in the laboratory at the University of Guelph, they were prepared for DNA extraction.

All rock samples were trimmed to remove the external portions of the sample that were in contact with the core barrel during extraction and also handled during packing in the field and unpacking at the University of Guelph. All the procedure involved in the trimming and crushing were performed using sterile techniques, according to following protocol:

Rock crushing for DNA extraction in the laboratory

1) bench tops and crushing equipment were wiped with a 70% ethanol or methanol solution
2) a propane burner was turned on to create a sterile working area
3) crushing cells and all the equipment that would have contact with the samples were washed with soap and/or sprayed with Eliminase (Fisher Cat# 04-355-32, Decon Laboratories), rinsed with deionized water and flame sterilized
4) all work from was done close to a flame and as quickly as possible to minimize die off of anaerobic microorganisms
5) bags were cut open and rock sample wrapping was opened carefully and the rock piece was placed inside a sterile stainless steel pot and trimmed using a sterile chisel
6) using a flame sterilized spatula, a trimmed piece of rock was placed inside a sterile crushing cell with the bottom plate on and then the top plate was then placed on
7) the sample was crushed using the rock crusher
8) the cell was then opened and with the flame sterilize spatula, the crushed material was transferred to a sterile 7 oz. (207 mL) Whril Pack bag (Nasco, VWR Cat# CA11216-200) and stored at -20^{o}C until processed

DNA has been extracted from 1 g of crushed rock using the Mo Bio PowerSoil DNA extraction kit (Cat#12888-100) following the manufacturer's protocol.

All the rock crushing procedures are performed in the sample preparation laboratory in the Axelrod building at the University of Guelph. After extraction, DNA was stored at -20^{o}C until processed. The DNA extraction was performed at Dr Kari Dunfield's laboratory in the Land Resource Science Department at the University of Guelph.

PCR ASSAYS

The extracted DNA templates will amplified using the following primer sets (Table 1). PCR amplifications targeted 16S rDNA genes using universal primer sets for general Bacteria (8f/1541r) and Archaea (1Af/1100Ar). Specific primers for detection of some dechlorinating microorganisms will also be performed using primer sets for *Dehalococcoides* (Dhc1200F/Dhc1271R), *Sulfurospirilum*, *Dehalobacter*, and *Geobacter* species (other specific primer sets will be chosen for further analyses of other important groups of dechlorinators, such as the sulphate-reducing bacteria). After PCR amplification, 5 µl of PCR products will be run on 1% ethidium bromide stained agarose gels to check for the presence of amplification products.

PCR reactions and conditions on Table 2 will be used. During PCR amplifications, positive and negative controls will be used according to current laboratory practice. For positive controls, DNA templates obtained from dechlorinating cultures (gently donated by Melanie Duhamel from the University of Toronto) will be used.

Table 1 - PCR Primers, Annealing Temperatures and Target Organisms

Name	Sequence	Annealing (°C)	Use	Reference
1Af	5'-TCY GKT TGA TCC YGS CRG AG-3'	55	Universal *Archaea* (PCR - 16S rDNA)	(Embley *et al.* 1992) + (Einen et al. 2008)
1100Ar	5'-TGG GTC TCG CTC GTT RCC-3'			
8f	5'-AGA GTT TGA TCC TGG CTC AG-3'	55	Universal *Bacteria* (PCR - 16S rDNA)	(Loffler *et al.* 2000; Weisburg *et al.* 1991)
1541r	5'-AAG GAG GTG ATC CAG CCG CA-3'			
Arc340f–GC	5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC CTA CGG GGY GCA SCA G-3'	53.5	*Archaea* (PCR-DGGE - 16S rDNA)	(Nicol *et al.* 2003)
Arc519r	5'-TTA CCG CGG CKG CTG-3'			
Bac341f-GC	5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3'	55	*Bacteria* (PCR-DGGE - 16S rDNA)	(Muyzer *et al.* 1993)
Bac534r	5'-ATT ACC GCG GCT GCT GG-3''			
Arc931f	5'-AGG AAT TGG CGG GGG AGC A-3'	64	>94% *Archaea* (qPCR - 16S rDNA)	(Einen et al 2008)
Arcm1100r	5'-BGG GTC TCG CTC GTT RCC-3'			
Bac338f	5'-ACT CCT ACG GGA GGC AGC AG-3'	61	>95% *Bacteria* (qPCR - 16S rDNA)	(Einen et al 2008)
Bac518r	5'-ATT ACC GCG GCT GCT GG-3'			
Dhc730F	5'-GCG GTT TTC TAG GTT GTC-3'	58	*Dhc* sp. (PCR - 16S rDNA)	(He *et al.* 2003; Loffler *et al.* 2000) Ballersdt et al 2003
Dhc1350R	5'-CAC CTT GCT GAT ATG CGG-3'			
Dhb477f	5'-GAT TGA CGG TAC CTA ACG AGG-3'	62	*Dehalobacter restrictus Dehalobacter* strain TCA1	(Grostern and Edwards 2006)
Dhb647r	5'-TAC AGT TTC CAA TGC TTT ACG G-30'			
Sulf114f	5'-GCT AAC CTG CCC TTT AGT GG-3'	59	*Sulfurospirillum multivorans, S. halorespirans* PCE-M2 *S. deleyianum*	(Duhamel and Edwards 2006)
Sulf421r	5'-GTT TAC ACA CCG AAA TGC GT-3'			
Geo73f	5'-CTT GCT CTT TCA TTT AGT GG-3'	59	*Trichlorobacter thiogenes, Geobacter* strain SZ	(Duhamel and Edwards 2006)
Geo485r	5'-AAG AAA ACC GGG TAT TAA CC-3'			

