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Abstract: Batch microcosm reactor studies using aquifer sand collected from a perchlorate-contaminated site were conducted in order to determine if perchlorate can be treated using in situ biodegradation stimulated by an organic electron donor, acetate, and to measure degradation rates over a range of environmental variables. The addition of acetate as an organic substrate stimulated rapid perchlorate degradation after a lag phase, presumably resulting from microbial acclimation. The lag phase was eliminated after previous exposure or after microbes consumed the oxygen and the reactors went anaerobic. Perchlorate degradation was effective when greater than 50 mg/L acetate was added, at temperatures greater than 20°C, and when the pH was between 4 and 8. Various perchlorate and acetate concentrations had different effects on the biodegradation process. Most of the studies were conducted at relatively high (mg/L) concentrations. To investigate removal at lower concentrations, a study with an initial perchlorate concentration of 50 µg/L was conducted, and this indicated that final treatment concentrations were reduced below the California maximum contaminant level of 6 µg/L. Chloride was the ultimate end product of perchlorate degradation, and measurements indicated that chloride formation accounted for 95.5 to 100% of the perchlorate degraded.

Contents

Figures and Tables

Figures

Tables

Preface

Funding for this research work was from the Research, Development and Engineering Command (RDECOM)-Armament Research, Development and Engineering Center (ARDEC) to U.S. Army Engineer Research and Development Center (ERDC), Environmental Laboratory (EL) in Vicksburg, MS.

This research effort was directed by Dr. Victor F. Medina, Environmental Processes and Engineering Division (EPED), Environmental Laboratory (EL), ERDC. Agnes Morrow, Environmental Processes and Engineering Division (EPED), Environmental Laboratory (EL), ERDC conducted the research. They were assisted by Barbara Extine, who at that time, was employed by Applied Research Associates, Inc. Roy Wade and Sally Yost, EL, provided in-house reviews.

This study was conducted under the direct supervision of W. Andy Martin, Branch Chief, EP-E, and under the general supervision of Dr. Richard E. Price, Division Chief, EPED, and Dr. Elizabeth C. Fleming, Director, EL.

COL Gary E. Johnston was Commander and Executive Director of ERDC. Dr. Jeffery P. Holland was ERDC Director.

1 Introduction

Perchlorate uses

Perchlorate (ClO_4^-) is the soluble anion associated with the solid salts of ammonium, potassium, and sodium perchlorate. Large-scale production of ammonium perchlorate began in the United States in the mid-1940s when it began to be used as an energetic booster or oxidant in solid rocket propellant for ballistic missiles and anti-tank rockets (Urbansky 1998). It is a national technical asset integral to the Nation's strategic defense system and space exploration.

Perchlorate can also be present as an ingredient or an impurity in road flares, lubricating oils, finished leather, fabric fixer, dyes, electroplating, aluminum refining, manufacture of rubber, paint and enamel, and in paper and pulp processing (as an ingredient in bleaching powder).

Ammonium perchlorate is used in certain fireworks, the manufacture of matches, as a component of air bag inflators, and in analytical chemistry to preserve ionic strength (Interstate Technology and Regulatory Council (ITRC) Perchlorate Team 2005). Perchlorate has been used as an additive in cattle feed, in magnesium batteries, and as a component of automobile air bag inflators (Motzer 2001). It is found in fertilizers, particularly in Chilean nitrate-based products, where the perchlorate was naturally formed (Urbansky et al. 2001; Ericksen 1983; Schumacher 1960). It was also found in other fertilizers, where it was probably a filler additive.

Sodium hypochlorite solutions used in water and wastewater treatment plants have also been identified as a potential source of perchlorate contamination (U.S. Environmental Protection Agency (USEPA) 2007). Perchlorate has long been considered relatively harmless from an environmental perspective, but over the past 10 years, concern has increased regarding its presence as a groundwater, surface water, and soil contaminant.

Ammonium perchlorate has a limited shelf life and must periodically be replaced in munitions and rockets, or in inventory. This has led to the disposal of large volumes of the compound since the 1940s in Nevada, California, Utah, and likely other states (USEPA 1999, Strategic Environmental Research and Development Program (SERDP) 2006).

Disposal and demilitarization of solid rocket motors from large propulsion systems is a major task facing the Department of Defense (Ground-Water Remediation Technologies Analysis Center (GWRTAC) 2001).

Perchlorate in the environment

Perchlorate has been found in drinking water supplies throughout the United States. It has been identified in surface water and groundwater in Texas, Arkansas, Maryland, New York, California, Utah, and Nevada (USEPA 1999). Ammonium perchlorate produced at facilities formerly located in Henderson, NV (near Las Vegas) has contaminated Lake Mead and the Colorado River, which are important potable water sources for California and Arizona (Renner 1998). Other sites that have received a great deal of media attention include the Massachusetts Military Reservation (MMR) (Clausen et al. 2004), Eastern Sacramento County, near Aerojet General Corporation (California Department of Health Services (CADHS) 2002), throughout Southern California (CADHS 2002), and Western Texas (Christen 2003). Researchers have found perchlorate in lettuce (Hogue 2003), supermarket milk (Erickson 2003), and even human breast milk (Kirk et al. 2005, Dasgupta et al. 2008).

In 1998, the USEPA placed perchlorate on its Contaminant Candidate List for possible regulation. In 1999, the USEPA required drinking water monitoring for perchlorate under the Unregulated Contaminant Monitoring Rule (UCMR). In February 2005, the USEPA established an official reference dose (RfD) of 0.7 micrograms of perchlorate per kilogram of body weight per day (µg/kg/day). The RfD is a scientific estimate of a daily exposure level that is not expected to cause adverse health effects in humans. USEPA's new RfD translates to a Drinking Water Equivalent Level (DWEL) of 24.5 micrograms of perchlorate per liter (µg/L). A DWEL assessment assumes that all of a contaminant comes from drinking water, and it gives the concentration of a contaminant in drinking water that will have no adverse effect. A DWEL has a margin of safety, so that exposures above it are not necessarily considered unsafe (USEPA 2005); for example, the State of California (CADHS 2006) has a maximum contaminant level (MCL) of 6 µg/L, a value identical to the California Public Health Goal (California Office of Environmental Health Hazard Assessment (OEHHA) 2004). Massachusetts has proposed a regulatory standard of 2 µg/L (Massachusetts Department of Environmental Protection (MDEP) 2006).

Perchlorate mitigation

The most promising technologies to remove perchlorate from water are ion exchange (Batista et al. 2000) and biological reduction (Coates et al. 1999; Logan et al. 2001; Kroon and van Ginkel 2004). Although successful, ion exchange only separates perchlorate from water, it does not destroy it, and then the material must be disposed. The waste streams from these systems consist of caustic or saline regenerant solutions with high concentrations of perchlorate (Gingras and Batista 2002).

Biodegradation has the advantage of destroying the perchlorate. Bacteria capable of perchlorate degradation appear to be widely present in nature at concentrations ranging from one to thousands of bacteria per gram of water, wastewater, aquifer material, and soil (Wu et al. 2001, Smith et al. 2009). Some bacteria use perchlorate as an electron acceptor for cellular respiration, and, in the process, perchlorate is degraded completely to chloride ions. Most perchlorate-respiring microorganisms are capable of functioning under varying environmental conditions and use oxygen, nitrate, and chlorate as terminal electron acceptors. A widely accepted perchlorate-reducing pathway for microbial reduction is shown in Equation 1 (Nozawa-Inoue et al. 2005; Rikken et al. 1996) where perchlorate is transformed into chlorate, then chlorite, finally to chloride ion and oxygen.

$$ClO_4^- \rightarrow ClO_3^- \rightarrow ClO_2^- \rightarrow Cl^- + O_2 \quad (1)$$

A column study simulating in situ bioremediation of perchlorate using acetate as an organic substrate showed degradation of perchlorate was rapid, with a minimal lag phase, once acetate was added to the column. By 72 hours, 99.5% removal of the perchlorate occurred (from 10 to <0.05 milligrams per liter [mg/L]) within the first 15 centimeters (cm) of the column (Medina et al. 2006).

The purpose of this research was to build upon the Medina et al. (2006) study by investigating perchlorate biodegradation under different environmental conditions in order to develop engineering parameters and to optimize the performance of previous technologies. The approach was to simulate reactions in an in situ bioremediation application in laboratory reactors. A series of experiments was conducted in laboratory reactors (microcosms) under closed anaerobic conditions with naturally occurring microorganisms and a variety of environmental operation parameters.

These parameters included various acetate concentrations, pH variation, a select range of temperatures, and varying perchlorate concentrations.

2 Literature Review

Effect of perchlorate on the environment

Perchlorate may be released into the environment in the form of a number of different salts, including ammonium perchlorate, potassium perchlorate, sodium perchlorate, and others. All are highly soluble in water, though the solubility of the different salts varies. Perchlorate may also be released into the environment in the form of a liquid, such as in solution with water as concentrated brine or as perchloric acid. The perchlorate ion's high solubility in water coupled with its limited tendency to adsorb to most soil surfaces leads to high mobility in aqueous environments. Perchlorate can persist in the environment for many decades under typical groundwater conditions because of its resistance to reactions with other available constituents in the subsurface. Once perchlorate is dissolved, these characteristics lead to the formation of long and persistent contamination plumes when released into either groundwater or surface water. Dilution and precipitation are considered to be the two most important processes influencing the fate and transport of perchlorate in natural aqueous environments (ITRC 2002). Biodegradation of perchlorate in groundwater will not occur unless significant levels of organic carbon are present, oxygen and nitrate are depleted, and perchlorate-degrading bacteria are present. The combination of high solubility, low sorption, and lack of degradation tends to create plumes that are large and persistent (Sellers et al. 2006). Through their roots, plants can take up soil moisture that contains perchlorate in solution, and several ecological studies have demonstrated the tendency of some plants to concentrate the perchlorate in plant tissues (Urbansky et al. 2000; Ellington et al. 2001).

Perchlorate exposure

Chemicals may enter the human body in several ways, known as "routes of exposure," including ingestion, dermal (skin) absorption, and inhalation. The primary route of human exposure to perchlorate is through ingestion of perchlorate-contaminated water and/or food. Human studies have indicated that ingested perchlorate is readily and completely absorbed from the gastrointestinal tract and excreted rapidly, primarily in the urine (Cheng et al. 2006). Ingestion of perchlorate-contaminated drinking water is one of the major exposure routes of concern, although ingestion of

contaminated food and human milk are other potential sources of exposure (Gibbs et al. 1998).

When compared to ingestion, skin absorption and inhalation of perchlorate can be considered negligible exposure pathways. The compounds most readily absorbed through the skin are primarily organic chemicals. Because perchlorate is an inorganic compound and completely ionized in water, the potential for dermal absorption of perchlorate while bathing and washing is minimal (Mattie et al. 2006).

Perchlorate particles can be suspended in the air and can be inhaled by individuals working in areas where perchlorate is manufactured (Lamm et al. 1999). Although release of perchlorate to the atmosphere is possible during the launching of solid propellant rockets, setting off fireworks (Wilkins et al. 2007), and open detonation of old propellant, no published data were found on levels of perchlorate in the ambient air. Since perchlorate is not volatile, there is little or no risk of inhalation exposure from domestic use of perchlorate-contaminated water (USEPA 2002).

Public water systems, monitored under the UCMR, have detected perchlorate concentrations in several drinking water sources across the United States. In California, perchlorate concentrations (11-270 μg/L) have been found in more than 350 of the approximately 6,700 public drinking water sources (CADHS 2005). The Texas Commission on Environmental Quality has detected perchlorate concentrations (0.5-58.8 μg/L) in an area exceeding 30,000 square miles in western Texas (Jackson et al. 2003; Christen 2003). Extensive testing of public water supplies in Massachusetts has shown several drinking water sources to have perchlorate (MDEP 2006). The occurrence of perchlorate in Japan in the Tone River Basin has been recently detected. Perchlorate was found at high concentrations in the upper Tone River and its tributary, the Usui River, and the maximum concentrations were 340 and 2,300 μg/L, respectively (Kosaka et al. 2007).

