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In-Situ Bioremediation of Perchlorate in Groundwater and Soil

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In-Situ Bioremediation of Perchlorate in Groundwater and Soil

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Chemical and Environmental Engineering

by

Liyan Jin

September 2012

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ABSTRACT OF THE DISSERTATION

In-Situ Bioremediation of Perchlorate in Groundwater and Soil

by

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Doctor of Philosophy, Graduate Program in Chemical and Environmental Engineering
University of California, Riverside, September 2012
Dr. Mark R. Matsumoto, Chairperson

Historical, uncontrolled disposal practices have made perchlorate a significant
threat to drinking water supplies in the United States. In-situ bioremediation (ISB)
technologies are cost effective and provide an environmental friendly solution for treating
contaminated groundwater and soil.

In situ bioremediation was considered as an option for treatment of perchlorate in
groundwater and soil in Lockheed Martin Corporation's Beaumont Site 2 (Beaumont,
CA). Based on the perchlorate distribution and concentration in the ground, in-situ
remediation within the site was divided into three parts (groundwater plume, source area
groundwater remediation and vadose zone soil remediation). In both groundwater
remediation studies, biological reduction of perchlorate was readily achieved within one
week by amending electron donating substrates. In both column studies, perchlorate
reductions were observed within two weeks operation and emulsified oil substrate (EOS)
had the best performance as electron donor in terms of effectiveness and longevity in
both column studies. In the vadose zone remediation study, perchlorate degradation did

not occur in 20% moisture content microcosms. However, perchlorate reduction occurred under saturated conditions with the same soil and both EOS and glycerin as electron donors.

To investigate the impact of soil moisture condition on perchlorate remediation, soil microcosm studies were conducted with different soils under different mass water contents. Optimum soil moisture content for perchlorate bioremediation varied significantly in different soils. Anaerobic respiration processes other than denitrification were all limited by unsaturated moisture condition in the soil. Dominant electron acceptor in the unsaturated microcosms was oxygen. However, eliminating oxygen in the soil system using an anaerobic chamber did not result in perchlorate reduction. Addition of humic acid as an electron shuttling mediate reduced soil redox potential significantly but was not able to promote perchlorate reduction under unsaturated condition.

In-situ bioremediation of perchlorate was readily achieved in groundwater but it was more challenging in vadose zone. Soil moisture was identified as a key factor in perchlorate remediation and optimum moisture condition in different soil had large variation. The results from this research provide basis for designing and optimization of in-situ bioremediation of perchlorate in different soils and groundwater.

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Introduction

Perchlorate contamination has become a widespread problem since its discovery in drinking water supplies throughout southern western United States in 1997 [1-4]. Perchlorate has potentially serious health effect for babies and young children. Perchlorate may cause mental retardations in young children by interrupting thyroid function [4]. USEPA placed perchlorate on the contaminant candidate list in 1998 and set an interim health advisory level of 15 μg/L [5]. California has a maximum contaminant level (MCL) of perchlorate of 6 μg/L for drinking water, which became effective October 2007 [6]. More recently, the Office of Environmental Health Hazard Assessment (OEHHA) proposed Public Health Goal (PHG) for perchlorate in drinking water from 6 μg/L to 1 μg/L [7].

Many researchers have proven that microorganisms can degrade perchlorate to chloride in anaerobic conditions with sufficient electron donors [8-13]. Bacterial species that are capable of dissimilatory perchlorate reduction have strong diversity and ubiquity. They have been isolated from many different environments including soils [12, 14-16]. Thus, in-situ bioremediation of perchlorate is a promising approach to clean up perchlorate in groundwater and soil.

In situ bioremediation was considered as an option for treatment of perchlorate in groundwater and soil at Lockheed Martin Corporation's Beaumont Site 2. High concentration of perchlorate was detected in the vadose zone soil (100 mg/kg) and saturated zone groundwater (70 mg/L) in the source areas. Perchlorate (up to 500 μg/L)

was also found in groundwater plume close to the southern property boundary. Remediation within the site was divided to three parts and different strategies were proposed for each part of perchlorate remediation. Creation of an in situ bioremediation zone and direct injection of electron donating substrate were proposed to treat perchlorate in groundwater plume and source area groundwater, respectively. Two in-situ treatment scenarios (one time flooding and continuous recirculation modes) were proposed to elevate moisture condition and deliver electron donors to vadose zone soil.

In the first portion of this research (Chapter 2), feasibility studies were conducted to evaluate the proposed in-situ remediation strategies. The objectives of this part of the research were to:

- Verify the potential of perchlorate bioremediation in different parts of the site,

- Identify optimum electron donating substrates for each strategy,

- Investigate the effectiveness of nutrient addition on perchlorate in situ remediation, and

- Investigate perchlorate reduction kinetics in each strategy.

The suitability of different electron donating substrates was initially evaluated in microcosm studies with and without nutrient addition. Based on the results from the microcosm study, optimal electron donors were screened out and column tests were conducted with selected electron donors to simulate each treatment scenarios to obtain kinetic information and also evaluated possible impact on environments. Test results provided information for assessing the reactivity and longevity of each electron donors under the conditions expected in field application.

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Based on the first portion of this research, perchlorate reduction in vadose zone soil was more challenging than that in saturated zone. Perchlorate reduction was readily achieved under saturated soil while no perchlorate remediation was achieved in unsaturated soils in both microcosm studies and column studies. In Chapter 3, it was hypothesized that soil moisture condition is a key factor in perchlorate bioremediation. To study the impact of soil moisture on perchlorate remediation in vadose zone soil, microcosm studies were conducted three different soils under different soil mass water content. The impact of moisture on microbial activities are complicate because changing moisture would also result in changes of other properties in soils such as pH, salinity, nutrient transport, oxygen availability and redox potential. Various possible mechanisms behind the limiting impact were investigated. In this part of research, possible mechanisms behind the moisture effect were also discussed based on the microcosm results. Results from this part of research can be applied to designing and optimizing perchlorate remediation processes in different vadose zone soils to decrease the operating time frame and the costs.

In Chapter 4, the effect of soil biogeochemical properties on perchlorate bioremediation was studied. Perchlorate bioremediation is a microbial anaerobic respiration process. Oxygen and nitrate are well known competitive electron acceptors of perchlorate in groundwater and drinking water remediation processes [12, 13]. Soil has more complicated physiochemical properties and has more competitive respiration processes than water systems. In this portion of the research, dominant electron pathways were investigated along perchlorate reduction under saturated condition and

unsaturated condition in microcosm studies. A bioaugmentation approach was also evaluated in the microcosm studies. Later, humic acid was selected as an electron shuttling substrate and the effect of humic acid on soil redox potential and perchlorate bioremediation was studied by conduction soil microcosms.

In summary, the overall objectives of this portion of the study were to:

- Evaluate the in-situ perchlorate bioremediation technologies to treat perchlorate in groundwater and vadose zone soil.

- Study the impact of soil moisture on perchlorate bioremediation in vadose zone soil and investigate possible mechanisms behind the impact.

- Identify possible limiting factor in perchlorate bioremediation in vadose zone soil and evaluate possible solutions to overcome the limitation.

From this research, a comprehensive understanding was obtained for perchlorate remediation in vadose zone soil at different geochemical conditions. Outcomes from this research will provide the basis to diagnose limiting factors in perchlorate bioremediation, design and optimize perchlorate remediation processes and predict the environmental impact of remediation on vadose zone soil.

Chapter 1   Background

1.1   BIOREMEDIATION OF PERCHLORATE

1.1.1   Perchlorate

Perchlorate is both a naturally occurring and man-made chemical that can disturb thyroid functions in mammals. Most naturally occurring sources of perchlorate are geographically limited to arid environments. In contrast, man-made perchlorate compounds have been widely used for a wide variety of commercial and military uses such as airbag initiators for vehicles, solid propellant rockets and missiles [1, 2]. However, most of the perchlorate manufactured in the United States is used as the primary ingredient of solid rocket propellant [3].

Perchlorate was first found in drinking water wells in eastern Sacramento County in California, near Aerojet General Corporation's facility in 1997 [4]. Since then, perchlorate has been found in surface water, groundwater, and potable water wells in 42 states of the US [5]. Perchlorate contamination is now considered to be a widespread concern in the United States with the development of more sensitive analytical methods that can detect it in soil and groundwater [6]. During the past five years, 296 active and standby perchlorate sources were detected in California alone [4]. Perchlorate has also been detected in various foods including lettuce, crops, milk, and alcoholic beverages [7]. Historical unregulated disposal of perchlorate containing waste is predominant source of perchlorate contamination.

The primary route of human exposure to perchlorate is via ingestion of perchlorate-contaminated water and food [8]. One of the more serious human health effects of perchlorate observed in scientific studies is disruption of thyroid hormone production. The thyroid produces two principal hormones, triiodothyronine and thyroxine, which help regulate the body's metabolism and physical growth. The perchlorate ion is similar in radius and charge to the iodide ion and can competitively inhibit iodide uptake by the thyroid and disrupt the normal function of the thyroid [8].

Perchlorate (ClO_4^-) is a negatively charged ion that non-volatile and highly soluble in water. Perchlorate is a strong oxidizing agent with standard redox potential +1.38V. However, it is quite stable under normal atmospheric conditions because of its high activation energy, $120kJ\ mol^{-1}$ [9].

Once a perchlorate compound is released into the environment, it readily dissolves in water, and gets transported through bulk movement of water and mixing processes. It persists in the environment for many decades. When the flow is low or if water becomes stagnant, perchlorate tends to diffuse into clay layers in an aquifer [8]. In addition, in terms of drinking water, it is not removed by conventional water treatment processes. Due to these concerns, USEPA placed perchlorate on the contaminant candidate list (CCL) in 1998 [10]. Although no federal drinking water standard for perchlorate has been established yet, several states have set advisory levels ranging from 1 µg/L to18 µg/L for perchlorate in drinking water. California has a maximum contaminant level (MCL) of perchlorate of 6 µg/L in drinking water [11].

1.1.2 Bioremediation of Perchlorate

Several technologies have been used to treat perchlorate contamination in drinking water, groundwater, and soil. Ion exchange is the most frequently used ex situ treatment technology for perchlorate in water [6]. Granular activated carbon and membrane treatment technologies are also available for perchlorate removal [12-15]. However, most of physical-chemical treatment technologies are expensive and not applicable for in-suit remediation [6, 16, 17]. Biological reduction of perchlorate is a promising technology because this technology is relatively cost-effective and environmentally compatible. Many researchers have proven that microorganisms can degrade perchlorate to chloride and oxygen in anaerobic conditions with sufficient electron donors and these microorganisms are ubiquitous in environment [18-23].

Bacterial species that are capable of dissimilatory perchlorate reduction have been isolated from many different environments, including sewage sludge, pristine soils and hydrocarbon-contaminated soils, aquatic sediments, paper mill waste sludges, farm animal waste lagoons and underground gas storage [22, 24-26]. The majority of these organisms have been classified as gram-negative and in the Proteobacteria class [27]. The most widely accepted perchlorate reduction pathway is $ClO_4^- \rightarrow ClO_3^- \rightarrow ClO_2^- \rightarrow O_2 + Cl^-$ which was proposed by Rikken in 1996 [28]. In general, perchlorate reducing bacteria are facultative anaerobes, capable of utilizing oxygen, nitrate, and perchlorate as terminal electron acceptors [29].

1.1.3 The effect of environmental factors on perchlorate bioremediation

Oxygen and nitrate are more favorable than perchlorate as electron acceptors in mixed cultures and pure cultures of perchlorate reducing bacteria [23, 29-31]. The sequence of utilization of various electron acceptors found in a perchlorate environment is shown in Figure 1.1 [32]. It has been reported that dissolved oxygen concentrations of just 2 mg/L are enough to inhibit perchlorate reduction and perchlorate reduction commences only when nitrate was completely removed [29]. In Choi *et al.*'s research, reduction of nitrate was favored over perchlorate, in a biofilm reactor which had been enriched under perchlorate-reducing conditions for 10 months [31]. Perchlorate reducing bacteria can utilize wide variety of organic substrates including lactate, acetate, ethanol, glycerin, succinate, vegetable oils, and plant-produced electron donors [22, 27, 33]. Inorganic substrates such as hydrogen, zero valent iron, sulfur have also been studied as electron donors for perchlorate reduction [34-36]. Utilization of electron donors is strain dependent with acetate being the most widely used electron donors in laboratory.

Aerobic Respiration	Denitrification	Perchlorate Reduction	Sulfate Reduction	Methanogenesis
$O_2 \rightarrow H_2O$	$NO_3^- \rightarrow N_2$	$ClO_4^- \rightarrow Cl^-$	$SO_4^- \rightarrow S^{2-}$	$CO_2 \rightarrow CH_4$

0-100

+800   Redox, mV   -250
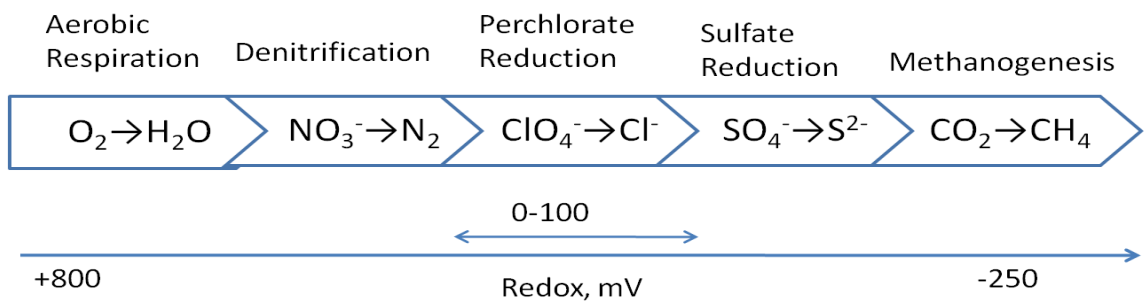
Figure 1.1 Sequence of Utilization of Electron Acceptors

Macro and trace elements are important factors that may limit perchlorate degradation. Evans *et al.* reported that total Kjeldahl nitrogen was a significant factor

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limiting perchlorate biodegradation in soil at a large brownfield site [37]. Analysis of the purified perchlorate reductase from chlorate-respiring strain GR-1 revealed the presence iron, molybdenum, and selenium and it was deduced that iron, molybdenum, and selenium are important in perchlorate remediation [38].

Perchlorate reduction by mixed culture occurs throughout the pH range from 5.0 to 9.0 and perchlorate reduction rates are significantly different at various pHs with a maximum rate (specific substrate removal rate of 0.02 h^{-1}) at pH 7.0 [39]. However, the effect of pH on the growth of perchlorate reducing bacteria is not well understood. The effect of temperature on perchlorate removal was investigated in an anaerobic biological contactor by Dugan. He concluded that $10^{o}C$ was a threshold temperature below which microbial activity, including perchlorate reduction, decreased dramatically [40].

Inhibition effect of salinity has been investigated in several studies to treat high-strength perchlorate wastewaters from ion exchange regenerant streams. In Park *et al.*'s research, it was observed that the perchlorate-reducing microbes that are dominant in low salinity environments are not well suited for treating high-strength wastewater stream due to their inhibition by high concentration of salt. However, microorganisms in fresh water could be acclimated to high-salinity wastewater and effectively reduce high concentrations of perchlorate [41]. In a later study, the authors also found that perchlorate wastewaters using either acclimated return activated sludge or anaerobic digester sludge cultures is feasible up to 3% or 4% NaCl [42]. High salinity inhibition effect was also studied by Chung *et al.* in hydrogen-based membrane biofilm reactor [43].

Bruce *et al.* reported that perchlorate reducing bacteria strain CKB has a salinity optimum of 1% NaCl [26].

1.1.4 Perchlorate bioremediation in vadose zone soil

Perchlorate has been deposited in vadose zone soils via disposal and spills [44]. Significant amount of perchlorate also occurs naturally in vadose zone soil. It has been reported that up to 1 kg ha^{-1} of natural perchlorate is present in diverse unsaturated zones of the arid and semi-arid southwestern United States [45]. Recently, increasing efforts have been placed on the perchlorate remediation in soils due to the realization that it can be a persistent source for groundwater contamination through infiltration.

Nozawa-Inoue *et al*. conducted microcosm tests with unsaturated soil (20% soil moisture content) to examine perchlorate remediation potential by native microbial communities in vadose soil. According to the results, bioremediation of perchlorate by native microbial communities may be feasible when enhanced by adding electron donors in the vadose zone. However, lag periods of reduction varied considerably with different electron donors [44]. Evans *et al.* reported that gaseous electron donors such as hydrogen and ethanol were able to promote nitrate and perchlorate reduction in vadose zone soil and that moisture content was an important factor [46]. Gal *et al.* examined the potential of perchlorate biodegradation in different depths (1, 15, 20 and 30m) of vadose zone soil. Perchlorate was completely reduced in a soil slurry (1:10, soil: basal solution), with acetate as the carbon source, in sediment samples from three (1, 15 and 30m) of the four depths examined. Sediment sample from 20 m below land surface had low viable microbial communities and water content, and high perchlorate concentration and this

may be the reason of the failure of perchlorate degradation [47]. Several full scale and

pilot scale projects were also conducted to degrade perchlorate in vadose zone soil and

were summarized in Table 1.1. Although perchlorate bioremediation in vadose zone soil

by native microbial communities was observed in these studies, perchlorate removal rates

had a large variation, from 50 μg/kg-soil per day to 6 to 7 mg/kg-soil per day.

Table 1.1 Summary of vadose zone bioremediation studies

Site	Initial perchlorate concentration	Amendment	Moisture content (%)	pH	Performance
Brownfields Site [37]	Maximum perchlorate concentration was 316,000 μg/kg	500 mg/kg glycerin with 50~100 mg-N/kg DAP	15~17	NA	Median destruction rate was about 200 μg/kg-soil/day.
Longhorn Army Ammunition Plant [48]	Maximum perchlorate concentration was 300,000 μg /kg	Horse manure; chicken manure; Ethanol	Maintained in saturated condition	NA	Maximum rates of perchlorate removal at the top layer during the start of the test were in the range of 6-7 mg/kg-soil/day.
Road flare manufacturing facility in Santa Clara Valley, California [49]	Average perchlorate concentration in soil sample was 7000 μg/kg	Calcium magnesium acetate and citric acid	Field capacity (10% by volume)	NA	Perchlorate concentrations declined from 7000 μg/kg to a geometric mean of 126 μg/kg within six weeks. Perchlorate removal rate was approximately 160 μg/kg-soil/day.[1]
Manufacturing facility in the western U.S. – ARCADIS [50]	Maximum perchlorate concentration was 13,000 μg/kg	Corn syrup and ethanol	Temporarily saturated	NA	Perchlorate concentrations were reduced by 81to 93% in eight month. Perchlorate removal rate is approximately 50 μg/kg-soil/day.[1]
Microcosm test by Nozawa-Inoue et al. [44]	Approximately 110,000 g/kg	Acetate; Hydrogen	20%	Initial soil was 7.2 ±0.4 and 6.8 to 8.5 during	Perchlorate removal rate was 2.7mg/kg-soil/day with acetate and 1.68mg/kg-soil/day with hydrogen.[1]

[1] Perchlorate removal rate was estimated using equation:

$$R = \frac{Initial\ perchlorate\ concentration - final\ perchlorate\ concentration}{incubation\ time}$$

				the experime nt	
Microcosm test by Evans *et al.* [46]	23mg/kg	H_2 and CO_2; Ethanol	9.5%	Final pH was 9.4	Perchlorate removal rate was 0.3mg/kg-soil/day with hydrogen and 0.8mg/kg-soil/day with ethanol.[1]
Microcosm test by Gal *et al.*[47]	70 mg/L in soil slurry	Acetate and trace elements.	Soil slurry (1:10, soil: basal solution)	NA	8.7mg/L-soil slurry/day.[1]

1.2 THE EFFECT OF MOISTURE ON BIOCHEMICAL PROCESSES

Soil moisture is an important factor that can control microbial biomass and activities [51-54]. Effects of moisture on microbial activity are likely to be complicated and unpredictable because of many possible interactions among the bacteria, solute transport, nutrient and oxygen availability, pH, and other parameters within the soil environment.

1.2.1 Soil water potential

Water within the soil moves in the direction of decreasing water potential which is the specific potential energy of soil water relative to that of water in a standard reference state. Total soil water potential in the vadose zone is the sum of gravitational, matric and osmotic potential [55]. The gravitational potential at each point is determined by the elevation of the point relative to a reference level. The matric potential in soil is caused by the capillary and adsorptive forces (matric suction) from soil matric. The osmotic potential is caused by the solutes in soil water. Total water potential in the vadose zone has a negative value due to the matric potential and osmotic potential.

Water potential is usually used to describe and predict water movement in the soil and to describe availability of water for the plant and soil microbes [55]. Different soil may have different water availability under the same mass water due to the matric suction or osmotic effects. Thus, water potential can be a better indicator than mass water content for the dehydration stress of microbial communities in soil.

1.2.2 Effect of moisture on soil microbial activity

Microbial activity can be inhibited by low water availability due to the dehydration and limited solute transport in soil. As the water content decreases, water films on soil surfaces become thinner. Thus, dissolved substrates in the soil water have more tortuous paths in diffusing to cells, which reduces the substrate flux in soil [52, 56]. Based on Stark *et al.*'s research, the effect of soil moisture on nitrification activity is mostly caused by limited substrate diffusion at high water potentials (>-0.6 MPa), whereas adverse physiologic effects associated with cell dehydration are the most limiting factor at low water potentials [52]. Or *et al.* also reported that the effect of soil pore geometry and moisture condition is on the diffusion pathways to the microbial colonies. Complex diffusion pathways in soil have a critical role in maintaining diversity and coexistence of microbial species in soils [56]. Different microorganisms have different tolerances to the water stress. It has been reported that a water potential lower than -2 MPa kills a proportion of soil microbes but remaining microbes adapt to the water potential[55].

The effect of soil moisture on microbial activity is not always positive. According to Rockhold's review, when a porous medium is close to the saturated

condition, the diffusion coefficient of oxygen in the gas phase declines exponentially with water content [57]. Due to the limited oxygen supply, total microbial respiration is inhibited when the soil water content reached saturation [51].

Under limited oxygen supply, some microorganisms such as denitrifiers can develop anaerobic respirations mechanisms to survive. It has been reported that denitrifiers in soil respond quickly to the moisture conditions [58, 59]. Anaerobic biochemical reactions are generally affected by soil moisture conditions. Reductive dechlorination of polychlorinated biphenyls in sediments also is significantly suppressed when the moisture content is reduced from 87% (slurry) condition to 37% (low moisture sediments) [54]. Population size of dechlorinators and sulfate-reducing bacteria are not affected by reduced moisture content while methanogens in the slurry are about two orders of magnitude higher in populations than that in low moisture sediments [54]. The reduction of sediment moisture decreased the dechlorination rate but did not inhibit the growth of PCB dechlorinators [54].

1.3   BIOGEOCHEMICAL REACTIONS IN SOIL

Perchlorate can be reduced to chloride by indigenous soil microbes with sufficient electron donors. Most perchlorate reducing bacteria are facultative anaerobes that can utilize oxygen, nitrate, and perchlorate as electron acceptors. Oxygen and nitrate are well known competitive electron acceptors of perchlorate in groundwater and drinking water remediation processes. Soil has more complicated physiochemical properties and may have more competitive redox pathways than aquatic systems.

1.3.1 Soil ORP and the effect on microbial activity

Soil reduction-oxidation status is an important parameter on biogeochemical process in soil. The redox characteristics of natural environmental systems are usually defined in terms of the redox potential. Redox potential is the measured potential versus the standard hydrogen electrode (Eh) [60]. However, defining and measuring ORP in soil is difficult because of the heterogeneity and instability of soil. It is said that thermodynamic equilibria rarely occur in soil systems [61].

Microbial activity can be significantly affected by undesirable redox conditions. In a computer-monitored and feedback-controlled bioreactor, methanogenesis stopped when redox potential rose from a value of -260mV to -100mV, but resumed after redox potential returned to initial range [62].

Soil microbial communities have developed different strategies to adapt to changes in soils. Different redox status may indicate different dominant electron acceptors in soil. Microbial communities are able to develop different metabolic pathways to adapt to changing redox conditions. Main terminal electron acceptors in soil include oxygen, nitrate, Mn^{4+}, Fe^{3+}, sulfate and carbon dioxide. These electron acceptors are used by soil microorganisms in a sequence, which follows thermodynamic theory [65-67] (see Table 1-2). Perchlorate reduction is usually observed at an ORP range between 0 to -100mV.

Table 1.2 Dominant redox reactions under different soil redox potential

Measured Redox Potential Range E_h (mV)	Observation
380~320	Oxygen depletion[66]
280~220	Nitrate and Mn (IV) reduction[66]
180~150	Fe(III) Non-detectable[66]
0~-100	Perchlorate reduction
-120~-180	Sulfate depletion[66]
-200~-280	Carbon dioxide depletion[66]

Sequential reduction of different terminal electron acceptors is explained by the outcompeting ability of different organisms for electron donors. Lovley *et al.* reported that addition of Fe (II) to sediments repressed sulfate reduction by 86 to 100% and in sulfate-depleted sediments Fe(III) additions also inhibited methane production. Fe (III) reducers in sediments were able to utilize lower concentrations of hydrogen and acetate than sulfate reducers and methanogens. So it was concluded that Fe (III) reducers can inhibit sulfate reduction and methane production by outcompeting available electron donors[68].

Later, competition for electron donors among different group of organisms was also studied by Achtnich [65]. Addition of nitrate inhibited Fe (II) production and sulfate

reduction in low organic carbon sediments but the inhibitions were overcome by addition

of H_2, acetate, or a mixture of fatty acids. Methane production was inhibited by nitrate,

Fe(III) and sulfate in sediment with nature organic carbon[65]. Inhibition of methane

production responded well to decreasing hydrogen concentrations in sediments[65].

Inhibition effect of nitrate on perchlorate reduction was extensively studied in

several researches. Chaudhuri *et al*. reported that expression of chlorite dismutase in *D.

Suillum* was regulated by nitrate and observed that perchlorate reduction by *D. Suillum*

only occurred after depletion of nitrate. However, another perchlorate reducer

Dechloromonas agitata strain CKB was able to concurrently reduce nitrate and

perchlorate[29]. Choi reported that perchlorate reduction by suspensions of perchlorate

reducers was decreased by 30% by addition of 2mM nitrate when sufficient acetate was

present and decreased by 70% when acetate was limited [31]. Most of inhibition effects

of competitive electron acceptors in soils are caused by outcompeting electron donors.

Inhibition effects can be overcome by providing sufficient electron donors.

1.3.2   Humic substrances in soil

Humic substances (HS) are formed from the biochemical weathering of plant and

animal material in soil [69]. Chemically defining HS is very difficult because of their

large chemical heterogeneity and geographical variability [69]. HS can be divided into

three groups: humins, humic acids, and fulvic acids based on their solubilities. Humins

are the fraction which is insoluble at all pHs, humic acids are insoluble at pHs below pH

2.0, and fulvic acids are soluble at all pHs. HS are redox-active compounds that can

serve as electron donors or electron acceptors in various microbial respirations[70].

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The iron reducing bacteria Geobacter *metallireducens* are able to grow using HS as the terminal electron acceptors and acetate as electron donor [71, 72]. Coates *et al.* reported that reduced forms of HS can serve as electron donors for anaerobic bacteria such as denitrifiers and that these HS oxidizing bacteria are ubiquitous and diverse in environmental samples [70]. In soils and sediments, HS can serve as electron donor as well as serve as a catalyst in many anaerobic respirations[73]. Several researchers have proven that HS can stimulate solid phase of Fe(III) reduction by serving as electron shuttles [71, 74-76]. Biodegradation of carbon tetrachloride by anaerobic granular sludge was stimulated by addition of both humic acids and AQDS up to 6 fold in term of first order degradation rate [77]. More recently, biodegradation of trichloroethene was also enhanced by addition of humic acid (HA) in Ying Zhang's research. TCE removal efficiency increased with increasing HA concentration in the range between 250 and 500mg/L. He concluded that humic acid could stimulate TCE degradation by serving as an electron shuttle between *Dehalococcoides* and TCE [78].

1.4   SUMMARY

In-situ bioremediation can be a promising approach to clean up perchlorate in groundwater and vadose zone soil. Although bacterial species that are capable of reducing perchlorate are ubiquitous in soil, specific biogeochemical conditions are required to stimulate perchlorate respiration in the ground.

Limiting factors for perchlorate bioremediation include oxygen, low amount of electron donor, low amount of nutrients, lack of water, high salinity, and pH. Moisture condition is an important factor that affects microbial activity and substrate mass transfer

in soil. In addition, soil water content significantly affect oxygen diffusion rate which is critical in creation of anaerobic condition. Thus it can be hypothesized that soil moisture is a key factor in perchlorate bioremediation in soil.

To achieve in situ bioremediation, perchlorate reducers need to outcompete with other soil microorganisms for limited substrates supply. Common biological reactions in soils are denitrification, ferric reduction and sulfate reduction.   Soil redox potential can be a good indicator to evaluate soil geochemical conditions for perchlorate bioremediation and identify dominant electron pathways in soil.

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Chapter 2   In-Situ Bioremediation of Perchlorate in Groundwater and Vadose Zone Soil

2.1   INTRODUCTION

Perchlorate contamination has become an important environmental issue since its discovery in drinking water supplies throughout southern western United States in 1997 [1-4]. One of the more serious human health effects of perchlorate observed in scientific studies is disruption of thyroid hormone production. This health effect is especially serious for babies and young children because it may cause mental retardation [4].