Table 2 - PCR Reactions and Thermalcycling Conditions

Primer set	Vol. (µL)	PCR buffer	$MgCl_2$ (mM)	dNTP (µL)	Taq (U)	Primers (nM)	Thermalcycling (°C/min)				
							Denat	Denat	Annealing	Extention	Final Extention
1Af/1100Ar	20	1×	2.0	300	1.25	400	94/5m	25 cycles			72/5
								94/1	55/1	72/2	
8f/1541r	20	1×	2.5	250	2.5	250	94/3m	30 cycles			72/7
								94/.75	55/.5	72/1.5	
Arc 340fGC/Arc519r	25	1×	1.5	250	1.0	310	94/5m	35 cycles			72/7
								94/1	53.5/1	72/1.5	
Bac 341fGC/534r	25	1×	2.0	250	1.25	360	94/5m	35 cycles			72/2
								94/1	20 touchdown cycles from 65 to 55/1 + 15 cycles of 55/1	72/2	
Dhc 730F/1350R	20	1×	1.6	200	0.5	210	94/3m	30 cycles			72/5
								94/.25	50-58/.75	72/1.5	
Dhb 477f/647r	20	1×	2.0	300	0.5	400	94/5	35 cycles			72/5
								94/1	62/1	72/2	
Sulf 114f/421r	20	1×	2.0	300	0.5	400	94/5	35 cycles			72/5
								94/1	62/1	72/2	
Geo 73f/485r	20	1×	2.0	300	0.5	400	94/5	35 cycles			72/5
								94/1	62/1	72/2	

PCR-DGGE Assays

PCR-DGGE assays will be performed in order to evaluate the Bacterial fingerprints in the samples using primers (341f-GC/534r) (Table 1). After PCR amplification with the DGGE primers, 3 to 5 µl of PCR product were run on 1% agarose gel stained with ethidium bromide to verify the presence of amplification product.

DGGE assays will be performed according to established protocols (Duhamel *et al*. 2004; Lima and Sleep 2007) using denaturing gradient from 30 to 60%, 0.5×TAE buffer in a Bio-Rad equipment. After DGGE, bands representing the range of bacterial diversity in the samples will be excised, soaked overnight in double distilled and autoclaved water and re-amplified through PCR with the DGGE primer set (341f-GC/534r). PCR products will be purified using the UltraClean™ PCR Clean-Up Kit from Mo Bio (Cat# 12500-100) and sent for sequencing with the reverse primer (534r) at the Genomics Facility of the Department of Integrative Biology, University of Guelph.

CLONING AND SEQUENCING

Cloning will be performed on a few samples of interest using the TOPO TA cloning kit from Invitrogen. Samples for cloning will be chosen according to the results of DGGE and the evidence of the presence of dechlorinating microorganisms in the samples.

Cloned plasmids will be isolated with the QIAprep® Plasmid Miniprep kit from Qiagen (Cat# 27106) according to the manufacturer's protocol. Plasmids will be sequenced at the Genomics Facility of the University of Guelph.

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Addendum

Characterization of the Microbial Populations from Fractured Rock Cores
Santa Susana Field Laboratory, Ventura County, California – USA

Prepared by: ***Gláucia Lima, Ph.D., Amanda Pierce, M.Sc., Beth Parker, Ph.D.***

Prepared for: ***The Boeing Company, NASA and DOE***
Santa Susana Field Laboratory – Treatability Study Plan

Date: **July 15th 2011**

This document was prepared in response to the following General and Specific Comments made by the Department of Toxic Substances Control (DTSC) dated of April 29th 2011. The DTSC comments will be addressed in sessions A.1 to A.4, as follows:

A.1 – Data Analysis and Reporting
A.2 – Tasks performed and at glance – dates and progress
A.3 – Reviewed Introduction and Motivation for the Proposed Study
A.4 – Rock core sample preservation methods

A.1 – Data Analysis, Publication and Reporting

This session addresses the DTSC General Comment 1: Data evaluation methods and how the results will be reported are not presented in the Work plan, and need to be developed and submitted in the work plan addendum.

A.1.1 – Data Analysis

Molecular biology results are analyzed according to their nature, using image processing software for gel image analyses, i.e. DGGE fingerprints, bioinformatics package for microarray and sequencing databases (to be determined according to the technology adopted).