Studies conducted by Sanchez and Krieger (2004) revealed that accumulation of perchlorate takes place in some plants, mostly in leafy greens. Studies on lettuce have shown that accumulation occurs mostly in outer leaves and not in consumable portions, such as the head. Studies reported that, when lettuce was irrigated with perchlorate-contaminated water (0.2–5 mg/L), total perchlorate concentrations within the leaves, stems, and roots ranged from 248–1559 mg/kg (Susarla et al. 1999). The

Food and Drug Administration (FDA) found that collected samples of various types of lettuce (e.g., romaine, red leaf, green leaf, and iceberg) from fields or packing sheds contained average perchlorate concentrations ranging from 7.76 to 11.9 µg/kg (USFDA 2004). In addition to these studies, information obtained by the Environmental Working Group (a private nonprofit environmental group) indicated that perchlorate in eight samples of lettuce from a California grower ranged from 0.110–6.9 mg/kg (Lunder and Sharp 2003).

Besides foodstuff crops, perchlorate has been detected in some animal feed crops, dairy, and meat. Alfalfa, a beef cattle and dairy cow feed, tested at 109–555 µg/kg for samples from the Imperial Valley and 146-668 µg/kg from Yuma (Sanchez and Krieger 2004). In addition, perchlorate has been detected in milk. Researchers from Texas Tech University measured perchlorate concentration up to 6.4 µg/kg in samples of supermarket milk (Kirk et al. 2005). The California Department of Food and Agriculture measured perchlorate at an average of 6.05 µg/kg in milk samples. Milk samples (whole, 1% fat, fat-free, and organic) collected at grocery stores in 14 states were found to contain an average perchlorate level of 5.76 µg/kg (USFDA 2004).

Bioremediation of perchlorate

Microbial reduction of perchlorate has been identified as a feasible method of remediation of contaminated environments. However, prior to 1990, very little was known about the diversity or ubiquity of microorganisms that can grow by dissimilatory chlorate or perchlorate respiration (Logan 1998). Although microbial reduction of chlorate has been known for more than 70 years, this metabolism was associated with nitrate-respiring organisms, and chlorate was assumed to be a coincidental substrate for the nitrate reductase. However, this assumption could not explain the presence of specialized enzymes, such as the chlorate reductase C purified from *Proteus mirabilis*, which could only use chlorate as a substrate (Kengen et al. 1999). Now it is known that specialized microorganisms have evolved that can couple growth to the anaerobic reduction of chlorate or perchlorate and completely reduce these compounds to chloride. Most of the known (per)chlorate reducing bacteria (CRB) are facultatively anaerobic or microaerophilic and some, but not all, alternatively respired nitrate, supporting the suggestion that perchlorate reduction is unrelated to nitrate reduction. A central step in the reductive pathway of perchlorate or chlorate

that is common to all CRB is the dismutation of chlorite, which is mediated by the enzyme chlorite dismutase (CD) (Chaudhuri et al. 2002).

Van Ginkel et al. (1995) demonstrated qualitatively that chlorate respiring microorganisms were present in a variety of environments such as wastewaters, rivers, sediments, and soils. This conclusion is supported by both microbial isolations and a multitude of microcosm studies showing that the addition of an appropriate electron donor (i.e., energy source) to a site sample causes perchlorate degradation without the addition of exogenous bacteria (Coates et al. 1999; Hatzinger 2002).

A wide range of perchlorate-reducing bacteria has been isolated; many of them are members of the newly identified genera *Dechloromonas*, *Dechlorospirillum*, and *Azospira* (formerly *Dechlorosoma*) (Xu et al. 2003). All of the bacteria isolated to date are facultative anaerobes, i.e., organisms that can grow in either the presence or the absence of oxygen, provided proper nutrients are available in the medium. Using this metabolic versatility, these organisms are capable of degrading perchlorate, chlorate, and in most cases, nitrate. For perchlorate, these bacteria use an organic substrate or, in some cases, hydrogen gas as an electron donor and use the perchlorate molecule as a terminal electron acceptor. The bacteria oxidize the organic substrate to carbon dioxide (or sometimes an intermediate) and reduce perchlorate initially to chlorate and then chlorite and finally to chloride and oxygen (Van Ginkel et al. 1996; Kengen et al. 1999). The enzyme perchlorate reductase is known to carry out this initial two-step reaction. A second enzyme, chlorite dismutase, subsequently disproportionates chlorite to chloride (Cl^-) and oxygen (O_2) (Coates et al. 1999).

A wide range of different electron donors has been shown to promote the biological reduction of perchlorate by individual strains and/or in environmental microcosms. These substrates include fatty acids (e.g., acetate, citrate, lactate), mixed and pure sugars (e.g., molasses, glucose), protein-rich substrates (e.g., whey, casamino acids), alcohols (e.g., ethanol), vegetable oils (Henry et al. 2003), and hydrogen gas (Logan 1998). However, the specific substrates utilized as energy sources are strain and site specific.

3 Material and Methods

Aquifer material collection and storage

The aquifer material used in this study was a sandy material collected from a confined groundwater aquifer by the Santa Clarita Valley Water District in Santa Clarita, CA, which was contaminated with low levels of perchlorate below 6 µg/L. After collection, the aquifer material was placed in a sealed 5-gallon bucket and transported to the Engineer Research and Development Center (ERDC), Environmental Laboratory, Hazardous Waste Research Center, Vicksburg, MS. The material was stored in a dry area in the laboratory for about 30 days before testing. Aquifer material pH was 8-8.5.

Sample preparation

For each experimental study, a bulk amount of the aquifer material was removed from the 5-gallon buckets and sieved through a 1.7-millimeter (mm) sieve to separate out larger material. Seventy grams of the aquifer material was placed into a 125-milliliter (mL) serum bottle (microcosm reactor) and filled to capacity with 125 mL of 10 mg/L perchlorate and 1000 mg/L acetate solution. All headspace was eliminated at this point. The serum bottles were sealed with a blue butyl 20-millimeter (mm) septum and capped with 20-mm flip-off aluminum seal caps. The perchlorate and acetate solutions were autoclaved at 150 ^{o}C for 3 hours before each experiment was set up, to rid solution of any microorganisms. This resulted in only microbes from aquifer material introduced into the microcosms. The samples were conducted in triplicate sets to test for sample variability.

To ensure good mixing within the serum bottles, the samples were shaken manually for one minute daily. Sampling was performed every three to five days unless analytical results indicated rapid perchlorate degradation. A Becton Dickinson 10-mL luer-lock tip with a B-D sterile precision glide 18-gauge 1.5 needles was used to remove 0.5 mL of aqueous sample from the serum bottles. The sample was placed into a 0.5-mL Dionex polyvial and capped with polyvial filter caps. Afterward the vials were placed in Dionex automated sampler 0.5-ml cassettes. The samples were analyzed on a Dionex DX 500 Ion Chromatograph for perchlorate. In the perchlorate breakdown study, chlorate, chlorite and chloride were analyzed.

Chemicals

Sodium perchlorate anhydrous (minimum purity of 99%) was purchased from EM Science in Gibbstown, NJ and used to prepare perchlorate samples. A Barnstead Nanopure32 Infinity Ultra Pure Water System supplied the deionized water to prepare all solutions. A high concentration stock solution (500 mg/L) was prepared and stored in the refrigerator at 4°C. Dilution from the stock solution was used to set up the low-level concentration needed in the experiments. A mid-level check standard (10 mg/L) was periodically injected during analyses of the samples for quality assurance. A blank (deionized water) was injected with each data set to monitor sample carryover.

The sodium acetate was purchased from Sigma Aldrich in St. Louis, MO (Sigma Ultra, 99% purity) and used as the food source in the microcosm reactors. A high concentration stock solution (25,000 mg/L) was prepared and stored at 4°C. Subsequent standards were diluted from the stock using deionized water as needed.

The sodium nitrate was purchased from Aldrich Chemical in Milwaukee, WI (99% purity) and used in the nitrate study. A high concentration solution (5120 mg/L) was prepared and stored at 4°C. Subsequent dilution was prepared from the 5120 mg/L solution.

Analysis of perchlorate and breakdown products

Experiments were conducted with initial perchlorate concentrations of 10 mg/L and 50 µg/L. These experiments required different analytical approaches. Perchlorate concentrations (for experiments with initial concentrations of 10 mg/L) were determined using a Dionex DX 500 Ion Chromatograph (IC) equipped with a 10 µL injection loop and a Dionex IonPac AS11 column. The sample volume was 0.5 milliliters (mL). The isocratic eluent for the IC was 33.5 millimolar (mM) of sodium hydroxide (NaOH) and the flow rate was 1.50 mL/min. Perchlorate and breakdown products eluted within approximately 7–15 min, and the laboratory reporting limits were 0.050 mg/L.

Low-level perchlorate concentrations (for experiments with initial concentration of 50 µg/L) were determined using a Dionex DX-100 IC equipped with a Dionex Ion Pac AS16 column and a 500-µL loop. The sample volume for analyses was 5 mL. The eluent (100 mM NaOH) was set

at a flow rate of 1.0 mL/min. Perchlorate and breakdown products eluted within a retention time of roughly 15 min. A laboratory reporting limit of 1 µg/L was determined for this configuration based on the low standard.

Statistical method

Statistical analyses of the experimental data were performed using Systat Software Inc. (SSI) Sigma Plot® 10.0 statistical software and Microsoft Excel. Analysis of Variance (ANOVA) tables were generated in which a probability level of <0.05 was considered to be statistically significant for the data sets. One-way ANOVA using the Holm-Sidak Method comparison procedure was used. All tests showed the calculated P value to be <0.001 in the ANOVA tables. The mean of the triplicate reactors was calculated and sample variations are shown as the standard deviation represented by error bars shown in the graphs.

4 Experimental Design

Table 1. Summary of experimental design.

Study	Perchlorate (mg/L)	Acetate (mg/L)	Time (Days)	pH	Temperature (°C)
Initial Batch	10	1000	17	7-8.5	25
Acclimation	10 Target	no additional	7	7-8.5	25
Acetate Concentration Comparison	10	1-1000	30	7-8.5	25
Perchlorate Concentration Comparison	1-1000	1000	63	7-8.5	25
Temperature Comparison	10	1000	64	7-8.5	5-50
pH Variation	10	1000	33	2-14	25
Breakdown Products	10	1000	23	7-8.5	25
Low level Perchlorate	50 µg/L	100	20	7-8.5	25
Nitrate	10	1000	21	7-8.5	25

Initial and acclimation study

The purpose of this experiment was to determine if acetate would stimulate biodegradation of perchlorate in the reactors and to study the role of prior contaminant exposure on the lag phase associated with degradation. The perchlorate degradation study used two triplicate reactor sets. One set consisted of an initial concentration of 10 mg/L perchlorate and 1000 mg/L acetate added to 70 grams of aquifer material. The other set of reactors served as a control containing only perchlorate and aquifer material. Both reactor sets were monitored for 17 days.

After the perchlorate concentration reached concentrations below the laboratory reporting limit (0.05 µg/L) in the acetate reactors, they were respiked with concentrated perchlorate to give a target concentration of 10 mg/L, and the degradation of perchlorate was monitored. After the perchlorate concentration again reached non-detect levels, reactors were respiked a second time. Sequential analyses were done to monitor the degradation of perchlorate in the reactors.

Acetate concentration comparison study

Solutions for this experiment were prepared with the following acetate concentrations: 1, 5, 10, 50, 100, 500, and 1000 mg/L, with initial perchlorate concentration of 10 mg/L to monitor the microbes' tolerance range for an organic substrate. Perchlorate removal was monitored for 30 days.

Perchlorate concentration comparison study

The purpose of this study was to determine the relation of the perchlorate degradation rate with various perchlorate concentrations. The test perchlorate concentrations included 1, 10, 50, 100, 250, and 500 mg/L, with an acetate concentration of 1000 mg/L. Monitoring was over a 63-day period.

Temperature comparison study

This experiment studied the effect of temperature on the rate of perchlorate removal. Reactors were incubated in controlled temperature chambers and monitored separately at temperatures of 5, 10, 25, 30, 40, and 50 ^{0}C for 64 days. These studies were performed in microcosms with 70 grams of aquifer material and 125 mL of solution with 1000 mg/L acetate and 10 mg/L perchlorate.

pH variation study

This experiment consisted of six reactor sets with the perchlorate (10 mg/L)/acetate (1000 mg/L) solution adjusted to the following pHs: 2, 4, 6, 8.4, 11, and 14. Concentrated nitric acid and sodium hydroxide were used for the pH alterations. The nitric acid and sodium hydroxide were added to the reactors with a medicine dropper. Measurements of pH levels were performed using an Accumet Research AR50 dual channel meter. The reactors were sampled for 33 days, after which no appreciable changes occurred in several reactor sets.