Once perchlorate is released into the environment, it persists for many decades because it's quite stable in the environmental conditions [4]. In addition, in terms of drinking water, it is not removed by conventional water treatment processes. Due to these concerns, USEPA placed perchlorate on the contaminant candidate list in 1998 [5]. Although no federal drinking water standard for perchlorate has been established, USEPA has an interim health advisory level of 15 μg/L. California has a maximum contaminant level (MCL) of perchlorate of 6 μg/L in drinking water which became effective October 2007 [6]. More recently, the Office of Environmental Health Hazard Assessment (OEHHA) proposed Public Health Goal (PHG) for perchlorate in drinking water from 6 μg/L to 1 μg/L [7].

Ion exchange is the most frequently used technology to remove perchlorate in water, particularly for drinking water applications [17]. However, this technology is relatively expensive and only applicable in ex-situ treatments. Based on a study

conducted by Burge and Halden, ion exchange costs $2100,000 dollars per kg perchlorate treated [18].

In contrast, in-situ bioremediation (ISB) technologies are more cost effective and provide an environmental friendly solution for treating contaminated groundwater and soil. ISB can be applied to areas that are inaccessible or hard to be excavated. As an in situ technology, ISB eliminates the need for "pump and treat", resulting in lower maintenance/operation costs than other ex situ treatment technologies.

In ISB treatment, perchlorate is utilized as a terminal electron acceptor in redox reactions carried out by bacterial perchlorate reducers and degraded to harmless chloride in anaerobic conditions in the presence of a corresponding electron donor [2, 12, 14, 19, 20]. Bacterial species that are capable of reducing perchlorate have been isolated from many different environments including soils [12, 14-16]. Thus, there is a potential of perchlorate bioremediation by indigenous microbes in soils.

Perchlorate contamination has been detected in groundwater and soil in Lockheed Martin Corporation's Beaumont Site 2 (Beaumont, CA). In situ bioremediation was considered as an option for treatment of perchlorate. Perchlorate concentration in groundwater ranges widely depending on location as shown in Figure 2.1. In the downstream plume, perchlorate concentrations were reported to be on the order of 500 μg/L. In addition, up to 68,000 μg/L have been detected in the groundwater underlying the source area and up to 100,000 μg/kg perchlorate in source area vadose zone soils.
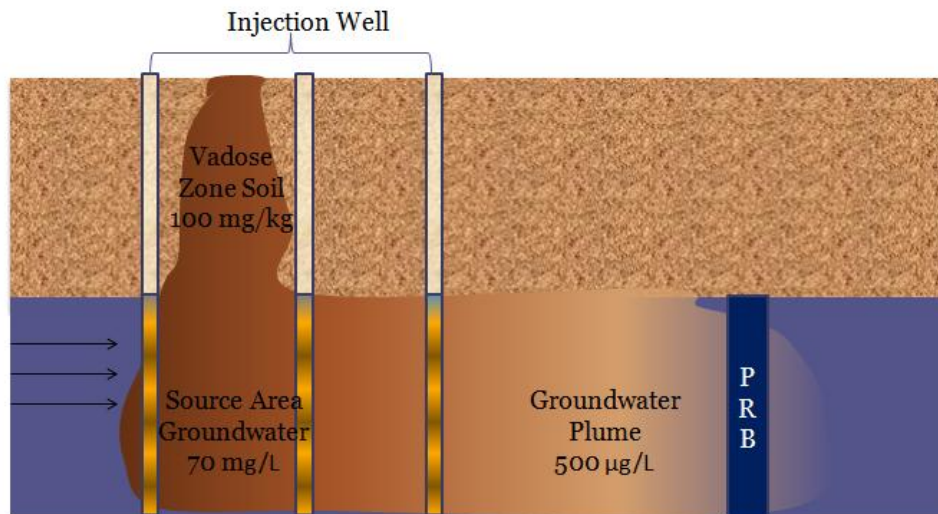
Figure 2.1 Perchlorate distribution and schematic of treatment plan in Lockheed Martin Corporation's Beaumont Site 2

Based on bench scale microcosm tests, perchlorate reducers existed in both vadose and saturated zone soil samples collected from the site. However, the presence of perchlorate reducers alone does not guaranty reduction of perchlorate. Stimulation of perchlorate reduction requires specific environmental conditions. Providing an adequate source of electron donor amendments to drive microbial respiration is needed to create and sustain anoxic conditions and stimulating biological reduction of perchlorate.

Different strategies were considered to deliver electron donors and achieve perchlorate remediation in each section of the site. A schematic of perchlorate distribution and treatment plan is shown in Figure 2.1. Creation of a bioremediation zone was proposed to treat perchlorate in groundwater plume. Perchlorate in groundwater can be reduced to chloride when it passes through a reactive zone in which an electron donor is introduced in the presence of perchlorate reducers. Ideal electron donor substrates should readily available to perchlorate reducers but also has high adsorption to soil so that it can sustainably donate electrons in the bioremediation zone.

To remediate the perchlorate in the groundwater underlying the source area, injection of electron donating substrate into the saturated zone was proposed. Important considerations in this endeavor are the effectiveness of electron donors in terms of perchlorate reduction and the ability to distribute throughout the desired area within the subsurface.

In contrast, perchlorate remediation within the vadose zone is more challenging. Low moisture conditions could be a limiting factor since the site is located at semi-arid area. Further, anoxic conditions needed for perchlorate reduction are difficult to maintain at unsaturated soils due to the air filled pores in soils. Two in-situ treatment scenarios (one time flooding and continuous recirculation modes) were proposed to elevate moisture condition and deliver electron donors to vadose zone soil.

In this study, suitability of different electron donors to stimulate in situ reduction of perchlorate in saturated and vadose zone of the site was investigated using soil microcosm studies. Based on results from microcosm study, optimal electron donors were screened out and column tests were conducted with selected electron donors to simulate each treatment scenarios to obtain kinetic information and also evaluated possible impact on environments. Test results provided information for assessing the reactivity and longevity of each electron donors under the conditions expected in field application.

2.2   MATERIALS AND METHODS

Based on the perchlorate distribution at Lockheed Martin's Beaumont Site 2, perchlorate bioremediation was divided into three parts: permeable bioremediation zone

for groundwater plume, source area groundwater bioremediation and vadose zone bioremediation. To evaluate remediation strategies, corresponding microcosm studies and column studies were conducted for each part of remediation project.

2.2.1 Materials

Groundwater and soils used in this research were collected from Lockheed Martin Corporation's Beaumont Site 2 (Beaumont, CA). Groundwater used in bioremediation zone and source area microcosm studies was collected from a potential bioremediation zone and the aquifer underlying the identified source area. Corresponding aquifer soil samples from these areas were also collected for microcosm studies and column studies. Soil used in the vadose zone experiments was collected from 20 feet depth in source area vadose zone. All soil and groundwater samples were stored at 4°C before experiment.

In bioremediation zone study, Emulsified Oil Substrate (EOS) and EHC were used as electron donating substrates and EOS, glycerin, high fructose corn syrup, acetic acid, and sodium acetate were selected in source area remediation studies. A soybean based product, EOS was obtained from EOS Remediation, Inc. (Raleigh, NC) and contained 59.8% (by weight) soybean oil. Soy bean oil is a long lasting substrate which has been used as electron donor to promote bioremediation of perchlorate, and chlorinated solvents in many studies [21, 22]. Glycerin used in this study was manufactured by U.S. Glycerin (Kalamazoo, MI). High fructose corn syrup was supplied by Sweetener Products Co (Vernon, CA). Acetic acid and sodium acetate were purchased from Fisher Scientific. EHC (Adventus Americas Inc., Bloomingdale, IL) is a substrate that combines a plant-based carbon/energy source to stimulate microbial

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activity with a zero-valent iron component to rapidly generate and sustain reducing conditions. Compost and mulch were obtained from a local supplier.

2.2.2 Bioremediation zone for groundwater plume

2.2.2.1 Microcosm test

Microcosm tests were conducted to identify effective and long lasting electron donor substrates in bioremediation zone approach. Three substrates (EOS, EHC and compost) were initially selected in this experiment based on their physical properties. Groundwater and soil were collected down gradient from the Lockheed Martin Corporation's Beaumont Site 2 (Beaumont, CA). Groundwater quality and soil properties are summarized in Table 2.1.

All experiments were conducted in the 250 mL glass serum bottles; 50g of soil and 200ml of groundwater were added into each bottle at the ratio of 1:4 (w/w) with the exception of the set with compost as the amendment, where soil was replaced by the same amount of compost. EOS and EHC were fed at the dosages of 0.3% (w/w) and 0.1% (w/w) of soil, respectively. Perchlorate reduction experiments were performed both with and without nutrients added. For nutrient amendments, 1 g/L of diammonium hydrogen phosphate $((NH_4)_2HPO_4)$ was added.

Table 2.1 Groundwater quality and soil properties in suspected source area and potential bioremediation zone.

	Water sample (mg/L)		Soil sample (mg/kg)	
	Source	Bioremediation zone	Source	Bioremediation zone
Perchlorate	64.1	0.505	18	0.026
pH (unitless)	7.76	7.71	8.8	9.00
Total Organic	2.62	1.01	28.1	<10.7

Carbon Hardness (as CaCO$_3$)	240	242	-	-
Total Dissolved Solids	839	990	-	-
Total Kjeldahl Nitrogen	0.462	0.35	8.37	48.6
Nitrate (as N)	8.6	8.2	-	-
Total Phosphorus	0.107	0.0245	0.869	0.278
Total Sulfur	20.1	58.5	-	-
Chloride	305	186	-	-
Sulfate	55.6	176	18.7	40.6
Calcium	73.5	81.0	-	-
Magnesium	13.7	9.64	-	-
Potassium	3.47	0.733	-	-
Sodium	187	240	-	-
Arsenic	<0.0400	<0.0400	<4.63	< 4.29
Iron	<0.0666	<0.0400	22,300	13,900
Manganese	0.0325	<0.0300	417	231

Controls were prepared without any amendment addition to quantify the natural

perchlorate degradation or loss. All of the bottles were sealed with a septum cap, and the

headspace was purged with nitrogen gas before starting the experiment. The microcosms

were mixed manually three times a day to promote mixing. All amended and control

microcosms were prepared in triplicate and at room temperature. Water samples were

withdrawn through the septum cap and filtered through 0.22 µm filter for perchlorate and

nitrate analysis. Nitrate is a common anion in groundwater and it is favored over

perchlorate as electron acceptor in many perchlorate remediation systems [13, 23-25].

2.2.2.2 Column test

Column studies were conducted to simulate flow through perchlorate remediation

in a bioremediation zone. Based on microcosm results, EOS and EHC were selected as

electron donors. Column reactors used in this research were made of polyvinyl chloride (PVC) pipe (2 inch ID). To investigate the perchlorate reduction kinetic, different length (6-inch, 12-inch, 18-inch and 24-inch) of columns were packed with three different soil mixtures: (1) control with site soil only; (2) EOS (0.3% w/w)-amended soil; and (3) EHC (0.1% w/w)-amended soil. At the start-up period, all the columns were operated in an up-flow mode at a flow velocity of 0.5 ft/d which is close to the groundwater flow underlying the site. The flow rate was increased to 1.0 ft/d, and later 2.0 ft/d depending on the treatment performance. Effluent samples were taken periodically for perchlorate, pH, nitrate and TOC analysis.

2.2.3   Source area groundwater remediation

2.2.3.1 Microcosm test

EOS, glycerin, HFCS, acetic acid, and sodium acetate were considered as electron donor amendments for source area groundwater remediation. Two different concentrations of each amendment were tested in microcosms with or without additional nutrient. Source area groundwater (200 mL) was placed into 250 mL glass bottles together with 50g of the aquifer soil. As recommended by the manufacturer, EOS, glycerin and HFCS were added at 0.1 and 0.5% (w/w, electron donor / groundwater). Acetic acid was added at concentrations of 280 and 1,440 mg/L. Sodium acetate was at concentrations of 1,000 and 5,000 mg/L. $(NH_4)_2HPO_4$, 1 g/L, was added to the solution for nutrient-amended microcosms. A microcosm control was prepared using soil only

without substrate addition to quantify potential perchlorate degradation/loss from natural attenuation.

The flasks were sealed with a septum cap and the headspace was purged with nitrogen gas to maintain an anaerobic condition. The microcosms were mixed manually three times per day to promote mixing the solid substrate with the groundwater. All amended and control microcosms were run in triplicate and at room temperature. Water samples were withdrawn through the septum cap and filtered with 0.22 μm filter for later analysis.

2.2.3.2 Column test

To investigate degradation kinetics of high concentration of perchlorate in source area and evaluate possible environmental impact, column tests were conducted with source area groundwater and soil. After evaluating the effectiveness of selected amendments on perchlorate treatment performance by conducting microcosm tests, EOS and glycerin were selected as electron donor in column tests. Three sets of PVC columns (2 inch ID) with the lengths of 6-inch, 12-inch, 18-inch and 24-inch were constructed to access the performance of packing with (1) site soil only (control); (2) EOS (0.3% w/w)-amended soil; and (3) glycerin (0.3% w/w)-amended soil. EOS and glycerin were mixed with the soil before it was packed into the column. Columns were operated in an up-flow mode to prevent air trapping in the pore spaces. During the start-up period, all the columns were operated at a flow rate of 0.5 ft/d. Since the addition of nutrients had a limited effect on perchlorate reduction using EOS or glycerin in the microcosm test, nutrients were not added in the column tests. Samples were collected at selected times

for perchlorate, nitrate, pH, and TOC analysis. Individual samples were also collected for metal analysis.

2.2.4 Source area vadose zone soil remediation

Vadose zone soil used in this study was a sandy loam collected from 20 feet depth from the Beaumont, CA perchlorate contaminated site. The soil contained 100mg/kg perchlorate and 16.3mg/kg nitrate-N. Initial soil mass water content (MWC) was 11%; initial organic content was 2.83 % and initial pH was 8.5±0.2. The soil was passed through a 2-mm sieve and stored at 4°C until the experiment was conducted.

2.2.4.1 Microcosm test

Glycerin and EOS were initially used as electron donors in soil microcosms. Microcosms were prepared in 40-ml serum bottles with PTFE/silicone septa and screw thread cap under two different soil moisture conditions (20% MWC and saturated). To prepare the 20% MWC microcosms, soil was premixed with each electron donor and tap water (dechlorinated) to adjust soil moisture content to 20% and electron donor concentration to 5g/kg. Soil for control microcosms was also prepared with a 20% MWC through the addition of tap water. Dry equivalent premixed soil (40 g) was added into each bottle and headspace was flushed with nitrogen gas.

In saturated microcosms, 40 g dry equivalent soil and 26 mL of electron donor solutions were added into each bottle. Electron donor solutions were made by dissolving glycerin or EOS in tap water (dechlorinated). The electron donor concentration in each microcosm was 5 g/kg dry soil. Soil was mixed with the electron donor solution by

vortex mixer; head space was flushed with nitrogen gas. Control microcosms were set up without adding any electron donor. Twelve microcosm bottles were setup for each condition. For each sampling time point, triplicate microcosms were sacrificed to measure soil perchlorate, nitrate, pH, and TOC concentrations.

2.2.4.2 Column test

Column tests were performed to determine the effectiveness of two treatment scenarios of perchlorate remediation in vadose zone soil, batch mode and recirculation mode, with EOS or glycerin as electron donor. Columns were made up of 2-inch clear PVC pipe, 6 inches in length. For each experimental condition, six sacrificial columns were packed with vadose zone soil to 1.3 g/mL bulk density which is close to the soil density in the field. Soil columns were amended with the electron donor by applying different substrate solutions either in batch mode or recirculation mode. The substrate solution consisted of local (Riverside) dechlorinated tap water containing EOS or glycerin at a concentration of 5 g/L and 20 mg/L of diammonium phosphate. Dechlorinated tap water was used as substrate solution for control columns.

Batch mode column tests were used to simulate soil flooding, followed by natural drainage (see Figure 2.2). Substrate solutions (300 mL or approximately two pore volumes) were pumped upward into the packed vadose zone columns at a rate of about 1 mL/min. Once the solution was added and the columns were saturated, the effluent port was opened and the column was allowed to drain. Collected leachate was analyzed for perchlorate, nitrate, pH, and TOC. Once the columns were drained, the effluent ports were closed and the columns were left with the top open to the atmosphere. Every

sampling time point, columns were destructed and the soils were homogenized. Representative soil samples from each column were measured for perchlorate, nitrate, pH, TOC, and moisture content at 0.5, 1, 2, 4, and 8 weeks.

Recirculation mode testing was used to simulate a recirculation approach in which an initial application of electron donor is applied to the surface and allowed to migrate into the underlying groundwater (see Figure 2.2). In this approach, the vadose zone soil is maintained at saturated conditions by pumping the underlying groundwater and applying it over the surface. Substrate solution were applied on a recirculating basis at a rate of about 0.1 mL/min, which is equivalent to about one pore volume per day. The solution was pumped from and returned to a reservoir to simulate the recirculation process. Columns were also sampled on a sacrificial basis. Representative soil samples from each column were measured for perchlorate, nitrate, pH, TOC, and moisture content at 0.5, 1, 2, 4, and 8 weeks. Also, recirculated water from the reservoir was analyzed for perchlorate, nitrate, pH, and TOC.
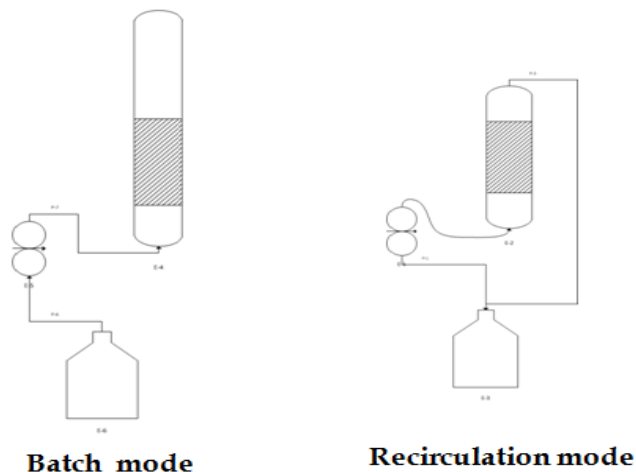
Figure 2.2 Schematic of two in situ treatment scenarios for perchlorate in vadose zone soil.

2.2.5   Analytical methods

2.2.5.1 Water Analysis

Perchlorate in water sample was analyzed using a Dionex 1000 Ion chromatograph (Dionex Corp.,Sunnyvale,CA,USA) with an IonPac[®] AS 16 analytical column (4×250 mm) and AG 16 guard column (4×50 mm). Nitrate was determined by an IonPac[®] AS 14 analytical column (4×250 mm) and AG 14 guard column (4×50 mm). The detection limits for perchlorate and nitrate were 4 μg/L and 100 μg/L (as N), respectively. In addition, all the other analyses for the parameters were conducted according to the EPA standard methods.

2.2.5.2 Soil Analysis

Perchlorate in soil samples were extracted from 10 g dry equivalent soil by vortexing with 20 ml of deionized water for 2min. The extracts were centrifuged at 5000×g and filtered through 0.2 μm filter to remove soil particles. This extraction method

was able to recover 98±4% of perchlorate in soils. The extracts were also used to analyze dissolved part of nitrate and TOC in soil samples. Ion chromatography (Dionex, ICS 1000) system was used to measure perchlorate and nitrate with IonPac AS16 column and IonPac AS14 column.

2.3   RESULTS AND DISCUSSIONS

2.3.1   Bioremediation zone for groundwater plume

The initial perchlorate concentrations were 505 μg/L and 26 μg/kg in the groundwater and soil, respectively. Perchlorate has poor adsorption on soils and readily transported through bulk movement of water [5]. The amount of perchlorate detected in the soil may be due to the perchlorate in soil pore water. The groundwater pH was 7.7, which is favorable for biological treatment. Nitrate concentration in the groundwater was 40 times that of perchlorate. Nitrate is well known competitive inhibitor of perchlorate bioremediation. Since nitrate is more favorable as electron acceptor in environment, the electron donor will be consumed by nitrate reduction before perchlorate reduction occurs. Sufficient electron donor is required to overcome the inhibition effect of nitrate [13, 23-25]. The total organic carbon concentration in the groundwater was 1.01 mg/L, which was not sufficient to achieve perchlorate reduction. Low levels of TKN and phosphorus in the water sample may not be enough to support microorganism growth. Nutrient-amendment was considered when conducting the microcosm experiments.

2.3.1.1 Bioremediation zone microcosm test results

2.3.1.1.1 Impacts of amendment and nutrient addition on perchlorate reduction

EOS, EHC and compost were selected as electron donors for the microcosm tests to investigate the effectiveness of reducing perchlorate in bioremediation zone. EOS is formulated with biodegradable vegetable oil and it has been widely used in anaerobic bioremediation [21, 22]. EHC is a controlled-release electron donor source which consists of integrated carbon and zero valent iron (ZVI). Compost is a widely used as fertilizer to conditioning soils. Compost can release organic carbons such as humus that can be utilized as electron donor for microbial respirations [26]. The results of perchlorate reduction using different electron donors are shown in Figure 2.2 and Figure 2.3.
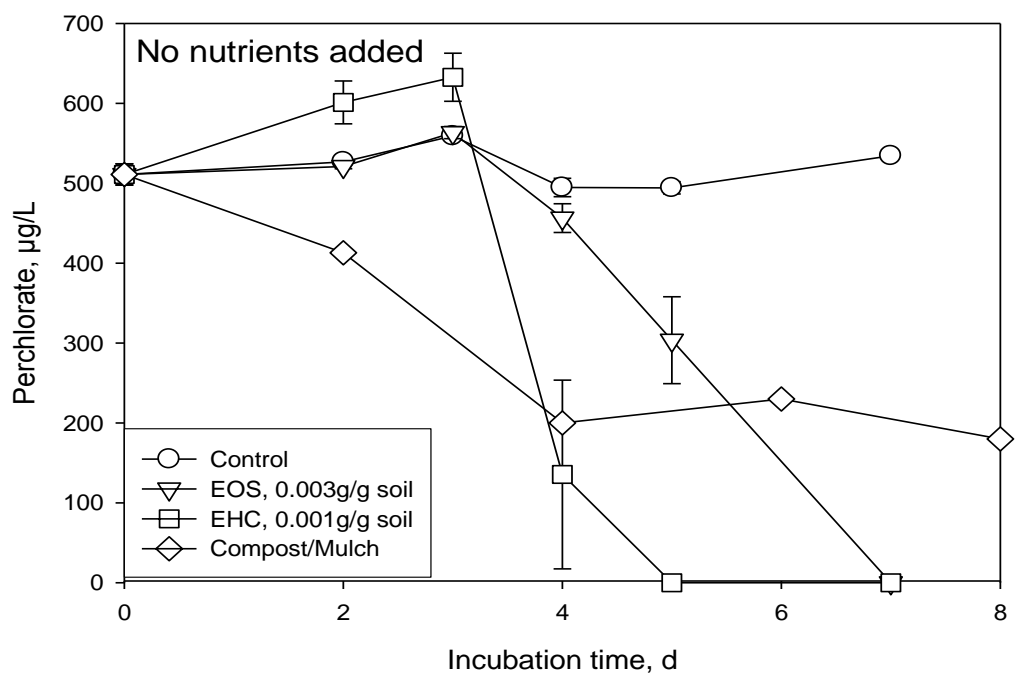
Figure 2.3 Perchlorate reduction in bioremediation zone microcosms without additional nutrients
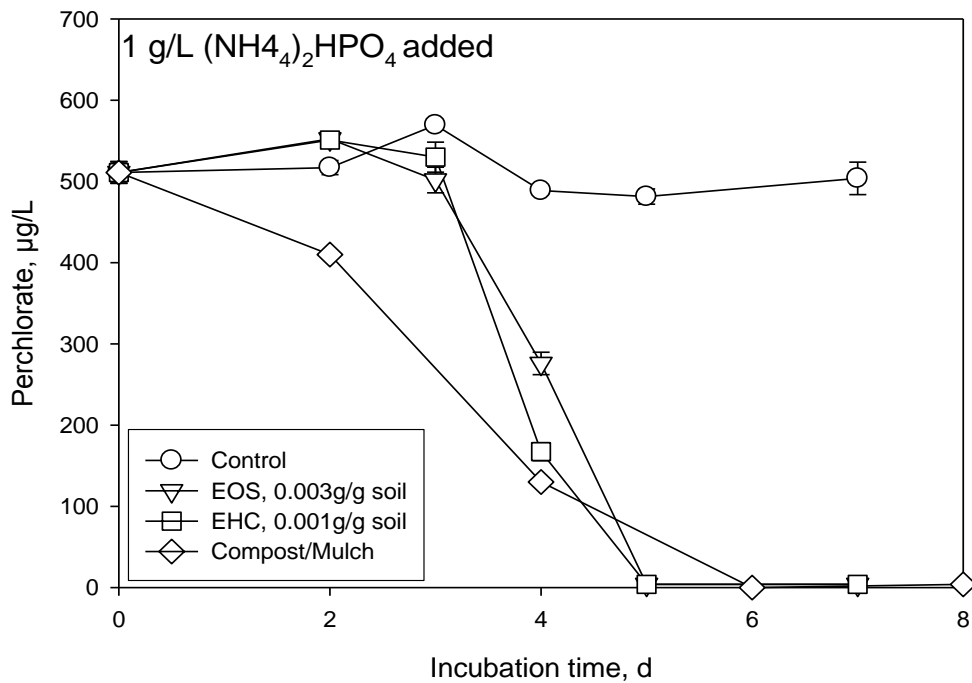
Figure 2.4 Perchlorate reduction in bioremediation zone microcosms with nutrients

In the controls, there was no removal of perchlorate either in the absence or presence of nutrients. Natural organic matter in soil and groundwater was not sufficient to achieve perchlorate reduction. Perchlorate reduction was observed in all other microcosms with different electron donor amendments. Thus, it was concluded that soil samples from the site contained perchlorate reducers and that EOS, EHC and compost successfully stimulated perchlorate reduction in microcosms. For both the treatments with and without nutrient addition, the best performance among all the electron donors chosen in this study was EHC treatment, which achieved 100% removal efficiency after 5 days. Complete removal was achieved in 7 days with EOS and 64.8% removal efficiency was achieved after 8 days with compost amended soil.

$(NH_4)_2HPO_4$ was added to supply additional nutrients to promote microorganism growth in barren conditions. Based on the results listed in Table 2.2, addition of nutrient did not enhance the removal rate of EHC treatment, but did increase perchlorate removal rates using EOS and compost amended treatment.

Table 2.2 Perchlorate reduction rate with different electron donors with or without nutrients.

Electron donor	Reduction rate (μg/L/d)	
	w/o Nutrients	w/ Nutrients
EOS	142.0	187.0
EHC	314.2	262.9
Compost/Mulch	39.76	90.05

2.3.1.1.2   Impacts of nitrate on perchlorate reduction

Nitrate is a more favorable electron acceptor compared to perchlorate in perchlorate bioremediation systems. As shown in Figure 2.4 and 2.5, nitrate reduction in both controls with nutrient absence and presence was observed after a 3-day lag period. Denitrification in control microcosms may have been due to the natural organic matter in groundwater and soil.