Percent similarities among microbial communities are determined from DGGE fingerprints using clustering analysis with Gene Directory software v. 2.01a (Syngene) with Jacquard similarity coefficient and unweighted pair group method with arithmetic mean (UPGMA). Relative band positions (1% tolerance) are used for band identification. After cluster analyses is done, results will be tabulated and principal component analyses (PCA) will be performed considering community structures and environmental parameters, such as contaminant concentrations, sample type etc. For this, a commercially available statistical software program, i.e. SAS, Matlab or R, will be used depending on the complexity of the data and the statistical tests to be performed.

A.1.2. Reporting

It is expected that at least one graduate student develops his/her thesis within this project, generating a thesis in the School of Engineering Department at the University of Guelph. Also, abstracts will be submitted to conferences, such as the Battelle Bioremediation Symposium, and to scientific journals, i.e. Environmental Science and Technology. It is expected that minimum of 2 papers and 2 presentations will result from the proposed project.

A full data report will be provided at the completion of the project. Interim reports will be provided via meetings between UofG and the various stakeholders, including DTSC, at a frequency to be determined as results and progress are made. These data are being continued as part of a research program lead by Dr. Beth Parker, post-doctoral fellow Dr. Gláucia Lima, support staff, and graduate students. This study and related site data will be used to prepare peer-reviewed journal papers and presented at scientific meetings and conferences.

A.2 –Tasks: past and future – dates and progress

This session addresses the DTSC General Comment 2. The text varies between past and future tense, making it difficult for the reader to clearly understand what work has been done versus what will be performed. i.e.: has the DNA extraction already been performed?

Rock coring was performed at SSFL between November 2008 and May 2009. As described in the standard operational procedure in the original proposal (pages 3 and 4), when samples arrive in the laboratory, they are stored in the cold room (+ 4°C) until they are crushed under sterile conditions (see page 5 – "Rock crushing for DNA extraction in the laboratory"). The crushed aliquots are placed in sterile Whril Pack bags and stored at -20 °C freezer until DNA extraction. After DNA is extracted, a working aliquot is stored at -20 °C and original templates at -80 °C.

Table A.1 show the tasks that have been already performed and what is planned.

Table A.1 – Tasks performed and planned

	Task description	Beginning	End	Accomplished
1	Rock core sample collection from C12, C13, C14 and C15)	Nov/Dec 2008	March 2009	100 %
2	Rock core crushing under sterile conditions (C12, C13, C14 and C15)	13/May/2009	8/May/2009	100 %
3	Crushed rock core samples preserved at -20°C	13/May/2009	To present date	100 %
4	DNA extraction C12, C13, C14	Summer 2009	Summer 2009	100 %
5	DGGE C12, C13, C14 samples	Spring 2011	Spring 2011	90 %
6	DNA extraction from C15 samples	March 2011	Summer 2011	10 %
7	DGGE C15 samples	Fall 2011	Fall 2011	0 %
8	Cloning of selected samples from C15	Fall 2011	Fall 2011	0 %

A.3 – Motivation for the Proposed Study – Evidence of microbial degradation at the site

This session addresses the DTSC Specific Comment 1: Introduction, page 2: The basis for the statement that "complete degradation is likely occurring in many areas and that it may be an important influence on TCE attenuation" is not fully supported in the Work Plan.

This is a revised version of the INTRODUCTION section from the original proposal (page 1) with added evidence of complete dechlorination ongoing at the site.

The purpose of this document is to outline the goals, objectives and methods that are being applied for DNA extraction and molecular biology analyses on rock core samples obtained from boreholes C12, C13, C14 and C15 from SSFL, California. This will provide information on the indigenous microbial community at a level of detail that has yet to be found in the scientific literature. This has relevance to the assessment of natural attenuation of subsurface contaminants in the Chatsworth Formation at SSFL or potential for biostimulation.

There is abundant evidence from extensive groundwater and rock core sampling that TCE degradation occurs in the Chatsworth Formation, which have demonstrated that TCE suffers both total and partial dechlorination, with mainly *cis*-DCE detected in groundwater samples from 42 of 122 wells sampled by Pierce (2005), and minor occurrences of *trans*-DCE, 1,1-DCE and vinyl chloride (VC) (Pierce 2005). Rock-core samples also demonstrated the existence of TCE daughter products (*cis*-DCE) within the rock matrix (Hurley 2003; Hurley *et al*. 2007). Compound specific isotope analyses (CSIA) performed in groundwater samples from 122 wells at the site (Pierce 2005) detected shifts in the isotope signatures of TCE and cis-DCE compatible to biodegradation. Complete dechlorination was detected as well and it is attributable to microbially mediated processes (Pierce 2005). Using the Snap Sampler™, Zimmerman (2010) found additional evidence of biological and abiotic dechlorination of TCE in groundwater from a sub-set of the monitoring wells sampled by Pierce (2005), with concentrations of VC, ethene, and acetylene much higher than those observed in 2005. Acetylene—a product of the abiotic degradation of TCE (Lee and Batchelor 2002)—was detected in some of the field samples together with high concentrations of vinyl chloride (VC), ethane and ethane in (Zimmerman 2010). Redox conditions determined by Zimmerman (2010) in the sub-set of monitoring wells were predominately Fe(III)-reduction with local occurrences of NO_3^{2-}-, Mn(IV)-, SO_4^{2-}-reduction, and methanogenesis.

