CHAPTER 15. Biodegradation of Perchlorate

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ABSTRACT

Perchlorate (ClO_4^-) has been detected in some states in the U.S and become an environmental concern since the late 1990's. Perchlorate properties, contamination, and health effects were introduced in this paper. Perchlorate biodegradation degradation were introduced and summarized. According to recent studies, perchlorate respiring bacteria are present in natural environments, providing possibility for perchlorate biodegradation. Compared to other perchlorate remediation techniques, biodegradation is cost effective and clean. A commonly accepted pathway of perchlorate reduction was successfully used by several researchers. Several studies indicated that perchlorate biodegradation is pseudo-first order kinetics. Metabolism of perchlorate respiring bacteria was introduced and summarized. Effecting factors of perchlorate biodegradation was also summarized in this paper.

KEYWORDS

Perchlorate, contamination, biodegradation, metabolism, kinetics

INTRODUCTION

Source of perchlorate contamination

In 1985, perchlorate contamination was discovered in wells at California Superfund sites; however, perchlorate contamination of water sources nationwide was not recognized until 1997. Today, more than 11 million people have perchlorate in their public drinking-water supplies at concentrations of at least 4μg/L. It is estimated that more than 15.9 million kg of perchlorate has been discharged into the environment since 1950's (Motzer, 2001). In recent years, perchlorate is becoming an environmental concern in the United States because of its stability, persistent and toxicity. Also its high solubility and low kinetic lability towards reduction make perchlorate treatment difficult. Perchlorate is typically found in the form of perchlorate acid and salts such as ammonium perchlorate, sodium perchlorate and potassium perchlorate. Among these chemicals, ammonium perchlorate (NH_4ClO_4) is the most prevalent form of this compound, and it has been widely used in rocket propellants, fireworks, flares and munitions. Perchlorate compounds are also used in a number of other manufacturing operations, including electroplating, production of pharmaceuticals, paints and enamels, and tanning and leather finishing (USEPA, 2005). Several uses of perchlorate are listed in Table 1. The only naturally occurring perchlorate is found in nitrate fertilizer deposits in Chile (Urbansky and Brown, 2003; and Logan et al., 2001).

Table 1. Examples of Perchlorate Uses (USEPA, 2005)

Air bag initiators for vehicles	Flash powder for photography
Bleaching agent Leather tanning	Chemical laboratories in analytical testing
Oxygen generators	Ejection seats
Electroplating operations	Electropolishing
Engine oil testing propellant in rocket engines	Etching of brass and copper
Road flares	Fireworks
Paints and enamels	Production of matches
Perchloric acid production and use	

Perchlorate was first manufactured in the U.S. in 1908 at the Oldbury Electrochemical plant in Niagara Falls, New York. Manufacture of ammonium perchlorate began in the 1940s, primarily for use by the defense industry and later by the aerospace industry. Other perchlorate containing salts were more common before 1953. Over the years, the number of perchlorate manufacturers has varied. Before the mid-1970s, there were at least five perchlorate manufacturing plants in the U.S., but from 1975 through 1998, only two plants manufactured the compound (American Pacific in Henderson, Nevada, and then in Cedar City, Utah, and Kerr-McGee in Henderson, Nevada). Currently there is only one U.S. manufacturer of ammonium perchlorate, American Pacific's Western Electro Chemical Company (WECCO) Plant in Cedar City, Utah. Perchlorate continues to be used in a variety of operations. According data from EPA (USEPA, 2005), there were more than 100 perchlorate users located in 40 states as of April 2003.

