Microbial Degradation of Perchlorate: Principles and Applications

Jianlin Xu, Yanguang Song, Booki Min, Lisa Steinberg, and Bruce E. Logan*

Department of Civil and Environmental Engineering
The Pennsylvania State University
University Park, PA 16802

ABSTRACT

Perchlorate (ClO$_4^-$) release into the environment has occurred primarily in association with its manufacture and use in solid rocket propellant. When released into groundwater, perchlorate can spread over large distances because it is highly soluble in water and adsorbs poorly to soil. Two proven techniques to remove perchlorate from drinking water are anaerobic biological reactors and ion exchange. In this review, we focus on the application of microbiological systems for degrading perchlorate. Some bacteria can use perchlorate as an electron acceptor while oxidizing a large range of substrates. Perchlorate-respiring bacteria (PRB) are widely distributed in the environment, and are enriched at perchlorate-contaminated sites. We review the pathways by which PRB degrade perchlorate, and the different biological treatment processes that have been developed to remove perchlorate from water sources. We also discuss the effects of alternate electron acceptors in the water, such as oxygen and nitrate, on perchlorate removal. Although many different biological treatment systems using PRB have so far only been proven at the bench scale, all pilot scale tests performed to date with a few of these systems have been successful. The success of these perchlorate bioreactor tests indicates that biological treatment is a suitable method for soil remediation and water treatment of perchlorate-contaminated water.

Key words: perchlorate; application; percolate-respiring bacteria; bioremediation

INTRODUCTION

Perchlorate (ClO$_4^-$) is a highly oxidized (+7) chlorine oxy-anion manufactured for use as the oxidizer in solid propellants for rockets, missiles, explosives, and pyrotechnics (Urbansky, 1998; Gullick et al., 2001; Logan, 2001a). Approximately 90% of all perchlorate salts are manufactured as ammonium perchlorate for use in rocket and missile propellants. The periodic replacement and use of solid propellant has resulted in the discharge of more than 15.9 million kg of perchlorate salts into the environment since the 1950s (Motzer, 2001). Perchlorate salts are highly soluble in water. Sodium perchlorate has a solubility of about 2 kg/L, allowing large amounts to be readily transported through surface and ground waters. The U.S. EPA has identified perchlorate users and

*Corresponding author: Department of Civil and Environmental Engineering, 212 Sackett Bldg, Penn State University, University Park, PA 16802. Phone: 814-863-7908; Fax: 814-863-7304; E-mail: blogan@psu.edu
manufacturers in 44 states, and perchlorate releases in at least 20 states (U.S. EPA, 2002). Such perchlorate releases are estimated to have affected the drinking water of 15 million people.

Perchlorate can be detected by many methods including ion-selective electrodes, ion chromatography, capillary electrophoresis, HPLC, and spectrophotometry (Urbansky, 1998). In 1997, the California Department of Health first reported an ion chromatographic method that was capable of detecting perchlorate concentrations of 4 \( \mu \text{g/L} \). Since then, ion chromatography has been the most commonly used detection method for perchlorate. Perchlorate is only known to occur in the natural environment in Chilean caliche, a material that is used for some fertilizers (Ericksen, 1983). The EPA developed a method for measuring perchlorate concentrations in fertilizers (Collette et al., 2001), and has used this method to survey large numbers of fertilizers and related materials. It was concluded that most fertilizers did not contain perchlorate, and therefore, that fertilizers did not contribute to the extensive environmental perchlorate contamination that has been observed (Urbansky et al., 2001).

Although there is currently no federal drinking water standard for perchlorate, perchlorate has been included on the federal Contaminant Candidate List (U.S. EPA, 1998). High concentrations of perchlorate are known to affect the function of the thyroid gland in humans by inhibiting the uptake of iodide (Wolff, 1998), but direct epidemiological evidence that indicates that perchlorate is toxic to exposed humans is lacking (Crump et al., 2000; Li et al., 2001; U.S. EPA, 2002). Recent studies have indicated that low concentrations of perchlorate significantly inhibit iodide uptake in humans and animals (Lawrence et al., 2000; OEHHA, 2002; U.S. EPA, 2002). Perchlorate contamination of the environment poses a threat to indigenous wildlife as well as human health. Smith et al. (2001) have found perchlorate contamination of wildlife and vegetation at concentrations that can pose a threat to the normal growth and development of amphibian populations (Golman et al., 2002a, 2002b). The Office of Environmental Health Hazard Assessment in California EPA has proposed a Public Health Goal of 6 \( \mu \text{g/L} \) for perchlorate in drinking water (OEHHA, 2002). In a recent perchlorate risk assessment draft report, the U.S. EPA (2002) proposed a draft reference dose of 0.03 \( \mu \text{g/kg} \) of body weight per day, which could produce a drinking water equivalent level of 1 \( \mu \text{g/L} \) to protect human health. Based on this information, the California Department of Health Service in California decreased the action level for perchlorate in drinking water from 18 to 4 \( \mu \text{g/L} \) (DHS, 2002a). In New Mexico, the action level was set at 1 \( \mu \text{g/L} \) (www.clin.org/studio/perchlorate-060402).