Even though field results from Hurley (2003), Pierce (2005), and Zimmerman (2010) suggest that microbial reductive dechlorination is a major degradation pathway, lab microcosm studies by Darlington et al. (2008) failed to demonstrate complete dechlorination of TCE. Microcosms demonstrated microbial production of *cis*-DCE, but, in contrast to the field results, no dechlorination to ethene was found. A bacterium from the microcosms was positively identified as *Pseudomonas* sp., which could cometabolic carry TCE biodegradation to *cis*-DCE (Darlington 2008). Other microorganisms, i.e. *Dehalococcoides* spp. (Maymo-Gatell *et al*. 1997), which could degrade TCE to ethene, were not detected by Darlington (2008). The lab microcosm studies by Darlington et al. (2008) were performed as batch tests using crushed rock samples and water from wells, both obtained from SSFL. These experiments were set up as live and autoclaved microcosms. They used samples from the microcosms actively degrading TCE and demonstrated that a *Pseudomonas* sp. was possibly the microorganism responsible for TCE to cis-DCE pathway. Darlington et al. (2008) suggested that iron-bearing minerals in the Chatsworth Formation

could be responsible for abiotic degradation of TCE and cis-DCE, and they estimated the extent of this transformation to be limited to 25%, based on laboratory test conditions and results.

The results found in microcosm studies by Darlington (2008) could reflect a limitation of microcosm enrichments of properly reflecting field microbiology and heterogeneities in microbial distributions, resulting in failure to detect important biodegraders. Besides, the use of groundwater samples for laboratory enrichments do not indicate where degradation reactions are taking place, if in the fracture spaces or within the rock matrix blocks in between fractures. Determining if biodegradation within the matrix blocks is occurring requires the analysis of rock-core samples. DNA extraction and microbial community characterization of rock core samples are the subject of the present proposal. Future studies at the site will also involve analyses of groundwater samples for comparisons with our findings in the rock matrix. This will be important for assessing the influence of biodegradation on contaminant transport and fate in sandstones and implications of engineered remediation technologies on *in-situ* conditions. The rock-core microbial studies described here are intended to elucidate the indigenous microbial community.

A.4 – Rock core sample preservation methods

This session addresses the DTSC Specific Comment 2: Rock Core Microbial Sample Collection Procedure. pages 2-4: Describe how have core samples been preserved. Clarify whether testing is to be conducted on DNA that was previously extracted or if DNA extraction will be part of the proposed test.

A.4.1 – Rock Core Preservation

Once the core is laid on the PVC at the sampling table, it is immediately logged, with visual identification of fractures and lithology, photographing, and samples collection. Samples are collected for physical properties, VOC analyses and DNA extraction. Samples for DNA extraction are preserved according to Item 4 in page 3 (C12, C13, C14) and Item 2 in page 4 (C15), which are transcribed here.

Item 4 from page 3 of the original document describes how samples from coring locations C12, C13 and C14 were preserved, as transcribed here:

> *(Page 3)*
> *4) once split, each rock core microbial sample was wrapped in packaging used to prevent exposure to oxygen*
> *a. each split sample was removed from the core using a **NEW** pair of nitrile gloves and a **NEW** (torn directly from the roll still contained in the box) piece of aluminum foil*
> *b. the sample was wrapped in the clean sheet of aluminum foil (preferably, with the shiny side on the outside)*
> *c. then the sample was vacuum packed using a food grade plastic bag and a vacuum sealer*

Rock core samples from C15 were preserved according to item 2 in page 4 of the original document (transcribed here). The procedure changed from the previous core holes because we decided to adopt an industrial vacuum sealer with a nitrogen purging system, instead of the domestic food saver vacuum packing equipment used previously.

> *(Page 4)*

2) *once the samples/pucks were broken out of the core they are packaged using the following procedure to maintain in situ REDOX conditions (presumably anoxic at depths below the water table)*
    a) *the samples were removed from the core using a NEW pair of nitrile gloves and a NEW (torn directly from the roll still contained in the box) piece of aluminum foil*
    b) *the sample was wrapped in the clean sheet of aluminum foil (with the shiny side of the foil on the outside)*
    c) *a sample ID label was applied to the foil and labels indicating which end of the sample represented a fracture plane and which end is up/top are also applied to the foil*
    d) *the foil wrapped sample was then placed in a plastic vacuum sealable bag*
    e) *the bag was then placed in the vacuum sealer for nitrogen purging, evacuation, and sealing*
        i. *the vacuum sealer were set to run 2 nitrogen purge/evacuation cycles*
    f) *the sample vacuum packed in the plastic bag was then be placed in a metalized bag (oxygen barrier) and placed in the vacuum sealer*
        i. *the vacuum sealer was set to run 2 nitrogen purge/evacuation cycles*
        ii. *once evacuated and sealed, a second (duplicate) sample ID label should be applied to the outside of the metalized bag for easy scanning during packaging*

A.4.2 – Molecular Biology Tests for C12, C13, C14 and C15

All molecular biology tests (Table A.1 items 5, 7, 8 and 9) to be performed in rock core samples from C12, C13, C14 and C15, obtained between the Fall of 2008 and March 2009 for this research proposal, as indicated in Table A.1. We started some of the molecular analyses started as soon as DNA was extracted from C12, C13 and C14, because of the need to verify if DNA was recovered from the samples and if we would have to modify our methods for the remaining samples from the site.