Perchlorate has been found in the groundwater and surface waters in several western states, including California, Utah, Nevada and Arizona. Colorado River and Lake Mead have been contaminated due to the nearby industrial facilities. Low level perchlorate contamination has been detected in the following states: Maryland, New York, Nebraska, Kansas, North Carolina and Iowa (Urbansky and Brown, 2003). Previous research reported that perchlorate concentrations in Utah ranged from 4 to 200 µg/L in groundwater wells on the property of a rocket motor manufacturer. In Henderson, Nevada, water samples taken from the site of the former Pacific Engineering & Production Company of Nevada (PEPCON) rocket fuel plant, which exploded in 1988, showed perchlorate concentrations ranging from 51.4 to 630 mg/L. Samples drawn from 50 wells near ammonium perchlorate manufacturer Kerr-McGee Chemical Corporation, showed perchlorate levels as high as 3.7 g/L in the groundwater (Urbansky, 1998).. In 1999, the Californian Department of Health Services (DHS) added perchlorate to its list of unregulated contaminants for which monitoring is required (UCMR). From 1997 through October 4, 2005, DHS investigated perchlorate contamination sampling for 7,073 drinking water sources, and the results showed that 395 sources in 12 counties reported two or more perchlorate detections (Table 2). While these sources are primarily groundwater wells, other sources containing water from the Colorado River have also reported perchlorate findings. More detailed information about perchlorate contamination in California is available online (http://www.dhs.ca.gov/ps/ddwem/chemicals/perchl/monitoringupdate.htm). Figure 1 depicts 31 pilot or full scale EPA perchlorate projects national-wide.

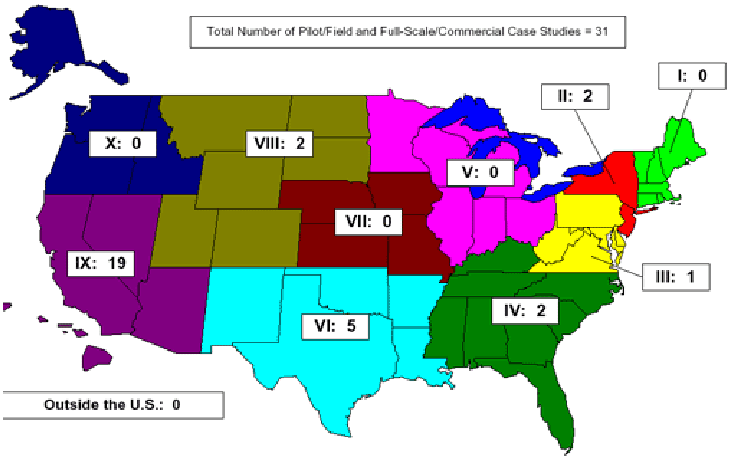

Figure 1 Total number of pilot/full scale perchlorate projects

Data collected by EPA's FFRRO for several monitoring sites national wide showed that for each site identified, the compilation includes data about perchlorate concentrations in drinking water, groundwater, surface water and soil. The maximum concentrations reported were as follows: drinking water was 811 µg/L; groundwater was 3,700,000 µg/L; surface water was 120,000 µg/L; and soil was 2,000 mg/kg (USEPA, 2005).

Table 2 Drinking water sources with two or more perchlorate detections in California (USEPA, 2005)

County	No. of sources	No. of systems	Peak Conc**.** µg/L
Los Angeles	149	39	159
San Bernardin	89	18	820
Riverside	74	9	73
Orange	33	9	10.7
Sacramento	21	5	400
Tulare	8	4	11
Santa Clara	6	4	8.5
San Diego	5	3	7
Ventura	4	2	20
Imperial	4	1	6
Sonoma	1	1	5
Stanislaus	1	1	3.3
TOTAL	395	96	--

Regulatory of perchlorate by different states

Currently, there is no federal or state drinking water standard - also known as a maximum contaminant level (MCL) for perchlorate contaminations in the US. The California DHS is using 6µg/L advisory level for drinking water. Also, as shown in Table 3 some other states have set a maximum advisory level.