In the three electron donor amended treatments, 100% removal of nitrate was achieved within four days or less without obvious lag periods. Nitrate depletion was followed by perchlorate reduction in most of microcosms. This result was consistent to the findings of other researchers [27-29]. Thus, lag periods in perchlorate reduction can be predicted by the nitrate reduction rate and nitrate concentration. Among three

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microcosm sets, compost amended microcosms had best denitrification rate. Although

perchlorate reduction rate in compost microcosm was lower than EOS and EHC

microcosms, perchlorate reduction occurred earlier than the other two. The addition of 1

g/L $(NH_4)_2HPO_4$ did not significantly affect nitrate reduction in all microcosms.
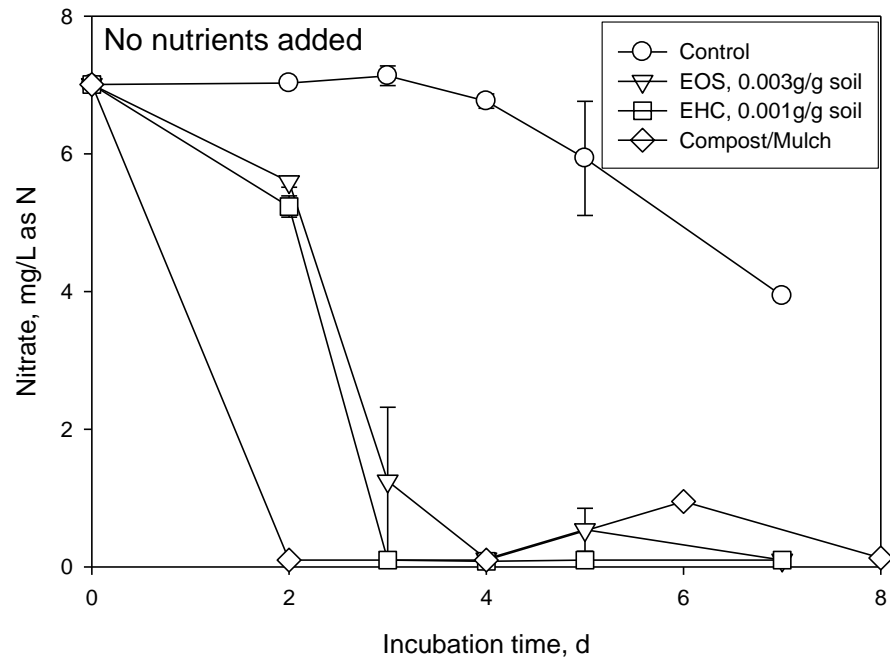
Figure 2.5 Nitrate reduction in bioremediation zone microcosms with no nutrients added
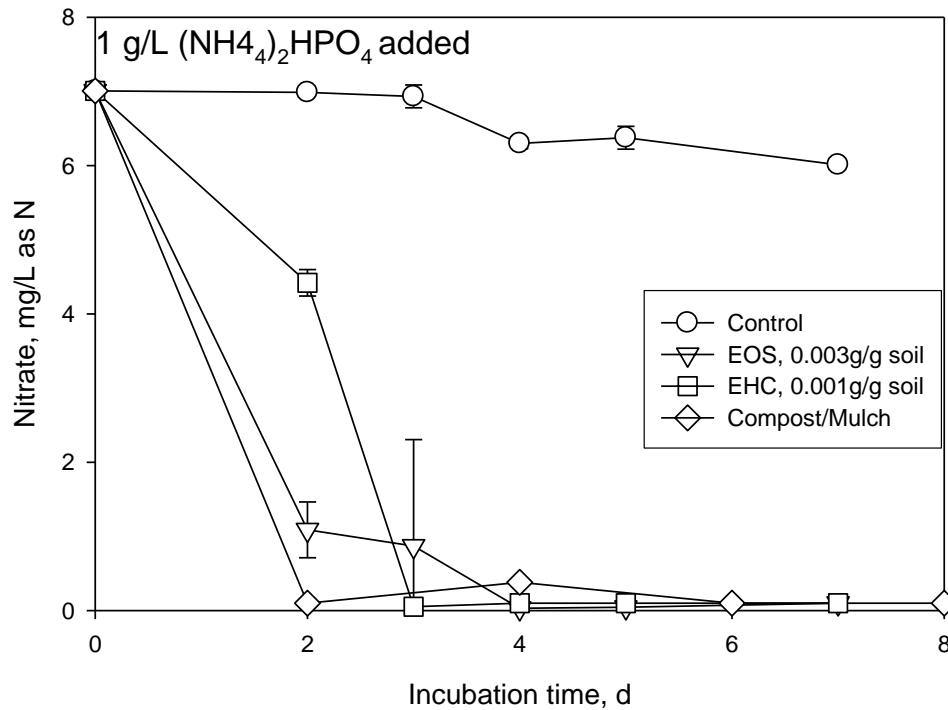
Figure 2.6 Nitrate reduction in bioremediation zone microcosms with 1 g/L (NH₄)₂HPO₄

2.3.1.2 Bioremediation zone column studies

2.3.1.2.1   Perchlorate degradation kinetics in soil columns

Based on the microcosm study, EOS and EHC were selected as amendment for bioremediation zone column study. Groundwater containing 500 ± 50 μg/L of perchlorate and 8.6 mg/L nitrate as N was fed upward into soil columns of different lengths (6, 12, 18, 24 inches) at the rate of 0.5 ft/d. After two weeks of operation, perchlorate reduction was achieved in all columns amended with EOS and EHC. Results, shown in Figures 2.6, were the average of 20 days performance after two weeks lag period. It appears that the 6-inch column, which had a 24-hr EBRT at rate of 0.5 ft/d, had sufficient reaction time to

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degrade 500 μg/L of perchlorate to non-detectable levels in both EOS and EHC amended

columns. No difference in EOS and EHC amended columns was observed in terms of

effectiveness of perchlorate reduction. No perchlorate reduction was observed in control
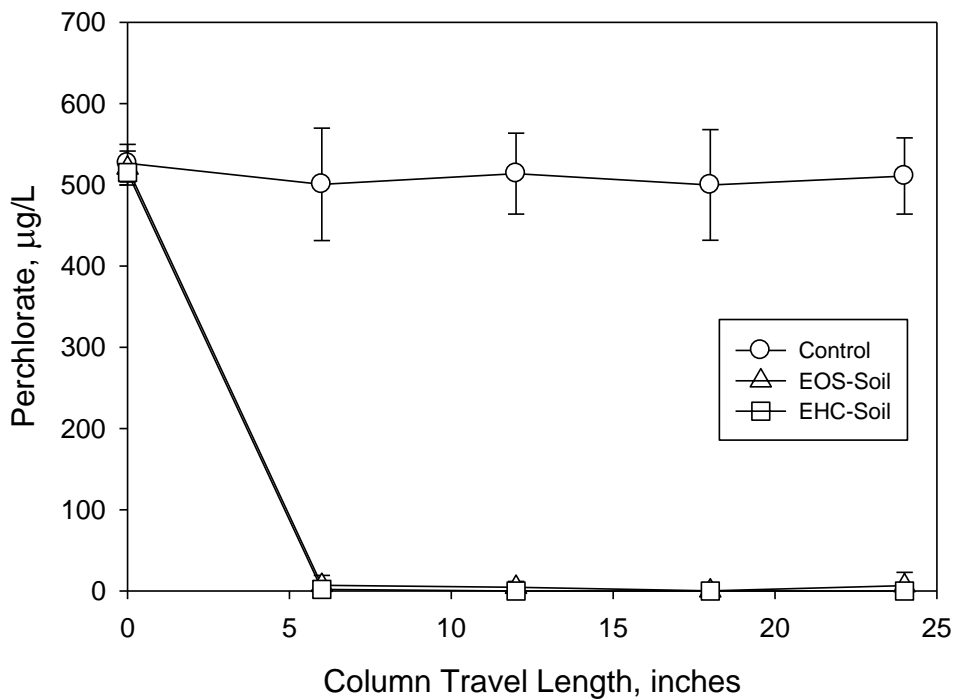
columns.

Figure 2.7 Perchlorate degradation in 6, 12,18 and 24 inches columns at Darcy velocity of 0.5 ft/day. Control columns were packed with soil sample without addition of electron donor.

To further investigate perchlorate reduction kinetics, groundwater feed was

increased to 1ft/d.   Removal efficiency at this condition had some variations from time to

time. The results shown in Figure 2.7 are the average of 28- and 14-day performance in

EOS and EHC amended columns. Perchlorate was degraded to none-detectable level in

both 24 inch columns (48h EBRT). Perchlorate breakthrough was observed in 6, 12 and 18 inch columns at different times, which was related to the length of the column. In the 6-inch EHC column, perchlorate reduction was not observed at a rate of 1ft/d. In the 12 inch columns (24h EBRT), 16 and 60% of perchlorate breakthrough were observed at flow rate of 1ft/d. Perchlorate reduction rate in soil columns decreased significantly after the flow rate was increased to 1ft/d.

Unexpected decrease in perchlorate reduction rate is possibly caused by the limited electron donors in soil columns. When the column operation was started, sufficient electron donor was available in the soil columns. At this condition, perchlorate reduction was only controlled by availability of perchlorate. As time goes on, electron donors (EHC and EOS) premixed with soil was gradually degraded or washed out. Perchlorate reduction rate may also be limited by available electron donor concentration. It can be readily expected that reduction rate would decrease with operating time.
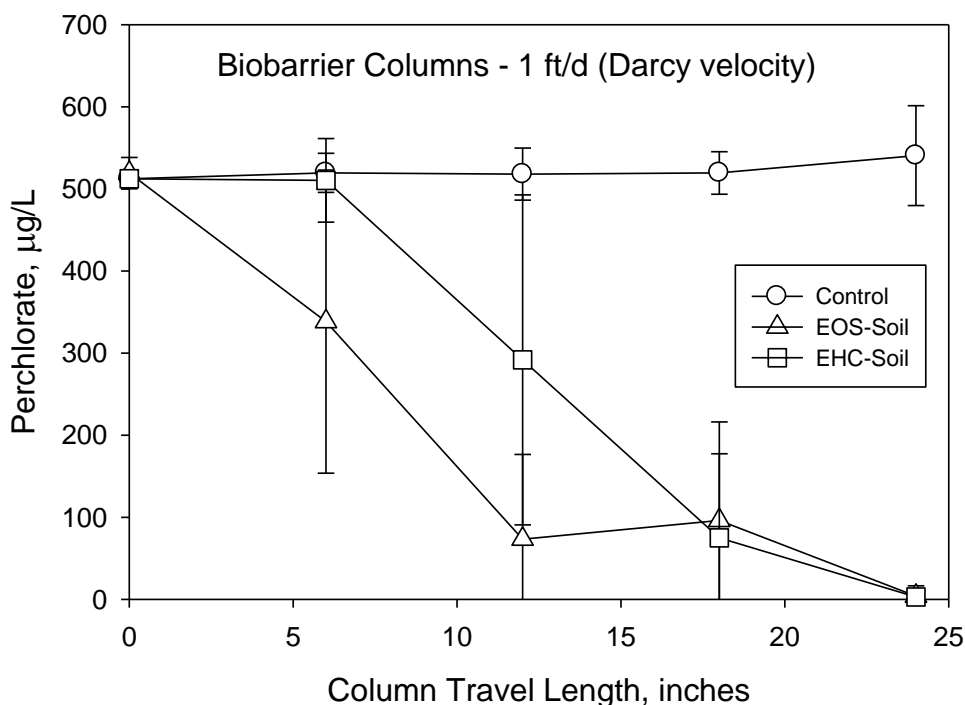
Figure 2.8 Perchlorate degradation in different length columns at Darcy velocity of 1 ft/day.

2.3.1.2.2   Longevity of EHC and EOS amended columns

During the first 45 days of operation, the flow rate was maintained at 0.5 ft/d in all the columns. In the EHC-amended soil columns, although perchlorate removal was observed in the first two weeks for the 12-inch, 18-inch and 24-inch columns, perchlorate concentration in the effluent kept increasing thereafter. The longevity of the EHC-amended column was much shorter than expected. A possible reason was that the column had been prepared about one month before this experiment started. So those columns were replaced by a set of newly prepared columns on Day 22. After several days of fluctuation, complete removal of perchlorate was achieved in all columns, and

perchlorate concentration of the effluent was below the detection limit of 4 μg/L. The flow rate was doubled to 1.0 ft/d on Day 46. Perchlorate breakthrough was observed in all the EHC columns. After increasing the flow rate to 2.0 ft/d, there was no reduction in perchlorate. Therefore, the flow rate was reduced to 0.5 ft/d to investigate whether the system would return to the initial performance. No removal was observed in the 6-inch column, but partial or complete removal was observed in the 12-inch, 18-inch and 24-inch columns in the first few days after the adjustment. Then perchlorate in the 12-inch and 18-inch columns gradually increased to the influent level, but perchlorate remained non-detectable in the 24-inch column until the end of the test. Depletion of EHC amendment might be the reason causing the different performances between the four columns.
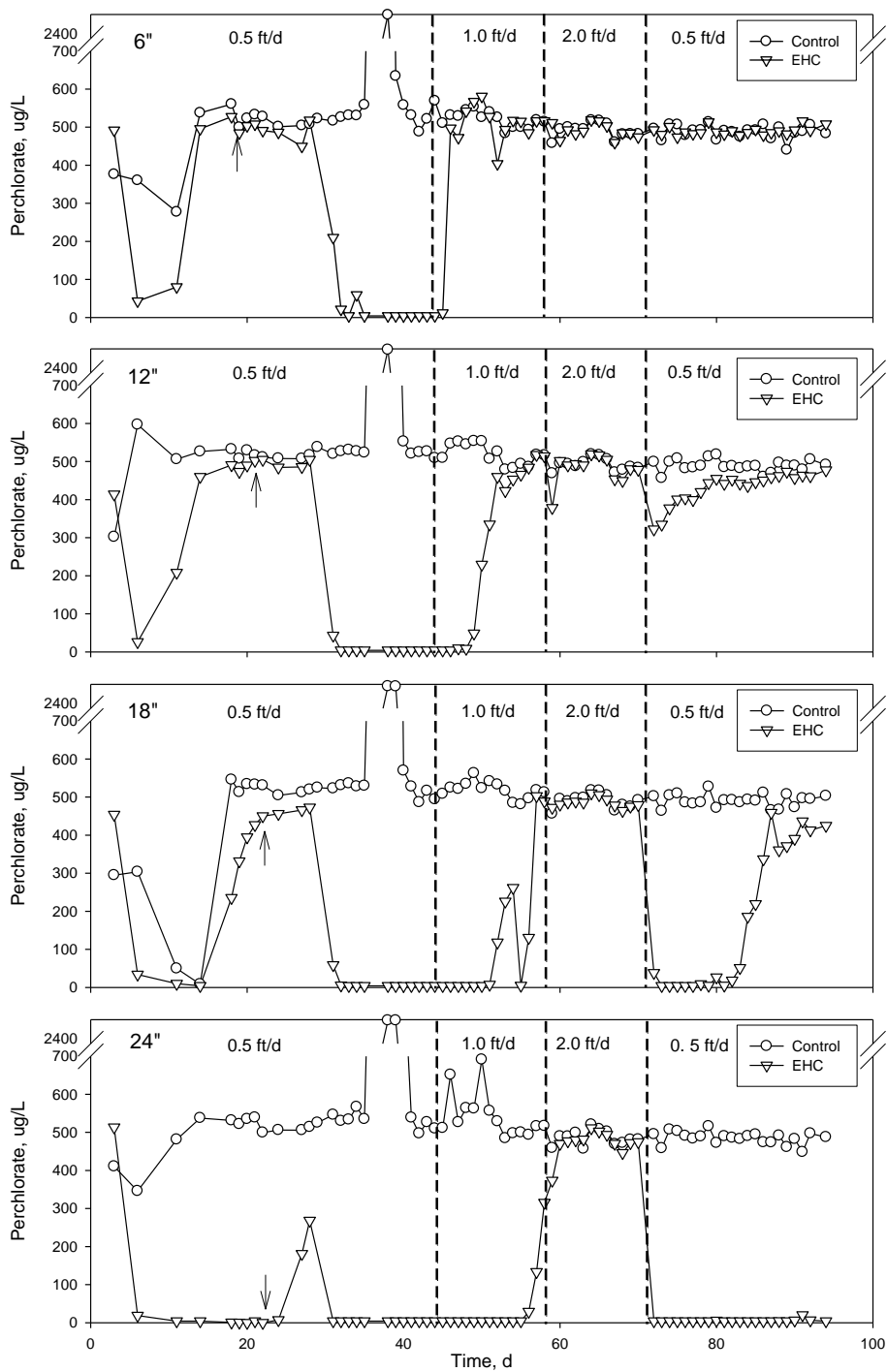
Figure 2.9 Perchlorate reduction in control and EHC-amended soil columns. Arrow symbol indicated the day new columns were initiated.
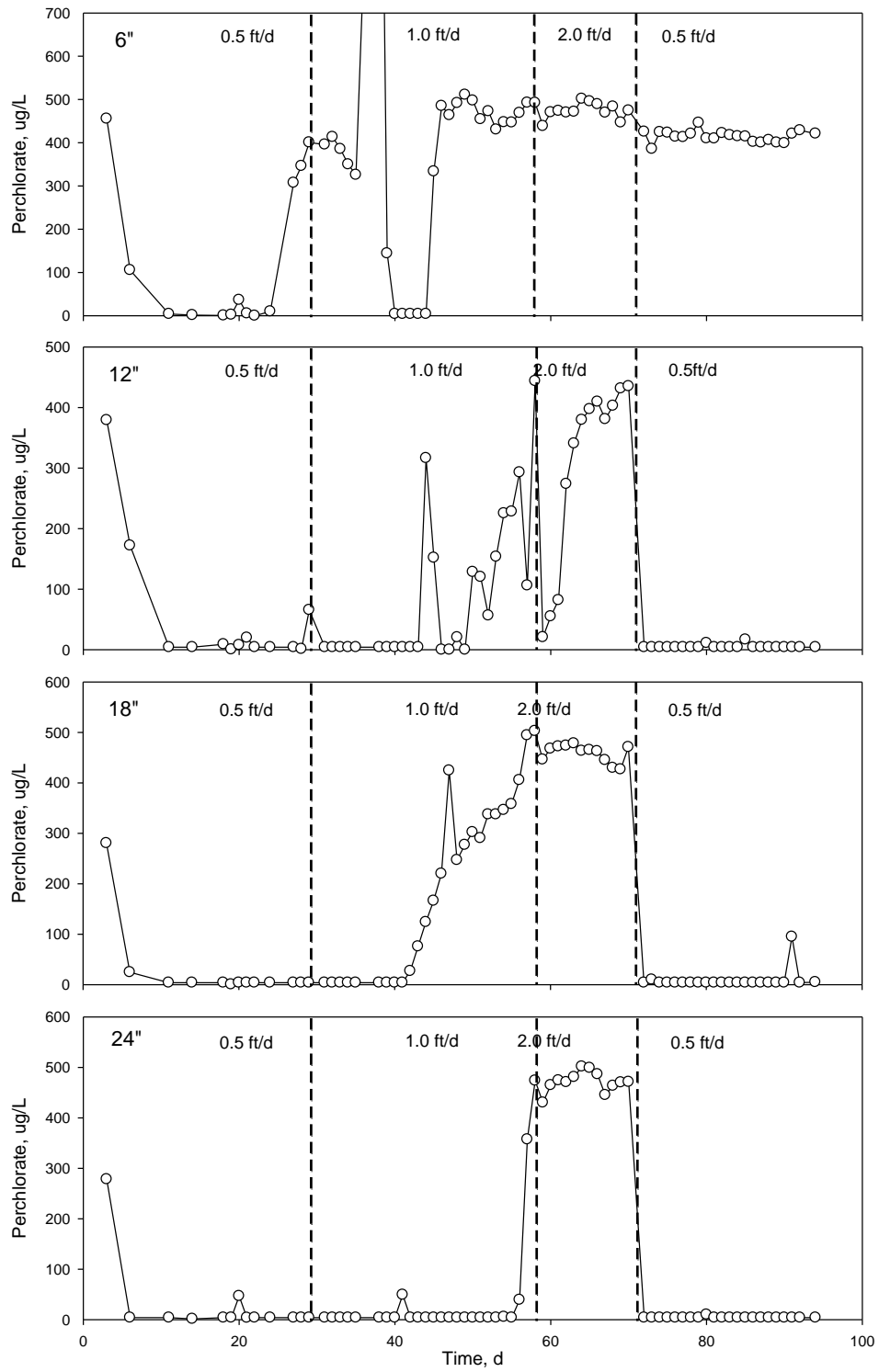
Figure 2.10 Perchlorate reduction in EOS-amended soil columns.

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In the EOS-amended columns, complete removal of perchlorate was achieved within two weeks when operated at the flow rate of 0.5 ft m/d. When the flow rate was doubled, perchlorate breakthrough was noticed in the 6-inch length column first, followed by the 12-inch and 18-inch columns. Measurable perchlorate was only found much later for the 24-inch column when it was running at a 1.0 ft/d flow rate. After increasing the flow rate to 2.0 ft/d, the effluent perchlorate matched the feed level in all the columns. Unlike EHC-amended soil columns, no perchlorate was detected in the columns except the shortest one (6-inch) after returning the flow to 0.5 ft/d, which indicated insufficient retention time for that column. The significant differences in the performance between EHC and EOS can be explained by the properties of the substrates. Compared to EHC, EOS adsorbs more strongly to the soil and leaches out at a slower rate than EHC.
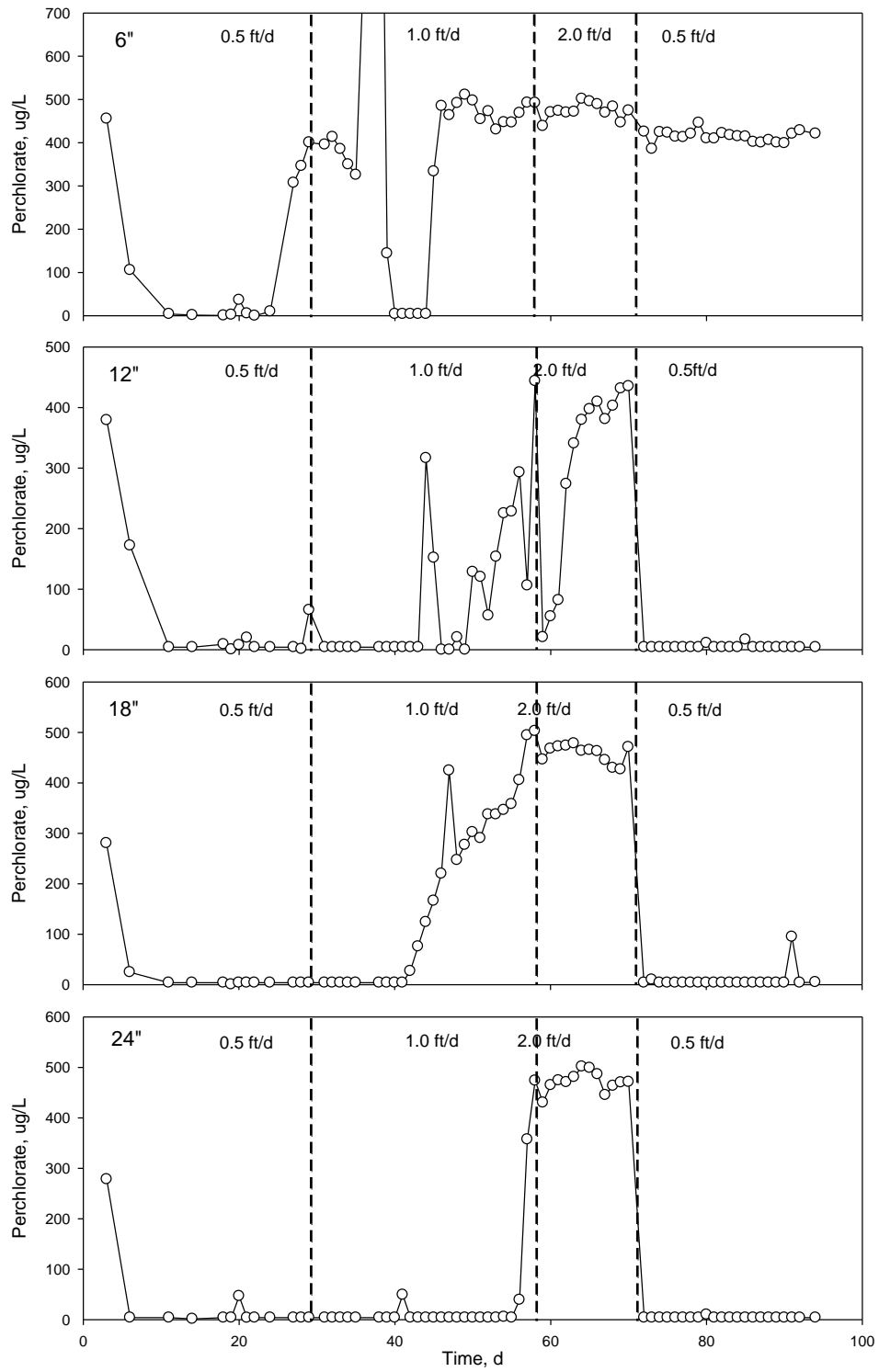
Figure 2.11 Perchlorate reduction in EOS-amended soil columns.

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5.4.2.2.3 Water Quality Evaluations

The effluent water quality analyses for nitrite, arsenic, iron and manganese are summarized in Table 2.3.

No nitrite and arsenic were observed in all the column experiments. However, increased concentrations of iron and manganese were noticed relative to the background levels in all the effluents. The elevated iron concentration was reasonable for EHC-amended columns. EHC is a mixture of nutrients and zero-valent iron so the addition of EHC will be a potential source of iron. Another possible source of iron is soil minerals. In an anoxic condition, iron reducing bacteria can readily reduce ferric iron in soil minerals to ferrous iron which is more soluble in water [21]. More details about ferric iron reduction in soil columns will be discussed in Chapter IV. Although these results indicated a potential for metals to leach from the bioremediation zone, they can be immobilized by either adsorption to the aquifer matrix or by precipitation with other ions after migrating downstream where the redox condition increases [22].

Table 2.3   Secondary water chemistry analyses for amended barrier columns.

Parameter	MDL[a], mg/L	EOS amended soil, mg/L	EHC amended soil, mg/L
Nitrite as N	0.09	ND	ND
Arsenic	0.07	ND	ND
Iron	0.15	0.5	4.0
Manganese	0.07	0.14	0.77

[a]MDL-Method Detection Limit.

[b]ND - Non Detectable.

2.3.2   Source area groundwater remediation

The source area groundwater contained approximately 64,000 µg/L perchlorate (shown in Table 2.1). The pH of the groundwater and soil were 7.8 and 8.8. Nitrogen was present mostly in the form of nitrate, which was 8.6 mg/L as N in groundwater sample. The total organic carbon concentrations were 2.62 mg/L in the groundwater sample and 28.1 mg/kg in the soil sample, which was not sufficient to deplete oxygen and achieve anoxic condition and stimulate perchlorate reduction. The TKN level was 8.4 mg/kg and 0.46 mg/L in soil and groundwater, respectively. Total phosphorus was detected at trace levels in both soil and groundwater samples. These results indicate the macronutrients were present at relatively low levels and additional nutrients might be needed to support the growth of perchlorate degraders.

2.3.2.1 Source area groundwater microcosm results

To stimulate perchlorate biological reduction, addition of a suitable electron donor is required. Perchlorate reducing bacteria can utilize wide various organic substrates. Suitable candidate for electron donor should be effectively stimulate perchlorate reduction, readily dissolve in water, have long persistent after injection and have less impact on environment after remediation. Based on these requirements, five organic substrates EOS, glycerin, high fructose corn syrup (HFCS), acetic acid (HAc) and sodium acetate were selected in microcosm studies. Microcosm tests were conducted with each electron donor at two different concentrations and with or without nutrient addition.

2.3.2.1.1   Effect of different electron donors in different dosages

Source area microcosm results are reported in Figure 2.10 through 2.11. Reduction of perchlorate will not start until the oxidation reduction potential (ORP) decreases to -150 mV [31], which requires the depletion of dissolved oxygen and nitrate. In general, perchlorate degradation will experience a lag period. During these lag periods, soil microbes utilize oxygen and nitrate as electron acceptors, which are preferable in environment compared to perchlorate.

As can be seen from Figure 2.10, all amendments except HAc were able to stimulate perchlorate reduction under lower dosage and without nutrient. Failure of perchlorate reduction in HAc amended microcosms may have been to the low pH associated with HAc. Addition of HAc decreased pH in the microcosm from 7.6 to 4.7. Perchlorate degradation had similar kinetics in EOS, glycerin and NaAc amended microcosms. Perchlorate reduction started after a 7-day lag period; complete degradation occurred within 11to 14 days. HFCS amended microcosms had a longer lag period and lower reduction rate than the other three electron donors.
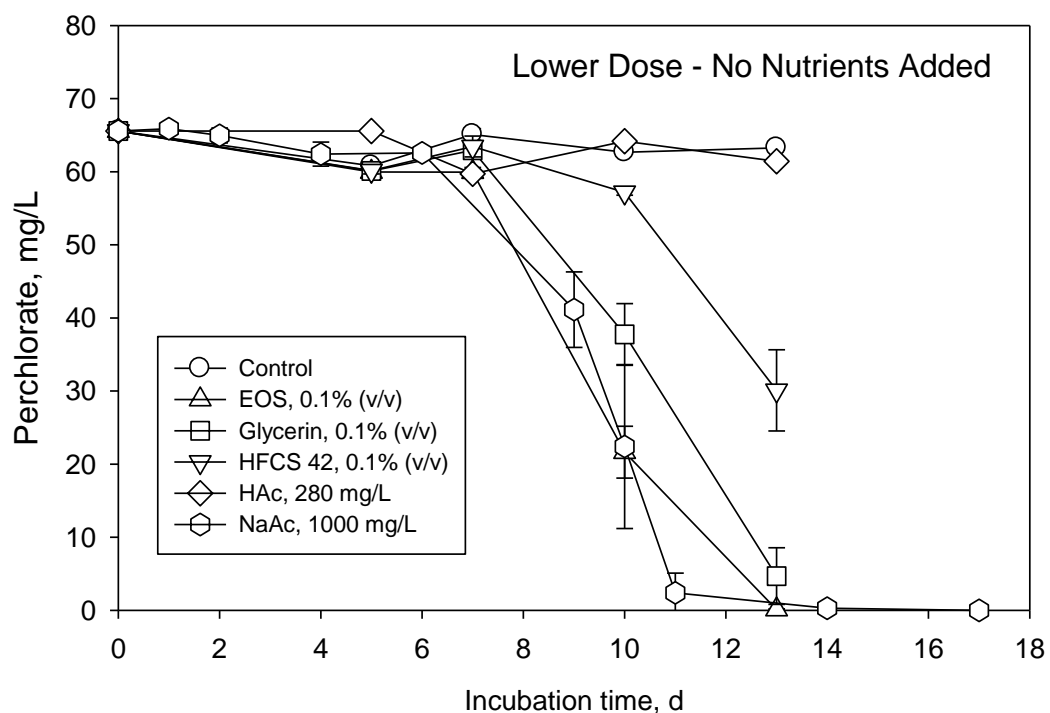
Figure 2.12 Perchlorate degradation in source area microcosms with lower dosage of different electron donors.

Under higher dose conditions (0.5%, v/v), perchlorate reduction in EOS and glycerin microcosms had similar results with lower dose ones (0.1%, v/v). Perchlorate degradation with NaAc at the higher concentration had a lag period that was 3 days longer than with lower dosage. It is possible that perchlorate reduction was delayed by increased salinity due to the addition of NaAc.