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7. Zimmerman, L. K. 2010. New Opportunities for Groundwater Hydrochemistry and Degradation Investigations Provided by the Snap Sampler®. University of Guelph, Guelph, Ontario, Canada.

Attachment C

Laboratory Evaluation of Biostimulation to Treat Chlorinated Ethenes in the Chatsworth Formation (Revised Work Plan, with Addendum)

Laboratory Evaluation of
Biostimulation to Treat Chlorinated Ethenes in the Chatsworth Formation, Santa Susana Field Laboratory, Ventura County, California

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1.0 Background

MWH has identified enhanced biological reduction as one of the in situ mass transformation technologies worthy of further consideration for potential deployment at the Santa Susana Field Laboratory (SSFL). The purpose of this work plan is to outline two stages of testing at the laboratory scale that will provide the information needed to determine if further testing at the field scale is warranted.

Field and laboratory evidence (2) strongly supports the conclusion that reductive dechlorination of trichloroethene (TCE) to *cis*-1,2-dichloroethene (cDCE) is an on-going process at SSFL. Natural organic matter in the groundwater and/or sandstone provides the electron donor needed for this process. Using samples from microcosms that actively reduced TCE to cDCE, Darlington (1) enriched for the dechlorinating microbes by transferring them to a defined mineral salts medium and supplying hydrogen as the electron donor and acetate as a carbon and energy source. Analysis of the enrichment culture by denaturing gradient gel electrophoresis suggested that a *Pseudomonas* sp. was most closely associated with dechlorination of TCE to cDCE.

At SSFL, the extent of further biotic reduction of cDCE to vinyl chloride (VC) and ethene appears to be limited. Only trace levels of VC and ethene have been detected in field samples. In microcosms that exhibited robust reductive dechlorination of TCE to cDCE, no significant further reductive dechlorination was observed (2). Two conditions likely explain the absence of reductive dechlorination beyond cDCE. First, reductive dechlorination of cDCE to ethene generally requires redox levels (e.g., less than -100 mV) that are lower than what is typical at most sampling locations at SSFL. Table 8-7 in the Draft SSFL Groundwater RI Report shows that the mean E_h value collected from well samples across the site is 387 mV, ranging from 51 to 629 mV. Although the amount of naturally occurring biodegradable organics appears to be sufficient to support TCE reduction to cDCE, it is not sufficient to create the lower redox environment generally associated with high rates of cDCE reductive dechlorination (4). Second, it is likely that the microbes required for high rates of cDCE reduction to ethene, i.e., *Dehalococcoides*, are either lacking in the SSFL subsurface, or are present in extremely low numbers. Since these microbes thrive best under low redox conditions, the absence of *Dehalococcoides* activity is expected. Unless a population of *Dehalococcoides* develops, significant mass destruction of TCE and cDCE by reductive dechlorination to ethene is not likely to occur at SSFL.

Biostimulation is one of the most common forms of enhanced biological reductive dechlorination that is used to overcome the limitations described above. Biostimulation involves the addition of a substrate to the subsurface where it undergoes fermentation and generates hydrogen, which is generally regarded as the universal electron donor for chlororespiration. Acetate is also a by-product of the fermentation process. Although acetate has limited usefulness as an electron donor for reductive dechlorination of cDCE and VC, it is a required source of carbon for growth of *Dehalococcoides*, so its formation during fermentation is also a desirable outcome. A wide variety of substrates are available for use in biostimulation, including organic acids (e.g., lactate), carbohydrates (e.g., corn syrup or molasses), and emulsified vegetable oil. Some substrates are "fast-acting," i.e., they undergo rapid fermentation and release high levels of hydrogen, while others are "time-release," i.e., they undergo slow rates of hydrolysis and fermentation and

therefore release hydrogen over longer periods of time and at lower concentrations. The latter are preferred for sites where remediation is expected to take several years or longer, in order to avoid addition of substrate at frequent intervals.

In addition to reductive dechlorination, a combination of abiotic and biotic transformation processes may be a significant fate process for chlorinated ethenes at SSFL. Darlington et al. (2) demonstrated the conversion of ^{14}C-labeled cDCE (as well as TCE) to soluble products and $^{14}CO_2$ in autoclaved microcosms. In live microcosms, $^{14}CO_2$ was the predominant product from [^{14}C]cDCE and [^{14}C]TCE. Darlington (1) speculated that iron-containing minerals in the Chatsworth sandstone are responsible for the abiotic transformation of cDCE and TCE; microbes then complete the transformation by oxidizing the products to CO_2. In those experiments, however, the extent of transformation was limited to approximately 25% or less of the cDCE. The reason for this limitation was not evaluated. One possibility is that the transformation capacity of the minerals was due to a lack of reductant that may be required to re-reduce them to an active state. A growing body of evidence suggests that abiotic transformation of chlorinated ethenes can be facilitated by producing low redox conditions; a low cost way to do so ***is*** through biostimulation.