Table 3. State Advisory Levels for Perchlorate (USEPA, 2005)

State	Advisory Level (µ g/L)	Comment
Arizona	14	1998 health-based guidance level; based on child exposure; to be reviewed after EPA issues final Reference Dose (RfD)
California	6	California EPA (Cal EPA) is anticipating a proposed maximum contaminant level (MCL) in 2005
Massachusetts	1	Precautionary recommendation to local water districts for children and at risk populations
Maryland	1	None
New Mexico	1	only for monitoring drinking water screening level
New York	5 and 18	5 µg/L for drinking water planning level; 18 µg/L for public notification level
Nevada	18	Public notice standard for contaminated groundwater
Texas	17 and 51	17 µg/L for residential protective cleanup level (PCL); 51µg/L for industrial/commercial PCL

Health effects of perchlorate

Perchlorate is known to target the thyroid, bone marrow, and muscle at high concentrations (Urbansky, 1998; and USEPA, 2005). The most commonly observed health effect of perchlorate is on or through the thyroid gland, because the perchlorate ion is similar in size to the iodide ion and can therefore be taken up in place of it by the thyroid gland (Urbansky, 2002). This can result in decreased production of thyroid hormones, which are needed for prenatal and postnatal growth and development, as well as for normal metabolism and mental function in the adult (http://www.dhs.ca.gov/ps/ddwem/chemicals/perchl/perchlindex.htm). A key concern about health effects of perchlorate is that impairment of thyroid function in pregnant women can affect fetuses and infants and can result in delayed development and decreased learning capability. However, the effect chronic exposure to very low concentrations of perchlorate over a long period of time has not been known yet (Urbansky, 1998).

Primary pathways of perchlorate intake of humans include ingestion of contaminated drinking water and food. Recent studies have showed perchlorate detection in milk, lettuce and bottled water (USEPA, 2005). When vegetables like lettuce are irrigated with perchlorate contaminated water of grow in soil that has been previously exposed to perchlorate containing water or fertilizer. The US Food and Drug Administration (FDA) data showed that perchlorate has been detected in red lettuce, green leaf lettuce, and iceberg lettuce in the following states, Arizona, California, New Jersey, Texas and Florida, with perchlorate concentration range of 1.2 to 129µg/L. Milk samples was collected at retail from various regions of the country. Perchlorate levels ranged from 3.16 to 11.3µg/L in 101 out of 104 samples, with perchlorate not quantifiable in three samples. The mean is 5.76µg/L for the 104 samples. Bottled water samples were collected at retail locations nationwide and included artesian water, well water, distilled water, drinking water, purified water, and spring water. Results of 51 bottled water samples show non-quantifiable levels of perchlorate in 49 samples. Two spring water samples were found to have 0.45µg/L and 0.56µg/L of perchlorate. The detailed data can be viewed on FDA's website (http://www.cfsan.fda.gov/~dms/clo4data.html).

In 1999, the EPA Interim Guidance recommended that agency risk assessors and risk managers continue to use the provisional reference dose (RfD) range of 0.0001 to 0.0005 mg/kg-day (http://www.epa.gov/fedfac/documents/perchlorate_qa.htm). In January 2005, the National Research Council (NRC) of the National Academy of Science (NAS) found that daily ingestion of up to 0.0007 milligrams of perchlorate per kilogram of body weight can occur without adversely affecting the health of the most sensitive populations. The NRC published the results of perchlorate impact on human health in a reported titled: "Health Implications of Perchlorate Ingestion." The detailed report is available online (http://www.nap.edu/books/0309095689/html). According to NRC's calculation, the daily ingestion of up to 0.0007 µg of perchlorate per kilogram of body weight will not have adverse effect on the health of the most sensitive population (USEPA, 2005).

Perchlorate kinetics

Inorganic perchlorate salts are extremely soluble, with potassium perchlorate an exception. Selected chemical and physical properties are listed in Table 4.