The higher dosage of HFCS appears inhibit perchlorate reduction. While the lower dosage showed 50 $\pm$ 9.2% removal, the higher dosage had 6.8 $\pm$ 1.1 % removal instead. The reason for performance drop after increasing HFCS dosage can be contributed to the pH. At the lower dosage, the final pH at Day 13 was 7.0, which was

similar to the starting. At the higher dosage of HFCS, the final pH dropped to 5.7 ± 0.06, which is not favorable for perchlorate bioremediation [32]. Significant pH drop in higher dosage HFCS microcosm may have occurred due to over accumulation of fermentation products such as lactic acid.
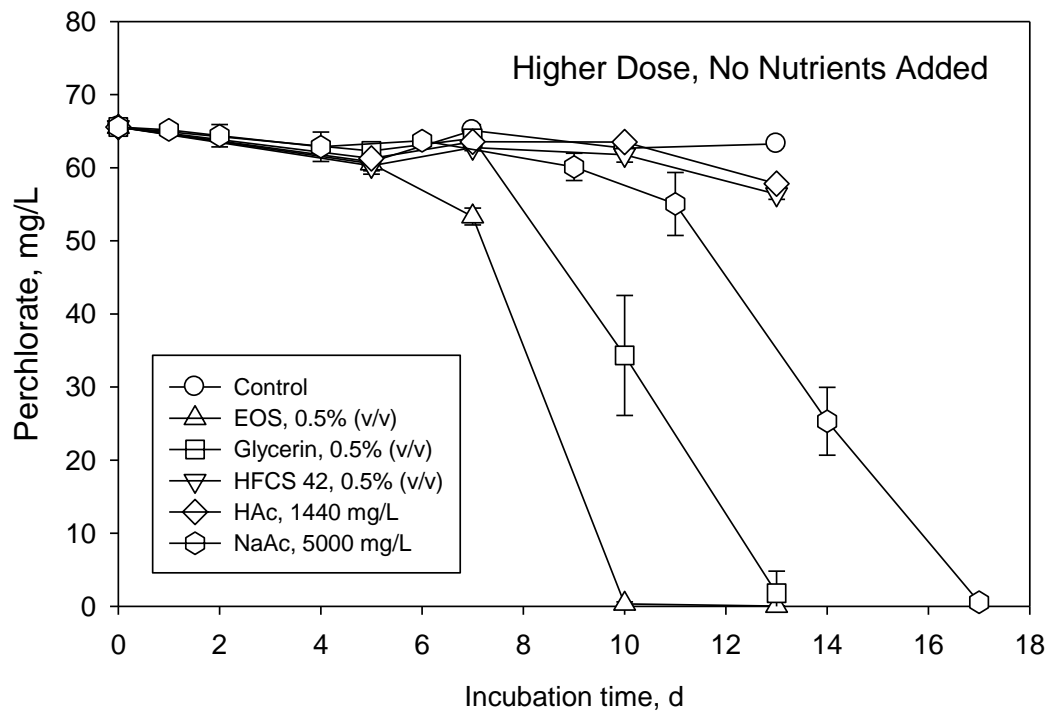
Figure 2.13 Perchlorate degradation in source area microcosms with higher dosage of different electron donors.

In conclusion, EOS, glycerin and sodium acetate had better performance in this set of microcosm studies. Lower dosage of electron donors was sufficient to stimulate and completely degrade 64mg/L perchlorate in groundwater within 14 days. Increasing electron donor in EOS and glycerin microcosms had limited effect on perchlorate

reduction while it had an inhibitory effect in the acetate microcosms. Acetate is the most widely used and accepted electron donor in perchlorate bioremediation [12, 33, 34]. Although no research reported the inhibition effect of acetate on perchlorate reduction, inhibitions of acetate on other microbes have been reported. Propionate degradation in an upflow anaerobic sludge bed was severely inhibited by the addition of 50 mM acetate to the influent[35]. In a feed controlled batch fermentation, the addition of various concentrations of sodium acetate to the growth medium resulted in a logarithmic decrease, with respect to acetate concentration. The inhibition effect of 5000 mg/L of acetate on perchlorate reduction was not severe and the mechanism behind the effect was not clear. But in a continuous flow system, excess dosage of electron donor may be required. Due to this concern, EOS and glycerin were selected in the further study.

2.3.2.1.2 Effect of nutrient addition

To determine whether nutrients should be added, microcosm results for EOS and glycerin are summarized in Figure 2.12 and Figure 2.13 respectively. At both lower and higher dosages EOS microcosms, the addition of nutrient decreased the lag period by 1 to 2 days while the effect on degradation rate was marginal. Nutrient addition only shortened the lag period in lower dosage glycerin microcosms. No significant differences were observed with or without nutrient microcosms in higher dosage glycerin microcosms. Nutrient addition seemed beneficial for perchlorate degradation but the effects were limited. Completed removal was observed in all EOS and glycerin amended microcosms. On the other hand, when the electron donor was consumed in the field,

addition of DAP could induce nitrite and nitrate formation within the groundwater due to nitrification of the ammonium in the nutrient. Hence, no nutrients were added in the column tests.
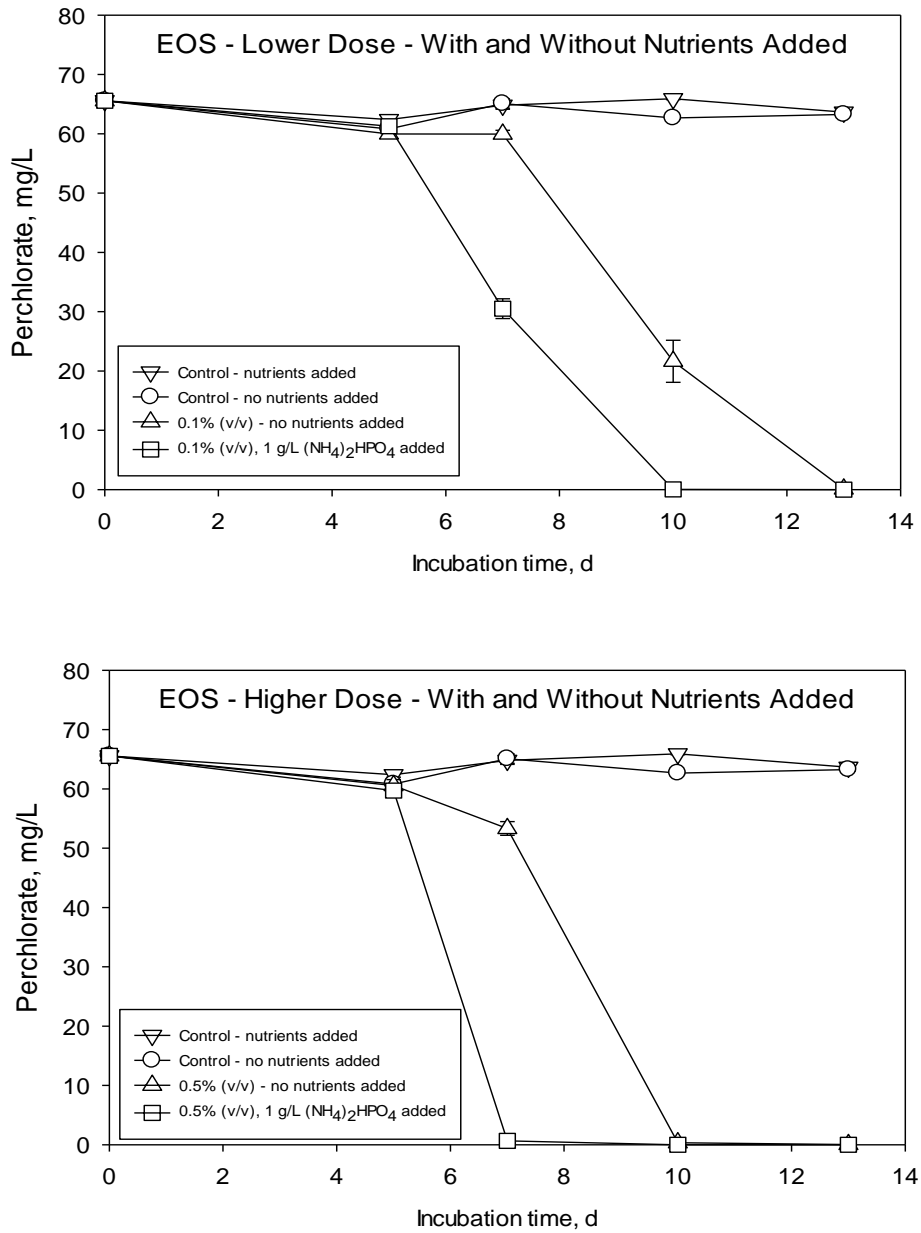
Figure 2.14 Perchlorate reduction in EOS-amended source area microcosms (Top: Low dose; Bottom: High Dose)
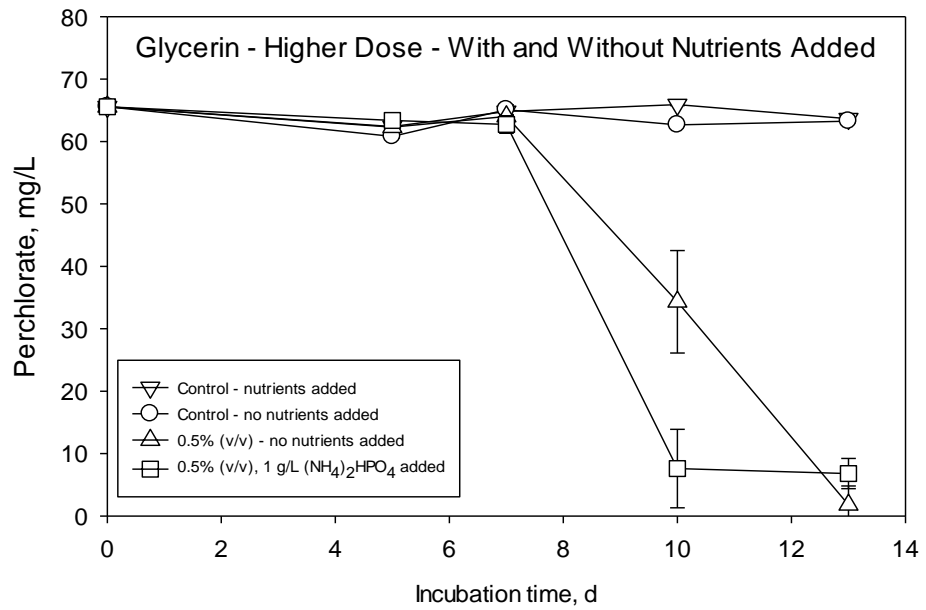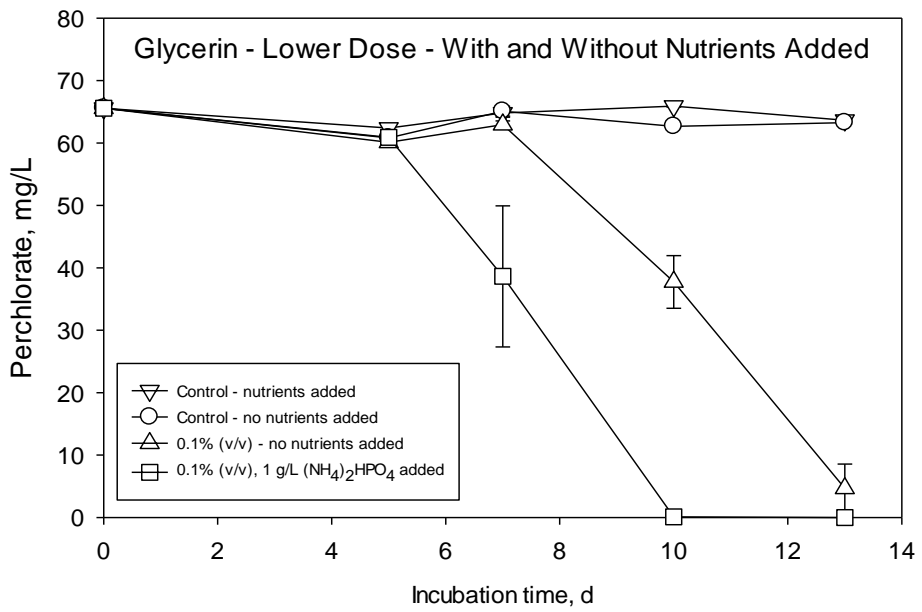
Figure 2.15 Perchlorate reduction in glycerin amended source area microcosms (Top: Low dose; Bottom: High Dose)

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2.3.2.1.3   Water quality changes

Apart from the perchlorate reduction, the secondary water quality, which resulted from the substrate addition, should be carefully considered before implementing anaerobic bioremediation treatment. Because biodegradation of perchlorate can only happen under reducing conditions, the metals such as arsenic, iron and manganese existing either from the natural environment or the addition of substrate will become more soluble, and thus the mobility of these metals will increase. In addition, nitrite, which is regulated by EPA due to its adverse health effects, may be formed as an intermediate product of nitrate reduction. Initial and final water quality analyses for these constituents are provided in Tables 2.4, Table 2.5 and Table 2.6.

As expected, no nitrite was formed during reduction of nitrate treated with EOS and glycerin. But it was found in the control microcosms at the end of the test. Arsenic, iron and manganese were all below the detection limit in the control and glycerin microcosm, but minor solubilzation of manganese appeared to occur at the reducing conditions in the EOS microcosms (Table 2.6).

Table 2.4 Initial-final water analyses for 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

Table 2.5 Initial-final water analyses for EOS-amended 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	1.7	ND	4.7	ND
Arsenic	0.070	ND	ND	ND	ND
Iron	0.15	ND	ND	ND	ND
Manganese	0.070	0.083	0.39	ND	0.22

Table 2.6 Initial-final water analyses for glycerin-amended 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

2.3.2.2 Source area groundwater column results

Column tests were conducted to 1) verify the feasibility of in situ bioremediation technology in the contaminated site; 2) investigate degradation kinetics of perchlorate in the groundwater system; 3) further evaluate selected electron donor amendments in continuous flow system. Based on the microcosm study, EOS and glycerin were selected as amendment for source area groundwater column study and 0.3% w/w ratio was selected. Groundwater containing 64mg/L of perchlorate was feed upward to different length (6, 12, 18, 24 inches) of soil columns, which were premixed with EOS or glycerin at the rate of 0.5 ft/d. Column tests results are summarized in Figure 2.14 through Figure 2.16. As expected, there was no concentration change in all lengths of control columns during the entire operation time which indicated no natural attenuation occurred.
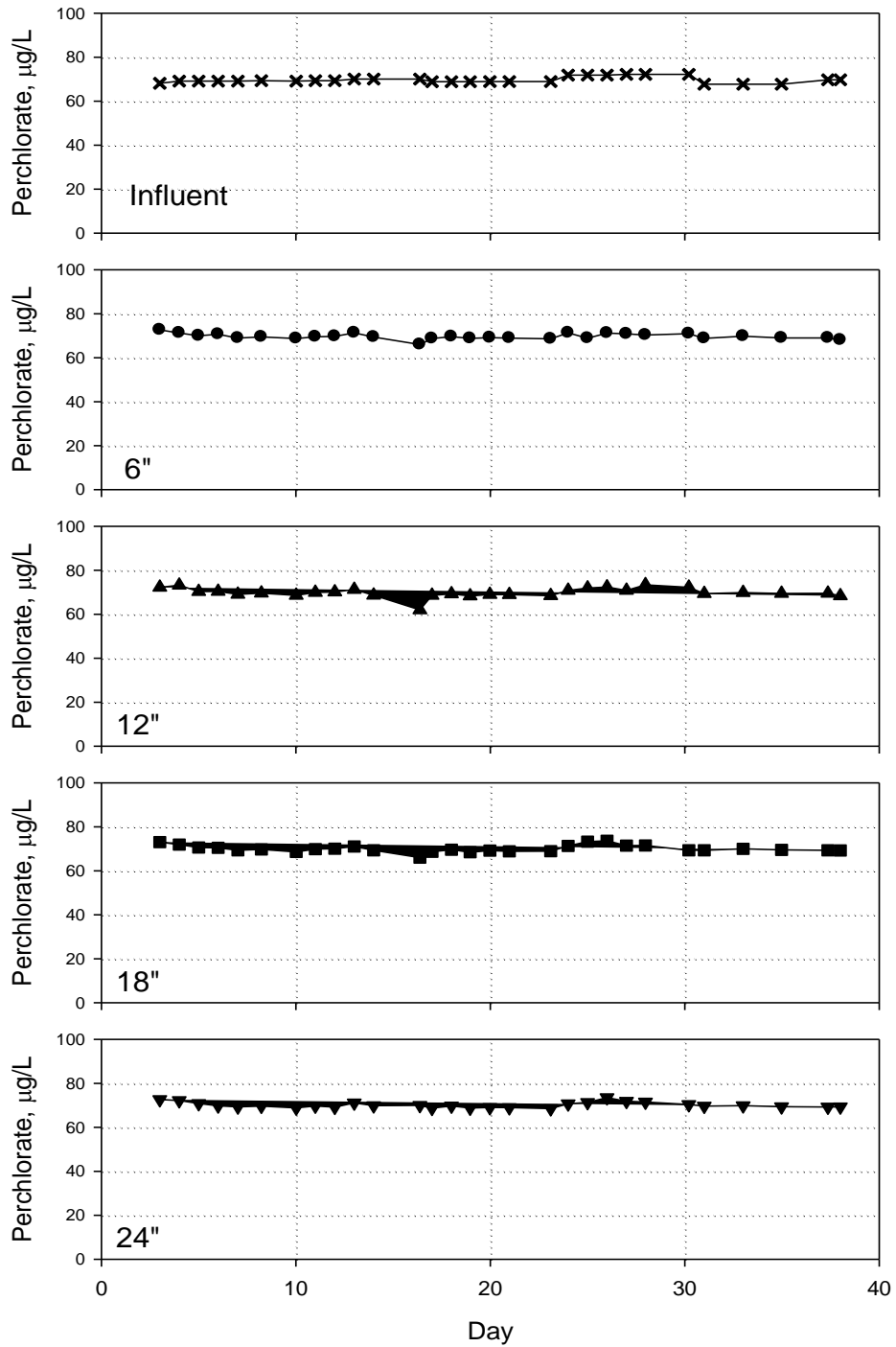
Figure 2.16 Perchlorate reductions in source area control columns.

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2.3.2.2.1 Perchlorate reduction in EOS amended columns

In the EOS-amended soil columns (Figure 2.15), perchlorate reductions started slowly in all columns during the first two weeks, but reached maximum removal efficiency by the third week. In the 6-inch column, maximum reduction of perchlorate was of 45% on Day 16 and other columns (12, 18 and 24 inches) reached 100% removal efficiency on day 24, 20 and 20. Removal efficiencies in 6, 12, 18 inches columns maintained at stable levels for 30, 35 and 60 days and decreased gradually with time.

Perchlorate concentration profiles at selected sampling times during the column tests are shown in Figure 2.16. Perchlorate reduction remained constant in the 24-inch column after Day 20 over the 120 days operation. Perchlorate reduction rates in the 6, 12, and 18 inch EOS-amended columns decreased with time, which may due to the less available electron donor and nutrients in soil columns that occurs over time. Perchlorate reduction can be readily stimulated by addition of EOS in groundwater flow condition. An EOS dose of 0.3% w/w was enough to provide electron donor sustainably for effective perchlorate reduction up to 120 days. An EBRT of 2 days was sufficient to achieve complete remediation of 64 mg/L perchlorate in groundwater.
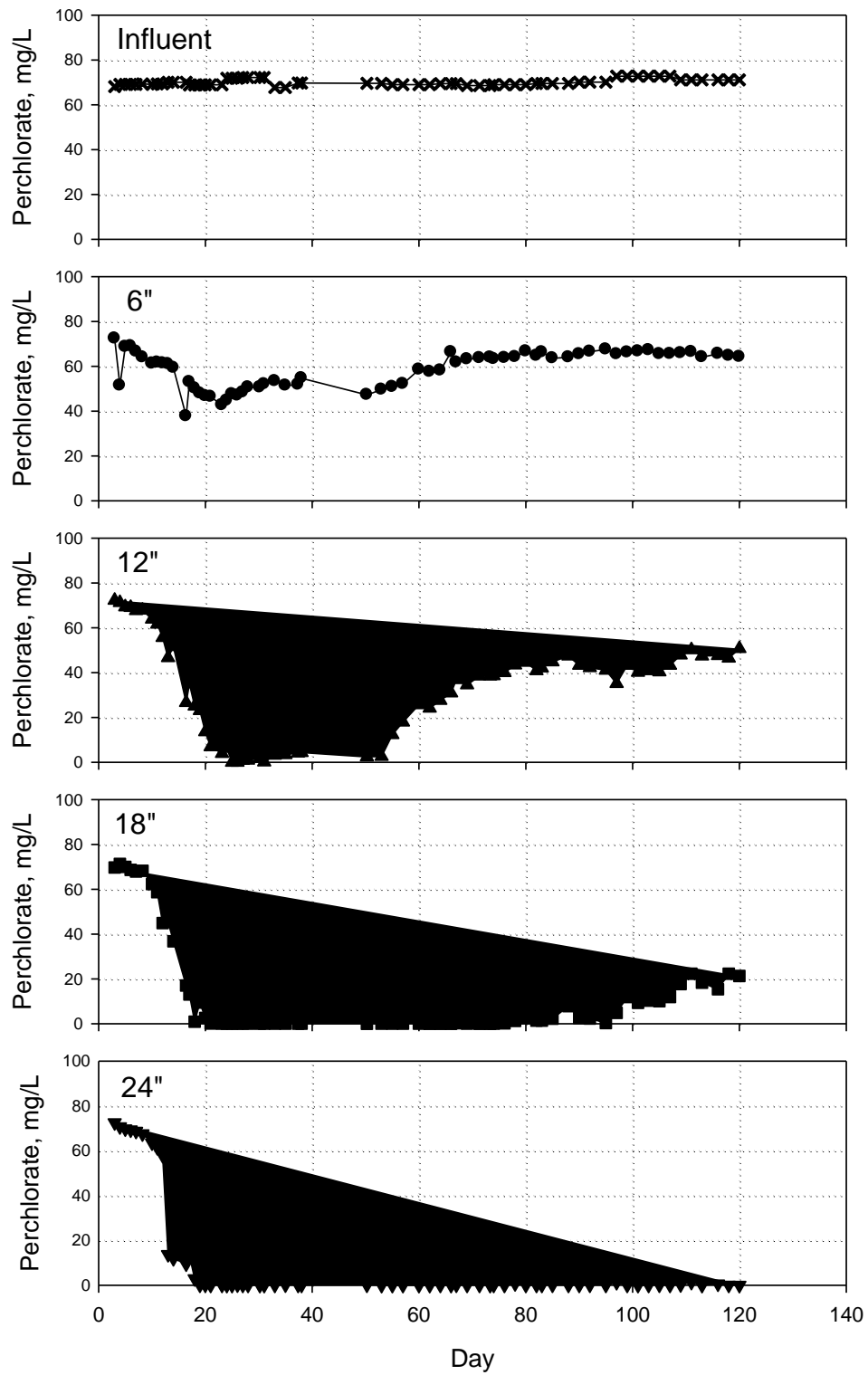
Figure 2.17 Perchlorate reduction in source area EOS-amended columns
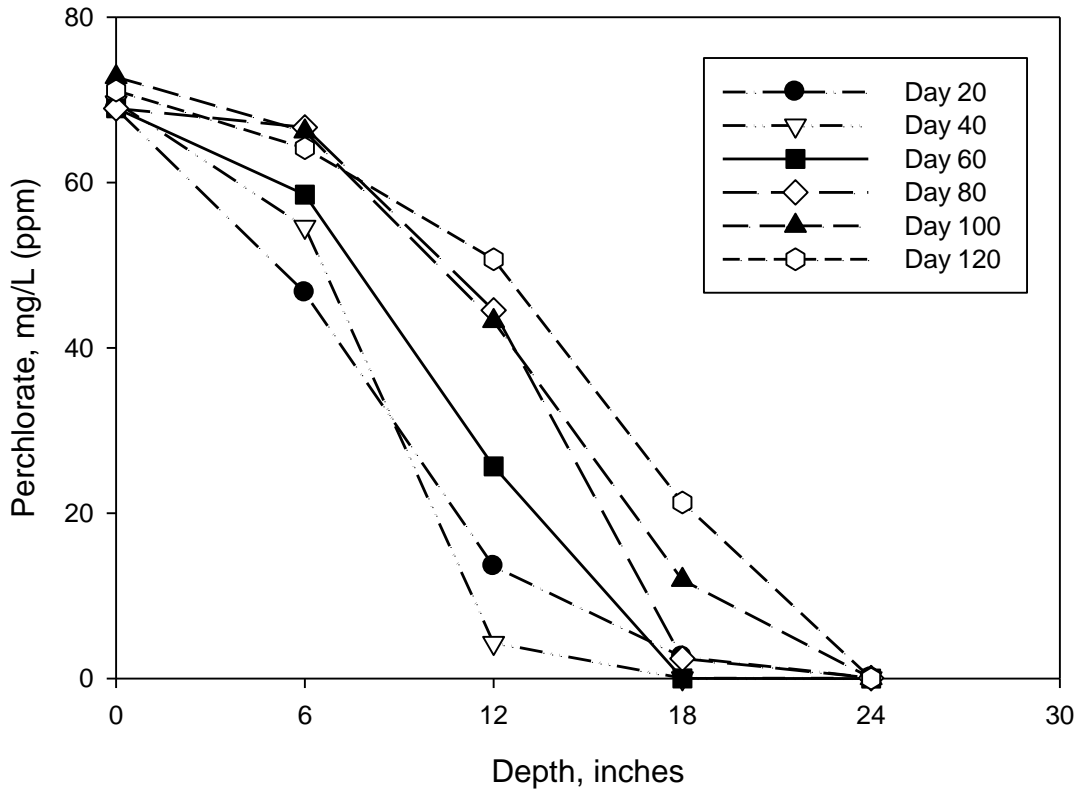
Figure 2.18 Perchlorate reduction profiles in EOS-amended source area columns

2.3.2.2.2 Perchlorate reduction in glycerin amended columns

Results of perchlorate reduction in glycerin-amended soil are reported in Figure 2.17. Glycerin was mixed with the soil at a concentration of 0.3 % (w/w) prior to being packed into the column. However, unlike the EOS-amended soil columns, where perchlorate reduction was either initiated or completed within 20 days, the glycerin-amended column did not remove perchlorate during the first 24 days. Thus, glycerin was added directly into the influent. The concentration of glycerin in the influent varied depending on the treatment performance. There was no concentration change in the

influent samples taken from the feed tank during this testing period. Detailed operation conditions are as follows.

At Day 25, 300 mg/L of glycerin was added into the source area groundwater influent. This concentration of glycerin is equivalent to five times the stoichiometric amount needed for perchlorate and nitrate degradation. After adding glycerin in the influent, a rapid decrease in perchlorate concentration in the effluent was observed in 12-inch, 18-inch and 24-inch columns, and complete removal of perchlorate was reached at Day 51, Day 32, and Day 32, respectively. Although the perchlorate degradation in the 6-inch column was relatively slow compared with others, a maximum of 86% removal was reached.

At Day 53, glycerin addition was temporarily stopped. Reduction of perchlorate was completely lost in all the columns.

At Day 68, 120 mg/L of glycerin was added into the source area groundwater influent. Reduction of perchlorate was resumed in all the columns, and was maintained at above 71% removal in both the 18-inch and 24-inch columns.

At Day 96, 60 mg/L of glycerin was added into the source area groundwater influent, which is about the stoichiometric amount needed for perchlorate and nitrate biodegradation. Perchlorate reduction gradually decreased in all the columns until the end of the testing.

It appears that glycerin premixed in soil columns washed out before the required lag period for perchlorate reducers. After continuously injecting glycerin, perchlorate reduction was initiated within two weeks, which was similar with lag period in EOS

columns. Perchlorate reduction decreased rapidly after the glycerin injection was stopped and no removal was observed in all columns after one week operation at this condition. It can be concluded that limited amount of glycerin could be adsorbed in soil columns and perchlorate reduction cannot be achieved by one time injection of glycerin.