Perchlorate breakdown products study

This study investigated the formation of chlorate, chlorite, and chloride as the perchlorate degraded. Aquifer material for this study came from a previously spiked reactor that had reached non-detectable perchlorate concentrations. Previous analysis indicated that the aquifer material had

residual levels of perchlorate breakdown products (chloride and chlorate), which obscured efforts to measure breakdown product concentrations. Therefore, the aquifer material underwent a rinsing procedure to remove these background concentrations. The aquifer material was carefully stirred with a spatula and rinsed seven times with de-ionized water, while decanting the clear water each time. The aquifer material was allowed to settle before decanting; in some cases centrifuging was needed to remove excess water. After the rinsing process, the material was analyzed for background concentrations.

The aquifer material was then placed in the reactors containing 10 mg/L of perchlorate and 1000 mg/L of acetate. Perchlorate degradation and by-product evolution was monitored over a 25-day period.

Perchlorate low level study

This study investigated perchlorate degradation at much lower levels, with an initial level of 50 µg/L. Because the low level analysis uses a much larger sample volume (5 mL compared to 0.5 mL), triplicate sacrificial reactors were set up for each sampling time. The control set consisted of 70 g aquifer material and 125 mL of 50 µg/L perchlorate in de-ionized water, while the acetate reactor set consisted of 70 g of aquifer material and 125 mL of solution consisting of 50 µg/L perchlorate in 100 mg/L acetate (chosen based on the acetate concentration study). The reactors were monitored for 20 days.

Nitrate study

The purpose of this study was to investigate the effect of the presence of a competing electron acceptor on perchlorate degradation. Four reactor sets, each in triplicate, with various nitrate concentrations (0, 5.12, 51.2, and 512 mg/L) were used in this study. The reactors contained an initial perchlorate of 10 mg/L and 1000 mg/L acetate. Perchlorate degradation was monitored over a 21-day period.

5 Results

Initial and acclimation study

The initial study indicated that acetate can stimulate perchlorate removal. After an initial lag phase of 8 days, the perchlorate in the acetate reactor steadily degraded to non-detect levels (<0.05 mg/L) within 17 days (Figure 1). No loss was observed in the control reactor.

There will usually be a lag phase for the biological mass to grow and to acclimate to the new environmental conditions that resulted from the acetate addition or possibly from the need to consume dissolved oxygen that was in the spiked reactors. Error bars, depicting the standard deviation among the replicate reactors, show an interesting pattern. During the lag phase, the replicates had tight error bars. By day 11, as illustrated in Figure 1, the perchlorate concentrations approach the detection limits in the acetate reactor. During degradation, the error bars expanded, presumably due to differences in timing of degradation. The timing differences could have occurred from the non-heterogeneity of the aquifer material or in slight variation of experimental setup.

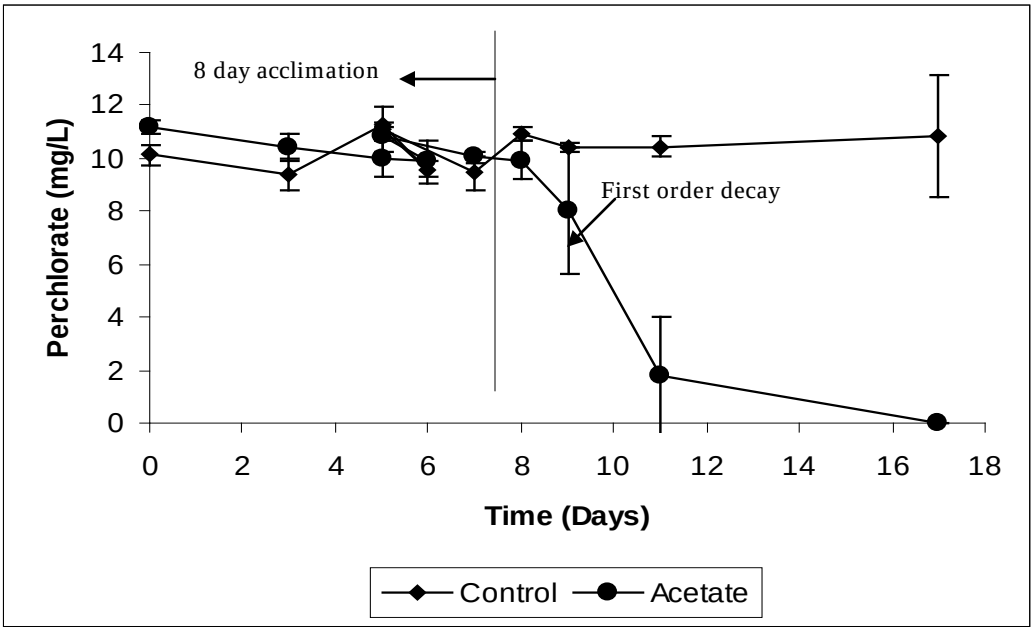

Figure 1. Perchlorate degradation over time with control reactor set vs. reactor set with acetate amendment.

To test whether the lag phase was due to microbial acclimation, the same reactor set was respiked with concentrated perchlorate to create an approximate concentration of 10 mg/L. After respiking the reactors, perchlorate degraded to non-detect levels (<0.05 mg/L) with a shorter lag time (Figure 2, respike 1). Reactors were amended once more (second respike at 10 mg/L perchlorate) and similar results were observed (Figure 2, respike 2). The first order reaction rate for respike 1 was 4.69 mg/L/day and 3.07 mg/L/day for respike 2. These results indicate that, once the microorganisms were acclimated to the contaminant and acetate or removal of all oxygen, the lag phase was reduced.

Acetate concentration comparison study

This study indicates that higher acetate concentrations resulted in faster perchlorate degradation, and degradation did not occur if acetate concentrations were below 50 mg/L (Figure 3). All the treatments had lag phases. This lag was probably due to the dissolved oxygen and the biomass growth that was in the test solutions prior to addition to the reactors. The 500- and 1000-mg acetate per liter treatments showed the perchlorate concentration was at non-detect levels (>0.05 mg/L) within 23 days. The 50- and 100-mg/L acetate concentration treatments took a little longer to reach non-detect levels, doing so in 30 days. All the treatments below 1, 5, and 10 mg/L of acetate did not show any significant perchlorate degradation. The one-way analysis of variance shows that there was a significant difference ($p \leq 0.05$) between the 1000 vs. 100 mg/L, 500 vs. 100 mg/L, and 50 vs. 100 mg/L studies.

Perchlorate concentration comparison study

Perchlorate degradation was tested with initial concentrations ranging from 1 to 500 mg/L. Perchlorate was degraded to a level below the detection limit (0.50 mg/L) for all starting concentrations of perchlorate (Figure 4). Lower concentration reactors reached non-detect perchlorate levels (0.05 mg/L) faster than the higher concentration reactors, as it appears that the higher concentration reactors had longer lag phases. The degradation rates were determined by analyzing trends after the lag phases. The linear (or zero order) degradation rates ranged from 0.22 to 28.74 mg/L/day and increased with increasing initial concentrations, indicating that the rate is concentration dependent. An average first order rate constant of 0.51 ± 0.19 day $^{-1}$ was determined for this concentration range.

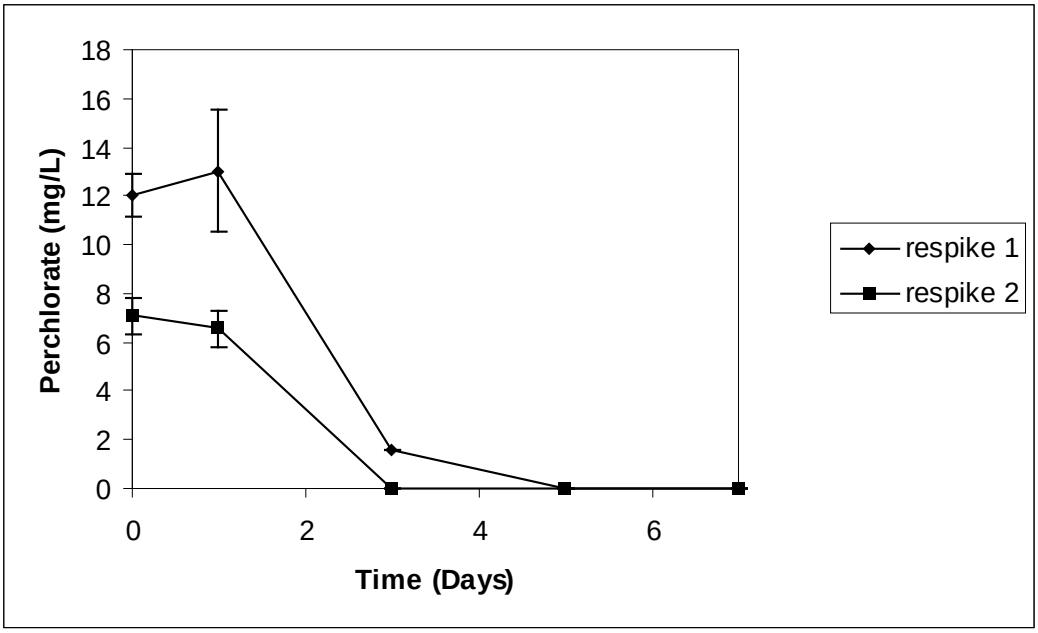

Figure 2. Perchlorate acclimation study: Comparison of perchlorate removal from previously exposed perchlorate contaminant (shorter lag phase).

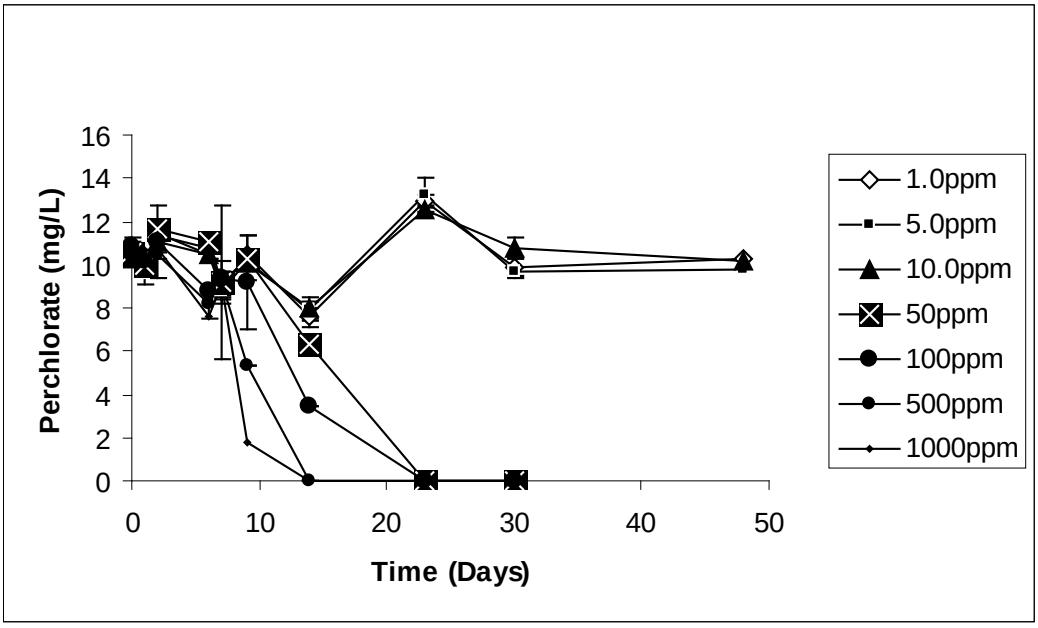

Figure 3. Comparison of perchlorate degradation with various acetate concentrations.