In the last 5 years, several reviews have been published on various perchlorate issues that include: bacterial degradation (Herman and Frankenberger, 1998; Logan, 1998); chemistry and analytical chemistry (Urbansky, 1998, 2000a, 2000b; Urbansky and Schock, 1999) toxicological studies and drinking water standards (Wolff, 1998; Urbansky, 2000a; Soldin et al., 2001; OEHHA, 2002; U.S. EPA, 2002); and contamination sources and occurrence data (Urbansky, 2000a; Cheremisnoff, 2001; Gullick et al., 2001; Logan, 2001a). However, there have been important advances made in the treatment of perchlorate-contaminated water since the microbiology and existing treatment technologies were reviewed in 1998 (Herman and Frankenberger, 1998; Logan, 1998). One of the most important developments since these reviews have been reports of microbial treatment processes capable of removing perchlorate down to levels expected to be suitable for drinking water (\(<4 \mu \text{g/L}\) ). These processes are the basis of several recent patents on biological treatment processes for perchlorate treatment (see Attaway et al., 1999; Coppola and McDonald, 2000; Frankenberger, 2000; Gaudre-Longerinas and Taucia, 2001; Logan, 2001b; Van Ginkel et al., 1999).

In the current review, we focus on recent developments that have improved our understanding of the bacteria responsible for perchlorate degradation, the pathways used for perchlorate degradation, and the treatment systems developed to use perchlorate respiring bacteria (PRB). We provide only a brief background on the chemistry, occurrence, health issues, and drinking water issues for perchlorate, and refer the reader to other more comprehensive studies and reviews on these subjects. We concentrate on topics related to microbial degradation of perchlorate because biological treatment is a particularly promising option for remediation of perchlorate-contaminated drinking water. Although these biological perchlorate treatment technologies are quite new, there are signs that such treatment systems will be accepted for use in water treatment plants. The California Department of Health Services recently approved the use of fluidized bed reactors for the treatment of perchlorate contaminated groundwater (DHS, 2002a), and biological denitrification has been used at one site in Oklahoma for the treatment of nitrate-contaminated groundwater (www.pall.com).

PECHLORATE RESPIRING BACTERIA

Ubiquity and diversity of perchlorate reducing bacteria

It has been known for several years that the ability to reduce perchlorate is not limited to a single bacterial species, although some of this earlier evidence of per-
chlorate degradation was inferred from information on biological chlorate degradation (Logan, 1998). Bacteria capable of chlorate reduction were found to inhabit a variety of environments including rivers, sediments, soils, and wastewater treatment plants (van Ginkel et al., 1995). Utilizing media with chlorate as the only electron acceptor, Coates et al. (1999) similarly found that the acetate-oxidizing chlorate reducing bacteria (CRB) represented a significant population whose abundance ranged from $2.31 \times 10^3$ to $2.4 \times 10^6$ cells per gram of samples obtained from a variety of sources, including pristine and hydrocarbon-contaminated soils, aquatic sediments, paper mill waste sludges, and farm animal waste lagoons. All 13 isolates obtained in this study were also capable of growth on acetate using perchlorate, leading to early speculation, via the abbreviation of (per)chlorate, that all CRB were also capable of perchlorate reduction (Coates et al., 1999).

