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In situ bioremediation of perchlorate in soil and groundwater

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IN SITU BIOREMEDIATION OF PERCHLORATE IN SOIL AND GROUNDWATER

A Project

Presented to the

Faculty of

California State University,

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In Partial Fulfillment

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in

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by

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ABSTRACT

This research project documents a technology assessment for in-situ bioremediation of perchlorate (ClO_4^-). The main goals are to compare methodologies, perform site characterization of soil and groundwater, and perform microcosm and bench scale tests. In this approach, perchlorate is reduced to chloride, requiring an electron donor. The suitability of electron donor amendments will be assessed for effectiveness in stimulating biological reduction of perchlorate in three applications: unsaturated (vadose zone) soils; source area groundwater; and plume edge groundwater.

This study involves two types of test systems: a) microcosms and b) columns. The main goals of the microcosm and column studies are to screen substrates based on cost, availability, and effectiveness for treatment of the vadose zone and groundwater; compare substrates using site-specific media and measure perchlorate reduction; collect longevity and general performance data in simulated biobarrier (groundwater) and vadose zone applications; and assess water quality parameters.

Soil and groundwater were collected from the project site source area and biobarrier area for use in microcosm and soil column experiments. Initial results showed that

perchlorate-reducing enzymes were present in the native soil. Several electron donors were tested with the soil and groundwater with and without addition of nutrients. Before, during, and after all tests, samples were analyzed for perchlorate, nitrate, and other chemical parameters.

Based upon the microcosm and soil column studies in biobarrier area, source area vadose zone, and source area saturated zone soils and groundwater, reduction of perchlorate was observed most consistently with the emulsified oil substrate (EOS) amendment.

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%	percent
µg/kg	micrograms per kilogram
µg/kg/day	micrograms per kilogram of body weight per day
µg/l	micrograms per liter
µS/cm	micro-Siemens per centimeter
As	arsenic
ASTM	American Society for Testing Materials
Ca	calcium
Cl^-	chlorine
ClO_2^-	chlorite
ClO_4^-	perchlorate
CO_2	carbon dioxide
CSM	conceptual site model
d	days
g	gram
g/l	grams per liter
mg/l	milligrams per liter
CEC	cation exchange capacity
CO_2	carbon dioxide
CSM	conceptual site model
DI	deionized water
DMSO	dimethyl sulfoxide
EHC	Redox Compound
EOS	Emulsified Oil Substrate

EPA	Environmental Protection Agency
FDA	Food and Drug Administration
Fe	iron
g/kg	grams per kilogram
H_2	hydrogen gas
HAc	acetic acid
HFCS	high fructose corn syrup
K	potassium
L	liter
L/d	liters per day
LPG	liquid petroleum gas
MCL	maximum contaminant level
Mg	magnesium
mg/kg	milligrams per kilogram
mL	milliliter
Mn	manganese
N_2	nitrogen
Na	sodium
NaAc	sodium acetate
NaAcetate	sodium acetate
$(NH_4)_2HPO_4$	diammonium hydrogen phosphate
NO_3^-	nitrate
ORP	oxidation-reduction potential
P	phosphorous
ppm	parts per million

PVC	polyvinyl chloride
QC	quality control
S	sulfur
SO_4^{2-}	sulfate
SO_3^{2-}	sulfide
TKN	total Kjeldahl nitrogen
TDS	total dissolved solids
TOC	total organic carbon
v/v	volume to volume
VOC	volatile organic compound
w/w	weight to weight

CHAPTER ONE

INTRODUCTION

Perchlorate salts, such as ammonium perchlorate, are used as oxidizing agents for the combustion of rocket fuel, explosives, and fireworks. Perchlorate salts have also been utilized as fertilizer, mined from natural nitrate deposits found in the U.S. and around the world. Other uses include industrial and medical (*1*).

Perchlorate generally refers to the anion (ClO_4^-) component of the salt. The main component of a perchlorate salt is its stable anion, which is highly soluble and mobile in groundwater (*1-5*). It has been a contaminant of concern in California since its detection in drinking water in the 1990s, which led to decommissioning hundreds of municipal wells in the San Bernardino and Riverside Counties (*4*). Since then, widespread perchlorate has been found in farm crops and livestock used for human consumption, which has been attributed to contaminated irrigation water (*1*).

Health effects caused by perchlorate include inhibition of thyroid function at levels of 7 micrograms per kilogram of body weight per day (µg/kg/day) with an uncertainty factor of 10, based upon consumption of two liters of drinking water with a concentration of 24.5

micrograms per liter (µg/l) per day by an average adult (*1,5*). In addition, it is thought to affect the development of the thyroid gland in children and fetuses at low levels (down to 1 µg/l) (*1,6*). In a 2008 study the Food and Drug Administration (FDA) estimated that the U.S. population ingests from 0.08 to 0.39 µg/kg/day from food (*1*).

The current Maximum Contaminant Level (MCL) for perchlorate in California drinking water is 6 µg/l. In a 2008 preliminary determination, the United States Environmental Protection Agency (EPA) decided against establishing an MCL, and the EPA is currently seeking comments on additional approaches to analyze their data (*7*). The EPA concluded that an MCL was not necessary because less than one percent of drinking water systems nationwide reported detections. California's drinking water systems are monitored and treated if perchlorate detections are found. However, groundwater that is used as a source for drinking water needs to be adequately characterized to determine what type(s) of treatment are necessary. As a result of ongoing research, perchlorate in drinking water has been treated in several ways, from large scale pump-and-treat systems, to smaller scale bioreactors, and in situ remedial alternatives (*2,3*).

In situ bioremediation involves stimulating native microbes in soil and groundwater, which digest the contaminant and reduce it into less toxic components. In situ is defined as on site and comes from the Latin phrase meaning *in the place*. In situ bioremediation targets a source area of contamination without removing soil and groundwater. Because this type of treatment does not involve removal of contaminated soil and groundwater, it is very cost effective compared to other aforementioned methods of treatment.

Perchlorate is used as an oxidizing agent and is reduced to chlorite (ClO_2^-) before being completely reduced to chloride during the reaction. Reduction occurs as electrons are gained; thus, anaerobic bacteria require reducing conditions and an electron donor.

Perchlorate-reducing bacteria possess a specialized enzyme located in the periplasm that transfers the electron during the reaction. The most commonly known enzymes are chlorite dismutase and perchlorate reductase. With the latter, electrons are transferred from the membrane via a cytochrome to the perchlorate reductase. With the chlorite dismutation, accumulation of toxic chlorite is alleviated. The chlorite dismutase gene has a proximal operon that encodes perchlorate reductase (*8*).

Organisms that possess the specialized enzymes are phylogenetically diverse with members in *Proteobacteria*. Many members with the perchlorate reductase enzyme are in either the genus *Dechloromonas* or *Dechlorosoma* (*9*); however, other pathways exist in many other types of bacteria classified as microbial dimethyl sulfoxide (DMSO) reductase family of molybdenum enzymes. It should also be noted that many nitrate-reducing bacteria can also reduce chlorate; however, perchlorate reduction does not occur with all types of chlorate-reducing bacteria (*8*). When nitrate and perchlorate are present together, as is often the case, nitrates are generally reduced before perchlorate. For example, the nitrate-reducing strain, *Dechlorosoma suillum*, was shown to reduce nitrate before perchlorate reduction began. In contrast, *Dechloromonas agitate* reduced nitrate and perchlorate concomitantly (*9*).

Statement of Purpose

The purpose of this project is to conduct a technology assessment for in situ bioremediation of perchlorate in soil and groundwater. For this thesis project, it was hypothesized that by setting up a dynamic approach to follow from the beginning to end of the project (cradle-to-grave), the site can be remediated in

the most cost effective manner, thus reducing the impact to the underlying groundwater aquifer. The cradle-to-grave process is applied to site characterization and bioremediation, and focuses on all aspects of the process. For this study, the suitability of pre-selected electron donor amendments will be assessed for effectiveness in stimulating biological reduction of perchlorate in three in situ applications: unsaturated soils; source area groundwater; and downgradient plume edge groundwater.

This project includes an assessment for in situ bioremediation technologies using native bacteria from the site soil and groundwater. The technology assessment involves two types of test systems: microcosm (bench-scale) and soil column (field scale) studies. This document presents the methodologies and results of the ongoing site characterization and microcosm and soil column studies.

Project Scope and Objectives

Perchlorate can be reduced to non-toxic chloride via either biotic (the focus of the microcosm and soil column studies) or abiotic pathways, requiring the availability of an electron donor and nutrients. The aspects about this study that are focused on are: 1) what types of

electron donors and nutrients will be successful degrading perchlorate; and 2) is this type of bioremediation feasible for the project site, given its complex hydrogeology and high-level source area contamination?

The objective of this project is to study the conditions necessary to reduce perchlorate in soil and groundwater, and to design and implement a field-scale bioremediation system. Geochemical conditions and a variety of amendments will be evaluated for the feasibility of in-situ perchlorate bioremediation in the San Timoteo badlands area of Riverside County, California, herein referred to as the site. As part of this project, microcosm tests and column studies were conducted. While this project is ongoing, the most currently available results will be utilized to facilitate preliminary remedial system design and construction specifications.

The suitability of pre-selected electron donor amendments will be assessed for effectiveness in stimulating biological reduction of perchlorate in three applications:

1) Unsaturated (vadose zone) soil from the source area;
2) Source area groundwater;

3) Plume edge groundwater (a plume interception biobarrier application intended to intercept and treat contaminated groundwater as it passes through).

This study will involve two types of test systems: bench scale, or microcosm, and field-scale, or soil column tests. Microcosm tests are performed on a bench scale with small flasks in order to screen as many substrates as possible, and soil columns are performed as field-scale experiments using columns packed with soil from the site.

The main goals of the microcosm and column studies are to screen substrates based on cost, availability, and effectiveness for treatment of the vadose zone and groundwater; compare substrates using site-specific media and measure perchlorate reduction; collect longevity and general performance data in simulated biobarrier (groundwater) and vadose zone applications; and assess water quality parameters affected by the amendments tested.

Proposed Biobarrier Design

A biobarrier is proposed be installed at the site in an area at the leading edge of the plume near the southern property boundary. This will consist of either a

vertical trench or a cluster of injection wells perpendicular to the groundwater flow direction. A trench will be constructed and filled with a permeable medium (pea gravel, compost, and/or mulch). Electron donor and nutrient amendments will be added thus creating optimal conditions for biological removal of the dissolved perchlorate as it passes through the biobarrier. In addition, well clusters will be evaluated for treatment of soil and/or groundwater treatment at the source areas via direct injection and extraction techniques.

Project Organization

The project has been organized into six main tasks: 1) create a technology team to conduct experiments; 2) conduct background research for similar studies; 3) prepare the procedures for the experiments to follow; 4) perform site characterization of two possible soil source areas and downgradient groundwater; 5) conduct microcosm tests and column studies; and 6) utilize the results to facilitate final design and construction of the remedial systems.

Project Staff

The first phase of this research project was to collaborate with a team of academic and industry professionals. My career in environmental consulting has

me in the forefront of performing site characterization and remedial system design, and University of California Riverside (UCR) has provided a team to perform the microcosm and soil column experiments. Our team of UCR professors, graduate, and undergraduate students performing experiments is referred to in this document as the perchlorate technology team. In addition to the perchlorate technology team, Tetra Tech, Inc. has a team of scientists interpreting and reporting results.

Project Background

Background Research

Other sites in which in situ treatment of perchlorate has been performed successfully were compared to the project site. Studies were queried and screened for technologies used, effectiveness, and similar site conditions. During the documentation review, studies were found that had similar site conditions or treatment technologies.

Evans & Trute (*10*) documented methods for testing removal of nitrate and perchlorate by using gaseous amendments in microcosms and soil columns. By using an anaerobic version of hydrocarbon bioventing, hydrogen and ethyl acetate gases were added to soil as electron donors in order to achieve complete nitrate removal and up to

39% perchlorate removal in soil column and microcosm studies.

Nozawa-Inoue, Scow, and Rolston (*11*) published methods for using several liquid amendments in bench and field scale studies and measured the effects of acetate and hydrogen as electron donors for native bacteria in vadose zone soil. The methods used by Nozawa-Inoue et al. included a comparison of results with kinetic data and identification of the species of perchlorate-reducing bacteria found. Evans & Trute and Nozawa-Inoue et al. used detailed procedures for microcosm and soil column studies under similar conditions; therefore, they form the basis of the procedures for the laboratory studies in this project. For this study, microcosm and soil column experiments were performed on both saturated and vadose zone soils, as outlined (*10,11*).

In addition to the soil column and microcosm studies, several documents are referenced for the use of a biobarrier, which is a permeable reactive substance that allows groundwater to pass through, treating the contamination. A biobarrier is planned to be constructed at the site in an area downgradient of the source areas in order to intercept the plume from migrating off of the property. The biobarrier can be comprised of liquid amendments including emulsified oil substrate (*12,13*), or

solid material, such as zero-valent iron (*14*) or mulch (*15,16*). All of these materials and techniques were evaluated during this project. Other studies explore other treatment technologies, such as bioreactors that are used to pump and treat groundwater (*17*). As this feasibility study was initiated, a draft technical memo was documented to outline procedures (*18*), using the background research to provide laboratory procedures for the microcosm and soil column experiments.

Site Background

Several field mobilizations have taken place to characterize the site since about 2003; however, due to client confidentiality, only summarized results were used in preparation of the conceptual site model (CSM) for this project (Chapter 2). The exact location of the site and any images identifying the site cannot be disclosed.

In order to assess the presence of water affected by perchlorate downgradient of the site and characterize the hydrogeology, several phases of site characterization have been conducted. My role at the site has been as a Geologist, characterizing and sampling site features. With respect to this project, my research includes investigating the southern downgradient plume edge, two phases of characterization at a second source area on-site (*19*), and on-going characterization of the primary

source area. The work presented herein will be used as a basis for the design and construction of remedial systems at the site.

In soil samples collected from 2007 to 2008, perchlorate has been detected up to approximately 220,000 micrograms per kilogram (µg/kg) at the primary source area and 134,000 µg/kg at the secondary source area (*19*). Methylene chloride and other volatile organic compounds (VOCs) have been detected at the secondary source area up to 220,000 µg/kg. Groundwater results have shown detections of up to 700,000 µg/l of perchlorate (*20*).

Project Limitations

The project site and property owners in this study are confidential. Any references to work performed at the site reflect the phase of work performed without referring to the site name or client. This project is an ongoing assessment of remedial technologies; however, only documents submitted for regulatory approval are considered public domain. Therefore, only the public documents are used to reference in this project.

All laboratory microcosm and soil column testing were performed at UCR, including all analytical testing with the exception of initial off-site analytical laboratory testing. Work performed by the perchlorate

technology team is attached as Appendix A, with permission by UCR.

CHAPTER TWO

CONCEPTUAL SITE MODEL

The following section describes a brief overview of the most current CSM. Figure 1 depicts the physical setting of the site.

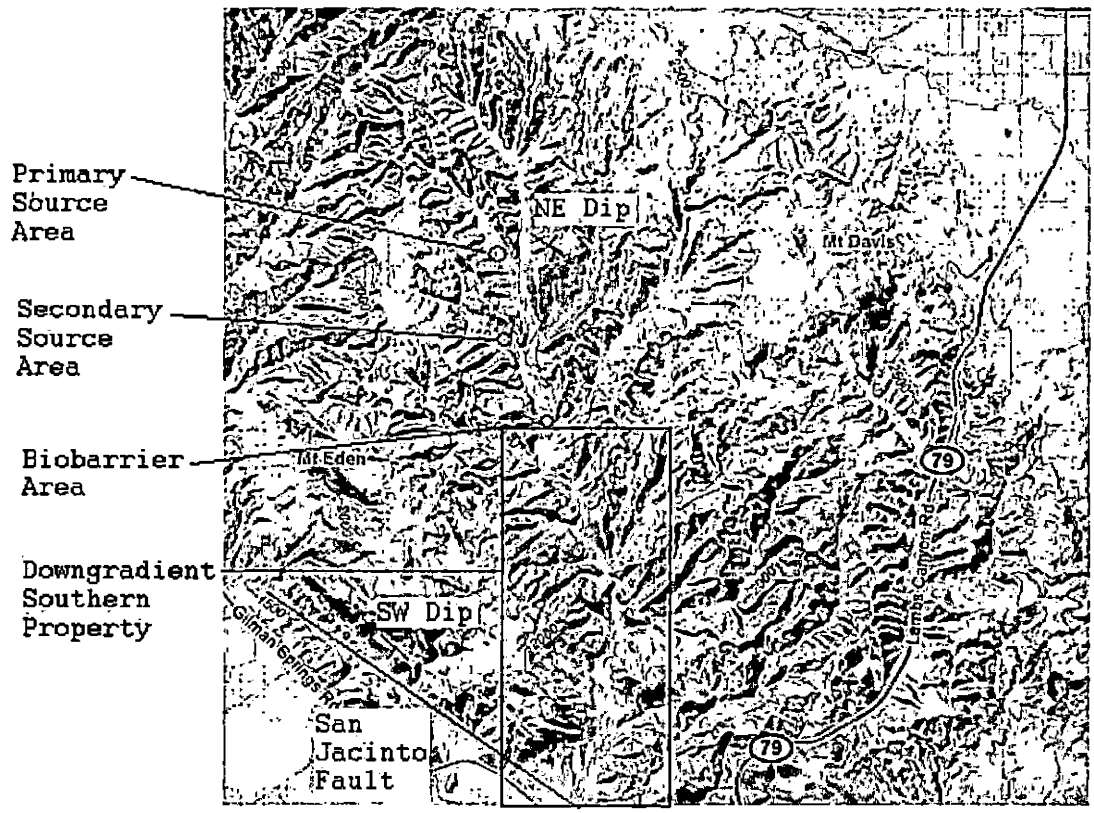


Figure 1. Physical Setting

United States Geological Survey (USGS). (2009). El Casco Quadrangle. California 7.5-Minute Series.

This model has been updated with the most recent available information about the site. This section includes discussions on the characterization of the contaminated plume, contaminant distribution and migration, and geologic and hydrogeologic properties. Interpretation of this data is also presented in a discussion of the two source areas on the site.

Contaminant Distribution

Detected perchlorate at the site ranges from approximately 700,000 µg/l in deep-zone groundwater near the primary source area to 500 µg/l at the southern border of the property. VOCs in groundwater do not appear to be migrating throughout the site; however, perchlorate in groundwater has been shown to be very mobile throughout monitoring since 2004.

There are two principal source areas in westerly canyon arms that drain into the main canyon (Figure 1). At the primary source area, perchlorate is found as high as 220,000 µg/kg at 20 feet below ground surface (bgs), but due to the complex hydrogeology, the source area for groundwater is actually 90 feet bgs in the upgradient direction. This is thought to be the result of the perchlorate on the hillside mobilizing with infiltrating precipitation as it percolates through the less weathered

material, eventually mixing with deeper groundwater (Figure 2).

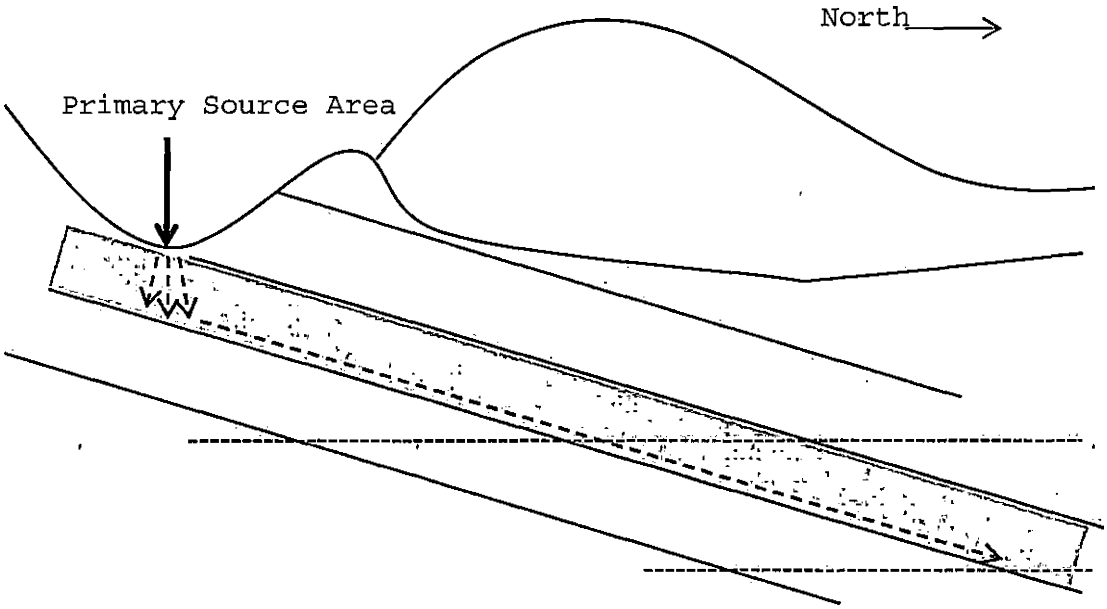


Figure 2. Cross Section of Primary Source Area

Legend:

Surface Topography

Approximate Dip of Bedding

Permeable Lithologic Unit

Pathway of Perchlorate Migration in Soil

Historic Groundwater Level Lower Level

Current Groundwater Level

By reviewing past groundwater levels regionally, it was determined that this had occurred after long term drought and heavy groundwater pumping resulted in lower water levels. A north-dipping sandstone unit acted as a preferential pathway for percolating water and perchlorate to migrate in the bedding towards the groundwater table. As perchlorate was precipitated out, a trail of contaminated soil was left behind. When water levels rose after several subsequent rainy seasons, the majority of the mobilized perchlorate in soil remained in the deep zone soil. The soil was submerged as water levels rose, becoming dissolved and migrating downgradient (toward the south).

At the second source area, perchlorate in groundwater was observed to be entering the main canyon from a small side canyon and mixing with downgradient shallow groundwater contamination. This explained some previous data discrepancies in wells located towards east and west sides of the main canyon, where the westerly wells have always had higher results (*21*). The resulting mixing of contaminated groundwater plumes from the two canyon branches is similar to a tributary mixing with a river. For example, where the Milk River and Missouri River meet, the turbid water of the Milk River slowly

mixes downstream with the larger Missouri River, forming a cloudy side of the Missouri for miles.

Downgradient groundwater contamination within the southern portion of the site is considerably shallower than elsewhere on the project site. This also supports the presence of groundwater barriers downgradient of the site. A possible groundwater barrier exists between the San Timoteo and Mt. Eden formations (i.e. granitic intrusions and/or additional faulting). The plume of impacted groundwater is terminated near the geologic contact between the San Timoteo and Mt. Eden formations, located south of the site. In addition, several faults across the canyon form groundwater barriers which result in areas with relatively higher groundwater levels, referenced from ground surface.

Physical Setting

The site is located in the San Timoteo badlands area of Riverside County, California, which is within the Peninsular Ranges geomorphic province (*22*) and mapped in the Santa Ana Quadrangle (*23*). The topography is characteristic of badlands with steep hills and canyons carved by surface water from sporadic, although infrequent heavy rain events. Drainage on the site follows the main canyon downslope toward the south, where

it is discharged to the Menifee Valley, flowing inland toward the Lake Elsinore Reservoir. The Menifee Valley also has a westerly-sloped drainage into the Santa Ana Watershed.

Site Geology

The geology consists of Quaternary alluvium overlaying the Pleistocene San Timoteo formation. Unconformably underlying the San Timoteo formation is the Mt. Eden formation, which is underlain by igneous and metamorphic bedrock. The alluvium is composed mainly of sands and silts with gravel, and the San Timoteo formation underlying the alluvium consists of siltstones and silty sandstones with some mudstones. The composition of the San Timoteo formation show depositional environments of low to high energy, as shown in the assemblage of fine-grained strata to coarse, gravelly units. In contrast, Mt. Eden sandstones represent an erosional environment, typically sharing the same granitic mineralogy as its parent bedrock.

The San Timoteo formation within the vicinity of the site is an uplifted fault block in a compression zone located between major splays of the San Jacinto and San Andreas strike-slip fault zones. The compression formed a broad anticline plunging roughly east-west, with the bedding dipping gently toward the north-northeast at the

site. The downgradient property to the south shows a reversal in dip direction, toward the south-southwest on the other side of the anticline, where it is terminated by the San Jacinto fault. Several other fault splays may exist on this property, along with irregular folding of the strata through the middle of the anticline, where underlying igneous and metamorphic rocks are exposed. The igneous portion generally forms the oldest part of the anticline formation.

The geology at the downgradient site consists of coarse, granitic sandstones of the Mt. Eden Formation and igneous and metamorphic rocks underlying the Mt. Eden Formation. Mt. Eden sandstones may be more permeable than the San Timoteo sandstones and siltstones, but permeability decreases with depth as density and the amount of fines increase. The contact between Mt. Eden and San Timoteo Formations is toward the north of the site; however, this area is inaccessible by vehicles and drill rigs.

Site Hydrogeology

As water percolates slowly through the alluvium into the weathered San Timoteo formation, a semi-permeable aquiclude is formed by less weathered, thus more competent and less permeable, San Timoteo sedimentary rocks. The low porosities and permeabilities of the units

lead to very low hydraulic conductivity values; however, some beds are more porous and permeable, allowing percolating water or groundwater to flow preferentially. Different strata of groundwater exist within the alluvium, weathered and less weathered San Timoteo formation, and Mt. Eden formation; although, the hydrologic units are connected at the project site.

Shallow groundwater flows toward the south beneath the site, generally following the topography of the canyon, with limited amounts of recharge from surrounding hills. In the primary source area canyon, shallow water slowly percolates through the alluvium and weathered rock, mixing with water beneath that is semi-confined beneath the hills.

This is because over time, the more competent material in the hills was weathered less than the material in the small canyons. The more competent material forms semi-confining barriers for shallow groundwater. However, the groundwater is still able to flow through the more competent semi-confining material, as it is forced deeper. Thus, when water in the more competent material beneath the hills reaches the less competent rock and alluvium, the positive pressure forces it upward, where it mixes with percolating water from sporadic seasonal rains.

The groundwater potentiometric surface is subject to variation based on seasonal and long-term weather patterns. While seasonal changes do not affect water levels in the canyons greatly, long-term droughts and wet seasons (greater than 10 years in duration) can cause the groundwater levels in the alluvium and weathered rock to change substantially.

As the main canyon widens toward the south, groundwater depth decreases. Mechanisms such as faulting and folding can create groundwater barriers where water levels are shallow on one side, and then drop down in the downgradient direction. Further downgradient on the southern property, groundwater surfaces to springs and is percolated back into the shallow groundwater zone past the obstruction.

Due to major groundwater obstructions, the groundwater present in the southern portion of the downgradient site may not be hydrologically connected to the shallow groundwater from the project site. By observing surficial geology and geomorphology, it was determined that the primary source of groundwater in the southern property is connected to a canyon toward the east. Located upgradient is a landfill, and following this canyon to the east, it appears to have been carved out along a geologic contact with less permeable igneous

rocks on the southern side. Therefore, water coming into this canyon enters on the south side of the groundwater barrier formed by the San Timoteo/Mt. Eden contact and then follows topography and bedding toward the south.

As the groundwater downgradient of the project site encounters this barrier on the southern property, it enters into the Mt. Eden Formation sandstones and conglomerates. Since the beds dip toward the north-northeast, if the groundwater enters a less permeable zone (i.e. coarse sandstone) and encounters a barrier in the downgradient direction, then it may essentially drain out into the coarser Mt. Eden Formation and follow the direction of the bedding planes.

This would effectively be a reversal of groundwater direction in a deeper unit under the site. No monitoring wells have been installed deep enough to observe this; however, former extraction wells have gone through both units into the fractured igneous and metamorphic bedrock, which is the deepest known hydrologic unit beneath the site. More investigation and well installation/sampling is underway and must be completed before any final conclusions are made.

The southern property boundary at the site was determined to be suitable as a possible biobarrier site because of the shallow groundwater located in loose

alluvium overlying permeable weathered San Timoteo sandstones. This section is located upgradient of the Mt. Eden Formation contact, and is likely to be in close proximity to another groundwater barrier which forces the groundwater into a shallow zone on top of well cemented Sat Timoteo sandstones at approximately 70 feet bgs. Groundwater in this area is found at shallow depths near 15 feet bgs, which is ideal for a biobarrier application consisting of a well cluster or trench filled with permeable material. As site investigation continues, additional area(s) may be found to be suitable for biobarrier application(s).

CHAPTER THREE

MATERIALS AND METHODS

Prior to site remediation, activities conducted at the site have included several phases of characterization. Figure 3 presents a flowchart summarizing the site activities and microcosm and soil column experiments. The laboratory microcosm and bench-scale soil column studies are described in this section. Site soil and groundwater were obtained from near the primary source area of contamination and in a downgradient location, in order to simulate in-situ conditions during the microcosm and column studies.

The initial process for microcosm tests included extracting groundwater and collecting soil at the two areas of the site. Collection of soil and groundwater from the southern property boundary and the source area were conducted in April and May 2008. Materials collected were used in the laboratory studies conducted by the perchlorate technology team. Additional site characterization has been underway since September 2008 (Figure 3).

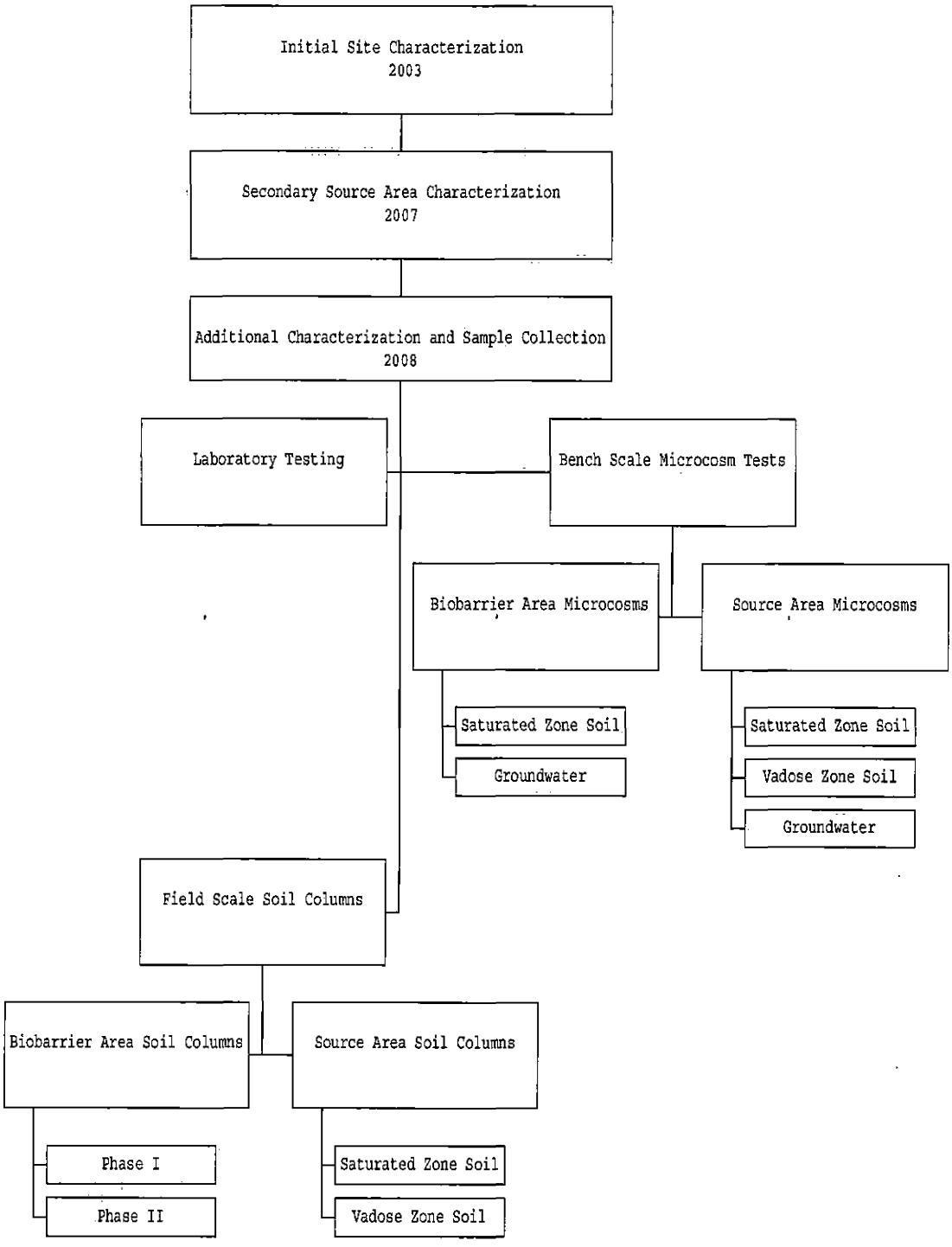


Figure 3. Process Flow Chart

Field Methodology

Table 1 summarizes field parameters collected, and chemical and biological parameters from off-site laboratory analyses that were used to establish baseline conditions for the experiments. Groundwater parameters were recorded using a calibrated field multi-meter and a water level probe as the well was purged, and groundwater samples were collected after the parameters had stabilized. Samples for analytical and geotechnical testing were sent to EMAX Laboratory, Inc. (groundwater chemical parameters), Environmental Geotechnical Laboratory, Inc. (soil physical parameters), TestAmerica (soil chemical parameters), and BioInsight LLC (microbiological testing).