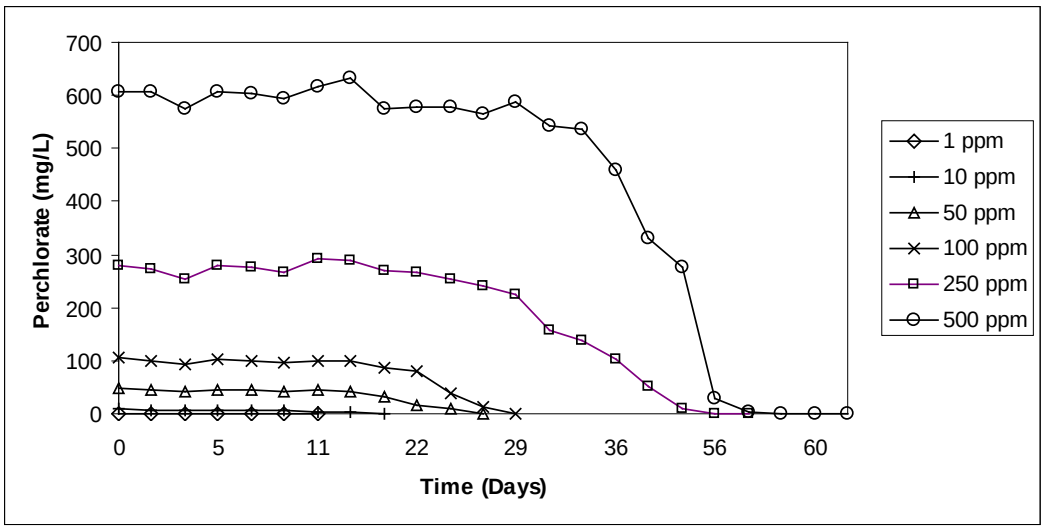

Figure 4. Various initial perchlorate concentration effects on rate of degradation.

In general, first order kinetics is sufficient to describe concentration-dependent degradation rates. However, Michaelis-Menton kinetics, which account for rate constant changes with changing contaminant concentration, can provide more refined degradation rate information. Therefore, the data were analyzed to investigate if Michaelis-Menton kinetics could provide a good description of contaminant removal. The Michaelis-Menton equation is: $v_o = \frac{V_m S_o}{K_m + S_o}$

where So is the initial contaminant (substrate) concentration (mg/L), *vo* is the reaction velocity (mg/L/hr), which is equivalent to the zero order reaction rate, at So, *Vm* is the maximum reaction velocity (mg/L/hr), and *Km* is the half-saturation constant (mg/L/hr). The data were plotted using a standard Eadie-Hoffstee plot, which yielded a *Vm* of 20.35 mg/L/day and a *Km* of 91.45 mg/L/day. The Michealis-Menton fit in comparison with the actual data was good at the lower concentrations but underestimated degradation rates at high concentrations (Figure 5).

Temperature comparison study

The temperature data are presented in Figure 6. Perchlorate was removed to non detect levels at temperatures ranging from 10 to 50°C. After 63 days, there was no observable degradation in the 5°C study.

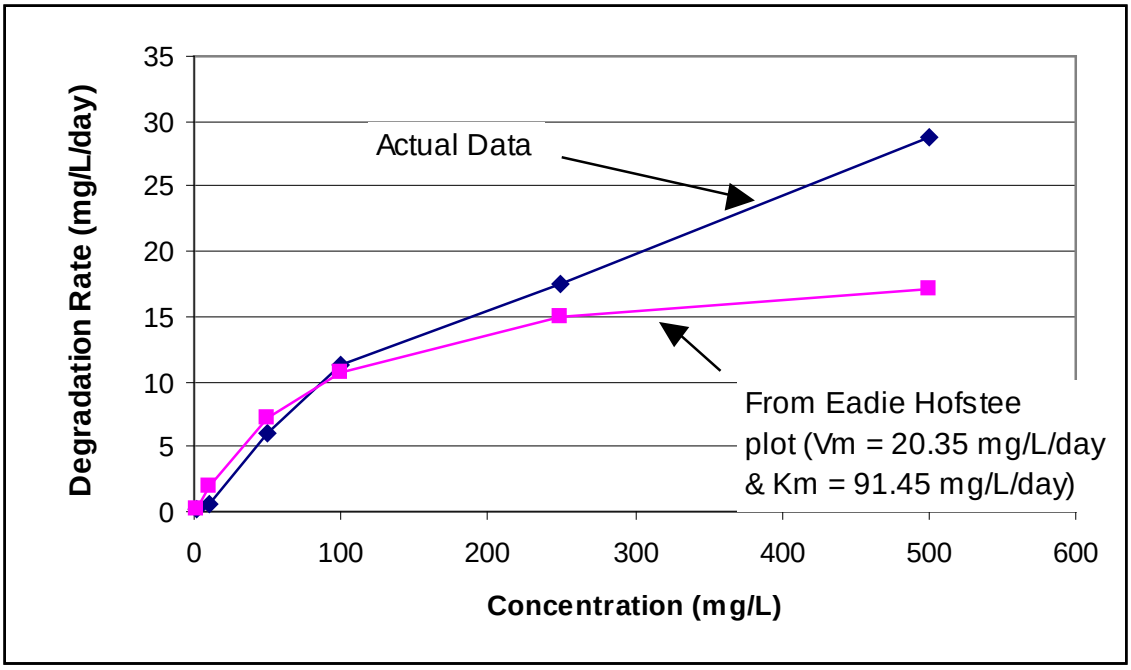

Figure 5. Zero order degradation rate (reaction velocity) versus concentration, and fit by Michaelis-Menton parameters derived from an Eadie-Hofstee plot.

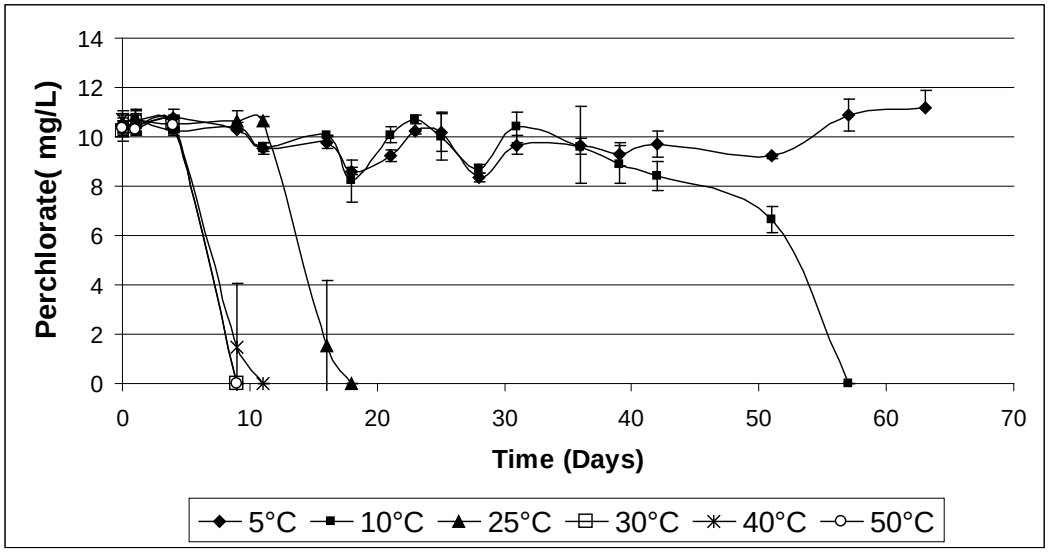

Figure 6. Effect of temperature on initial perchlorate concentration 10 mg/L removal over time.

For the remaining temperature studies, lower temperature reactors appeared to produce longer lag times. In the 10°C reactors nothing was observed until after the 40 days lag phase and the perchlorate was below non-detect in approximately 57 days. In the 25°C reactor the degradation started in about 12 days and was at non-detectable level within 18 days. The 30-50°C reactors started the perchlorate process after 4 days and

degradation was complete within 9-11 days. These results show that perchlorate removal increased with temperature and was not limited at temperatures up to 50°C. The lower temperature reactors produced longer lag times.

Table 2. Zero and first order reaction rates of perchlorate biodegradation in 1000-mg/L acetate at varying initial perchlorate concentrations.

Concentration (mg/L)	Zero Order Reaction Rate (mg/L/day)	First Order Reaction Rate (1/day)
1.0	0.22	0.41
10.0	0.51	0.27
50.0	6.09	0.64
100	11.20	0.49
250	17.52	0.45
500	28.74	0.80
Average	10.71	0.51
Standard Deviation	11.02	0.19

pH variation study

This experiment tested the effect of pH variation on perchlorate degradation. This study consisted of six reactor sets ranging from pH 2.0 to 14. The initial perchlorate concentration was 10 mg/L and with an acetate concentration of 1000 mg/L. As anticipated, no appreciable perchlorate degradation was detected in the pH 2.0, pH 11, and pH 14 reactors over the 40 day study (Figure 7). After a lag phase, the pH 4.0 reactors reached non-detectable levels (0.05 mg/L) within 18 days and the pH 6.0 and 8.4 reactors reached non-detectable levels within 24 days. The pH 4.0, 6.0 and 8.4 reactors all resulted in complete perchlorate degradation within 31 days.

Perchlorate breakdown products study

This study was conducted to monitor the breakdown products of perchlorate with previously spiked aquifer material that went through a vigorous rinsing process in order to remove background concentrations. Previously spiked soil was used to shorten the lag phase, but the rinsing process might have eliminated some of the microbes. The initial spike concentration of perchlorate was 97.72 micromolar (µmol/L) or (76%), and the initial chloride concentration was 30.10 µmol/ or 24% with no chlorate

or chlorite present (Figure 8). After a lag phase, the perchlorate concentration decreased to 57%; while the chlorate concentration increased from non-detectable levels to 0.02 mg/L, and chloride concentrations increased to 43%. Chlorite was not detected. After 25 days, perchlorate was below detection limit (3%). Chloride concentration increased to 3.27 mg/L, which accounted for greater than 95% of the initial perchlorate added to the reactor (Figure 8). The perchlorate had mostly degraded to chloride.

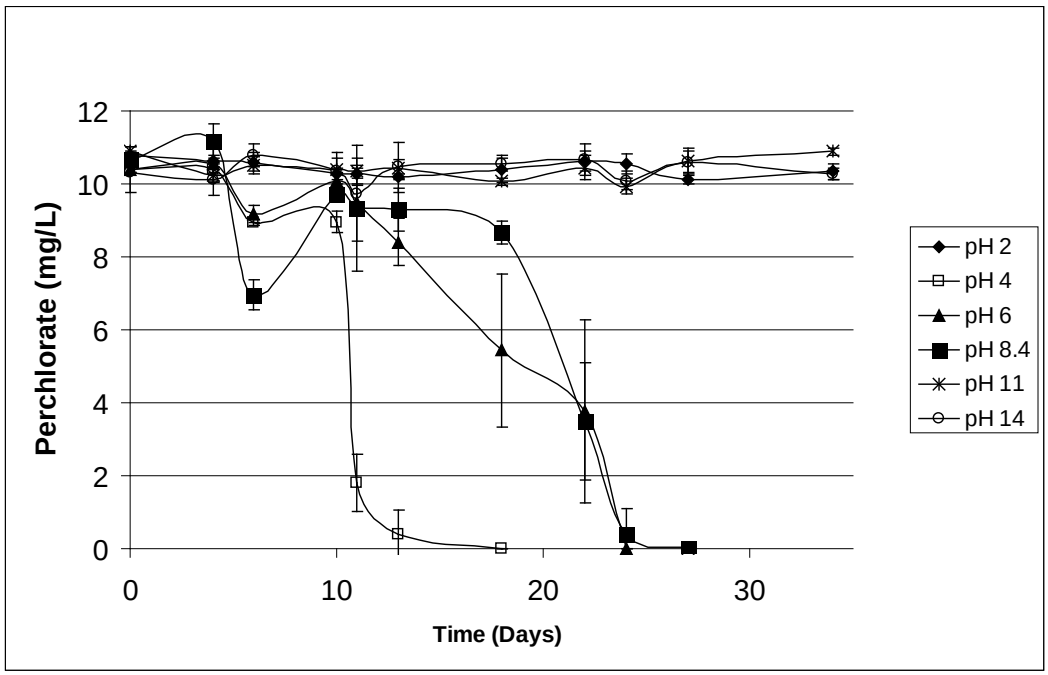

Figure 7. Effect of pH range 2.0-14 on perchlorate degradation.

