Nevada Environmental Response Trust
Henderson, Nevada

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Emeryville, California

In partnership with
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Fort Collins, Colorado

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IN-SITU BIOELECTROCHEMICAL LABORATORY-SCALE TREATABILITY STUDY WORKPLAN

NEVADA ENVIRONMENTAL RESPONSE TRUST SITE HENDERSON, NEVADA

RAMBOLL

In-Situ Bioelectrochemical Laboratory-Scale Treatability Study Work Plan

Nevada Environmental Response Trust Site
(Former Tronox LLC Site)
Henderson, Nevada

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I certify that this document and all attachments submitted to the Division were prepared at the request of, or under the direction or supervision of NERT. Based on my own involvement and/or my inquiry of the person or persons who manage the system(s) or those directly responsible for gathering the information or preparing the document, or the immediate supervisor of such person(s), the information submitted and provided herein is, to the best of my knowledge and belief, true, accurate, and complete in all material respects.

Office of the Nevada Environmental Response Trust

Le Petomane XXVII, Inc., not individually, but solely in its representative capacity as the Nevada Environmental Response Trust Trustee

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Date: 5/7/18

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Responsible Certified Environmental Manager (CEM) for this project

I hereby certify that I am responsible for the services described in this document and for the preparation of this document. The services described in this document have been provided in a manner consistent with the current standards of the profession and, to the best of my knowledge, comply with all applicable federal, state and local statutes, regulations and ordinances.

_____ 5/7/2018
_____

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Principal

Date

Certified Environmental Manager
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Date	**May 7, 2018**
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ACRONYMS AND ABBREVIATIONS

C_{org}	Organic carbon
CCH	Center for Contaminant Hydrology
cld	Chlorite dismutase
COH	City of Henderson
CSU	Colorado State University
DBP	Disinfection by-product
DI	Deionized
DNA	Deoxyribonucleic acid
DoD	Department of Defense
DC	Direct current
EPA	Environmental Protection Agency
FS	Feasibility Study
GW	Groundwater
H_2	Hydrogen
HCO_3^-	Bicarbonate
M	Molar
MMO	Mixed metal oxide
NDEP	Nevada Division of Environmental Protection
NERT	Nevada Environmental Response Trust
O_2	Oxygen
O&M	Operation and maintenance
ODBP	Organic disinfection by-product
pcrA	Perchlorate reductase
PEM	Polymer-electrolyte membrane
PI	Principal Investigator
PVC	Polyvinyl chloride
qPCR	Quantitative polymerase chain reaction
RAOs	Remedial Action Objectives
RI	Remedial Investigation
RI/FS	Remedial Investigation and Feasibility Study
RNA	Ribonucleic acid

rRNA	Ribosomal ribonucleic acid
RT	Reverse transcription
SERDP	Strategic Environmental Research and Development Program
Ti	Titanium
Ti-MMO	Titanium-coated mixed metal oxide
TIC	Total inorganic carbon
TOC	Total organic carbon
UNLV	University of Nevada Las Vegas
ZVI	Zero-valent iron

1. INTRODUCTION

On behalf of the Nevada Environmental Response Trust (NERT or "the Trust"), Ramboll US Corporation (Ramboll), in partnership with Colorado State University (CSU), has prepared this In-situ Bioelectrochemical Laboratory-Scale Treatability Study Work Plan (Work Plan) to evaluate electrochemical methods for promoting and/or enhancing in-situ biological reduction of perchlorate and chlorate in groundwater impacted by former operations at the NERT site in Henderson, Nevada (the "Site").[1] More specifically, this Work Plan presents the technical approach and scope of work for conducting initial laboratory-scale testing to evaluate the feasibility of coupling electrochemical (a.k.a electrolytic) hydrogen generation with naturally-occurring biological perchlorate reduction in groundwater under Site-specific conditions.

Along with other treatability and pilot studies NERT has and/or is planning to conduct, this Work Plan has been prepared to support remedy selection as part of the Remedial Investigation and Feasibility Study (RI/FS). Upon completion of this laboratory-scale treatability study and based on study results, the Trust may propose a field test of this treatment technology.

1.1 Technical Summary and Study Rationale

Bioelectrochemical remediation, as contemplated here, is a combination of two well-documented and proven technologies paired in stepwise fashion. The first step involves the application of a low voltage direct current to a set of submerged electrodes that promotes the hydrolysis of water to generate pure hydrogen gas (H_2) at the cathode and oxygen gas (O_2) at the anode. The second step is the biological reduction of perchlorate and other oxidized contaminants by anaerobic microorganisms, using the generated H_2 as an electron donor. The use of H_2 as an electron donor to stimulate the biological reduction of perchlorate is a demonstrated approach (e.g., Nozawa-Inoue, et al., 2011; Wan et al, 2016) and has previously been used by Ramboll as part of an in-situ remedy for perchlorate-impacted groundwater (Warner, et al., 2008). The electrochemical stimulation of microbial perchlorate reduction has been demonstrated by numerous studies (e.g., Thrash, et al, 2007; Butler, et al, 2010; and Sevda, et al, 2018) and researchers at CSU have prior expertise coupling electrochemical processes with biological treatment for other aqueous-phase contaminants such as 1,4-dioxane and chlorinated ethenes (e.g., Jasmann, et al. 2017).

In-situ H_2 generation is also being evaluated as part of Ramboll's *Galleria Road ZVI-Enhanced Bioremediation Treatability Study Work Plan* dated September 29, 2017 (approved by NDEP on October 26, 2017) (Ramboll 2017) wherein zero-valent iron (ZVI) is being tested for its performance under Site-specific conditions in generating H_2 in-situ and thereby promoting and/or enhancing biological reduction of perchlorate and chlorate. The bioelectrochemical approach described in this work plan represents a different means of generating H_2 in-situ, but is similarly intended to promote biological reduction of perchlorate and chlorate. While H_2 generation is the common step of each approach, both merit testing because the methods and materials of construction are expected to be

[1] For the purposes of this Work Plan, the "Site" as referenced herein includes the entirety of the NERT Remedial Investigation (RI) Study Area.

markedly different between the two approaches, which may dictate the implementability of one versus the other during the technology screening conducted as part of the FS.