Table 4 Chemical and physical properties of selected perchlorate salts (USEPA, 2005)

Property	Ammonium Perchlorate	Sodium Perchlorate	Potassium Perchlorate
Formula	NH_4ClO_4	$NaClO_4$	$KClO_4$
Formula Weight	117.49	122.44	138.55
Color/Form	White, orthorhombic crystals	White, orthorhombic crystals; white, deliquescent crystals	Colorless crystals or white, crystalline powder; colorless, orthorhombic crystals
Density	1.95 g/cm^3	2.52 g/cm^3	2.53 g/cm^3
Solubility	200 g/L of water at 25 ^{o}C	209.6 g/100 mL of water at 25 ^{o}C	15 g/L of water at 25 ^{o}C

The perchlorate tetrahedron itself is structured such that the four oxygen atoms surround the central chlorine atom, effectively blocking reductants from directly attacking the chlorine. The perchlorate anion is soluble and very mobile in aqueous systems. It can persist in the environment for many decades under typical groundwater and surface water conditions because of its resistance to react with other available constituents. While perchlorate is thermodynamically a strong oxidizing agent, with chlorine in the +7 oxidation state, it is a kinetically sluggish species, such that its reduction is generally very slow, rendering common reductants ineffective.

Depending on the reductant, perchlorate can be reduced to either chlorate or chloride. The corresponding reactions and standard reduction potentials are indicated as below (Urbansky, 1998):

$$ClO_4^- + 8H^+ + 8e \Leftrightarrow Cl^- + 4H_2O \qquad E^0 = 1.287V$$

$$ClO_4^- + 2H^+ + 2e \Leftrightarrow ClO_3^- + H_2O \qquad E^0 = 1.201V$$

$$2H_2O \Leftrightarrow 4H^+ + O_2 \qquad -E^0 = -1.229V$$

The first work to show reduction of perchlorate by a metal cation was done about 100 years ago by Rothmund (Urbansky, 1998). He showed that Ti(III), V(II), and Cr(II) all reduce perchlorate to chloride in acidic aqueous solution at ambient temperature.

King and Garner (King and Garner, 1954) published the results of the first thorough kinetic investigation of the reaction of vanadium(II) and vanadium(III) with perchlorate, as shown in the following reactions.

$$8V^{2+} + ClO^{4-} + 8H^+ \rightarrow 8V^{3+} + Cl^- + 4H_2O$$

$$8V^{3+} + ClO^{4-} + 4H_2O \rightarrow 8VO^{2+} + Cl^- + 8H^+$$

$$8VO^{2+} + V^{2+} + 2H^+ \rightarrow 2V^{3+} + 2H_2O$$

Available techniques for perchlorate degradation

Due to its fundamental physical and chemical nature, the perchlorate anion does not respond to typical water treatment techniques, therefore treatment of perchlorate contamination in water is complicated. Currently, technologies used for treating perchlorate contamination in drinking water, groundwater and soil include the following approaches:

Physical process
- Membrane based techniques (mainly Reverse Osmosis and Electrodialysis)
- Ion exchange
- Precipitation
- Liquid Phase Carbon Adsorption

Chemical process
- Chemical and Electrochemical reduction
- Permeable Reactive Barrier

Bioremediation
- Bioreactor
- Composting
- In Situ Bioremediation
- Phytotechnology

Among the above technologies, bioremediation methods may prove to be the most practical approach. Table 5 summarized the total number of recent EPA projects about perchlorate treatment.

Table 5 Number of perchlorate treatment projects by EPA in recent years (USEPA, 2005)

Technology	No. of Projects	
	Full-Scale	Pilot-Scale
Ion Exchange	14	3
Granular Activated Carbon	2	2
Permeable Reactive Barrier	2	1
Electrodialysis	0	2
Reverse Osmosis	0	0
Bioreactor	4	5
Composting	1	3
In Situ Bioremediation	1	10
Phytotechnology	0	1
TOTAL	24	27
Bio-based technologies	6	19

As shown in Table 5, ion exchange and bioremediation are the most frequently used technologies in perchlorate remediation. Other removal technologies like granular activated carbon and reverse osmosis have limited application. However, there are some potential limitations to ion exchange technology. For example, spent regenerating solution from ion exchange resins usually has high saline (7-10%) perchlorate contaminated brines (Logan, et al., 2001), which requires further treatment. The treated water from ion exchange system may also contain increased chloride levels and thus be corrosive to the treatment equipment (USPEA, 2005). Used resin from ion exchange system also needs treatment prior to disposal, increasing the treatment cost.