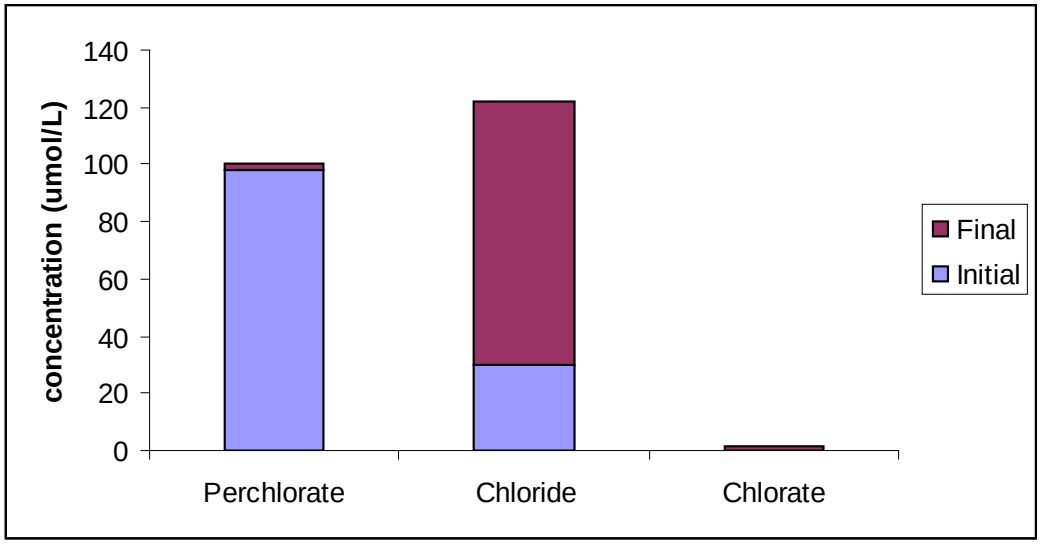

Figure 8. Initial and final mass of perchlorate, chloride, and chlorate.

Perchlorate low-level study

The previous studies were conducted with perchlorate concentrations that were in the parts per million; however, there are many perchlorate contaminated sites where the Interim Drinking Water Health Advisory level is 15 µg/L, and for California it is 6 µg/L. In California, perchlorate concentrations ranging from 11 to 270 µg/L have been detected in more than 350 of the approximately 6,700 public drinking water sources (CADHS 2005). Therefore, an experiment was conducted using a target perchlorate concentration that represented the concentration observed in drinking water sources samples of 50 µg/L with a treatment target of 6 µg/L, which is the State of California MCL (CADHS 2006). One reactor was fortified with 100 mg/L of acetate; the other, which served as a control, had no acetate added. The reactor that was amended with the acetate had an initial lag phase of 9 days followed by rapid perchlorate degradation, which reached a final concentration of less than 6 µg/L (Figure 9). The control reactor had no degradation. The first order degradation rate constant derived for the portion of the curve where the perchlorate was present degraded at 0.28/day (after the 9 days lag phase).

Nitrate study

Nitrate is another compound that can be used as an electron acceptor in the absence of oxygen. As such, nitrate may compete with perchlorate for reaction with the electron donor, in this case acetate. To test this competition, four reactor sets with 1000 mg/L acetate, nitrate concentrations ranging from 0 to 512 mg/L, and perchlorate concentrations at 10 mg/L were prepared and sampled for 22 days. No appreciable difference in the perchlorate degradation was found between the reactor sets, suggesting that competition by nitrate did not occur under the tested conditions (Figure 10).

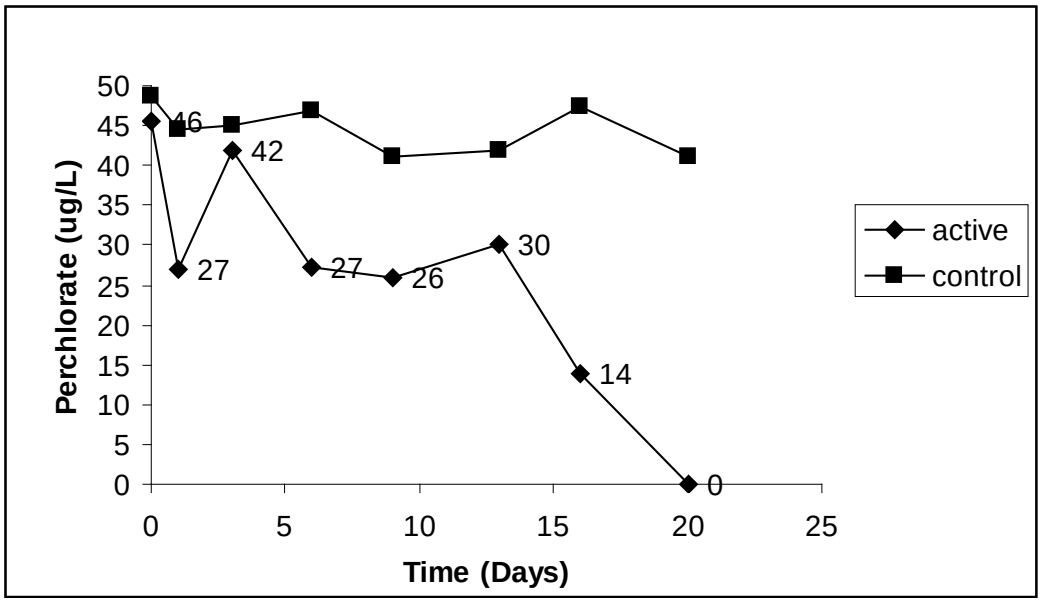

Figure 9. Perchlorate concentration 50 µg/L reactor with 100 mg/L of acetate added (active) vs. a control reactor (control) with no acetate. Final perchlorate concentrations in acetate reactor were below CA MCL of 6 µg/L.

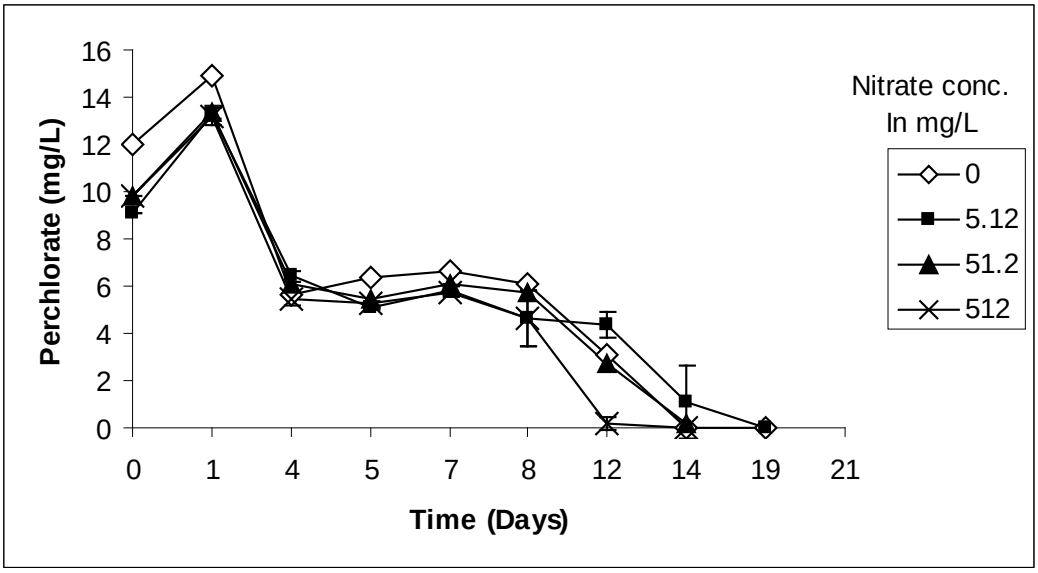

Figure 10. Effect of variation of nitrate concentration on perchlorate degradation.

6 Discussion

Results from the microcosm reactors experiments presented above indicate that perchlorate is readily biodegraded under a broad range of environmental conditions. The addition of an electron donor (e.g., acetate) was critical to stimulating degradation. Without acetate, perchlorate removal was minimal at best.

The lowest acetate concentration tested was 1.0 mg/L; however, the 50-1000 mg/L stimulated perchlorate degradation. An acetate concentration of 10 mg/L did not yield appreciable perchlorate degradation after 30 days. Therefore, an acetate concentration threshold existed between 10 and 50 mg/L where perchlorate degradation was not observed. Although higher acetate concentrations did seem to result in faster perchlorate removal, the difference was not especially great. For most field applications, lower acetate concentrations would be more cost-effective and could have a smaller impact on the geochemistry of the aquifer. However, if sufficient acetate is added, bioremediation is possible.

Lag phases were observed in most experiments. These periods of minimal degradation ranged from 8 to 30 days (500 mg/L condition for the perchlorate concentration study). Of particular interest in design was that, in many cases, changes in environmental conditions resulted in changes in lag phases, and that the lag phase frequently was a key factor controlling the time needed to degrade the contaminant to non-detectable levels. These results suggest that understanding the site conditions to properly address the lag phase may be a critical design factor, particularly in the early stages of establishing biological activity.

Once biological activity was established, new pulses of perchlorate were degraded with a shorter lag phase. Therefore, concern about lag phase may be minimal once biological activity has been established. Once biological activity was established, the perchlorate removal rates were comparable to those found in other reductive transformation studies by Medina et al. (2006).

Various factors, including pH, temperature, acetate concentration, and perchlorate concentration, were tested to study their effect on perchlorate

degradation rate over time. pH had a liberal range in which effective perchlorate degradation occurred, degradation being inhibited only at extreme conditions. In this study, nitrate did not have any competitive interaction with acetate on perchlorate degradation, which is encouraging given that nitrate contamination is widespread throughout the United States. According to Choi and Silverstein (2008), cultures enriched with nitrate as the electron acceptor did not reduce perchlorate immediately. This indicates that there are chlorate-reducing bacteria that do not utilize nitrate. Temperature, however, could turn out to be a sensitive factor. Perchlorate was effectively removed at temperatures as low as 10°C, although the lag phase increased with increasing temperature. However, no measurable degradation occurred at 5°C over 40 days. This indicates that in situ biodegradation of perchlorate may be limited in cold environments where groundwater temperatures are below 10°C. Temperature affects kinetics, and lower temperatures result in lower reactions and biological growth. Results may vary because soil as microbial species will probably also vary.

The perchlorate concentration of 10 mg/L was used for most of the experiments in this study. However, many sites have been identified with perchlorate concentrations less than 1 mg/L. In the low-level study, after degradation, perchlorate concentrations reached levels below the California MCL of 6 µg/L. This level is also lower than the EPA level of 24 µg/L. It appears that bioremediation can be effective at reaching these low regulatory levels.

7 Conclusions

In summary, the following conclusions were obtained from this study:

- Perchlorate degradation was stimulated with an acetate amendment. However, degradation was delayed by a lag phase.
- After acclimation of the microorganisms, degradation of newly added perchlorate occurred rapidly.
- Acetate concentrations ranging from 50 mg/L and higher stimulated perchlorate removal in the microcosm reactors.
- Extreme pH (2 or below and greater than 11) inhibited the perchlorate degradation process.
- Chloride was the primary breakdown product of perchlorate. Increases in chloride concentration stoichiometrically accounted for greater than 99.5% of the degraded perchlorate . A small amount of chlorate was also formed as an intermediate.
- An experiment conducted at 50 μg/L achieved perchlorate concentrations below the MCL of 6 μg/L set by the California Department of Health Services. The Federal regulatory levels are still higher at 24 μg/L.
- Nitrate, a potential competitor with perchlorate as an electron acceptor, did not measurably affect the rate of perchlorate removal in this study.

References

Batista, J. R., F. X. McGarve, and A. R. Vieira. 2000. The removal of perchlorate from waters using ion-exchange resins. In *Perchlorate in the Environment*, ed. E. T. Urbansky, 135-45. New York: Kluwer Academic/Plenum Publishers.

California Department of Health Services (CADHS). 2002. California Experience with Perchlorate.*www.dhs.cahwnet.gov/ps/ddwem/chemicals/perchl/perchlinder.htm*

_____. 2005. Perchlorate in California Drinking Water: Monitoring Update. *http://www.dhs.ca.gov/ps/ddwem/chemicals/perchl/monitoringupdate.htm*.

