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IN SITU BIOREMEDIATION OF PERCHLORATE IMPACTED GROUNDWATER

FINAL TECHNICAL REPORT

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1. PROJECT BACKGROUND

Groundwater contamination related to the production, handling and use of rocket propellants such as ammonium perchlorate has been identified as a widespread problem at United States Department of Defense (DOD), Department of Energy (DOE), National Aeronautic and Space Administration (NASA) and defense contractor facilities. Based on Environmental Protection Agency (EPA) reports, perchlorate has been manufactured, handled and/or used in 44 states, and has been detected in groundwater or surface water in at least 15 states (U.S. EPA, February 1999). The majority of these sites are rocket manufacturers and testing facilities associated with DOD and NASA.

The states of California, Nevada and Utah have established a provisional action level (PAL) of 18 μg/L for perchlorate in drinking water, the presence of which requires the timely development of robust, reliable and cost-effective treatment technologies for large volumes of perchlorate-impacted groundwater. In California alone, perchlorate has been detected in more than 51 public water supply systems, including 20 that exceed the PAL. Nationwide cleanup costs for perchlorate-impacted groundwater are expected to be in the billions of dollars, the costs of which may jeopardize major DOD and propulsion contractor production programs and mission readiness.

Remediation of perchlorate-impacted groundwater is made difficult by the physical and chemical properties of perchlorate. In groundwater, perchlorate is fairly unreactive, particularly at low concentrations (Urbansky, 1997, 1998). It does not sorb strongly, and tends to behave in similar fashion to chloride, which is often considered a conservative tracer. Ion exchange, reverse osmosis, electrochemical reduction and bioremediation have been identified as potential technologies for treating perchlorate-contaminated groundwater. Of these, bioremediation appears to be the most promising and cost effective technology as it has the potential to transform perchlorate to innocuous end products (see **Figure 1**) rather than transferring it to another waste stream (e.g., impacted resin or brine) that requires further treatment or disposal. Ex situ

bioreactors are currently being used at various facilities to treat perchlorate-impacted groundwater for the purposes of plume migration control. However, this approach does little to address continuing perchlorate impacts to groundwater from source areas, or to reduce the overall duration and cost of remedial efforts at perchlorate-impacted sites. By comparison, in situ bioremediation may provide a cost-effective approach for both containing perchlorate plumes, as well as destroying the perchlorate source areas that are driving these expansive plumes at many DOD and related sites.

Previous research (e.g., Coates et al., 1999; Logan et al., 1998; Cox et al., 2000) supports the potential for in situ bioremediation to remediate perchlorate-impacted sites. However, more information is needed regarding the ubiquity of perchlorate-degrading bacteria and their geochemical tolerances to assess the wide-spread applicability of in situ bioremediation as a technology for DOD use. On this basis, SERDP funded multiple research projects to evaluate the ubiquity of perchlorate-degrading bacteria in the environment and to assess the widespread applicability of in situ bioremediation for perchlorate impacted groundwater at DOD and related facilities. This report provides a compilation of the research program conducted by GeoSyntec Consultants (GeoSyntec) and the University of Toronto (UT) to address the technology information needs for in situ bioremediation of perchlorate.

2. RESEARCH PROGRAM OBJECTIVES

The overall goal of our research program was to develop a robust, reliable and cost-effective in situ treatment technology for groundwater containing perchlorate and possibly other common co-contaminants such as nitrate and chlorinated solvents. To address this goal, research was conducted to address the following specific project objectives:

1. Evaluate the ubiquity of perchlorate-degrading bacteria in subsurface environments at various impacted DOD and/or defense contractor facilities, and identify bacteria responsible for perchlorate reduction at each test site (Task 1);

2. Evaluate the capabilities of varying isolated perchlorate-degrading bacteria, and assess process applicability over a range of groundwater conditions (e.g., perchlorate concentrations, high nitrate or sulfate environments) to identify factors that promote and sustain, or limit/inhibit, successful perchlorate bioremediation (Task 2);

3. Evaluate process suitability for treatment of mixed contaminant plumes containing perchlorate and chlorinated solvents (Task 3); and

4. Conduct a small-scale field pilot test to demonstrate that perchlorate can be biodegraded under field conditions at a candidate test site having groundwater impacted by perchlorate, and generate initial design and cost data for technology demonstration and validation under a follow-on DoD program (Task 4).

To accomplish these objectives, our research program was divided into laboratory and field components. The laboratory component (Tasks 1 through 3) was conducted primarily by UT, while the field component (Task 4) was conducted primarily by GeoSyntec. The following sections present the technical approach & project accomplishments for each of these components of the overall program.

3. LABORATORY PROGRAM: TECHNICAL APPROACH & PROJECT ACCOMPLISHMENTS

Laboratory microcosm studies were initiated in the summer of 2000 to: i) evaluate the ubiquity of perchlorate-degrading bacteria in subsurface environments; ii) assess the capabilities of perchlorate-reducing bacteria in subsurface environments of varying geochemistry; and iii) assess the potential for joint treatment of perchlorate and common co-contaminants. The laboratory studies were conducted by Dr. Elizabeth Edwards at UT. Theses studies were completed in December 2001. The following subsections summarize the technical approach and methodology of the laboratory component of the research program, and present the key accomplishments of the laboratory program.

3.1 Site Selection

Soil and/or groundwater was collected from six different perchlorate-impacted facilities nationwide for use in the laboratory research program. The test facilities were selected following a screening of approximately 20 sites either known or suspected to contain perchlorate. Site selection was based on a variety of criteria, including:

- degree of characterization of perchlorate impacts to soil and groundwater (sites with documented/characterized impacts were preferred);
- site accessibility and on-site or local support for sample collection;
- degree of potential/eventual remedial needs (sites likely to benefit from technology development were preferred);
- ability to fund soil and groundwater collection through in-kind contributions;
- suitability of site conditions and support for future (Task 4) field pilot testing; and
- permission to document/use study results.

Through the screening, the following six test sites were selected for the initial assessment of ubiquity of perchlorate degraders:

1. Aerojet Superfund Site, California
2. Edwards Air Force Base, California
3. U.S. Navy, Allegany Ballistics Laboratory (ABL), West Virginia
4. U.S. Navy, San Nicolas Island (SNI), California
5. Former Perchlorate Manufacturing Facility, Nevada
6. Inactive Rancho Cordova Test Site (IRCTS), California

The locations of the six facilities are shown in **Figure 2**. Brief descriptions of each test site, their remedial needs, and sample collection procedures (soil and groundwater) are provided in Appendix A.

3.2 Assessment of Ubiquity of Perchlorate Degrading Bacteria (Task 1A)

To evaluate the ubiquity/activity of perchlorate-degrading bacteria for each test site, a series of control and electron donor treatment microcosms were constructed. The construction, incubation, sampling and analysis methodology was essentially the same for each test site, with the exception that soil was not available/used for some test sites (as indicated in Appendix A). All soil manipulations were conducted independently in an anaerobic chamber (glovebag) to preserve (as best as possible) the subsurface redox conditions. To construct the microcosms, aquifer material was removed from the core liners, sieved to remove large particulates, and homogenized in a clean, sterile bag. Treatment and control microcosms were constructed by filling 250 mL (nominal volume) sterile glass bottles with 60 g of homogenized aquifer material and 180 mL of related groundwater, allowing some headspace (about 40 mL) for gas (carbon dioxide; CO_2) production. The microcosms were amended with 0.15 ml of resazurin to monitor redox conditions (resazurin is clear under anaerobic conditions but turns pink if exposed to oxygen). Treatment microcosms were amended with either acetate, molasses, oleate or canola oil as electron donors, at approximately 1.5 times the stoichiometric amount required to reduce the starting concentration of perchlorate in the microcosms. Active

controls were not amended with any electron donors, and were used to assess the rate and extent of intrinsic perchlorate reduction in the test site materials. The aquifer material for the sterile control microcosms was autoclaved for 60 minutes at 121°C on three consecutive days and poisoned with mercuric chloride (1 ml of a 5% solution) and/or sodium azide (0.5 ml of a 5% solution). All microcosm bottles were sealed with mininert™ caps to allow repetitive sampling and re-spiking of electron donors or perchlorate, as needed, to sustain metabolic/degradation activities. All treatments and controls were constructed in triplicate.

Table 1 summarizes the starting conditions and test regimes for each test site. Starting perchlorate concentrations ranged from 0.25 to 660 mg/L (both from Site 5 in Nevada). A perchlorate concentration in the range of 100 mg/L was typically tested for each site for comparative purposes. The treatment goal for each test site was generally the PAL of 18 µg/L (0.018 mg/L). Treatment and control microcosms were incubated in the anaerobic chamber for periods varying up to 120 days, depending on the test site and condition. For selected treatments showing successful perchlorate biodegradation, microcosms were re-spiked with perchlorate at higher concentrations (e.g., 500 mg/L) to evaluate potential concentration effects on degradation rates and extents.

For each test site, groundwater samples were collected on a semi-weekly to weekly basis (depending on observed level of activity) from each control and treatment microcosm, and analyzed for perchlorate, chloride, nitrate, nitrite, sulfate, phosphate and acetate. Acetate analyses served to monitor electron donor availability in both the acetate and molasses treatments, as the molasses was typically metabolized to yield acetate, which was subsequently consumed during perchlorate reduction. All anions were measured using a Dionex ion chromatograph (IC) instrumented with an AS16 column and using the perchlorate analysis method provided to UT by Dionex. Method detection limits for the various test sites depended on the sample injection loop size used with the IC, and were generally as follows: Aerojet, 0.010 mg/L; Edwards AFB, 0.018 mg/L; ABL, 0.012 to 0.018 mg/L; SNI, 0.018 mg/L; Site 5, 0.12 to 0.017 mg/L; and IRCTS, 0.015 to 0.018 mg/L.

The following specific conclusions were drawn from these microcosm studies:

1. Perchlorate biodegradation was observed at all test sites, indicating that the distribution of perchlorate-biodegrading bacteria in subsurface environments is widespread (ubiquitous).

2. Perchlorate biodegradation was stimulated/accelerated through the addition of a range of inexpensive organic carbon-based electron donors, including acetate, molasses, oleate, and canola oil.

3. Perchlorate biodegradation was observed over a wide range of starting concentrations, from as low as 0.25 mg/L to in excess of 660 mg/L.