More recent studies have provided evidence that not all CRB are PRB, although the converse is true, that all PRB are CRB. Wu et al. (2001) used perchlorate or chlorate in anaerobic growth medium to compare the abundance of PRB and CRB in different environmental samples. They found that when perchlorate was the sole terminal electron acceptor in the medium, the number of PRB in a pristine soil was up to 1,000-fold lower than that found using chlorate in the medium to enumerate CRB for the same samples (Wu et al., 2001). This provided indirect evidence that not all CRB were PRB, and that PRB were less abundant than CRB in these environments. Confirmation that not all CRB were PRB was provided by three different studies. Only 8 of 10 CRB isolated from wastewater were found to be capable of respiring perchlorate (Logan et al., 2001c). In another study, it was mentioned that a microorganism was isolated that was capable of using chlorate, but not perchlorate (Coates et al., 2000). Wolterink et al. (2002) isolated a CRB strain Pseudomonas chloritidismutans AW-1 from an anaerobic Pseudomonas chloritidismutans treating wastewater containing chlorate, and found that this strain did not reduce perchlorate. These studies indicate that while most CRB are able to degrade perchlorate, there is a subset of CRB that cannot use perchlorate as an electron acceptor for cell respiration.

**Perchlorate reducing isolates**

Many PRB have now been isolated (Table 1). These PRB are all Gram-negative, facultative anaerobes. Analysis of the 16S rRNA sequences of tested strains indicated that all isolates were members of the class Proteobacteria. The majority of these PRB, including GR-1, perlace, KJ, PDX, HZ, JDS5, and 15 other strains (Achenbach et al., 2001), are located in the β-subclass of Proteobacteria. Achenbach et al. (2001) proposed two new genera, *Dechloromonas* and *Dechlorosoma*, for these β-subclass lineages, which represent the predominant PRB in the environment. The phylogenetic tree in Fig. 1 shows the general positions of several PRB in comparison to other bacteria.

### Table 1. Perchlorate-respiring bacterial isolates.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vibrio dechloralticans</em> Cuznesove B-1168</td>
<td>Korenkov et al. (1976)</td>
</tr>
<tr>
<td><em>Wolinella succinogenes</em> HAP-1</td>
<td>Wallace et al. (1996)</td>
</tr>
<tr>
<td><em>Dechloromonas</em> agitata CKB</td>
<td>Bruce et al. (1999)</td>
</tr>
<tr>
<td><em>Dechloromonas</em> sp. SIUL</td>
<td>Coates et al. (1999); Coates et al. (2000)</td>
</tr>
<tr>
<td><em>Dechloromonas</em> sp. MissR</td>
<td>Coates et al. (1999); Coates et al. (2000)</td>
</tr>
<tr>
<td><em>Dechloromonas</em> sp. CL</td>
<td>Coates et al. (1999); Coates et al. (2000)</td>
</tr>
<tr>
<td><em>Dechloromonas</em> sp. NM</td>
<td>Coates et al. (1999); Coates et al. (2000)</td>
</tr>
<tr>
<td><em>Dechlorosoma</em> sp. SDGM</td>
<td>Coates et al. (1999); Coates et al. (2000)</td>
</tr>
<tr>
<td><em>Dechlorosoma</em> sp. PS</td>
<td>Coates et al. (1999); Coates et al. (2000)</td>
</tr>
<tr>
<td><em>Dechloromonas</em> sp. JM</td>
<td>Miller and Logan (2000)</td>
</tr>
<tr>
<td><em>Dechloromonas</em> sp. HZ</td>
<td>Zhang et al. (2002)</td>
</tr>
<tr>
<td><em>Dechlorospirillum anomalous</em> WD</td>
<td>Michaelidou et al. (2000)</td>
</tr>
<tr>
<td><em>Dechloromonas</em> sp. KJ</td>
<td>Logan et al. (2001c)</td>
</tr>
<tr>
<td><em>Dechlorosoma</em> sp. PDX</td>
<td>Logan et al. (2001c)</td>
</tr>
<tr>
<td><em>Dechlorosoma</em> sp. GR-1</td>
<td>Rikken et al. (1996)</td>
</tr>
<tr>
<td><em>Dechlorosoma</em> sp. perlace</td>
<td>Herman and Frankenberger (1999)</td>
</tr>
<tr>
<td><em>Citrobacter</em> sp. IsoCock1</td>
<td>Okeke et al. (2002)</td>
</tr>
<tr>
<td><em>Dechloromonas</em> sp. JDS5</td>
<td>Shrou and Parkin (2002)</td>
</tr>
</tbody>
</table>
Electron donors and acceptors used by PRB for growth

All PRB are capable of dissimilatory reduction of chlorate to chloride for energy and growth, and many PRB can also reduce nitrate. For both perchlorate and chlorate, reduction does not occur in the presence of a high concentration of dissolved oxygen. Most isolates can use oxygen and many can respire using nitrate as a terminal electron acceptor. HAP-1 was initially reported to be an obligate anaerobe (Wallace et al., 1996), but in a later study it was reported that it was a microaerobic organism (Wallace et al., 1998), although no further details on oxygen tolerance were provided. It seems reasonable that PRB should be able to use, or at least tolerate, dissolved oxygen as it is produced during the decomposition of chlorate and perchlorate. Although many PRB are capable of complete denitrification, CKB does not grow on nitrate (Bruce et al., 1999), and Cuznesove B-1168 (Korenkov et al., 1976) and HAP-1 (Wallace et al., 1996) reduce nitrate only to nitrite and do not produce ammonia or nitrogen gas.