_____. 2006. Drinking Water Notification Levels [accessed on line]. *http://www.dhs.ca.gov/ps/ddwem/chemicals/AL/notificationlevels.htm*.

California Office of Environmental Health Hazard Assessment (OEHHA). 2004. Public Health Goal for Perchlorate in Drinking Water, Office of Environmental Health Hazard Assessment. *http://www.oehha.ca.gov/water/phg/index.html*.

Chaudhuri, S. K., S. M. O'Connor, R. L. Gustavson, L. A. Achenbach, and J. D. Coates. 2002. Environmental Factors that Control Microbial Perchlorate Reduction. *Applied and Environmental Microbiology* 68: 4425-30.

Cheng, Q., F. Liu, J. E. Canas, and T.A. Anderson. 2006. A Cleanup Method for Perchlorate Determination in Urine. *Talanta* 68: 1457-62.

Choi, H., and J. Silverstein. 2008. Inhibition of perchlorate by nitrate in a fixed biofilm reactor. *Journal of Hazardous Materials* 159: 440-45.

Christen, K. 2003. Perchlorate mystery surfaces in Texas. *Environmental Science & Technology* Online. *http://pubs.acs.org/subscribe/journals/esthag-w/2003/-oct/science/kc_perchlorate.html*.

Clausen, J., J. Robb, D. Curry, and N. Korte. 2004. A Case Study of Contaminants on Military Ranges: Camp Edwards, Massachusetts. *U.S. Environmental Pollution* 129: 12-21.

Coates, J. D., U. Michaelidou, R. A. Bruce, S. M. O'Connor, J. N. Crespi, and L. A. Achenbach. 1999. Ubiquity and Diversity of Dissimilatory (Per)chlorate-Reducing Bacteria. *Applied and Environmental Microbiology* 65: 5234-41.

Dasgupta, P. K., A. B. Kirk, J. V. Dyke, and S-I. Ohira. 2008. Intake of iodine and perchlorate and excretion in human milk. *Environmental Science and Technology* 42:8115-8121.

Ellington, J. J., N. L. Wolfe, A. G. Garrison, J. J. Evans, J. K. Avants, and Q. Teng. 2001. Accumulation of Perchlorate in Tobacco Plants and Tobacco Products. *Environmental Science and Technology* 35: 3213-18.

Erickson, B. 2003. Perchlorate found in Texas Milk. *Environmental Science & Technology* Online. http://pubs.acs.org/subscribe/journals/esthag-w/2003/oct.

Ericksen, G. E. 1983. The Chilean nitrate deposits. *American Science* 71: 366-374.

Gibbs, J. P., R. Ahmad, K. S. Crump, D. P. Houck, T. S. Leveille, J. E. Findley, and M. Francis. 1998. Evaluation of a Population with Occupational Exposure to Airborne Ammonium Perchlorate for Possible Acute or Chronic Effects on Thyroid Function. *Journal of Occupational and Environmental Medicine* 40: 1072-82.

Gingras, T. M., and J. R. Batista. 2002. Biological Reduction of Perchlorate in Ion Exchange Regenerant Solutions Containing High Salinity and Ammonium Levels. *Journal of Environmental Monitoring* 4: 96-101.

Ground-Water Remediation Technologies Analysis Center (GWRTAC). 2001. *http://www.perchlorateinfo.com/perchlorate.html*.

Hatzinger, P. B. 2002. In Situ Bioremediation of Perchlorate. Final Report to the Strategic Environmental Research and Development Program, Project CU-1163.

Henry, B. M., D. R. Griffiths, J. R. Gonzales, and P. E. Haas. 2003. Strategies Using Vegetable Oil for Enhanced Bioremediation of Chlorinated Solvents. Presented at the 7th International In Situ and On-Site Bioremediation Symposium, Orlando, FL.

Hogue, C. 2003. Of Lettuce and Rocket Fuel. *Chemical & Engineering News* 81: 11.

Interstate Technology and Regulatory Council (ITRC). 2002. A Systematic Approach to In-Situ Bioremediation in Groundwater, Including Decision Trees on Situ Bioremediation for Nitrates, Carbon Tetrachloride, and Perchlorate. http://www.itrcweb.org/users/isb-8r.pdf.

Interstate Technology and Regulatory Council (ITRC) Perchlorate Team. 2005. Perchlorate: Overview of Issues, Status, and Remedial Options. Washington, DC: Interstate Technology & Regulatory Council.

Jackson, W. A., K. Tan, and T. Anderson. 2003. Distribution, Degradation, and Uptake of Perchlorate (PC) in Contaminated Stream Sediments. Presented at the 7th International In Situ and On-Site Bioremediation Symposium, Orlando, FL.

Kengen, S. W. M., G. B. Rikken, W. R. Hagen, C. G. Van Ginkel, and A. J. M. Stams. 1999. Purification and Characterization of (Per)chlorate Reductase from the Chlorate-Respiring Strain GR-1. *Journal of Bacteriology* 181: 6706-11.

Kirk, A. B., P. K. Martinelango, K. Tian, A. Dutta, E. E. Smith, and P.K. Dasgupta. 2005. Perchlorate and Iodide in Diary and Breast Milk. *Environmental Science & Technology* 39: 2011-17.

Kosaka, K. M., Asami, Y. Matsuoka, M. Kamoshita, S. Kunikane. 2007. Occurrence of perchlorate in drinking water sources of metropolitan area in Japan. *Water Research* 41: 3474-3482.

Kroon, A. G. M., and C. G. Van Ginkel. 2004. Biological Reduction of Chlorate in a Gas-Lift Reactor Using Hydrogen as an Energy Source. *Journal of Environmental Quality* 33: 2026-2029.

Lamm, S. H., L. E. Braverman, F. X. Li, K. Richman, S. Pinoand, and G. Howearth. 1999. Thyroid Health Status of Ammonium Perchlorate Workers: A Cross-Sectional Occupational Health Study. *Journal of Occupational and Environmental Medicine* 41: 248-60.

Logan, B. E. 1998. A Review of Chlorate- and Perchlorate-Respiring Microorganisms. *Bioremediation Journal* 2: 69-79.

Logan, B. E., J. Wu, and R. F. Unz. 2001. Biological Perchlorate Reduction in High-Salinity Solutions. *Water Research* 134.67.99.64.

Lunder, S., and R. Sharp. 2003. Suspect Salads: Toxic Rocket Fuel Found in Samples of Winter Lettuce. The Environmental Working Group *www.ewg.org/reports/-suspectsalads*

Massachusetts Department of Environmental Protection (MDEP), 2006. Perchlorate Toxicological Profile and Health Assessment. Office of Research and Standards, Boston, MA.

Mattie, D. R., J. Strawson, and J. Zhao. 2006. Perchlorate Environmental Occurrence, Interactions and Treatment. *Perchlorate Toxicity and Risk Assessment*. Springer US: 169-96.

Medina, V. F., A. Weathersby, M. Jones, and A. Morrow. 2006. Column Study Simulating In Situ Bioremediation of Perchlorate Using Acetate as an Organic Substrate. *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management* 10: 102-7.

Motzer, W. E. 2001. Perchlorate: Problems, Detection, and Solutions. *Environmental Forensics* 2: 301-11.

Nozawa-Inoue, M., K. M. Scow, and D. E. Rolston. 2005. Reduction of Perchlorate and Nitrate by Microbial Communities in Vadose Soil. *Applied Environmental Microbiology* 71: 3928-34.

Renner, R. 1998. Perchlorate-tainted wells spur government action. *Environmental Science & Technology News* 32: 210A.

Rikken, G. B., A. G. M. Kroon, and C. G. Van Ginkel. 1996. Transformation of perchlorate into chloride by a newly isolated bacterium. *Applied Microbiology Biotechnology* 45: 420-26.

Sanchez, C., and R. I. Krieger. 2004. Occurrence of Perchlorate in Vegetable Crops Produced in the Lower Colorado River Region. Risk Forum presentation, U.S. Department of Agriculture, Office of Risk Assessment and Cost-Benefit Analysis. *www.usda.gov/agency/oce/oracba/forum.htm*.

Schumacher, J. C. 1960. Perchlorates: Their properties, manufacture and uses. *American Chemical Society Monograph* 146. New York: Reinhold Publishing.

Sellers, K., K. Weeks, W.R. Alsop, S.R. Clough, M. Hoyt, B. Pugh, and J. Robb. 2006. Perchlorate: Environmental problems and solutions. Taylor & Francis/ Boca Raton, FL: CRC Press: 226.

Smith, D. J., C. E. Oliver, W. L. Shelver, T. Caesar, and R. C. Anderson. 2009. Chlorate metabolism in pure cultures of Escherichia coli 0157:H7 pretreated with either nitrate or chlorate. *Journal of Agricultural and Food Chemistry* 57:10216-10224.

Strategic Environmental Research and Development Program (SERDA). 2006. *Engineered intrinsic bioremediation of ammonium perchlorate in groundwater*. ER-1562.

Susarla, S., N. L.Wolfe, and S.C. McCutsheon. 1999. Perchlorate Uptake in Lettuce Seedlings. Symposia Papers presented before the Division of Environmental Chemistry, American Chemical Society, New Orleans, LA. August 22-26.

Urbansky, E. T. 1998. Perchlorate Chemistry: Implications for Analysis and Remediation. *Bioremediation Journal* 2: 81-95.

Urbansky, E. T., M. L. Magnuson, C. A. Kelty, and S. K. Brown. 2000. Perchlorate uptake by salt cedar (*Tamarix ramosissima*) in the Las Vegas Wash riparian ecosystem. *Science Total Environment* 256: 227-32.

Urbansky, E. T., S. K. Brown, M. L. Magnuson, and C. A. Kelty. 2001. Perchlorate Levels In Samples of Sodium Nitrate Fertilizer Derived from Chilean Caliche. *Environmental Pollution* 112: 299-302. Available at: *http://www.clu-in.org/download/contaminantfocus/perchlorate/EPClO4.pdf*

United States Environmental Protection Agency (USEPA). 1999. Region 9 perchlorate. *http://www.epa.gov/safewater/ccl/perchlor/r9699fac.pdf*

_____. 2002. Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization. NCEA-1-0503 (External Review Draft), Washington, DC.

_____. 2005. EPA Sets Reference Dose for Perchlorate. USEPA press release, 18 Feb 2005. URL: *http://yosemite.usepa.gov (40)*.

_____. 2007. Drinking Water: Regulatory Determinations Regarding Contaminants on the Second Drinking Water Contaminant Candidate List – Preliminary Determinations. *Federal Register* 72 FR 24016.

_____. 2008. Federal Facilities Restoration and Reuse, Perchlorate. Available at: *http://www.epa.gov/swerffrr/documents/perchlorate.htm*

U.S. Food and Drug Administration (USFDA). 2004. Exploratory Data on Perchlorate in Food. Center for Food Safety and Applied Nutrition, Office of Plant and Dairy Foods. *www.cfsan.fda.gov/~dms/clo4data.html*.

Van Ginkel, C. G., C. M. Plugge, and C. A. Stroo. 1995. Reduction of chlorate with various energy substrates and inocula under anaerobic conditions. *Chemosphere* 31: 4057-66.

Van Ginkel, C. G., G. B. Rikken, A. G. M. Kroon, and S. W. M. Kengen. 1996. Purification and Characterization of Chlorite Dismutase: A Novel Oxygen-Generating Enzyme. *Archives of Microbiology* 166: 321-26.

Wilkins, R. T., D. D. Fine, and N. G. Burnett. 2007. Perchlorate in a Municipal Lake following Fireworks Displays. *Environmental Science & Technology* 41: 3966-71.

Wu, J., R. Unz, H. Zhang, and B. E. Logan. 2001. Persistence of perchlorate and the relative numbers of perchlorate and chlorate respiring microorganisms in natural waters, soil and waste water. *Bioremediation Journal* 5: 119-30.

Xu, J. Y., Y. Song, B. Min, L. Steinberg, and B. E. Logan. 2003. Microbial Degradation of Perchlorate: Principles and Applications. *Environmental Engineering Science* 20: 405-22.

Appendix A: Summary Tables from Experiments

Table A1. Acetate concentration comparison study.