4. Biodegradation typically reduced perchlorate concentrations below the PAL of 0.018 mg/L, making in situ bioremediation an appropriate technology for site remediation.

5. Biodegradation half-lives generally ranged from 1 to 7 days in treatment microcosms from the various sites (**Table 2**).

6. Short acclimation periods (i.e., days to weeks) were observed before onset of perchlorate reduction at most sites, likely related to the time required to deplete available oxygen in the microcosm groundwater, and in some cases, the time to reduce nitrate concentrations.

7. Nitrate reduction typically occurred prior to the onset of perchlorate reduction. At some sites, these reduction activities appeared to occur together. At other sites, nitrate and perchlorate reduction occurred in strict sequence.

8. Perchlorate reduction preferentially occurred over sulfate reduction at all sites except IRCTS in California, where sulfate reduction occurred prior to or coincident with perchlorate reduction. At the other sites, sulfate

concentrations remained stable and only declined following perchlorate and nitrate depletion, provided that electron donor remained in the microcosms.

Figures 3a to **3i** present the results of the Task 1 ubiquity studies.

3.3 Enumeration of Perchlorate-Reducing Microorganisms (Task 1B)

For each test site showing perchlorate biodegradation activity as part of Task 1A (assessment of ubiquity), microbial enumerations were conducted to estimate the most probable number (MPN) of perchlorate-reducing bacteria per unit groundwater. Enumerations were conducted using a three-tube MPN technique, in liquid medium, using acetate as the only added electron donor and perchlorate as the only added electron acceptor.

The results of the MPNs are presented in **Table 3,** expressed as the number of acetate-oxidizing, perchlorate-reducing organisms per milliliter (mL) of groundwater or gram (g) of soil. **Table 3** shows that organisms capable of reducing perchlorate were detected at most sites. Microbial counts for groundwater ranged from 150 to 2.3×10^6 organisms per mL of groundwater. These results are in general agreement with MPN values estimated by Coates et al. (1999). Of note, the sites with the lowest MPNs were the pristine site in Ontario and the source area site (>600 mg/L perchlorate) at the Former Perchlorate Manufacturing Facility in Nevada. Given that only one pristine site was evaluated for this study, it is difficult to determine whether prior exposure to perchlorate can be correlated with higher MPNs. With the source area site, it is possible that the elevated concentrations of perchlorate and other anions (chloride, sulfate, chlorate, nitrate) may be inhibitory, resulting in lower microbial biomass. However, perchlorate reduction was observed in microcosms from this source site (**Figure 3e**), indicating that the perchlorate reducers are active, despite low numbers.

3.4 Isolation & Identification of Perchlorate-Reducing Microorganisms (Task 1C)

Following enumerations, attempts were made to isolate perchlorate-reducing microorganisms from each site. Microbial colonies were isolated using a two generation plate-to-tube methodology. Samples from microcosms were streaked onto agar plates prepared with a standard medium containing 2.5 mM sodium acetate and 1 mM sodium perchlorate. Plates were poured under aerobic conditions and subsequently incubated and manipulated in an anaerobic glovebox. Growth of colonies on the plates was typically observed within two weeks, whereafter, colonies were transferred to medium containing perchlorate (1 mM) and acetate (2.5 mM). Following complete degradation of the perchlorate, a sample of the culture was re-streaked on new plates and resulting colonies were transferred to liquid medium (as above). Total genomic DNA was then extracted from the second generation cultures using standard procedures, and the 16sRNA was amplified using Polymerase Chain Reaction (PCR) techniques. Sequencing of the PCR products was conducted at the Ontario Cancer Institute's sequencing facility (Princess Margaret Hospital, Toronto, Ontario) using a Beckman Coulter CEQ 2000 automatic sequencer. The PCR primers 27f and 1492r were used as the sequencing primers. For the eight isolates that were included in subsequent studies, internal primers were designed to determine the entire sequence. A forward primer was designed to anneal with the 16s rDNA of organisms in the Azospirillium genus at base pair number 581 of Azospirillium lipoferum (X79730). Another primer was designed to anneal with sequences of organisms related to the Dechloromonas genus at base pair 567 of Dechloromonas SIUL (AF170356). **Table 4** summarizes the primers used for these activities.

The results of the isolations and identifications indicate that all of the organisms isolated belong in the Beta or Alpha subgroup of the proteobacteria class. **Figure 4a** presents a phylogenetic tree of organisms isolated for this study, referred to by Site name. For comparison, **Figure 4b** presents a phylogenetic tree of organisms within the

proteobacteria class isolated through previous studies (e.g., Coates et al., 1999). Most of the organisms isolated during this study were found to be either closely related to the Azospirillium genus or the Dechloromonas genus. Although previous researchers have isolated organisms from the Beta subclass, only one organism related to Azospirillium lipoferum has been previously documented. The relatively large proportion of isolates related to A. lipoferum isolated in this experiment could be due to biases in the isolation or amplification procedure. Of note, an evolutionary distance estimation indicates that there is an 18% difference in the sequences of the Azospirillium genus and the Dechloromonas genus. However, within the groupings there were much smaller differences between sequences. For example, within the Dechloromonas groups, there was a 1.5% maximum difference between the organisms, while there was a 0.9% difference within the Azospirillium.

3.5 Geochemical Tolerances and Capabilities of Perchlorate-Degrading Bacteria (Task 2)

A variety of studies were conducted using both isolates (from Task 1) and mixed perchlorate-degrading cultures, to assess the basic metabolic capabilities of perchlorate-degrading bacteria, and their geochemical tolerance ranges (e.g., perchlorate concentration ranges, competing electron acceptor concentrations) in simulated groundwater environments. The following subsections describe these activities.

3.5.1 Electron Acceptor Utilization Trials - Isolates

Experiments were conducted to examine the ability of the isolates to use common groundwater anions other than perchlorate as electron acceptors, including sulfate, nitrate and ferric iron. **Table 5a** summarizes the treatments that were constructed for each isolate. Results of this trial are summarized in **Table 5b**.

The results lend support to the hypothesis that perchlorate degrading organisms have a limited range of electron acceptors that they can use. Five of the seven organisms isolated could effectively reduce nitrate. All isolates related to the Dechloromonas species reduced nitrate, including the Edwards AFB isolates, which are closely related to the Dechloromonas strains NM and CL, which cannot utilize nitrate (Coates *et al*., 1999). However, two of the Azospirillium isolates (from SNI and the perchlorate manufacturing site source area) could not reduce 10 mM nitrate. The seven isolates generally could not reduce sulfate or ferric iron. These results are in agreement with the findings of Coates et al. (1999).

3.5.2 Perchlorate Concentration Thresholds - Isolates

Experiments were conducted to determine perchlorate concentration thresholds for the various isolates in terms of biodegradation, to assess differences in the capabilities of the various perchlorate-degrading microorganisms. **Table 6a** summarizes the treatments that were constructed for each isolate. Results are summarized in **Table 6b**.

Results of the experiment indicate that all of the isolates tested can reduce perchlorate concentrations that are relevant to groundwater contamination. The highest groundwater concentration reported for the various test sites was 7 mM. However, higher concentrations were tested to determine if certain isolates may be more suitable to treat concentrated brine solutions from ion exchange systems, or to degrade diluted effluent from rocket wash water. All of the isolates could reduce perchlorate concentrations up to 20 mM. Furthermore, isolate IRCTS Alpha can reduce perchlorate concentrations of 50mM. Interestingly, the perchlorate concentrations at IRTCS were not significantly higher than other sites from which these organisms were isolated. None of the isolates could degrade perchlorate concentrations of 75 mM.

For comparison, it has been reported that Wolinella succinogenes HAP-1 can degrade 77.5 mM (Wallace, 1996), and strain CKB can reduce up to 20 mM (Coates, 1999). The data from this trial suggest that in situ bioremediation should be an appropriate remedial approach for perchlorate source areas, where concentrations are commonly in the hundreds of mg/L and in some cases in the 1000s of mg/L.

3.5.3 Salt Tolerance - Isolates

Limited experiments were conducted to evaluate whether the isolates can biodegrade perchlorate in environments with elevated salt concentrations. To conduct these trials, isolates were transferred into medium containing 1% sodium chloride (172 mM), 250 mg/L perchlorate (2.5 mM), and 295 mg/L acetate (5 mM).

Results indicated that none of the isolates tested could degrade perchlorate in medium with a 1% NaCl concentration. However, these data are in apparent contrast to data recently presented by Logan (2001), showing perchlorate reduction ability in selected cultures in excess of 10% salinity.

3.5.4 Nitrate, Perchlorate and Sulfate Use – Mixed Cultures

During the Task 1 microcosm studies, the concentrations of perchlorate and potentially competing electron acceptors were monitored over time. Review of these data allows development of preliminary conclusions on the order and rate of use of the different electron acceptors. The initial concentrations of the different electron acceptors in microcosms from the different sites, and the order of electron acceptor use observed is shown in **Table 7**. Based on these data, there were two different types of apparent interactions between nitrate and perchlorate reduction.

- *Simultaneous reduction of nitrate and perchlorate:* At sites 1 and 3, nitrate and perchlorate reduction appeared to occur simultaneously.

- *Nitrate reduction before perchlorate reduction:* At sites 4, 5a, 5b, 5c, 6a and 6b, nitrate reduction was consistently observed prior to perchlorate reduction.

Three different types of interactions were observed between sulfate and perchlorate reduction.

- *No sulfate reduction:* No sulfate reduction was observed over varying incubation periods in microcosms from sites 3, 5a and 5b. These sites had sulfate concentrations ranging from 0.5 mM to 17 mM.

- *Sulfate reduction after perchlorate reduction:* This reaction, which was expected to be the default sequence based on energy yields of the various reactions, was observed in microcosms from sites 1, 2, 5c and 6b.

- *Simultaneous reduction:* Simultaneous sulfate and perchlorate reduction was observed in microcosms from sites 4 and 6a. These sites had varying concentrations of both perchlorate and sulfate.