In some cases PRB have been tested for their ability to use other electron acceptors such as metals and sulfate. GR-1 can utilize Mn (IV) as an electron acceptor. Most PRB and CRB cannot reduce sulfate (Riken et al., 1996; Wallace et al., 1996; Bruce et al., 1999; Coates et al., 1999; Herman and Frankenberger, 1999; Michaelidou et al., 2000; Wolterink et al., 2002). The only report of CRB (Acinetobacter spp.) capable of growth using sulfate was provided by Stepanyuk et al. (1992), but the ability to respire perchlorate was not tested and these bacteria could not use nitrate. PRB have not been found to be capable of using other electron acceptors, including: Fe (III), selenate, malate, and fumarate (Coates et al., 1999).

Both heterotrophic and autotrophic PRB have been isolated. Acetate has been most frequently used as a single substrate for heterotrophic perchlorate reduction (Korenkov et al., 1976; Wallace et al., 1996; Bruce et al., 1999; Coates et al., 1999; Herman and Frankenberger, 1999; Logan et al., 2001c), but hydrogen or formate was required as an electron donor for the growth of HAP-1 on acetate (Wallace et al., 1996). Perchlorate reduction by Citrobacter sp. IsoCock1 (Okeke et al., 2002) was sustained on acetate, but yeast extract was found to improve growth. A wide variety of organic substrates, including alcohols and carboxylic acids, can be used as growth substrates by PRB although the use of these substrates is strain-dependent. Dechloromonas sp. JM (Miller and Logan, 2000), a strain isolated from the bacterial consortium in an autotrophic packed-bed biofilm reactor, reduced perchlorate using dissolved hydrogen, but could not grow using hydrogen as the sole electron donor. Two autotrophic isolates, Dechloromonas sp. HZ (Zhang et al., 2002) and Dechloromonas sp. JDS5 (Shrout and Parkin, 2002), have recently been isolated that can grow in a minimal inorganic medium using perchlorate, hydrogen, CO2, and nutrients.

Nutritional requirements for PRB

There is no detailed information on the best medium to use for PRB or what trace nutrients or metals are needed for growth. Several research groups have used a phosphate buffer system (Attaway and Smith, 1993; Rikken et al., 1996; Wallace et al., 1996; Herman and Frankenberger, 1999; Logan et al., 2001c; Wu et al., 2001), while others (Bruce et al., 1999; Coates et al., 1999) have used a bicarbonate-buffered freshwater medium amended with a complex vitamin solution. There is no evidence that these additional vitamins are necessary for PRB growth, but in one study it was shown that several PRB strains could not grow without the trace metal solution (Michaelidou et al., 2000). Iron, molybdenum, and selenium appear to be important for PRB growth and perchlorate degradation. Perchlorate reductase purified from GR-1 was found to contain 11 mol of iron, 1 mol of molybdenum, and 1 mol of selenium per mol of heterodimer (Kengen et al., 1999). Bender et al.

**Figure 1.** Neighbor-joining phylogenetic tree based on the 16S rRNA sequences of PRB (Dechloromonas and Dechlorosoma) and others. Bootstrap values (100 replicates) are indicated on the nodes. The prealigned 16S rRNA sequences in TREECN format (Van de Peer and De Wachter, 1994) were downloaded from the rRNA WWW Server (HTTP://rrna.uia.ac.be/)
(2002) also reported that molybdenum was important for PRB growth. Wallace et al. (1996) used yeast extract and peptone in their medium for HAP-1. Zhang et al. (2002) found that yeast extract improved the growth of the autotrophic PRB strain HZ, but that yeast extract was not needed for growth.

It is likely that the different trace nutrients used for laboratory media are not needed for bioremediation of low perchlorate concentrations in natural systems. As explained below, several field studies have achieved perchlorate degradation only through the addition of an oxidizable substrate (acetate, ethanol, etc.), nitrogen, and phosphorus (Green and Pitre, 2000; Hatzinger et al., 2000; Logan et al., 2001a; Evan et al., 2002; Min et al., 2003).