Although biostimulation is used increasingly at sites contaminated with chlorinated ethenes, this approach has not yet gained as widespread adoption at sites with fractured rock. This type of environment poses a significantly greater challenge to distribution of the electron donor. Nevertheless, biotic reductive dechlorination may still be a feasible approach. Reductive dechlorination activity may be beneficial in at least two ways. First, the potential exists for the development of a biofilm in the fractures where the majority of the groundwater flows. Chlorinated ethenes that diffuse out of the rock matrix will be subjected to reductive dechlorination as long as a sufficient supply of electron donor can be maintained. A time-release substrate that adsorbs to the sandstone should be used, to prevent it from being easily washed out with the groundwater. Although activity in the fractures may not impact the majority of the mass of chlorinated ethenes found in the rock matrix, it could serve to prevent any further migration of the compounds as they diffuse out. Furthermore, while the likelihood of microbes penetrating significantly into the rock matrix is small, this possibility should not be ruled out entirely. Second, as mentioned above, the creation of highly reducing conditions may facilitate the transformation of cDCE and TCE via abiotic pathways that, combined with microbial activity, yield CO_2 and Cl^- as ultimate end products.

One limitation to a remediation process that yields CO_2 and Cl^- as products is the difficulty in documenting the process in situ. An emerging tool for this purpose is monitoring for the enrichment of $\delta^{13}C$. Measurement of $\delta^{13}C$ has become increasingly common to ascertain the extent of biodegradation. However, it is less commonly used for processes that involve abiotic transformation such as the one envisioned for the SSFL. Obtaining data on $\delta^{13}C$ enrichment in a laboratory-controlled experiment will provide an important opportunity to document cDCE transformation in situ, via pathways other than reductive dechlorination.

2.0 Work Plan Objectives

The objectives of the proposed work plan address the main issues outlined above, pertaining to the feasibility of using enhanced reductive dechlorination in the SSFL subsurface:

1) Using batch microcosms, determine the effect of biostimulation on the rate of TCE reduction;

2) Using batch microcosms, determine if biostimulation enhances transformation of TCE and cDCE via pathways other than reductive dechlorination by quantifying the products formed from [^{14}C]TCE, as well as the extent of δ^{13}C enrichment; and

3) Using flow through columns, determine the extent of biofilm formation in response to biostimulation, as well as the products formed from TCE based on δ^{13}C enrichment.

3.0 Experimental Approach

3.1 Microcosm Evaluation of Biostimulation and Product Formation

The first and second objectives will be addressed in a microcosm study. Microcosms will be prepared with sandstone and groundwater from an adjacent well. The treatments will include:

- No amendments
- Biostimulation with lactate
- Biostimulation with emulsified vegetable oil
- Biostimulation with HRC-X
- Water controls
- Autoclaved controls

Lactate will be used as a positive control, i.e., nearly all of the mixed cultures that reductively dechlorinate TCE are able to use lactate as an electron donor. Emulsified vegetable oil and HRC-X are both "long-lasting" electron donors that have been used in fractured rock.

Prior to preparing the microcosms, the groundwater pH will be checked. If it is outside of the range considered optimal for reductive dechlorination (i.e., 6-8), consideration will be given to adjustment. Resazurin will be added to the groundwater (1 mg/L) to provide a colormetric indication of the redox level.

The same methods described by Darlington et al. (2) will be used to prepare the microcosms, using 160 mL glass serum bottles and Teflon-faced septa held in place with aluminum crimp caps. Samples of a rock core will be crushed at Clemson University using a hand-operated hydraulic press. The microcosms will be assembled in an anaerobic chamber. After sealing, the microcosms will be removed from the chamber and the headspaces will be purged with high purity nitrogen gas to strip out hydrogen present in the atmosphere of the anaerobic chamber. Then, TCE will be added using TCE-saturated water to an initial concentration of approximately 1 mg/L. The initial amount of electron donor added will be based on stoichiometric reduction of

nitrate and sulfate (if present) plus 100 times the stoichiometric amount needed for reduction of the TCE to ethene, thereby ensuring a considerable excess.

The fate of TCE will be determined by adding approximately 0.50 µCi/bottle of [^{14}C]TCE to all of the treatments; this is equivalent to 1.11×10^6 disintegrations per minute. This amount of ^{14}C activity ensures that it will be possible to detect the TCE transformation products. Similar amounts have been used with success in other studies performed in the Freedman laboratory (2, 3, 5, 6). Higher amounts are unnecessary for the objective and begin to raise concerns with disposal of the microcosm contents. To minimize the risk of adding soluble ^{14}C contaminants from the stock solution, the [^{14}C]TCE will be injected into the microcosms in the gas phase after purification on a gas chromatograph, as previously described (2). Because the labeled material has a high specific activity (i.e., approximately 1-5 mCi/mmole), adding 0.50 µCi/bottle will not appreciably increase the total concentration of TCE or cDCE in the microcosms. For example, at a specific activity of 5 mCi/mmol, adding 0.50 µCi/bottle of [^{14}C]TCE will increase the TCE concentration by approximately 15%.