Soil and groundwater samples were delivered to the perchlorate technology team at UCR. Approximately 50 gallons of soil were collected from the vadose zone and beneath the water table in the weathered San Timoteo Formation at the source area, and ten gallons from the biobarrier site. About 140 gallons of biobarrier area water were purged, for use in the microcosm and column studies. Fifteen gallons of water each were used for initial microcosm tests of the biobarrier area and primary source area.

Table 1. Summary of Testing Parameters

Field Parameters (Groundwater)	Offsite Laboratory (Groundwater)
pH; Dissolved Oxygen; Electrical Conductivity; Oxidation-Reduction Potential; Temperature; Turbidity; Water Level Drawdown	Alkalinity; Perchlorate; TDS & TOC; Cl^-, SO_4^{2-}, NO_3^-; Ca^{2+}, Mg^{2+}, Na^{1+}, K^{1+}; Nitrogen (ammonia, TKN); Fe, Mn, As, Total S; Soluble and Total P

Geotechnical Laboratory (Soil)	Analytical Laboratory (Soil)	Microbiological Laboratory
Total Porosity; Permeability; Moisture Content & Density (ASTM D5084/EPA 9100); Grain Size Distribution [ASTM D422(ii)]	pH; As, Fe, Mn, Total P; Perchlorate; Sulfate, Sulfide (SO_4^{2-} , SO_3^{2-}); Total Kjeldahl Nitrogen (TKN); Total Organic Carbon	Cation Exchange Capacity (CEC); Plate Count

The remaining 110 gallons were used for the soil columns, with 50 additional gallons of water bimonthly from the biobarrier and source area locations while laboratory studies were conducted.

Laboratory Methodology

Soil and groundwater samples were collected for offsite analysis of a variety of parameters (Table 1). Soil samples capped in steel sleeves were sent to a geotechnical laboratory for physical parameters. Additional soil and groundwater samples were delivered for a suite of analytical, geotechnical, and microbiological testing. The microbiological samples were enumerated and quantified for perchlorate reducing bacteria enzymes in soil, while the analytical testing covered a suite of chemical analyses for soil and groundwater.

Microcosm Testing Methodology

During the microcosm phase, selected amendments were screened in terms of effectiveness, in order to gain an understanding of the potential rate of treatment. Nutrient requirements, pH effects, and changes to geochemical water quality parameters were assessed.

The microcosm experiments followed the methodology from Evans and Trute (*10*) and Nozawa-Inoue et al. (*11*) for saturated zone and vadose zone soil experiments, respectively. Initial experiments by the perchlorate technology team included testing of perchlorate and other anions, cations, field moisture capacity, and moisture content. If the perchlorate baseline analyses were not as high as previous results for the soil, then laboratory-grade perchlorate was added to the soil to bring it up to 4,000 µg/kg, which was the highest detected amount of perchlorate detected near the primary source area before additional characterization began in September 2008.

Soil was sieved to remove coarse particles greater than one-quarter inch, and in the case of drying during the process; moisture was added by using deionized water (DI) to return the sample to baseline conditions. Each batch had one control sample with no amendments, and other soils collected from the site were mixed with the amendments, in such a way as to represent field conditions and minimize moisture loss.

Liquid and gaseous amendments were evaluated in the microcosm and soil column experiments. Table 2 lists the amendments utilized. The utilization of liquid and gaseous amendments allows flexibility for the final remedial system design and may determine whether a liquid

or gas injection system will be required. Gas may be beneficial for vadose zone treatment because it may enhance biodegradation in the soil without transporting contamination in solution to the groundwater for treatment. Liquid, on the other hand, may have a larger radius of influence beneath the water table.

Table 2. Electron Donor Amendments used in Initial Microcosm/Column Tests

Ethyl or Sodium Acetate	Glycerin
$LPG/CO_2/H_2$	Acetic Acid
Emulsified Vegetable Oil	Compost, gravel, and mulch
Reduction Potential Compound	High Fructose Corn Syrup

Nutrients and Electron Donors

Electron donor amendments consisted of a variety of readily soluble, sparingly soluble, and gaseous substrates. Amendments were prepared with and without nutrient addition consisting of 1 gram per liter (g/l) of diammonium hydrogen phosphate [$(NH_4)_2HPO_4$]. A soil control sample was tested alongside the amended microcosms in order to determine if natural attenuation was occurring. Gaseous electron donors were introduced into the

headspace after each microcosm was sealed. These amendments included ethyl acetate and liquid petroleum gas (LPG) with carbon dioxide (CO_2) and hydrogen gas (H_2).

Liquid amendments added directly to the soil included EOS, EHC, glycerin, HFCS, and acetic acid (Table 2). EHC is a commercially available substrate that combines a plant-based carbon/energy source to stimulate microbial activity with a zero valent iron component to rapidly generate and sustain reducing conditions by lowering the redox potential (Eh; also known as ORP). EOS is also a commercially available product, composed of emulsified vegetable oils which provide food to stimulate biodegradation. EOS products contain mixtures of soybean oil (food grade), long chain fatty acids, fast release soluble substrate, and food additives, emulsifiers, and preservatives.

Saturated Zone Soil Microcosms

The first phase of microcosm testing included the biobarrier area soils from the saturated zone and groundwater. Vadose zone microcosms from near the soil source area were performed as the second phase, and saturated zone source area microcosms were the third phase.

Each microcosm (3 per amendment and 3 controls) consisted of one amendment and 200 grams (g) of soil and

was sealed in a flask using air-tight septa caps. Laboratory-grade nitrogen (N_2) gas was used to purge out headspace. The flasks were incubated in a light and temperature-controlled environment.

Sampling for perchlorate biodegradation in the flasks was performed destructively by breaking the seal after incubation for up to three selected time periods (t=0, 2 weeks, and subsequent times depending on observed biodegradation rates). 10g of representative soil was extracted and placed in a shaker (*11*) or vortex mixer (*10*) with 10 milliliters (mL) of DI water for up to 6 hours. Initial total organic carbon (TOC) and measurement from the final sampling round (t=4 weeks) were analyzed and headspace was monitored for gaseous substrates using a gas chromatograph (GC).

Groundwater Microcosms

Source area and biobarrier area groundwater microcosms follow similar methodology. Each test includes a control sample consisting of site water with no nutrient addition. Nutrient addition includes 10g of diammonium hydrogen phosphate added per 10 liters (L) of site water. 200 mL of water for each test is added to 50g of site soil, followed with 0.2 and 1.0 mL of each amendment (EOS, glycerin, HFCS, and acetic acid). Approximately 10g of soil was withdrawn on days 2, 3, 4,

5, and 7 and analyzed for perchlorate, pH, and ORP. After all samples were taken, supernatant was decanted after centrifugation and stored for other possible analyses, such as general minerals.

Source Area Vadose Zone Soil Microcosms

For the vadose zone microcosms, all tests were the same; however, DI water was utilized since no groundwater is present in the vadose zone. Each microcosm (3 per amendment and 3 controls) consisted of one amendment and 200 g of soil sealed in a 250-mL flask. Sampling was performed for up to three selected time periods (t=0, 2 weeks, and subsequent times depending on observed biodegradation rates).

Vadose zone soil was used from the first sample collection and the soil was spiked to 4,000 µg/kg. This soil was approximately 9% moisture upon receipt, with a field capacity of 35%. For a second phase, microcosms were brought up to 15% and 25% moisture using tap water. Because the soil at 25% moisture was observed to be cohesive clay with very low permeability, only soluble amendments were tested, which included glycerin, sodium acetate, and HFCS. All source area vadose zone microcosms also included the gaseous amendments, ethyl acetate, and LPG/H_2/CO_2, and a control (no amendments). Each microcosm amendment was tested with and without nutrient addition.

Following phase 2 vadose zone microcosms, a third phase began in order to check whether perchlorate-degrading bacteria were present and could be stimulated. The third phase was conducted by saturating the soil with sodium acetate as an electron donor. Sodium acetate was amended at a dosage of 500 mg/kg, in separate microcosms with and without nutrient addition. After each time interval, analyses were conducted after sacrificially sampling the microcosms, which included pH, nitrate, nitrite, and TOC.

Soil Column Methodology

Upon completion of the first phase of biobarrier area microcosm testing, microcosm data was evaluated, and the data was utilized to refocus the soil column methodology. Soil columns were built and tested utilizing soil and groundwater from the biobarrier and primary source areas. The purpose of the soil columns is to provide an indication of what field performance we might expect to see during the final testing phase at the site.

Biobarrier area column studies were performed in two phases, utilizing EOS, EHC, and a mixture of compost, gravel, and mulch, all of which were selected after completion of the microcosm tests. Nutrients were only added to the soil columns if poor performance was

observed. All soil column tests were performed in accordance with American Society for Testing and Materials (ASTM) methodology (*24*). This procedure entails collecting aqueous leachate from the materials inside a column apparatus.

Biobarrier Area Soil Columns

During the first phase, 6-inch diameter polyvinyl chloride (PVC) pipe with a length of 2 feet was used to construct the column. Figure 4 presents the typical construction of the soil column apparatus. Sampling ports were installed on the pipes in 6-inch intervals to permit sampling along the internal flow path. Columns were packed with site soil, saturated with site groundwater, and a total of 2 liters of site groundwater per day was pumped in thereafter from the bottom up (to reduce unsaturated soil pore space) at a rate of 0.31 liters per day (L/d). Since site groundwater velocities and hydraulic conductivities values are very low, this low flow rate (0.31 L/d) was chosen to represent the high end of actual site conditions.

For the second phase of Biobarrier area soil columns, the column designs were modified to better suit the conditions. Three sets of four parallel 2-inch diameter PVC pipes were used instead of 6-inch diameter.

These were constructed in lengths of 12 inches, 18 inches, and 24 inches (Figure 4).

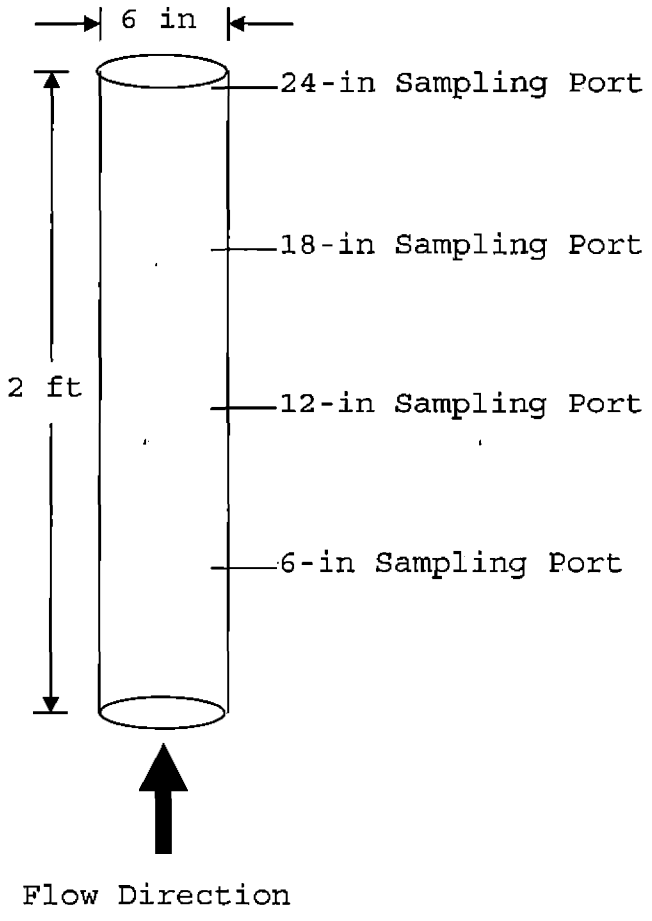


Figure 4. Typical Soil Column Apparatus

A fourth pipe was used as a control for each experiment. These tests included the addition of EOS, EHC, and the gravel, compost, and mulch mixture. Using

clustered sets of smaller pipes reduced the amount of groundwater needed for the tests. Similar to the first phase, the columns were packed and saturated, but flow rates varied from 0.31 to 1.24 L/d (0.5 to 2.0 feet per day).

Source Area Saturated Zone Soil Columns

Source area column studies were performed in two phases, utilizing EOS and glycerin, after determining that sodium acetate would have a detrimental effect on the groundwater by adding additional salt ions. In addition, these were determined to be the best performing substrates during the microcosm and soil column tests prior to this phase of the study (*16*). In both phases, nutrients (consisting of diammonium phosphate) were added to the soil columns only if poor performance was observed. It should also be noted that EOS is a commercial product manufactured with nutrients, thus added nutrients were not needed.

During the first phase, 6-inch diameter polyvinyl chloride (PVC) pipe with a length of 2 feet was used. Sampling ports were installed on the pipes in 6-inch intervals to permit sampling along the internal flow path. Columns were packed with site soil, saturated with site groundwater with a perchlorate concentration of 60,000 µg/l, and 2 liters of groundwater per day were

pumped from the bottom up. Site groundwater velocities and hydraulic conductivities values are very low so a low flow rate (0.31 L/d) was used in order to represent actual site conditions.

During the second phase, three sets of four parallel 2-inch PVC columns were constructed in lengths of 24, 18, 12, and 6 inches (*25*). Procedures were the same as the first phase soil columns.

Source Area Vadose Zone Soil Columns

Additional soil collected in October 2008 from the source area vadose zone was used for this phase of column studies; however, since the concentration was higher (220,000 vs. 4,000 µg/kg), the column lengths and widths were adjusted. Six-inch long columns with 2-inch diameter were packed with site soil from the 2008 sample collection. Substrate solutions consisted of tap water with a 0.5% volume to volume (v/v) ratio of electron donor amendments and 20 mg/l of nutrients (diammonium phosphate).

Before implementation of the vadose zone soil columns, the procedures needed to be re-strategized based on observations from the vadose zone microcosms. The microcosm tests were used to assess the most successful treatment technique that was further evaluated in soil columns. Based on the results, it was determined that

adding a donor/water solution would be a more effective option than gaseous amendments for in-situ treatment of the vadose zone soils. This was primarily based on the soil properties with moisture added and the inability for gaseous donors to be effective in such conditions.

In April 2009, two alternative bench scale options were proposed for the modified approach to the source area vadose zone soil columns (*26*). The modified plan was to complete the column testing with up-flow gravity drained tests and continual flooding tests in the columns using vadose zone soil from the source area. Since the laboratory application of the vadose zone soil columns requires the addition of water, the field application will also require injection of water into the soil source area. Additional controls will need to be designed to prevent mobilization of residual perchlorate in the vadose zone into the groundwater table.

Column tests were proposed to be completed to simulate in situ vadose zone treatment by adding water and electron donors under two treatment scenarios: a batch mode (treatment scenario 1) or a recirculating mode (treatment scenario 2). In addition, separate microcosm tests were proposed for the vadose zone soils (*26*).

In treatment scenario 1, the purpose was to simulate flooding, followed by drainage. Two pore volumes of water

(300mL) were pumped from the bottom up through the soil columns at 1 mL per minute. One cycle was completed using amendments (0.5% v/v of EOS/glycerin and 20 mg/l diammonium phosphate) mixed with the influent water. One control column was used with tap water only. Effluent samples were collected at designated sampling times (0, 0.5, 1, 2, 4, and 8 weeks) and analyzed for perchlorate, nitrate, pH, and TOC. Columns were left open to gravity drain after sampling.

In Scenario 2, the purpose was to simulate a recirculating approach with only an initial application of electron donors and nutrients. In this application, the electron donor is added to the surface and allowed to migrate through the contaminated soil to the underlying groundwater. Vadose zone soils in the column are maintained at constant saturated conditions with this scenario. The same donor/water solutions were applied, and one pore volume per day was recirculated at a rate of 1 mL per minute. Samples and recycled water were collected at designated sampling times (0, 0.5, 1, 2, 4, and 8 weeks) and analyzed for perchlorate, nitrate, pH, and TOC.

Sampling and Analysis

Daily samples were collected from sample ports and tested to assess the performance of the columns. In order

to keep flow disruption to a minimum while sampling, low flow rates were obtained using syringes in the sampling ports. The extracted water samples were then analyzed for geochemical parameters. These included perchlorate, pH, oxidation-reduction potential (ORP), TOC, anions (nitrate, nitrite, sulfate, and sulfide), and metals (arsenic, manganese, and iron).

Analytical sampling was performed at t=0, 0.5, 1, 2, 4, and 8 weeks. All samples were analyzed at the UCR laboratory. Samples were tested for pH by EPA Method 150.1, ORP by Standard Method 2580, TOC by EPA 415.1, perchlorate by EPA 314.1, and nitrate by ASTM D4327-03. Soil analyses were performed by homogenizing the column material before a representative subsample was taken (*25*). The soil extraction procedure followed Nozawa-Inoue et al. (*11*).

Quality control sampling included matrix spike/matrix spike duplicates (for accuracy) at one out of every 20 samples and commercial reference standards for proper calibration. Microcosm replicate samples were also conducted by analyzing in triplicate. All data and observations were documented in laboratory composition books.

CHAPTER FOUR

RESULTS AND CONCLUSIONS

Results of the biobarrier area microcosm and soil column experiments were completed and summarized in a technical memo in November, 2008 (*18*). The source area saturated zone and vadose zone column studies were documented in August 2009 (*25*). The results of the biobarrier area and source area groundwater and vadose zone microcosm/soil column experiments are summarized in tables and charts included in Appendix A. The following sections present a summary of the data and a discussion of the results from the experiments.

Initial Field and Laboratory Results

Biobarrier Area Soil and Groundwater

During sample collection the field instrument showed that biobarrier groundwater pH readings were neutral, ranging from approximately 7 to 8 (6 to 8.5 is desirable to favor bioremediation), and dissolved oxygen and ORP indicated aerobic groundwater. Samples sent to UCR had cation and anion results that were within normal ranges, and sulfate was slightly elevated, at 175 milligrams per liter (mg/l) in groundwater and 48 milligrams per kilogram (mg/kg) in soil. Total Kjeldahl Nitrogen (TKN)

was detected in initial biobarrier area soil samples at 48.6 mg/kg but only at 0.35 mg/l in groundwater. This is the sum of free ammonia and organic nitrogen compounds. Total phosphorous was detected at trace levels, but ortho-phosphate was not detected. This is the biologically available form of phosphorous. Metals were not detected in the biobarrier groundwater, but are present in the soils within normal ranges.

Initial groundwater samples collected at the biobarrier location had perchlorate detected at approximately 500 µg/l and nitrate (NO_3^-) at 7.5 milligrams per liter (mg/l). In addition, the samples had an alkalinity of 280 to 300 mg/l, a pH of 7.7, and electrical conductivity of 280 micro-Siemens per centimeter (µS/cm). A total dissolved solids (TDS) value was calculated to be approximately 200 mg/l based on the electrical conductivity. It should be noted that the field reading of electrical conductivity was approximately 1,316 µS/cm. The calculated conductivity reading differed from the field instrument reading and led to an imbalanced TDS analysis with regard to cations and anions; therefore, the initial laboratory analysis was rejected. This was resolved with additional analyses for electrical conductivity and TDS, which yielded 1321

µS/cm and 902 mg/l, respectively, and commercial laboratory analyses which showed TDS at 990 mg/l.

Analytical results from commercial laboratories were validated using a program that analyzes field and laboratory quality control (QC) samples to ensure data integrity. While commercial analytical and QC results contain confidential client information and are too long to be included, results are summarized and tabulated in Appendix A.

Source Area Groundwater and Soil

Initial perchlorate in the source area groundwater was detected at 57,800 µg/l. The groundwater pH from the source area was 7.8 and the soil from the aquifer was 8.8. Since it is favorable to have a pH between 6 and 8.5, this soil pH may be higher than the optimal range. The groundwater sample had a TOC of 2.62 mg/l and the soil had a TOC of 28.1 mg/kg. Since nitrate was present as the predominant nitrogen form, an aerobic environment is indicated. With these TOC values and an oxidized environment, biodegradation of the perchlorate is not favorable. This is thought to be the reason that perchlorate persists in the site soils (25).

Cations and anions were detected 56 and 19 mg/l in groundwater and soils, respectively. Reduced sulfides from bioremediation may enhance precipitation of metals,

preventing migration, if reduction occurs. Only low-level iron and manganese were found in the soils tested.

Groundwater samples had a very low TKN at 0.46 mg/l, and nitrate as nitrogen was detected at 8 to 9 mg/l. Since nitrate reduction is usually favored before perchlorate reduction, this and the sulfate bioreduction need to be taken into account when designing a field system. Ortho-phosphate, the predominantly biological form of phosphorous, was reported at a low level (0.13 mg/l) in groundwater but was below the detection limit in soil. It was concluded that low-level macronutrients were present (*25*).

Source Area Vadose Zone Soil

In the vadose zone soil collected first, the soil pH was 9.3, which is above the favorable range for bioreduction to occur. Total phosphorous was low, at 1.0 mg/kg, with ortho-phosphate being 40% of the total phosphorous. A concentration of TOC was reported as 102 mg/kg, which is not favorable for biological activity. However, low levels of macronutrients are present, as shown by the ortho-phosphate (*25*).

The initial collection of source area vadose zone soil yielded soil that was unexpectedly non-detect for perchlorate and was spiked to 4,000 µg/kg using a perchlorate reference standard. Conversely, soil was

reported from the analytical laboratory at a value of 220,000 µg/kg once resampled from the source area. The second soil sample collected from the vadose zone was not tested for the other parameters.

Microbiological Soil Analysis

Samples sent for microbiological testing were reported positive for the chlorite dismutase enzymes found in perchlorate reducing bacteria, with an enumeration of about 9,300 cells/g in site soil. As previously mentioned, chlorite dismutase is found in the cell walls of perchlorate-reducing bacteria (*8*). Therefore, perchlorate-reducing bacteria are present in site soils at the biobarrier area and source area; however, geochemical conditions do not favor biological activity.

Microcosms and Column Studies

Biobarrier Area Microcosm and Soil Column Results

Biobarrier area microcosms were completed after 10 days of testing, with some substrates yielding 100% reduction in less than 10 days. A summary of results including detailed graphs from the biobarrier microcosms can be found in Appendix A. Nitrate reduction preceded perchlorate reduction where both anions were present. Control samples, with no electron donors and nutrients

added, remained stable with no perchlorate reduction. During the testing, metals were detected in microcosms with compost/mulch only, and the substrates EOS and EHC had no mobilization of metals. It should be noted that the solubilized metals from the compost/mulch columns actually came from the substrate itself and not the soils, since no soils were used in these experiments.

Phase I columns were tested with compost/gravel/mulch amended with EOS and EHC, added during the initial construction of the columns. After 4 months, complete perchlorate reduction was observed in the first 12 inches of the columns. In addition, nitrate was reduced in the first 6 inches of the columns. The pH values dropped from 7.6 to 6.4 due to the presence of humic acids from the compost/mulch. Anaerobic conditions were maintained throughout the column studies. TOC readings dropped from over 1,000 to about 50 mg/L during the experiments. It should be noted that metals were elevated above background levels in the effluent samples.

Phase II soil columns had complete reduction of perchlorate with either EOS or EHC, but not in the control. As shown in the graphs (pages 113-115, Appendix A), a spike of perchlorate concentration occurred, but this was due to a mistake in the laboratory. Source area groundwater, with a much higher concentration, was

accidentally used as the influent instead of the biobarrier area groundwater.

Complete reduction of perchlorate was observed within the first 6 inches of the columns with EOS with an influent flow rate of 0.5 ft/day, but when the influent flow rate was raised to 1.0 and 2.0 ft/day, elevated perchlorate was detected in the sample ports at 6, 12, and 18 inches; thus, perchlorate reduction was not occurring in the same intervals. When flow rates were reduced back to 0.5 ft/day, perchlorate reduction occurred again (page 114, Appendix A).

Initial results from EHC columns were inconclusive, most likely due to the fact that they were prepared one month before the tests began. As a result, a second set of EHC columns were prepared for the column tests. Perchlorate reduction occurred within the first 12 inches of the columns at a low flow rate.

When the velocity was raised, elevated perchlorate was detected at 6, 12, and 18 inches, and when the velocity was lowered again, perchlorate reduction was restored, but only for one of the four columns, with the most length (24 inches). Within the columns, pH values remained stable; however, effluent pH values were lowered to approximately 6.5. Conditions in the columns remained reducing; however, sulfate and sulfide concentrations

were shown to be lower during the testing. Increased metals were observed above background in effluent, but not as high as detected in effluent from the phase I biobarrier area soil columns.

Source Area Microcosm and Soil Column Results

In the initial source area microcosm studies, perchlorate reduction ranged from 2% to 100% in 10 days, with the best results from EOS with nutrients added in 7 days; in addition, 100% nitrate reduction was achieved. Bacteria with EOS reduced the perchlorate in 5 days with nutrient addition and 7 days without nutrients. For comparison, EOS columns were reduced 100% in 5 to 7 days in the biobarrier area microcosms.

Limited perchlorate reduction occurred with HFCS at the lower dosage after 13 days. With sodium acetate, perchlorate reduction was delayed at the higher dosage. In contrast, there was little difference between lower and higher doses of glycerin. The worst performance was with acetic acid; however, its poor performance was attributed to a low pH which was below the optimum range for perchlorate reducing bacteria, thus these may need to be run again with a pH buffer in future studies. Use of a higher dosage of amendments was concluded to be unnecessary based on the results.

Phase I columns were tested with EOS, glycerin, and a control column. After 105 days, preliminary results showed that up to 100% perchlorate reduction was observed in 24 inches of the EOS column, and 39% with glycerin. In addition, nitrate was reduced in the first 6 inches of the columns with EOS and glycerin. Anaerobic conditions were maintained throughout the column studies. TOC readings had a reduction of up to 78% during the experiments with EOS and glycerin; however, the glycerin reduction varied greatly and actually decreased to only 1.74% reduction (Appendix A).

Phase II Columns showed similar results. Perchlorate was reduced in EOS and glycerin columns but not in the control columns. In soil columns amended with EOS, perchlorate removal began gradually over the first two weeks, followed by more rapid degradation. After 20 days, perchlorate was reduced to the detection limit (<1 µg/l) in the effluent samples collected from the 18 and 24 inch columns, with the 12 inch columns nearly complete and about 35% reduction in the 6 inch columns. Reduction of perchlorate slowed after about 50 to 90 days. In addition, complete denitrification was observed in all of the EOS columns.

Glycerin was mixed with the soil at a ratio of 0.3%. After 20 days, no perchlorate reduction had been observed

yet. As a result, an additional 300 mL of glycerin was added to the influent after 25 days. This amount was determined to be more than ample for the biodegradation to occur (five times the stoichiometric amount. Perchlorate was reduced when the glycerin was added to the influent; however, reduction was not observed between 53 and 68 days, when glycerin supplementation was discontinued. Afterward, 120 mg/l (2 times the stoichiometric amount) of glycerin was used to supplement the influent, and this was reduced to 60 mg/l after 96 days. At only one times the stoichiometric amount necessary for the reaction to occur, biodegradation was significantly reduced for the remainder of the experiment.

It should be noted that nitrate was nearly consumed in the glycerin columns within the first 25 days. When the electron donor was consumed in the process, the denitrification rate decreased, thus inhibiting perchlorate reduction. After the 300 mL of glycerin was added (Day 25), complete denitrification occurred, allowing perchlorate degradation to commence.

Vadose Zone Microcosm and Soil Column Results

As previously mentioned, the initial vadose zone soil was tested and reported as non-detect (<10.7 µg/kg) for perchlorate in both UCR and commercial laboratory

analyses, so it was spiked to approximately 4,000 µg/kg for the microcosm and soil column testing. This was based on previous results from initial site investigation, in which one sample boring was located near this source area. The highest sample collected during this previous phase was 4,510 µg/kg.

With the initial soil collected that was reported negative for perchlorate, the microcosm studies were inconclusive. Perchlorate reduction was not observed or minimally observed from 40 to 80 days of testing, even when moisture was added to 15% and 25%. It is thought that because there was no perchlorate, the populations of perchlorate-reducing bacteria were too low to support perchlorate reduction in the spiked samples.

In order to test whether perchlorate-reducing populations were present or not, further microcosm testing was conducted with vadose zone soil saturated and amended with 500 mg/kg of sodium acetate, both with and without nutrients added. Perchlorate reduction was observed after 5 to 7 days with or without nutrients in the saturate soil microcosm tests. Near complete reduction was observed in as little as 6 days, with nutrient addition, and 9 days without nutrients.

Therefore, a favorable environment for biodegradation of perchlorate in the site vadose zone

soil was not duplicated in laboratory microcosm experiments with 15% and 25% moisture added. Measured soil moisture found to be optimal was at 64% moisture (saturated), compared to initial readings of only 9% and a field moisture capacity of 35% (the amount retained after allowed to drain).

Possible explanations as to why the unsaturated soil remediation was ineffective are being proposed for further investigation (25). Moisture content, pH, and salinity are the primary macro-variables for this study. Extracted water was tested for salinity after moisture content was added to 15% and 25%, yielding salinity results of 22,000 and 13,200 mg/l, respectively. This is within the range where biodegradation can occur; however, salinity was lower (5,000 mg/l) in water extracted from the saturated soils. Due to the amount of carbonates in the soil, it is thought that the initial pH of 9.3 might be raised when water is introduced into the soil pore space. Additional studies may need to be conducted using a pH buffer to lower the soil pH into the optimal range for biodegradation to occur (6 to 8.5).