Based on these data, nitrate reduction consistently occurs prior to or simultaneous with perchlorate reduction. From a thermodynamic point of view, the reduction of perchlorate and nitrate yield similar amounts of energy. Furthermore, most perchlorate-degrading organisms are denitrifying facultative anaerobes (Logan, 1998). Therefore, any electron donor delivery system designed to promote perchlorate reduction will have to consider the electron donor demand placed by nitrate. Similarly, the energy yield of oxygen is slightly better than that of perchlorate and nitrate and so oxygen demand must be included in donor requirements calculations. Fortunately, oxygen concentrations in groundwater are limited by solubility constraints, and so this demand may be small

compared to the demand posed by perchlorate. Nitrate concentrations in groundwater are also generally low and unlikely to pose undue demand on electron donor. However, sulfate concentrations in groundwater at many DoD and related propulsion contractor facilities in the southwest (California, Nevada, Utah, Arizona) are frequently in the hundreds of mg/L and in some cases in the thousands of mg/L. Stimulation of sulfate reduction in an in situ bioremediation system designed to treat perchlorate, as was observed in microcosms at sites 4 and 6a, would be highly undesirable, dominating electron donor demand (which would drive treatment cost), producing odorous hydrogen sulfides, and potentially inhibiting the desired perchlorate biodegradation activity. Of note, in situ bioremediation systems that are designed to supply an abundance of electron donor such as molasses or vegetable oil (independent of perchlorate stoichiometric considerations) may be highly undesirable at perchlorate sites having high sulfate concentrations.

3.6 Joint Biodegradation of Perchlorate and Chlorinated Solvents (Task 3)

A series of laboratory experiments were conducted to assess the potential to jointly biodegrade perchlorate and several common chlorinated solvents, since these compounds are frequently detected together in groundwater at DoD and related sites. To test process interactions in a controlled manner, several enriched mixed cultures developed in the laboratories at UT were used, including:

- An anaerobic dehalorespiring enrichment culture, referred to as KB-1, that has been documented to reductively dechlorinate PCE and TCE to ethene;

- Several perchlorate-reducing mixed cultures enriched during Task 1C from sites 1 (Aerojet) and 5 (Former Perchlorate Manufacturing Facility); and

- A mixed culture, capable of 1,2-dichloroethane (1,2-DCA) oxidation coupled to nitrate reduction, enriched from aquifer materials from a site in Louisiana.

Three specific questions were addressed in these experiments:

1. Does the presence of common chlorinated solvents such as trichloroethene (TCE) in groundwater inhibit perchlorate-reducing microorganisms?

2. Does the presence of perchlorate in groundwater inhibit the activity of microorganisms capable of dehalorespiration (i.e., TCE reduction to ethene)?

3. Can chlorinated solvents that are capable of serving as an electron donor in microbial metabolism (e.g., 1,2-DCA, dichloromethane [DCM], VC), promote the reduction of perchlorate, as has been shown for nitrate-reduction.

Experiments were conducted in glass bottles or vials containing anaerobic groundwater or defined, pre-reduced medium, amended with different concentrations of perchlorate, TCE, cis-1,2-dichloroethene (cis-1,2-DCE), vinyl chloride (VC) or 1,2-DCA, and inoculated with one or more of the microbial cultures listed above (3 to 10% inoculum). Each combination (or treatment) was tested in triplicate. Sterile controls were prepared, in which microbial activity was inhibited using mercuric chloride and sodium azide. Positive control cultures were established for each test, where the degradation of either perchlorate or chlorinated solvents was monitored in the absence of the potentially competing or inhibiting co-contaminant. In most cases, medium was

used rather than groundwater, to facilitate interpretation of the results. The results are summarized in the following subsections.

3.6.1 Effect of Chlorinated Ethenes on Perchlorate Reduction

The results of this trial indicate that the rate of perchlorate degradation by the perchlorate-reducing enrichment culture is unaffected by the presence of TCE (10 mg/L) or the dehalorespiring microbial culture (**Figure 5a**). These results suggest that perchlorate reduction can be readily accomplished under the mixed perchlorate-solvent plumes that exist at many DoD and related contractor sites.

3.6.2 Effect of Perchlorate on Chlorinated Ethene Reduction

The results of this trial confirmed that dehalorespiration of TCE is not adversely affected by the presence of perchlorate at concentrations ranging from 10 to 100 mg/L (**Figure 5b**). In addition, the rate of dechlorination relative to control cultures did not decrease after longer term exposure (> 3 months) to perchlorate. To further investigate the effect of perchlorate on chloroethene degradation, experiments were repeated with a focus on the VC to ethene dechlorination step, as this step is the most critical for TCE detoxification (VC is a known carcinogen). The results of this trial confirmed that:

- With an excess of electron donor (methanol plus acetate mixture) and well-established cultures, perchlorate reduction occurred before VC dechlorination to ethene. In this situation, perchlorate (100 and 500 mg/L) was degraded rapidly (within 4 and 9 days, respectively) and prior to initiation of VC dechlorination;

- During perchlorate degradation there was a lag or slow VC degradation, after which VC was dechlorinated to ethene (after perchlorate reduction);

- The rate of VC dechlorination, once initiated after perchlorate reduction, was not strongly affected by initial perchlorate concentrations (100 and 500 mg/L); and

- Repeated spikes of both perchlorate and VC showed similar trends (perchlorate reduction always before VC dechlorination).

Figure 5c illustrates the effect of perchlorate on ethene production from VC. The higher the perchlorate concentration, the longer the time to degrade the perchlorate, and hence the longer the lag before the onset of ethene production.

3.6.3 Chlorinated Solvents as Electron Donors for Perchlorate Reduction

Laboratory and field studies have shown that several mono- and di-chlorinated solvents such as 1,2-DCA, DCM and VC can serve as electron donors coupled to reduction of electron acceptors such as nitrate (Gerritse et al., 1999; Cox et al., 2000). Given the similarities between nitrate- and perchlorate-reduction, and given that groundwater at a variety of DoD and related contractor sites is impacted by perchlorate and chlorinated solvents, microcosm studies were conducted to assess whether chlorinated solvents could be used as electron donors in perchlorate reduction. To conduct these evaluations, 1,2-DCA was added to a perchlorate-reducing mixed culture from site 5 (Former Perchlorate Manufacturing Facility), while perchlorate was added to a mixed culture (from a site in Louisiana) capable of 1,2-DCA oxidation coupled to nitrate reduction. As shown in **Figure 5d**, the addition of 1,2-DCA to the perchlorate culture did not stimulate significant perchlorate reduction relative to the sterile control, while acetate addition promoted complete perchlorate reduction. Similar results were observed with perchlorate addition to the 1,2-DCA degrading culture from the Louisiana site. Specifically, the addition of perchlorate to the 1,2-DCA-oxidizing culture did not stimulate anaerobic oxidation of 1,2-DCA, whereas nitrate addition

promoted complete degradation of the 1,2-DCA (**Figure 5e**). These data suggest that while perchlorate-reducing bacteria may be able to use a variety of carbon substrates as electron donors, chlorinated solvents may not be suitable.

4. FIELD PROGRAM: TECHNICAL APPROACH & PROJECT ACCOMPLISHMENTS

Field pilot testing activities were initiated in the summer of 2001 to: i) confirm the ability of indigenous bacteria to biodegrade perchlorate to target treatment levels; ii) identify operational factors that will affect successful implementation of the technology for field deployment; and iii) generate preliminary design information for technology implementation under a follow-on technology validation program such as ESTCP. The following subsections summarize the approach and methodology for the pilot test, and present the pilot test results.

4.1 Pilot Test Site Selection

Candidate sites for the pilot test included the six test sites initially screened as part of the Task 1 ubiquity study (see Section 3.1). In addition, the Jet Propulsion Laboratory (JPL) facility in Pasadena was added as a candidate for pilot testing, at the request of the U.S. Navy. **Figure 2** shows the location of the seven candidate sites. Site information was reviewed for each of the seven facilities to identify the best candidate site for the pilot test. A screening process was developed to facilitate site selection based upon three categories: technical, logistical, and financial factors. **Table 8** presents the scoring for each candidate test site; scores of 1 (least appropriate) to 5 (most appropriate) were given for each criteria. Based on this screening process, the Aerojet Superfund Site in Sacramento, California had the highest total scoring (67 of 70 possible points) as well as the highest scoring for each of the three categories. A detailed description of the screening process, and Site conditions at the pilot test site, are provided in the pilot test workplan (GeoSyntec, 2001).

4.2 Pilot Test Site Background

4.2.1 Site Description

Perchlorate is present in soil and groundwater at the Aerojet Superfund Site as a result of the production and testing of solid rockets for DOD use. The pilot test area (PTA) selected at the Aerojet facility is located within a perchlorate plume that originates from a former disposal/burn area. Chlorinated solvents, consisting predominantly of TCE, are also present in the groundwater in this area. The perchlorate groundwater plume is approximately 5,000 feet long and approximately 3,000 feet wide in the vicinity of the PTA. The main impacted aquifer is located at a depth of about 100 feet below ground surface (bgs). The plume is currently captured at the boundary of the facility (about 2,000 feet downgradient from the pilot test location), and the extracted groundwater is treated by air-stripping to remove the VOCs. Perchlorate is not currently being treated by the ex situ system, and the VOC-treated groundwater is re-injected to the aquifer on site. Treatment of perchlorate in this groundwater plume, either using ex situ treatment (fluidized bed bioreactor or ion exchange) or through in situ bioremediation, is expected to be required in the very near future. **Figure 6** presents the location of the Aerojet facility within California, the location of the PTA at the facility, and the locations of existing groundwater monitoring wells in the vicinity of the PTA.

4.2.2 Site Geology and Hydrogeology

The Aerojet site is located on fluvial deposits from the ancestral American River. Three distinct river terraces are recognized across the Site and the PTA is located on the youngest of these terraces. The geologic materials beneath the PTA have been previously divided into four main aquifer units, designated as Aquifers A, B, C and D. Aquifer A is an unconfined aquifer predominantly composed of unconsolidated sand and gravel to depths of about 85 feet in the PTA. Aquifer B is also predominantly composed of sand and gravel, but contains several low-permeability beds. Aquifer C is

predominantly sand and silty sand with gravel and some sandstone. Aquifer D (the deepest aquifer) is located well below the area of influence of the pilot test (and perchlorate impacts). **Figures 7a** and **7b** present geologic cross sections of the PTA, oriented perpendicular and parallel to groundwater flow, respectively. Perchlorate impacts in the PTA are primarily confined to Aquifer B. Accordingly, all PTA wells were screened within Aquifer B, at a depth interval of about 85 to 100 feet bgs. **Figure 7c** provides a potentiometric map for Aquifer B. Groundwater flows to the west-northwest with a horizontal hydraulic gradient of about 0.008 feet per foot.