As more has been learned through the completion of the various phases of the NERT RI, it has become evident that the subsurface conditions vary widely throughout the NERT RI Study Area. Therefore, achieving the Remedial Action Objectives (RAOs) may require an overall remedial alternative comprised of different technologies deployed at strategic locations, implemented in concert, and targeting conditions that take advantage of the individual technologies' strengths and limit exposure of their weaknesses. The bioelectrochemical approach described herein has characteristics which may make it a component of such a remedial alternative, one that may be well-suited to areas exhibiting long-term, low-level or variable-level mass flux. However, it should be emphasized that a field test application of a bioelectrochemical approach is not being proposed at this time. Rather, this Work Plan proposes an initial evaluation of the technology with the following aims:

1. Demonstrate the feasibility of the technology at the laboratory scale;
2. Evaluate the range of geochemical and hydraulic conditions, to the extent practicable in a laboratory study, under which bioelectrochemical treatment may be a feasible approach; and
3. Provide information to assess potential field applications (e.g., a modular in-well H_2 generator, a trenched system, etc.) for testing bioelectrochemical treatment at a strategic location within the NERT RI Study Area.

If ultimately transitioned to and demonstrated effective in the field, bioelectrochemical treatment has a number of benefits. Electrochemical H_2 production offers the potential for sustainable, long-lasting generation of H_2 at relatively low direct current inputs. Thus, the long-term implementation of such a treatment would have a comparatively low environmental footprint and accompanying low life-cycle costs. Produced electrochemically, the H_2 production rate is controllable via the amount of current applied thus allowing it to be adaptable to changing conditions, e.g., reducing H_2 production to adapt to reducing max flux or increasing H_2 production in response to higher transient groundwater flows. As discussed in Section 2, previous studies performed by CSU on electrochemical treatment observed production of organic molecules that can serve as additional electron donors and/or sources of organic carbon for bacterial cell growth. Total electron donor generated per unit mass of installed material (i.e., the electrodes) is high since the ultimate source of H_2 is from the electrolysis of water, the source of organic molecules is existing inorganic carbon, and the sole driving force is applied current. Moreover, the performance of the electrodes can be monitored remotely using simple electrical measurements. Thus, disturbance and access to the Site can be minimized. Overall, if demonstrated effective, bioelectrochemical treatment could provide a highly cost-effective long-term treatment for perchlorate and chlorate in groundwater with low impact to future land use.

This laboratory-scale treatability study will attempt to address the potential challenges that could limit or reduce the effectiveness and/or implementability of bioelectrochemical treatment. These challenges may include mineral precipitation and subsequent reduced longevity of the electrodes; lower than expected perchlorate reduction rates due to insufficient organic carbon; and difficulty delivering adequate H_2 while maximizing

electrode lifespans. However, the potential benefits of bioelectrochemical treatment as a modular, sustainable, and adaptable long-term remedy make an initial evaluation of this technology appropriate at this time.

1.2 Treatability Study Approach

The proposed approach for this treatability study, prior to field-based testing, consists of a laboratory-scale program, which will serve as a proof-of-concept for assessing the technology under anticipated Site-specific conditions and providing information necessary for potential future field testing and implementation.

This treatability study is intended to compliment the other treatability and pilot studies NERT has and/or is planning to conduct in order to provide a range of treatment options that can be assembled into remedial alternatives during the FS. Because there are some technical similarities between this bioelectrochemical treatability study and the ZVI treatability study (discussed in Section 1.1), and because the two studies are more or less being conducted contemporaneously, these studies have been planned in close coordination. For example, to enhance the quality of the data generated, the universities involved in the two treatability studies—CSU and the University of Nevada Las Vegas (UNLV)—have aligned laboratory methods where possible and are planning to work collaboratively as the treatability studies progress.

The laboratory-testing program proposed herein will be phased so that information from preceding steps will be used to inform subsequent steps; however, where appropriate to do so, steps will be performed in parallel to reduce, to the extent practicable, the duration of the study. The laboratory-scale testing program consists of the following steps:

1. **Batch reactor testing** with groundwater from the Site will be performed to evaluate the electrochemical production of H_2 and other potential electron donors such as formate and acetate.
2. **Column reactor testing** with soil from the Site will be performed to evaluate the perchlorate reduction process under dynamic conditions.
3. **Sand tank prototype testing** will be conducted under simulated Site conditions to evaluate performance of a prototype H_2 delivery system that could be utilized in the field.

1.3 Laboratory-Scale Testing Objectives

The objectives of the laboratory-scale testing described by this Work Plan are to:

1. Assess the applicability of bioelectrochemical reduction of perchlorate and chlorate under Site-specific conditions; and, if supported by preliminary results and requested by the Trust
2. Obtain design parameters and other necessary information for a field test application of the treatment.

This laboratory-scale testing program has been designed to allow some steps to be performed in parallel for timeline efficiency without undermining evidence-based decisions. For example, after acquiring critical data from the column testing the sand tank testing may begin in parallel with the end of the column testing. Similarly, at the beginning of the sand tank testing phase, sufficient information may have been acquired

to begin preliminary planning for a potential field test application (e.g. reactor design, material orders, location selection, permitting, etc.) before completion of the sand tank testing. Recommendations for accelerating the testing program, eliminating tests, and/or performing tests in parallel will be presented in the monthly updates to the Trust and NDEP/EPA in accordance with Section 4.