It should be noted that the initial vadose zone soil used in the microcosm testing was collected in an area near the primary source area. The targeted soil contamination is a narrow diffuse plume extending

downward. This location sampled in April 2008 was less than 100 feet away from the actual source area, but the soil was reported to be non-detect for perchlorate. Additional soil from the source area vadose zone was collected in October 2008 during ongoing site characterization to delineate the horizontal and vertical extent of perchlorate-affected groundwater and soil. The maximum concentration of this soil was reported with 220,000 µg/kg at a depth of 20 feet bgs. Although the source area vadose zone soil microcosms were inconclusive, the vadose zone source area soil columns were conducted utilizing the contaminated soil collected in October 2008.

Column studies focused on utilizing saturated conditions of the soils in order to maximize perchlorate reduction. Electron donors were added in a batch mode, with amendments added to the soil, or a recirculating mode, with amendments added to the influent water. Sodium acetate was successful reducing perchlorate in the saturated microcosms; however, it was determined to be a potential harmful additive to the quality of the groundwater if used at the site (by adding additional ions into solution). Therefore, EOS and glycerin were tested in the columns, with an unamended control.

Before starting the column tests, additional microcosm testing was performed using EOS and glycerin added to the site soil collected from the source area. Both EOS and glycerin were found to be successful degrading perchlorate in these microcosms, with EOS or glycerin added at 0.5% weight to weight (w/w) with 20 mg/l of diammonium phosphate. Therefore, column studies were commenced using EOS and glycerin as electron donor amendments.

The batch application soil columns had little to no perchlorate degradation observed; moreover, approximately 30% to 40% of the perchlorate in the soil was leached out as a result of the batch application of water. Therefore, it was determined that this approach would lead to increased mobilization of perchlorate from the soil to the groundwater, resulting in higher groundwater contamination. As with the microcosms at 15% and 25% moisture, saturated conditions were not maintained. The batch application allows water to drain through the soil, resulting in decreasing moisture content from 40% to 15% over eight weeks. In contrast, the batch application method resulted in complete denitrification within one week. Therefore, either the species present may be denitrifying bacteria that do not reduce perchlorate, or the bacteria present were able to overcome limitations

for denitrification but were not able to overcome limitations for perchlorate reduction.

Rapid perchlorate and nitrate reduction were observed in the recirculation application soil columns whether amendments were used or not. The control column was observed to reduce perchlorate although reduction was limited compared to amended columns; however, a reducing trend was observed. This may be a result of high organic content of the soil, which was measured at 2.1% versus less than 1% in initial soil collected. It was concluded that the organic matter already present in the contaminated soil may have provided adequate electron donors for perchlorate biodegradation. In addition, recirculating the water through the columns provided sustainable conditions for this reduction to occur in saturated soils.

Perchlorate reduction was shown to be consistent with the microcosm tests while using the recirculation approach. This appears to be due to the fact that the recirculation application is able to keep soils at or near full saturation for the entire testing period. In contrast, during the batch method application, saturated conditions could not be maintained.

Discussion

Biobarrier Area

In the groundwater at the site, the conditions are generally aerobic, based upon field readings and laboratory results. For perchlorate biodegradation to occur, the groundwater must be in reducing and anaerobic conditions. In the microcosm and soil column studies, the addition of the substrates caused the pH of the water to be reduced.

Because reducing conditions needed to be created, this may pose a possible threat to the local environment by mobilizing metals in the groundwater. This was evaluated during the column studies as a potentially harmful effect of the biobarrier application. Metals were mobilized from compost/mulch columns due to their presence within the compost/mulch. In the second phase, metals were shown to be mobilizing from the soil with EOS and EHC; however, the levels were lower than the compost/mulch.

It is expected that reduced soluble metals will be precipitated out as conditions return to oxidizing downgradient of the biobarrier, after perchlorate reduction is complete. Therefore, although the mobilization of metals may be a local problem, it is not expected to affect the quality of the groundwater

permanently, as the metals are expected to attenuate as they migrate and groundwater returns to aerobic conditions. Although, this will need to be evaluated further in the field.

Reducing conditions were maintained by both EOS and EHC throughout the biobarrier area column studies, and biodegradation was observed with both. The appropriate amounts of the amendments, when applied in the field, will need to be adjusted in order to maintain reducing conditions until perchlorate is completely reduced. Since the soil columns were sealed, they remained in reducing conditions; however, the field application is not a sealed system. As a result, this will need further field evaluation.

Source Area

Source area saturated zone and vadose zone microcosm and soil studies were successful degrading perchlorate with EOS and glycerin as electron donors; however, the most consistent results were observed with EOS. Glycerin needed to be reapplied in microcosm tests, leading to the conclusion that it is consumed during the reaction. If used in the field, an appropriate amount of glycerin would need to be reapplied during remedial system operations. Sodium acetate was shown to be successful degrading perchlorate in microcosm testing; however, due

to the addition of salt ions into the water, it was determined that this would be detrimental to groundwater quality in a field application.

The batch and recirculating application methods of electron donors were evaluated during the source area vadose zone soil column experiments. As with the vadose zone microcosm results, it was shown that perchlorate reduction was maintained only when the vadose zone soil was completely saturated. Since only the recirculation approach was able to maintain saturation, this will be the approach used to add water and amendments to the source area soil in the primary source area of the site.

Field application of a recirculating remedial system would entail a network of injection and extraction wells that enable targeting a narrow zone of contaminated soil in a north-dipping bed. Therefore, very careful drilling and logging would be necessary in order to screen within the same geologic unit. For example, with injection wells applying recirculated water with amendments, the injection well would need to be placed above the source area, allowing infiltrated water and electron donors to saturate the source area. As the water drains out, it follows the dip of the bedding as it percolates toward the water table. An array of extraction wells can be placed in this unit north of the source area, extracting

water from deeper in the permeable zone. This application would also minimalize contaminated water percolating into the water table, thus eliminating a possible source of secondary contamination inadvertently caused by the treatment approach.

It should be noted that due to the lack of reduction observed in one set of vadose zone microcosms, the dynamic approach was used to refocus the testing. However, data gaps exist with respect to effects of soil moisture, pH, salinity, as well as other possible factors. While it was determined that the microcosms were successful under saturated conditions, additional studies can be done to assess how biodegradation is influenced with changes in soil moisture, and the resulting changes to salinity and pH. Additional studies are proposed to experiment with vadose zone conditions that would effectively promote biological activity thus reducing perchlorate (27). As part of the proposed additional experiments, chemical amendments will be assessed to alter the existing vadose zone conditions in order to make it favorable for biodegradation to occur.

Conclusions

Based upon the microcosm and soil column studies in all three applications (biobarrier area, source area

vadose zone, and source area saturated zone soils), reduction of perchlorate was observed most consistently with the EOS amendment (a commercially available product composed of emulsified vegetable oils). The biobarrier area soil columns had slightly higher metals mobilized from the compost/mulch columns, which were attributed to the media. Therefore, we believe that the best results may be achieved by utilizing EOS as electron donors for this site. Since no additional nutrients needed to be added, combined with the fact that reapplication may not be necessary, this substrate would be cost effective for the site.

Because similar results were shown with EOS and EHC in terms of kinetics in response to varying velocities of groundwater (Appendix A), we were not able to determine which was more effective during this phase of column studies. However, the longevity of EHC was observed to be slightly less than EOS in the columns tested. For the biobarrier area, both EOS and EHC were shown to be effective in reducing perchlorate concentrations, and mobilization of metals was expected to be attenuated as conditions return to aerobic. Source area soils, with higher perchlorate levels, may encounter differences in terms of longevity and performance between EOS and EHC. It was anticipated that more testing would be necessary

in order to make final conclusions. Due to limits in budgeting and time constraints often associated with projects of this size, EOS was chosen for further comparison in the source area microcosm and soil column studies.

As an electron donor, EOS consistently showed positive results with near complete perchlorate and nitrate reduction in most applications. While the initial source area vadose zone microcosms were not successful degrading perchlorate, EOS was shown to be effective in similar microcosms. Saturated conditions were maintained and soil from the source area with high-level contamination was used in these tests that were successful at reducing nitrate and perchlorate.

With respect to the hypothesis, we believe that this project shows that a dynamic framework can be designed to be successful in the cradle-to-grave process of in situ site remediation. This project documented several phases of investigation and laboratory microcosm and soil studies, including changes necessary to refocus technologies for better results. It should be noted however, that in order to be brief, not every aspect could be documented in detail in this study. This framework of site characterization and laboratory and field scale experiments can be used at other sites

because it takes into consideration changes to approaches in a dynamic fashion, based on real-time results.

At the project site, this ongoing process first included site characterization, which started in 2003. Using data from ongoing site characterization and plume delineation, we proposed treatment techniques and then collected soil and groundwater from the primary soil source area and proposed biobarrier treatment area located downgradient. Background research was performed targeting similar technologies and treatment techniques that can be screened for use at our site.

The soils and groundwater were first screened against multiple electron donors in microcosm tests, using methodologies obtained from the background research. Then the soil columns were performed using site soils, groundwater, and the best performing electron donors from the microcosm tests. In conclusion, while the site has complex hydrological and geochemical conditions, this study screened technologies and substrates and determined that EOS would be the most effective electron donor amendment for the site in both biobarrier and source area applications.

EOS can be applied to the final biobarrier design by constructing a trench in the downgradient area where groundwater is shallow (about 15 feet below ground

surface), and mixed with gravel, compost, and mulch. This would make a permeable reactive barrier designed to treat contaminated groundwater as it passes through. If field conditions require a deeper application for the biobarrier, a network of injection wells can be used in place of a semi-permeable barrier. Additional field testing will be required in order to determine the best approach for remedial system installation.

At the source area, a network of injection and extraction wells will be used to circulate water and EOS through the vadose zone. To treat groundwater, existing monitoring wells can be converted into injection wells for EOS amendment. For the actual field application, pilot tests need to be conducted in order to determine the appropriate radius of influence for both vadose zone infiltration and groundwater injection and extraction applications.

In conclusion, this study shows how this dynamic process can be utilized to characterize the site and treat the contamination using bacteria found naturally in site soil and groundwater. As part of the dynamic approach, treatment technologies were refocused using results from previous phases of investigation and laboratory experiments. Additional laboratory and field testing will be commenced upon completion of all soil

column experiments, and the data summarized herein will be utilized throughout the design and operation of the remedial systems. In addition, because this project was successful setting up the dynamic framework of perchlorate bioremediation, the dynamic approach used in this study has been proposed to be utilized at another site in a different hydrogeological setting contaminated with perchlorate (27).

APPENDIX A

MICROCOSM AND SOIL COLUMN DATA

Initial Groundwater Quality

Water Quality Analyses

		Wet Chemistry (mg/L)					Nutrients (mg/L)						Alkalinity (mg/L)		
Sample Name	Perchlorate (µg/L)	pH (pH units)	Total Organic Carbon (TO	Electroconductivity (µS/c	Hardness (mg/L as CaCO	Total Dissolved Solids	Ammonia (as N)	Total Kjeldahl Nitrogen (	Nitrate as N	Ortho-Phosphate (Solubl	Total Phosphorus	Total Sulfur (Test Americ	Total Alkalinity (as CaCO	Bicarbonate (as $CaCO_3$)	Carbonate (as $CaCO_3$)
Biobarrier Area Groundwater:															
Testing Lab	502	6.76 f	1.01	1316 f	242 c	990	0.0911 BJac	0.35	8.2	<0.0200	0.0245 Jeq	53.5	280	280	< 1.00
Testing Lab-DUP	580	7.71	1.05		243 c	1070	0.112 Ba	0.271	8.17	<0.0200	0.0223 Jeq	58.3	300	300	< 1.00
UCR	505	7.71		1321	234	902			8.62	<0.5					
Source Area Groundwater:															
Testing Lab	57,800		2.62			670	0.135	0.462	90	0.126	0.107	20.1	97.5	97.5	<1.0
UCR		7.76				839									

Notes:

-- - Not analyzed
µg/L - micrograms per liter
mg/L - milligrams per liter
MDL - Method Detection Limit
B - The sample result is less than 5 times (10 times for common organic laboratory contaminants) the amount of blank contamination. The result is considered not to have originated from the environmental sample, because cross-contamination is suspected.
J - The analyte was positively identified and the result is usable; however, the analyte concentration is an estimated value
a - The analyte was found in the method blank
c - By calculation
e - A holding time violation occurred
f - Field measurement
q - The analyte detection was below the practical quantitation limit.

		Anions (mg/L)		Cations (mg/L)				Trace Metals (mg/L)			VOCs (µg/l)	
Sample Name	Perchlorate (µg/L)	Chloride	Sulfate	Calcium	Magnesium	Potassium	Sodium	Arsenic	Iron	Manganese	Acetone	Methylene Chloride
Biobarrier Area Groundwater:												
Testing Lab	502	186	176	81.0	9.64	0.733 Jq	240	< 0.0400	<0.0400	<0.00300	–	–
Testing Lab-DUP	580	186	175	81.1	9.74	0.700 Jq	237	< 0.0400	<0.0400	<0.00300	–	–
UCR	505											
Source Area Groundwater:												
Testing Lab	57,800	305	55.6	73.5	13.7	3.47	187	<0.0400	0.0666 Jq	0.0325		
UCR												

Notes:

– - Not analyzed
µg/L - micrograms per liter
mg/L - milligrams per liter
MDL - Method Detection Limit
B - The sample result is less than 5 times (10 times for common organic laboratory contaminants) the amount of blank contamination. The result is considered not to have originated from the environmental sample, because cross-contamination is suspected.
J - The analyte was positively identified and the result is usable; however, the analyte concentration is an estimated value
a - The analyte was found in the method blank
c - By calculation
e - A holding time violation occurred
f - Field measurement
q - The analyte detection was below the practical quantitation limit.

		Soil Results (mg/kg unless otherwise noted)											
Sample ID: Location and Depth	Zone	Cation Exchange Capacity (meq/kg)	Arsenic	Iron	Manganese	Perchlorate (µg/kg)	pH (units)	Ortho-Phosphate (Soluble P)	Total Phosphorus	Total Sulfide	Sulfate	Total Kjeldahl Nitrogen (TKN)	Total Organic Carbon (TOC)
Biobarrier Soil:													
SB2-7-15'	Aquifer	210	< 4.29	13,900	231	26.4	9.00 Je	< 0.214	0.278 Jq	< 4.00	40.6	48.6	< 10.7
SB2-7-15-DUP		180	< 4.29	14,600	240	14.2 Jq	8.98 Je	< 0.215	1.48	< 4.00	32.1	43.7	11.8 Jq
SB2-7-29.5-30.5'	Aquifer												
Source Area Soil:													
15B-20-21'	Vadose	160	< 4.27	20,700	369	<10.7	9.30 Je	0.395 Jq	1.02 Jq	16	5.28 Jq	17.6 Ba	102
15B-70-71'	Aquifer	120	< 4.63	22,300	417	18000	9	< 0.232	0.869 Jq	20	18.7	8.37 Ba	28.1

Notes:

-- - Not analyzed
µg/kg - micrograms per kilogram
meq/kg -
mg/kg - milligrams per kilogram
MDL - Method Detection Limit (Note that MDL varies depending on a variety of factors including amount of soil used, dilution factor, etc.)
NA - Not applicable
B - The sample result is less than 5 times (10 times for common organic laboratory contaminants) the amount of blank contamination. The result is considered not to have originated from the environmental sample, because cross-contamination is suspected.
J - The analyte was positively identified and the result is usable; however, the analyte concentration is an estimated value
a - The analyte was found in the method blank
e - A holding time violation occurred
f - The duplicate/replicate sample's relative percent difference was outside of control limits
q - The analyte detection was below the practical quantitation limit.

		Biochemical Results			Geotechnical Results						
Sample ID: Location and Depth	Zone	Presence of Chlorite Dismutase Gene	Quantification (cells/g)	Polymerase Chain Reaction (PCR) Identificatio	Soil Type (USCS)	Moisture Content (%)	Dry Density (pcf)	Specific Gravity	Total Porosity (%)	Effective Confined Pressure (psi)	Saturated Hydraulic Conductivity (cm/sec)
Biobarrier Soil:											
SB2-7-15'	Aquifer	Positive	1,600	–	SM	12.4	121.4	2.677	27.32	9.9	6.2E-06
SB2-7-15-DUP											
SB2-7-29.5-30.5'	Aquifer	Positive	138	–	SM	9.0	115.3	2.681	31.08	17.5	2.5E-05
Source Area Soil:											
15B-20-21'	Vadose	Positive	9300	–	ML	9.6	107	2.749	37.62	11.1	1.9E-05
15B-70-71'	Aquifer	Positive	16000	–	CL	16.9	113	2.761	34.41	43.1	1.5E-07

Notes:

– - Not analyzed

µg/kg - micrograms per kilogram

meq/kg -

mg/kg - milligrams per kilogram

MDL - Method Detection Limit (Note that MDL varies depending on a variety of factors including amount of soil used, dilution factor, etc.)

NA - Not applicable

B - The sample result is less than 5 times (10 times for common organic laboratory contaminants) the amount of blank contamination. The result is considered not to have originated from the environmental sample, because cross-contamination is suspected.

J - The analyte was positively identified and the result is usable; however, the analyte concentration is an estimated value

a - The analyte was found in the method blank

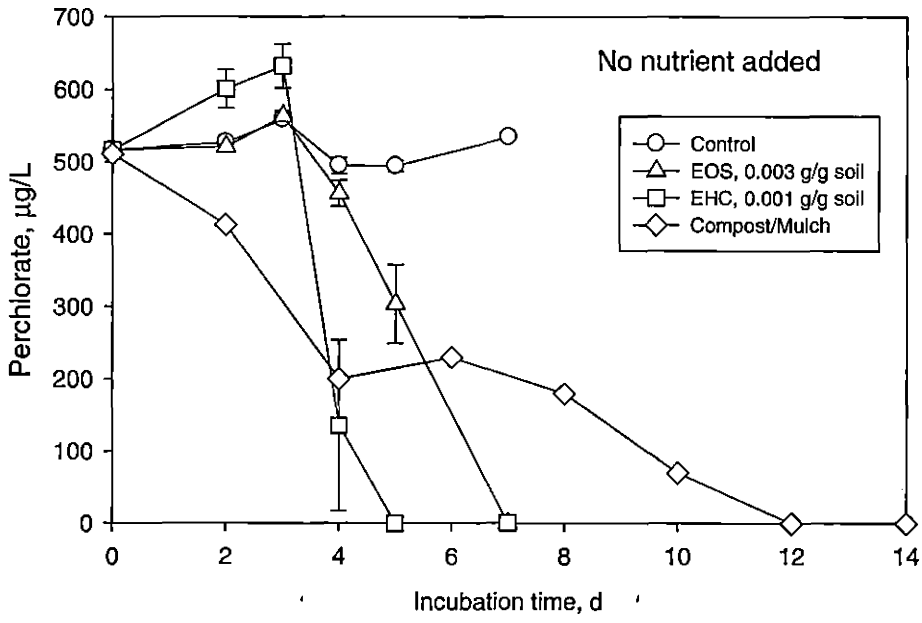
e - A holding time violation occurred

f - The duplicate/replicate sample's relative percent difference was outside of control limits

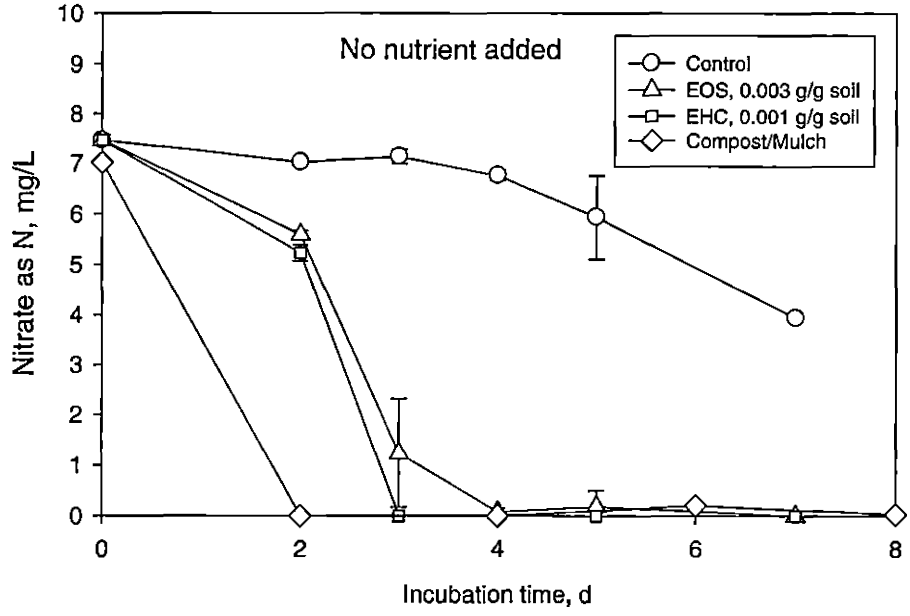
q - The analyte detection was below the practical quantitation limit.

Biobarrier Microcosms

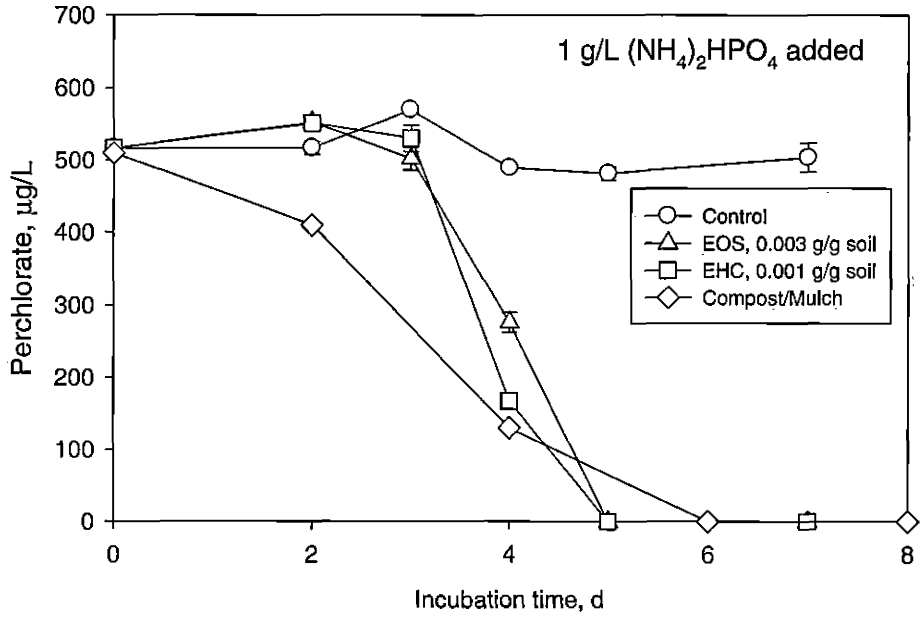
Perchlorate Reduction: No Nutrients Added



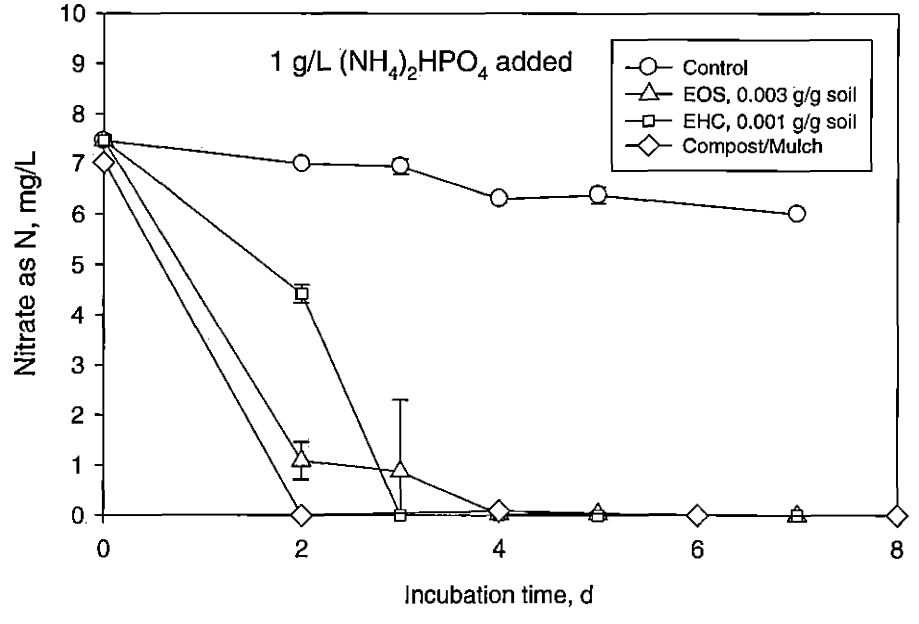
Nitrate Reduction: No Nutrients Added



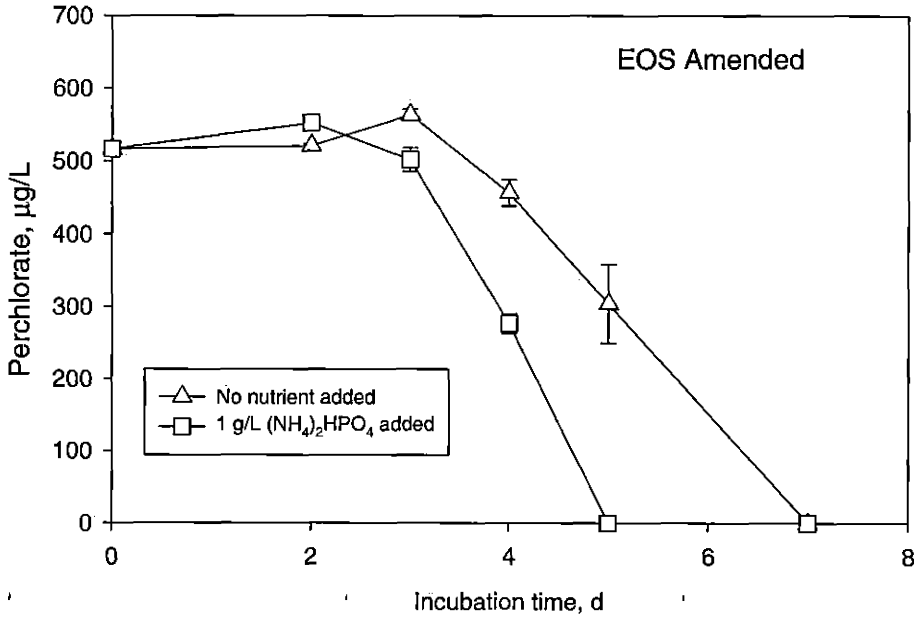
Perchlorate Reduction: 1 g/L $(NH_4)_2HPO_4$ Added



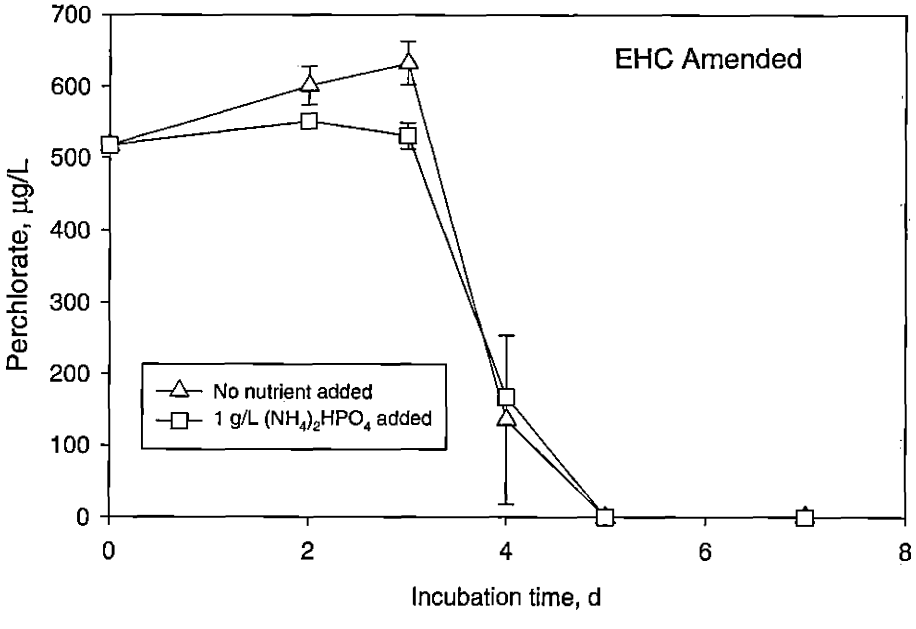
Nitrate Reduction: 1 g/L $(NH_4)_2HPO_4$ Added



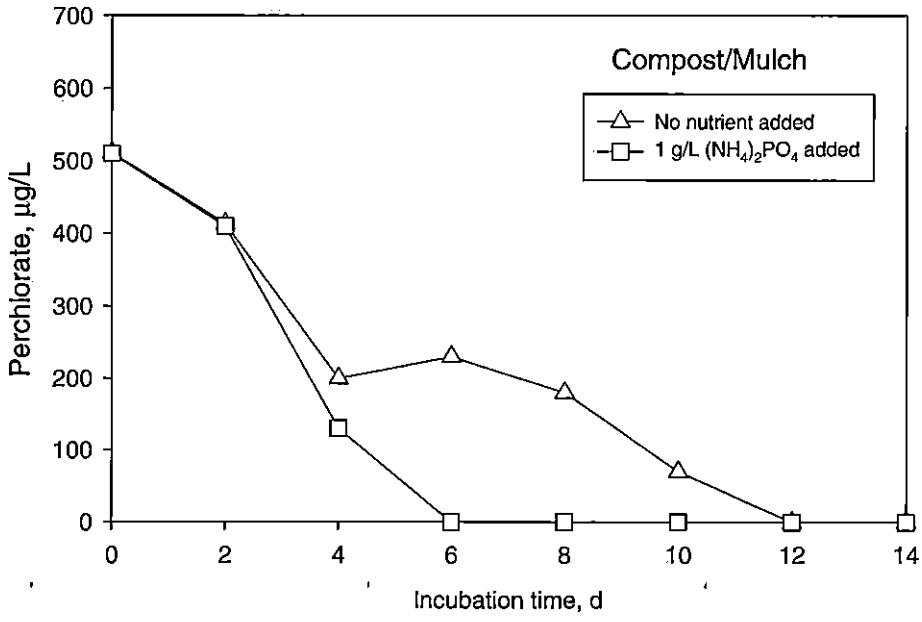
Perchlorate Reduction: EOS – With and Without $(NH_4)_2HPO_4$ Added