4.3 Pilot Test Design

The pilot test involved the use of an active biobarrier design, whereby groundwater was extracted using multiple pumping wells, amended with electron donor (ethanol), and recharged to the aquifer via a single recharge well, to promote perchlorate biodegradation in situ. In this manner, the system operated as a single-pass biobarrier, rather than as a recirculation loop. To improve well coverage for this pilot test (within the project budget), pilot test infrastructure used in a previous Aerojet bioremediation pilot test was used. This pilot test was conducted from May 2000 to April 2001. The original PTA consisted of a single electron donor delivery/recharge well (denoted 4385) and 3 downgradient monitoring wells (denoted 3601, 3600 and 100) located along the prevailing groundwater flowpath at distances of 15, 35 and 65 feet downgradient from the recharge well. For the previous pilot test, the PTA was bioaugmented with dehalorespiring bacteria (KB-1) in December 2000, to evaluate the ability of bioaugmentation to improve the rate and extent of TCE dechlorination to ethene in Aerojet groundwater. Therefore, while the focus of the SERDP pilot test was to demonstrate perchlorate biodegradation, the fate of TCE in the PTA groundwater was also assessed.

To evaluate the locations of the extraction wells and several new performance monitoring wells, a simplified numerical groundwater flow and transport model was developed (using Visual MODFLOW) for the PTA. The domain was 3,000 feet by 3,000 feet to ensure that the model boundaries were far enough away from the PTA to have no significant effect on the simulations. The vertical dimension of the domain was from ground surface (about 150 ft amsl) down to sea level. Groundwater flow across a hydraulic gradient of 0.008 ft/ft was simulated with constant head boundaries at either end of the domain. The aquifer was simulated to have a horizontal hydraulic conductivity of 30 ft/day and a vertical hydraulic conductivity of 3 ft/day. Pumping wells were simulated with 20 foot screened intervals in the B Aquifer.

Results of the modeling indicated that two new extraction wells, spaced at a distance of 200 feet on other side of the single central injection well, and pumping at 10 gpm each, would be capable of capturing the core of the perchlorate plume in the area. The extracted groundwater would be combined and recharged (at 20 gpm) via the single recharge well. **Figure 8** shows the results of the groundwater flow simulation at PTA scale. The particle tracking simulations show the area influenced by the extraction wells (about 600 feet width). Each arrowhead represents a groundwater travel time of 2 weeks. The particle tracks were used to confirm the suitability of using existing monitoring wells for pilot test performance monitoring. According to particle tracks, groundwater recharged via well 4385 would be expected to reach performance monitoring wells 3601, 3600, 100 and 3618 (new) within about 2, 6, 21 and 56 days, respectively, while particle arrival at a new transgradient well (3617) located 50 feet from recharge well 4385 was estimated to be 35 days. Based on these travel estimates, the system residence time was expected to be long enough to allow complete biodegradation of perchlorate in the single-pass biobarrier system. Bromide tracer test results, as described in Section 4.6, were subsequently used to validate and refine the PTA model.

4.4 PTA Installation, Instrumentation & Operation

The PTA was installed and instrumented in August through October 2001. **Figure 9** presents the layout of the groundwater extraction, electron donor delivery and performance monitoring wells in the PTA in plan view. The two new 6-inch diameter groundwater extraction wells were denoted 3619 (east) and 3620 (west). Existing well 4385 (8-inch diameter) was used as the electron donor delivery/recharge well. Existing wells 3601, 3600 and 100 were used as downgradient performance monitoring wells. Two new wells (2-inch diameter) were installed (under Aerojet supervision, following standard protocols) to improve the performance monitoring network: well 3618, located 100 feet downgradient from recharge well 4385; and well 3617, located 50 feet from recharge well 4385.

Figure 10 provides a schematic of the electron donor delivery system. The system was operated by extracting groundwater from wells 3619 and 3620 at a rate of approximately 10 gpm each using submersible Grundfos Reddiflo-3 pumps. The extracted groundwater was directed through a filter system to remove particulates, followed by a series of in-line monitoring electrodes designed to measure pH, ORP, and perchlorate concentrations in the re-circulating groundwater. The re-circulating groundwater then passed a flow sensor that measured the groundwater flow rate and provided feedback control to the extraction well pumps to maintain steady extraction rates. The re-circulating groundwater was amended with electron donor (see Section 4.7) using a metering pump, and the amended groundwater was re-injected to Aquifer B via well 4385. System operation was controlled using a programmable logic controller (PLC) and personal computer. **Figure 11** provides a screen-snapshot of the computer system interface that controlled system operation. The control system recorded the groundwater recirculation rate, individual electrode measurements, and water levels in the injection and extraction wells at 30-minute intervals. The extraction wells were instrumented with low-level water sensors/pump-shutoffs to limit drawdown and protect the pump. Similarly, the injection well was instrumented with a high-level

sensor/pump-shutoff to prevent overflow at the injection well. The PLC and computer were housed in a locked, air-conditioned control shed. Electron donor and tracer solutions were contained in an explosion-proof housing with secondary containment, positioned behind the control shed.

4.5 Baseline Geochemical Characterization

Several baseline groundwater sampling events have been conducted for the PTA, including: i) May 2000, prior to initial Aerojet pilot testing activities at this test site; ii) October 2001, prior to bromide tracer testing for this SERDP pilot test; and iii) November 2001, following the SERDP bromide tracer test and immediately prior to electron donor addition. Through these events, samples were collected for baseline analysis of:

- Field parameters (dissolved oxygen [DO], oxidation-reduction potential [ORP], pH and temperature);
- Perchlorate and associated degradation products (e.g., chlorate, chloride);
- Volatile organic compounds (VOCs);
- Inorganic parameters (bromide, nitrate, nitrite, and sulfate);
- Dissolved hydrocarbon gases (DHGs; methane, ethane, ethene);
- Dissolved metals;
- Biological oxygen demand (BOD) and chemical oxygen demand (COD);
- Sulfide; and
- Volatile fatty acids (VFAs; acetate, propionate).

Samples were collected following standard sampling protocols for the site, and were submitted to either Aerojet's Analytical Laboratory (AAL) for standard parameters or to Pioneer Technology Center (PTC) in Mississauaga, Ontario for non-standard analyses (chlorate, sulfide, DHGs and VFAs). **Table 9** summarizes the parameters that were analyzed as part of the baseline characterization, analytical

laboratories that conducted the analyses, and details regarding the analytical method, container size and type, preservation method, and sample holding times.

Table 10 summarizes the results of the baseline geochemical characterizations. For most parameters, concentrations measured in November 2001 (prior to electron donor addition) were similar to those measured in May 2000 prior to initiating bioremediation pilot testing activities at the site. The November 2001 baseline groundwater chemistry can be summarized as follows:

- Perchlorate concentrations in the extraction wells ranged from 2.1 to 13 mg/L, resulting in a combined influent perchlorate concentration in the range of 8 mg/L. Perchlorate concentrations in the monitoring wells ranged from 3.9 mg/L (at far downgradient well 3618) to 8.0 mg/L. Chlorate was not detected at any PTA wells above its PQL (1 mg/L).

- TCE was the dominant VOC detected in the influent and PTA groundwater, at approximately 1,700 μg/L. PCE, 1,2-DCE and 1,1-DCE were also consistently detected in the groundwater, at concentrations in the range of 35, 25, and 55 μg/L, respectively. VC was not detected above its PQL (0.5 μg/L) in the influent groundwater, but was detected in several downgradient monitoring wells (100 and 3618) due to the previous Aerojet pilot test.

- Carbon tetrachloride (CT), chloroform (CF), 1,2-DCA and 1,1-dichloroethane (1,1-DCA) were detected in the influent at concentrations ranging from 5 to 15 μg/L.

- Redox conditions in the PTA were typically aerobic and oxidizing, with ORP values in excess of 100 mV, and DO values in excess of 1.5 mg/L. The sole exception was well 100, in which the ORP remained reduced (-52 mV) from the previous Aerojet pilot test.

- The pH of groundwater at all PTA wells was near neutral, ranging from 6.88 to 7.29.

- The concentrations of DHGs (ethene, ethane, methane) were generally below PQLs in the PTA, with the exception of well 3618, which appeared to still be slightly affected by the previous pilot test at the site.

- Nitrate concentrations in the new extraction wells ranged from 20 to 28 mg/L (about 23 mg/L in the combined influent). By comparison, nitrate concentrations in downgradient monitoring wells 3600, 100 and 3618, even after several weeks of recharge without electron donor, ranged from 0.78 to 6.0 mg/L.

- Sulfate concentrations ranged from 10 to 15 mg/L, which is consistent with historic (pre- pilot testing) concentrations in the PTA groundwater.

4.6 Hydraulic Characterization (Tracer Testing)

Conservative tracer testing was initiated on 2 November 2001 to calibrate the PTA numerical model and refine estimates of PTA residence time and breakthrough at each monitoring well, and to allow estimation of perchlorate biodegradation rates. For the tracer test, sodium bromide (stock solution in de-ionized water) was added as a daily one hour pulse (same method as electron donor addition) for 14 consecutive days, to achieve a target time-weighted average concentration of 100 mg/L as bromide. Breakthrough of the conservative tracer at the monitoring and extraction wells was monitored by collecting samples on a daily to semi-weekly basis from each of the wells. Samples were analyzed on-site (for screening purposes) using a bromide ion-specific electrode (ISE) method, and were submitted to Aerojet's Analytical Laboratory (AAL) for laboratory confirmation analysis by ion chromatography (IC).

Figure 12 shows the results (IC data) of the bromide tracer test conducted to characterize PTA hydraulics. Maximum breakthrough concentrations in wells 3600, 3617 and 100 (35, 50 and 65 feet from the injection well) represented about 100%, 76% and 72% of the injected concentrations, confirming that concentration changes attributable to physical processes (dilution, dispersion, matrix diffusion) along the primary downgradient flowpath are minimal, and that observed mass losses at these wells are primarily due to biodegradation. The maximum bromide concentration at Well 3618 (100 feet from the injection well) represented about 40% of the injected concentration, confirming that this well was also on the flowpath, but the breakthrough curve indicated that significant dispersion of the added bromide pulse occurred over the 100 foot distance. Bromide concentrations at well 3601, located 15 feet from the injection well, showed significant variability related to the pulse addition methodology. The data suggest that this well was located too close to the recharge well (at the injection rate of 20 gpm) to provide useful performance data, and therefore well 3601 was not used for subsequent biodegradation performance assessment.