Actate Degradation Study							
Time (days)	**0.00**	**1.00**	**2.00**	**7.00**	**14.00**	**23.00**	**30.00**
1.0 ppm	9.94	10.30	10.77	10.01	10.80	10.16	10.63
	10.24	10.35	10.99	10.33	10.43	9.87	10.40
	9.70	10.58	10.44	10.95	10.60	9.52	9.73
avg	9.96	10.41	10.74	10.43	10.61	9.85	10.25
stdev	0.27	0.15	0.28	0.48	0.18	0.32	0.46
5.0ppm	9.42	10.40	10.56	10.80	10.21	9.90	9.61
	9.75	10.95	10.88	9.55	10.62	10.41	10.02
	9.54	10.05	9.92	10.07	10.74	8.79	9.63
avg	9.57	10.47	10.45	10.14	10.53	9.70	9.75
stdev	0.17	0.46	0.49	0.63	0.28	0.83	0.23
10 pppm	10.03	10.22	10.56	9.69	10.09	10.78	9.70
	9.89	10.17	10.88	10.08	10.08	10.66	10.40
	9.64	10.88	9.92	10.49	10.61	10.93	10.55
avg	9.85	10.42	10.45	10.08	10.26	10.79	10.22
stdev	0.20	0.40	0.49	0.40	0.30	0.13	0.45
50 ppm	9.46	10.37	10.24	10.77	9.52	6.29	0.00
	10.27	10.11	9.74	10.48	8.77	7.31	0.00
	10.25	10.37	10.22	9.70	9.17	5.29	0.00
avg	9.99	10.28	10.07	10.32	9.15	6.30	0.00
stdev	0.46	0.15	0.29	0.55	0.38	1.01	0.00
100 ppm	10.06	10.38	10.75	9.49	10.09	5.93	0.00
	9.85	9.84	7.97	9.27	9.09	2.17	0.00
	10.61	9.95	7.74	9.32	8.38	2.16	0.00
avg	10.17	10.06	8.82	9.36	9.18	3.42	0.00
stdev	0.39	0.29	1.67	0.12	0.86	2.18	0.00
500 ppm	10.44	10.92	9.84	8.16	1.24	0.00	0.00
	10.01	10.05	9.03	7.97	7.28	0.00	0.00
	10.30	10.44	8.62	8.37	7.53	0.00	0.00
avg	10.25	10.47	9.16	8.17	5.35	0.00	0.00
stdev	0.22	0.43	0.62	0.20	3.56	0.00	0.00
1000 ppm	10.43	10.84	9.57	7.62	0.00	0.00	0.00
	10.86	10.99	8.76	7.85	5.20	0.00	0.00
	10.58	10.45	8.85	7.31	0.00	0.00	0.00
avg	10.62	10.76	9.06	7.59	1.73	0.00	0.00
stdev	0.21	0.28	0.45	0.27	3.00	0.00	0.00

Table A2. Perchlorate concentration comparison study.

	1.0 ppm				
Time (days)	Rep 1	Rep 2	Rep 3	avg	stdev
0	1.00	1.00	1.00	1.00	0.00
1	0.79	0.68	0.88	0.78	0.10
3	0.79	0.68	0.88	0.78	0.10
5	0.71	0.67	1.02	0.80	0.19
8	0.59	0.56	0.85	0.67	0.16
10	0.37	0.11	0.40	0.30	0.16
11	0.00	0.00	0.00	0.00	0.00

	10 ppm				
Time (days)	Rep 1	Rep 2	Rep 3	avg	stdev
0	8.35	8.35	8.35	8.35	0.00
1	7.85	7.81	7.58	7.75	0.15
3	7.54	7.25	7.55	7.44	0.17
5	7.79	7.93	8.20	7.98	0.21
8	7.24	6.93	7.44	7.20	0.26
10	5.39	5.84	5.62	5.62	0.23
11	4.10	5.08	5.25	4.81	0.62
17	3.40	4.41	4.43	4.08	0.59
19	0.00	0.00	0.00	0.00	0.00

	50 ppm				
Time (days)	Rep 1	Rep 2	Rep 3	avg	stdev
0	47.09	47.09	47.09	47.09	0.00
1	44.60	47.47	43.97	45.35	1.86
3	38.15	43.76	41.80	41.23	2.85
5	44.56	46.79	45.92	45.76	1.12
8	42.26	46.80	44.36	44.47	2.27
10	39.77	43.22	41.92	41.64	1.74
11	41.45	46.62	44.24	44.10	2.59
17	38.88	45.81	43.13	42.61	3.49
19	27.09	38.24	34.92	33.41	5.72
22	13.28	14.74	16.99	15.00	1.87
24	8.55	6.55	10.68	8.59	2.07
26	0.00	0.00	0.00	0.00	0.00

	100 ppm				
Time (days)	Rep 1	Rep 2	Rep 3	avg	stdev
0	104.51	104.51	104.51	104.51	0.00
1	98.14	102.45	100.74	100.44	2.17
3	90.99	92.70	91.40	91.70	0.90
5	99.68	103.30	102.34	101.77	1.88
8	95.11	101.18	99.58	98.62	3.15
10	93.49	95.98	96.29	95.26	1.53
11	97.70	104.54	100.29	100.84	3.45
17	96.03	99.44	103.56	99.68	3.77
19	83.35	87.84	88.84	86.68	2.92
22	80.83	83.72	75.72	80.09	4.05
24	69.29	40.43	4.22	37.98	32.60
26	31.88	4.22	0.00	12.03	17.31
29	0.00	0.00	0.00	0.00	0.00

	250 ppm				
Time (days)	Rep 1	Rep 2	Rep 3	avg	stdev
0	278.14	278.14	278.14	278.14	0.00
1	276.30	272.87	268.80	272.66	3.75
3	250.16	255.18	255.58	253.64	3.02
5	275.81	277.39	281.81	278.34	3.11
8	268.49	279.03	279.91	275.81	6.35
10	271.50	261.81	267.98	267.09	4.91
11	292.19	295.04	292.19	293.14	1.64
17	283.99	295.74	291.26	290.33	5.93
19	270.75	271.02	269.18	270.31	1.00
22	265.85	269.67	263.97	266.50	2.91
24	265.04	257.31	241.51	254.62	11.99
26	257.86	234.34	232.98	241.73	13.99
29	267.79	210.04	200.99	226.28	36.24
30	243.87	112.02	117.42	157.77	74.62
32	226.87	89.05	98.32	138.08	77.03
36	141.59	82.35	86.06	103.33	33.19
46	66.68	34.78	51.08	50.85	15.95
49	12.59	7.53	10.35	10.16	2.53
56	1.48	0.38	0.59	0.81	0.58
57	0.00	0.00	0.00	0.00	0.00

	500 ppm				
Time (days)	Rep 1	Rep 2	Rep 3	avg	stdev
0	605.87	605.87	605.87	605.87	0.00
1	604.52	605.62	606.21	605.45	0.86
3	570.84	567.22	582.00	573.35	7.70
5	595.14	620.09	600.62	605.28	13.11
8	598.82	607.13	609.75	605.23	5.71
10	594.86	587.78	597.32	593.32	4.95
11	616.21	618.88	615.44	616.84	1.81
17	629.61	632.01	638.91	633.51	4.83
19	582.02	576.16	569.89	576.02	6.06
22	592.23	575.84	564.35	577.47	14.01
24	584.37	587.73	566.01	579.37	11.69
26	570.94	568.86	557.90	565.90	7.01
29	591.34	595.38	573.36	586.69	11.72
30	535.22	549.47	547.34	544.01	7.69
32	526.05	550.69	535.72	537.49	12.42
36	471.73	461.81	446.72	460.08	12.59
46	305.95	354.34	328.48	329.59	24.21
49	285.30	273.06	269.82	276.06	8.16
56	47.26	11.35	29.63	29.41	17.96
57	4.22	1.38	7.48	4.36	3.06
59	0.50	0.00	0.26	0.25	0.25
60	0.00	0.00	0.00	0.00	0.00
63	0.00	0.00	0.00	0.00	0.00

Table A3. Temperature comparison study.

	5°C				
Time (days)	**Rep 1**	**Rep 2**	**Rep 3**	**avg**	**stdev**
0	9.72	10.70	10.65	10.36	0.55
1	10.27	10.69	10.37	10.45	0.22
4	10.31	11.00	10.96	10.76	0.39
9	10.32	10.31	10.34	10.32	0.02
11	9.27	9.57	9.76	9.53	0.24
16	9.92	9.96	9.46	9.78	0.28
18	8.73	8.67	8.42	8.61	0.17
21	9.48	9.00	9.27	9.25	0.24
23	10.27	10.12	10.26	10.22	0.08
25	9.39	10.25	10.96	10.20	0.78
28	8.18	8.39	8.51	8.36	0.17
31	9.68	9.26	10.07	9.67	0.41
36	10.63	7.88	10.48	9.66	1.55
39	9.80	9.23	8.89	9.31	0.46
42	10.19	9.14	9.74	9.69	0.53
51	9.30	9.13	9.22	9.22	0.09
57	11.63	10.40	10.69	10.91	0.64
63	10.40	11.80	11.29	11.17	0.71
66	11.40	10.95	10.85	11.07	0.29
67	11.44	11.45	10.84	11.24	0.35

	10°C				
Time (days)	**Rep 1**	**Rep 2**	**Rep 3**	**avg**	**stdev**
0	10.20	10.79	10.48	10.49	0.30
1	10.95	10.42	10.68	10.68	0.27
4	10.15	10.45	10.09	10.23	0.19
9	10.28	10.44	10.57	10.43	0.15
11	9.73	9.60	9.38	9.57	0.17
16	10.04	10.09	9.99	10.04	0.05
18	8.11	7.43	9.11	8.22	0.85
21	10.41	9.77	10.06	10.08	0.32
23	10.53	10.91	10.71	10.71	0.19
25	10.85	9.00	10.14	10.00	0.93
28	8.65	8.87	8.52	8.68	0.18
31	10.79	10.72	9.65	10.39	0.64
36	9.95	9.33	9.54	9.61	0.32
39	9.64	8.09	8.91	8.88	0.78
42	8.71	8.76	7.71	8.39	0.59
51	6.30	7.26	6.41	6.66	0.52
57	0	0	0	0	0

	25°C				
Time (days	**Rep 1**	**Rep 2**	**Rep 3**	**avg**	**stdev**
0	10.51	10.17	11.07	10.58	0.45
1	11.02	10.76	10.14	10.64	0.46
4	10.13	10.81	10.62	10.52	0.35
9	11.10	10.51	10.33	10.65	0.40
11	10.45	10.76	10.69	10.63	0.16
16	0.00	0.00	4.60	1.53	2.66
18	0.00	0.00	0.00	0.00	0.00

	30°C				
Time (days)	**Rep 1**	**Rep 2**	**Rep 3**	**avg**	**stdev**
0	10.23	10.19	10.28	10.23	0.05
1	10.76	10.04	10.92	10.57	0.47
4	10.21	10.80	10.56	10.52	0.30
9	0.00	0.00	0.00	0.00	0.00

	40°C				
Time (days)	**Rep 1**	**Rep 2**	**Rep 3**	**avg**	**stdev**
0	10.74	10.91	10.47	10.71	0.22
1	10.18	10.36	10.75	10.43	0.29
4	10.56	10.77	10.37	10.57	0.20
9	4.48	0.00	0.00	1.49	2.59
11	0.00	0.00	0.00	0.00	0.00

	50°C				
Time (days)	**Rep 1**	**Rep 2**	**Rep 3**	**avg**	**stdev**
0	10.36	10.06	10.64	10.35	0.29
1	10.01	10.35	10.48	10.28	0.25
4	10.81	10.54	10.15	10.50	0.33
9	0.00	0.00	0.00	0.00	0.00

Table A4. pH variation study.