Based on the recent EPA report (USEPA, 2005), much of the treatment technology research is looking at various aspects for bioremediation of perchlorate. Table 6 gives some examples of EPA projects of perchlorate bioremediation in 2004.

Table 6 Recent researches on biotechnologies for perchlorate treatment (USEPA, 2005)

Organization	Method	Objective
Atlantic Research Corp. Gainesville, VA	In-situ anaerobic bioremediation	Determine if subsurface conditions can be adjusted to create an in situ, anaerobic, bioremediation system capable of reducing perchlorate and VOCs. Stimulate VOCs and perchlorate reducing bacteria
ATK Tactical Systems, LLC Elkton, MD	Enhanced anaerobic biodegradation	Reduce perchlorate and chlorinated solvent in shallow groundwater
Atlantic Research Corp. Camden, Arkansas	In-situ and ex-situ bioremediation	Evaluate accelerated anaerobic reduction of perchlorate in soil and groundwater

BIODEGRADATION OF PERCHLORATE

Potential of perchlorate biodegradation

Perchlorate-degrading microorganisms have been found to be widespread in the subsurface environment (Logan et al., 2001; Coates et al., 1999; and O'Connor et al., 2002) and present at many, if not most, perchlorate contamination sites. And bacteria capable of perchlorate degradation appear to be widely distributed in nature at concentrations ranging from one to thousands of bacteria per gram of water, wastewater, sediment, and soil. The bacteria that degrade perchlorate are diverse. Almost all of the perchlorate degrading bacteria fall within new species' classification based on a 16s rDNA classification scheme (Logan, 2001). Recent studies on the microbial diversity, biochemical mechanism and kinetics of perchlorate bio-reduction to non-toxic chloride and oxygen have been reported (Logan, 1998; Coates et al., 1999; Miller and Logan, 2000; and Kim and Logan, 2001). Several reactor technologies have been shown to treat perchlorate, for which four patents have been obtained. Table 7 lists treatment methods and bioreactor systems for degrading perchlorate in both pure and mixed cultures.

Table 7 Bioreactor systems for degrading perchlorate (Logan, 2001)

Reactor type	Substrate	Microorganisms	Perchlorate (ppm)
Suspended growth	Protein nutrients[a]	Wollinella succinogenes HAP-1 in mixed culture	7750
Fixed bed	BYF-100[b]	Wollinella succinogenes HAP-1 in mixed culture	500, 1500
Fixed bed	Acetate	Perc1ace	0.13, 0.738, 100
Fixed bed	Acetate	Mixed culture	20, 22.5
Fixed bed	Acetate	*Dechlorosomas* sp. KJ	19.6
Fixed bed	Hydrogen	Mixed culture	0.740
Fixed bed	Hydrogen	Mixed culture	0.70
Fluidized bed	Ethanol	Mixed culture	6–13

The listed treatment methods have been used to degrade perchlorate at influent concentrations ranging from 0.13 to 7750 ppm. [a] Aged brewer's yeast, cottonseed protein, or whey powder.

[b] Naturally occurring protein (54%), peptides, free amino nitrogen, vitamins, and trace elements.