Considering continuous injection of 120 mg/L, which is two times the stoichiometric value, was suitable to remove 64mg/L perchlorate in groundwater within 2 days or EBRT. Although EOS and glycerin had excellent performance in microcosm study, EOS was the most suitable electron donor amendment for in situ bioremediation of perchlorate in high concentration source area groundwater.
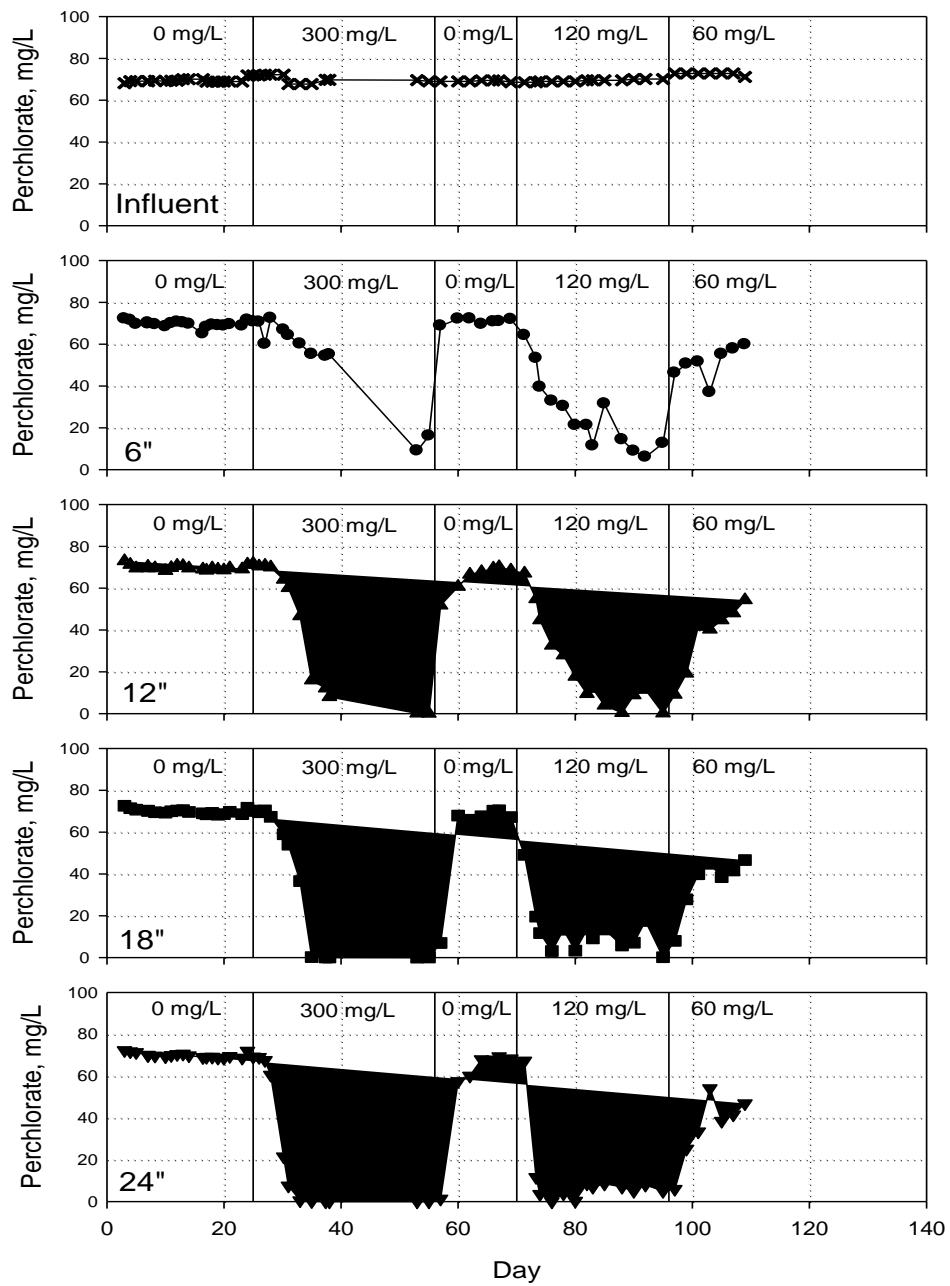
Figure 2.19 Perchlorate reductions in source area glycerin-amended columns. Values across the top of each graph indicate the concentration of glycerin in the influent

2.3.3 Source area vadose zone soil remediation

2.3.3.1 Vadose zone soil microcosms

Microcosm studies were conducted to investigate favorable soil moisture condition and electron donors for perchlorate degradation in vadose zone soil. Soil microcosms were initially prepared at 20% (w/w) moisture content. EOS and glycerin were diluted in chlorine free tap water and mixed with soils to provide electron donors and adjust moisture condition to a desirable level.

Based on the results shown in Figure 2.18, no perchlorate reduction was observed in any microcosms within 5 weeks incubation time. Most of the nitrate was degraded within one week in EOS and glycerin amended microcosms. In the bioremediation zone and source area groundwater microcosms, perchlorate reduction started right after nitrate depletions. In these soil microcosms, perchlorate reduction did not begin even after 4 weeks of nitrate depletion. It is possible that the incubation time was not sufficient to stimulate perchlorate reduction in soil microcosms. Since the microcosms were conducted in sacrificial bottles, no more samples were available for further monitoring. However, comparing these results with the time frame of bioremediation zone and source area microcosm studies, 5 weeks incubation time was twice that in the other microcosms. As found in the prior studies, electron donor is the most critical factor in perchlorate reduction. Final TOC in control, EOS and glycerin microcosms were 30, 680 and 800 mg/kg respectively which were sufficient for perchlorate reduction.
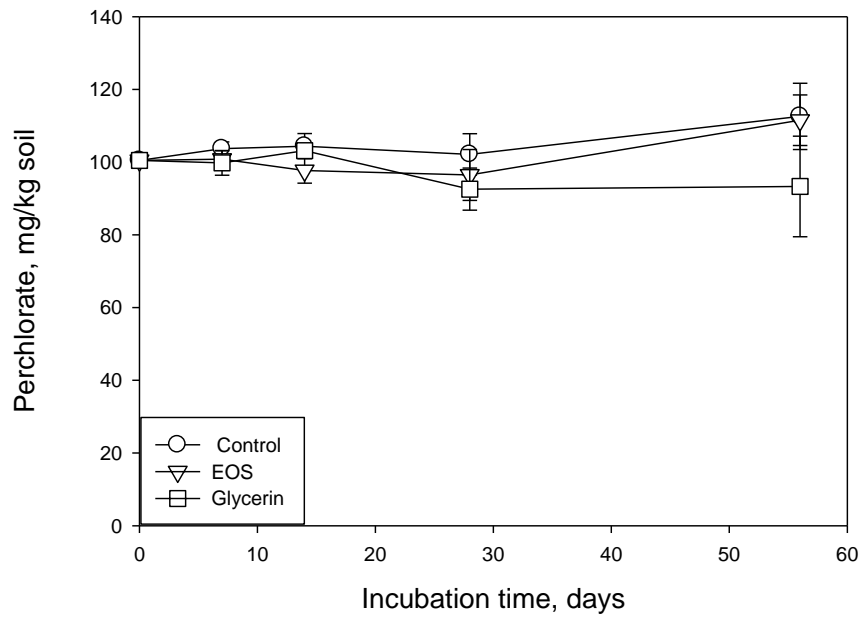
Figure 2.20 Perchlorate reduction in vadose zone soil microcosms under 20% mass water content.
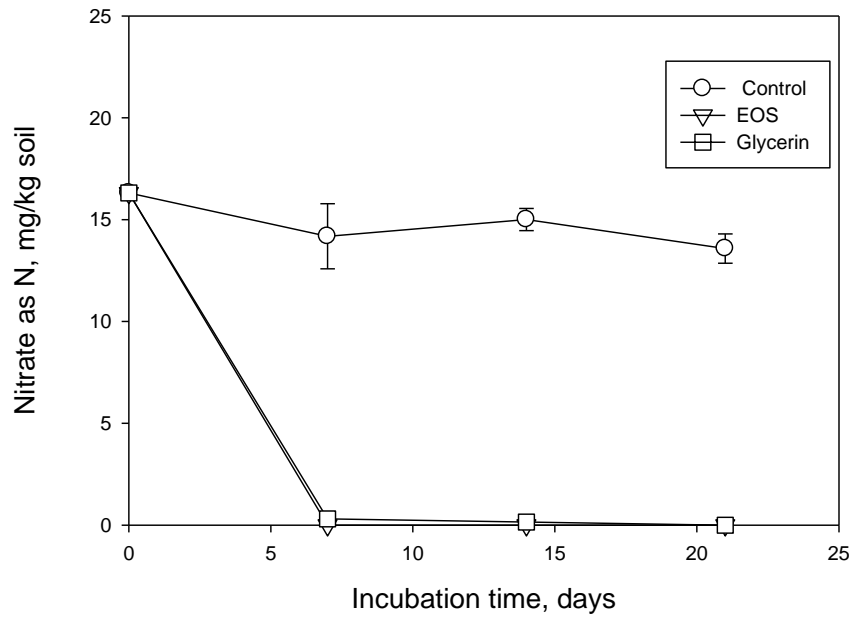
Figure 2.21 Nitrate reduction in vadose zone soil microcosms under 20% mass water content.

Final pH values in these microcosms varied from 7.9 to 8.5. Although the pH of soil microcosms was not optimum for perchlorate removal, perchlorate reduction was observed at pH value of 9.8 in Evans's soil microcosms [36]. The soil used in this part of research was collected from vadose zone of the contaminated site while the other soils were all collected from saturated zones. Although it is believed that perchlorate reducers are ubiquitous, it does not guaranty that the soil used in vadose zone microcosms containing them. To verify if perchlorate reducers exist in the vadose zone, another soil microcosm study was conducted. Since perchlorate reduction was observed in most of groundwater studies, it was believed that increasing moisture condition could accelerate perchlorate reduction. Thus the second soil microcosms were prepared under saturated conditions (65% w/w) and other parameters were controlled in the same condition with previous soil microcosms.

Perchlorate and nitrate reduction in saturated microcosms are shown in Figure 2.20 and 2.21. Most of the nitrate degraded within a week with EOS or glycerin as electron donors. No nitrate degradation was observed in control microcosms. As expected, perchlorate reductions were initiated right after nitrate depletion in EOS or glycerin amended microcosms. No perchlorate degradation was observed in control microcosms. Perchlorate degradation rate with EOS was 8 times higher than that with glycerin. Effect of different electron donors was more significant in soil microcosms than groundwater microcosms. It was concluded that the vadose zone soil had the potential of perchlorate biodegradation and addition of EOS or glycerin enhanced nitrate and perchlorate reductions by endogenous microorganisms in the vadose zone soil. The

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reason for the failure of perchlorate in unsaturated microcosms was not clear. Moisture

content is an important soil parameter that affects biogeochemistry of soil such as organic

carbon decomposition, ORP, microbial density. It is possible that moisture content could

be the direct limiting factor or it indirectly controlling perchlorate reduction by altering

other soil properties.
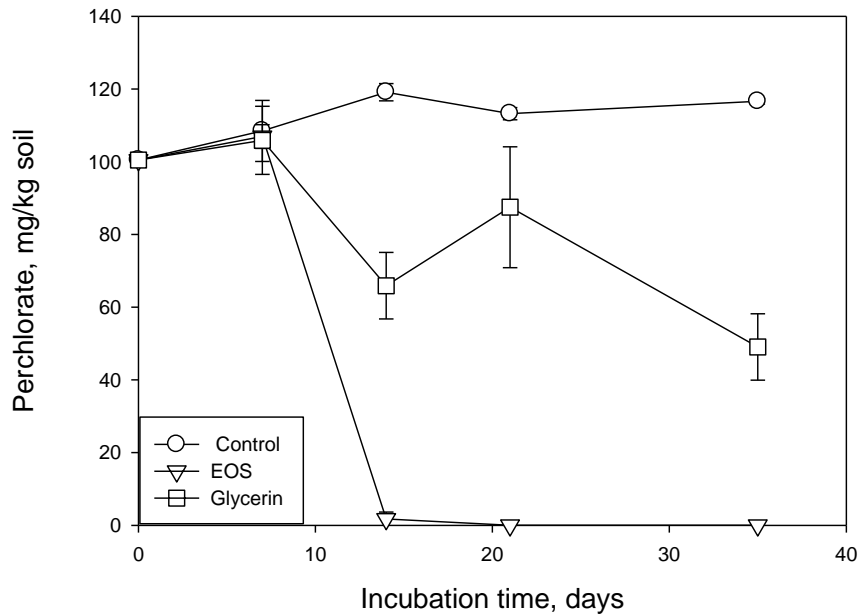
Figure 2.22 Perchlorate reduction in vadose zone soil microcosms under saturated condition.
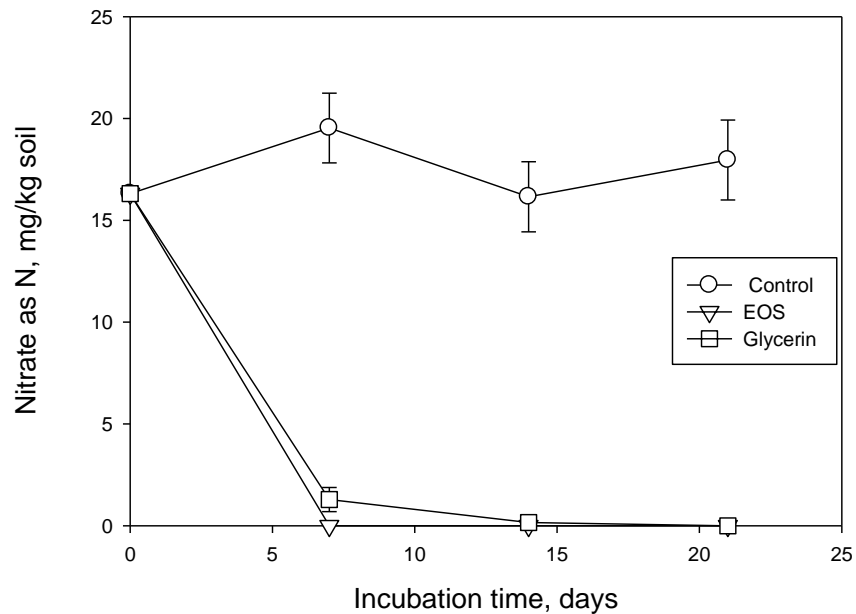
Figure 2.23 Nitrate reduction in vadose zone soil microcosms under saturated condition.

2.3.3.2 Vadose zone soil column studies

Column tests were conducted to evaluate two in-situ treatment scenarios for perchlorate in vadose zone soil. In the batch mode column test, $40\pm8\%$ of perchlorate was leached out from soil columns by one time application of two pore volumes of substrate solutions. Thus initial perchlorate concentration in soil columns were around 60 mg/kg but had variations among the different sacrificial columns.

Based on the results shown in Figure 2.23 and compensating for the perchlorate collected in the leachate, little or no perchlorate reduction was observed in all columns. Nitrate reduction was completed in one week in all columns except for control columns. Availability of electron donors were monitored by measuring dissolved part of organic carbon in soil samples which were shown in Figure 2.24. TOC in the glycerin amended

column decreased with time and at the end of the test TOC value were similar with that in control column. In EOS amended columns, TOC had a rapid decrease in the first week. After a week, TOC increased slightly until week 4. Due to the relatively high adsorption coefficient of EOS, it was difficult to quantify electron donors left in these columns by dissolved TOC. However, it was confirmed that there was sufficient electron donor left at the end of this test.

As discussed in the microcosm studies, perchlorate reduction in this soil preferred saturated conditions. Although moisture condition in soil columns was increased to field capacity, it gradually decreased to 15% in all columns. The failure of perchlorate reduction in batch mode column might be due to the low moisture conditions. After nitrate depletion, no perchlorate reduction was observed while TOC was continuously consumed. It appears that significant amount of electron acceptor other than nitrate and perchlorate exist in soil columns. Because the top ends of these columns were open to the atmosphere, oxygen could diffuse into soil columns and decreasing moisture condition in soil columns might accelerate the process. It is also possible that other electron acceptors such iron and manganese exists in soil minerals. Whether these electron acceptors inhibit perchlorate reduction is not clear.
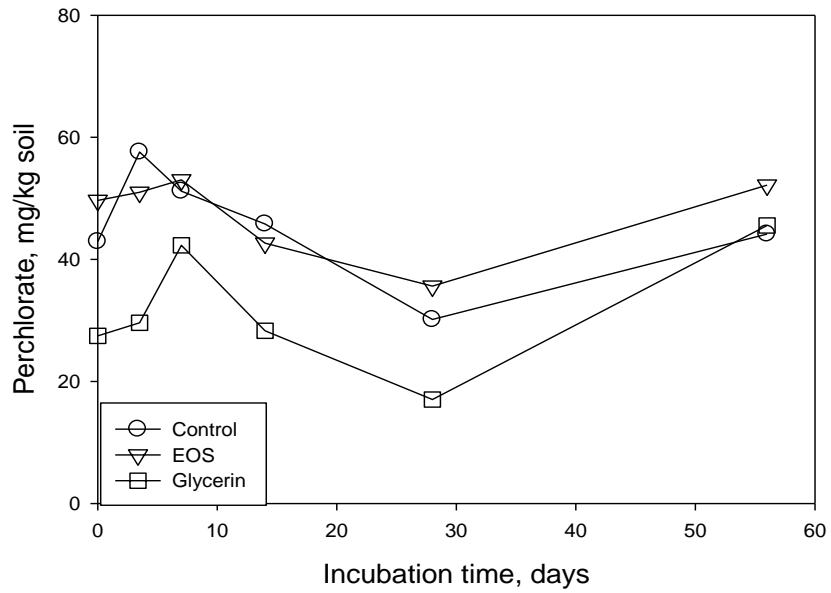
Figure 2.24   Perchlorate in the leachate from different batch mode sacrificial columns.
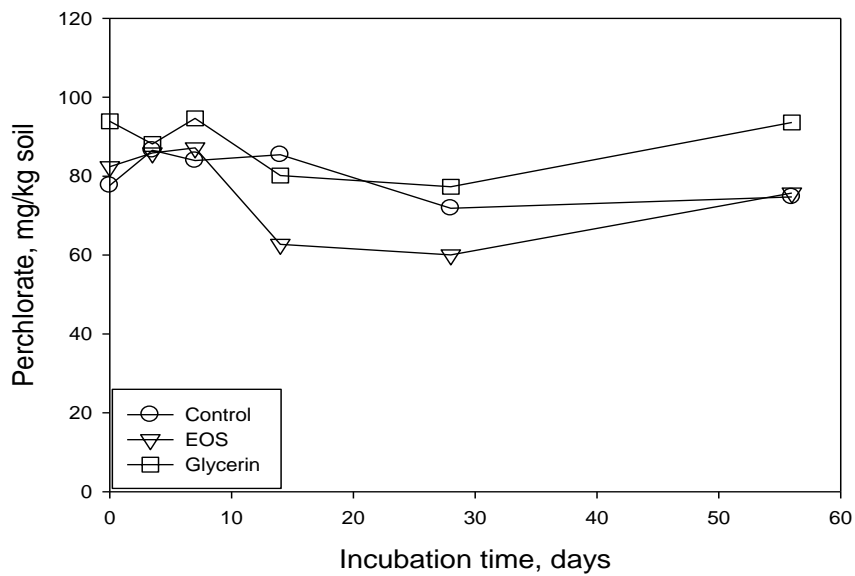
Figure 2.25 Total perchlorate in batch mode columns with different incubation times (after compensation).
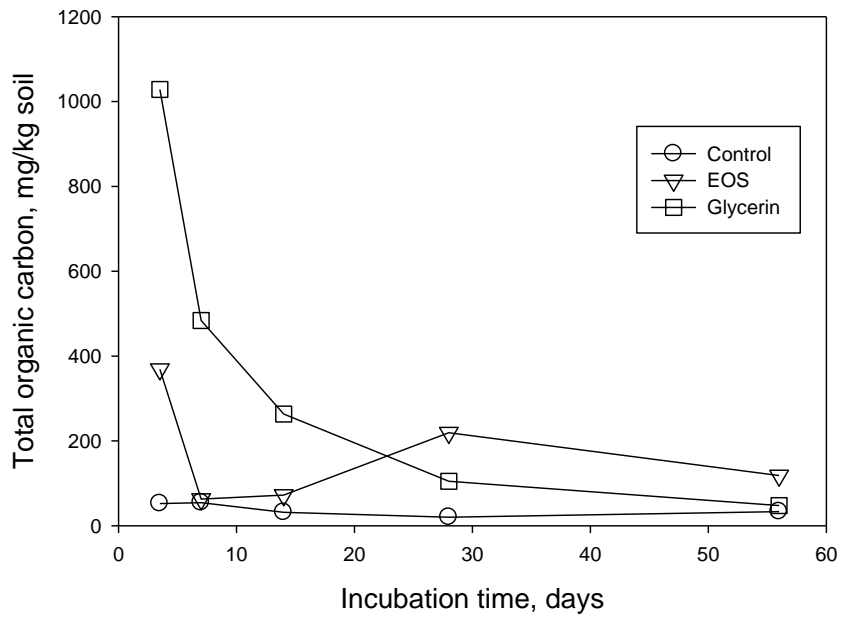
Figure 2.26 Decomposition of dissolved organic carbon in batch mode columns
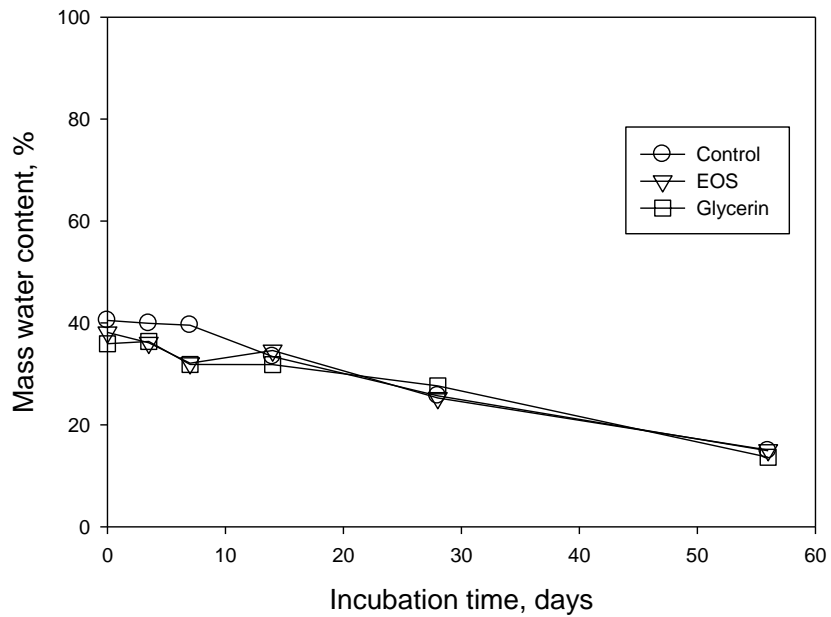
Figure 2.27 Mass water content of soil packed in batch mode columns at different incubation time

In recirculation mode column test, perchlorate in soil columns, soil pore water and recirculation water were analyzed and the total perchlorate in soil systems was calculated using Equation 2.1.

$$\boldsymbol{Total\ perchlorate} = \frac{C_S M_S + C_{PW} V_{PW} + C_{RW} V_{RW}}{M_S} \qquad \textbf{Equation 2-1}$$

Where Cs= perchlorate concentration in soil, mg/kg;
        Ms= mass of soil in each column, kg;
        C_{PW}= perchlorate concentration in pore water, mg/L;
        V_{PW}= Volume of pore water in each column, L;
        C_{RW}= perchlorate in recirculated water, mg/L;
        V_{RW}= Volume of recirculated water in each column, L

After 7 days operation, most of the perchlorate (>98%) had leached out from the soil and disolved into the soil pore water and recirculated water. Nitrate was reduction was completed within 4 days in all soil columns. Perchlorate reduction in the soil column system was shown in Figure 2.26. Perchlorate reduction was observed after 7 and 14days lag period in glycerin and EOS amended columns, respectively. Perchlorate reduction was completed within 21 days in both EOS and glycerin amended columns. Perchlorate reductions with EOS had similar rate in recirculation columns and saturated microcosms. In contrast, perchlorate reduction rate in glycerin amended columns was 4 times higher than that in the corresponding microcosms. Possible explanation is that external mass transfer resistance in recirculation columns is much lower than that in saturated microcosms since the soil and water in microcosms were stagnant.

Complete perchlorate reduction was also observed in control columns, which was most likely the result of the presence of sufficient natural organic carbon in this soil. As

shown in Figure 2.27, dissolved organic carbon (DOC) in the control column increased after 4 days of operation. The vadose zone soil contained 2.8% of soil organic component (SOC). It is possible that most of SOC in the soil was initially non-dissolved organic carbon. After the recirculation flow saturated the soil, it accelerated mass transfer in soil columns and biochemical reactions such as hydrolysis [37, 38]. Thus organic compounds in soil broke down to more soluble and bio-available molecules. It has been reported that water content is an important factor in DOC release from soils. In a microcosm test with a south-central Ontario peatland, DOC concentration and aromaticity increased with peat water content [39].

DOC in EOS amended columns had similar trends with the control columns. EOS is readily soluble while it also easy to adsorb to soil particles. Even though the same dosage of EOS and glycerin were added to the columns, DOC in EOS was only one third of that in glycerin. It appears that at the beginning of the operation, significant amount of EOS was adsorbed to the soil. After few days, some of EOS was degraded to smaller molecular which might have high solubility and contributed to DOC. Unlike batch mode column test, most of the electron donor in columns was not consumed after 56 days incubation time. Based on the column study results, the continuous recirculation approach was shown to be more effective than one time flooding scenario. However, at sites with limited or no water and electricity available, one time flooding scenario is more desirable.

Based on this study, however, it appears that moisture condition is somehow limiting the perchlorate degradation in unsaturated microcosms and batch mode columns.

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These results led to the questions, "How much moisture is required for perchlorate reduction and the mechanism behind this limiting effect? Can it be overcome by adjusting other parameters?" Further research was required to address these questions.
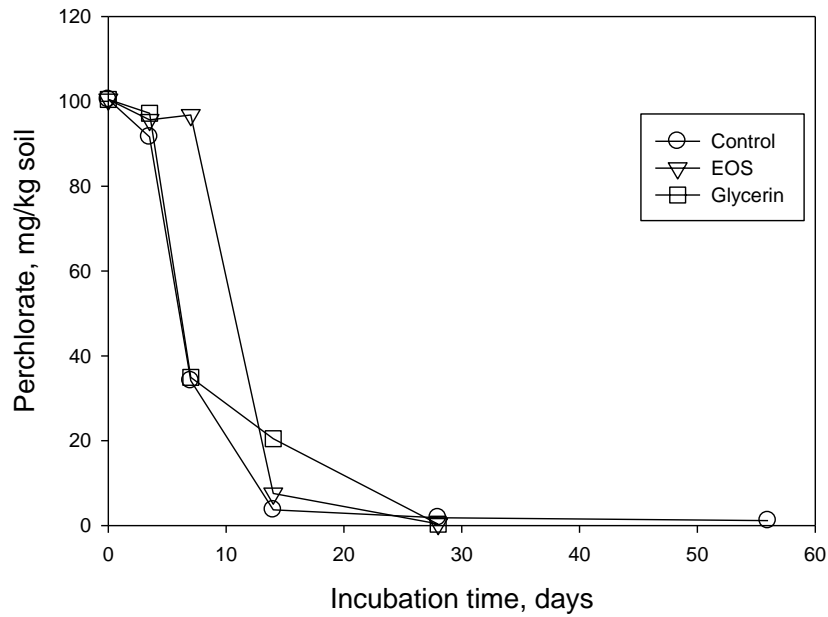
Figure 2.28 Total perchlorate in recirculation mode columns at different incubation time (after compensation).
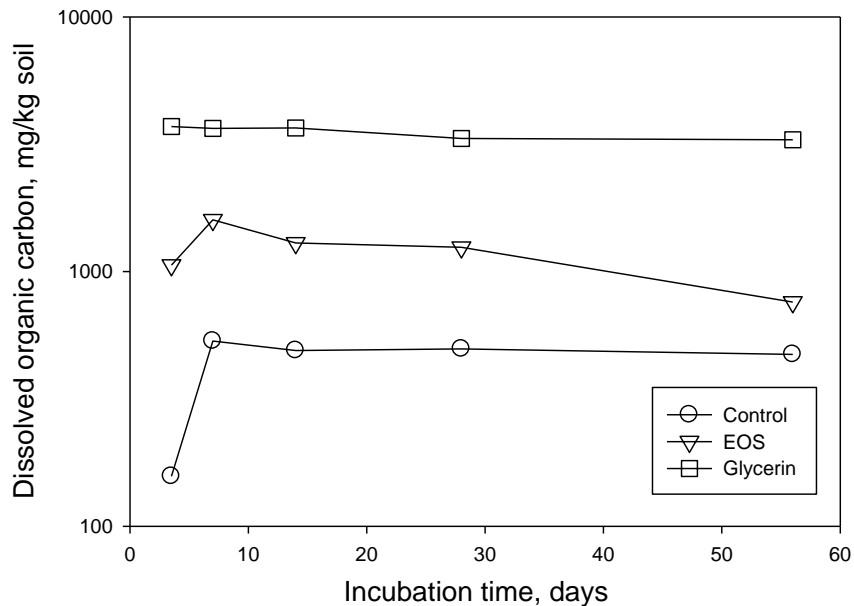
Figure 2.29 Decomposition of dissolved organic carbon in recirculation mode columns

2.4   SUMMARY

In the bioremediation zone study, complete perchlorate removal was observed within one week in EOS- and EHC-amended microcosms. Soil samples collected from the contaminated site contained perchlorate reducers and both EOS and EHC can readily stimulate perchlorate reduction in soil microcosms. The addition of 1 g/L $(NH_4)_2HPO_4$ nutrient had no or little effect on perchlorate reduction in microcosms. Perchlorate reduction was also achieved in soil column study which was simulating groundwater flow through bioremediation zone. Perchlorate reduction was initiated after two weeks operation in both EOS and EHC columns. Perchlorate bioremediation through soil columns was not suppressed by relatively high level of nitrate in influent groundwater.

Perchlorate reduction rates decreased with operation time. However, a 24-hour EBRT was sufficient to completely degrade 500 μg/L perchlorate for at least 90 and 60 days in EOS and EHC amended columns, respectively. In terms of longevity, EOS had better performance than EHC in this column study. Elevated level of iron and manganese was noticed in all amended column effluents. Since anoxic conditions were maintained in the soil columns by addition of electron donors, iron and manganese could also be utilized as electron acceptor by iron reducing bacteria and manganese reducing bacteria.

In source area groundwater remediation study, EOS, glycerin and sodium acetate were shown in microcosm tests to be effective in stimulating biological reduction of perchlorate among the five selected substrates (EOS, glycerin, high fructose corn syrup, acetic acid and sodium acetate). The rates of reduction were relatively similar for EOS, glycerin and sodium acetate, with complete reduction observed in the microcosms between 7 and 18 days. To reduce the cost and minimize salt addition, EOS and glycerin were chosen for column testing. In the column tests, the treatment with EOS amendment had significant advantages over using glycerin amendment. EOS-amended soil can achieve a nearly complete perchlorate reduction with 24 hour EBRT over 120-day operation with one time addition into the soil. In contrast, glycerin should be added on a continual basis at a five times stoichiometric amount into the influent to achieve a similar treatment performance as using EOS amendment.

In source area vadose zone soil remediation study, no perchlorate degradation was occurred in 20% moisture content microcosms while nitrate in soil depleted within one week. In the second microcosms study, perchlorate reduction observed under saturated

microcosms with EOS and glycerin as electron donors. Perchlorate degradation rates in

EOS microcosms were 8 times higher than that in glycerin microcosms under saturated

condition. Perchlorate degradation only occurred in recirculation mode columns where

soil was maintained at saturated conditions.