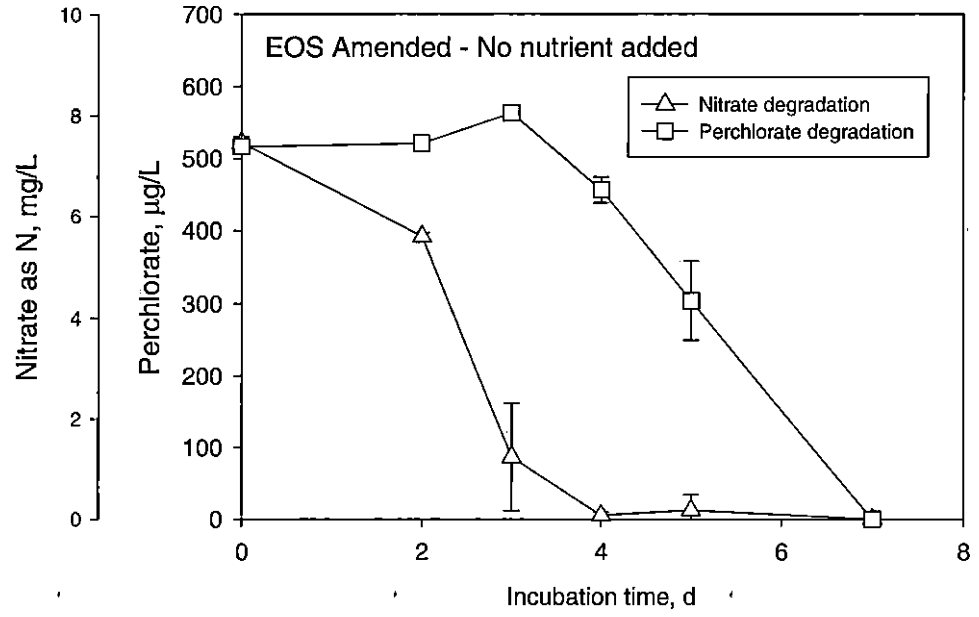
Perchlorate Reduction: EHC – With and Without $(NH_4)_2HPO_4$ Added

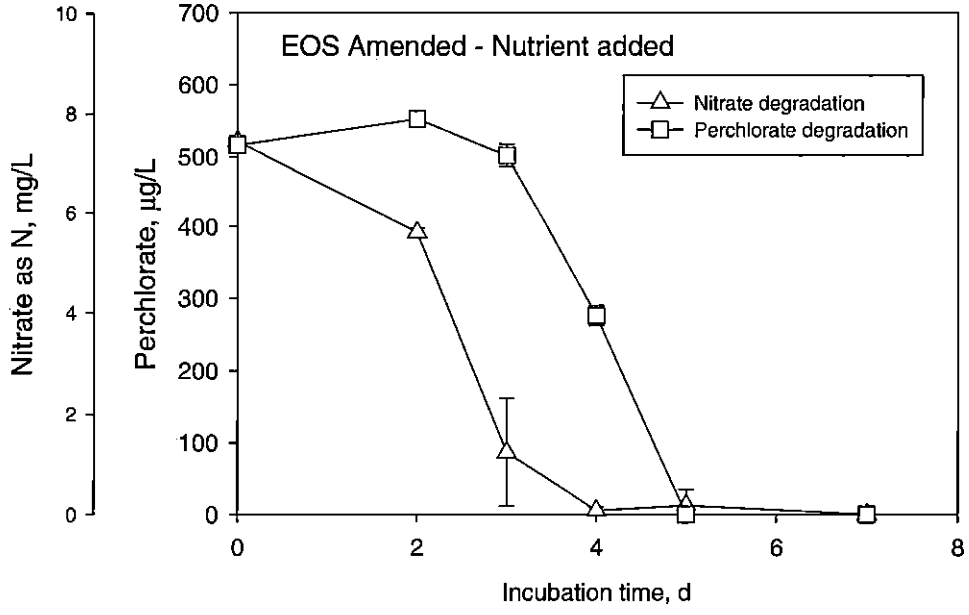


Perchlorate Reduction: Compost Mulch – With and Without $(NH_4)_2HPO_4$ Added

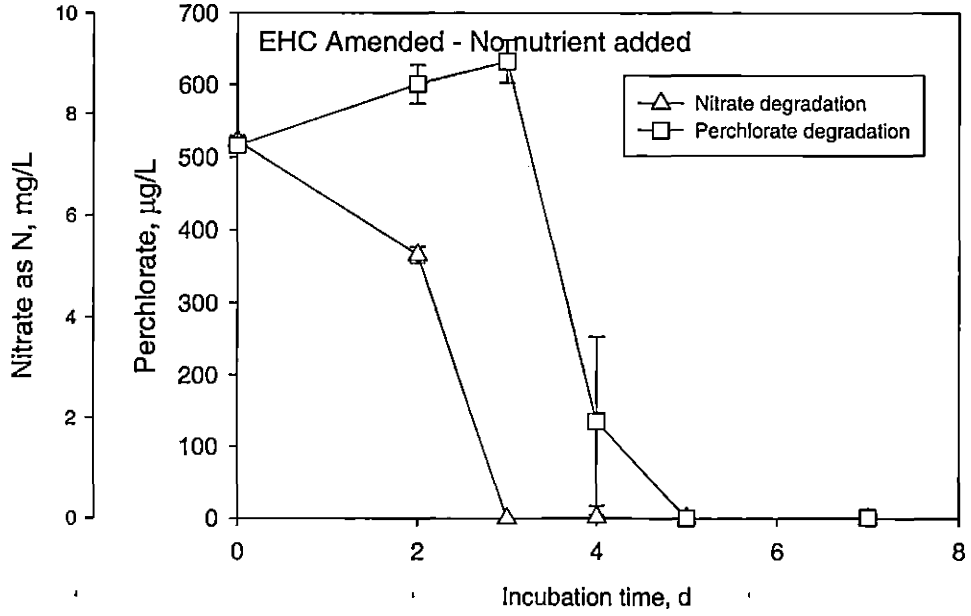


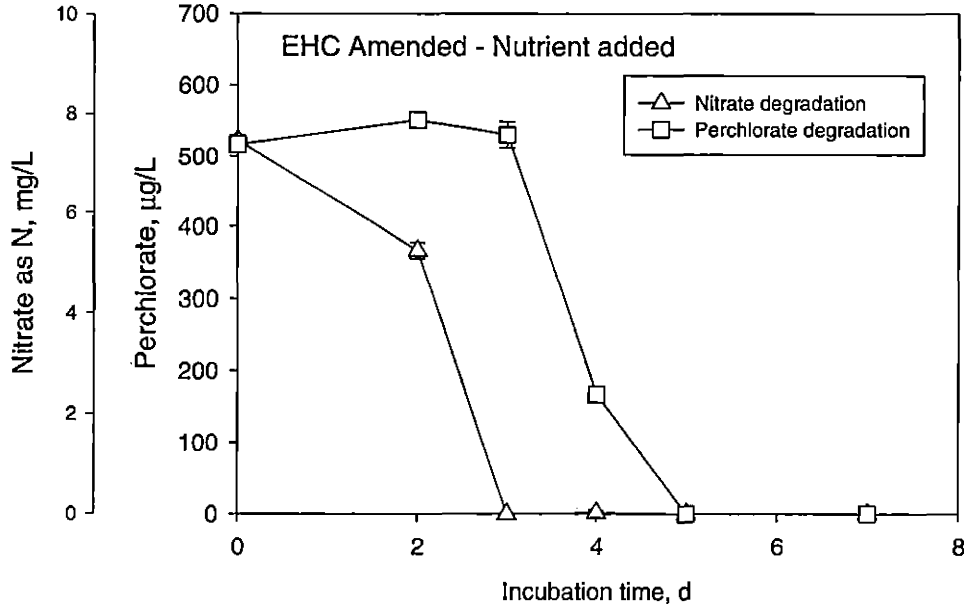
Perchlorate vs Nitrate Reduction: EOS With/Without $(NH_4)_2HPO_4$ Added



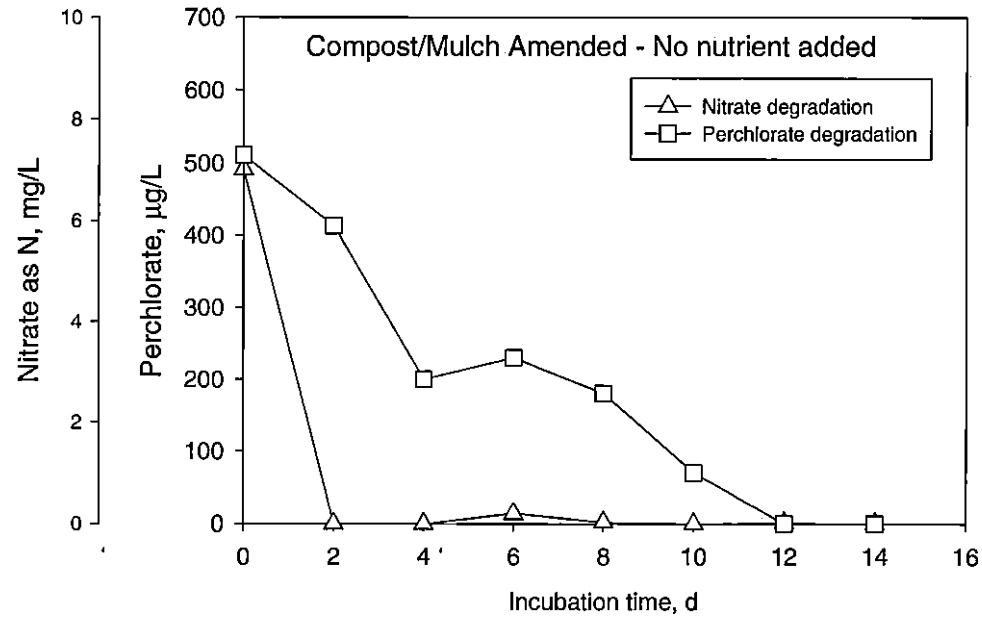


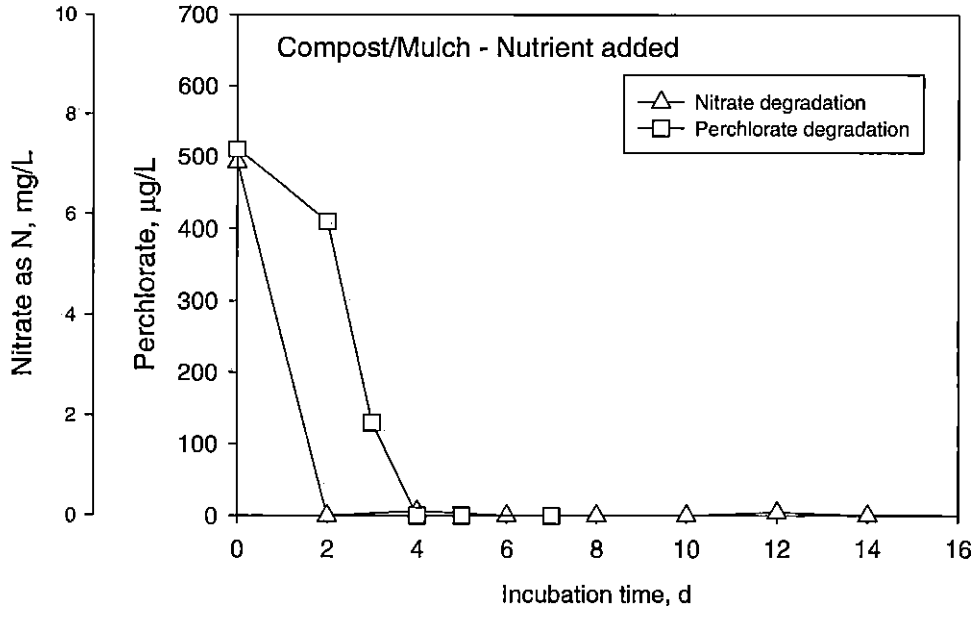
Perchlorate vs Nitrate Reduction: EHC With/Without $(NH_4)_2HPO_4$ Added





Perchlorate vs Nitrate Reduction: Compost/Mulch With/Without $(NH_4)_2HPO_4$ Added





Initial-Final Analyses

Control Microcosms

Parameter	MDL, mg/L	Without nutrient		With nutrient	
		Initial, mg/L	Final, mg/L	Initial, mg/L	Final, mg/L
Nitrite as N	0.090	0.31	2.2	ND	1.5
Arsenic	0.070	ND	ND	ND	ND
Iron	0.15	ND	ND	ND	ND
Manganese	0.070	ND	ND	ND	ND

EOS Microcosms

Parameter	MDL, mg/L	Without nutrient		With nutrient	
		Initial, mg/L	Final, mg/L	Initial, mg/L	Final, mg/L
Nitrite as N	0.090	0.53	ND	0.60	ND
Arsenic	0.070	ND	ND	ND	ND
Iron	0.15	ND	ND	ND	ND
Manganese	0.070	ND	ND	ND	ND

EHC Microcosms

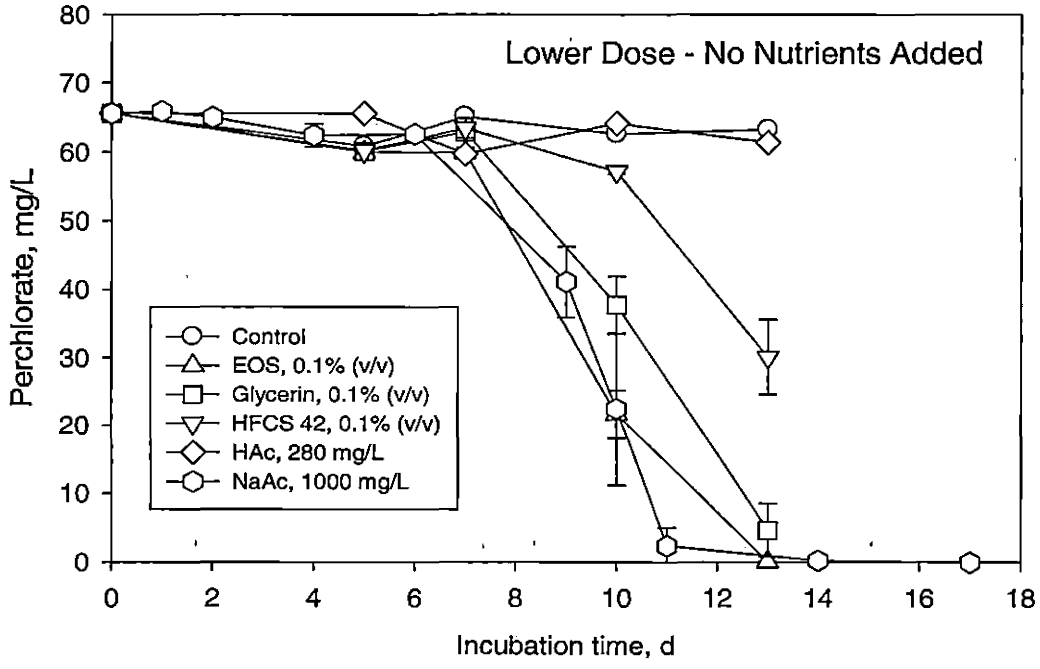
Parameter	MDL, mg/L	Without nutrient		With nutrient	
		Initial, mg/L	Final, mg/L	Initial, mg/L	Final, mg/L
Nitrite as N	0.090	0.57	ND	1.1	ND
Arsenic	0.070	ND	ND	ND	ND
Iron	0.15	ND	ND	ND	ND
Manganese	0.070	ND	ND	ND	ND

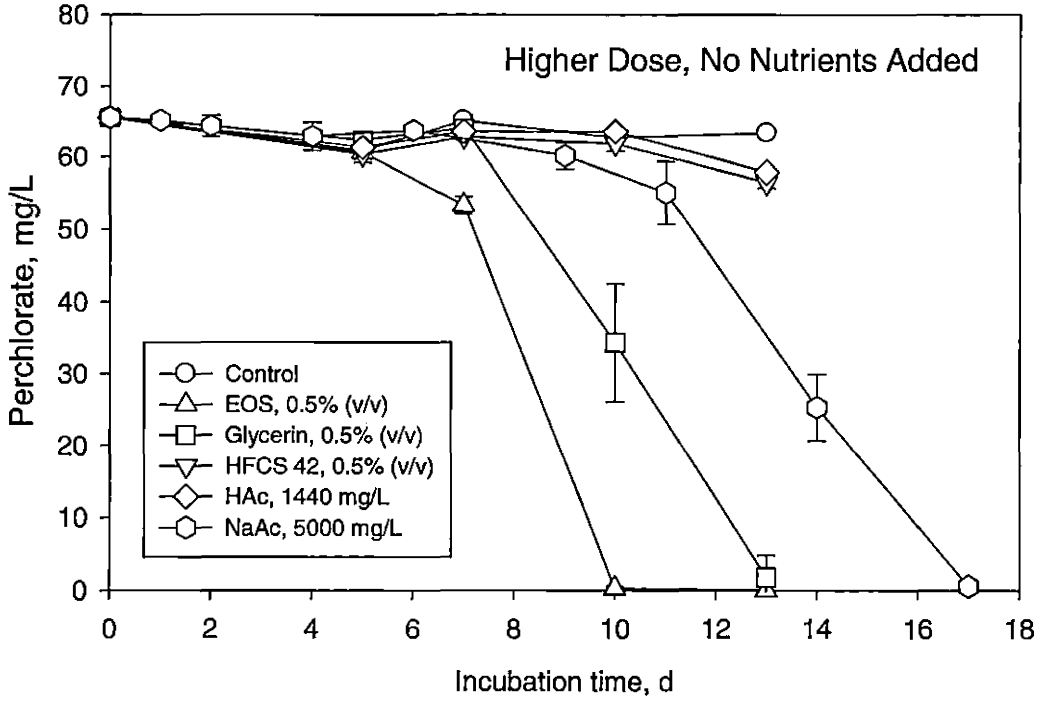
Compost/Mulch Microcosms

Parameter	MDL, mg/L	Without nutrient		With nutrient	
		Initial, mg/L	Final, mg/L	Initial, mg/L	Final, mg/L
Nitrite as N	0.090	ND	ND	ND	ND
Arsenic	0.070	ND	ND	ND	ND
Iron	0.15	0.76	27	0.54	17
Manganese	0.070	0.035	1.5	0.034	1.1

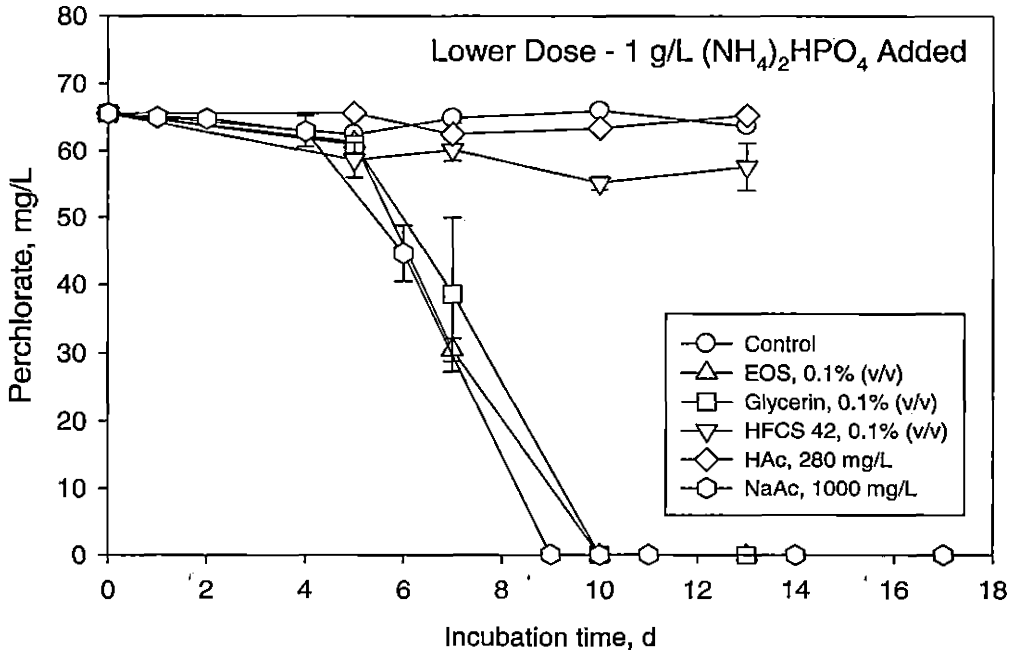
Source Area Microcosms

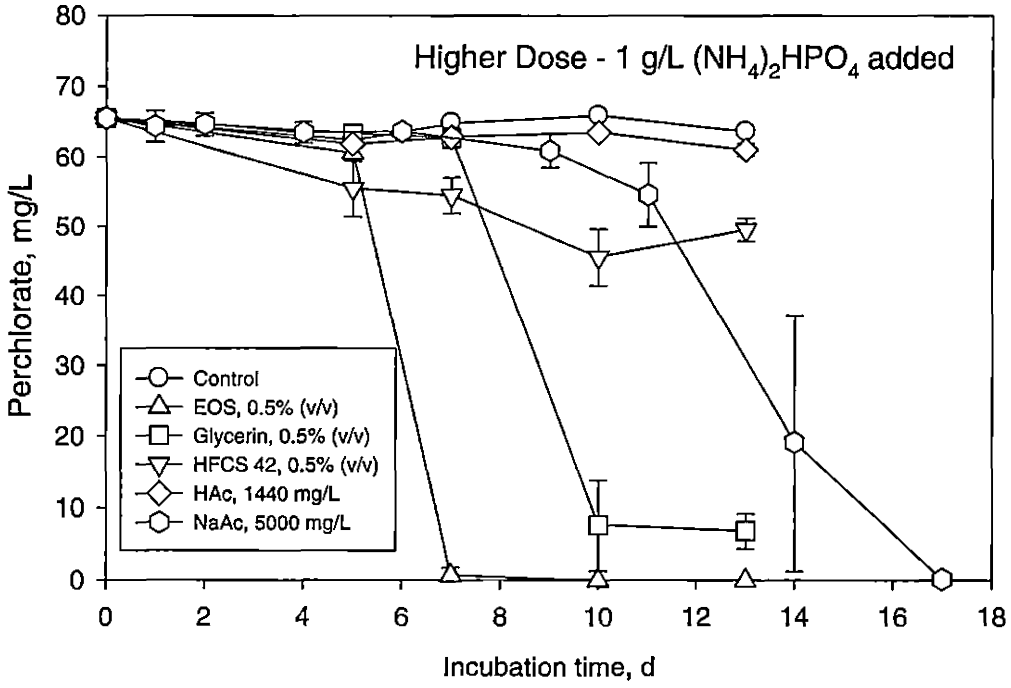
Source Area Groundwater Microcosms, No Nutrients Added
(Top: Low Dosage; Bottom: High Dosage)



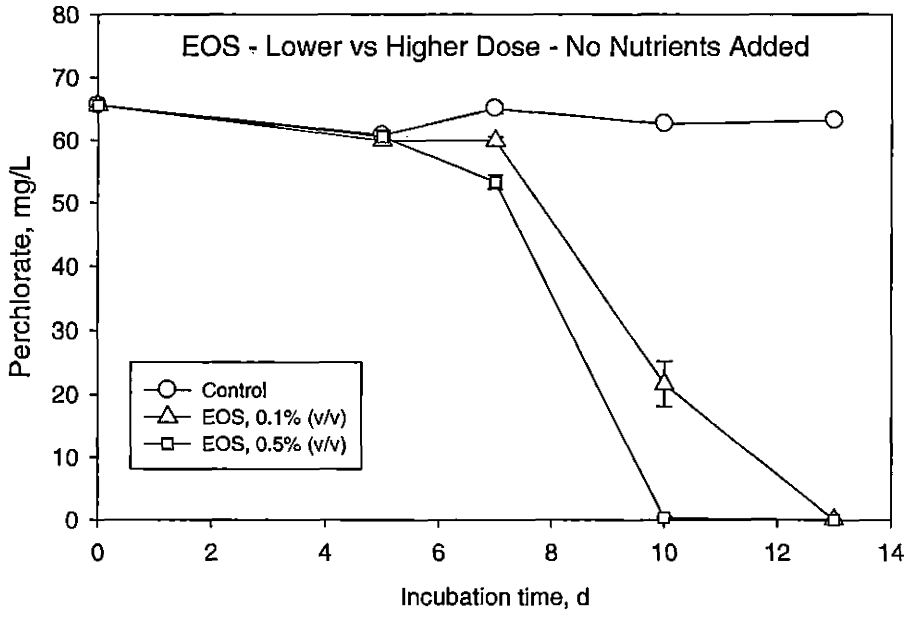


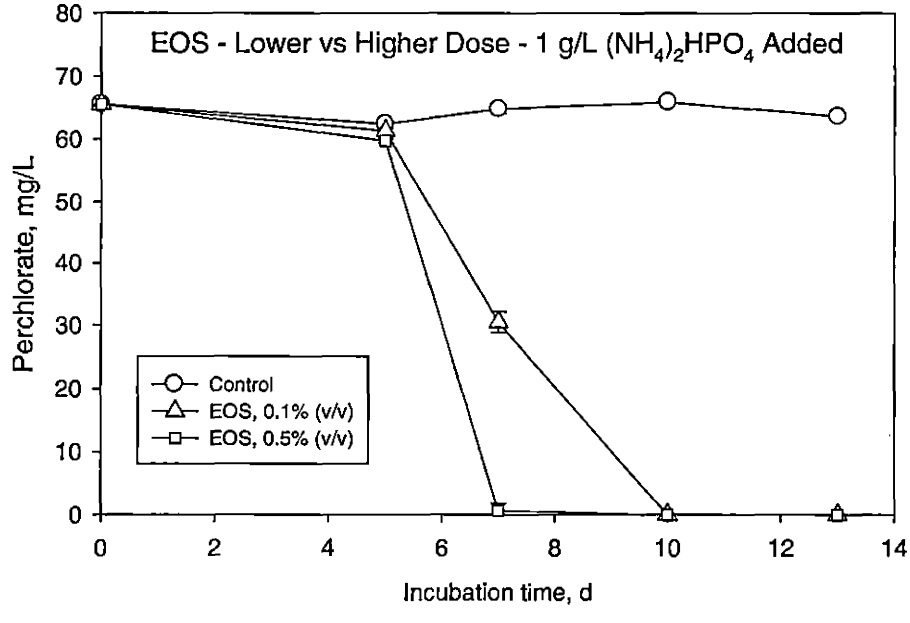
Source Area Groundwater Microcosms, Diammonium Phosphate Added (Top: Low Dosage; Bottom: High Dosage)



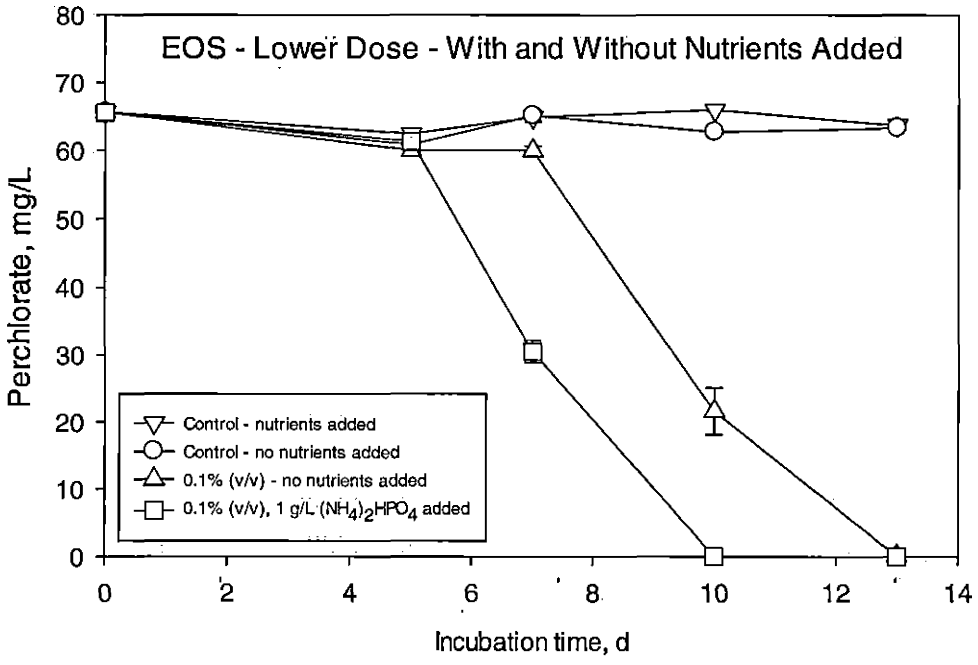


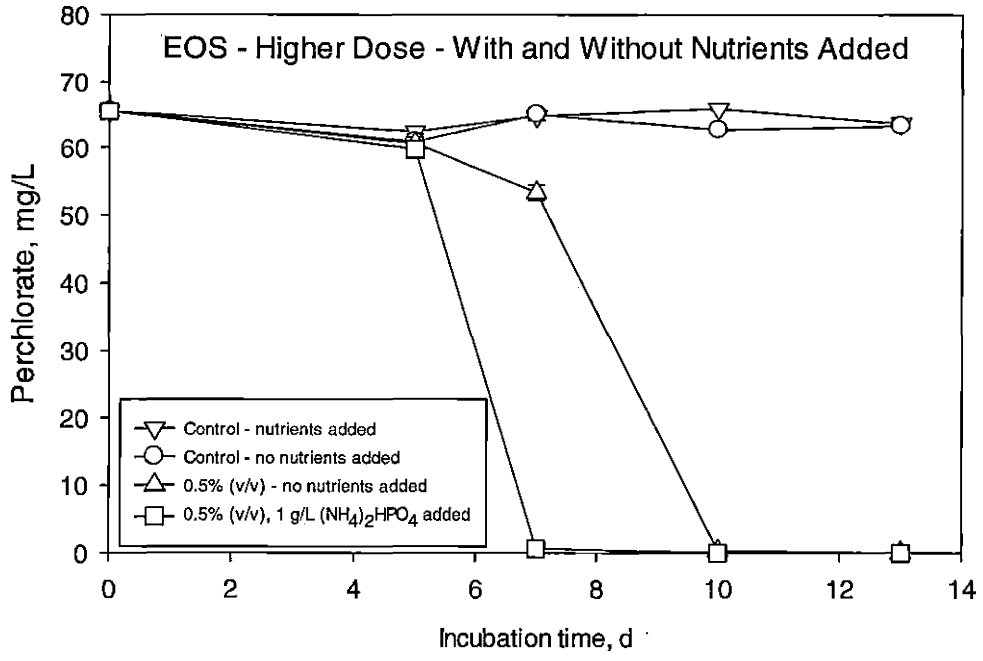
Perchlorate Reduction: EOS – Dose Effect