Ex-situ and In-situ bioremediation options for perchlorate reduction

Fluidized and fixed bed biological reactors are typical ex-situ remediation device and some of them have been constructed in the laboratory (Kim and Logan, 2001; Miller and Logan, 2000; and Herman and Frankenberger, 1999). Fluidized bed reactors (FBRs) have proven to be an effective reactor for ex-situ perchlorate treatment in groundwater. In a fluidized-bed reactor, the support medium (usually GAC or sand) is kept suspended and mixed in the reactor by using a high recycle flow rate. The biomass on the packing is kept uniform in the reactor by mixing, and biomass thickness is controlled by shear and particle collisions. In the study reported by Hatzinger et al. (Hatzinger, et al, 2002), an ethanol-fed FBR consistently reduced perchlorate concentrations from influent levels averaging 770 µg/L to below the analytical reporting limit (< 4 µg/L). The FBR also consistently treated nitrate from 33.2 mg/L to non-detectable levels (< 0.4 mg/L). The high biomass maintained within an FBR system, its ability to efficiently handle high groundwater flow rates and changing concentrations of perchlorate and nitrate, and effective mechanical devices to prevent biological fouling and channeling make the FBR an optimal reactor design for perchlorate treatment in groundwater. The main system disadvantage is the pumping cost for maintaining high recycles rates.

In a fixed-bed bioreactor, the biofilm support medium (sand or plastic media) does not move during water treatment. The main disadvantage of fixed-bed reactor is that biomass buildup on the packing requires periodical backwashed to prevent clogging (Logan, 2001). However, both fluidized and fixed-bed reactors have been proven to be cost effective methods to reduce perchlorate (Herman and Frankenberger, 1999; Miller and Logan, 2000; and Kim and Logan, 2001).

In addition to ex-situ biotechnologies such as the FBR, in-situ treatment of perchlorate-contaminated groundwater through electron donor addition is likely to be a viable remediation option at many sites. In situ bioremediation of perchlorate-contaminated waters eliminates the need for aboveground treatment. Anoxic conditions necessary for in situ perchlorate treatment can be established either by creating permeable reactive biobarriers containing high concentrations of organic matter, or by injecting substrates into the ground. Laboratory data suggest that perchlorate-reducing bacteria are widely occurring in natural environments, including groundwater aquifers. These organisms can be stimulated to degrade perchlorate to below detection through addition of specific electron donors. Initial pilot tests of in-situ perchlorate bioremediation conducted at the Aerojet site in California are showing promising results (Logan, 2001)

American Water Works Association Research Foundation (AWWARF) founded bench and pilot scale tests indicate that biologically active carbon (BAC) filtration can effectively remove low levels of nitrate and perchlorate under anaerobic conditions with the addition of an electron donor. Nitrate reduction can also enhance perchlorate reduction kinetics, making BAC filtration particularly attractive for combined nitrate-perchlorate remediation.

The disadvantage of using an organic feed is that the unoxidized substrate could remain in the drinking water. Residual substrate can stimulate bacterial growth in water distribution systems and contribute to the formation of disinfection byproducts in water disinfected with chlorine.

Metabolism of bacteria in degrading perchlorate

Most perchlorate-respiring bacteria are capable to use oxygen, nitrate, and chlorate (ClO_3^-)—but not sulfate—as a terminal electron acceptor (Bardiya and Bae, 2005). Perchlorate can be successively degraded to chlorate and then chlorite (ClO_2^-) by a novel chlorate reductase respiratory enzyme. $ClO_4^- \rightarrow ClO_3^- \rightarrow ClO_2^- \rightarrow Cl^- + O_2$ is a commonly accepted pathway of perchlorate reduction (Urbansky, 1998; Logan et al., 2001; Xu and Logan, 2003; Song and Logan, 2004; and Nozawa-Inoue et al., 2005). Some of the bacteria are able to reduce perchlorate in the presence of nitrate (Logan, 1998). In the presence of an appropriate food source (electron donor) and under redox conditions, these bacteria have the capability to reduce perchlorate. Injection or placement of a food/carbon source (electron donor) into the contaminated aquifer is necessary to promote growth of the desired bacteria and effect perchlorate degradation. Injected carbon sources that have been used or considered include acetate, corn syrup and edible oils. Key biological treatment parameters would include redox, dissolved oxygen, perchlorate and parent/by-product concentrations, carbon source concentration, microbiological growth, etc.