A total of 12 bottles will be prepared for each treatment. At intervals of approximately two to three months, one set of triplicate bottles will be sacrificed to determine the distribution of ^{14}C products. The selection of sampling intervals will be based on the activity observed using routine GC headspace measurements (at approximately two week intervals).

For the second objective, a parallel set of microcosms will be prepared using the same set of treatments. However, [^{14}C]TCE will not be added. At the same intervals when bottles are sacrificed to determine the distribution of ^{14}C products, bottles without the ^{14}C added will be sent to the University of Waterloo for analysis of δ^{13}C enrichment.

Time frame: Eighteen months

3.2 Evaluation of Biostimulation in Continuous Flow Columns

The third objective will be addressed in six continuous flow columns that will contain chunks of sandstone (approximately 3 cm in diameter), rather than crushed rock. The rock will be loaded into columns and SSFL groundwater will be pumped through at velocities comparable to what occurs in fractures. Electron donor (selected based on the results of objective #1) will be added to five of the columns; the sixth will be used as an unamended control. The amount of donor added to each column will be varied, in order to evaluate the effect of dose on the length of time that TCE degradation can be sustained.

The columns will be monitored routinely for reductive dechlorination of TCE using 5 mL samples taken from the sampling ports. Effluent samples will be analyzed periodically for the extent of δ^{13}C enrichment.

At the conclusion of column operation, samples of the rock pieces will be removed from the columns and used to determine the relative distribution of microbes on the surface of the rock versus the number that penetrated the rock matrix. Quantification of microbes will be based on the polymerase chain reaction (qPCR) using universal bacterial primers. After rinsing the

surface of the rocks to remove unattached microbes, more aggressive methods will be used to extract DNA from the surface of the rock. The surface area directly exposed to the flowing groundwater will be measured in order to normalize the qPCR results in terms of the attached bacteria per m^2. Rock pieces will then be crushed and DNA will be extracted to estimate the extent of microbes within the rock matrix. Samples will be taken along the length of the column, to establish the distribution as a function of distance from the point of groundwater addition.

A schematic of the columns is shown in Figure 1. Each column will consist of an acrylic tube (50 cm x 3.8 cm internal diameter). The inlet will consist of 0.635 cm thick circular plate of acrylic machined and cemented to the end of the column. A 0.635 cm hole in this base plate (drilled and tapped) will serve as the inlet. A 3.5 cm diameter stainless steel screen (50 mesh) will be placed inside the column, over the inlet hole, to retain any small pieces of rock that may block the inlet.

A flange will be machined and attached to the outlet end of the column and secured by three screws (0.318 cm). An O-ring (5.5 cm) will be placed into a machined grove in the flange to form a seal between the flange plate and the outlet plate. A 0.635 cm hole will be drilled and tapped into the outlet plate. Holes (15.9 mm) for sampling ports will be drilled at 2.4, 4.9, 10.1, 15.2, 20.1, 30.5, and 39.6 cm along the length of the column, measured from the inlet end.

The groundwater will be transferred to an 80 L Tedlar bag, which is expected to provide sufficient capacity for the duration of the experiment. Neat TCE will be added to provide 1 mg/L. The outlet of the Tedlar bag will be attached to a 40 cm length of Viton tubing. A manifold will be constructed using 0.794 mm polyethylene Tees to create six branches of tubing. A 40 cm length of the Viton tubing will extend from the manifold through six cartridges on a peristaltic pump (Cole Parmer, model #7519-06). The outlet end of the Viton tubing will be fitted over a 20-22 cm length of 0.159 cm stainless steel tubing, which will be stepped up to a 5 cm length of 0.318 cm stainless steel tubing using an adapter. This tubing will be connected to a 0.318 cm union Tee. The male branch of the tee will then be fitted with a Teflon-faced rubber septum, through which column inlet samples will be withdrawn. The outlet end of the Tee will be connected to an adapter, which will screw into a 0.635 cm stainless steel nipple (with NPT threads) and connect the valve to the inlet plate of the column.

The outlet end of the column will be fitted with a nylon male pipe adapter. A short length of tygon tubing will connect the pipe adapter to a 500 mL Erlenmeyer flask, which will collect the column effluent.

Sampling ports will be constructed by placing 0.20 cm Teflon-faced red rubber septa over the 3 mm holes and securing them in place with 5.7 cm hose clamps that will encircle the septa and the column. A 0.635 cm hole will be drilled in the hose clamps to allow access to the septa. A 3.2 cm long stainless steel needle (16 gauge) will be inserted through each septum and hole and into the center of the column. A small piece of glass wool will be placed inside the needles to minimize the chance of clogging. Female-to-male Luer stopcocks will be attached to the seven stainless steel sampling needles to seal the ports when not in use (Figure 1).