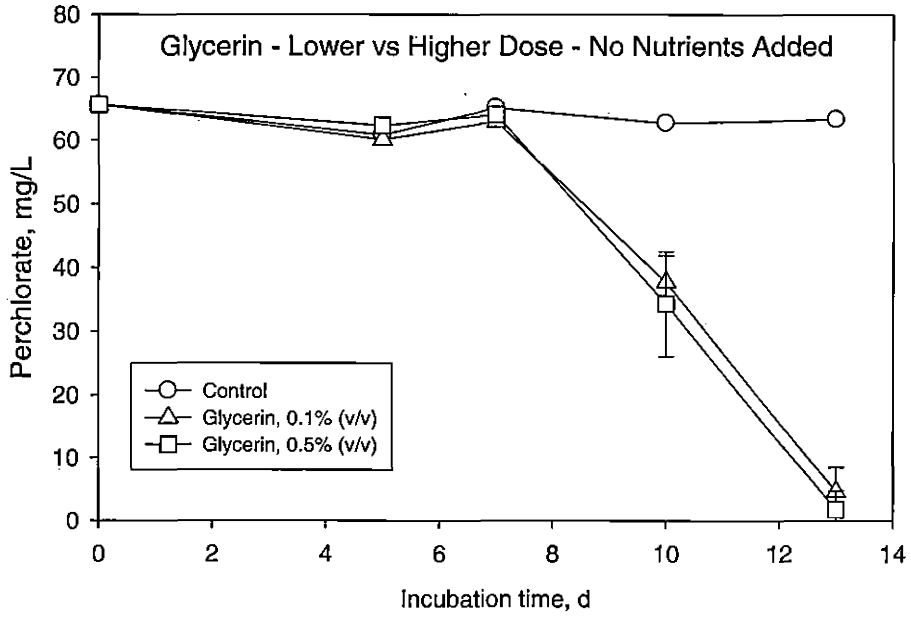


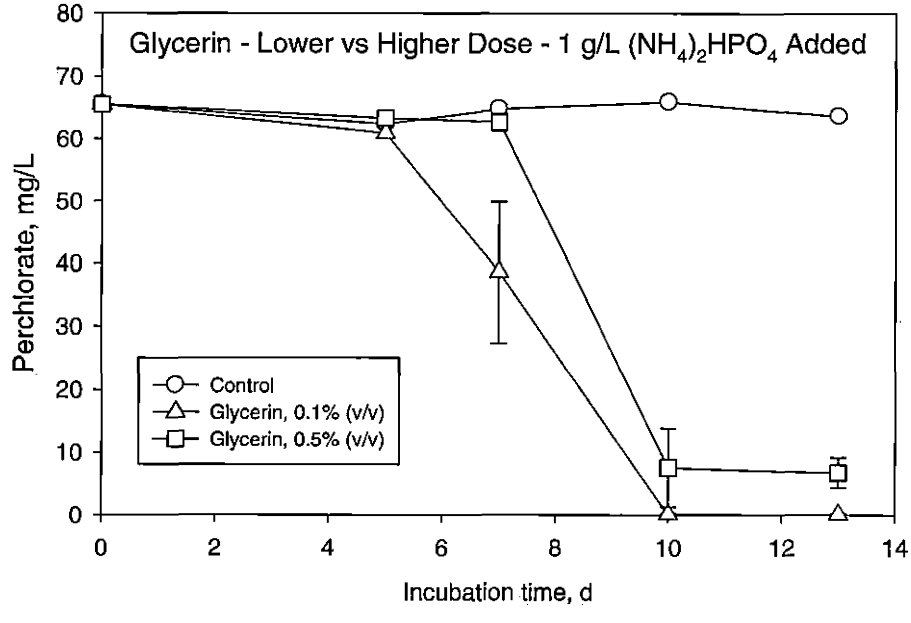
Perchlorate Reduction in EOS Amended Source Area Microcosms
(Top: Low Dosage; Bottom: High Dosage)



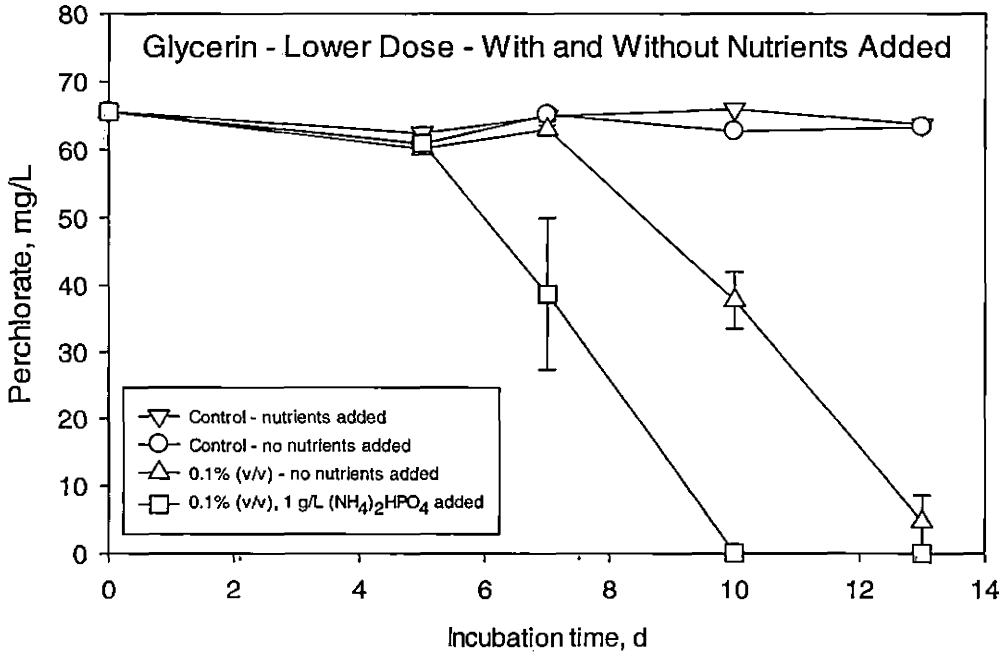


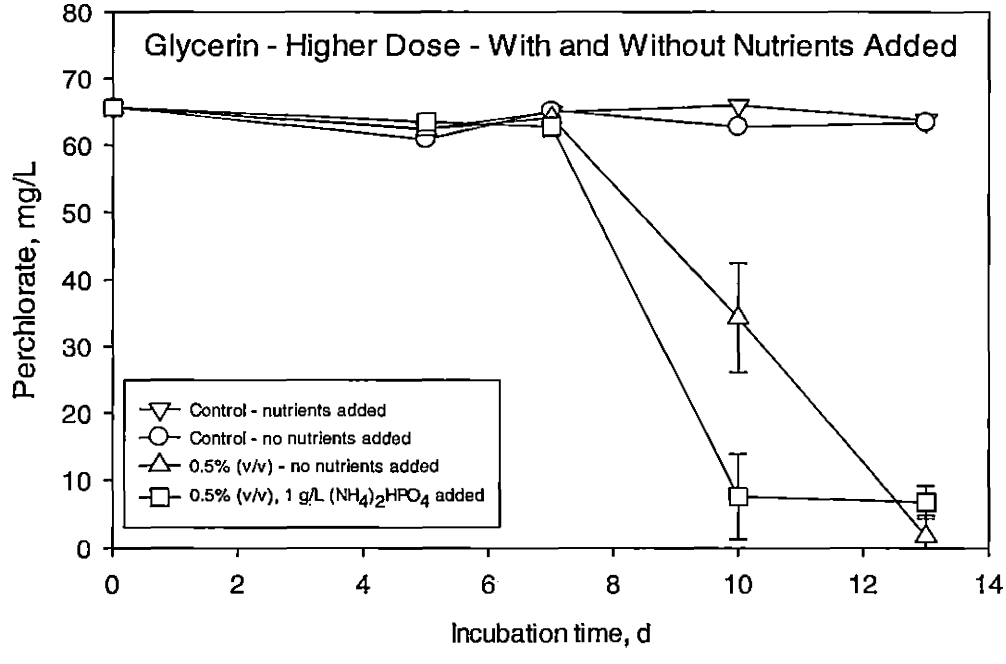
Perchlorate Reduction: Glycerin – Dose Effect



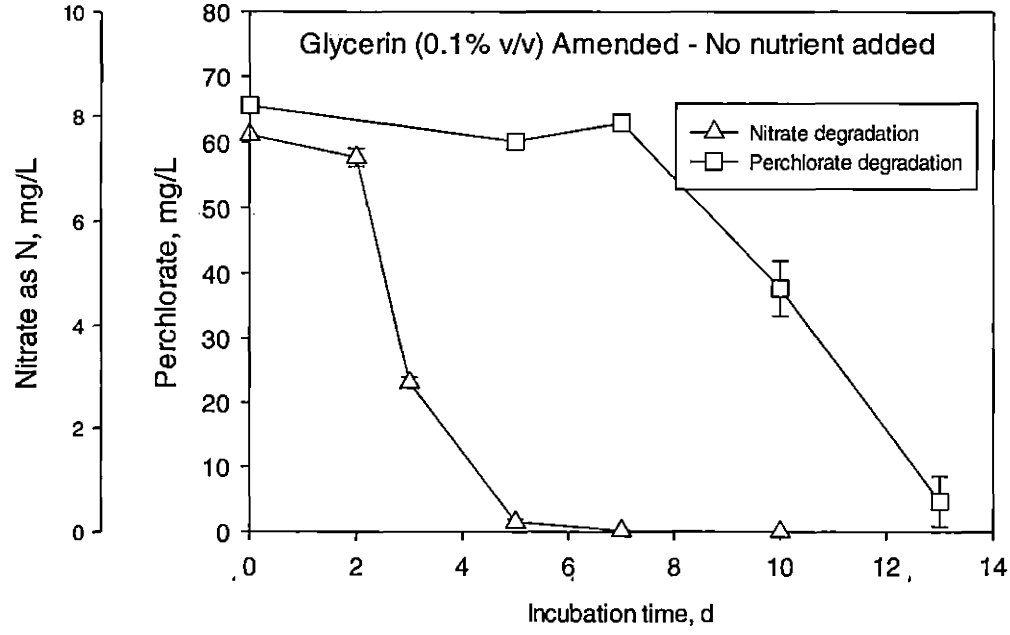


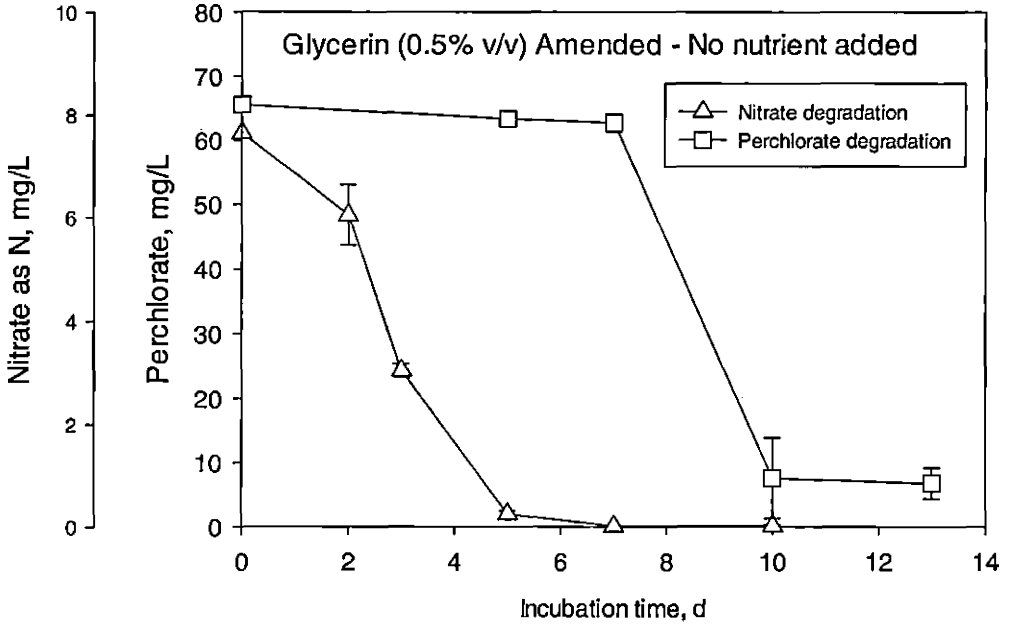
Perchlorate Reduction in Glycerin Amended Biobarrier Microcosms (Top: Low Dosage; Bottom: High Dosage)



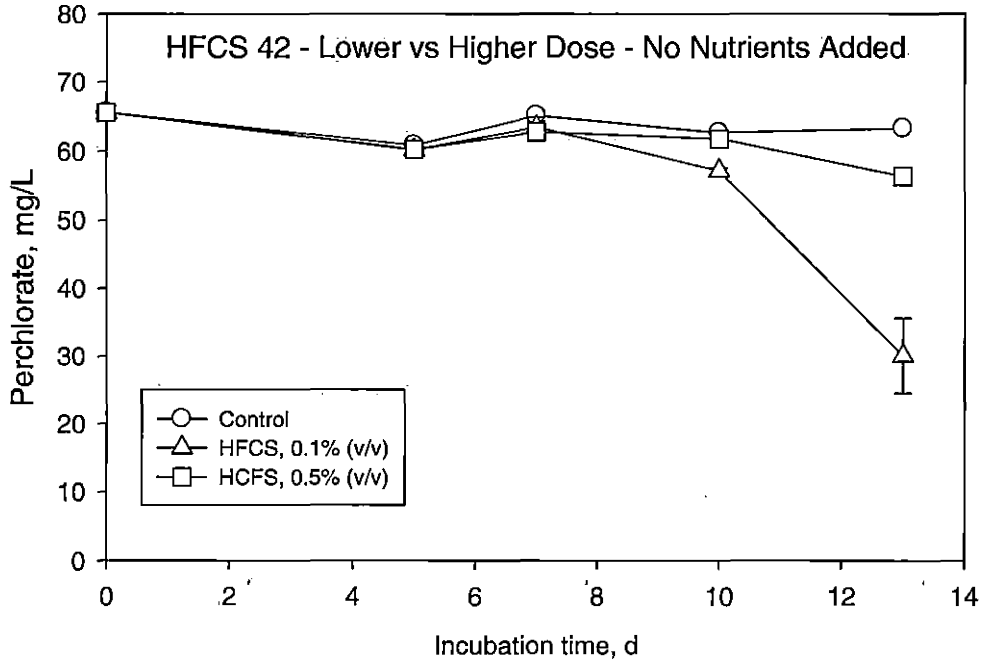


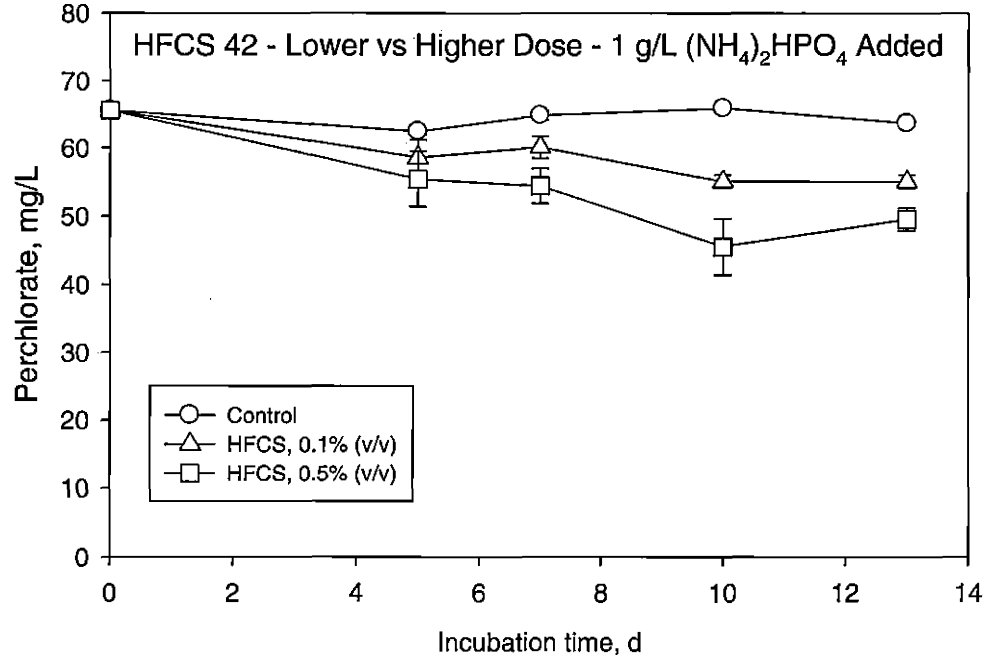
Nitrate and Perchlorate Reduction in Glycerin Amended Source Area Microcosms



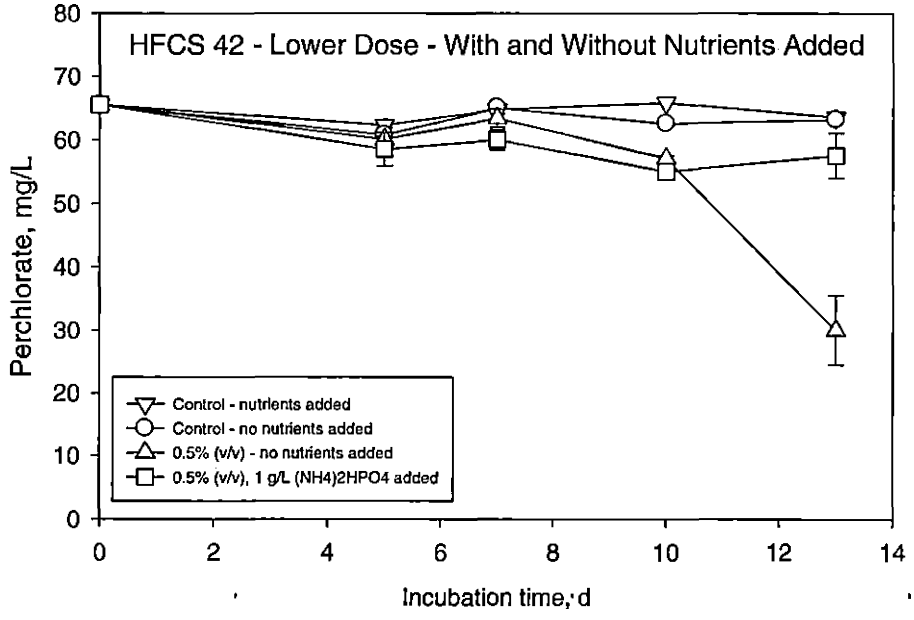


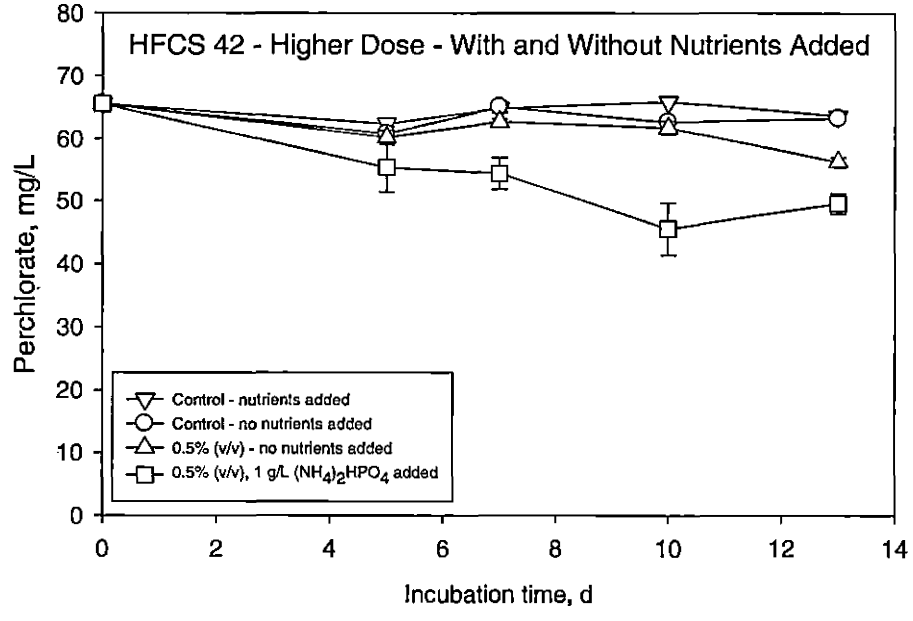
Perchlorate Reduction: High Fructose Corn Syrup – Dose Effect



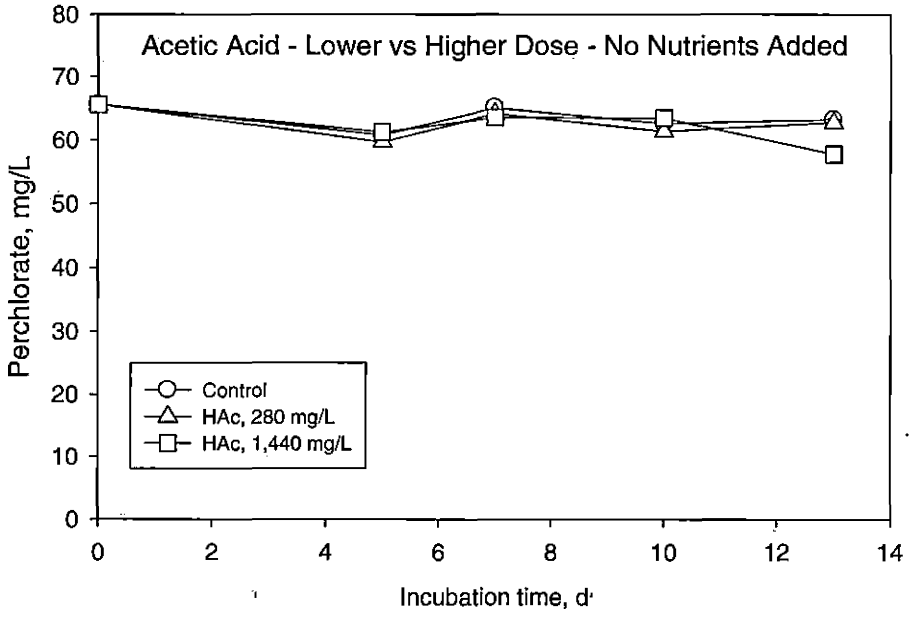


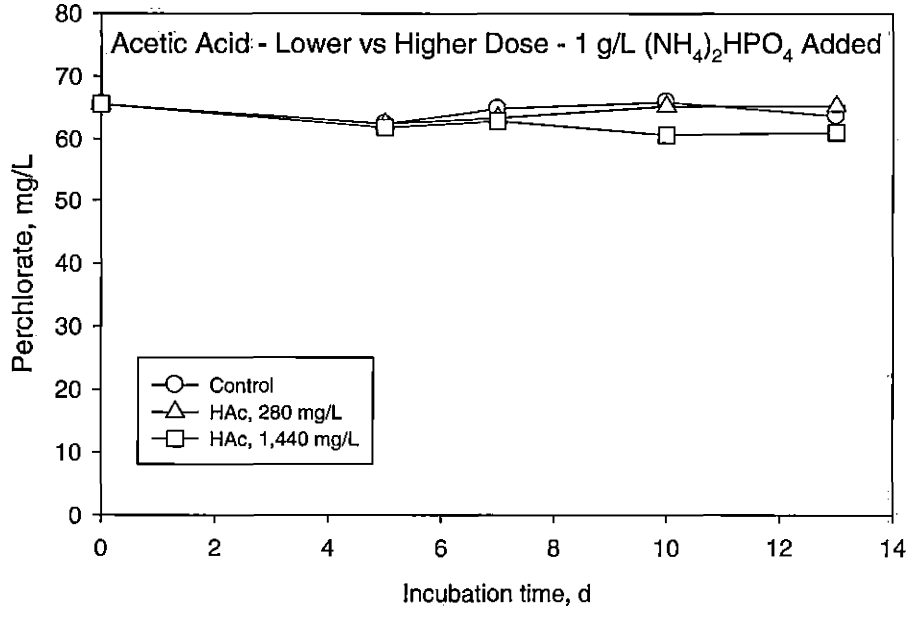
Perchlorate Reduction: High Fructose Corn Syrup – Nutrient Effect



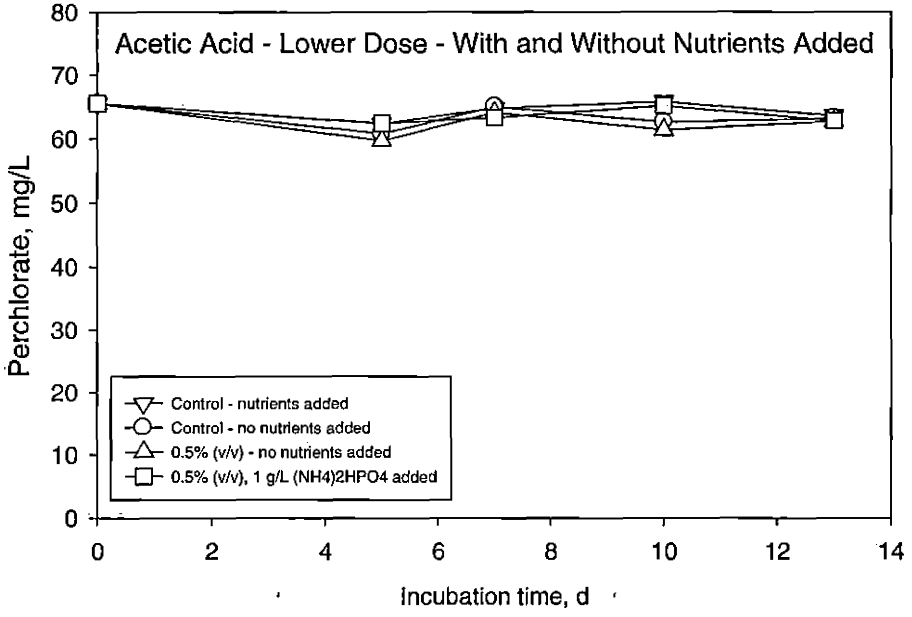


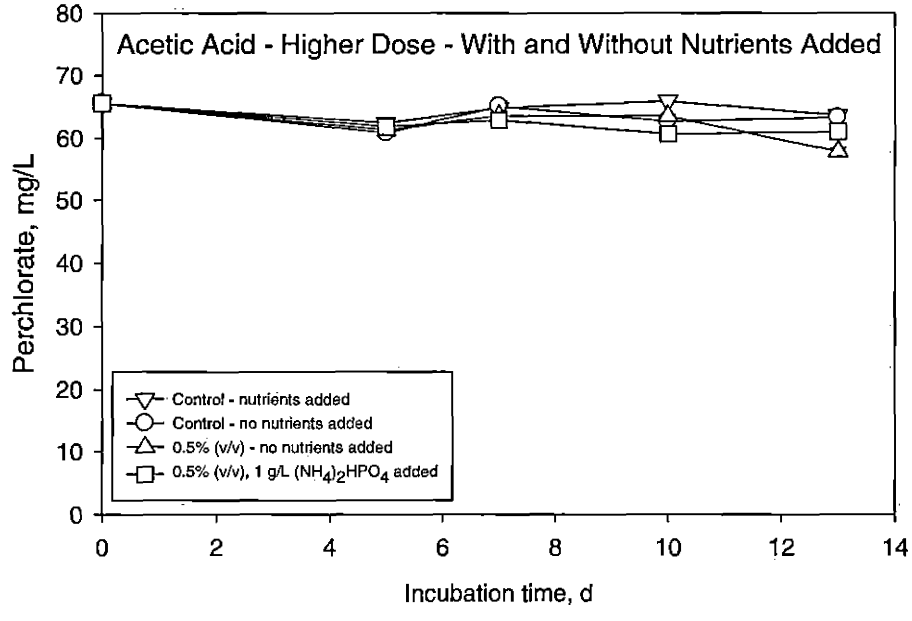
Perchlorate Reduction: Acetic Acid – Dose Effect



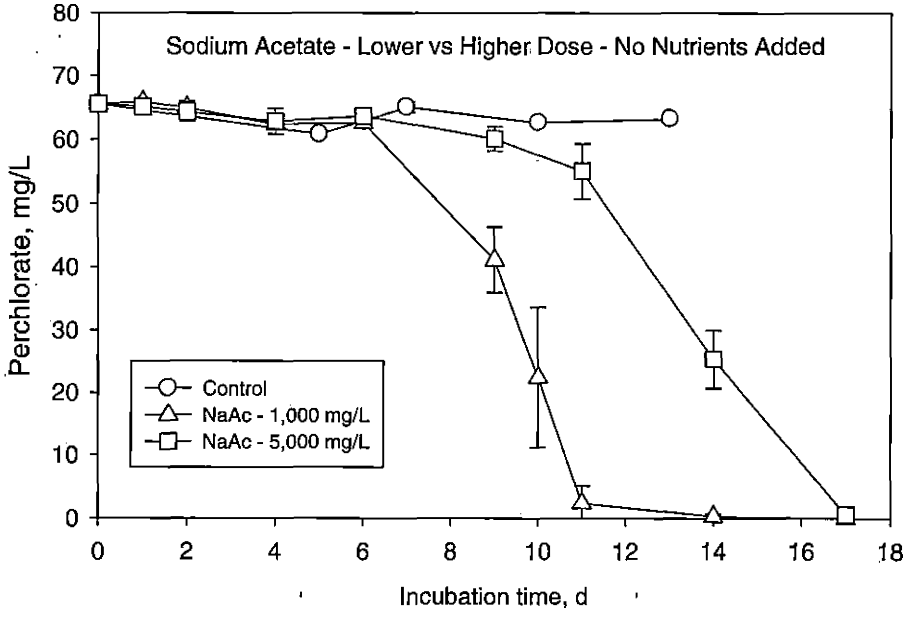


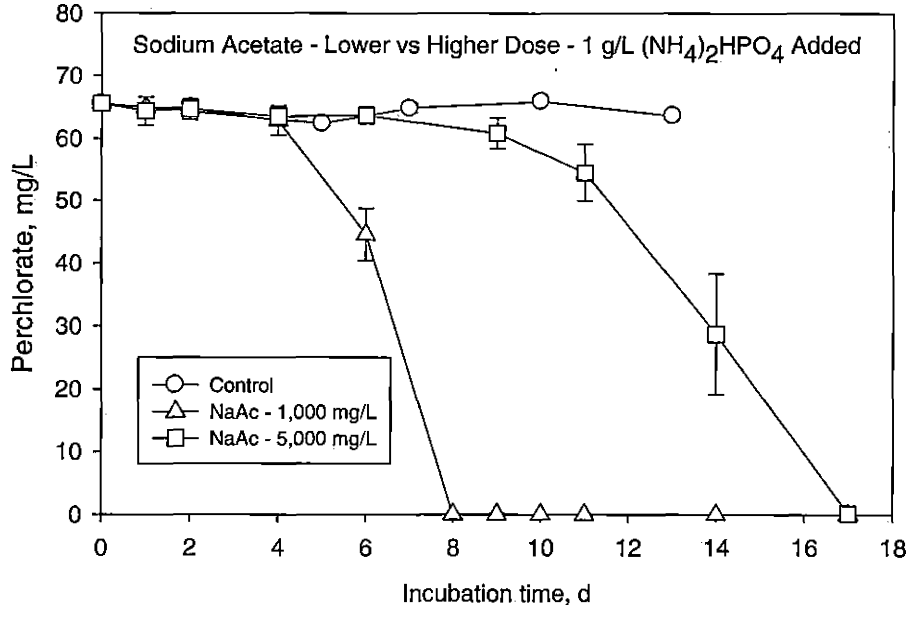
Perchlorate Reduction: Acetic Acid – Nutrient Effect