According to Logan's observation, some perchlorate respiring bacteria can have very high growth rates. The maximum observed growth rates of two isolates (*Dechlorosomas* sp. KJ and PDX) using perchlorate were 0.14 h^{-1} and 0.21 h^{-1} respectively, which were found to be only slightly less than or equal to growth rates using oxygen (0.27 and 0.28 h^{-1}, respectively) or chlorate (0.26 and 0.21 h^{-1}, respectively). Also, the bacteria growth rates dependent on the perchlorate concentration in feed water. Overall perchlorate removal rates are a first-order function of perchlorate concentration in the fixed-bed reactors (Logan, 2001, and Logan et al., 2001). Figure 2 describes the pathway used by bacteria to degrade perchlorate.

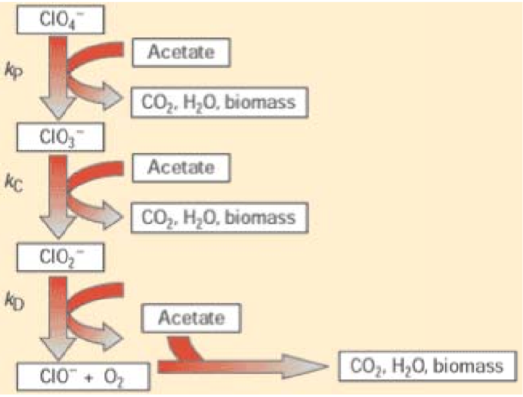

Figure 2 Pathway used by bacteria to degrade perchlorate.
Chlorite and chlorate do not accumulate in water because kD>>kC>kp (Logan, 2001)

Kinetics of perchlorate degradation

There are a few perchlorate biodegradation kinetics data for bacterial isolates other than maximum growth rate. Several papers reported that under certain conditions, perchlorate biodegradation kinetics can be described by Monod kinetics (Logan et al., 2001; Logan et al., 2001; and Nozawa-Inoue et al., 2005).

Effect and cost analysis of biodegradation of perchlorate

The factors that influence perchlorate remediation options at these sites are varied, and include the characteristics of the perchlorate plume (i.e., concentration, depth, extent), the presence of co-contaminants (such as chlorinated solvents and heavy metals, and existing systems to remove these contaminants), the necessity to protect public or private drinking water wells from plume migration, regulatory requirements, and cost. In cases where well protection is required, where existing pump-and-treat systems are already in place, or where plumes are very deep and/or expansive, ex-situ treatment is likely to be the bioremediation option of choice. Conversely, in well-defined source areas, shallow or narrow zones of contamination, and/or regions where drinking water is unlikely to be affected, in-situ perchlorate treatment may prove to be the best treatment option.

Because there is some question about the public's acceptance of using bacteria to treat drinking water, the technology has been used for contaminated soil, wastewater and groundwater. In 2002, Californian Department of Health Services (DHS) Water Treatment Committee recommended a conditional acceptance of biological treatment (fluidized bed reactor) for the removal of perchlorate in a drinking water supply. The recommendation in based on a treatability study pf the full scale fluidized bed reactor system treating perchlorate contaminated groundwater at the Aerojet facility in Rancho Cordova, California. All recent studies reducing perchlorate concentrations in contaminated water to acceptable drinking water levels have used acetic acid/acetate or hydrogen gas as substrates (Logan, 2001). Acetic acid is relatively inexpensive but, unlike alcohols and sugars, it is not lost to fermentation. It remains to be seen if the public would accept this for addition to drinking water supplies. Packed-bed reactors using acetic acid have been extensively tested in the laboratory and are currently being evaluated in the field at the pilot scale.