Based on the bromide tracer breakthrough curves in **Figure 12**, the average travel times for non-retarded particles to reach downgradient performance monitoring wells 3600, 100 and 3618 were estimated to be 5, 10 and 38 days, respectively, which is reasonably consistent with the travel times predicted by the model (5, 21 and 56 days, respectively). By comparison, the average travel time for non-retarded particles to reach transgradient performance monitoring well 3617 was only 5 days, as compared to 35 days predicted by the model. Therefore, the PTA model was revised to provide a "best-fit" to the observed travel times.

In addition to bromide breakthrough at the monitoring wells, bromide was detected at extraction well 3620. The maximum breakthrough concentration observed was about 5% of the injected concentration. This confirms that the biobarrier provided effective capture/treatment over the full 200-foot western portion of the PTA, from well 4385 to 3620. Bromide was not detected significantly above background concentrations at

extraction well 3619 in the eastern portion of the biobarrier, suggesting that the groundwater extraction rate at well 3619 was not high enough during the pilot test to provide complete 200 foot lateral coverage on the eastern portion of the biobarrier. Review of the site geology in **Figure 7a** (data not available prior to pilot testing) indicates that the aquifer materials differ in the eastern and western portions of the biobarrier. Specifically, in the western portion (from well 4385 to 3620), the target aquifer consists largely of sands, with some gravels, whereas in the eastern portion (from well 4385 to 3619), the geology consists predominantly of gravels. The apparent lack of bromide capture by well 3619 is likely related to the presence of higher permeability materials in this eastern portion of the biobarrier, and an insufficient extraction rate at this well to achieve capture. Optimization of extraction rates in future biobarriers is recommended to improve biobarrier effectiveness.

4.7 System Operation & Performance Monitoring

4.7.1 Electron Donor Addition

Ethanol was selected as the electron donor for the pilot test because: i) it has been determined to be one of the most cost-effective electron donors for large scale use; and ii) it does not adversely impact groundwater quality other than redox and alkalinity. Other electron donors (e.g., molasses, lactate) have been shown to have the potential to contribute metals, cations or anions to groundwater. Since the PTA at the Aerojet site is located in proximity to a drinking water supply aquifer, the introduction of non-native constituents such as metals or cations (e.g., sodium) has been deemed unacceptable by water quality authorities.

Ethanol addition was accomplished using a pulsed-addition mode (one hour pulse per day) to minimize microbial fouling. The amount of ethanol required to treat the average perchlorate and TCE influent concentrations was estimated based on

stoichiometric considerations, as shown in **Table 11.** Specifically, sufficient ethanol was added to reduce oxygen (5 mg/L), nitrate (23 mg/L), perchlorate (8 mg/L), sulfate (13 mg/L) and TCE (1.7 mg/L) in the PTA influent groundwater. Based on these average influent concentrations, the ethanol electron donor demand was estimated to be 17 mg/L. To account for uncertainty and biomass production, a 3-fold safety factor was applied to this concentration, and therefore the time-weighted average (TWA) ethanol addition concentration was 50 mg/L.

4.7.2 Biofouling Control

Control of biofouling in the electron donor delivery well was conducted using chlorine dioxide, a chemical biocide commonly used to disinfect drinking water, and to prevent biofilm formation in ex situ treatment systems, cooling towers and industrial applications. Unfortunately, installation of the pilot-scale chlorine dioxide generator was not completed in time to meet the schedule for electron donor delivery, and therefore, electron donor addition was conducted for approximately one month without biofouling control. This allowed biofouling of the delivery well to occur unchecked, resulting in rising waterlevels in the recharge well over the pilot test. As a result, system extraction/recharge flow rates were reduced on several occasions to maintain system operation. **Table 12** provides a summary of operating details for the pilot test, including chlorine dioxide dosing events and concentrations, and groundwater extraction/recharge rates. **Figure 13a** presents water level elevation trends in recharge well 4385 over the pilot test, and denotes recharge flow rates and chlorine dioxide dosing events. **Figure 13b** presents water level elevation trends in all PTA wells. As shown, water levels were stable in all wells over the pilot test, with the exception of the recharge well.

4.7.3 Performance Monitoring

Performance monitoring of groundwater chemistry consisted of weekly measurement of field parameters, and weekly to bi-weekly collection of groundwater samples from the influent (4385) and PTA Wells 3600, 3617, 100 and 3618 for analysis

of perchlorate, anions, VFAs, VOCs and DHGs (ethene, ethane, methane) by the methods identified in **Table 9**. Field parameter measurements were conducted on-site using specific electrode methods. Laboratory analyses were conducted by either AAL or PTC, as discussed in Section 4.5. VOC analyses conducted by AAL did not differentiate the cis- and trans- isomers of 1,2-DCE; however, analyses by PTC during the previous Aerojet pilot test differentiated the isomers and confirmed that the 1,2-DCE reported by AAL was consistently cis-1,2-DCE. Therefore, concentrations reported herein as 1,2-DCE are inferred to be the cis-1,2-DCE isomer.

4.8 Redox Trends in the PTA

Figures 14a and **14b** present trends in ORP and DO conditions in the PTA groundwater over the duration of the pilot test. Redox data are also summarized in **Table 13**. As indicated in Section 4.5, initial redox conditions in the PTA groundwater were generally aerobic and oxidizing, with DO concentrations exceeding 1.5 mg/L and ORP values exceeding 100 mV. Over the duration of the pilot test, the influent DO concentrations (well 4385) were consistently aerobic, ranging from 3.4 to 7.4 mg/L, while the ORP was consistently oxidizing, ranging from 56 to 258 mV.

Following addition of electron donor, ORP values in performance monitoring wells 3601, 3600 and 100 quickly (within 6 to 9 days) became reducing (**Table 13**), with ORP values declining to and stabilizing between –50 and –100 mV through the remainder of the pilot test. ORP values also declined at transgradient well 3617, and became reducing by Day 16. While ORP values at downgradient well 3618 declined, they generally remained oxidizing through the end of the pilot test (**Table 13**). DO concentrations in wells 3601 and 3600 generally declined below 1 mg/L, suggesting the development of anoxic conditions. While declines in DO concentrations were observed in groundwater at downgradient well 3618, concentrations generally remained above 1 mg/L, reflecting the return to background redox conditions downgradient from the PTA.

4.9 Perchlorate Biodegradation Results

Figure 15a presents trends in perchlorate concentrations in the PTA groundwater over the duration of the pilot test. As previously indicated, perchlorate concentrations in the extraction wells differed in magnitude, with concentrations at well 3619 ranging from 2.1 to 3.0 mg/L over the pilot test, while concentrations at well 3620 ranged from 12 to 14 mg/L over the pilot test. As a result, the influent perchlorate concentration (a 50:50 blend from wells 3619 and 3620) ranged from 7.1 to 8.3 mg/L for the pilot test.

Following electron donor addition, perchlorate concentrations declined rapidly, with little to no acclimation period. For example, perchlorate concentrations at well 3600 declined from 7.8 mg/L to <0.004 mg/L (the PQL) within 9 days from the start of electron donor addition, and remained below the PQL (with only one exception) for the duration of the pilot test. At well 100, perchlorate concentrations declined from 6.3 mg/L to <0.004 mg/L by Day 29 and remained below the PQL. At transgradient well 3617, perchlorate concentrations declined from 8.0 mg/L to <0.004 mg/L by Day 20 and remained below the PQL. Perchlorate concentrations at downgradient well 3618 also declined from 3.9 mg/L to 0.15 mg/L by the end of the pilot test (72 days), and concentrations were continuing to decline. Given the degree of dispersion observed for bromide at this well, it is expected that perchlorate concentrations would eventually decline below the PQL at 3618, but that this would require several additional months. Perchlorate concentrations showed an overall declining trend at well 3601, but as discussed in Section 4.6, the data from this well showed significant variability related to the short travel time and the electron donor pulse addition methodology.

To estimate perchlorate biodegradation rates in the PTA, first-order degradation half-lives were approximated using the influent data (Well 4385) as the initial concentration, and data from monitoring wells 3600, 3617 and 100 as the final concentrations. Travel times of 5, 5 and 10 days, derived for these wells from the bromide tracer test, were used as the elapsed time for the respective wells. Using these data, the perchlorate biodegradation half-lives during the early portion of system operation at 20 gpm (prior to the first reduction in system recharge rate) were estimated to be in the range of 0.5 to 1.2 days. These rates are consistent with half-lives calculated from previous pilot tests at the Aerojet facility (0.2 to 1.8 days, McMaster et al., 2001).

Consistent with the reduction of perchlorate, chloride concentrations in the downgradient monitoring wells showed an increase relative to the influent concentration (**Table 14**; **Figure 15b**). On a stoichiometric basis, the reduction of 8 mg/L of perchlorate would result in the production of about 3 mg/L of chloride in the groundwater. Influent chloride concentrations averaged 35 mg/L over the 72-day pilot test period, whereas the concentrations of chloride in groundwater at performance monitoring wells 3600, 100 and 3617 increased during the first two weeks of electron donor addition and averaged 39, 39 and 37 mg/L, respectively. These chloride increases (4, 4 and 2 mg/L) are consistent with the increases expected from perchlorate reduction. The slightly higher chloride concentrations in wells 3600 and 100 relative to transgradient well 3617 can likely be attributed to the chloride contribution from dechlorination of the TCE. For example, the dechlorination of 1.7 mg/L TCE (the average influent concentration) to DCE would be expected to contribute about 0.5 mg/L of chloride to groundwater at well 3617, whereas complete dechlorination of the TCE to ethene would be expected to contribute about 1.5 mg/L chloride to groundwater at wells 3600 and 100. For wells 3600 and 100, the chloride increase observed is within 10% of the chloride increase expected, whereas at well 3617 the chloride increase observed is within 25% of the chloride increase expected (both results are considered excellent for field applications).