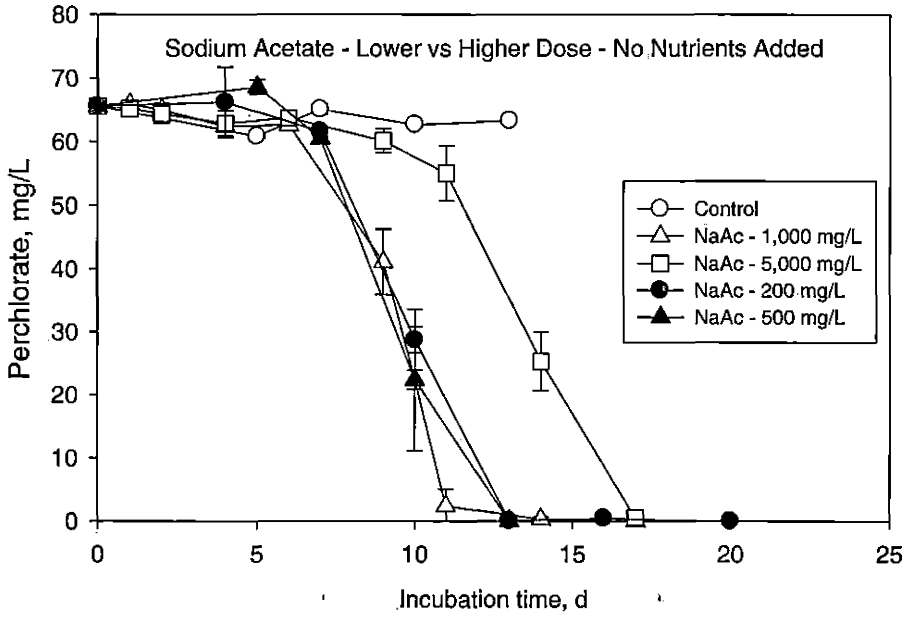


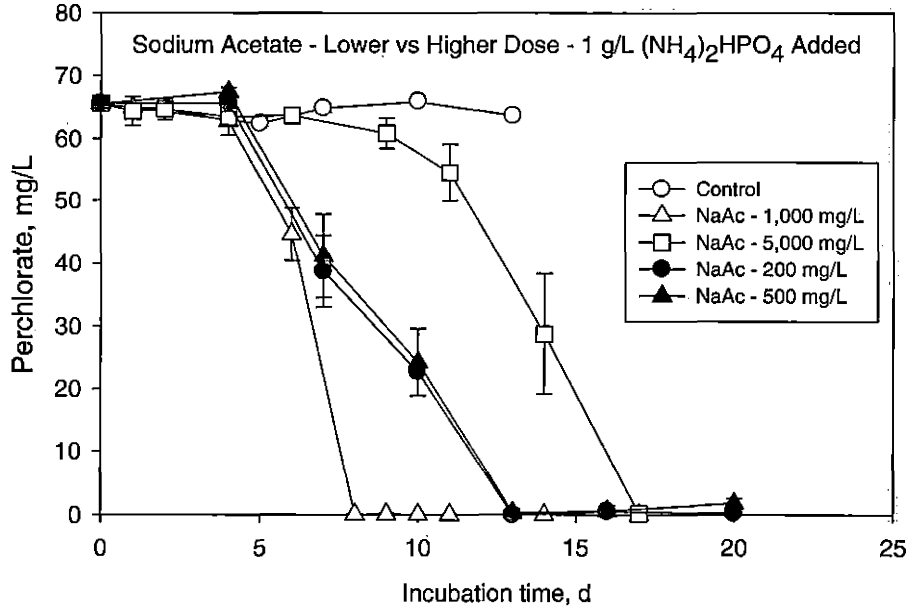
Perchlorate Reduction: Sodium Acetate – Dose Effect



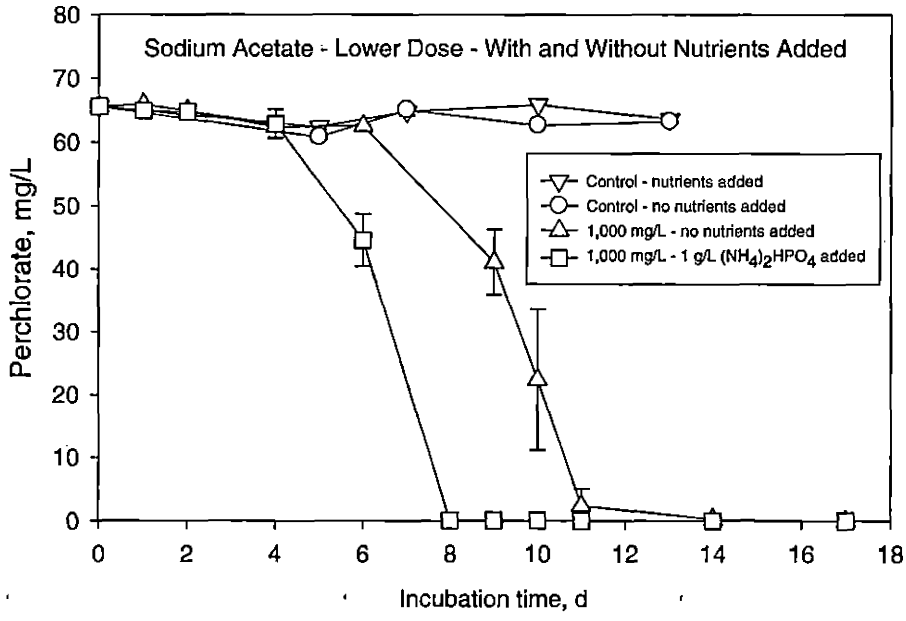


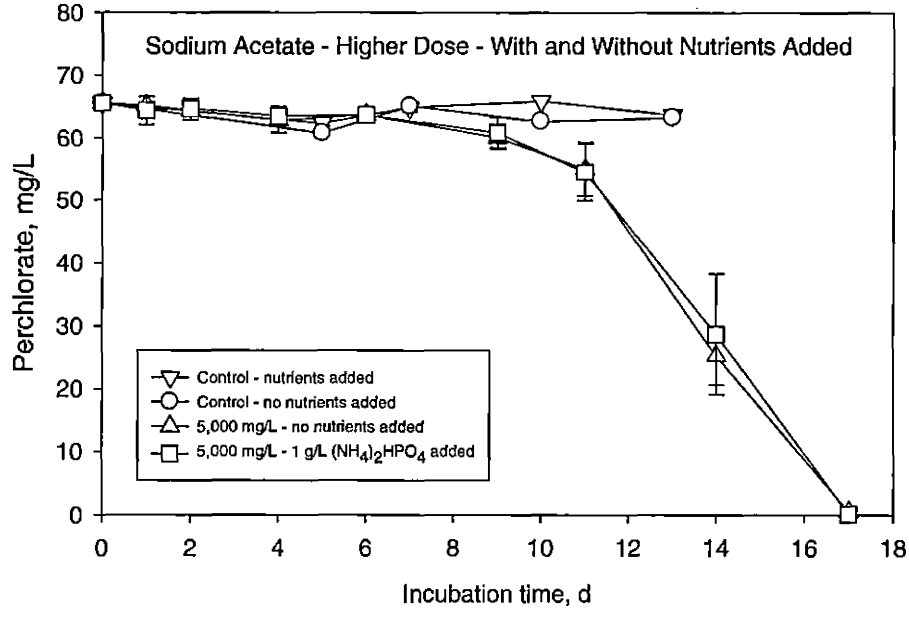
Perchlorate Reduction: Sodium Acetate – Dose Effect (Amended)



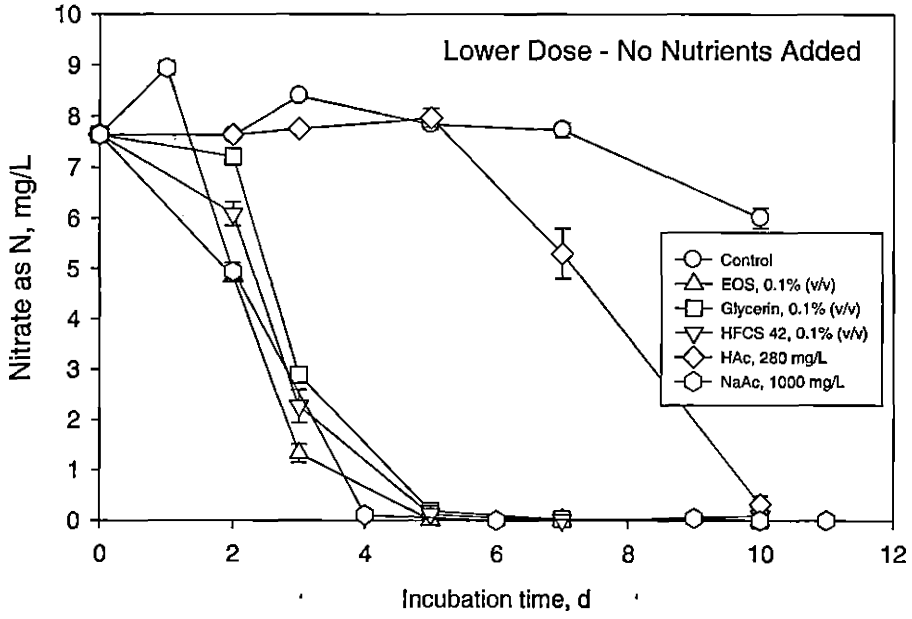


Perchlorate Reduction: Sodium Acetate – Nutrient Effect

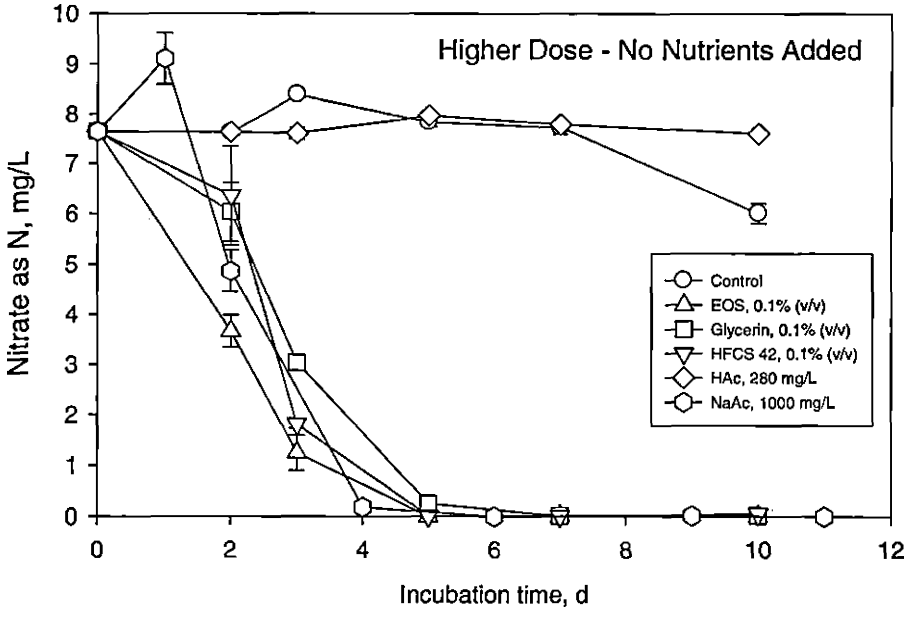




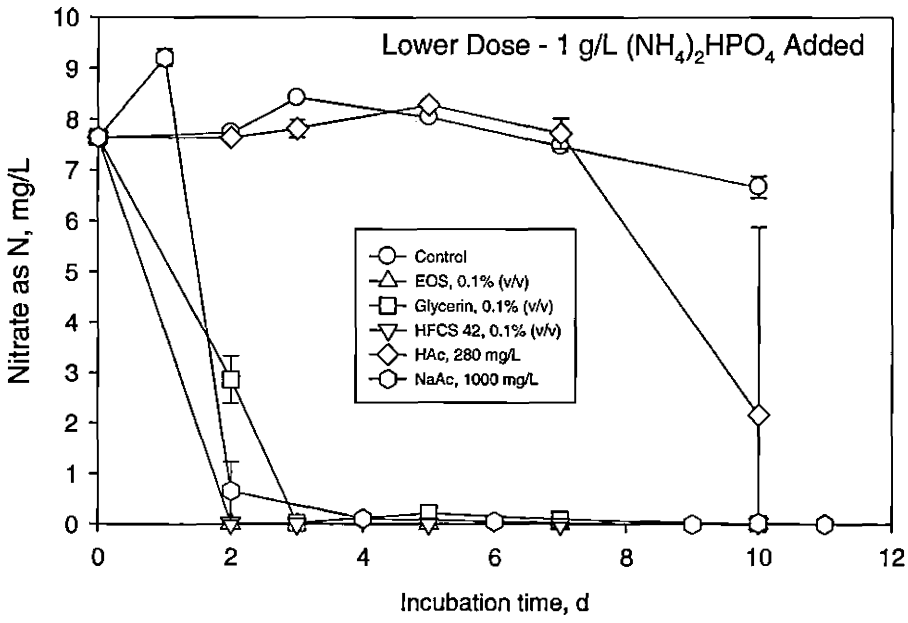
Nitrate Reduction: Lower Dose – No Nutrients Added



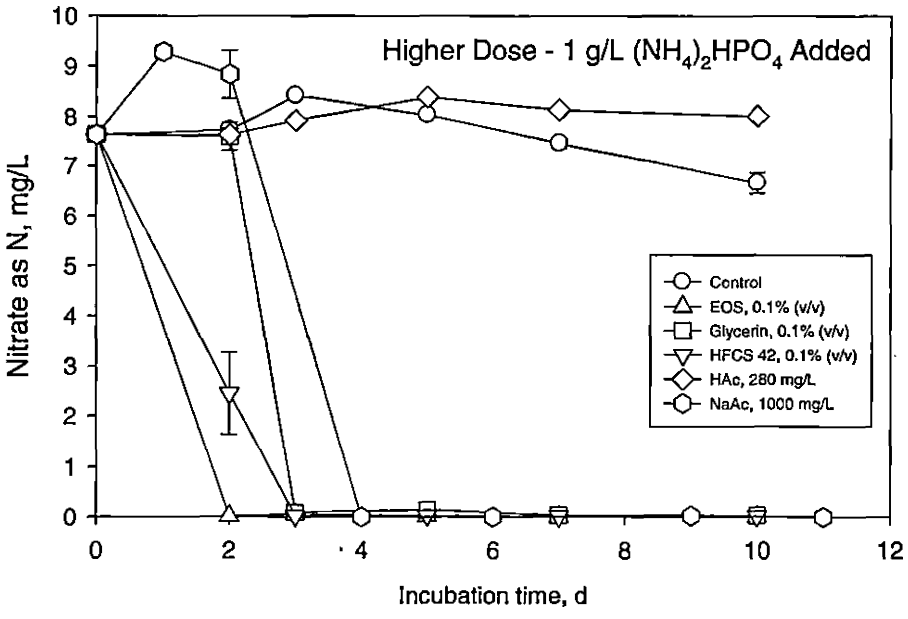
Nitrate Reduction: Higher Dose – No Nutrients Added



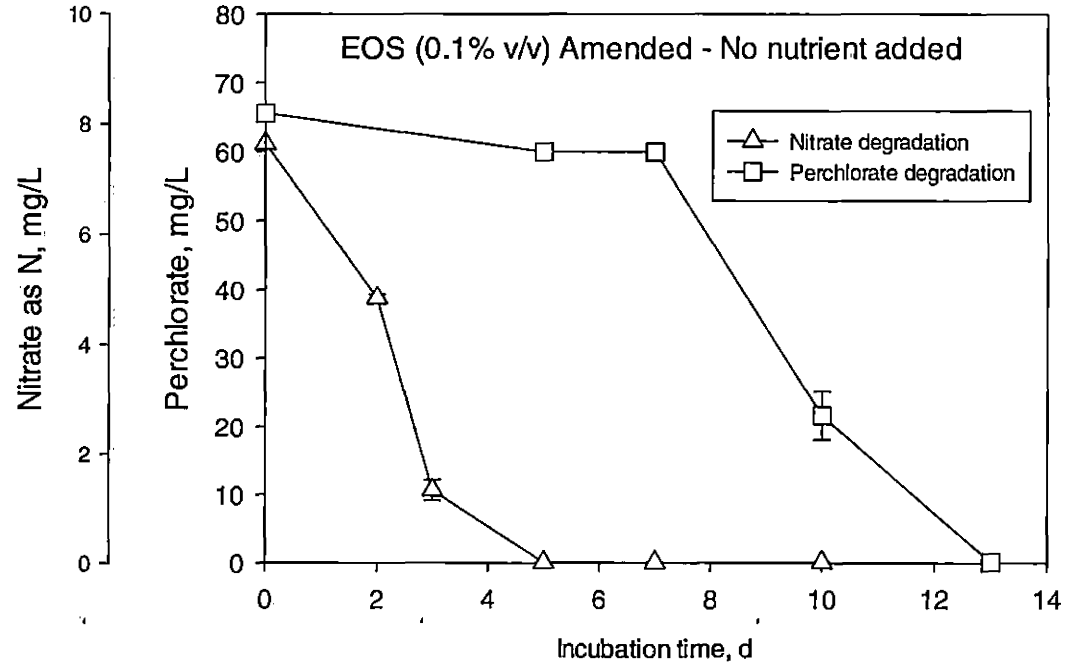
Nitrate Reduction: Lower Dose – Nutrients Added

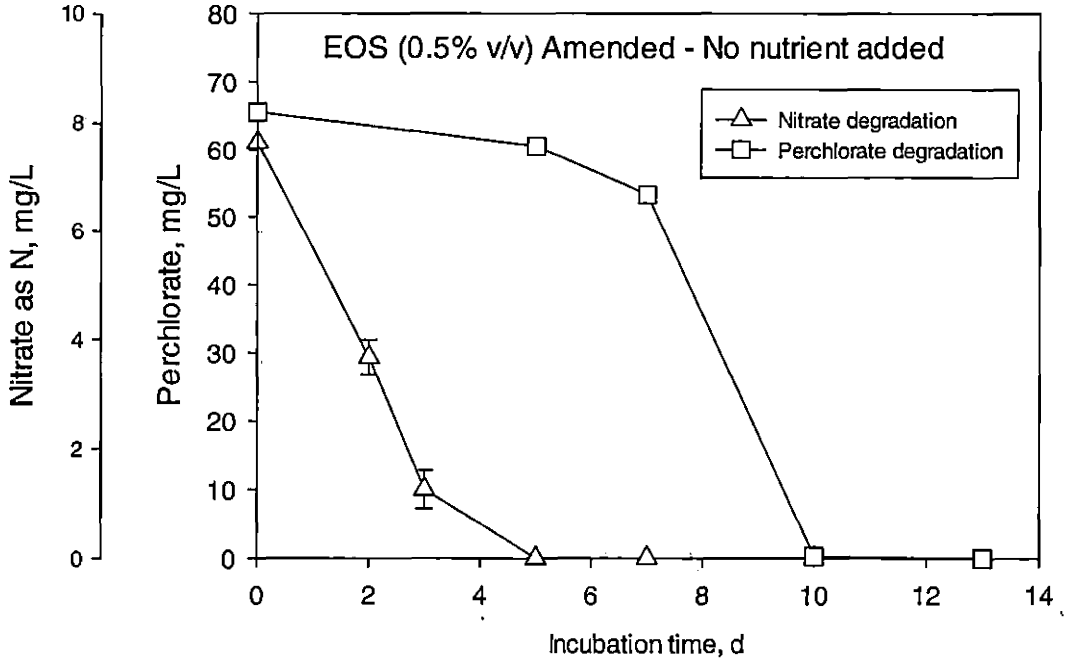


Nitrate Reduction: Higher Dose – No Nutrients Added

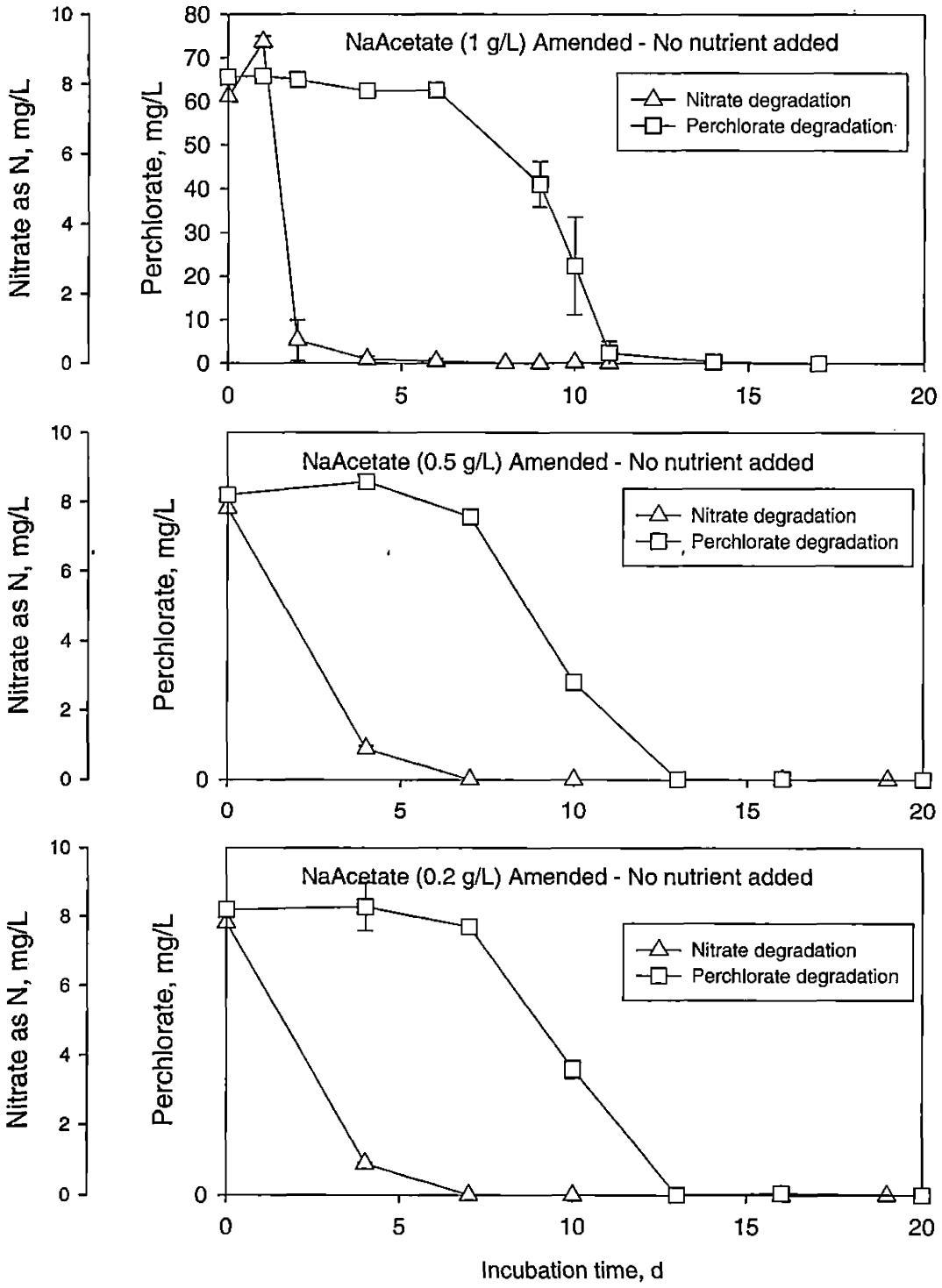


Nitrate and Perchlorate Reduction in EOS Amended Source Area Microcosms





Perchlorate vs Nitrate Reduction: NaAcetate Without $(NH_4)_2HPO_4$ Added



Initial-Final Analyses

Control Microcosms

Parameter	MDL, mg/L	Without nutrient		With nutrient	
		Initial, mg/L	Final, mg/L	Initial, mg/L	Final, mg/L
Nitrite as N	0.090	ND	1.2	ND	1.8
Arsenic	0.070	ND	ND	ND	ND
Iron	0.15	ND	ND	ND	ND
Manganese	0.070	ND	ND	ND	ND

EOS Microcosms – 0.1% (v/v)

Parameter	MDL, mg/L	Without nutrient		With nutrient	
		Initial, mg/L	Final, mg/L	Initial, mg/L	Final, mg/L
Nitrite as N	0.090	1.7	ND	4.7	ND
Arsenic	0.070	ND	ND	ND	ND
Iron	0.15	ND	0.52	ND	0.52
Manganese	0.070	0.083	0.39	ND	0.22

EOS Microcosms – 0.5% (v/v)

Parameter	MDL, mg/L	Without nutrient		With nutrient	
		Initial, mg/L	Final, mg/L	Initial, mg/L	Final, mg/L
Nitrite as N	0.090	0.92	ND	5.9	ND
Arsenic	0.070	ND	ND	ND	ND
Iron	0.15	ND	0.52	ND	ND
Manganese	0.070	0.077	0.60	ND	0.25

Glycerin Microcosms– 0.1% (v/v)

Parameter	MDL, mg/L	Without nutrient		With nutrient	
		Initial, mg/L	Final, mg/L	Initial, mg/L	Final, mg/L
Nitrite as N	0.090	0.60	ND	ND	ND
Arsenic	0.070	ND	ND	ND	ND
Iron	0.15	0.35	ND	ND	0.77
Manganese	0.070	ND	0.61	ND	0.37

Glycerin Microcosms – 0.5% (v/v)

Parameter	MDL, mg/L	Without nutrient		With nutrient	
		Initial, mg/L	Final, mg/L	Initial, mg/L	Final, mg/L
Nitrite as N	0.090	1.9	ND	7.2	ND
Arsenic	0.070	ND	ND	ND	ND
Iron	0.15	ND	ND	ND	0.52
Manganese	0.070	0.13	1.1	ND	1.5

HCFS Microcosms– 0.1% (v/v)

Parameter	MDL, mg/L	Without nutrient		With nutrient	
		Initial, mg/L	Final, mg/L	Initial, mg/L	Final, mg/L
Nitrite as N	0.090	0.59	ND	ND	ND
Arsenic	0.070	ND	ND	ND	ND
Iron	0.15	ND	0.15	ND	0.20
Manganese	0.070	ND	0.79	ND	0.41

HCFS Microcosms– 0.5% (v/v)

Parameter	MDL, mg/L	Without nutrient		With nutrient	
		Initial, mg/L	Final, mg/L	Initial, mg/L	Final, mg/L
Nitrite as N	0.090	2.5	ND	2.6	ND
Arsenic	0.070	ND	ND	ND	ND
Iron	0.15	ND	3.1	ND	15
Manganese	0.070	ND	4.9	ND	3.2

Acetic Acid Microcosms– 280 mg/L

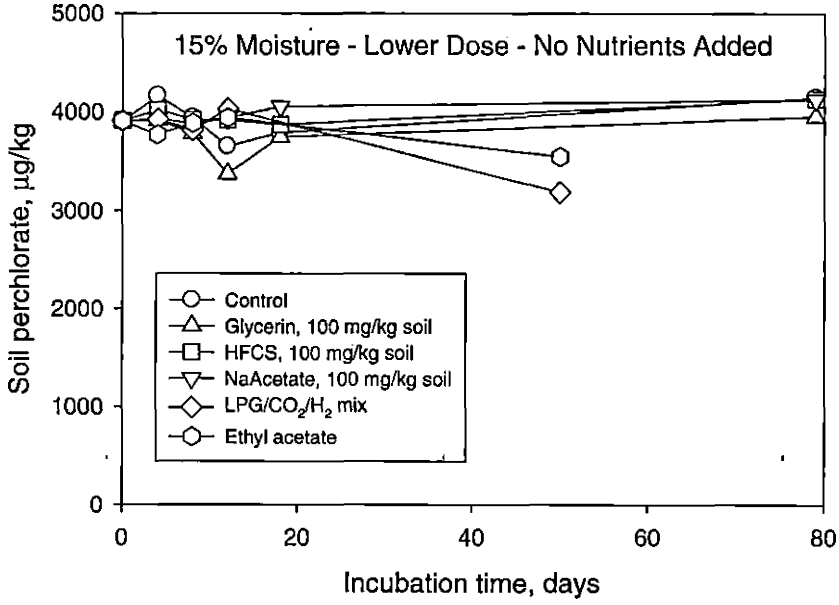
Parameter	MDL, mg/L	Without nutrient		With nutrient	
		Initial, mg/L	Final, mg/L	Initial, mg/L	Final, mg/L
Nitrite as N	0.090	ND	ND	ND	ND
Arsenic	0.070	ND	ND	ND	0.078
Iron	0.15	ND	ND	ND	ND
Manganese	0.070	ND	0.41	ND	0.19

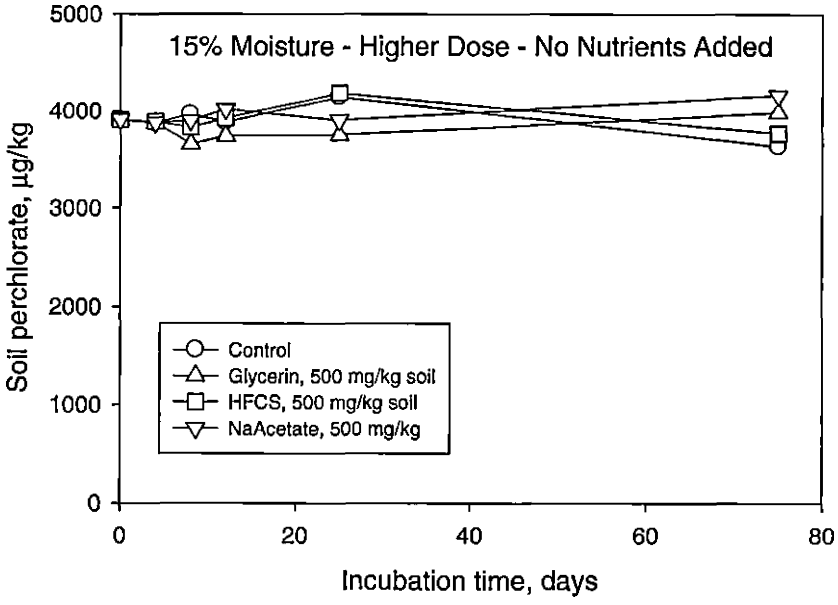
Acetic Acid Microcosms– 1,440 mg/L

Parameter	MDL, mg/L	Without nutrient		With nutrient	
		Initial, mg/L	Final, mg/L	Initial, mg/L	Final, mg/L
Nitrite as N	0.090	ND	ND	ND	ND
Arsenic	0.070	ND	ND	ND	ND
Iron	0.15	ND	0.43	ND	0.38
Manganese	0.070	ND	1.2	ND	0.88