THE PERCHLORATE DEGRADATION PATHWAY

Much of what is known about bacterial degradation of perchlorate has resulted from earlier studies on chlorate reduction. Quastel et al. (1925) found that chlorite was produced from chlorate by one strain of Escherichia coli without further reduction of chlorite (ClO$_3^-$). The failure of chlorate respiration in this strain was likely due to the toxic effects of chlorite and an absence of the enzyme chlorite dismutase. Later, it was found that some bacteria could reduce chlorite to chloride (Aslander, 1928; Bryan and Rohlich, 1954). The reduction of perchlorate to chloride by several species of heterotrophic bacteria was first demonstrated with the use of $^{36}$Cl-labeled perchlorate (Hackenthal et al., 1964). Hackenthal (1965) concluded that chloride was the product of perchlorate reduction by cell free extracts obtained from nitrate-adapted cells of Bacillus cererus. They also found that chlorate could be reduced by the same cell free preparation, and that it competitively inhibited perchlorate reduction. The first proposed perchlorate reduction pathway was ClO$_4^-$→ClO$_3^-$→ClO$_2^-$→Cl$_2$O$^-$→Cl$^-$ (Hackenthal et al., 1964; Hackenthal, 1965). The reduction of perchlorate or chlorate to chloride by bacteria was subsequently confirmed by other researchers (Korenkov et al., 1976; Malmqvist et al., 1991; Rikken et al., 1996; Coates et al., 1999; Wu et al., 2001).

Research on the perchlorate reduction pathway did not make further progress until a new enzyme, chlorite dismutase, was purified from the PRB strain GR-1 and found to produce oxygen from chlorite (Rikken et al., 1996; van Ginkel et al., 1996). Rikken et al. (1996) proposed a three-step mechanism of perchlorate reduction (Fig. 2) in which chlorate, chlorite, and dissolved oxygen were sequentially produced. This pathway, which is now widely accepted for bacterial respiration using perchlorate and chlorate, is: ClO$_4^-$→ClO$_3^-$→ClO$_2^-$→O$_2$ + Cl$^-$. Chlorite dismutase has been isolated from two other bacteria, Ideonella dechloratans, and strain CKB. Stenklo et al. (2001) purified chlorite dismutase from I. dechloratans that had catalytic properties that were similar, but not identical, to those found for the chlorite dismutase enzyme obtained from GR-1. Stenklo et al. (2001) determined the 22-residue N-terminal amino acid sequence for this enzyme and found no homologue in the protein sequence of the purified enzyme from I. dechloratans. Coates et al. (1999) also purified a chlorite dismutase from the strain CKB, which had characteristics similar to the enzyme purified from strain GR-1. The finding by Coates et al. (1999) that all 13 CRB isolates obtained in their laboratory could disproportionate chlorite into chloride and oxygen makes it likely that chlorite dismutase

![Figure 2. Perchlorate reduction pathway (adapted from Rikken et al., 1996)](image-url)

ENVIRON ENG SCI, VOL. 20, NO. 5, 2003
is the central enzyme for the dissimilatory reduction of perchlorate to chloride in PRB. The impact of nitrate on perchlorate reduction is important because nitrate is a common contaminant of perchlorate-contaminated waters. Many PRB are capable of partial or complete denitrification, and the presence of nitrate usually decreases the rate of perchlorate reduction. The similarities between the perchlorate reduction and denitrification pathways led to an early suggestion that the perchlorate and nitrate pathways were catalyzed by the same nitrate reductase (Hackenthal et al., 1964; Stouthamer, 1967). In addition, it was found that perchlorate reductase obtained from GR-1 reduced nitrate as well as chlorate and perchlorate (Kengen et al., 1999).

The concept of a shared enzyme for nitrate and perchlorate reduction is unlikely given more recent findings. It has been found that some PRB, for example Dechloromonas agitata CKB (Bruce et al., 1999), can respire using perchlorate but not nitrate. In a PRB capable of denitrification (strain perclace), it was found that nitrate and perchlorate reductases were located in different cell fractions (membrane and periplasmic fractions, respectively), indicating that these two enzymes were separate (Giblin and Frankenberger, 2001). Further evidence of completely separate denitrifying and perchlorate pathways was provided by Xu et al. (2002). These researchers found that perchlorate degradation and denitrification in Dechlorosoma sp. KJ and PDX were separately induced. When cells were grown on only perchlorate, there was minimal nitrate reduction. Also, there was no perchlorate or chlorate reduction when cells were initially grown on only nitrate. However, the presence of both perchlorate and nitrate in the medium stimulated the degradation of both electron acceptors. Their kinetic results were supported by whole cell protein profiles using SDS-PAGE that showed the appearance of bands in the gels at locations expected for perchlorate reductase, chlorite dismutase, and nitrate reductase under conditions that agreed with the kinetic studies (Xu et al., 2002).