	pH 2				
Time (days)	Rep 1	Rep 2	Rep 3	avg	stdev
2	10.80	11.00	10.49	10.77	0.26
4	10.00	10.94	10.95	10.63	0.54
6	10.37	10.88	10.57	10.61	0.26
10	10.33	10.08	10.37	10.26	0.16
11	10.44	10.18	10.25	10.29	0.13
13	10.11	10.21	10.26	10.19	0.08
18	10.12	10.82	10.29	10.41	0.36
22	10.31	10.74	10.83	10.63	0.28
24	10.32	10.47	10.87	10.55	0.28
27	10.10	10.02	10.25	10.12	0.12
34	10.09	10.48	10.45	10.34	0.22

	pH 4				
Time (days)	Rep 1	Rep 2	Rep 3	avg	stdev
2	10.45	10.28	10.48	10.40	0.11
4	10.33	10.59	10.26	10.40	0.17
6	8.91	9.04	8.88	8.94	0.09
10	8.98	8.65	9.22	8.95	0.29
11	2.73	1.31	1.41	1.81	0.79
13	1.16	0.00	0.00	0.39	0.67
18	0.00	0.00	0.00	0.00	0.00
22					
24					
27					
34					

	pH 6				
Time (days)	Rep 1	Rep 2	Rep 3	avg	stdev
2	10.39	10.32	10.53	10.41	0.11
4	10.77	10.30	10.60	10.56	0.24
6	9.00	9.47	9.06	9.18	0.25
10	10.37	9.94	9.94	10.08	0.24
11	10.68	8.87	8.87	9.47	1.05
13	9.07	8.37	7.79	8.41	0.64
18	7.27	5.89	3.16	5.44	2.09
22	6.25	3.79	1.26	3.76	2.50
24	0.00	0.00	0.00	0.00	0.00

	pH 8.4				
Time (days)	Rep 1	Rep 2	Rep 3	avg	stdev
2	10.84	10.46	10.73	10.68	0.20
4	10.77	11.06	11.69	11.18	0.47
6	7.15	6.47	7.26	6.96	0.43
10	9.89	9.65	9.69	9.74	0.13
11	10.32	7.34	10.32	9.33	1.72
13	8.66	9.48	9.79	9.31	0.59
18	8.81	8.32	8.90	8.68	0.31
22	2.55	5.34	2.55	3.48	1.61
24	0.00	1.20	0.00	0.40	0.69
27	0.00	0.00	0.00	0.00	0.00

	pH 11				
Time (days)	Rep 1	Rep 2	Rep 3	avg	stdev
2	10.76	10.99	10.93	10.89	0.12
4	10.00	10.33	10.41	10.25	0.22
6	10.40	10.76	10.34	10.50	0.23
10	10.27	10.90	10.00	10.39	0.46
11	10.74	10.19	10.10	10.35	0.35
13	10.52	10.61	10.12	10.42	0.26
18	10.00	10.03	10.17	10.06	0.09
22	10.33	10.82	10.17	10.44	0.34
24	9.96	9.70	10.13	9.93	0.22
27	10.92	10.22	10.71	10.61	0.36
34	11.00	10.82	10.87	10.89	0.09

	pH 14				
Time (days)	Rep 1	Rep 2	Rep 3	avg	stdev
2	11.00	9.99	9.99	10.33	0.58
4	10.23	10.49	9.64	10.12	0.44
6	11.00	10.41	10.94	10.78	0.33
10	9.99	10.67	10.41	10.36	0.34
11	9.62	9.99	9.59	9.73	0.22
13	11.25	9.98	10.16	10.46	0.68
18	10.70	10.57	10.33	10.53	0.19
22	10.90	10.94	10.15	10.66	0.44
24	10.40	9.95	9.94	10.10	0.27
27	10.87	10.61	10.29	10.59	0.29
34	10.10	10.32	10.38	10.26	0.15

Table A5. Perchlorate breakdown product study.

Days Elapsed	Acetate Conc. (mg/L)	Acetate Avg. Conc. (mg/L)	Stdev	Perchlorate Conc. (mg/L)	Perchlorate Avg. Conc. (mg/L)	Stdev	Chloride Conc. (mg/L)	Chloride Avg. Conc. (mg/L)	Stdev
0 Hour	117.22	117.14	0.12	9.62	9.72	0.15	1.21	1.07	0.20
	117.06			9.83			0.93		
0.73	102.67	107.90	4.74	7.78	8.03	0.23	0.87	1.01	0.25
	111.91			8.08			1.30		
	109.13			8.23			0.85		
1.00	102.77	103.01	2.52	8.07	8.03	0.46	0.72	0.55	0.24
	100.61			8.46			0.38		
	105.63			7.55					
2.00	87.84	90.63	2.77	8.66	8.94	0.25	0.42	0.32	0.09
	93.39			9.01			0.31		
	90.65			9.15			0.23		
7.02	101.41	101.56	0.13	8.35	8.45	0.15	0.95	0.94	0.02
	101.64			8.38			0.92		
	101.64			8.62			0.94		
8.02	61.37	62.64	1.31	7.81	8.09	0.55	0.89	0.73	0.14
	62.57			8.73			0.62		
	63.99			7.74			0.68		
9.00	61.53	63.40	1.63	7.97	8.46	0.42	0.75	0.71	0.05
	64.17			8.67			0.73		
	64.50			8.73			0.66		
10.06	62.37	61.65	0.74	8.57	8.47	0.41	0.64	0.67	0.03
	60.90			8.81			0.69		
	61.69			8.02			0.68		
11.02	60.87	61.10	0.80	7.61	8.11	0.47	0.72	0.71	0.10
	60.44			8.19			0.60		
	61.98			8.54			0.80		
14.06	55.86	55.19	1.95	4.78	5.02	0.22	1.57	1.35	0.31
	53.00			5.08			0.99		
	56.71			5.21			1.49		
15.02	54.30	53.99	0.45	2.99	3.26	0.25	2.64	2.52	0.19
	53.48			3.28			2.62		
	54.20			3.50			2.30		
16.02	46.28	46.51	0.57	1.71	1.90	0.19	2.60	2.66	0.06
	46.09			1.88			2.67		
	47.17			2.10			2.72		
17.02	41.33	40.88	0.43	0.71	0.74	0.03	3.12	3.13	0.09
	40.82			0.78			3.22		
	40.48			0.74			3.04		
17.77	29.81	30.14	0.69	0.37	0.36	0.02	2.40	2.49	0.12
	30.93			0.36			2.63		
	29.68			0.34			2.45		
21.73	9.60	8.92	0.60	0.00	0.00	0.00	3.22	3.08	0.15
	8.43			0.00			3.10		
	8.75			0.00			2.93		
22.04	5.09	4.59	0.79	0.00	0.00	0.00	2.74	2.87	0.12
	3.68			0.00			2.93		
	5.00			0.00			2.94		
23.02	1.99	1.86	0.13	0.07	0.07	0.04	2.89	2.91	0.08
	1.73			0.00			2.83		
	1.85			0.00			3.00		
24.02	1.59	0.83	0.66	0.00	0.00	0.00	3.37	3.17	0.19
	0.45			0.00			3.00		
	0.44			0.00			3.16		
25.02	0.77	0.50	0.24	0.00	0.00	0.00	3.37	3.27	0.09
	0.37			0.00			3.18		
	0.37			0.00			3.26		

Days Elapsed	Chlorate Conc. (mg/L)	Chlorate Avg. Conc. (mg/L)	Stdev	Chlorite Conc. (mg/L)	Chlorite Avg. Conc. (mg/L)	Stdev
0 Hour	0.00	0.00	0.00	0.00	0.00	0.00
	0.00			0.00		
0.73	0.00	0.00	0.00	0.00	0.00	0.00
	0.00			0.00		
	0.00			0.00		
1.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00			0.00		
	0.00			0.00		
2.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00			0.00		
	0.00			0.00		
7.02	0.07	0.03	0.03	0.00	0.00	0.00
	0.02			0.00		
	0.01			0.00		
8.02	0.04	0.02	0.01	0.00	0.00	0.00
	0.02			0.00		
	0.01			0.00		
9.00	0.03	0.02	0.01	0.00	0.00	0.00
	0.02			0.00		
				0.00		
10.06	0.03	0.03	0.01	0.00	0.00	0.00
	0.02			0.00		
	0.03			0.00		
11.02	0.02	0.01	0.00	0.00	0.00	0.00
	0.01			0.00		
	0.01			0.00		
14.06	0.00	0.02	0.01	0.00	0.00	0.00
	0.02			0.00		
	0.03			0.00		
15.02	0.07	0.04	0.03	0.00	0.00	0.00
	0.02			0.00		
	0.00			0.00		
16.02	0.03	0.03	0.02	0.00	0.00	0.00
	0.00			0.00		
	0.00			0.00		
17.02	0.03	0.03	0.02	0.00	0.00	0.00
	0.00			0.00		
	0.00			0.00		
17.77	0.05	0.03	0.03	0.00	0.00	0.00
	0.00			0.00		
	0.01			0.00		
21.73	0.02	0.02	0.01	0.00	0.00	0.00
	0.00			0.00		
	0.00			0.00		
22.04	0.04	0.04	0.02	0.00	0.00	0.00
	0.00			0.00		
	0.00			0.00		
23.02	0.02	0.02	0.01	0.00	0.00	0.00
	0.00			0.00		
	0.00			0.00		
24.02	0.00	0.00	0.00	0.00	0.00	0.00
	0.00			0.00		
	0.00			0.00		
25.02	0.11	0.11	0.06	0.00	0.00	0.00
	0.00			0.00		
	0.00			0.00		

Table A6. Nitrate study.

DAYS	0	0.21	1	4	7	8	12	14	21
5.12-1	8.73	13.17	6.58	5.03	3.77	3.96	0.00	0.00	0.00
5.12-2	8.20	13.46	6.29	5.11	5.45	4.77	2.15	0.00	0.00
avg	8.47	13.32	6.44	5.07	4.61	4.37	1.07	0.00	0.00
stdev	0.38	0.21	0.21	0.05	1.19	0.57	1.52	0.00	0.00
51.2-1	8.90	13.51	6.18	5.47	5.60	1.42	0.00	0.00	0.00
51.2-2	9.51	13.17	6.12	5.37	5.79	3.81	0.50	0.00	0.00
51.2-3	9.84	13.32	5.92	5.64	5.79	2.87	0.00	0.00	0.00
avg	9.42	13.33	6.07	5.49	5.73	2.70	0.17	0.00	0.00
stdev	0.48	0.17	0.14	0.14	0.11	1.20	0.29	0.00	0.00
512-1	9.46	12.85	5.72	5.21	5.62	0.49	0.00	0.00	0.00
512-2	8.87	13.72	5.37	5.20	4.87	0.11	0.00	0.00	0.00
512-3	9.84	13.10	5.21	5.38	3.31	0.00	0.00	0.00	0.00
avg	9.39	13.22	5.44	5.26	4.60	0.20	0.00	0.00	0.00
stdev	0.49	0.45	0.26	0.10	1.17	0.26		0.00	0.00
Control-1	8.07	10.41	3.93	4.37	4.39		0.26	0.00	0.00
Control-2	12.30	16.42	6.51	7.18	6.59		1.08	0.00	0.00
Control-3	13.47	17.99	6.57	7.43	7.31		7.86	0.00	0.00
avg	11.28	14.94	5.67	6.33	6.10		3.07	0.00	0.00
stdev	2.84	4.00	1.51	1.70	1.52		4.17	0.00	0.00

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14. ABSTRACT

Batch microcosm reactor studies using aquifer sand collected from a perchlorate- contaminated site were conducted in order to determine if perchlorate can be treated using in situ biodegradation stimulated by an organic electron donor, acetate, and to measure degradation rates over a range of environmental variables. The addition of acetate as an organic substrate stimulated rapid perchlorate degradation after a lag phase, presumably resulting from microbial acclimation. The lag phase was eliminated after previous exposure or after microbes consumed the oxygen and the reactors went anaerobic. Perchlorate degradation was effective when greater than 50 mg/L acetate was added, at temperatures greater than 20°C, and when the pH was between 4 and 8. Various perchlorate and acetate concentrations had different effects on the biodegradation process. Most of the studies were conducted at relatively high (mg/L) concentrations. To investigate removal at lower concentrations, a study with an initial perchlorate concentration of 50 µg/L was conducted, and this indicated that final treatment concentrations were reduced below the California maximum contaminant level (MCL) of 6 µg/L. Chloride was the ultimate end product of perchlorate degradation, and measurements indicated that chloride formation accounted for 95.5 to 100% of the perchlorate degraded.

15. SUBJECT TERMS

Acetate concentration
Lag phase
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Perchlorate degradation
pH measurements

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