1.4 Work Plan Organization

This work plan is organized as follows:

- Section 1 presents the overall objectives and organization of the Work Plan.
- Section 2 provides background information and a description of the proposed technologies.
- Section 3 describes the proposed laboratory-scale testing program.
- Section 4 describes the proposed reporting to be performed.
- Section 5 provides a proposed schedule for the laboratory-scale testing program.
- Section 6 lists citations for key documents referenced in this Work Plan.

2. TECHNOLOGY DESCRIPTION

As introduced in Section 1.1, bioelectrochemical remediation is a combination of two technologies: microbial perchlorate reduction and electrochemical H_2 generation. In this Section the two technologies are described to provide some technical background to support the proposed laboratory-scale testing program detailed in Section 3.

2.1 Microbial Perchlorate Reduction

The remediation of perchlorate-impacted groundwater using microbial processes is a proven approach that is well documented in technical literature (e.g., Chaudhuri et al., 2002). Different strains of naturally-occurring perchlorate reducing bacteria have been identified where these microbes utilize a variety of electron donors such as H_2, simple organic acids (e.g., formate and acetate) and alcohols, aromatic hydrocarbons, reduced humic substances, emulsified oils, ferrous iron, and hydrogen sulfide (Coates and Achenbach 2004). The H_2-utilizing bacteria can be either autotrophic (i.e., organisms can use inorganic carbon for cell growth) or heterotrophic (i.e., organisms require an external organic carbon source for cell growth). Note that Ucar, et al, 2016 found that heterotrophic bacteria reduced perchlorate twice as fast as autotrophic bacteria.

The enzyme involved in reducing perchlorate to chlorate (and subsequently chlorite) is perchlorate reductase (encoded by the pcrABCD genes), while chlorite is further reduced to chloride by chlorite dismutase (encoded by the cld gene) (De Long et al. 2012). However, chlorate and chlorite are rarely observed to accumulate during perchlorate reduction (Ucar et al. 2016). All identified perchlorate reducers have been shown to reduce chlorate as well, while there are chlorate reducers unable to respire on perchlorate (Coates and Achenbach 2004).

The reference list provided within this work plan provides several examples that demonstrate microbial reduction of perchlorate (e.g. Chaudhuri et al. 2002, Coates 2004, De Long et al. 2012). This list includes current examples where bioremediation of perchlorate-impacted groundwater has been demonstrated within other portions of the NERT RI Study Area (Ramboll 2017; Tetra Tech 2015b). The NERT project example includes the groundwater bioremediation treatability study performed between April 2015 and September 2016 within the vicinity of the City of Henderson (COH) Water Treatment Facility, mid-way between the NERT AWF and Seep Well Field (SWF).

2.2 Electrochemical Hydrogen Generation

The key objective of bioelectrochemical remediation is to generate H_2 at an appropriate rate and amount to promote sustained biological reduction of perchlorate dissolved in groundwater under Site-specific conditions. As introduced in Section 1.1, H_2 is readily produced electrochemically through the hydrolysis of water whereby H_2 is produced at the cathode and O_2 is produced at the anode according to:

$$2\ H_2O \rightarrow O_2 + 4\ H^+ + 4e^- \qquad \text{(Eq. 1)}$$

$$2\ H^+ + 2e^- \rightarrow H_2 \qquad \text{(Eq. 2)}$$

Figure 1 shows how these two reactions are central to the concept of electrochemical water treatment, which is the foundation of the bioelectrochemical approach. Oxidation and reduction of contaminants can be coupled with the anodic production of O_2 or the cathodic production of H_2, respectively. Figure 1 also shows concurrent cathodic

reduction of inorganic carbon naturally present in the aquifer, such as bicarbonate (HCO_3^-), to form low-molecular weight organics such as formate and acetate (Hara et al. 1995, Jitaru et al. 1997). While some contaminants require an oxidative pathway, perchlorate and chlorate require a reductive pathway, one that is mediated by perchlorate-reducing bacteria.

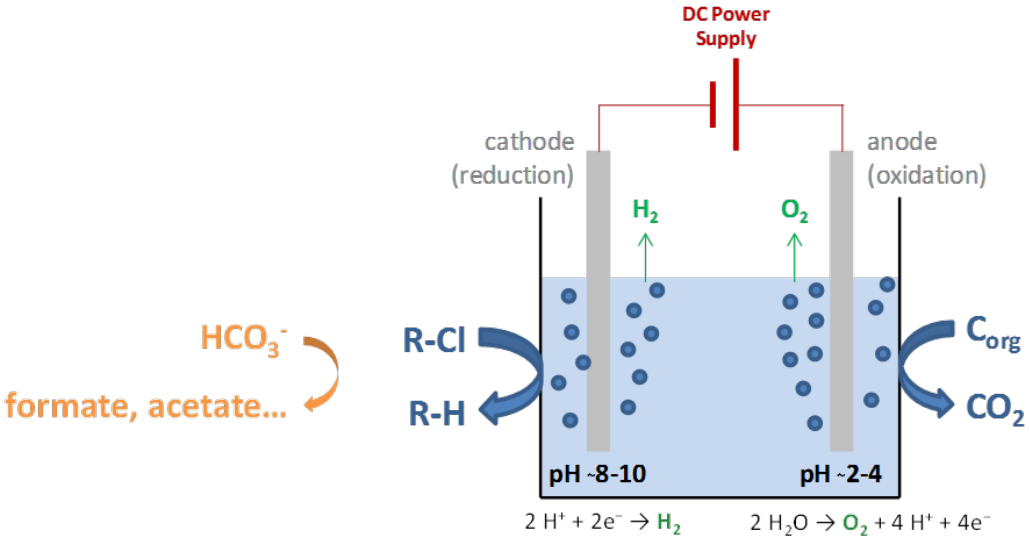

Figure 1: Conceptual process of electrochemical water treatment, showing concurrent cathodic reduction of inorganic carbon