In situ bioremediation of perchlorate in contaminated groundwater and soil is a

feasible and reliable technology. In both bioremediation zone and source area study,

EOS had the best performance as electron donor.   Perchlorate remediation can be

initiated within two weeks in groundwater remediation. Perchlorate reduction in vadose

zone soil preferred saturated condition which has more operation cost. Further research

is required to investigate limiting factor in perchlorate reduction under unsaturated

conditions.

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Chapter 3 Effect of Moisture Condition on Perchlorate

Bioremediation in Vadose Zone Soil

3.1 INTRODUCTION

Historical, uncontrolled disposal practices have made perchlorate a significant threat to drinking water supplies all over the United States. In the last five years (2006-2011), perchlorate has been detected in 296 active and standby sources and 91 systems of drinking water in California alone [40].

Most early research efforts (late 1990s to early 2000s) were focused on removing perchlorate from drinking water and groundwater because the primary route of human exposure to perchlorate is via ingestion of perchlorate-contaminated water and food [4].. Recently, increasing efforts have been placed on perchlorate remediation in vadose zone soil. Although perchlorate salts have high solubility in water and high mobility in soils, significant amount of perchlorate via disposal and spills trapped in vadose zone soil especially in locations where has limited precipitations such as Southern California [33, 41]. It has been reported that perchlorate also occurs naturally in diverse vadose zones of the arid and semi-arid southwestern United States [42]. Perchlorate in soils can be a persistent source of groundwater contamination. It may increase the operating time frame and associated costs for groundwater remediation.

In situ bioremediation is a promising technology to treat perchlorate in vadose zone soil. Several research studies and remediation projects have shown that vadose zone soils have the potential for perchlorate biodegradation [33, 36, 43, 44]. Nozawa-Inoue *et*

al. demonstrated that bioremediation of perchlorate by native microbial communities is feasible when enhanced by adding electron donors in the vadose zone [33]. Evans *et al.* reported that gaseous electron donors such as hydrogen and ethanol were able to promote nitrate and perchlorate reduction in vadose zone soil [36]. Gal *et al.* also reported the potential of perchlorate biodegradation in different depths of vadose zone soil [44].

In addition to these results, microcosm studies and column studies have been conducted to investigate feasibility of in-situ bioremediation technology for perchlorate in vadose zone soil as discussed in Chapter 2. Perchlorate was readily degraded to non detectable level within 4 weeks in both microcosm and column studies under saturated moisture condition. In contrast, little or no perchlorate reduction was observed in 20% mass water content (MWC) microcosms and batch mode columns (unsaturated condition) during 8 weeks.

It appears that perchlorate reduction is favored under saturated conditions (i.e, groundwater aquifers). However, there is a significant amount of perchlorate located within the vadose zone in arid or semi-arid areas. In these regions, maintaining saturated conditions dramatically increases the operation costs of a remediation project.

Soil moisture is an important factor that can control microbial biomass and activities [45-48]. Effects of moisture on microbial activity are likely to be complicated and unpredictable because of many possible interactions that may occur among the bacteria, solute transport, nutrient and oxygen availability, pH, and other parameters within the soil environment.

In the study presented in Chapter 2, it was observed that denitrification occurred readily in vadose zone soil within one week at a mass water content of 20%. However, little or no perchlorate degradation was observed at this same moisture content. It is possible that perchlorate reduction requires higher moisture condition than denitrification and other respiration process. These findings are inconsistent with the findings form Nozawa-Inoue *et al*. and Evans *et al*.'s research where perchlorate reductions were achieved in microcosms under mass water contents of 20% and 9.5% [33, 36].

Perchlorate reduction in unsaturated microcosms may also be limited by other factor such as toxic metals or salinity and that increasing moisture condition relieve the limiting condition by diluting it.

The soil used in previous microcosms was collected from a semi-arid area (Beaumont, CA). In a dry and hot climate, low moisture and salinity are the most important limiting factor for soil microbial activity and it may occur simultaneously [49]. In this case, the limiting effect of moisture may differ in different soils.

Another possible explanation is that higher moisture conditions can stimulate anaerobic respirations by limiting oxygen transfer. Oxygen is a common inhibitory factor in anaerobic respiration [23, 50-52]. Oxygen availability is mainly controlled by soil moistures. Increasing water content in soil was able to stimulate denitrification by limiting oxygen transfer into soils if there was sufficient electron donor exist [53-55]. Both perchlorate and denitrification are anaerobic process while they may have different level of oxygen tolerances. Thus, perchlorate reduction may require higher moisture condition and moisture requirement may be different in different soil textures.

The objectives of this research are to: 1) study the impact of moisture condition on perchlorate bioremediation in different soils; 2) investigate possible mechanisms for the moisture effect on perchlorate bioremediation; 3) investigate possible approaches to improving perchlorate removal in vadose zone soils.

3.2   BACKGROUND

To evaluate in situ perchlorate bioremediation approaches within the vadose zone, microcosm studies and column studies were conducted with vadose zone soil contaminated with 100 mg/kg perchlorate (see Chapter 2). Perchlorate reduction was not observed within 56 days incubation time with EOS or EHC as electron donors in soil microcosms under 20% mass water content (MWC). In contrast, perchlorate reduction was initiated after a 7-day lag period in both EOS and EHC amended microcosms under 65% MWC (saturated condition). Denitrification, a well-known competitive process of perchlorate reduction was completed within 7 days in both saturated and unsaturated moisture conditions.

In the column studies (see Chapter 2), little or no perchlorate reduction was observed within 56 days in batch mode columns with EOS or EHC as electron donating substrate in which soil moisture gradually decreased from 40% MWC to 15% MWC. Rapid degradation of organic carbon was observed during the incubation period in the soil columns while sufficient electron donor was left in the soils by the end of the experiment. Perchlorate reduction in recirculation mode columns (saturated condition) was completed within 21 days.

Based on that study, it appears that moisture condition is somehow limiting the perchlorate degradation in unsaturated microcosms and batch mode columns. However, nitrate reduction was not significantly affected by moisture condition in the range between 65% and 15% MWC.

A similar microcosm study was conducted by Nozawa-Inoue *et al*. with vadose zone soil collected from the top 0 to 15 cm in an agricultural field in Yolo County, California. Perchlorate reduction in soil microcosms under 20% MWC was observed after a 14-day lag period with acetate as electron donor; 110 mg/kg perchlorate was completely degraded within 40 days. It was hypothesized that the lag periods observed for perchlorate reduction were due to the presence of nitrate in the soil. However, different nitrate amendments did not affect perchlorate degradation lag periods in the experiment [33]. Evans *et al.* reported that addition of gaseous electron donors such as hydrogen and ethanol promoted nitrate and perchlorate reduction in a silty sand under 9.5 % MWC but no perchlorate reduction was observed in the soils under 7.5% MWC [36].

It appears that optimum moisture condition for perchlorate bioremediation is different in different soils. These results led to the questions, "How to determine the moisture requirement for perchlorate reduction in different soils and what is the mechanism behind this limiting effect?" Further research was required to address these questions.

3.3   MATERIALS and METHODS

3.3.1   Materials

Three soils were used in this part of research. Geochemical properties of three

soils are summarized in Table 3.1. Soil A was a sandy loam collected from 20 feet depth

in a perchlorate contaminated site located in Beaumont, California. The soil contained

100 mg/kg perchlorate and 16.3 mg/kg nitrate-N. Initial soil mass water content (MWC)

was 11%, initial organic content was 2.83% and the initial pH was 8.5±0.2. Soil B was

also a sandy loam collected from 20 cm below the soil surface in a lawn area in the UC

Riverside campus in Riverside, CA. Soil C was sandy soil collected from 20 cm below

the surface of a wastewater rapid infiltration treatment bed located in Colton. CA. No

perchlorate was detected in Soil B or Soil C as collected from the field. All soils were

passed through a 2 mm sieve. Soil B and Soil C were amended with 100mg/kg

perchlorate and air dried at room conditions. All soils were stored at 4°C until the

experiment was conducted.

Table 3.1 Geochemical Properties of Soils Used in Study

	Soil A	Soil B	Soil C
Texture	Sandy Loam	Sandy Loam	Sand
Location	Beaumont, CA	Riverside, CA	Colton, CA
Depth, m	6	0.2	0.2
Mass Water Content , %	10.9	6.5	0.4
Bulk density, kg/L	1.37	1.35	1.95
Pore Volume, %	0.57	0.55	0.41
Perchlorate, mg/kg	100±3	109±4	94±0.4
pH	8.5±0.2	8.4±0.2	7.5±0.2

3.3.2 Experimental Method

3.3.2.1 Investigation of limiting factor in unsaturated microcosms with soil A

To investigate bioavailable metals in the Soil A, water extractable part of metals were analyzed using an inductively coupled plasma (ICP) mass spectrometer. Soluble soil metal concentrations were obtained by mixing 20 mL of distilled water with 10 g of soil (1:2 solid/liquid ratio) on an orbital shaker for 1hr. Extractant was obtained by filtering the slurry through a 0.2-μm syringe filter. The pH of the extractant was adjusted to 2 by adding nitric acid. The sample was analyzed using Perkin-Elmer 7300DV inductively coupled plasma (ICP) optical emission spectrometer in the Department of Environmental Sciences in UCR.

Soil salinity of the extractant was determined by evaporation. Soil (100 g) was mixed with 200 mL of distilled water for 1 hour in an orbital shaker at 100 RPM. Extractant (100 ml) was obtained from the soil slurry by passed it through a glass-fiber filter disk (Whatman, Grade GF/B: 1.0 μm) and applying vacuum to a filtration apparatus. Fifty (50) ml of filtered solution were transferred to a clean and evaporating dish and placed in a drying oven at 180 ± 2 °C. Salinity of soil was calculated using equation1

$$mg\ total\ dissolved/kg\ soil = \frac{(A-B) \times 4 \times 1000}{soil\ weight, g} \qquad \textbf{Equation 3-2}$$

where A = weight of dried residue and dish, mg
B= weight of dish, mg

To investigate the effect of salinity on perchlorate reduction, a microcosm study using Soil A was conducted to observe perchlorate degradation rates with varying soil

salinity. Sodium acetate, (5 g/L) was used as electron donor in this microcosm. Microcosms were prepared using 40-ml serum bottles with PTFE/silicone septa and screw thread cap under five different salinities. Five electron donor solutions were prepared by dissolving 5g/L acetate and different amount of sodium chloride in tap water (chlorine free) to adjust salinity to targeting values (control, 1%, 2%, 3% and 4%). The solution for control microcosm was made up of sodium acetate and tap water only.

Soil (10 g of dry equivalent) and 30 mL electron donor solution were added to each serum bottles; headspace was flushed with nitrogen gas. Triplicate bottles were prepared for each experimental condition. For each sampling time point, 2 mL of soil solution were withdrawn through the septum; perchlorate in the solution was analyzed via ion chromatography (IC).

3.3.2.2 Effect of moisture condition on perchlorate bioremediation in different soils

The water holding capacity of the three soils was analyzed using the pressure plate method as described in Richard *et al.'s* research [56] and initial moisture content was measured by oven drying method.

This microcosm study was conducted with three different soils under different moisture conditions. All microcosms were prepared in 40-ml serum bottles with PTFE/silicone septa and screw thread cap; 2.5 g of sodium acetate per kg of soil was used as the electron donor. Soil A was incubated under 15, 20, 25, 30, 40 and 50% w/w mass water content. Soil B was prepared under 15, 20, 25, 30% w/w mass water content; Soil C was prepared under 5, 10 and 15% w/w mass water content. Soil was premixed with electron donor and tap water (chlorine free) to adjust soil moisture content to targeting

value in all microcosms except for 40 and 50% microcosms with Soil A. Premixed soil (10 g of dry equivalent) was added into each bottle and headspace was flushed with nitrogen gas. For each experimental condition, eighteen sacrificial microcosm bottles were setup. For the 40 and 50% Soil A microcosms, 10 g dry equivalent soil was placed in bottles first and then the required amount of electron donor solution were added into each bottle. Electron donor solutions were made by dissolving sodium acetate in tap water (dechlorinated). Soil and electron donor solution in each bottle was evenly mixed using vortex mixer and the head space was flushed with nitrogen gas. For each sampling time point, triplicate microcosms were sacrificed to measure soil perchlorate, nitrate, pH, and TOC concentrations.

3.3.2.3 Analytical methods

Perchlorate in soil samples were determined by extracting 10 g of dry equivalent soil with 20 ml of deionized water and vortex mixing for 2min. Extracts were obtained by centrifugation at $5000 \times g$ and filtered through 0.2-μm filter to remove soil particles. This extraction method was able to recover $98 \pm 4\%$ of perchlorate in soils. The extracts were also used to analyze dissolved part of nitrate and TOC in soil samples. Perchlorate in extracts was analyzed using a Dionex 1000 Ion chromatograph (Dionex Corp., Sunnyvale, CA, USA) with an IonPac® AS 16 analytical column (4×250 mm) and AG 16 guard column (4×50 mm). Nitrate was determined by an IonPac® AS 14 analytical column (4×250 mm) and AG 14 guard column (4×50 mm). The detection limits for perchlorate and nitrate were 4 μg/L and 100 μg/L (as N), respectively. In addition, all the other analyses for the parameters were conducted according to the EPA standard methods.

3.4   RESULTS AND DISCUSSIONS

3.4.1   Identifying possible inhibitory factors in perchlorate bioremediation under unsaturated conditions with soil A

Other than perchlorate, no other contamination was reported at the site. However, it is possible that the specific soil sample contain high level of metals that may limit perchlorate remediation under unsaturated condition. To investigate bioavailable metals in Soil A, water extractable soil metal content was determined using the extraction/ICP analysis as described above. Based on the results shown in Table 3-2, trace amounts of soluble Cu, Fe, Mn and Zn were detected from the soil.

At a soil moisture concentration of 20%, the projected concentrations of Cu, Fe, Mn and Zn in soil pore solution were 0.03, 0.25, 0.024, and 0.03 mg/L, respectively. Iron and manganese are abundant metal elements in soil and perchlorate reduction was achieved in a previous bioremediation zone column study (see Chapter 2) in which Fe and Mn concentrations were 4.0 and 0.77 mg/L respectively. Thus, iron and manganese are unlikely to be inhibitory factors.

Table 3.2 Water extractable metals in Soil A

Metals, mg/kg	Soil A	Method Detection Limit
Ag	ND	0.001
As	ND	0.008
Cd	ND	0.0007
Cr	ND	0.002

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Cu	0.006	0.001
Fe	0.050	0.001
Mn	0.0048	0.0002
Mo	ND	0.004
Ni	ND	0.002
Pb	ND	0.04
Se	ND	0.01
V	ND	0.009
Zn	0.006	0.0003

Copper is an essential micronutrient that is necessary in wide ranges of metabolic processes. However, a high level of copper is toxic to microorganisms and higher organisms. It has been reported that copper concentration in uncontaminated fresh water systems ranges from 0.2 to 30µg/L [57]. Different microorganisms have different tolerance levels of copper. Algal biomass gradually decrease when 0.005 to 0.01mg/L copper sulfate in added to freshwater lakes and 0.025 to 0.05 mg/L copper in drinking water killed 90% of coliforms within 2 to 6 days [57]. At a sub-lethal concentration of 0.001 mg/L, copper does not affect the bacterial CFU; however, $_{14}$C-glucose uptake and mineralization were reduced significantly in water column [57]. Granger reported that denitrification by cultures of WLB20 and *P. stutzeri* had the highest growth rates in a medium containing 0.6 µg/L of copper. Higher concentrations (7 µg/L and 60 µg/L) had lower growth rates, but were still higher than growth rates in copper-free conditions [58].

In the unsaturated microcosms, the copper concentration in soil solution was estimated to be 0.03 mg/L and rapid denitrification was observed at this condition, which is consistent with Granger's findings.

Currently, little information is available about metal tolerance of perchlorate reducers. Further research is required to investigate whether copper is a limiting factor in perchlorate bioremediation under unsaturated condition. Zinc is also a toxic metal to microorganisms. However, threshold inhibitory concentrations of zinc are higher than that for copper [59-62].

Total water extractable salt in Soil A was 640 ± 120 mg/kg. The salinity in soil pore solution at 20% mass water content was estimated at 3.2 g/L. Perchlorate reducers isolated from domestic wastewater treatment plants have been shown to tolerate salinity up to 3% (w/v) of NaCl after stepwise acclimation to high salinity [63, 64]. That study was conducted with pure cultures of perchlorate reducer whereas the general soil microbial community is more complicated. To achieve in-situ perchlorate bioremediation, perchlorate reducers in soil have to acclimate to the changing environment and outcompete with other microbes. Thus, a lag period is usually observed in perchlorate reduction in in-situ remediation systems.

To investigate the impact of salinity on the initiation of perchlorate reduction in soil, microcosm study was conducted under different salinity (1, 2, 3, 4% and control). As seen in Figure 3.1, perchlorate reduction in fresh water condition (control) started after 7 days incubation time and completed within 18 days. The 1% salinity had little or no inhibition effect on perchlorate reduction in saturated condition. Perchlorate reduction

in 2% salinity started after a 14-day lag period and the degradation rate was also

decreased compare to the control. However, no perchlorate reduction was observed in

the 3% and 4% salinity microcosms.
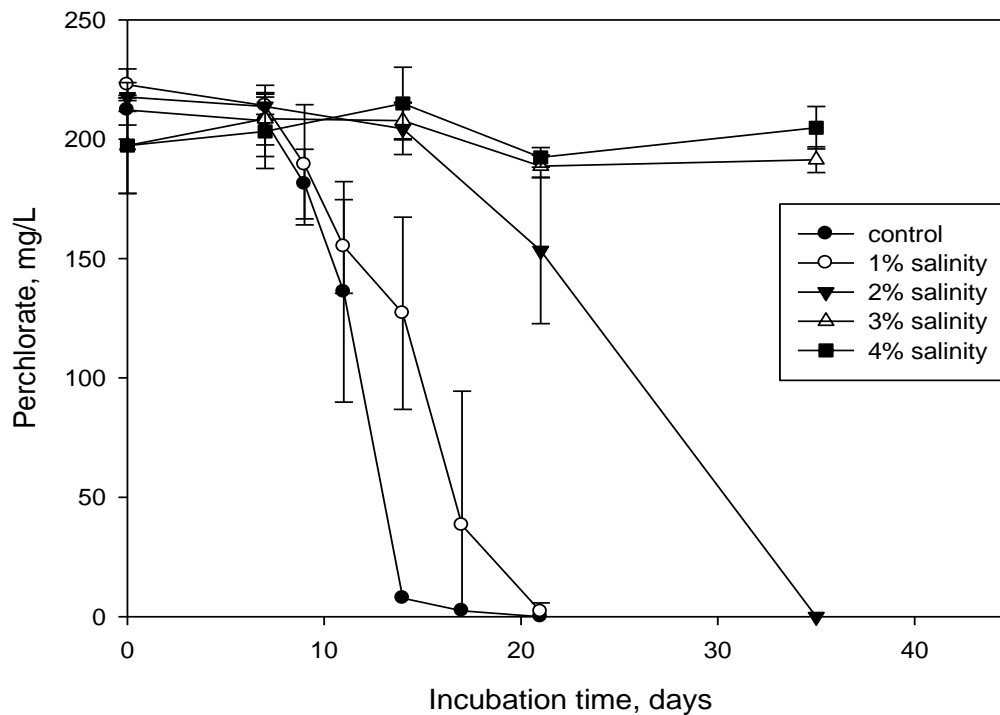
Figure 3.1 Perchlorate bioremediation under different salinity

When there is no water stress, perchlorate reducers were able to tolerate up to 2%

salinity with elongated lag period and decreased reduction rate. Salinity affects microbial

activity mainly by decreasing osmotic potential [49]. Under sub-lethal levels of salinity,

some microorganisms can adapt to changing salinity by accumulating osmolytes in cells

[49, 65]. The stress may decrease the microbial growth rates and activities in microbes

because synthesizing osmolytes requires large amounts of energy which can reduce the

energy available for growth. Although it has been reported that perchlorate reduction in a packed bed reactor was able to resist salinity up to 10% (w/v), it was validated that salinity could be a significant limiting factor in soil remediation. Osmotic pressure in 1, 2, 3 and 4% microcosm were estimated at -414, -828, -1239 and-1653 kPa, respectively using Morse equation [66]. The results of this current study are comparable with Polonenko's research in which -500 kPa of osmotic stress by NaCl significantly reduced the number of viable bacteria in soil columns[66].

As mentioned, EOS and glycerin were used as electron donors in unsaturated soil microcosms. Little or no salinity were introduced by electron donor amendment and the salinity in soil solutions can be estimated to be 0.32%. Thus, salinity is unlikely the limiting factor in the previous microcosm. Acetate is a widely used electron donor in perchlorate remediation. Applying electron donor such as acetate may increase the salinity and limit perchlorate reduction under unsaturated condition.

3.4.2 Perchlorate bioremediation in soils under different moisture conditions

To investigate the impact of moisture condition on perchlorate remediation in soil, perchlorate reduction in three different soils were monitored under different mass water contents. Soil characteristics were summarized in Table 3.1 and Figure 3.2.
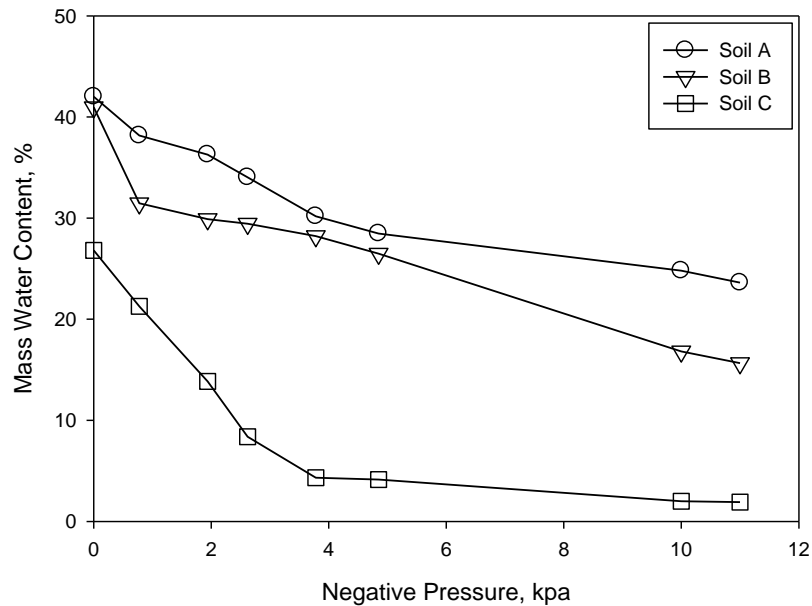
Figure 3.2 Water holding capacity of soils measured by pressure plate

Soil A was the soil collected from the perchlorate contaminated site (Beaumont, CA), while the other two soils were collected from Riverside and Colton were amended with 100mg/kg perchlorate. As shown in Table 3.1 and Figure 3.2, Soil A and Soil B had similar physical and chemical properties.

Perchlorate reduction in Soil A were initially monitored under 15, 20, 40 and 50% mass water content (MWC) with 2.5 g/kg acetate as the electron donor. Based on the results shown in Figure 3.3, perchlorate reduction in 40 and 50% MWC microcosms was initiated after a 7-day lag period and complete after 21 days. The field capacity of Soil A was 40 % MWC and 50% MWC was the completely saturated condition. Perchlorate reduction at 40% and 50% MWC had no significant differences. In contrast, no perchlorate reduction was observed at the 15% and 20% MWC microcosms. Additional

microcosm studies were conducted under 25 and 30% MWC. As it shown in Figure 3.4,

no perchlorate was degraded in both microcosms within 140 days incubation time.
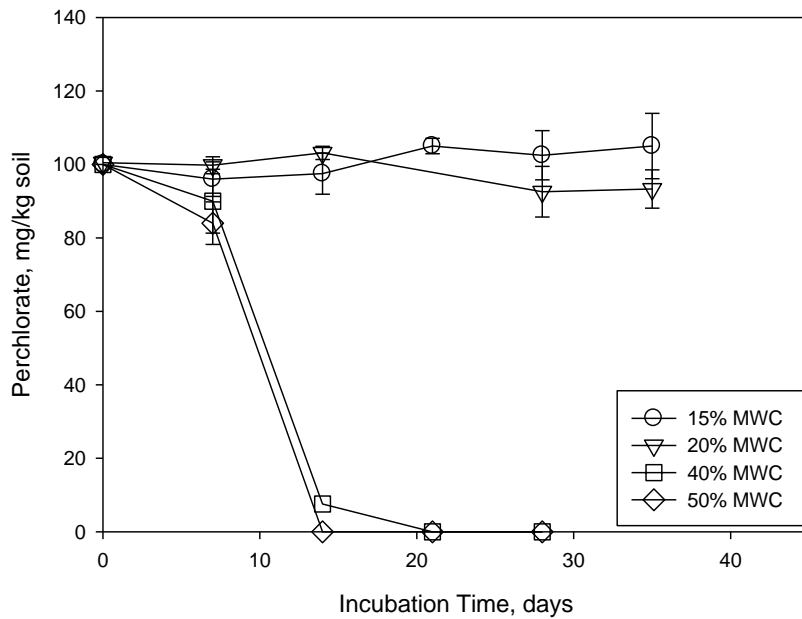
Figure 3.3 Perchlorate reductions in microcosms with soil A under 15, 20, 40 and 50% mass water content
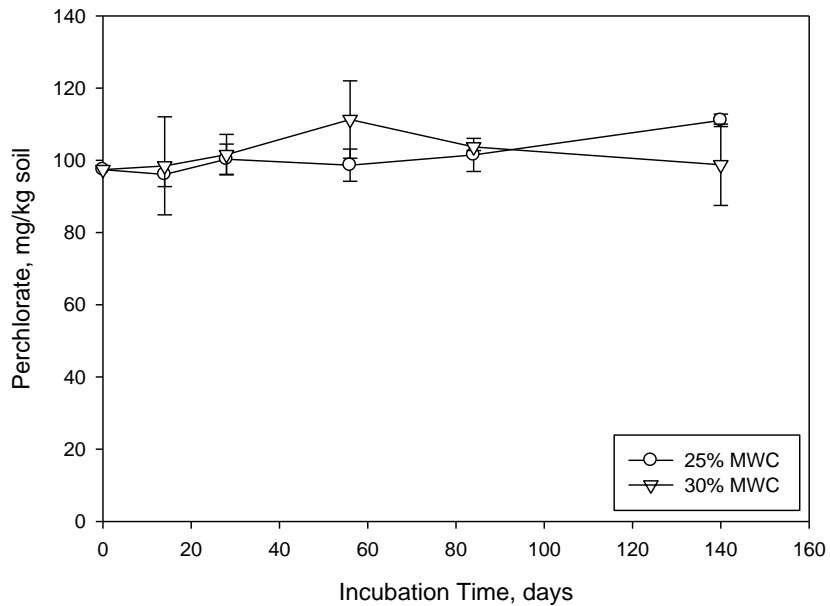
Figure 3.4 Perchlorate reductions in microcosms with soil A under 25 and 30% mass water content

Water stress is a major factor that limits microbial activity in hot and dry soil [66]. Water stress in a soil can be quantified by total soil water potential, which is the sum of matric, osmotic and gravitational potentials.

The gravitational potentials at each point of soil are determined by the elevation of the point relative to a reference level. The gravitational potentials in the microcosms were similar. The matric potential in soil is caused by the capillary and adsorptive forces (matric suction) from soil matric. Based on the soils' water-holding capacities shown in Figure 3.2, the matric potential of Soil A under 25 to 40% MWC were always higher than 11 kPa which is negligible compared with the osmotic potentials (-414 kPa and -260 kPa) in the microcosms.

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Water stress in the soil microcosms were mainly from osmotic potential while it was shown in previous study (see Figure 3.1) that perchlorate reduction can tolerate osmotic potential up to -828 kPa. It appears that water content is a limiting factor in perchlorate degradation in the microcosms; however, the limiting effect is not associated with cell dehydration.

Another mechanism for soil moisture effects on microbial activity is limiting substrate supply by altering diffusion pathway in soil [46, 67]. At lower water contents, the water film coating soil particle surfaces is thinner and the diffusion paths are more tortuous. Thus, the diffusion rate of dissolved substrate in soil matrices may decline with decreasing water content [46, 49, 67]. Substrate limitation may inhibit microbial growth and activity in soil. It has been reported that microbial activity in a silt loam was mainly inhibited by substrate limitation other than dehydration in higher moisture content (greater than 0.6 MPa) [46]. According Rockhold's review, effective substrate diffusion coefficient in the aqueous phase increases exponentially with aqueous saturation under low moisture conditions and reaches a plateau value under moderate moisture condition in a sand porous medium. When the porous medium is close to the saturated condition, the diffusion coefficient of oxygen in the gas phase declines exponentially with water content [67]. As water content increases, solute transfer reaches optimum condition and the oxygen supply rate decreases due to the decreasing air filled porosity. Thus, a saturated soil condition favors anaerobic processes while aerobic processes prefer a moderate moisture condition.