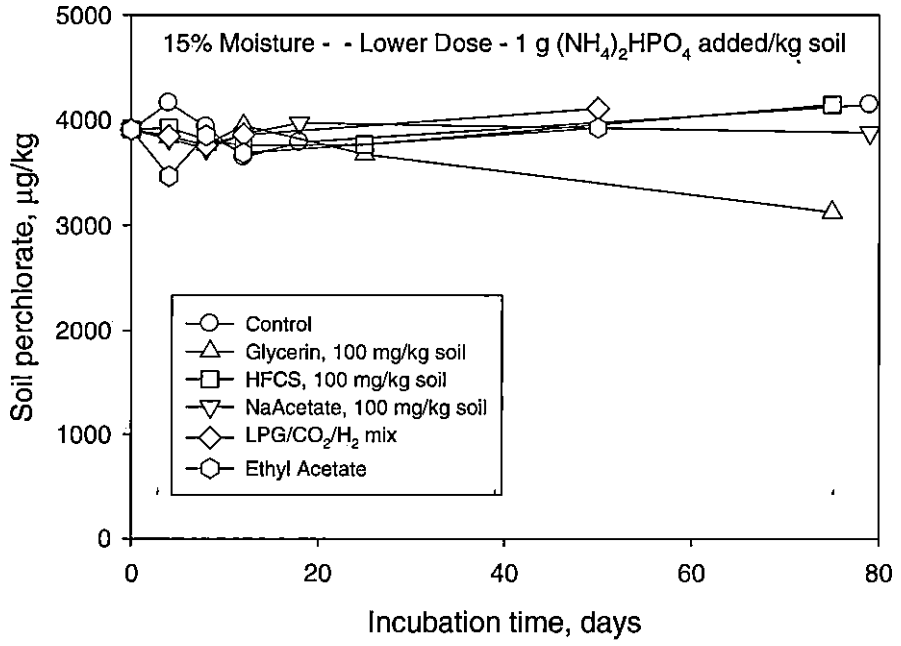
Vadose Zone Microcosms

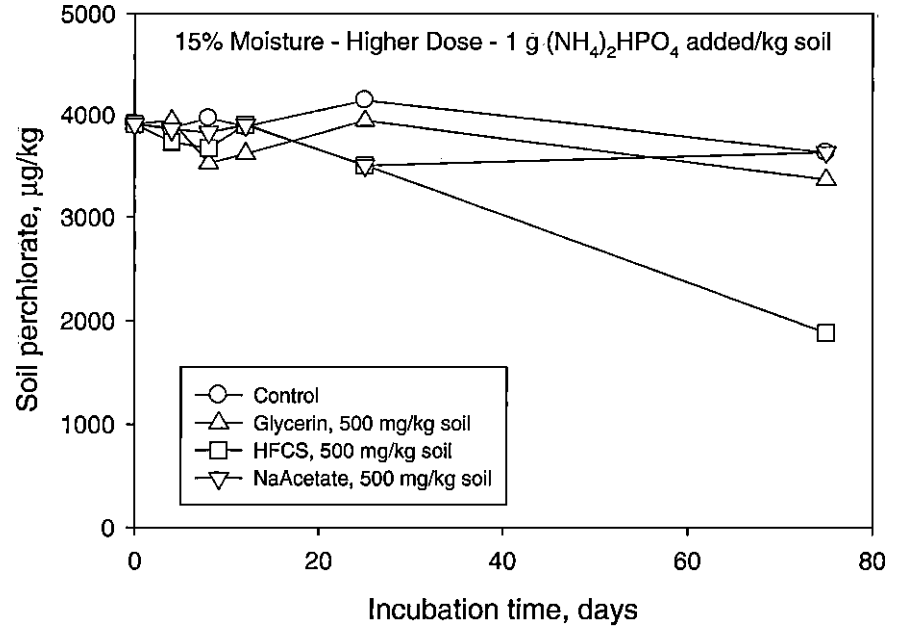
Vadose Zone Microcosms – 15% Moisture Content
No Nutrient Added, Soil Amended with Perchlorate – 4,000 µg/kg
Top: Gaseous and Soluble Donors (low dosage); Bottom: Soluble Donors (high dosage)



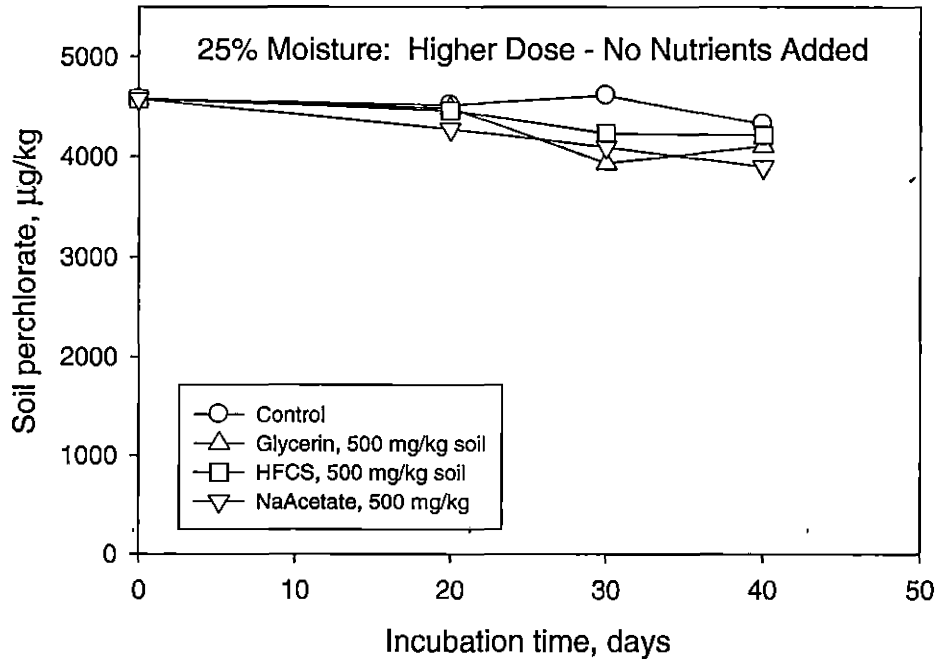


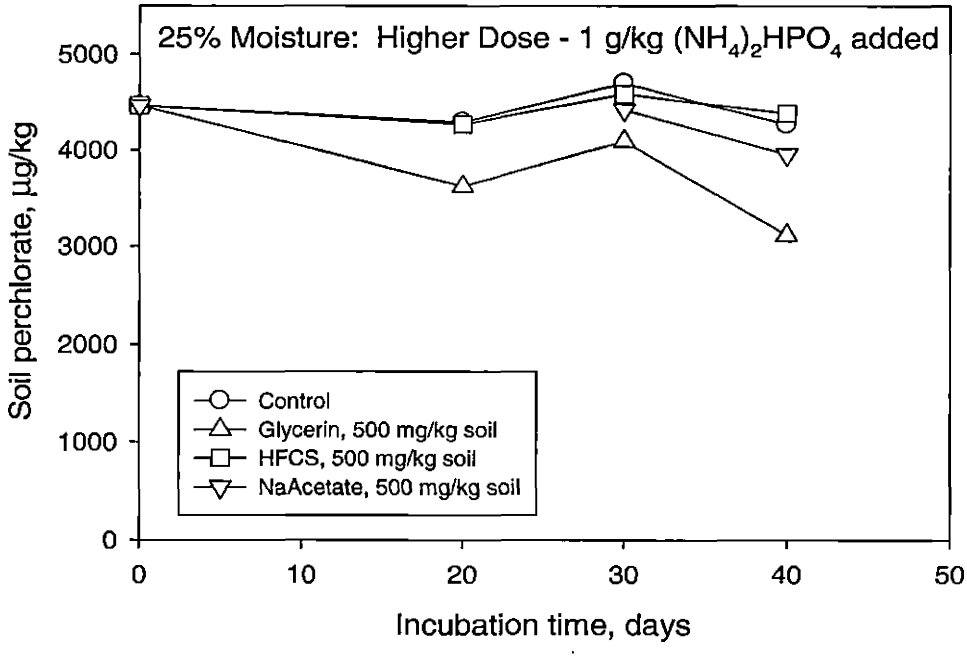
Vadose Zone Microcosms – 15% Moisture Content,
Diammonium Phosphate Added, Soil Amended with 4,000 µg/kg Perchlorate
Top: Gaseous and Soluble Donors (low dosage); Bottom: Soluble Donors (high dosage)



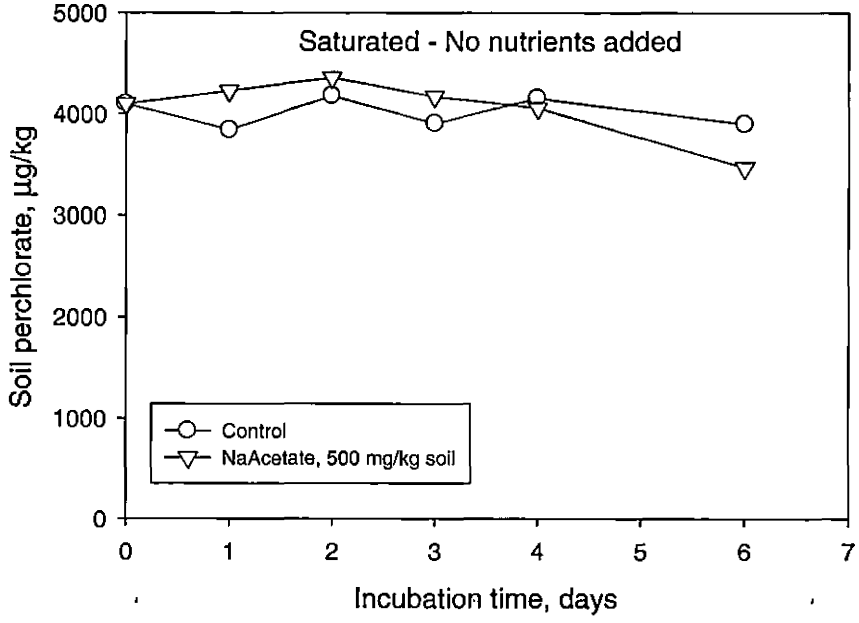


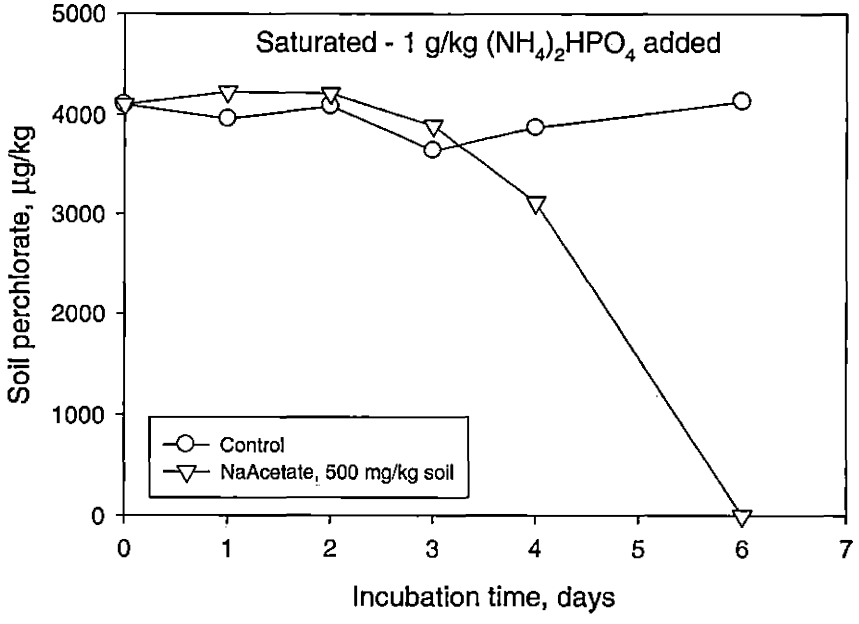
Vadose Zone Microcosms – 25% Moisture Content,
500 mg/kg (high dosage) of Soluble Electron Donor Added
Top: No nutrient added. Bottom: 1 g/L (NH4)2HPO4 added



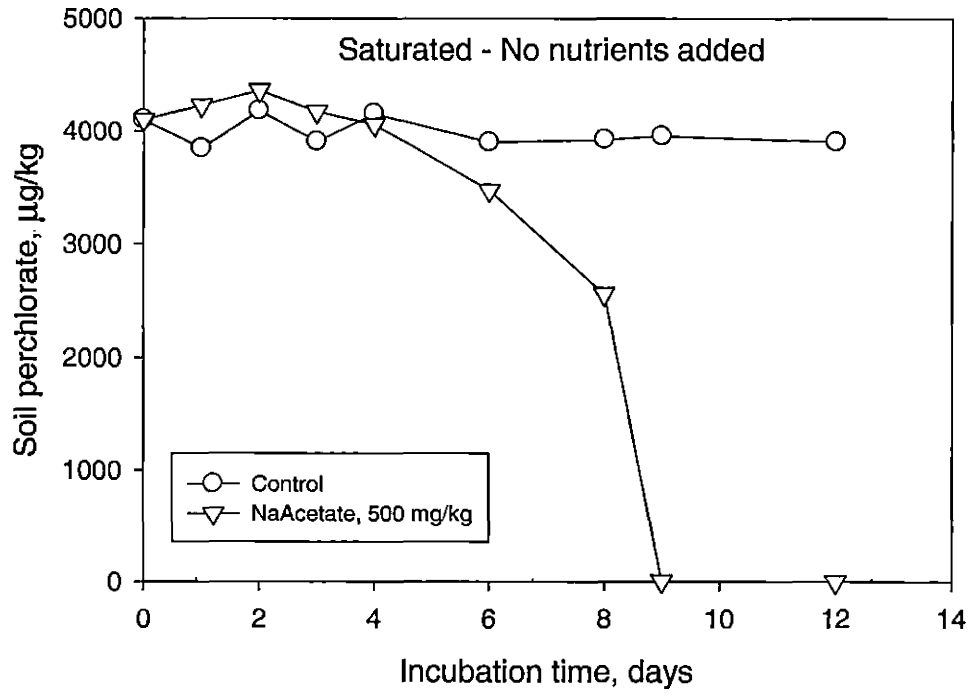


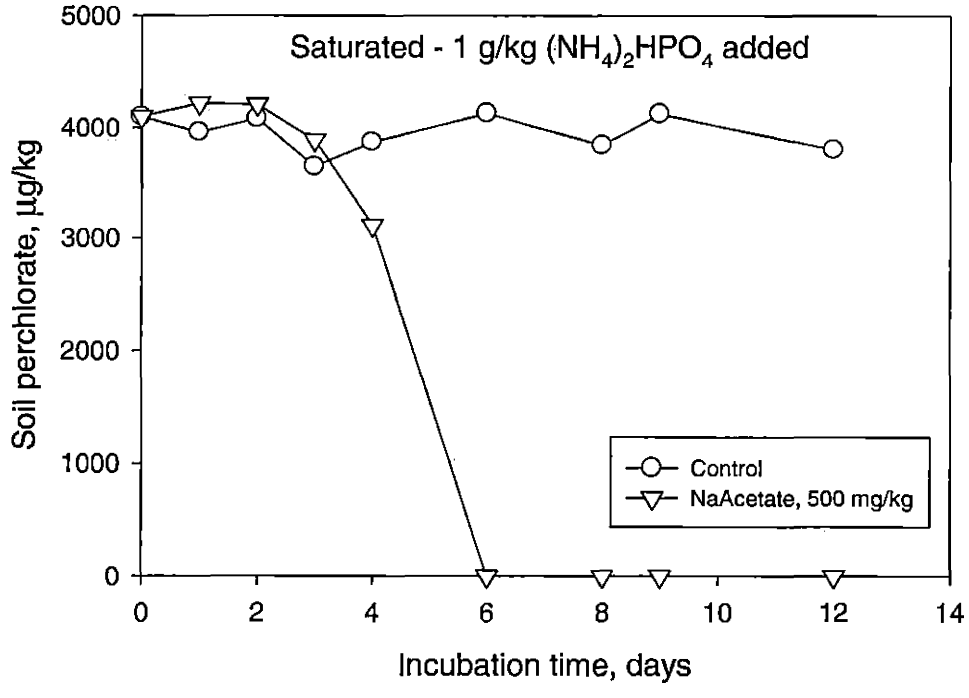
Saturated: Acetate – With and Without Nutrient Added



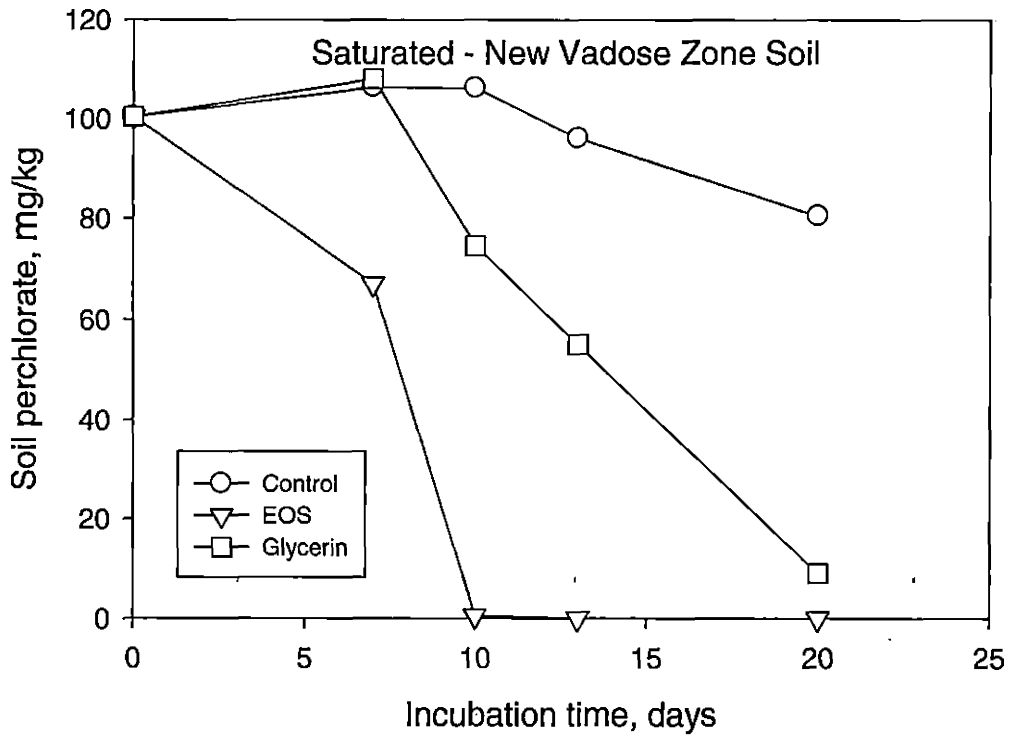


Vadose Zone Microcosms – Saturated,
500 mg/kg (high dosage) of Sodium Acetate Added



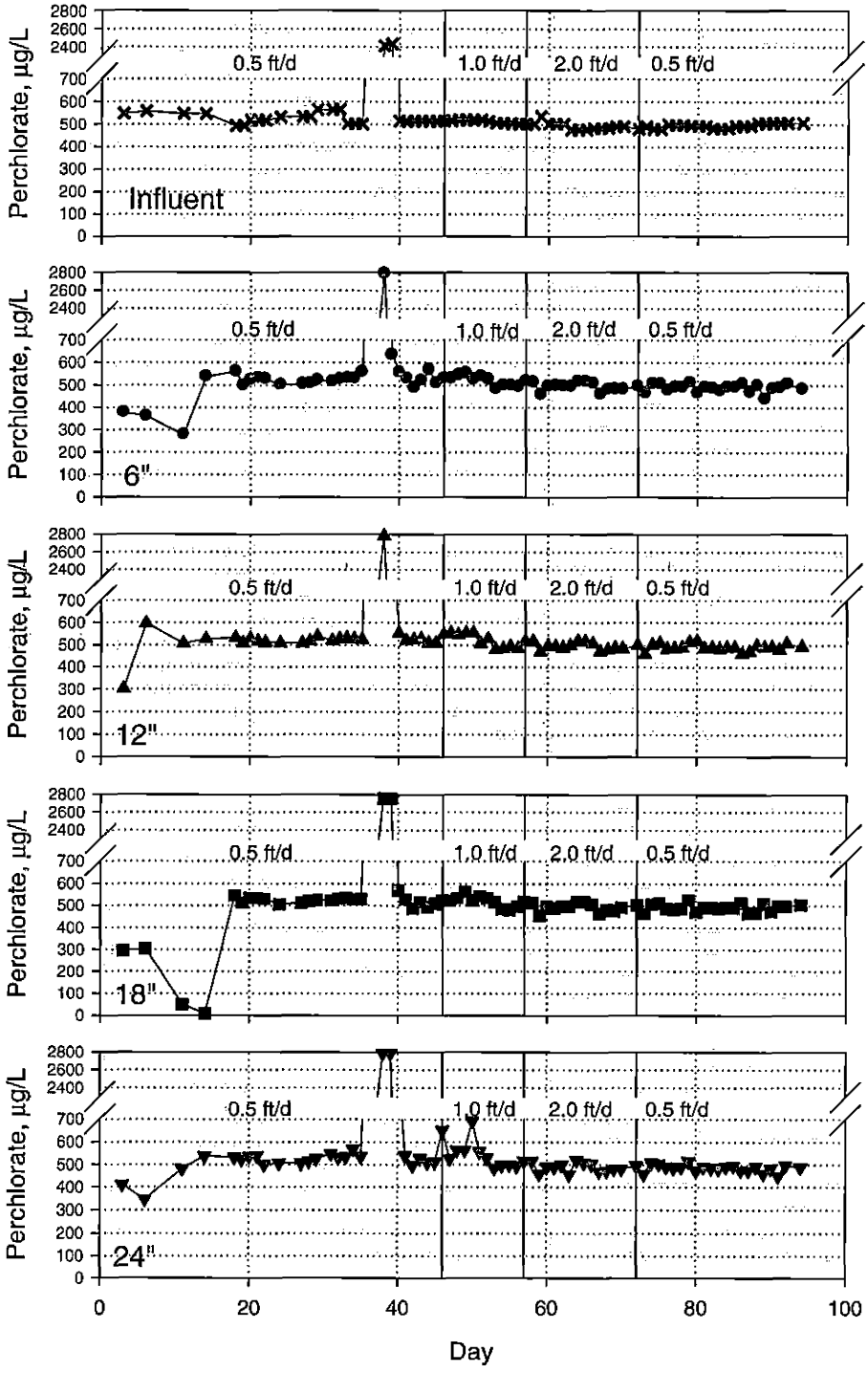


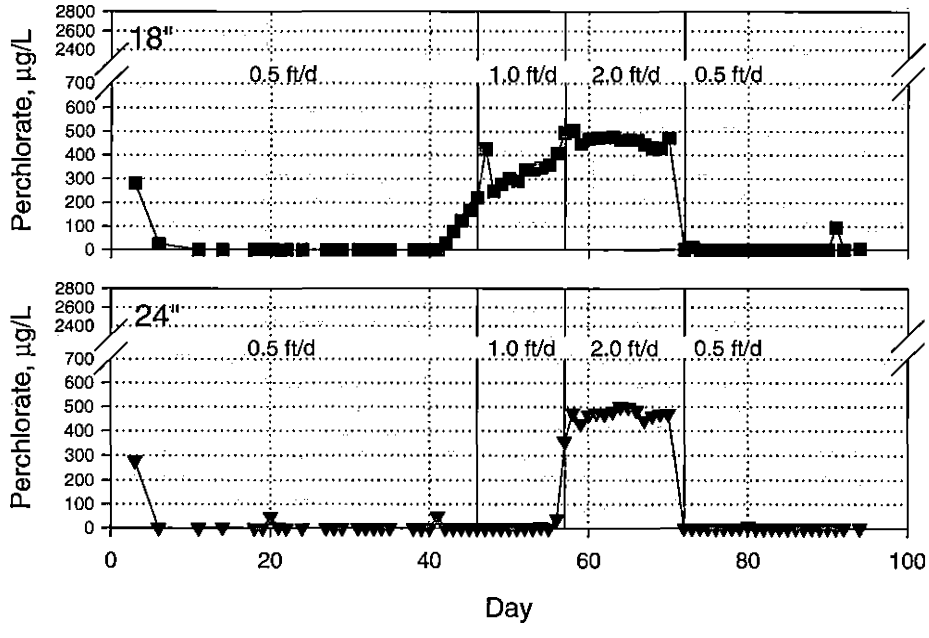
Vadose Zone Microcosms – Saturated,
New Vadose Zone Soil Sample, Donor Solution = 0.5% (w/w)



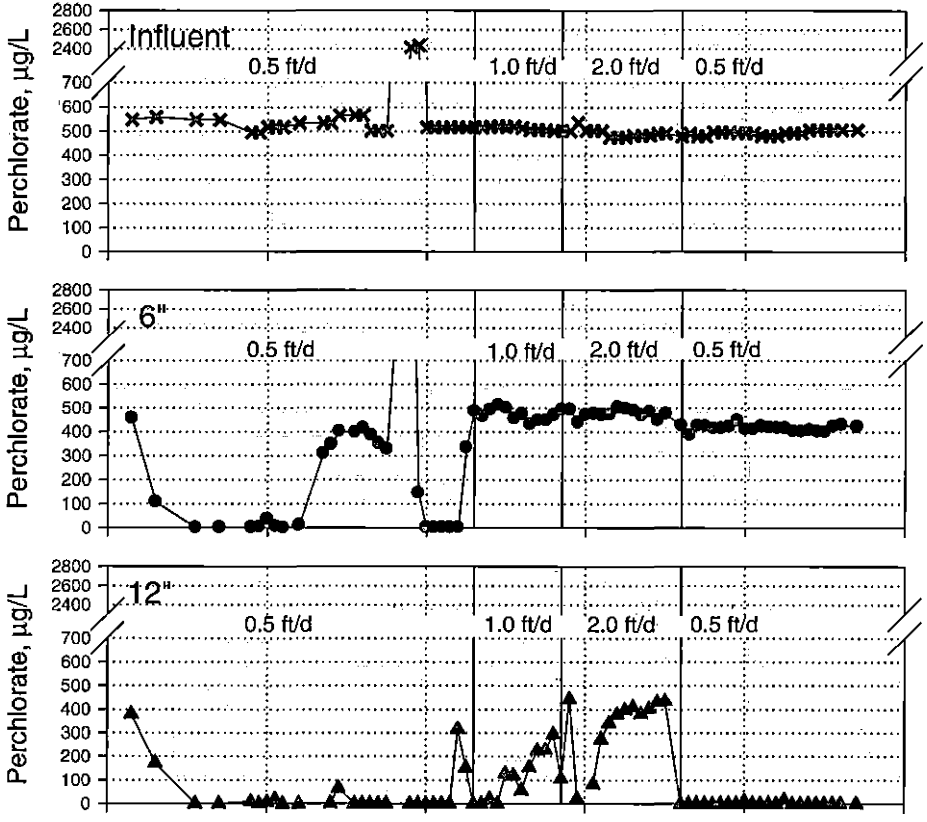
Biobarrier Columns

Control Columns

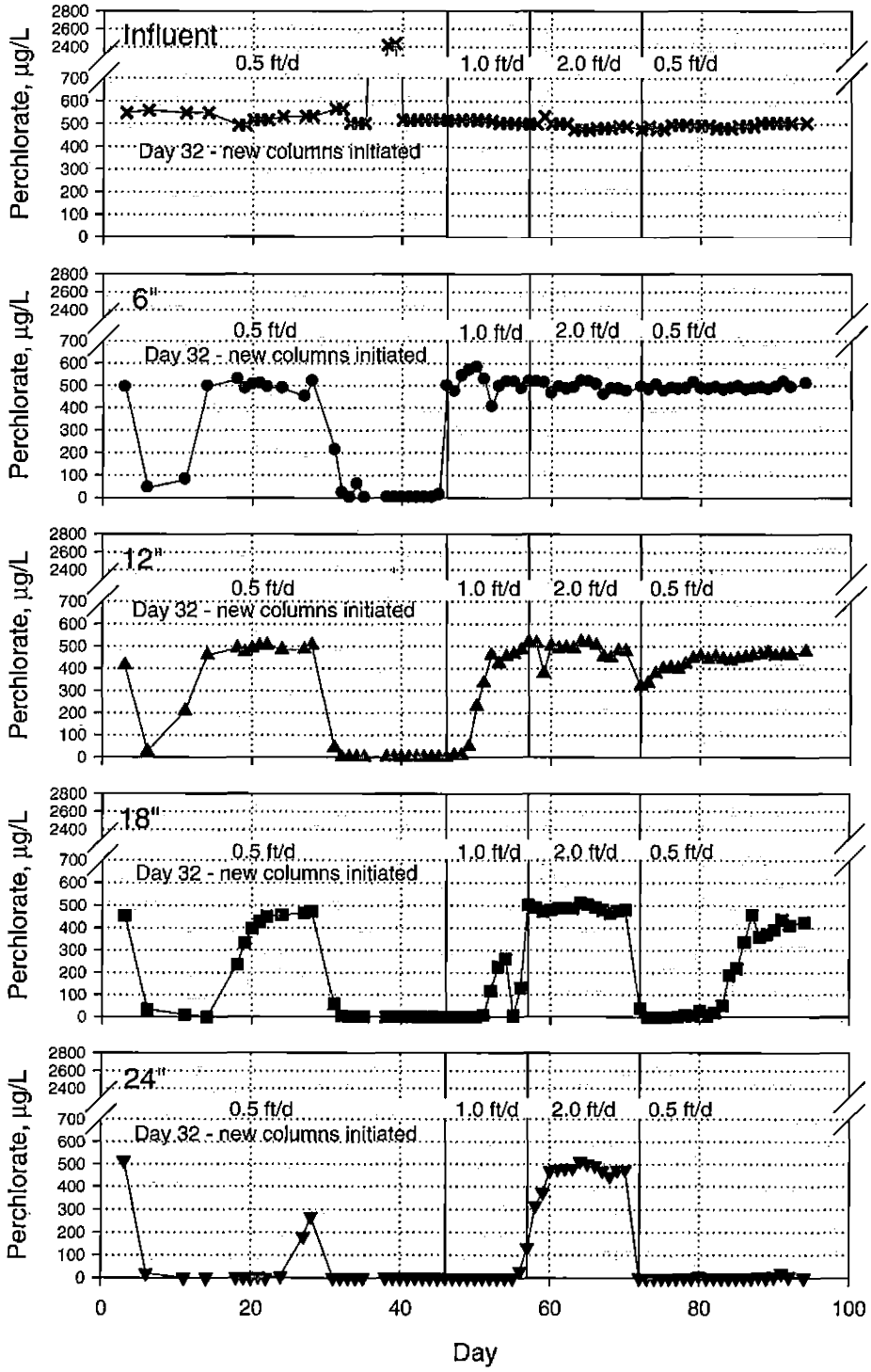



18"
24"
0.5 ft/d
1.0 ft/d
2.0 ft/d
0.5 ft/d
Perchlorate, µg/L
Day
2800
2600
2400
700
600
500
400
300
200
100
0
0
20
40
60
80
100

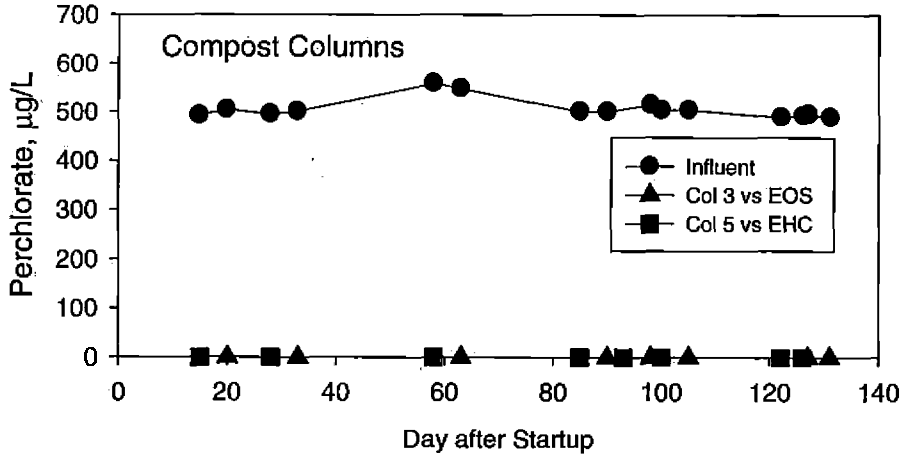
EOS Columns

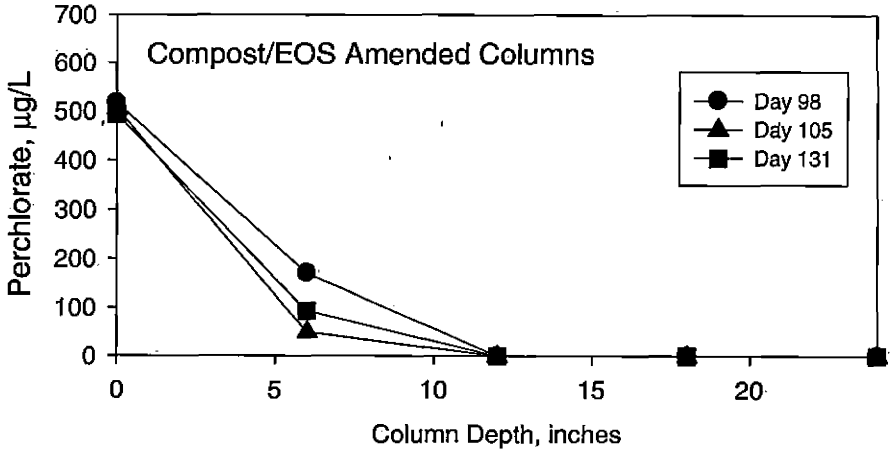

Influent
0.5 ft/d
1.0 ft/d
2.0 ft/d
0.5 ft/d
Perchlorate, µg/L
6"
12"
2800
2600
2400
700
600
500
400
300
200
100
0