Using the generated H_2 as the required electron donor, electrochemical generation of H_2 is combined with the microbial reduction of perchlorate to create the bioelectrochemical treatment technology (Figure 2). The viability of this combined treatment technology has been demonstrated in the laboratory by several studies (Thrash et al. 2007, Wang et al. 2014, Sevda et al. 2018). Sun et al. also demonstrated bioelectrochemical treatment of chlorinated compounds (2010) and nitrobenzene (2012) in-situ with a reactive cap delivery system. Additionally, CSU has previous experience with the bioelectrochemical treatment technology for other aqueous-phase contaminants such as 1,4-dioxane and chlorinated ethenes (Jasmann et al. 2017).

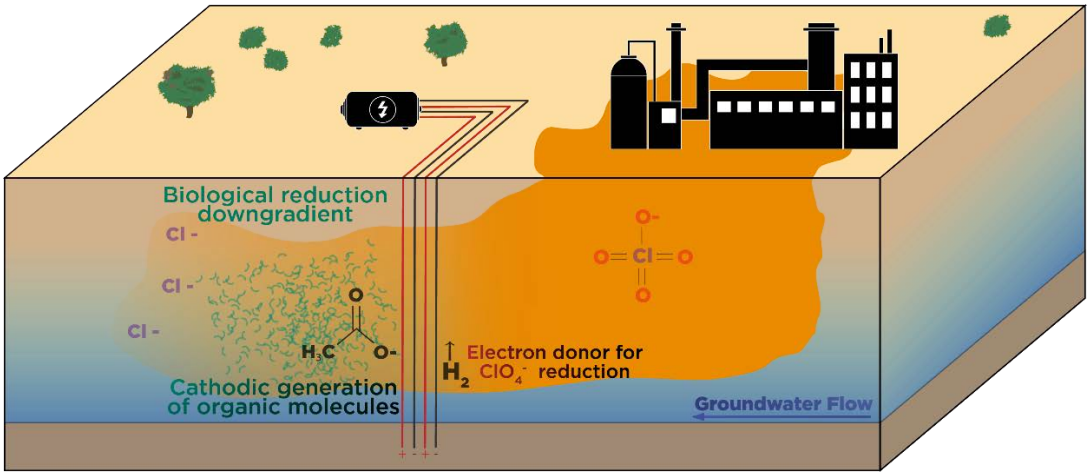

Figure 2: General conceptual model of bioelectrochemical treatment technology

The potential benefits of in-situ H_2 generation to promote biological perchlorate reduction over other electron donors have previously been documented in the *Galleria Road ZVI-Enhanced Bioremediation Treatability Study Work Plan* (Ramboll 2017) and include long-term release of electron donor and the potential for enhanced efficiency due to avoiding the growth of non-target bacteria competing for electron donor. The benefits of bioelectrochemical treatment are similar, but may also include additional potential benefits such as the ability to adjust H_2 generation to match variations in the influent contaminant composition and flux resulting in a modular and flexible system design, and offering more resilience to periodic dry conditions. The eventual effectiveness and implementability of the technology largely depends on water quality conditions (e.g., dissolved solids content and specific electrical conductivity), spatial delivery, and ability to limit electrode passivation (chemical formation of a thin unreactive coating on the electrode surface) and aging. The specific objectives of the testing program described herein are intended, in part, to assess the potential of realizing these benefits and limiting negative impacts under Site conditions.

Electrochemical H_2 generation can provide sustainable, long-lasting production of H_2 at relatively low direct power inputs. The electrodes to be used in this treatability study are dimensionally stable. While these electrodes do have a finite service life and may become passivated over time due to secondary mineral precipitation, previous field installations by the CSU project team have shown sustained operation over time periods of over 2 years (Sale et al. 2010a,b) at potentials that were much higher than are intended to be applied in this treatability study.[2] Electrode service life is expected to be significantly increased when applied to H_2 generation. The longevity of in-situ H_2 generation allows these systems to be implemented with low operation and maintenance (O&M) costs, low environmental footprints, and limited land disturbance and required access. Therefore, if demonstrated effective in this application, bioelectrochemical

[2] The study (Sale et al. 2010a,b) was ended at 770 days and the electrodes were still operating. The studied process was aimed at organic contaminant degradation, which happens at much higher potentials than H_2 generation. For H_2 generation, scaling is expected to be significantly reduced and service life of electrodes is expected to be significantly increased.

treatment could provide a highly cost-effective long-term treatment for perchlorate and chlorate in groundwater with negligible impact to future land use.

Similar to ZVI, electrochemical H_2 production alone may be sufficient to support autotrophic bacteria, which uses inorganic carbon naturally present in groundwater for metabolic synthesis of biomass. However, a potential major benefit of electrochemical treatment is concurrent cathodic reduction of inorganic carbon naturally present in the aquifer to form low-molecular weight organics such as formate and acetate (Figure 1) (Hara et al. 1995, Jitaru et al. 1997), which in turn may also support biological mediated perchlorate reduction as a supplement to available H_2 or when H_2 becomes limiting (Zhao et al. 2011). This synergistic effect of promoting both autotrophic and heterotrophic perchlorate reducers could make for a more robust biological community capable of higher perchlorate reduction rates compared with an autotrophic-only system.