It was also seen that decomposition of organic carbon occurred in the 25% and 30% MWC Soil A microcosms (see Figure 3.5). Degradation of organic carbon is a supportive evidence of microbial activity in the microcosms. It can be concluded that the effect of moisture on perchlorate bioremediation is not through dehydration or substrate limitations.
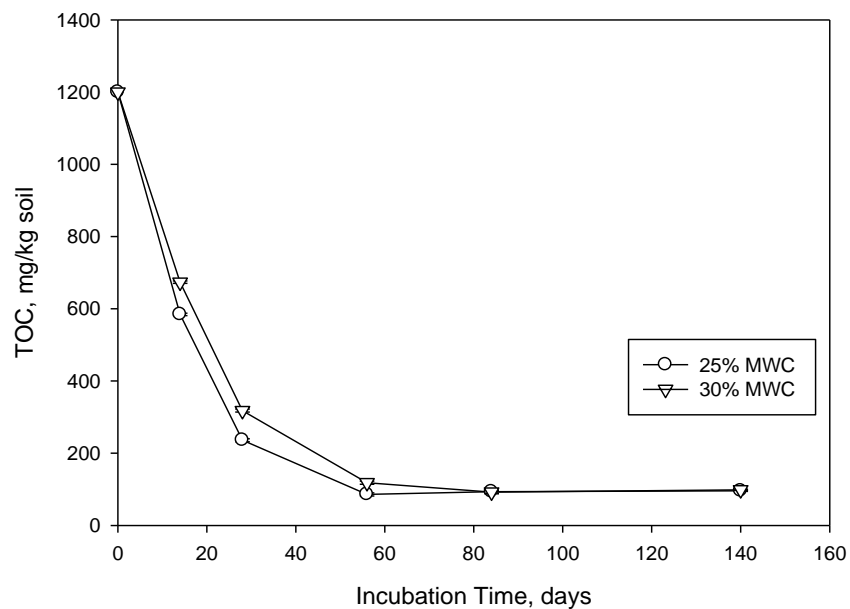
Figure 3.5 Decomposition of total organic carbon in soil A microcosms under 25 and 30% moisture conditions

Organic carbon in the microcosms was degraded rapidly right after incubation. Most of the organic carbon in the microcosms was acetate, which was amended into the soil as electron donor. The redox pathway of acetate can be written as shown in Equation 3-2 and Equation 3-3. Acetate serves as electron donor in most of the respiration process as shown in Equation 3-2. In this path way, acetate decomposition requires coupling electron acceptors to accept the electrons from acetate. *Methanogens* can also convert

acetate to methane and carbon dioxide as shown in Equation 3-3. In this pathway, acetate

can be considered as both electron donor and acceptor. However, methane production is

a strict anaerobic process and requires lower redox potential then perchlorate reduction

(see Table 3.3). Methane production will not occur prior to perchlorate reduction. This

hypothesis was confirmed by analyzing headspace of final microcosms. No methane was

detected in gas samples collected from headspace.

$$CH_3COO^- + 4H_2O \rightarrow 2CO_3^{2-} + 11H^+ + 8e^- \qquad \text{Eq 3-3}$$

$$CH_3COO^- + H_2O \rightarrow CO_3^{2-} + CH_4 + H^+ \qquad \text{Eq 3-4}$$
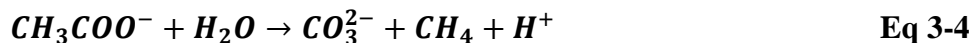
Table 3.1 Dominant electron acceptors in soils at different redox potentials.

Measured Redox Potential Range E_h (mV)	Observation
380~320	Oxygen depletion[68]
280~220	Nitrate and Mn (IV) reduction[68]
180~150	Fe(III) Non-detectable[68]
0~-100	Perchlorate reduction
-120~-180	Sulfate depletion[68]
-200~-280	Carbon dioxide depletion[68]

Organic carbon degraded in the first week may consumed by oxygen and nitrate.

Although head space of each microcosm were flushed with nitrogen gas, all of the

oxygen in the soil pores was not removed from the microcosms. Denitrification was observed in all soil microcosms and nitrate was depleted within one week. Anoxic condition might occur in some part of aggregates in soil microcosm. Both denitrification and perchlorate reduction require anaerobic condition but perchlorate reduction has lower level of tolerance to oxygen [24, 69, 70]. Based on the results of the microcosm testing, it appears that the anaerobic condition in the soil was suitable for denitrification but not for perchlorate reduction.

After the first week following electron donor amendment, organic carbon was consumed using oxygen as the electron acceptor. Oxygen is believed to slowly diffuse into the soil system. Due to the limited oxygen transfer and substrate, organic carbon decomposition rates were decreased with time. After 80 days, however, the organic carbon in soils decreased to background level. Perchlorate reduction in these microcosms could not be expected due to the depletion of electron donor after 80 days. Insufficient electron donor could be the reason of failure in perchlorate reduction. Whether higher dosage of electron donor can promote perchlorate bioremediation was discussed in next portion of this research (see Chapter 4).

Oxygen concentration in soil microsites depends on the oxygen diffusion rate and also the oxygen consumption rate [50, 71]. Different soils may have different threshold value of MWC for perchlorate bioremediation due to the different texture and different biogeochemical properties.

To further investigate the effect of moisture condition on perchlorate reduction in soils, additional two set of microcosms were conducted under different MWC with Soil B and Soil C. Results from microcosm with Soil B are shown in Figure 3.6.
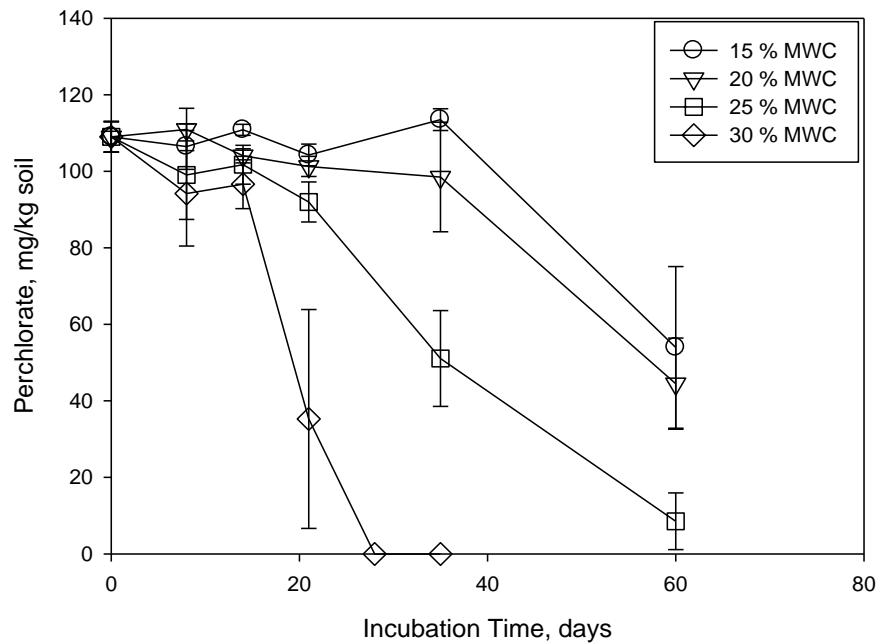
Figure 3.6 Perchlorate reductions in microcosms with soil B under 15, 20, 25 and 30% mass water content

Perchlorate reduction started after 14 days lag period and perchlorate was depleted within 28 days in 30% MWC. Perchlorate reduction in 25%, 20%, and 15 %M WC microcosms initiated after 12, 35, and 35 days, respectively. Air filled porosity in each microcosm were estimated with soil porosity and water filled porosity. As seen in the results shown in Figure 3.7, perchlorate degradation lag period had a strong correlation with air filled porosity in the range between 0.2 and 0.31. When air filled porosity increased from 0.31 to 0.37 (20% MWC to 15%MWC), no changes were noticed

in the length of lag period. Oxygen is a competitive inhibitor of perchlorate bioremediation. As MWC increased, the total amount of oxygen and oxygen transfer rate in microcosms decreased. Thus, anaerobic condition most likely occurs earlier at higher moisture conditions.

Perchlorate reduction had similar rates in the 15%, 20%, and 25% MWC microcosms while perchlorate reduction in the 30% MWC microcosms were twice the rate of the other microcosms. The effect of moisture content on perchlorate reduction rate was limited in the MWC range between 25 and 30%. The mechanism behind this effect is not clear.

Perchlorate reduction in the Soil B microcosms is comparable with Nozawa-Inoue's microcosm test. The soil used in Nozawa-Inoue et al.'s test was silt loam soil collected from the top 0 to 15 cm in an agricultural field [33]. Perchlorate degradation in acetate amended microcosms to 20% MWC started after 14 days and 100 mg/kg perchlorate was completely degraded within 40 days, which is similar to the results from 25% and 30% MWC Soil B microcosms.
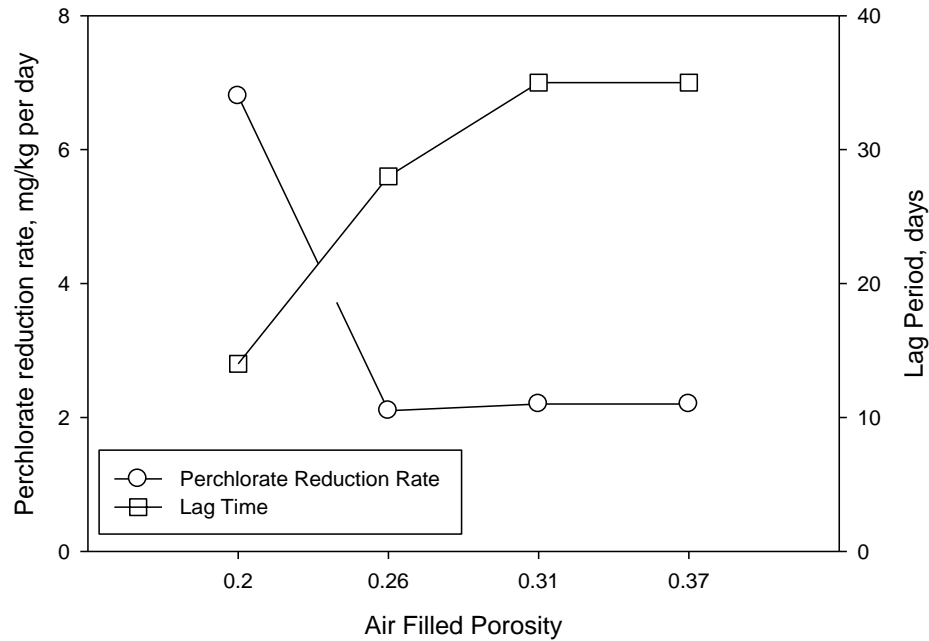
Figure 3.7 Lag period of perchlorate bioremediation and perchlorate reduction rate at different air filled porosity of soil B

In the Soil A microcosms, perchlorate reduction was only observed under 40 and 50% MWC, which represent near saturation and fully saturated conditions. The oxygen diffusion through soil pore was suspected as the main limiting factor in perchlorate reduction under lower moisture conditions. However, perchlorate reduction in Soil B occurred under MWC as low as 15% and no threshold value of MWC was detected in the range between 15% and 30% MWC. Soil A and B had similar texture and water holding capacity.

Oxygen diffusion rate in soil is mainly controlled by soil texture and water content while oxygen consumption rate is mostly depends on soil biomass and bioavailable organic carbon [50, 71]. It is possible that development of anaerobic

conditions in soils with similar physical properties may different due to different

biogeochemical properties. Soil B was collected from 20 cm below the surface of ground

while Soil A was collected from 6m depth of the vadose zone. Numerous researches

have shown that microbial biomass decreases with increasing depth in soil due to limited

organic carbon [72-74]. Oxygen depletion in a soil aggregate can be more readily

achieved with higher biomass. Gal *et al.* examined the potential of perchlorate

biodegradation in different depths of vadose zone soil. Perchlorate was completely

reduced in soil slurry from depths of 1, 15 and 30 m, while no perchlorate reduction was

observed in sediment sample from 20 m below land surface. The failure of perchlorate

degradation was explained by low viable microbial communities, low water content, and

high perchlorate concentration [44].

Although it has been validated that Soil A had potential of perchlorate reduction

under saturated condition, it is possible that anaerobic condition does not develop due to

lower oxygen consumption rate by soil biomass.

Perchlorate bioremediation results from Soil C microcosms are shown in Figure

3.8. Soil C was sand with a higher density and lower pore volume than Soil A and Soil B.

Soil C, a sandy soil, also had lower water holding capacity then the other two tested soils.

Soil C microcosms were conducted under lower MWC (5% to 15%) than the

other two soils. Perchlorate degradation in 15% MWC Soil C microcosms started after

14 days with complete reduction within 21 days. Perchlorate reduction rate in the 15%

MWC Soil C microcosms were similar to the rates of Soil A under 40% MWC and Soil B

under 30% MWC. No perchlorate reduction was detected in 5% and 10% MWC Soil C microcosms in 120 days.

In contrast, denitrification was completed in all microcosms within one week of incubation. Similar results were observed in Evan's research[36]. Significant perchlorate reduction was observed in silty sand under 9.5% MWC with ethanol as electron donor, while no perchlorate reduction were promoted under 7.5% MWC within 34 days [36].

Organic carbon in Soil C microcosms degraded rapidly without any lag time and completed within 60 days of incubation as shown in Figure 3.9. Decomposition rate of organic carbon decreased to 25% after 60 days. As discussed previously, most of the organic carbon degraded during incubation is coupled with oxygen reduction. As the readily available oxygen in the pore spaces is consumed, organic carbon reduction may be limited by the oxygen transfer rate. When oxygen in the microcosm depleted, organic carbon might not be further degraded. No significant differences in organic carbon decomposition were observed in all of the Soil C microcosms under different MWC. At the end of incubation, sufficient organic carbon (370 to 580 mg/kg) were left in all soil microcosms but perchlorate reductions were only observed in the 15% MWC microcosms. Perchlorate degradation in Soil C under different mass water content had similar trend with Soil A. The reason for the lack of perchlorate reduction under lower MWC microcosms with Soil A and Soil C is not clear. Higher MWC might be beneficial for perchlorate reduction by increasing solute transfer rate and limiting oxygen transfer. This hypothesis is validated by the Soil B microcosm results. However, this hypothesis

does not seem to apply to Soil A or Soil C. Other mechanisms may exist behind the

effect of moisture condition on perchlorate reduction in Soil A and Soil C.
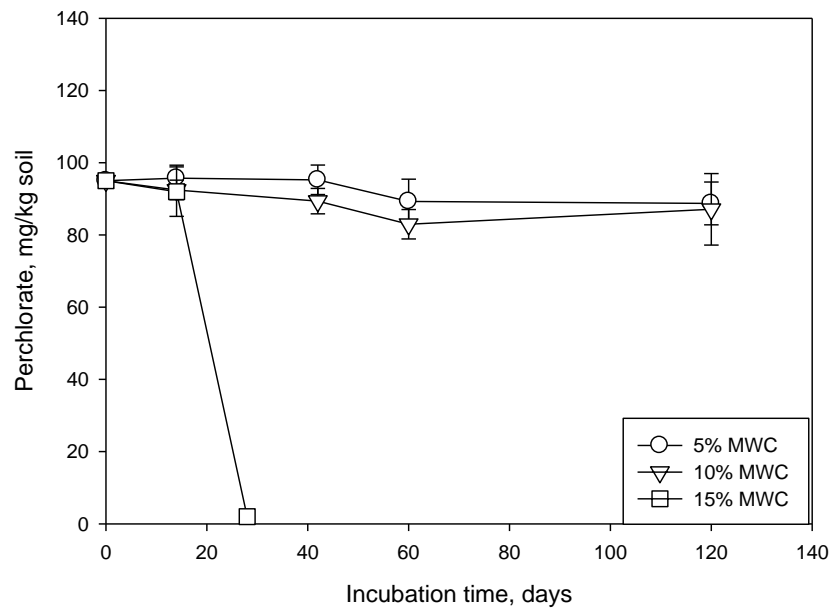
Figure 3.8 Perchlorate reduction in microcosms with soil C under 5,10 and 15% MWC
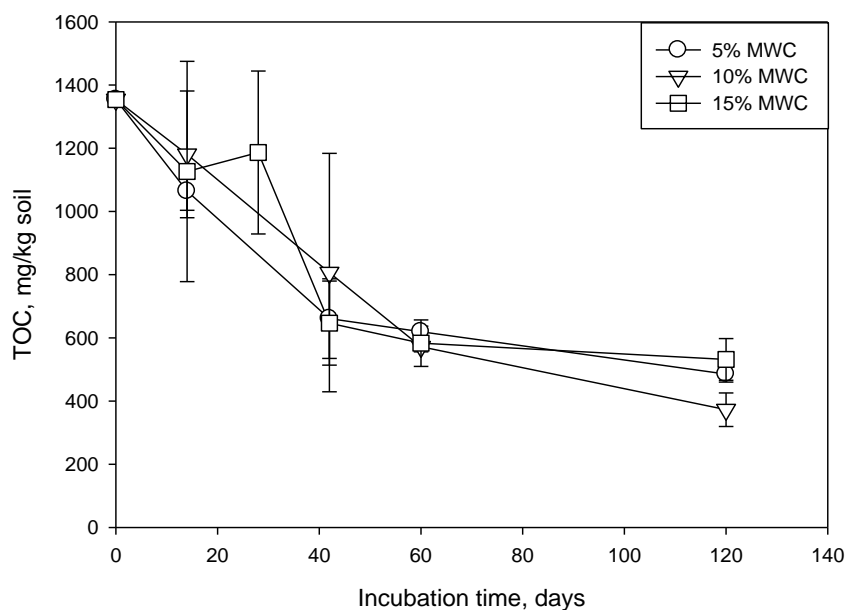
Figure 3.9 Decomposition of total organic carbon in soil C microcosms under 5, 10and 15% MWC

3.5   SUMMARY

Denitrification was completed within one week in all soil microcosms and was

not affected by different moisture conditions in the three soils investigated in this

research. Perchlorate reduction was observed in the 40% and 50% MWC microcosms

with Soil A and 15% MWC microcosms with Soil C. Perchlorate reduction in Soil A and

Soil C was completely suppressed by lower MWC (<40% and <15%, respectively). In

Soil B, perchlorate degradation was occurred in all MWC (15~30%) microcosms. The

lag periods of perchlorate degradation became longer and the reduction rate decreased

with decreasing MWC although it did not inhibit perchlorate reduction completely.

Optimum soil moisture content for perchlorate bioremediation varies significantly

in different soils. Threshold values of MWC may depend on soil textures and other

biochemical properties. The moisture effect on perchlorate reduction does not appear to be caused by cell dehydration.

Higher moisture conditions can readily promote anoxic condition by limiting oxygen diffusivity in the soil. Although no perchlorate reduction was promoted, organic carbons in the microcosms were continuously degraded even after nitrate depletion in Soil A and Soil C microcosms under lower MWC. This part of organic carbon might be consumed by oxygen while it is also possible that soils contain competitive electron acceptors other than oxygen, such as nitrate. Thus, investigation of the dominant redox pathways in soil can provide clues to further investigate perchlorate reduction in vadose zone soil and overcome the inhibition from low moisture.

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Chapter 4   Effect of Soil Biochemical Reactions on In-Situ Bioremediation of Perchlorate in Vadose Zone Soil

4.1   INTRODUCTION

Contamination of soil and groundwater due to historical uncontrolled disposal practices of perchlorate is a widespread problem in United Sates. In-situ bioremediation (ISB) is a process where pollutants are biologically degraded under natural conditions to harmless transformation product. ISB technologies are usually low-cost, low maintenance and sustainable approaches for the cleanup of various pollutants in soil and groundwater [1]. It has been reported that biological reduction of perchlorate can be readily stimulated in groundwater (saturated zone) by addition of electron donors such as acetate [2-7]. In addition, researchers have shown that perchlorate remediation within the vadose (unsaturated) zone by native microbial communities is possible [8, 9]. Several full scale and pilot scale projects were conducted to remediate perchlorate in vadose zone soil and perchlorate reduction was observed in all these studies. However, most of the remediation process was conducted ex-situ or saturated condition (slurry) and perchlorate removal rates ranging from 50 μg/kg-soil per day to 6-7 mg/kg-soil per day [8-12].

Stimulation of biological reduction of perchlorate in the ground is a complex process, which involves numerous biogeochemical components. As a result, perchlorate bioremediation efficiency may differ in different soil environments.
In the prior studies (Chapter 2 and Chapter 3), soil moisture was identified as a key factor that affect bioremediation rate of perchlorate in vadose zone. Perchlorate remediation was readily achieved and had higher reduction rate with higher moisture in all three soils tested.

Perchlorate reduction rate increased with increasing moisture in Soil B in the range between 15% and 30% of mass water content (MWC). However, perchlorate biological reduction was only observed in soil microcosms with higher mass water content (40% and 15%), which were close to the saturation and the field capacity in Soil A and Soil C respectively. Similar results were also observed in Cai *et al.*'s microcosm study in which perchlorate bioremediation was investigated with a gaseous electron donor [13]. Significant perchlorate reductions were observed in microcosms with higher MWC (15%) soil while little or no perchlorate degradation was detected in microcosms with lower MWC (7% to 13%) within 191 days [13]. The reason of failure in perchlorate reduction with low MWC was not discussed further. However, it was pointed out that optimum moisture for perchlorate bioremediation may vary at different sites. Even under the same mass water content, the water availabilities for microorganisms in different soil may due to the capillary effect and adoption effect from soil particles and soil organic matters. Thus, soil water potential rather than mass water content could be a good indicator of moisture requirement in perchlorate remediation [13].

Based on the earlier results from this research (see Chapter3), the optimum water potentials for perchlorate reduction also were found to vary in different soils and that the effect of moisture on perchlorate bioremediation was not through direct cell dehydration. Anaerobic bioremediation is more challenging in vadose zone soils due to the available oxygen in the soil pores [14-17]. Most anaerobic respirations cannot be initiated until oxygen in the soil aggregate depleted. Oxygen availability in soil can be limited by increasing soil water content. Soil water displaces air (oxygen) in the pores and also reduces the oxygen diffusion by increasing diffusion pathways in the soil [14, 15, 17].

The results of perchlorate reduction in Soil B can be well explained by this hypothesis in which lag period of perchlorate reduction decreased and reduction rate increased with increasing soil MWC.

In contrast, the effect of moisture on perchlorate remediation in Soil A differed significantly from Soil B even though two soils had similar physical properties. Other mechanisms might be behind the limiting effect of moisture on perchlorate reduction. Perchlorate reduction was not stimulated in Soil A microcosms with 15% and 20% MWC and Soil C microcosms with 5% and 10% MWC within 140 and 120 days. However, dramatic reductions of organic carbon were observed in these microcosms. This part of organic carbon can be consumed by oxygen in the soil. It is also possible that other anaerobic processes such as iron reduction or sulfate reduction in soils consumed the organic carbon.

Common terminal electron acceptors in soil include oxygen, nitrate, Mn^{4+}, Fe^{3+}, sulfate and carbon dioxide [18-20]. In situ bioremediation of perchlorate in soil requires specific environmental conditions to stimulate perchlorate respiration. During remediation, lag periods are often observed for microbial community to acclimate to the changing environment and compete with other soil microbes in environmental conditions. Most perchlorate cleanup efforts are focused on contaminated groundwater and drinking water. As a result, it has been well documented that oxygen and nitrate are competitive electron acceptors in perchlorate bioremediation. However, little is known about the competitive effect of other biochemical reactions in soil on perchlorate reduction such as ferric iron reduction process. On the other hand, perchlorate remediation may require similar geochemical conditions with other anaerobic reactions in soils. An investigation

of general biochemical reactions during soil incubation could be helpful to identify and overcome challenges in perchlorate soil remediation.

Objectives of this research are to 1) investigate electron pathways in unsaturated soil condition and identify competitive electron acceptors in perchlorate bioremediation in soil; 2) investigate the suitability of humic acid as electron shuttling substrate to lower the soil ORP and accelerate perchlorate remediation in unsaturated soil. Two hypotheses were made based on results from prior researches. First, competitive respiration processes in vadose zone soil inhibits perchlorate reduction. Increasing the population of perchlorate reducers in soil will stimulate perchlorate reduction under lower moisture condition. Second, anaerobic processes were generally inhibited in Soil A under lower moisture condition. Thus, it is hypothesized that addition of electron shuttling mediate (humic acids) can stimulate anaerobic processes. To achieve the research goals, several soil microcosm studies were conducted under different conditions.

4.2   MATERIALS and METHOD

4.2.1   Materials

The soil used in this experiment was the same soil used in Chapter 2 and Chapter 3, which was collected from a depth of 20 feet at the Lockheed Martin Corporation's Beaumont Site 2 (Beaumont, CA). The soil contained 100 mg/kg of perchlorate and 16.3 mg/kg of nitrate as N. Initial soil mass water content (MWC) was 10% and initial pH was 8.5 ± 0.2. Additional soil information is available in Chapter 3. The soil was passed through a 2-mm sieve and stored at 4^0C until the experiment was conducted.

Perchlorate reducing bacterial consortium was enriched from a soil sample (Soil A) described above. The soil sample (50 g) and nutrient medium (200 mL) containing 200 mg/L perchlorate and 1 g/L acetate were added into a 250-mL glass bottle with a septum cap. The sample was incubated at room temperature on a shaker table. After perchlorate degradation observed, supernatant (50 mL) from the bottle were transferred to fresh medium (150 mL) containing 1 g/L perchlorate and 2 g/L acetate. After perchlorate depletion was observed, the culture solution (50ml) was transferred to fresh medium. These steps were repeated to keep the activity of perchlorate reducers until the experiment was conducted.

Humic acid used in this research was Elliott Soil Humic Acid Standard from the International Humic Substances Society. Humic acid (100 mg) was dissolved in 500 mL deionized water and the pH was adjusted to 7 with sodium hydroxide. The humic acid solution (200 mg/L) was stored at 4^0C until the experiment was conducted.

4.2.2 Electron path ways in soils under saturated and unsaturated soils and the effect of bioaugmentation on perchlorate bioremediation in unsaturated soil

To investigate electron pathways in vadose zone soil during perchlorate remediation, three soil microcosm studies were conducted with Soil A. Two microcosm studies were conducted at MWCs of 20% and 50% with 2.5 g acetate per kg soil as the electron donor.

The setup procedure was the same as described in prior study (see Chapter 3). In the bioaugmentation study, perchlorate reducing bacterial consortium was amended to the soil to stimulate perchlorate reduction under 20% MWC. Perchlorate reducers were

harvested by centrifuging culture medium described in the previous paragraph. The cell
pellets were washed three times with deionized water and suspended using electron donor
(acetate) solution. Optical density in the final amendment solution was 0.2. Soil
moisture and electron donor concentration was adjusted to 20% MWC and 2.5 g/kg,
respectively, by adding amendment solution. The soil mixtures (10 g dry equivalent)
were added to individual 40-mL vials. As summarized in Table 4-1, 18 sacrificial
microcosms were prepared for each experimental condition. Triplicate microcosms were
scarified and analyzed for nitrate, perchlorate, ferric iron, ferrous iron, sulfate, and
acetate, which are common electron acceptors and electron donors in the microcosms.

Table 4.1 Summary of experimental conditions in electron pathway study

Microcosms	1	2	3
Mass water content, %	50	20	20
Electron donor (acetate), g/kg	2.5	2.5	2.5
Bacterial consortium	No	No	Yes
Number of sacrificial microcosms	18	18	18

4.2.3   Perchlorate reduction in unsaturated soil under strict anaerobic condition

Oxygen is the most favorable electron acceptor to microbes in aquatic and
terrestrial systems. It can be a critical factor that controls anaerobic processes in
groundwater and soil. To investigate anaerobic respiration process in unsaturated soil, a
soil microcosm study was conducted with under strict anaerobic condition to eliminate

competitive effect of oxygen. The microcosms were prepared with 2.5 g/kg acetate as the electron donor and a 20% MWC.

The procedures were the same as those with the 20% MWC microcosms outlined above. However, the whole experiment was conducted in an anaerobic chamber and microcosms were also stored in the chamber until it scarified for nitrate, perchlorate, ferric iron, ferrous iron, sulfate and acetate.

4.2.4 Effect of humic acid (HA) on soil ORP and perchlorate bioremediation

To examine whether HA can accelerate perchlorate bioremediation and other anaerobic process in unsaturated soil, a soil microcosm study was conducted with 20mg/kg HA as electron shuttling mediate [21-23]. This study was conducted with 2.5 g/kg acetate at a 20% MWC. The control experiments were performed in the same manner except that no HA was added. For each experimental condition, 19 sacrificial microcosms were prepared in 40-mL serum bottles. One sacrificial microcosm from each condition was used to monitor ORP in the soils during the incubation and other 18 microcosms were used to analyze nitrate, perchlorate, ferric iron, ferrous iron, sulfate and acetate. A sacrificial microcosm at 50% MWC was also prepared for investigate ORP changes in saturated soil.

4.2.5 Analytical methods

Perchlorate in soil samples were determined by extracting 10 g of dry equivalent soil with 20 mL of deionized water and vortex mixing for 2 min. Extracts were obtained by centrifugation at $5000 \times g$ and filtered through 0.2-μm filter to remove soil particles.

This extraction method was able to recover 98±4% of perchlorate in soils. The extracts were also used to analyze dissolved part of nitrate and TOC in soil samples. Perchlorate in extracts was analyzed using a Dionex 1000 Ion chromatograph (Dionex Corp., Sunnyvale, CA,USA) with an IonPac® AS 16 analytical column (4×250 mm) and AG 16 guard column (4×50 mm). Nitrate and sulfate were determined by an IonPac® AS 14 analytical column (4×250 mm) and AG 14 guard column (4×50 mm). The detection limits for perchlorate, nitrate and sulfate were 4 μg/L, 100 μg/L (as N) and 0.2 μg/L respectively. Irons in soil were extracted with 0.5N HCl solution (1:50 w/w). Ferrous iron and ferric iron in the extracts were analyzed using 1,10-phenanthroline method [24].