The six columns will be mounted side-by-side vertically on a frame using ring clamps. The Tedlar bag storing the groundwater will be supported by a plywood box. The bag will be placed face down to allow withdrawal of the groundwater at the low point of the bag. The testing will be conducted at room temperature, which will be recorded daily. The empty bed volume of each column will be determined by measuring the amount of water they hold without media present. The expected range is 576-581 cm^3 per column. The pieces of sandstone will then be added and bulk pore volumes will be measured based on the volume of water required to fill the columns.

Time Frame: Taking into account some method development for qPCR and allowing ample time to establish reductive dechlorination in the columns, we anticipate this effort will take 18 months.

4.0 References

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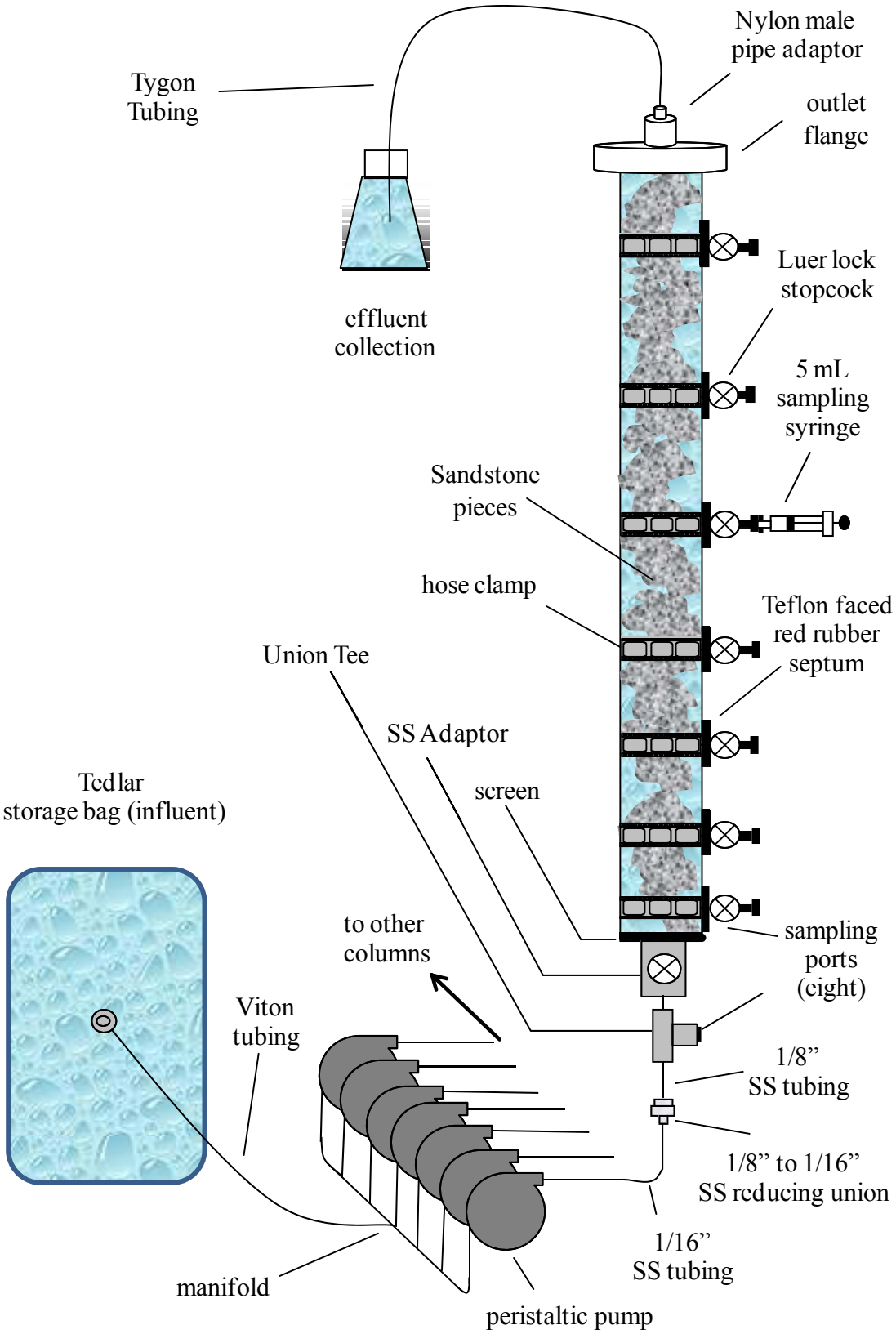

Figure 1. Schematic of the laboratory columns for objective #3.

5.0 Addendum: Plan for Data Evaluation

During the course of the project, data will be entered into Excel spreadsheets that will be updated each time new samples are evaluated. The spreadsheets will be distributed for review quarterly. Results for the microcosms and continuous flow columns will be summarized in graphical format.

At the conclusion of data collection, a final report will be prepared. A draft outline for the report follows:

I. Microcosms

I.A Introduction

I.B Materials and Methods
- I.B.1 Rock and Groundwater Collection and Processing
- I.B.2 Chemicals
- I.B.3 Experimental Design
- I.B.4 Microcosm Preparation and Monitoring
- I.B.5 Analytical Methods and ^{14}C Distribution
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