As a final note related to perchlorate reduction, chlorate (a potential perchlorate degradation intermediate) was not detected above its PQL (1 mg/L) in any of the monitoring wells during perchlorate reduction.

4.10 VOC Biodegradation Results

Coincident with perchlorate reduction, the addition of ethanol to the PTA groundwater promoted rapid and complete dechlorination of TCE (1.7 mg/L) to ethene within 35 to 65 feet from the electron donor delivery well. **Figure 16a** provides a comparison of the relative proportions (in μmoles/L) of TCE, 1,2-DCE, VC and ethene at the start of the pilot test, and at Days 44, 58 and 72 following initiation of ethanol addition. At the start of the demonstration (Day –1), TCE was the dominant VOC in the biobarrier influent and at all downgradient and transgradient performance monitoring wells. Dechlorination products present in wells 100 and 3618 were a relic from the previous pilot test in the area. By Day 58, ethene was the dominant product at wells located 35 and 65 feet downgradient, within the portion of the PTA that was previously bioaugmented with KB-1. By Day 72, steady state TCE and 1,2-DCE concentrations were below their respective MCLs at wells 3600 and 100, while VC concentrations had declined to 12 μg/L at well 100, and were continuing to decline. VOC concentrations were also continuing to decline at downgradient well 3618, the furthest downgradient well in the PTA. Based on the data summarized above, the calculated half-life for TCE dechlorination to cis-1,2-DCE under steady state conditions ranged between 1.3 to 3.7 days, while the half-life for complete TCE dechlorination to ethene ranged between 4.1 to 11 days.

Of note, transgradient well 3617 was located outside of the original area of influence of the bioaugmentation conducted during the initial Aerojet pilot test. As a result, TCE was only dechlorinated to 1,2-DCE along the flowpath to well 3617 over

the 72-day test period, confirming that bioaugmentation is likely required at the Aerojet site to achieve complete TCE reduction to ethene.

In addition to the dechlorination of TCE, several other VOCs in the PTA groundwater were biodegraded. For example, PCE (37 μg/L) and 1,1-DCE (65 μg/L) initially present in the groundwater were dechlorinated to below PQLs (0.5 μg/L) in the previously bioaugmented portion of the PTA (**Figures 16b** and **16c**, respectively). PCE dechlorination (via TCE to cis-1,2-DCE) was also observed in the non-bioaugmented transgradient portion of the PTA (i.e., well 3617) after an acclimation period of about 45 days, while dechlorination of 1,1,-DCE was not observed in the non-bioaugmented portion of the PTA (consistent with the 1,2-DCE results).

Degradation of both CT (6 μg/L) and CF (11 μg/L) was also observed in the PTA. CT degradation occurred rapidly, within 13 days in the bioaugmented portion of the PTA, and within 30 days in the non-bioaugmented portion of the PTA (**Figure 16d**). CF concentrations declined to non-detect in the bioaugmented portion of the PTA within 44 days, but remained elevated in the non-bioaugmented transgradient portion of the PTA (**Figure 16e**). While the KB-1 dehalorespiring culture has not been previously shown to be capable of mediating dechlorination of either CT or CF, it appears that the geochemical and/or microbiological conditions created within the bioaugmented portion of the aquifer are suitable for promoting CT and CF reduction, likely via dichloromethane (DCM) to CO_2. While the chlorinated ethenes and methanes were degraded in the PTA, the concentrations of the chlorinated ethanes 1,2-DCA (influent average of 11 μg/L) and 1,1-DCA (influent average of 7 μg/L) did not significantly decline over the pilot test.

4.11 Supporting Groundwater Chemistry

The following sections summarize the inorganic chemistry results for nitrate, nitrite, sulfate, sulfide, VFAs (acetate, propionate), methane, and dissolved metals (iron and manganese).

4.11.1 Nitrate and Nitrite

Figures 17a and **17b** present trends in the concentrations of nitrate and nitrite in the PTA groundwater. Influent nitrate concentrations averaged approximately 23 mg/L over the pilot test. Following electron donor addition, nitrate concentrations in wells 3600, 100 and 3617 immediately declined to less than the PQL (0.05 mg/L) within 6 to 9 days. Nitrate concentrations also declined at well 3618, reaching non-detect (<0.05 mg/L) by Day 44. Based on these data, the calculated biodegradation half-life for nitrate in the PTA was 0.6 to 0.7 days. Nitrite (a transient intermediate of nitrate-reduction) was initially present in groundwater in various PTA wells, likely reflecting slow nitrate reduction linked to decay of biomass in the PTA groundwater from the initial Aerojet pilot test. Following initiation of electron donor addition, nitrite was not detected in groundwater samples from wells 3600, 100 or 3617. Nitrite concentrations also declined below detection at well 3618 by Day 30. Nitrite was sporadically detected in groundwater samples from well 3601, likely due to insufficient residence time to completely reduce the nitrate in this 15 foot interval.

4.11.2 Sulfate and Sulfide

Figures 18a and **18b** present trends in the concentrations of sulfate and sulfide in the PTA groundwater. Influent sulfate concentrations averaged approximately 14 mg/L over the pilot test. Following electron donor addition, sulfate concentrations in wells 3600, 100 and 3617 declined to less than the PQL (0.05 mg/L) within 60 days.

Sulfate concentrations also declined at well 3618, reaching 2.1 mg/L by Day 72. Coincident with sulfate reduction, sulfide concentrations increased in wells 3600, 100 and 3617 to maximums of 1.1, 1.2 and 2.5 mg/L, respectively, at Day 30, but then declined to non-detect by Day 72 at each well. It is unclear whether sulfide was produced and precipitated with dissolved metals, or whether the variability was the result of analytical variability.

4.11.3 Acetate and Propionate

The metabolism of ethanol by acetogenic bacteria in aquifers often results in the production of acetate and propionate, which can subsequently be used by a variety of bacteria as carbon and energy sources, and as electron donors in the reduction of nitrate, perchlorate and/or chlorinated solvents. **Figures 19a** and **19b** present trends in the concentrations of acetate and propionate in the PTA groundwater. In general, the trend in acetate concentrations was production within the initial 35 to 65 feet of the PTA, as reflected by the increasing concentrations over time at wells 3600, 100 and 3617, followed by metabolism of these carbon substrates (to CO_2) in the downgradient PTA areas. Acetate concentrations were consistently below detection (3 mg/L) at downgradient well 3618. Similarly, propionate concentrations showed increasing trends at wells 3600 and 3617, but tended to decrease thereafter to well 100. As with acetate, propionate concentrations were consistently below detection (3 mg/L) at downgradient well 3618. Ethanol was rarely detected in groundwater samples in the PTA (PQL of 0.5 mg/L). These data suggest that ethanol is rapidly metabolized to acetate and propionate, which subsequently serve as slower-release electron donors promoting further desirable degradation reactions along the groundwater flowpath.

4.11.4 Methane

Figure 20 presents trends in the concentrations of methane in the PTA groundwater. As expected, methane concentrations began to increase in the PTA at wells 3600 and 100 within several weeks. However, unlike the initial Aerojet pilot test, where methane concentrations increased to about 5,000 μg/L in the groundwater, methane concentrations during the SERDP pilot test remained below 100 μg/L at wells 3600 and 100, where the majority of the TCE dechlorination to ethene was occurring. These data suggest that the dehalorespiring microbial community, which is typically associated with methanogenic conditions, was operating at a highly efficient level, and that electron donor efficiency was very good. Dehalorespiring bacteria must often compete with methanogens and sulfate reducing bacteria for the hydrogen that is generated by electron donor metabolism. In inefficient systems, much of the electron donor is wasted on methanogenesis or sulfate reduction rather than the desired dehalorespiration reaction. In the Aerojet system, the low levels of methanogenesis suggest that electron donor usage is being primarily directed toward the desired degradation reactions for perchlorate, nitrate and TCE.

Methane was never detected over the 72 day pilot test in transgradient well 3617, confirming that the development of dehalorespiration activity and methanogenesis is limited at the Aerojet site unless bioaugmented. Methane concentrations actually declined over the pilot test at downgradient well 3618, reflecting the ability of the system to return to ambient conditions.

4.11.5 Dissolved Metals

Figures 21a and **21b** present trends in the concentrations of dissolved iron and manganese in the PTA groundwater. These were the only two metals to show increasing trends over the pilot test. Metals data are summarized in **Table 15**. The addition of

electron donor to the PTA groundwater promoted bacterial reduction and mobilization of both iron and manganese. Dissolved iron concentrations increased at wells 3600 and 100 to maximums of 0.8 and 2.9 mg/L, respectively. However, dissolved iron was not detected during the pilot test at downgradient well 3618, suggesting that the dissolved iron precipitates within the PTA within an acceptable distance (from 65 to 100 feet from the electron donor delivery well). Interestingly, dissolved iron was not detected at transgradient well 3617 over the pilot test. By comparison, dissolved manganese was produced in the PTA and detected at wells 3600, 100 and 3617. Once produced, concentrations did not decline before reaching downgradient well 3618. These data are consistent with previous pilot tests at the Aerojet site which show that manganese tends to persist in groundwater once formed. The best solution for manganese control is to optimize electron donor addition to prevent or limit its formation.

5. DESIGN & COST INFORMATION FOR FULL-SCALE ACTIVE BIOBARRIERS

The pilot test results indicate that in situ bioremediation is a technically feasible remediation alternative for groundwater impacted by perchlorate, present alone or in association with nitrate and chlorinated solvents. To assess the economic feasibility of in situ bioremediation using an active biobarrier approach (as depicted conceptually in **Figure 22**), a template site was created having conditions similar to the pilot test site, and a conceptual in situ bioremediation design was developed to allow detailed cost estimation for the approach.