A potential limitation to effective, sustained application of electrochemical H_2 generation, and one that will be evaluated during the course of laboratory-scale testing and future field testing, is mineral precipitation and subsequent passivation of the electrodes. The hydrolysis process can result in steep pH gradients in the immediate vicinity (within millimeters) of the electrodes (Aoki et al. 2013). While the pH gradients attenuate rapidly and generally not an issue for the bulk groundwater system, the pH gradient immediately surrounding the electrodes may lead to a carbonate shift and the occurrence of secondary mineral precipitation, particularly at the cathode terminal of the electrode array (Sale et al. 2010a). The chemical composition of groundwater (due in part to the presence of calcite, gypsum, and dolomite in the aquifer matrix) will be evaluated during treatability testing to assist with predicting the precipitation potential for this method. The testing also will provide information that can be used to design mitigation measures that would reduce the potential for mineralization at the electrode surface. Mitigation measures include polarity reversals which can be easily automated, pH adjustment, and sonication.

3. LABORATORY-SCALE STUDY DESIGN

3.1 Overview

The following sections describe the proposed laboratory-scale testing program, which will serve as a proof-of-concept study and provide information necessary for potential future field testing and implementation. Groundwater and soil samples from the Site will be used, to the extent practicable, for the experiments outlined in this Work Plan.

As mentioned in Section 1.1, the testing program consists of the following steps:

1. Batch reactor testing
2. Column reactor testing, and
3. Sand tank prototype testing.

Detailed specific objectives for each experimental stage are given in the relevant sections that follow.

3.2 Analytical Methods

Table 1 provides an overview of analytical methods that will be applied in the course of the laboratory-scale testing. The frequency of sampling and number of samples will be adjusted based on the progress of the tests.

Table 1: Analytical methods for groundwater and soil extract characterization.

Parameter	Method	Task
Anions (perchlorate, chlorate, chlorite, chloride, nitrate, nitrite, formate, acetate, sulfate, phosphate, etc.)	Ion chromatography	Baseline characterization, Batch, Column, Sand tank
Cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, etc.)	Ion chromatography	Baseline characterization
Dissolved H_2	Gas chromatography coupled with thermal conductivity detection	Batch, Column, Sand tank
Total inorganic carbon (TIC), total organic carbon (TOC)	TOC analyzer	Baseline characterization, Batch, Column, Sand tank
DNA and RNA (perchlorate reductase gene *pcrA*, chlorite dismutase gene *cld*)	Quantitative real-time polymerase chain reaction (qPCR)	Baseline characterization, Column, Sand tank
Microbial community analysis	Next-generation sequencing of 16S rRNA gene	Baseline characterization, Column, Sand tank
Microbial density	Protein concentration	Baseline characterization, Column, Sand tank
Metals (As, Cr, Fe, etc.)	Inductively coupled plasma mass spectrometry	Baseline characterization, Column, Sand tank
Protein biomarkers	2-dimensional liquid chromatography-tandem mass spectrometry	Column
Process parameters (pH, electrical conductivity, dissolved oxygen, ORP)	Various common laboratory-grade probes	Batch, Column, Sand tank

Notes:

For analysis of anions, soil samples will undergo sequential extraction with deionized (DI) water followed by centrifugation.

DNA will be extracted with the DNeasy PowerSoil Kit; RNA will be extracted via the RNeasy PowerSoil Total RNA Kit (both manufactured by Qiagen).

3.3 Site Soil and Groundwater

Site soil and groundwater samples for use in the laboratory testing program will be collected by Ramboll and shipped to CSU. At CSU, samples will be homogenized in an anoxic chamber under the exclusion of oxygen. Samples will be stored in a refrigerator at 4 degrees Celsius and will be analyzed for a baseline characterization, as identified in Table 1.

3.4 Batch Reactor Testing

Batch reactor testing will be performed to evaluate the electrochemical generation of H_2 and organic by-products. A divided electrochemical reactor will be designed in which the anode (and electrolytic fluids) is isolated from the cathode (and electrolytic fluids). The efficiency of the cathodic H_2 (and organic compounds) generation process will depend on various factors, such as water quality, electrode type, polymer-electrolyte membrane properties, and power input. Thus, the divided electrochemical reactor will be operated to evaluate the effects of these factors as described below.

3.4.1 Specific Objectives

The specific objectives of the batch testing are as follows:

- Test different membrane and electrode materials with respect to H_2, formate, and acetate generation rates, as well as energy efficiency;
- Obtain initial cathode surface area-normalized H_2, formate, and acetate generation rates that will also inform subsequent column testing design;
- Measure the mass flow rate of electrolytically generated H_2;
- Establish a pH range conducive to the desired microbial activity and contaminant reduction rate; and
- Evaluate membrane and electrode longevity.

Furthermore, this reactor will be used to generate feed for subsequent column testing.

3.4.2 Materials and Methods

A closed batch reactor will be built from acrylic plastic similar in design to previous models built at CSU (Figure 3) with a total volume of approximately two liters (final dimensions will be based on initial characterization). The reactor will house an anode, cathode and membrane, and hold ports for pH and conductivity measurements, gas venting and collection in Tedlar bags, and withdrawal of liquid phase (i.e., samples or column feed if used in continuous mode).

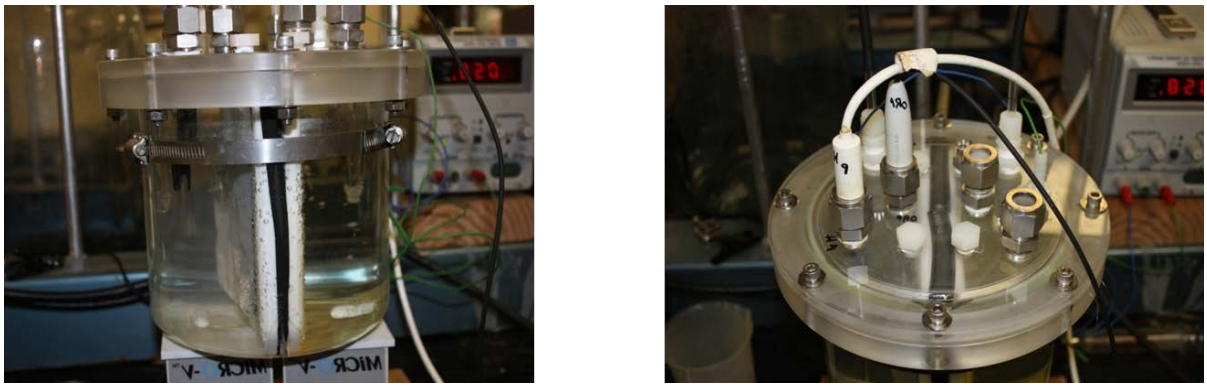

Figure 3: Example of a divided electrochemical batch reactor built at CSU.