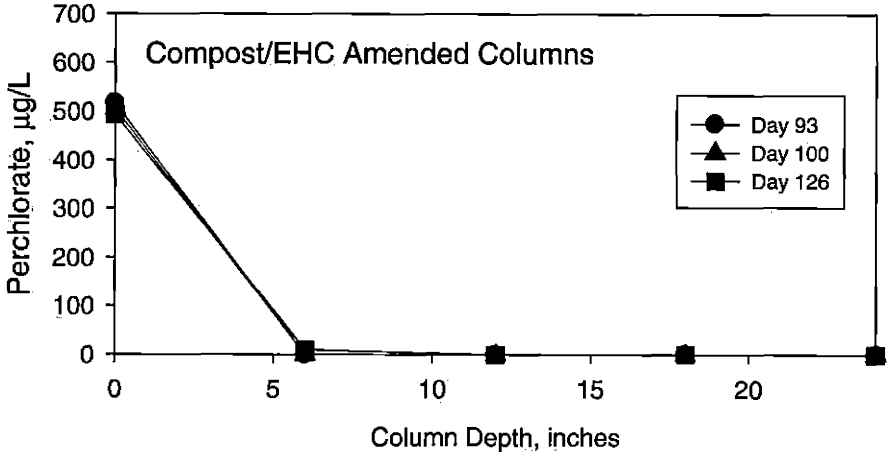
EHC Columns


Influent
6"
12"
18"
24"
Perchlorate, µg/L
0.5 ft/d
1.0 ft/d
2.0 ft/d
0.5 ft/d
Day 32 - new columns initiated
Day

Compost Columns

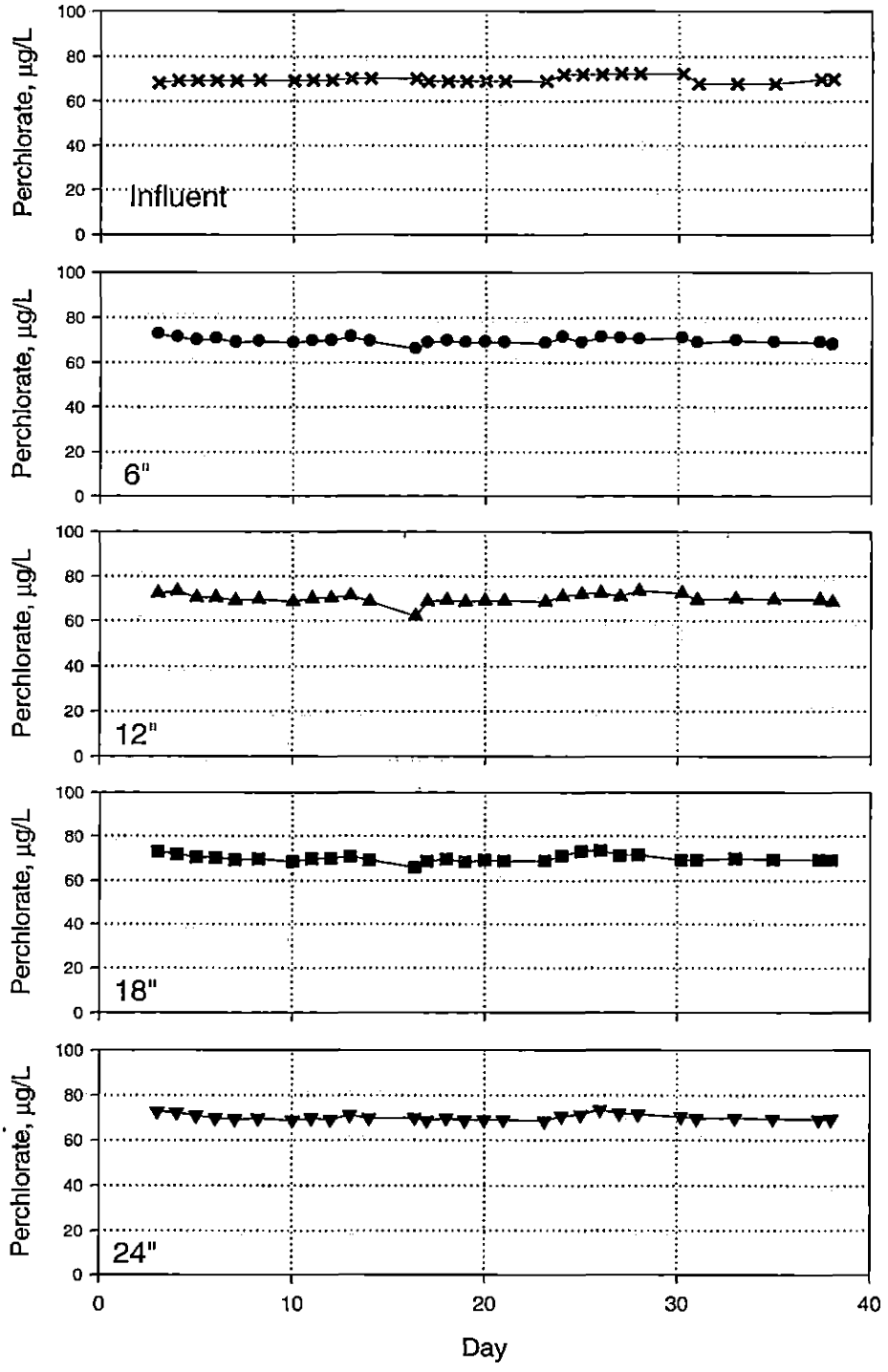




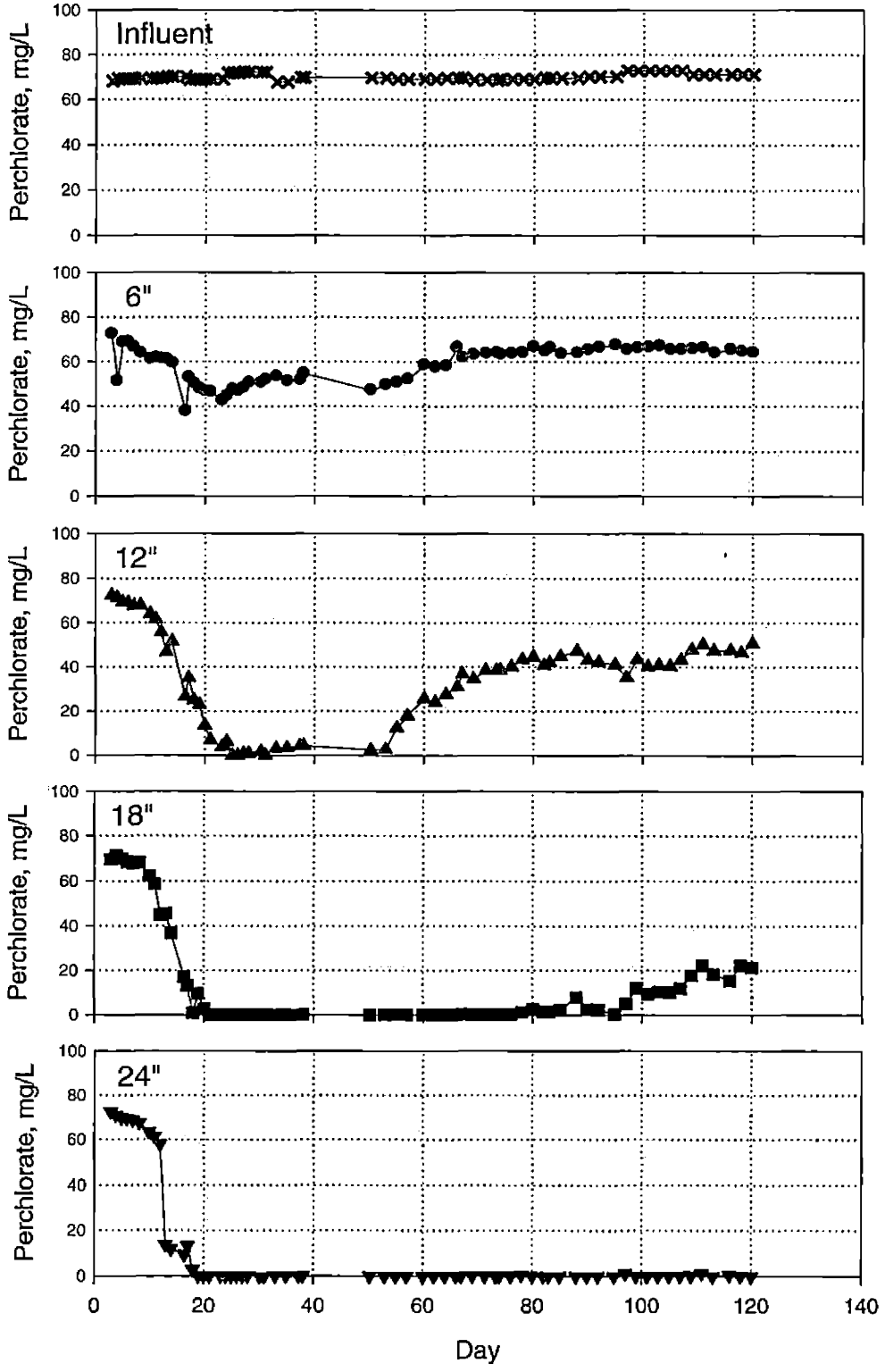


Source Area Soil Columns

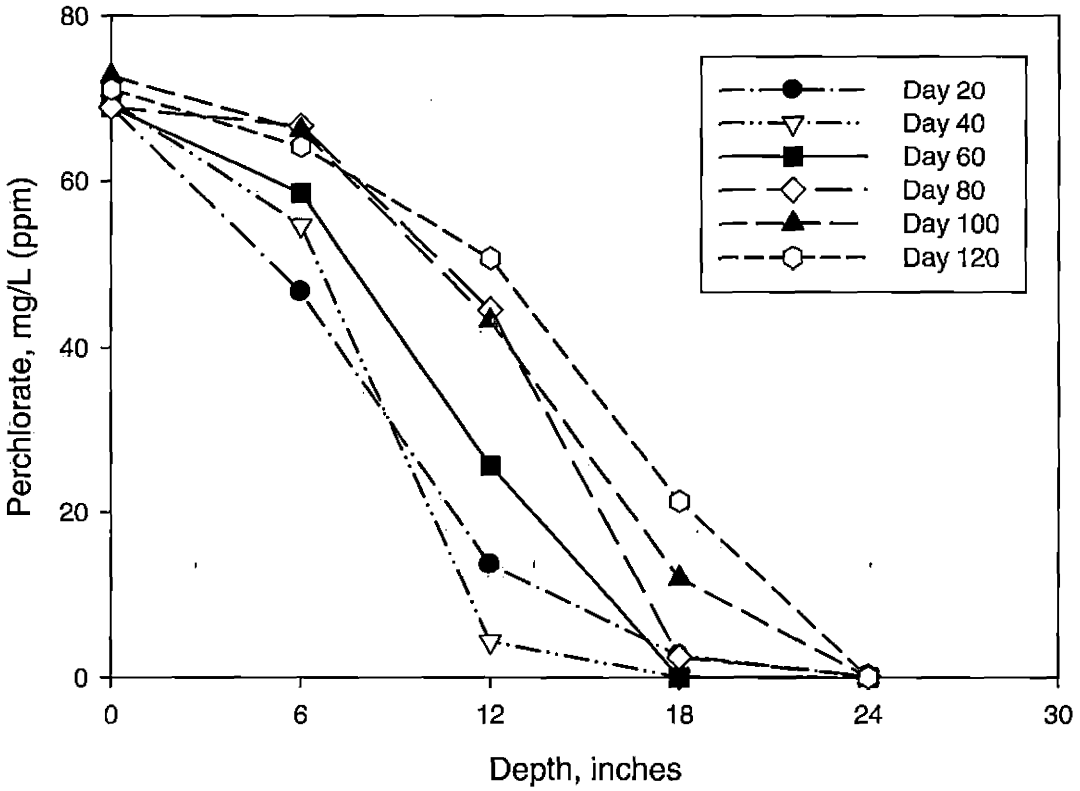
Perchlorate Reduction in Source Area Control Columns



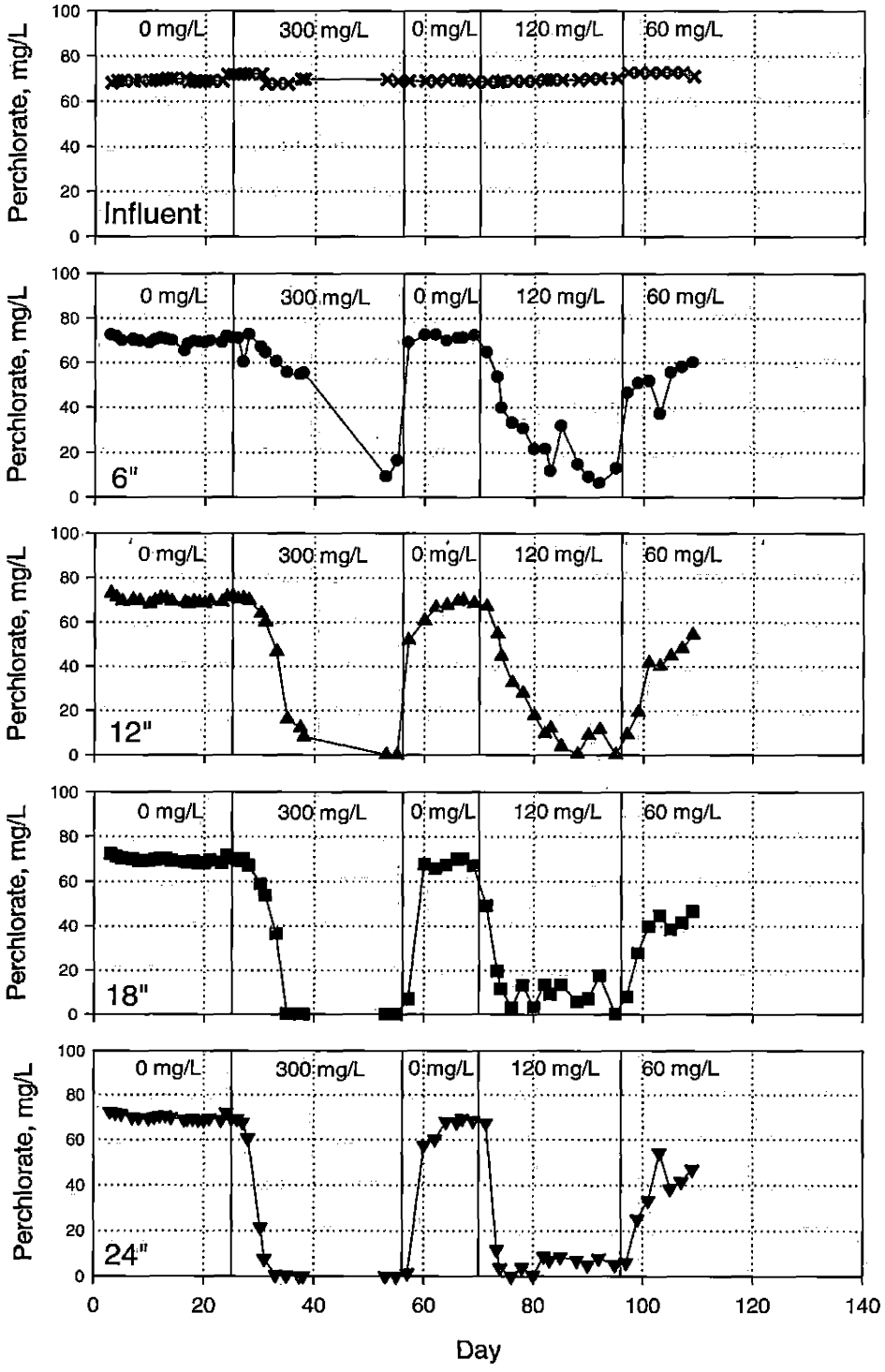
Perchlorate Reduction in Source Area EOS-Amended Columns



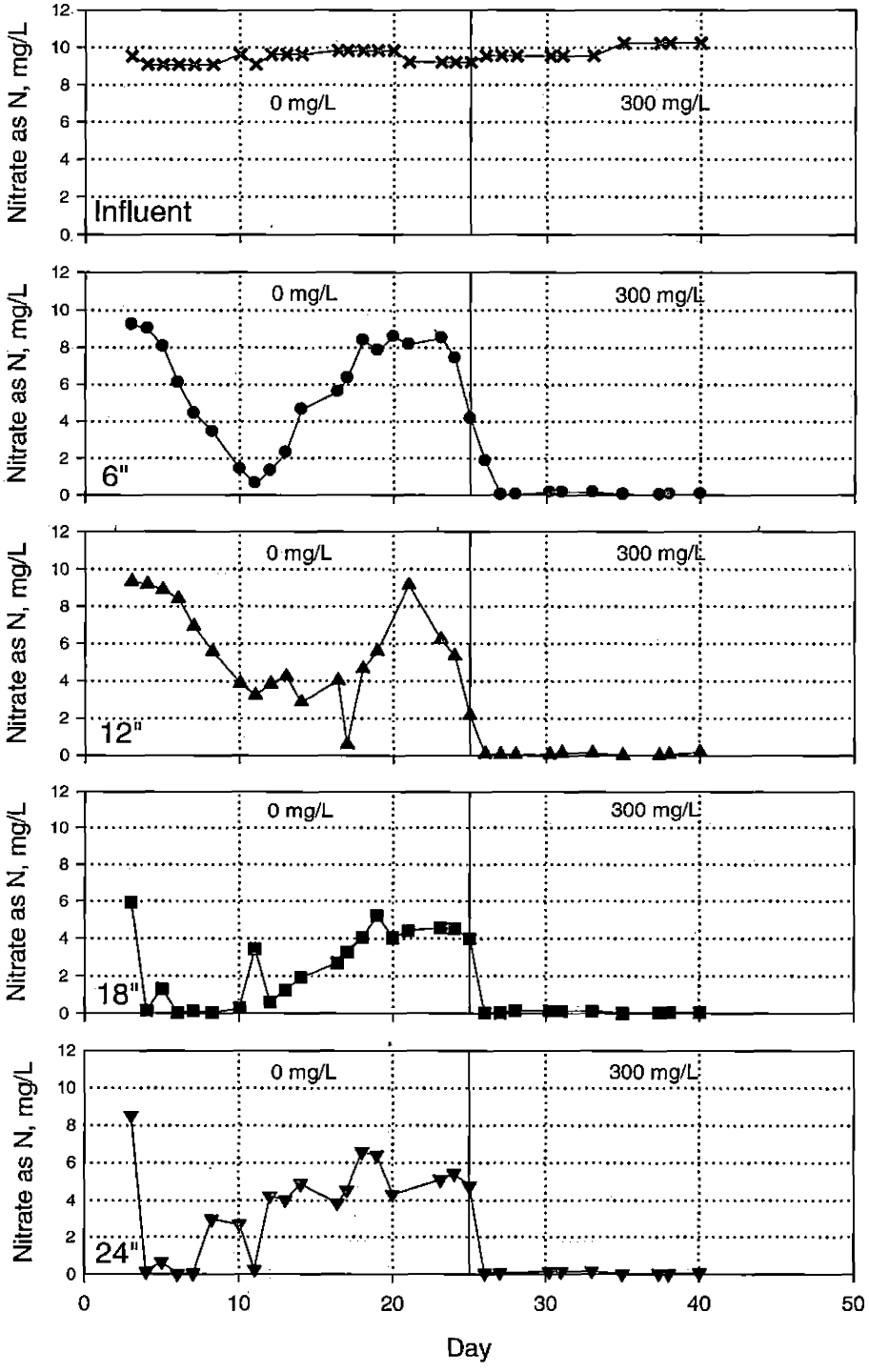
Perchlorate Reduction Profiles in EOS Amended Source Area Columns



Perchlorate Reduction in Source Area Glycerin-Amended Columns
Concentration Indicated is Amount of Glycerin Added to Influent

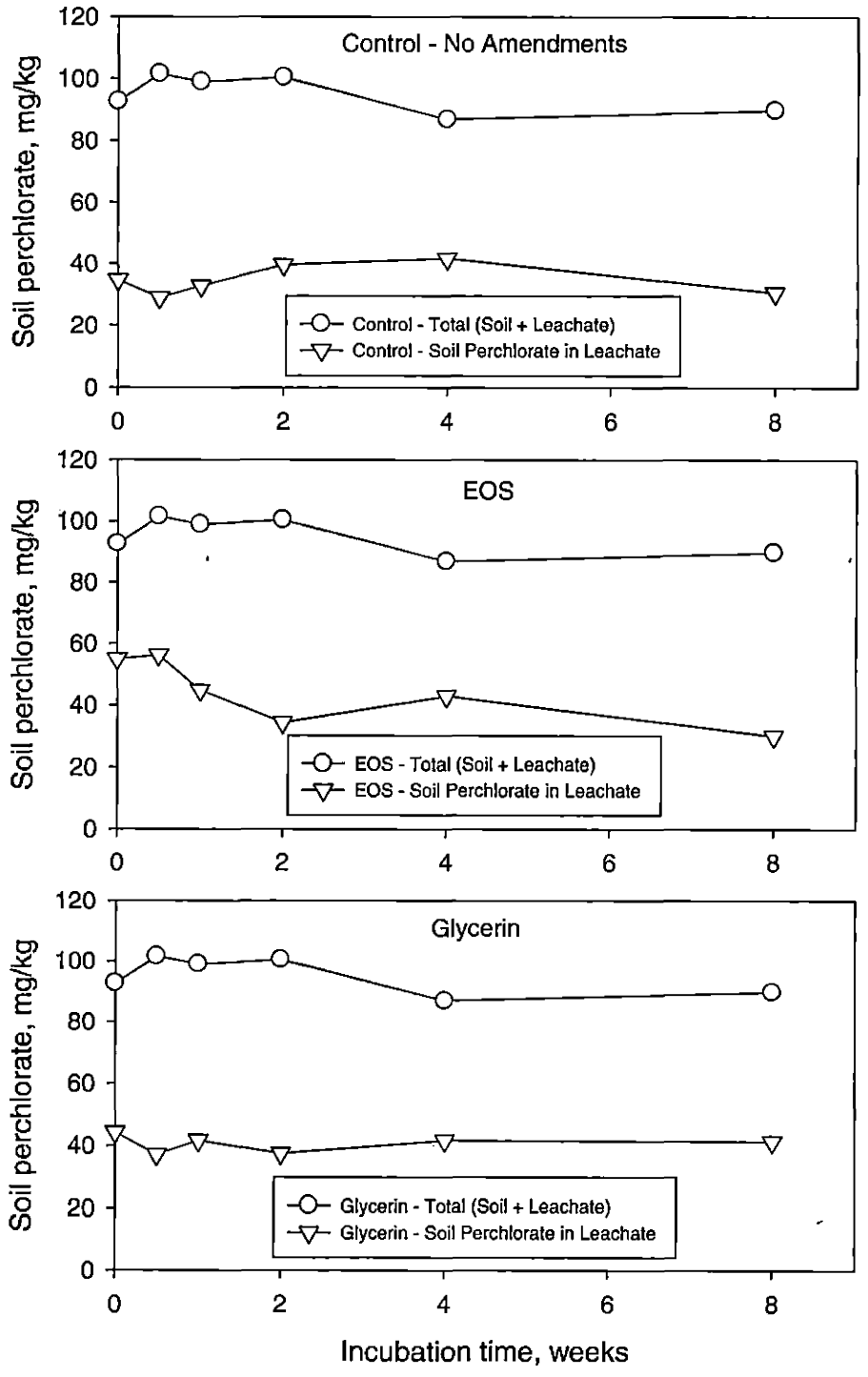


Nitrate Reduction in Source Area Glycerin-Amended Columns
Concentration Indicated is Amount of Glycerin Added to Influent

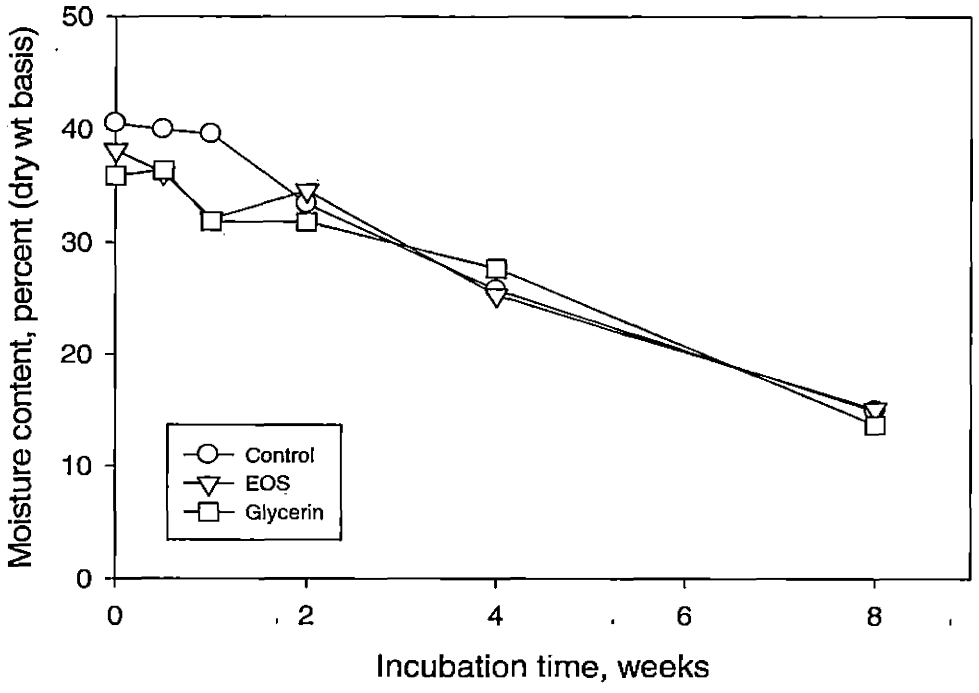


Vadose Zone Soil Column Data

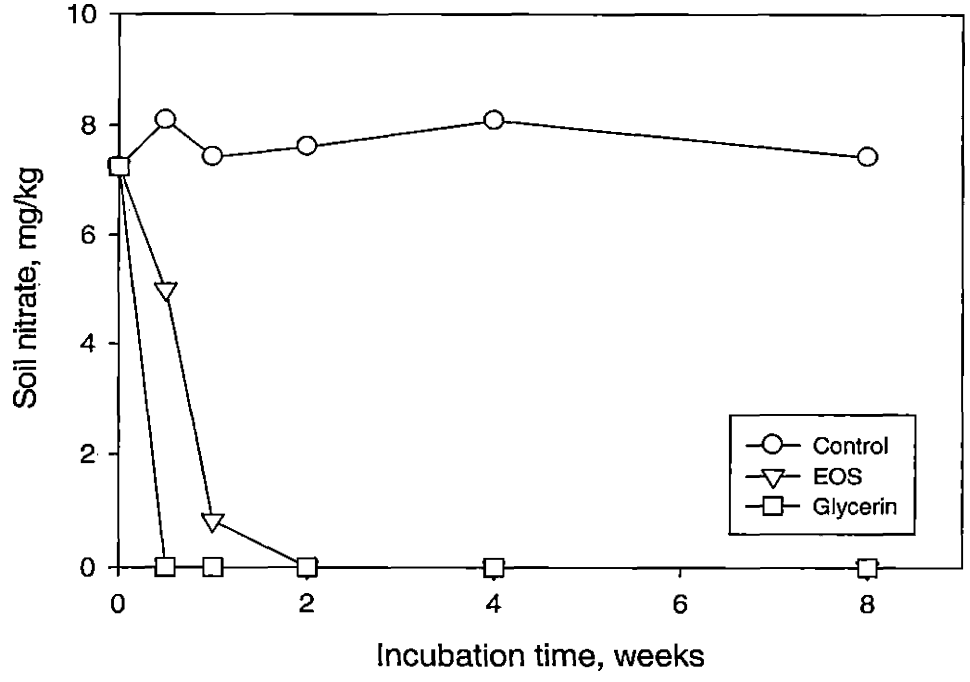
Perchlorate Results – Vadose Zone Columns – Batch Application (Scenario 1)



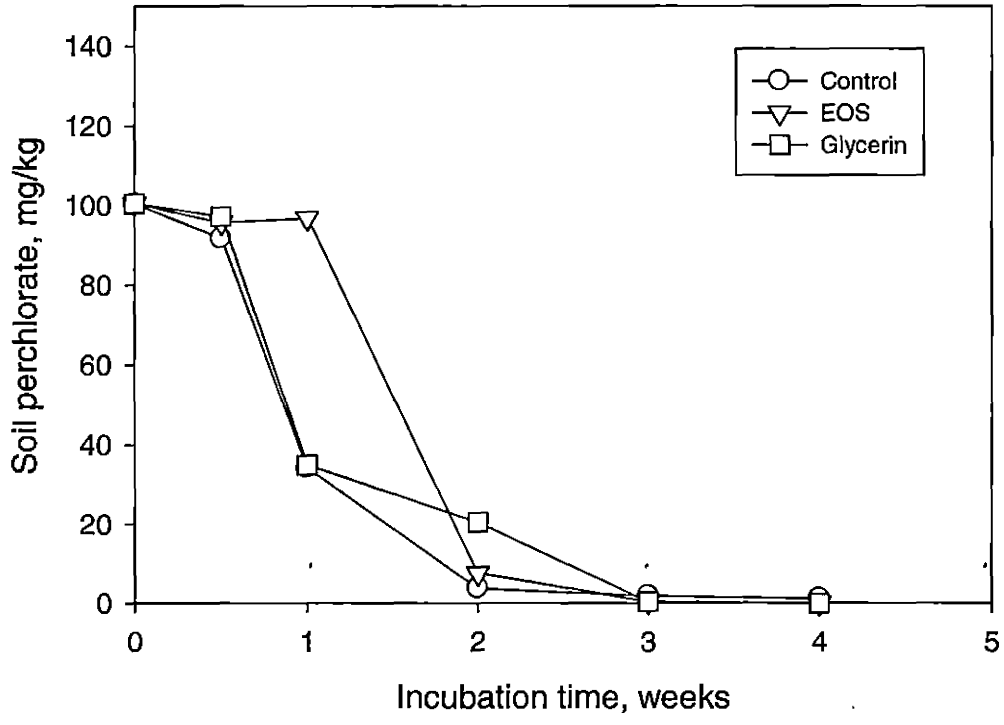
Moisture Content – Vadose Zone Columns – Batch Application (Scenario 1)



Nitrate Removal – Vadose Zone Columns – Batch Application (Scenario 1)



Perchlorate Results – Vadose Zone Columns – Recirculation Application (Scenario 2)



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