Table 16a summarizes the design basis for the in situ bioremediation system. Key design parameters selected for the template site were as follows:

- Plume width = 3000 feet
- Plume depth = 100 feet
- Saturated thickness = 60 feet
- Hydraulic conductivity (K) = 30 feet/day
- Hydraulic gradient = 0.008 ft/ft

Using these parameters, the estimated aquifer discharge (groundwater flux) through the treatment transect is 224 gpm. Constraining drawdown in the extraction wells to 25% of the saturated thickness (i.e., 15 feet), the theoretical maximum sustainable well yield would be 140 gpm. Assuming a conservative extraction well efficiency of 60% of theoretical maximum, each extraction well would be capable of achieving a maximum extraction rate of 84 gpm, and therefore 3 extraction wells (8-inch diameter, operating at 75 gpm each) would be required to provide effective capture of the plume discharge. The estimated capture zone for each extraction well is estimated to be slightly in excess of 1100 feet. Groundwater would be amended with electron donor (ethanol; TWA 50 mg/L) and recharged to the aquifer via two recharge wells

operating at 112 gpm each. To provide increased recharge capacity to allow for some biofouling, the recharge wells would be 10-inch diameter. Chlorine dioxide would be used for biofouling control of the recharge wells. Four performance monitoring wells would be required for system performance monitoring; two downgradient from each recharge well.

Table 16b presents a cost estimate (-30%/+50%) for the proposed in situ bioremediation design presented above. Costs include both estimated capital costs and annual O&M costs, as well as net present value cost projections for a 30-year operating term. Capital costs for the active biobarrier include: well installation; trenching, piping and electrical; system controls, pumps, valves and storage vessels; biofouling control system; system housing; and engineering design and startup. Operating and maintenance costs for the active biobarrier include: system operator (one day per week); electricity; electron donor costs; biofouling control reagents; annual recharge well rehabilitation (if required); sample collection and laboratory analysis; and annual reporting.

Based on the site conditions for the template site, the net present value (NPV; 30 years at 6%) for the full-scale active in situ biobarrier is estimated to be about $1.1M. Capital costs are estimated to be $650K, while annual O&M costs range from $78K to $90K. A significant component ($23K) of the annual O&M costs is allocated for physical rehabilitation of the electron donor/recharge wells, in the event that this is required. However, operation of the chlorine dioxide biofouling control system at an independent Aerojet pilot test site was able to maintain recharge rates at 150 gpm, and therefore this annual cost may not be required.

6. CONCLUSIONS

The combined results of this laboratory and field research program indicate that perchlorate can be readily biodegraded under subsurface conditions, and that in situ bioremediation is an appropriate technology for widespread use at DoD and related contractor facilities. Specific conclusions are provided below for each of the 4 tasks in the research program:

Task 1 – Assessment of Ubiquity of Perchlorate Degraders

The specific conclusions from Task 1 were:

1. Microorganisms capable of perchlorate reduction are present at each of the test sites evaluated, with population densities ranging from a low of 10^2 cells/mL to a high of 10^6 cells/mL of groundwater. There does not appear to be any significant correlation between population densities and known site conditions.

2. Perchlorate-reducing microorganisms were isolated from each test site and were generally found to be related to the Azospirillium genus or the Dechloromonas genus.

Task 2 - Geochemical Tolerances and Capabilities of Perchlorate-Degrading Bacteria

The specific conclusions from Task 2 were:

1. Most of the perchlorate-degrading isolates are able to use nitrate as an electron acceptor, but none of the isolates were clearly able to use sulfate or ferric iron as electron acceptors.

2. All of the isolates can reduce perchlorate concentrations up to 20 mM. An isolate from the IRCTS Alpha site can reduce perchlorate concentrations of 50 mM. These data suggest that in situ bioremediation should be an appropriate remedial approach for treatment of perchlorate source areas, where concentrations are commonly in the 100s of mg/L and in some cases in the 1000s of mg/L.

3. Results of nitrate-perchlorate interaction trial confirm that nitrate reduction occurs preferential to (or simultaneous with) perchlorate reduction.

4. Results of perchlorate-sulfate interaction trials suggest that, despite energy yield considerations, joint reduction of sulfate and perchlorate can occur in mixed microbial systems. These results may represent significant concerns for treating sites having high sulfate concentrations, since sulfate reduction can place significant demand on electron donor consumption and cost.

Task 3 – Joint Biodegradation of Perchlorate and Chlorinated Solvents

The specific conclusions from Task 3 were:

1. The presence of chlorinated VOCs such as TCE appears to have no effect on perchlorate reduction.

2. The presence of perchlorate appears to have little effect on dehalorespiration of TCE and VC, other than delaying the dechlorination reactions until perchlorate is depleted. Both reduction activities can be accomplished in the same system using the same electron donor, with perchlorate being reduced before VOC reduction.

3. 1,2-DCA, which has been shown to serve as an electron donor coupled to nitrate reduction, did not promote perchlorate reduction. Similarly, perchlorate did not promote anaerobic oxidation of 1,2-DCA.

Task 4 – Field Pilot Test of Perchlorate Biodegradation

The specific conclusions from Task 4 were:

1. Perchlorate biodegradation was readily initiated, without acclimation period, through the addition of ethanol as electron donor. Perchlorate concentrations in excess of 8,000 μg/L were consistently reduced to less than the PQL of 4 μg/L within 35 feet of the recharge well. Perchlorate biodegradation half-lives were rapid, ranging from 0.5 to 1.8 days.

2. Coincident with perchlorate reduction, TCE (~2 mg/L) was also dechlorinated to ethene in a portion of the aquifer that was previously bioaugmented with a dehalorespiring microbial consortium. The calculated half-life for TCE dechlorination to ethene was 11 days, which is very fast. CT and CF were also effectively treated, while 1,2-DCA was not.

3. Nitrate concentrations in the influent (~23 mg/L) were routinely reduced to <0.05 mg/L within 35 feet of the recharge well. The half-lives for nitrate were in the range of 0.6 to 0.7 days.

4. Ethanol was a highly effective and efficient electron donor for perchlorate, nitrate and TCE reduction. At only a 3:1 donor:acceptor ratio, the level of treatment success indicates that very little donor was wasted on non-required microbial processes. It appears that ethanol is rapidly metabolized to acetate and propionate in situ, which are subsequently used as electron donors and depleted within 100 feet of the recharge well.

5. The sole groundwater impact created by the in situ bioremediation technology appears to be mobilization and persistence of low levels (about 1 mg/L) of dissolved manganese.

Based on the results of the microcosm studies and field pilot test, it appears that accelerated (enhanced) in situ anaerobic bioremediation will be a widely applicable groundwater remediation technology for perchlorate-impacted DoD, DoE, NASA and related contractor facilities for both source remediation or control of perchlorate plumes.

7. TRANSITION PLAN

In order to successfully transition the in situ bioremediation technology to both federal and non-federal sectors, GeoSyntec has been employing a mutli-pronged approach. First, GeoSyntec has been highly active in advocating the use of the technology and educating site stakeholders, DoD groups, and regulatory agencies on the use of the technology. As a result, various DoD and related contractor sites have initiated laboratory and/or field demonstrations of the technology based on the emerging results from the SERDP study. These stakeholders include: Edwards AFB, Aerojet, The Boeing Company, Alliant Techsystems, American Pacific Corporation, Atlantic Research Corporation, and United Technologies Chemical Systems). While many of the advocacy presentations have been conducted directly to site stakeholders, GeoSyntec has also made multiple presentations to large groups at conferences, including:

- Remediation of Chlorinated & Recalcitrant Chemicals; May 2002 & 2000, Monterey, California;
- Groundwater Resources Association of California Perchlorate Workshop; April 2002; Baldwin Park, California;
- Department of Toxic Substances Control Perchlorate Technology Update; March 2002, Sacramento, California.
- In Situ and On-Site Bioremediation Symposium; June 2001, San Diego, California;
- Symposium on Engineering Geology & Geotechnical Engineering; March 2001; University of Las Vegas, Nevada.
- Pollution Prevention Perchlorate Technology Workshop; August 2000, San Antonio, Texas;
- SERDP/ESTCP Partners Conference; November 2000; Arlington, Virginia;

In addition to these presentations, four papers/extended abstracts were published (copies provided in Appendix B) containing information related to this study, including:

Cox, E.E. and S. Neville. 2002. "In Situ Remediation of Rocket Fuels in Groundwater: Where do we Stand Today?. In: Proceedings of the Groundwater Resources Association conference on Perchlorate, Baldwin Park, California, April 17, 2002.

Cox, E.E., M. McMaster and S. Neville. 2001. "Perchlorate in Groundwater: Scope of the Problem and Emerging Remedial Solutions. In: Proceedings of the 36th Symposium on Engineering Geology & Geotechnical Engineering, University of Las Vegas, Nevada, March 28-30, 2001.

McMaster, S. Neville, L. Bonsack and E.E. Cox. "Successful Demonstration of In Situ Bioremediation of Perchlorate in Groundwater". In: *Bioremediation of Inorganic Compounds,* Battelle Press. June 4-7, 2001.

Waller. A. D. Seepersad, E. Edwards and E. Cox. 2001. "In Situ Bioremediation of Perchlorate Contaminated Groundwater". In: *Bioremediation of Inorganic Compounds,* Battelle Press. June 4-7, 2001.

GeoSyntec is also currently working on a manuscript detailing the pilot test presented herein. Our intent is to submit the manuscript for consideration of publication in a peer-reviewed journal, as the first successful field demonstration of joint perchlorate and TCE bioremediation in a deep aquifer system. The contribution of SERDP to the research will be acknowledged, and reprints of the paper, if published, will be provided to SERDP and DoD agencies for distribution.

In addition to GeoSyntec's technology transition activities, we are currently working with the Naval Facilities Engineering Service Center (NFESC) to disseminate information on the technology throughout DoD. This will be accomplished through posting of information by NFESC on Denix, and on relevant web pages. GeoSyntec will also post an abbreviated version of this report on our web page, and will cross-link the web page to various perchlorate web sites. The "executive summary", complete with key graphics, will also be made available to SERDP and DoD agencies who may wish

to post the abbreviated, reader-friendly version of the report as a downloadable PDF. These efforts should help transition this technology within DoD and DOE. Information will also be provided to the EPA Technology Innovation Office for inclusion on their perchlorate treatment technology database CLU-IN, for broad dissemination.

Finally, GeoSyntec has been involved with both ESTCP and the United States Air Force Center for Environmental Excellence (AFCEE) in scoping the contents for a potential book on perchlorate treatment technologies. This book will include results from the initial SERDP perchlorate studies, as well as results from the upcoming field demonstrations to be conducted under the ESTCP program.

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Cox, E.E., M. McMaster and S. Neville. 2001. Perchlorate in groundwater: Scope of the problem and emerging remedial solutions. Symposium of Engineering Geology & Geotechnical Engineering. March 2001, Las Vegas.