DISCUSSION

Effect of nitrate on perchlorate biodegradation

Previous research demonstrated that most perchlorate respiring bacteria can utilize nitrate as a suitable electron acceptor (Okeke et al., 2002; Chaudhuri et al., 2002; Xu et al., 2004; Nozawa-Inoue et al., 2005; and Bardiya and Bae, 2005). According to the observation of Chaudhuri et al. (Chaudhuri et al., 2002), if a nitrate-grown culture of *D. suillum* (a perchlorate respiring bacterium) was transferred into fresh anoxic medium, nitrate reduction and growth were observed immediately. Apparent lag phase and chlorite dismutase (CD) activity were not observed. However, when a nitrate-grown culture of *D. suillum* containing equimolar amounts of perchlorate and nitrate was used, there was an extended lag period for both growth and respiration, and after that, nitrate reduction occurred prior to perchlorate reduction. Similarly, when a perchlorate grown culture was inoculated into fresh medium containing equimolar amounts of both nitrate and perchlorate, no lag phase was apparent, and again, nitrate reduction occurred prior to perchlorate reduction. Regardless of the electron acceptor utilized in the inoculum culture, neither CD activity nor perchlorate reduction was observed until nitrate was completely consumed. Their results agreed well with previous studies (Tipton et al., 2003; and Xu et al., 2004). These results indicated that nitrate is a competing ion in perchlorate reduction. On the other side, these results also demonstrated that co-reduction of both perchlorate and nitrate is possible. The differences in the molecular structure, configuration, and substrate binding domains of the nitrate reductases could be responsible for dominance of perchlorate reduction among heterotrophic denitrifying bacteria (Bardiya and Bae, 2005).

Effect of other electron donors on perchlorate biodegradation

Effect of sulfur and thiosulfate on perchlorate biodegradation: Since some of the sulfur utilizing bacteria are capable of denitrification, and many perchlorate reducers are denitrifiers, the consortium from the sewage treatment plant was first tested for autotrophic perchlorate reduction with thiosulfate as e^- donor (Bardiya and Bae, 2005). In the experiment of Bardiya and Bae (Bardiya and Bae, 2005), the bacteria failed to grow and reduce perchlorate with thiosulfate as e^- donor. Several inocula from different natural habitats, like sulfur, thiosulfate, $H_2 + CO_2$, were also tested $e^–$ donors. Unfortunately, none of the batercia could grow and reduce perchlorate with sulfur compounds as e^- donor. This observation indicated that reduced sulfur compounds such as sulfur and thoirulfate could not serve as e^- donors for perchlorate reduction.

Effect of acetate on perchlorate biodegradation: Under the experiment conditions of Bardiya and Bae (Bardiya and Bae, 2005), acetate was critical to perchlorate reduction. It was noticed that when acetate was omitted from the medium, no growth of perchlorate respiring bacteria was observed. In the presence of acetate, the reduction of perchlorate was stimulated. Also, the reduction of both perchlorate and nitrate was observed. However, when sulfur and thiosulfate were added into the medium, the reduction of perchlorate was inhibited.

Effect of oxygen on perchlorate reduction

Based on the perchlorate reduction pathway described earlier in this paper, oxygen is not favorable in the procedure. According to the observation of of Chaudhuri et al. (Chaudhuri et al., 2002), when D. *suillum* was grown anaerobically under inert gas protection with perchlorate as the only electron acceptor, perchlorate removal was rapid and CD activity was observed. However, if air was introduced after 21 h, perchlorate reduction was immediately inhibited. This finding agreed with previous studies (Coates et al., 1999). In their experiment of perchlorate respiring bacteria growth and CD expression under anaerobic conditions, CD activity and perchlorate reduction were not observed if the culture was grown aerobically, even at lower dissolved oxygen level. This result indicated that oxygen inhibited perchlorate reduction. However, when the O_2 content was replaced with inert gas, CD activity was inhibited immediately, but recovered after a lag phase of 4 h. These observations demonstrated that anaerobic condition could not induce the bacteria growth alone. This is because genetic regulation may also play an important role.

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