The cell membrane will be composed of Nafion, a polymer that has superior conductive properties at high thermal and mechanical stability (Kraytsberg & Ein-Eli 2014, and references therein).

Two types of mesh electrodes will be tested. The first type is Grade 1 titanium (Ti) as a durable, low-cost alternative. The second type is titanium coated with a mixed metal oxide (Ti-MMO), specifically IrO_2/Ta_2O_5. These electrodes are more expensive than pure Ti yet reach a higher current density and thus H_2 generation rate. A key objective of the testing program is to identify an appropriate H_2 generation rate for promoting the necessary microbial activity and thus using that information to select a cost-effective electrode material.

3.4.3 Batch Operation and Analyses

The membrane and electrode materials will be evaluated in various combinations within the divided electrochemical batch reactor. The cathodic chamber will be filled with Site groundwater, the anodic chamber with 0.2 molar (M) sodium sulfate electrolyte (i.e., equal to or greater than the electrical conductivity of the groundwater catholyte). Evaluation of each membrane/electrode combination will occur by applying direct current under potentiostatic conditions (i.e., constant potential). The power supply used (a.k.a. potentiostat) measures both voltage and current, but both will be verified independently with a multimeter. Power input will be determined by multiplying total current with resulting voltage. The rate of H_2 evolution will be assessed by measuring the volume of gas collected in a Tedlar bag over a certain amount of time (assuming that the concentration of dissolved H_2 is negligible based on reactor volume and relatively low aqueous solubility of H_2). Taken together, these data will allow for quantification of both cathode surface area-normalized and energy input-normalized H_2 generation rates. One combination of membrane and electrode materials will be chosen based on the testing results (and possibly other experimental observations such as membrane stability) to proceed with more detailed testing.

Next, the chosen membrane/electrode combination will be installed in the batch reactor and groundwater will be pumped through the reactor at a minimum of three volumetric flow rates at constant current (to be determined based on measurements in previous experimental evaluations). Aqueous phase H_2 informs about saturation levels for subsequent column testing, and thus will be measured. Any H_2 produced in excess will be captured in the Tedlar bag, allowing for a calculation of total H_2 produced. Anion concentrations will be tracked to quantify formate and acetate concentrations and quantify any (unanticipated) losses of perchlorate, chlorate and nitrate due to microbial activity (as their direct cathodic reduction is likely negligible). These data will allow for determination of H_2, formate and acetate generation and mass flow rates. Changes in pH and electrical conductivity will be recorded to ensure conditions conducive to microbial proliferation. Finally, total inorganic carbon (TIC) and total organic carbon (TOC) will be tracked throughout the experiments. TOC will serve to check whether any significant concentrations of other organic molecules besides formate and acetate are produced. TIC will serve to evaluate carbonate precipitation within the reactor, and thus indicate the potential for electrode passivation (and possibly membrane clogging). An additional indicator of electrode passivation is a decreasing potential at constant current. Thus, these parameters will be closely tracked throughout the batch testing and the following tests.

3.5 Column Testing

Flow-through column testing will be performed to demonstrate the general feasibility of bioelectrochemical perchlorate reduction under dynamic conditions. The information from the column testing will create the foundation for subsequent technology design and scale-up. Site soil will be used in these experiments.

3.5.1 Specific Objectives

The specific objectives for the column experiments are as follows:

- Quantify perchlorate, chlorate, and nitrate (competitive) reduction rates as well as H_2 oxidation kinetics at representative flow rates (to be determined);
- Test the effects of cathodically produced organics; and
- Elucidate impacts on microbial community dynamics.

3.5.2 Materials and Methods

Three column reactors will be built from clear PVC and run in parallel at ambient temperature. Similar to columns previously built and used at CSU (Figure 4), the columns will be equipped with ports for sampling of both liquid and solid samples along the flow path, as well as liquid samples at the inlets and outlets.

All columns will be filled with Site soil. Groundwater from the Site will be used if practical to do so based on volume requirements. If synthetic groundwater is to be used, it will be prepared similar to the original Site groundwater in ionic composition, alkalinity, pH and electrical conductivity (to be determined based on initial baseline testing).