Cox, E.E. and S. Neville. 2002. "In Situ Remediation of Rocket Fuels in Groundwater: Where do we Stand Today?. In: Proceedings of the Groundwater Resources Association conference on Perchlorate, Baldwin Park, California, April 17, 2002.

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APPENDIX A

TEST SITE INFORMATION

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TEST SITE INFORMATION

As indicated in Section 3.1, the following facilities were selected for testing as part of the laboratory research program:

1. Aerojet Superfund Site, California
2. Edwards Air Force Base, Operable Unit 5, California
3. U.S. Navy, Allegany Ballistics Laboratory, West Virginia
4. U.S. Navy, San Nicolas Island, California
5. Perchlorate Manufacturer, Nevada
6. Inactive Rancho Cordova Test Site, California

The locations of these test sites are shown in **Figure 2**. Brief descriptions of each test site and their remedial needs are provided below.

Aerojet Superfund Site, California: Perchlorate (approximately 12 mg/L) and chlorinated volatile organic compounds (VOCs; 2 to 3 mg/L) are present in soil and groundwater at the Aerojet Superfund Site as a result of the production and testing of solid and liquid rockets for DOD use. For this evaluation, groundwater was collected from a perchlorate plume originating from a former burn area. The plume is approximately 5,000 feet long and approximately 3000 feet wide. The main impacted aquifer is located at a depth of about 100 feet below ground surface (bgs). **Table 1** summarizes the baseline groundwater chemistry in the test area. The plume is currently captured at the boundary of the facility for treatment, and the extracted groundwater is treated to remove VOCs. Perchlorate is not currently being treated by the ex situ system, and the VOC-treated groundwater is re-injected to the aquifer on site. Treatment of perchlorate in this groundwater plume, either using an ex situ bioreactor or through in situ bioremediation, is expected to be required in the foreseeable future.

Edwards Air Force Base, Operable Unit 5 Site, California: Perchlorate is present in soil and groundwater at Operable Unit 5 (OU5) as a result of historic rocket manufacturing and testing activities at the former Jet Propulsion Laboratory facility. Perchlorate impacts to groundwater appear to extend vertically from the watertable at 125 ft bgs to a depth of approximately 250 feet bgs. The highest concentrations of perchlorate in groundwater in this area are in the range of 160 mg/L (at 128 ft bgs). The eastern (downgradient) edge of the perchlorate plume is co-mingled with a chlorinated solvent plume (primarily trichloroethene; TCE), with TCE concentrations up to 1,000 µg/L. Table 1 summarizes the baseline groundwater chemistry in the test area. Remediation of the perchlorate and VOC impacted groundwater in this area is anticipated to be required to comply with the California non-degradation policy.

U.S. Navy, Allegany Ballistics Laboratory, West Virginia: The Allegany Ballistics Laboratory (ABL) has historically been used for research, development and testing of ballistic missiles and/or perchlorate-based explosive materials. As a result of these testing activities, perchlorate is present in alluvial soils and groundwater at the Site, particularly in former open burn and detonation areas. Perchlorate is also suspected to be present in groundwater in fractured bedrock at the Site. The thickness of the alluvium is approximately 20 feet, with the groundwater table generally located at 10 to 15 feet bgs. Perchlorate concentrations in alluvial groundwater range up to 26 mg/L. The supporting groundwater chemistry is relatively unknown (see Table 1), but is suspected to contain chlorinated solvents in the range of 1 to 5 mg/L. A groundwater extraction and treatment system is currently operating to treat chlorinated VOCs in extracted groundwater; perchlorate is not treated.

U.S. Navy, San Nicolas Island, California: San Nicolas Island (SNI) has been used since the 1950s to test domestic and foreign missiles, many of which contain ammonium perchlorate as solid propulsion fuel. Soil and groundwater investigation activities at the Site have been limited, however perchlorate impacts to several natural springs have been reported. The springs are suspected to be located hydraulically downgradient from a Missile Firing Range (MFR) and an Exploded Ordinance Disposal

(EOD) area. Perchlorate concentrations in the springs vary seasonally from 5 to 20 μg/L. Water from the springs is used as part of the potable water supply for the island. The goal of in situ bioremediation at SNI would be protection of this drinking water supply from future perchlorate impacts. Little characterization of subsurface conditions has been conducted at SNI, and therefore, a field screening study was conducted to delineate shallow perchlorate impacts in the EOD area to define appropriate soils for microcosm testing. Table 1 summarizes the baseline chemistry of microcosms constructed from the EOD materials.

Former Perchlorate Manufacturing Facility, Nevada: Ammonium perchlorate was historically manufactured at this former facility in Nevada for use in explosives and solid rocket engines. Perchlorate impacts have been reported in several aquifers beneath the site to depths in excess of 250 feet. Perchlorate concentrations range up to 700 mg/L. Nitrate and sulfate concentrations are also elevated in several locations, at concentrations ranging up to 60 and 1700 mg/L, respectively. Chloride concentrations are in excess of 1,000 mg/L through much of the plume. The plume extends several miles downgradient of the site. The groundwater anion chemistry is highly variable throughout the plume, and therefore, soil and/or groundwater materials representing 3 separate locations (source area, plume core, and plume toe) were tested to assess the ubiquity/activity of perchlorate-degrading bacteria in the various differing geochemical environments. Table 1 summarizes the baseline groundwater chemistry for each of the test locations from this site.

Inactive Rancho Cordova Test Site, California: Perchlorate is present in soil and groundwater at the Alpha and Sigma Complex test sites at the Inactive Rancho Cordova Test Site (IRCTS) in California as a result of historic rocket and explosives manufacturing and testing activities. The available soil and groundwater chemistry data from the Site suggest that perchlorate is present at concentrations up to 45 mg/L at depths ranging up to 225 feet bgs. Soil and groundwater for the Alpha Complex trials were collected from approximately 120 feet bgs; materials for the Sigma trials were

collected from approximately 130 feet bgs. Table 1 summarizes the limited baseline groundwater chemistry data available for these sites.

Soil and Groundwater Collection

Attempts were made to collect both soil and groundwater impacted by perchlorate at each of the six test sites. However, due to difficulties in recovering soil materials at depth at several of the sites, microcosms from several sites (or subsites) had to be constructed using groundwater only. Sample collection (including drilling) was conducted primarily through in-kind contributions by each facility, the cost of which varied from approximately $5K to $20K per site. Brief descriptions of sample collection activities for each test site follow.

Aerojet Superfund Site, California: Microcosms were constructed using groundwater from an existing well located within the perchlorate plume near the site boundary where groundwater is currently being extracted for VOC treatment. Approximately 4 L of groundwater was collected, following well purging by standard protocols, directly into a sterile plastic container, minimizing headspace to the extent possible. The groundwater was then placed on ice and express-shipped to the research laboratory at UT. Attempts were made to collect soil from new boreholes drilled adjacent to the monitoring well sampled; however the aquifer geology (heaving sands and gravels) prevented sample recovery at two separate boreholes.

Edwards Air Force Base, Operable Unit 5 Site, California: Microcosms were constructed using soil and groundwater from a new borehole and monitoring well installed in the OU5 perchlorate plume. Approximately 5 Kg of saturated perchlorate-impacted aquifer material was collected from a depth of approximately 125 to 135 ft bgs. Soils were collected in sterile brass liners within a clean 2-inch split-spoon sampler. The brass liners were capped upon retrieval from the borehole, placed on ice, and express-shipped to UT. Approximately 8 L of groundwater was collected from the new well, following well development and purging, directly into a sterile plastic

container, minimizing headspace to the extent possible. The groundwater was placed on ice and express-shipped to the research laboratory at UT.

U.S. Navy, Allegany Ballistics Laboratory, West Virginia: Approximately 5 kg of saturated perchlorate-impacted aquifer material was collected from a new borehole, using methods similar to those described above for Edwards AFB. Approximately 8 L of groundwater was collected from an existing alluvial extraction well known to contain perchlorate at concentrations of up to 25 mg/L. Both the soil and groundwater were express-shipped on ice to UT.

U.S. Navy, San Nicolas Island, California: A limited perchlorate screening study was conducted to delineate perchlorate impacts to surface and near-surface soils at the EOD. Soils were collected on a grid pattern covering the EOD and from surrounding areas suspected to be impacted (based on visual observations and drainage patterns). Soil samples were express-shipped to the GeoSyntec laboratory for analysis by ion-specific electrode methods to assess relative degree of perchlorate impacts to the soils, and these results were later confirmed through analysis by ion chromatography methods. Soils containing elevated perchlorate (e.g., >10 mg/kg) were composited to provide adequate materials for the microcosm studies. Water samples for the microcosms were collected from one of the impacted surface springs located approximately 800 feet downgradient from the EOD. Water samples were collected directly into 4 L plastic carboys (minimizing headspace) and express-shipped to UT.

Former Perchlorate Manufacturing Facility, Nevada: Soil and/or groundwater was collected from 3 different locations within the groundwater perchlorate plume at the site. Each location represents different geological and chemical conditions, including the source (depth of 161 to 181 ft bgs), plume core (depth of 35 to 73 ft bgs), and plume toe (depth of 14 to 29 ft bgs). At each test location, groundwater was collected from an existing monitoring well (following well purging) directly into a 4L plastic carboy, minimizing headspace. Despite repeated (and costly) efforts, aquifer materials (gravels and coarse sands) could not be recovered from the source area borehole, and therefore source area microcosms were constructed using fresh groundwater and an existing soil core recovered (using different drilling methods) from the well where the groundwater was collected. For the plume core location, soil was collected using brass liners, as detailed for Edwards AFB. Aquifer materials could not be collected from the plume toe location due to drilling complications through caliche materials, and therefore silt and fine grained materials were collected from the bottom of the existing well during purging. All materials were express-shipped on ice to the UT research laboratory.

Inactive Rancho Cordova Test Site, California: Approximately 5 kg of soil was sampled from the saturated zone at each of the Alpha and Sigma test sites at IRCTS. Samples were collected into 4-inch diameter plastic liners, and express-shipped on ice to UT. Approximately 8 L of groundwater was collected from existing perchlorate-impacted monitoring wells at each of the test sites, and express-shipped on ice to UT.

APPENDIX B

TECHNICAL PAPERS