4.3   RESULTS AND DISCUSSIONS

4.3.1   Electron path ways in soils under saturated and unsaturated soils and the effect of bioaugmentation on perchlorate bioremediation in unsaturated soil

Soil is a complex system consists of minerals, organic matters water, air and microorganisms. In-situ perchlorate bioremediation requires specific environmental conditions for the perchlorate reducers to carry respiration using perchlorate as the electron acceptor and compete against other soil microorganisms. In prior study (Chapter 2 and Chapter 3), perchlorate reduction in unsaturated Soil A was not successful but significant reduction in TOC in soil microcosm was observed. It appears that some competitive respirations occurred in the microcosms.

To investigate electron pathways in soil microbial community and evaluate bioaugmentation approach in perchlorate bioremediation, microcosm studies were

conducted at three different conditions – 50% MWC, 20%MWC, and 20% MWC with bioaugmentation.

In Soil A, oxygen, nitrate, ferric iron, perchlorate and sulfate were identified as competing terminal acceptors. The terminal electron acceptors and possible half redox reactions in soil were summarized in Table 4.2. The dominant electron donor in soil was 2.5 g/kg acetate amended to the soil.

Microcosm results are shown in Figure 4.1 through Figure 4.4. In these figures, all terminal electron acceptors in soil were converted to electron equivalent based on the half reactions summarized in Table 4.2 and the ferrous iron was monitored instead of ferric reduction.

Table 4.2 Dominant electron acceptors and possible half reactions in Soil A

Electron acceptors	Units	Concentrations	Half reactions
Nitrate	N-mg/kg	7.8	$NO_3^- + 5e^- + 3\,H_2O \rightarrow 1/2\,N_2 + 6OH^-$
Ferric Iron	mg/kg	580	$Fe^{3+} + e^- \rightarrow Fe^{2+}$
Perchlorate,	mg/kg	100	$ClO_4^- + 8e^- + 4\,H_2O \rightarrow Cl^- + 8OH^-$
Sulfate	mg/kg	9.8	$SO_4^{2-} + 8e^- + 4H_2O \rightarrow S^{2-} + 8\,OH^-$

The first microcosm study was conducted under 50% MWC in which soil was completely saturated. Based on results shown in Figure 4.1, nitrate was depleted in soils within 7 days. Perchlorate reduction in the microcosms was initiated after a 14-day lag period and completed within 35 days. Significant increase in ferrous iron was observed after a 28-day incubation, which could be the result of biological ferric reduction. Sulfate

reduction was also observed in the microcosms within 35 days. Acetate decomposition

began right after incubation and it accelerated significantly after 21 days.   Electron donor

(acetate) oxidation during 35 days incubation was 16 times that of monitored electron

acceptors (sum of nitrate, perchlorate, ferric iron and sulfate reduction). Thus, other

electron acceptors or other electron pathways existed in the microcosms.
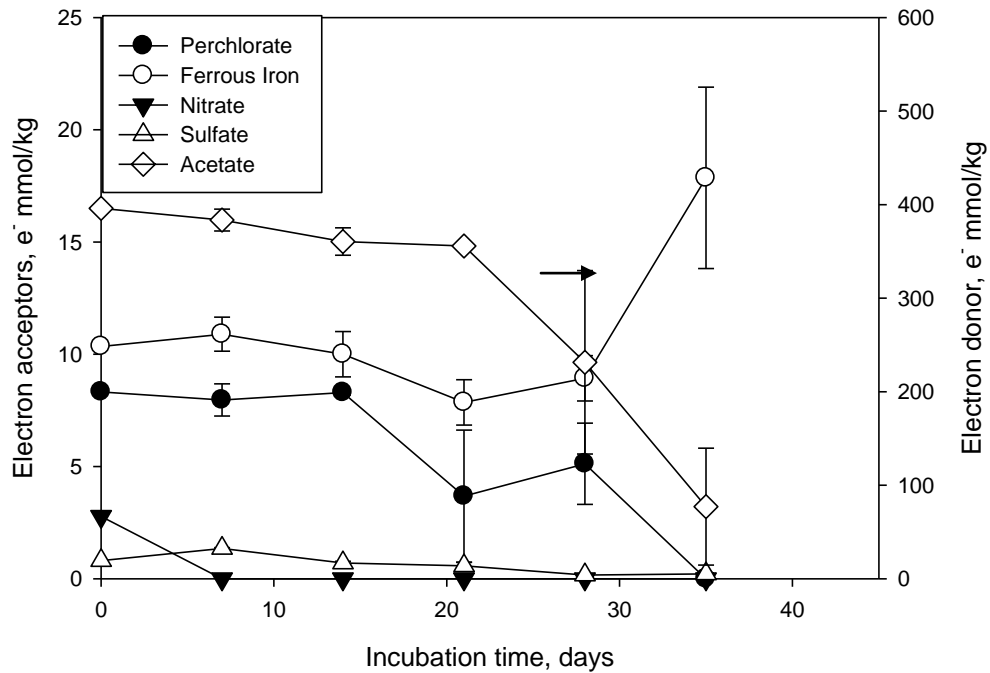
Figure 4.1 Reduction of dominant electron acceptors and electron donor (acetate) in saturated (50% MWC) soil.

It is observed that these electron acceptors consumed by soil microorganisms in a

sequence which follows the thermodynamic theory as shown in Table 4.3 [18-20].

Perchlorate reduction is usually observed at ORP range between 0 to -100mV in

groundwater [25]. It was expected that perchlorate reduction would occur after ferric

reduction and before sulfate reduction. Based on the results from this study, ferric

reduction and sulfate reduction started at similar period with perchlorate degradation.

ORP in soil is difficult to define and measure comparing with aquatic system because

thermodynamic equilibriums are rarely reached in soil systems[26]. As a result, favored

ORP for perchlorate reduction in soil may differ from that in aquatic system. On the

other hand, sequential reduction of different terminal electron acceptors was explained by

outcompeting ability of different organisms for electron donors in many researches [18,

19, 27, 28]. Unlike natural soils and sediments, sufficient electron donors were provided

for anaerobic process in soil microcosms. Thus, it is possible that the competitive effect

among different electron acceptors was not significant. Unlike other anaerobic process,

denitrification was observed without any lag period.

Table 4.3   Dominant electron acceptors in different oxidation reduction potential.

| Redox Potential Range E_h (mV)	Observation
380~320	Oxygen depletion[19]
280~220	Nitrate and Mn (IV) reduction[19]
180~150	Fe(III) Non-detectable[19]
0~-100	Perchlorate reduction [25]
-120~-180	Sulfate depletion[19]
-200~-280	Carbon dioxide depletion[19]

The second microcosm study was conducted under 20% MWC with acetate as electron donor. Based on the results shown in Figure 4.2, nitrate reduction was completed within 7 days, similar to the denitrification results in saturated soil shown in Figure 4.1. Denitrification in the soil was not affected by decreased moisture condition. In contrast, little or no perchlorate reduction was observed in unsaturated soil (20% MWC) within 35 days. In addition, neither ferric iron nor sulfate was reduced in the unsaturated soil. The results from this study were consistent with the sequence summarized in Table 4.2 in which denitrification in soil or sediments occurs before ferric reduction and sulfate reduction. It appears that the geochemical conditions required for denitrification are different from other anaerobic reactions in soil.

Acetate decomposition started without a lag period and decomposition rate increased significantly after 7 days. After 35 days, TOC in the soil microcosms decreased to background level. Thus, perchlorate reduction could not be expected with a longer incubation period. As mentioned before, acetate degraded in the first week may be consumed by oxygen and nitrate. After nitrate depletion, no other anaerobic process was observed in the soil. Thus, acetate consumed in the microcosms was coupled with oxygen, which is believed to slowly diffuse into the soil system.
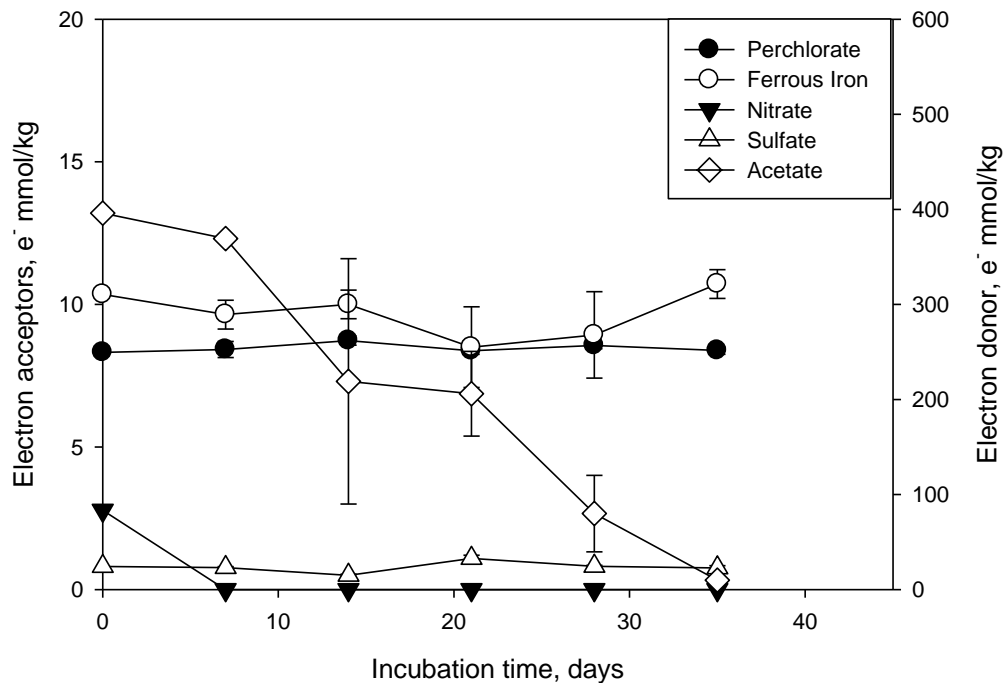
Figure 4.2 Reduction of dominant electron acceptors and electron donor (acetate) in unsaturated (20% MWC) soil.

To investigate the suitability of bioaugmentation in vadose zone soil, a microcosm study was conducted with perchlorate reducing bacterial (PRB) consortium enriched from the same soil sample. As seen in Figure 4.3, amending extra perchlorate reducers into the microcosms didn't stimulate perchlorate reduction within 35 days. During a 35-day incubation period, no ferric iron or sulfate reduction observed in the microcosms. Nitrate was depleted within 7 days in the microcosms. Acetate in the microcosms was degraded to background level within 20 days.
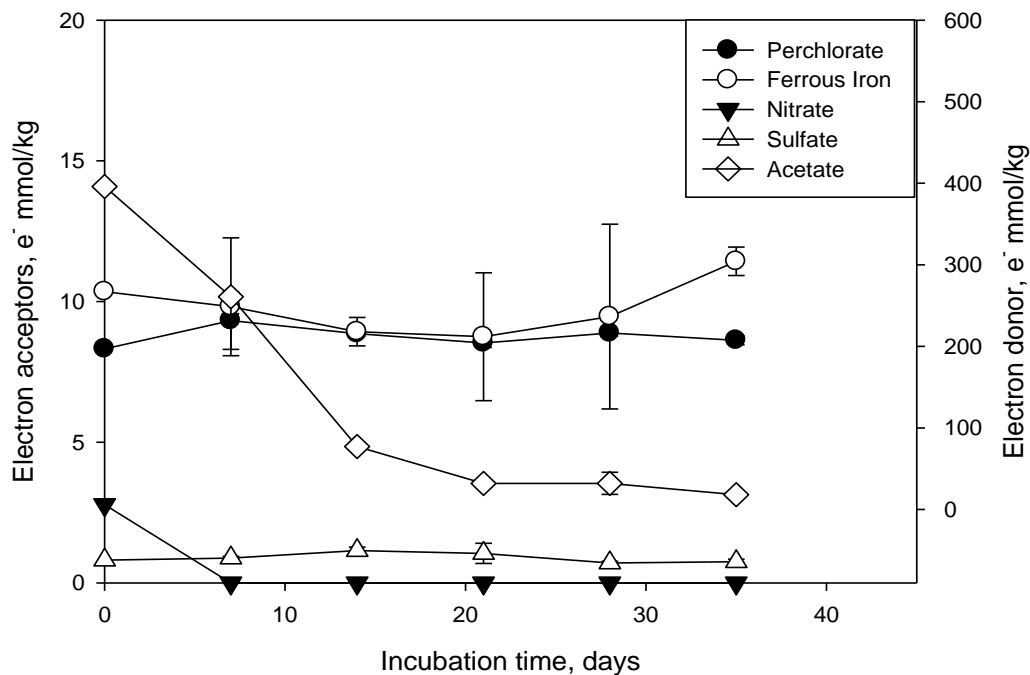
Figure 4.3 Reduction of dominant electron acceptors and electron donor (acetate) in unsaturated (20% MWC) soil amended with PRB.

Based on the results shown in Figure 4.4, acetate decomposition in the saturated soil (50% MWC) was significantly slower than that in the unsaturated soil (20% MWC). It appears that the overall microbial activity in saturated soil was lower than that in unsaturated condition. After a 20-day incubation period, anaerobic processes were developed and acetate decomposition rate was increased due to the anaerobic respirations.

Acetate decomposition was stimulated by PRB augmentation. In general, perchlorate reducing bacteria are facultative anaerobes, capable of utilizing oxygen, nitrate, and perchlorate as terminal electron acceptors. However, oxygen and nitrate are more favorable than perchlorate as electron acceptors in mixed cultures and pure cultures of perchlorate reducing bacteria [29-32]. Thus, amending perchlorate reducers could be

expected to accelerate oxygen consumption rate and denitrification in the soil, which was

confirmed by rapid acetate decomposition in soil. However, it did not stimulate

perchlorate reduction in the soil within 20 days. After the 20-day incubation no more

electron donor was available for perchlorate reduction in the soil. This result leads to a

question "whether perchlorate would be degraded eventually with sufficient electron

donor or complete exclusion of oxygen?"
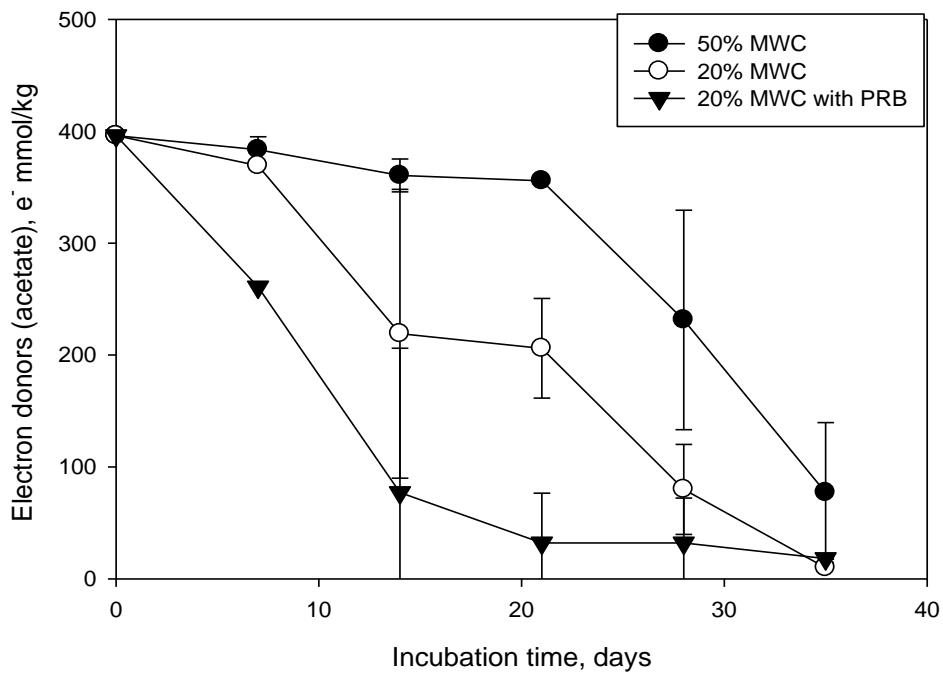
Figure 4.4 Acetate decomposition in soil microcosms under three different conditions.

4.3.2 Perchlorate reduction in unsaturated soil under strict anaerobic condition

To eliminate the effect of oxygen in perchlorate reduction in soil, a microcosm study was conducted in a strict anaerobic condition. As shown in Figure 4.5, denitrification was completed within 7 days, which is consistent with the results from previous microcosms. Little or no acetate decomposition was observed within 100 days without oxygen. Acetate consumed by denitrification process was not significant compared with the total acetate amended to the soil, confirming that most of the organic carbon consumed in previous unsaturated microcosms was oxidized by oxygen. Perchlorate reduction was not promoted within 100 days with sufficient electron donor, however.

It appears that anaerobic processes other than denitrification were inhibited in unsaturated soil condition and the inhibitory effect was not from oxygen or other competitive redox process.
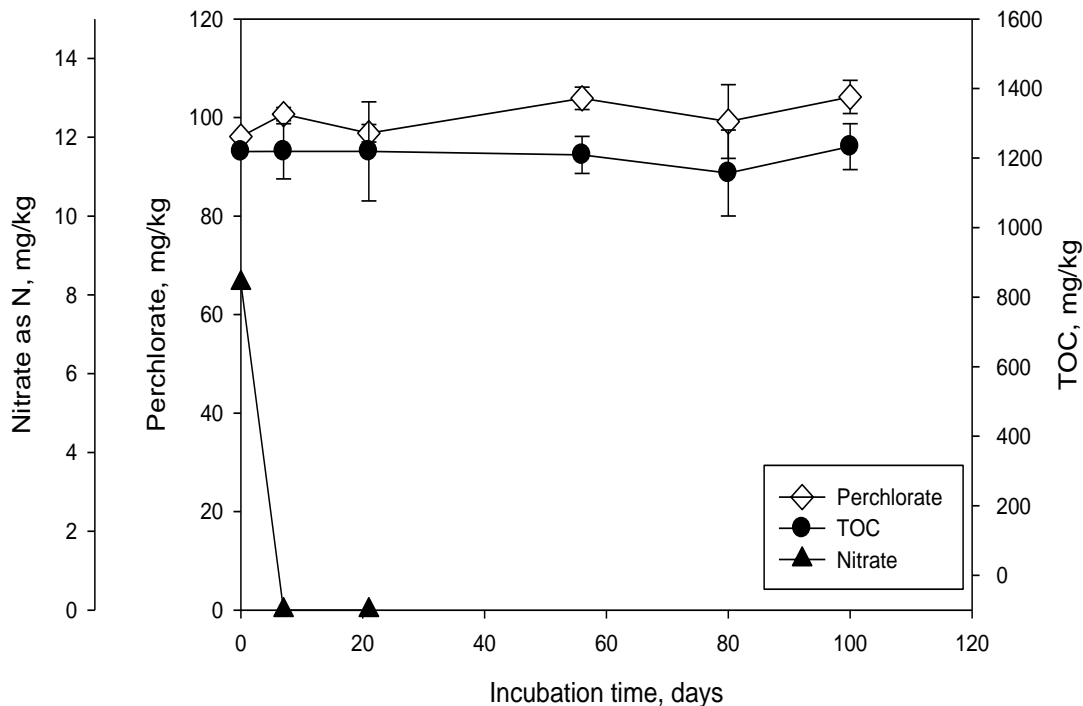
Figure 4.5 Perchlorate reduction in 20% MWC soil microcosms under anaerobic condition.

Biological respiration processes involve sequences of redox reactions in which electrons are transferred from electron donors and electron acceptors [33-35]. Some bacteria perform direct electron transfer in the cell while some microorganisms produce redox-active proteins to utilize extracellular solid or organic electron acceptors [36-39]. Thus, extracellular or intracellular electron transfer rate is an important factor in biological process.

It has been reported that addition of electron shuttling mediate such as anthraquinone-2,6-disulfonate (AQDS) and humic substrate were able to enhance solid phase of Fe (III), tetrachloride and TCE reduction by accelerating electron transfer rate

in several researches [23, 36, 37, 40-43]. Humic acid has reversible redox moieties, which are mostly quinones [38, 44]. Dissolved humic acid and other dissolved organic matter (DOM) is thought to be serving as electron shuttles in natural systems [38].

The soil sample used in this research was collected from 70 feet depth of a semi-arid area, which was low in DOM [45]. On the other hand, the soil used in Nozawa-Inoue et al.'s microcosm study was collected from top of the surface in which perchlorate reduction was observed under 20% MWC [8]. It has been well documented that organic matter in soil decreases with increasing depth [45-48]. It was also observed that soil flooding or dry-wet cycle increased DOM more than 50% in agricultural peat soils [49]. Thus, it is possible that saturated condition or higher DOM level can enhance electron transfer rate and stimulate perchlorate bioremediation. It was hypothesized that perchlorate remediation in Soil A under unsaturated condition was controlled by electron transfer rate and addition of electron shuttling substrate such as humic acid (HA) may stimulate perchlorate bioremediation.

4.3.3 Effect of humic acid (HA) on soil ORP and perchlorate bioremediation

To investigate the effect of humic acid (HA) and moisture condition on soil ORP, ORP was monitored in three microcosms: 1) saturated MWC, 2) 20% MWC with humic acid, and 3) 20% MWC without humic acid. In all three microcosms, 2.5 g/kg acetate was amended as electron donor.

As shown in Figure 4.6, soil ORP under saturated condition decreased rapidly right after incubation. After a 2-days incubation period, ORP in saturated soil reached plateau at -500 mV. ORP in the 20% MWC soil maintained at +200 mV for 3 days and decreased

rapidly and reached plateau at -100 mV within 2 days. Based on the results, soil moisture

condition had dramatic effect on the soil redox potential and it is consistent with

Cassondra et al.'s research results in which soil redox potential decreased exponentially

when the water table increased 5 cm in Everglades wetlands[50].

In the 20% MWC with humic acid microcosm, ORP decreased rapidly from +200 mV to

-200 mV within 5 days and then maintained at the same level for 10 days. After a 15-day

incubation, ORP in humic acid amended soil dropped again to -500 mv and reached

plateau at -500 mV.   Reductive environment was enhanced by addition of humic acid

under unsaturated condition. However, comparing with the results from saturated soil,

the effect of humic acid was not significant as saturated moisture on soil ORP.
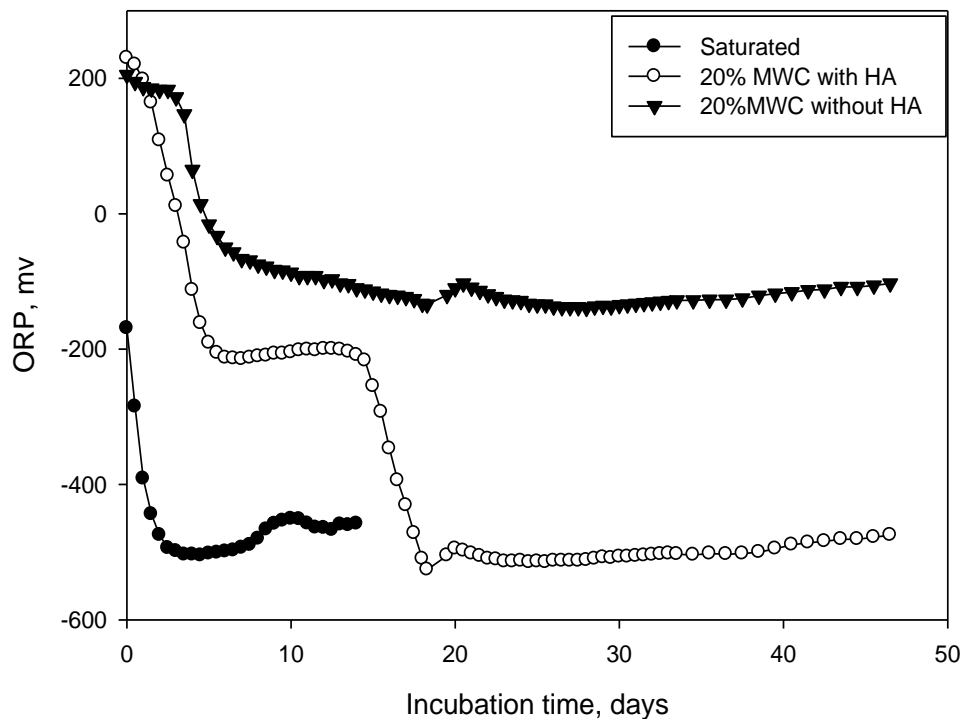
Figure 4.6 The effect of moisture condition and humic acid on soil ORP.

As shown in Figure 4.7, perchlorate reduction under unsaturated moisture condition was not initiated within 90 days with or without humic acid. Addition of humic acid was beneficial to develop reductive environments. However, it did not stimulate perchlorate reduction in unsaturated soil.

In contrast, perchlorate reduction under saturated condition was initiated after a two-week incubation period (see Figure 4.1). Based on Figure 4.6, perchlorate reduction was started after soil redox potential decreased to -500 mV. In groundwater system, perchlorate reduction was observed at an ORP range between 0 and -100 mV [25]. While the redox potential in unsaturated microcosm (acetate only) was maintained at -100 mV after 7 days, no perchlorate reduction was observed. It is possible that redox potential required for perchlorate remediation in terrestrial system is lower than groundwater system.

In contrast, redox potential in humic acid and acetate amended microcosm reached -500 mV after 20 days, which should low enough for perchlorate reduction. However, based on TOC results shown in Figure 4.6, most of electron donor in the microcosm had been consumed by oxygen and nitrate after 20 days. Thus, insufficient electron donor could be the reason of failure in perchlorate reduction in the microcosms. It is also possible that 20 mg/kg humic acid was not sufficient to enhance electron transfer in unsaturated soil. Higher dosage of humic acid or other electron shuttling substrates would be suggested in further research.
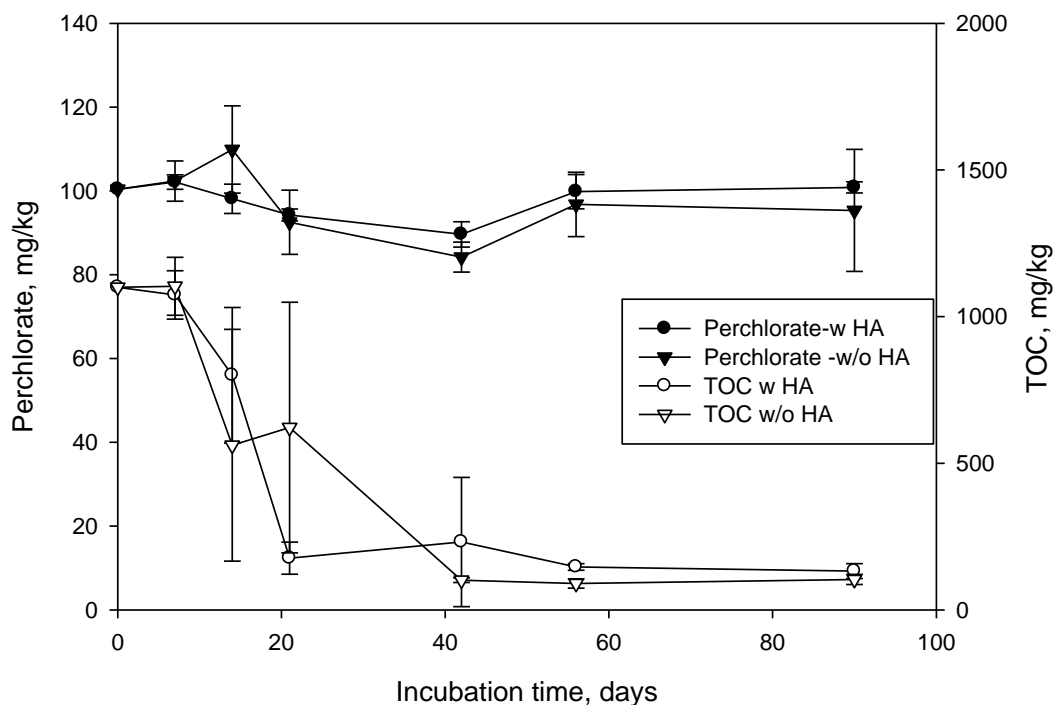
Figure 4.7 Perchlorate reduction with or without humic acid as electron shuttles in unsaturated soil.

4.4   SUMMARY

Oxygen, nitrate, perchlorate, ferric iron and sulfate were identified as dominant electron acceptors in the soil used in this research. Under saturated moisture conditions, nitrate reduction was first observed and completed within 7 days. Perchlorate reduction started after a 14-day incubation period. Ferric iron and sulfate reduction were also observed concurrently with perchlorate reduction. In contrast, no other anaerobic processes were observed except denitrification in unsaturated soil within 35 days. Nitrate reduction was not affected by soil moisture condition while most of the anaerobic process was limited by unsaturated moisture condition. Acetate decomposition rate in

unsaturated condition was higher than that in saturated condition. Most of the acetate consumed in unsaturated soil was coupled with oxygen reduction. Addition of perchlorate reducers to the soil didn't promote perchlorate reduction but it accelerated acetate decomposition.

Under strict anaerobic condition, nitrate reduction was completed within 7 days in unsaturated soil. However, no perchlorate reduction was observed within 100 days. After nitrate depletion, no acetate degradation was observed in the soil. Thus, electron donor was sufficient for perchlorate reduction and no other competitive process occurred in the unsaturated soil. The effect of soil moisture on anaerobic processes was explained by altering oxygen availability in soil microsite. Based on this research, it was clear that a mechanism other than oxygen limitation exists that is responsible for the effect of soil moisture content on perchlorate bioremediation.

It was hypothesized that perchlorate reduction and other anaerobic process in unsaturated soil was limited by electron transfer rate due to the low natural organic matter in the soil. Redox potential in saturated soil was significantly lower than that in unsaturated soil. Addition of humic acid as electron shuttling mediate decreased the soil redox potential but was not able to promote perchlorate reduction in unsaturated soil. Higher dosage of humic acid or other electron shuttling substrates would be suggested for future research.

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