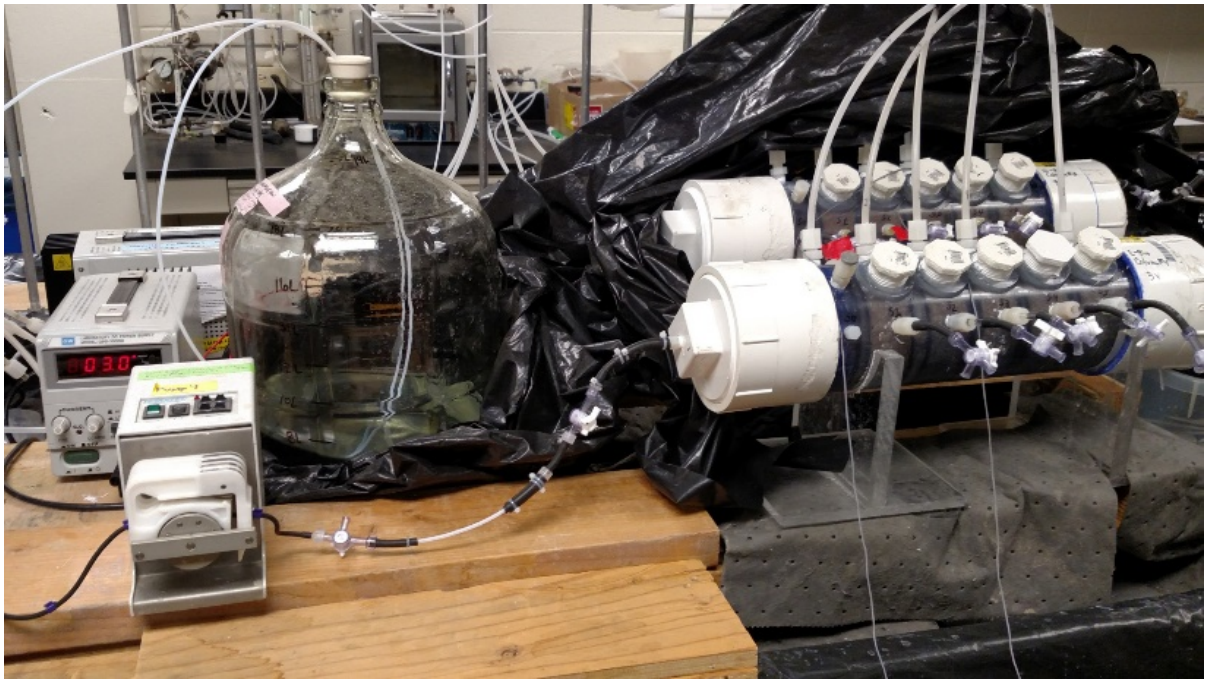

Figure 4: Example of bioelectrochemical column reactors built at CSU.

Two sets of column experiments will be run to compare perchlorate reduction rates under H_2 addition only (autotrophic) and under cathodic generation of H_2 and organics (mixotrophic), to understand the impact of different carbon sources. In the first set, water will be saturated with H_2 in a high-pressure equilibration vessel and supplied to the columns. In the second set, H_2 and organic compound-enriched feed will be generated in the batch reactor (variations in concentration will be taken into account and adjusted as necessary) and used as column influent.

3.5.3 Column Operation and Analyses

Flow-through column testing will be operated to demonstrate the feasibility of bioelectrochemical perchlorate reduction under dynamic conditions. The electron donor-enriched feed will be pumped through the columns at flow velocities within a range representative of Site conditions. Depending on the final dimension of the columns, Site-specific flow velocities, and reduction kinetics observed in previous experimental stages, liquid samples will be taken either along the flow path or at the column effluent (to be determined following batch testing) at varying flow velocities to determine electron acceptor reduction kinetics and electron donor oxidation kinetics. Throughout the experiment, aqueous samples will be analyzed for anions (perchlorate, chlorate, chlorite, chloride, nitrate, nitrite, formate, and acetate), dissolved H_2, TOC, TIC, and process parameters (pH, electrical conductivity, dissolved oxygen, oxidation-reduction potential) and microbial densities (via measurement of protein concentrations). Each setup will be run until four consecutive sampling events indicate steady-state conditions (±15 % variation), which will be used to obtain final kinetic data for each scenario.

In an effort to establish a desired target microbial community profile for subsequent tests, five core samples in triplicate along the column will be taken at the end of one select H_2 only and one select H_2 & organics experiment under steady-state conditions to evaluate microbial community dynamics. One sample representing (unstimulated) starting conditions will be collected and the DNA and RNA will be extracted and evaluated for the perchlorate reductase (pcrA) and chlorite dismutase (cld) genes using qPCR and reverse transcription qPCR (RT-qPCR) (to amplify the DNA and RNA in order to identify whether the target genetic sequences are present). Additionally, a molecular biomarker for autotrophic growth (cbbL/cbbM genes) will be monitored to validate that the generated H_2 is used as an electron donor for perchlorate reduction. This will provide information to refine the target H_2 production rate for subsequent tests. Furthermore, a total of eleven samples (one from the original Site soil, five from each of the H_2 only vs. H_2 & organics column experiments) will be chosen to perform microbial community analyses via next-generation sequencing of specific RNA segments to provide lines of evidence regarding the relative contribution of autotrophs vs. heterotrophs.

Finally, proteomic analyses (a technique to measure of protein abundance, which is the directly measured parameter that is used as an indicator for enzymatic activity) will be performed as a direct measure for autotrophic vs. heterotrophic activity. While DNA and RNA testing may provide information on organisms present and active, respectively, only proteomic analyses provide direct information on enzymatic activity. Several factors may influence microbial community composition resulting in functional redundancy and gene expression. Therefore, direct assessment of protein abundance for specific functions coupled with measuring particular metabolic byproducts results in an improved understanding of microbial function and activity, potentially enhancing stoichiometric and

kinetic models and yielding valuable insights for process monitoring, optimization, and troubleshooting. In combination, these analyses will help understand microbial dynamics in this system and aid in future field-scale technology design, implementation, and operation. Lastly, there is one planned sampling event of microorganisms present in the column solids to take place under steady state, representative conditions near the end of the column testing, which will undergo proteomic analyses.

3.6 Sand Tank Prototype Testing

After demonstrating the feasibility of bioelectrochemical perchlorate reduction under Site conditions and determining the kinetics of the reaction(s), a larger-scale sand tank prototype test will be conducted. Sand tanks of various sizes and geometries are available at CSU (Figure 5). The overall objective of the sand tank testing is to test scale-up performance, distribution/delivery effects, and dynamic effects in a two-dimensional system. The sand tank testing will provide data necessary to implementing a future field-scale treatability study.

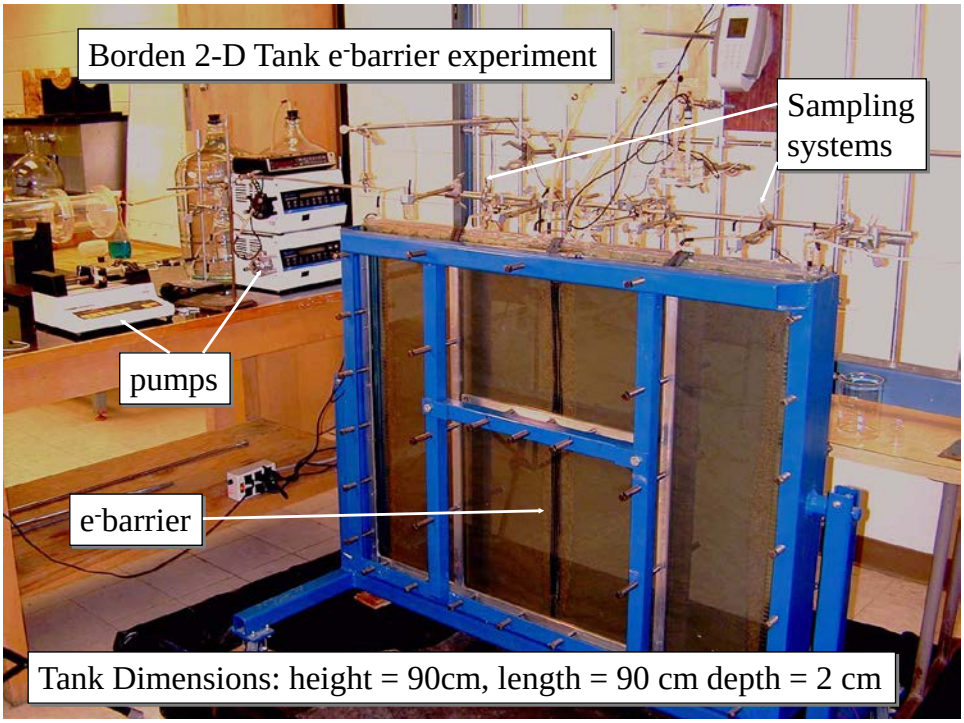

Figure 5: Example of a two-dimensional sand tank setup at CSU.

Supplementing the column testing, which is a one-dimensional test intended to quantify reaction kinetics and mechanisms, the sand tank prototype testing provides a small-scale setup closer in design to a potential future field-scale treatment testing. In addition to providing a two-dimensional scale-up of the column testing test conditions (allowing data pertaining to fate and transport of generated and infiltrated electron donors to be collected), the sand tank prototype testing will evaluate a proposed delivery system for the bioelectrochemical treatment. Implementation of bioelectrochemical treatment at a

Site can be based on a variety of designs; for instance, as a permeable reactive barrier, funnel-and-gate, or modular H2 generator coupled with gas sparging, although the exact design(s) to be evaluated will be chosen later. The primary drivers of the delivery system design, the performance of which will be tested in the sand tank prototype testing, will be data from the batch and column testing and expected Site conditions.

A final design of the sand tank prototype test will be developed based on expected Site conditions and findings of previous experiments (i.e., mass flow rates, reduction rates, impact of organic substrates). The preliminary concept is to run this prototype test for several weeks with two-dimensional spatial analysis of anions (perchlorate, chlorate, chlorite, chloride, nitrate, nitrite, formate, and acetate), dissolved H_2, TOC, TIC, microbial density, and process parameters (pH, electrical conductivity, dissolved oxygen, oxidation-reduction potential). The relative abundance and activity of perchlorate reducers will be measured (qPCR and RT-qPCR of pcrA and cld) as in the column testing, and microbial community structure will be assessed for a subset of the samples in order to understand how changing hydraulic and chemical conditions effect the microbial community structure and function. The data collected during the sand tank prototype test will inform the design of a subsequent field test (not included in this Work Plan) including the H_2 distribution/delivery mechanisms to be tested and the range of conditions appropriate for testing the technology.

4. REPORTING

Monthly status updates will be provided to the Trust and NDEP/EPA summarizing the progress and preliminary results of the laboratory-scale testing. Substantive changes to the schedule or scope of the laboratory-scale testing program will be presented in the monthly updates for approval. In addition, nearing the completion of the column testing, a proposed plan for performing the sand tank prototype testing will be presented in the monthly updates.

Following completion of the work described in Section 3, a report summarizing the laboratory-scale testing program will be prepared and submitted to NDEP/EPA. This report will include:

- Summary of analytical results collected for the laboratory-scale testing program;
- Summary of the laboratory-scale testing data evaluation and interpretation, including evaluation of the batch reactor testing, column testing, and sand tank prototype testing; and
- If warranted based on laboratory-scale testing results, a field test conceptual design and location (to be determined) will be included as an appendix to the report.

5. SCHEDULE

The schedule for the laboratory-scale testing is outlined in Table 2. Pending work plan approval and collection of Site groundwater and soil, the study is scheduled to begin in May 2018.

Table 2: Project schedule.

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9
Baseline Characterization	■								
Batch Testing	■	■	■	■					
Column Testing		■	■	■	■	■			
Sand Tank Studies						■	■	■	■
Progress Reports	■	■	■	■	■	■	■	■	
Field Study Workplan									■

Considering the laboratory work would extend through 2018, it is anticipated that the construction of a field test system could happen in early 2019 (if warranted). Subsequent field testing and performance monitoring would begin in the spring of 2019, to be concluded in September 2019.

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