It is still not clear if only a single enzyme is used by PRB for chlorate and perchlorate reduction, or if there are separate enzymes used for perchlorate and chlorate reduction. Research by Kengen et al. (1999) suggests only one enzyme is needed for chlorate and perchlorate respiration. They found that a single enzyme could catalyze both chlorate and perchlorate reduction, and that this oxygen-sensitive enzyme was located in the periplasm. However, the maximum reaction rates (measured with methyl viologen) for perchlorate (3.8 U/mg) were actually less than those with either nitrate (6.2 U/mg) or chlorate (11.3 U/mg) (Kengen et al., 1999). Because it appears likely that separate enzymes are used for nitrate and perchlorate (as explained above), it may also be that there are separate chlorate and perchlorate enzymes. Additional evidence for separate enzymes is provided indirectly by the fact that not all CRB are capable of respiration with perchlorate, although this question will require further research to resolve.

**MICROBIAL TREATMENT PROCESSES—BENCH SCALE PROCESSES**

The first bench-scale treatment system reported to degrade perchlorate was a suspended growth system developed to treat high concentrations of perchlorate (Attaway and Smith, 1993); this system has been adequately described in previous reviews (Herman and Frankenberger, 1998; Logan, 1998). More relevant for drinking water treatment and bioremediation studies are reports on fixed and fluidized bed bioreactors primarily designed to treat low concentrations of perchlorate, and laboratory batch tests to evaluate the feasibility of in situ bioremediation. These different bench scale studies are summarized in Table 2.

**Heterotrophic fixed-bed reactor studies**

The first reported fixed-bed bioreactor was an up-flow anaerobic reactor inoculated with a mixed culture containing Wollinella succinogenes HAP-1 (Wallace et al., 1998). This system was designed to treat high perchlorate concentrations (about 10%) found in rocket washout wastewaters. The reactor was made of acrylic tubing (1.17 m in length; 7.6 cm inside diameter) filled with diatomaceous earth pellets (mean pore diameter of 20 μm). The substrate for the mixed culture (BYF-100) consisted of 54% naturally occurring protein, peptides, free amino nitrogen, vitamins, and trace elements. Perchlorate was reduced to <300 mg/L over the entire 43-day period of operation at hydraulic retention times (HRT) and influent perchlorate concentrations of 1.17 h and 1500 mg/L, and 0.46 h and 500 mg/L, respectively. For both influent concentrations, the effluent concentration was below 100 mg/L (the detection limit in this system) for 95% of the samples taken during the reactor operation.

Perchlorate was found to be degraded to lower concentrations in a sand media bioreactor inoculated with perclace, which was isolated from wastewater biosolids (Herman and Frankenberger, 1999). The reactor was a glass column (2.8 cm diameter; 14 cm long) packed with sterilized, oven-dried sand (40 to 70 mesh size). Following a start up period, it was found that perchlorate could be degraded from a concentration of 130 μg/L to below the detection limit (4 μg/L) at an HRT of 3 h. It was also reported that a celite-packed bioreactor inoculated with the same isolate completely removed perchlorate from
738 μg/L at a flow rate of 1 ml/min (Giblin et al., 2000a). When the flow was increased to 2 ml/min, 92 to 95% of the perchlorate was removed.

Perchlorate concentrations of 20 mg/L were found to be completely removed in a sand-packed bioreactor (2.5 cm diameter; 28 cm long) inoculated with a perchlorate-degrading enrichment (Kim and Logan, 2000). The enrichment was developed with acetate and perchlorate using primary digester solids from a wastewater treatment plant. Perchlorate was removed to nondetectable levels (<4 μg/L) over 35 days, after a 10-day startup period, at HRTs ranging 18 to 51 min. The same sand column system was also used to examine perchlorate degradation rates achieved when the reactor was inoculated with a pure culture of Dechlorosoma sp. KJ vs. that obtained using a perchlorate-degrading enrichment (Kim and Logan, 2001). The reactor inoculated with the pure culture removed perchlorate from 20 mg/L to less than 4 μg/L at a much shorter HRT of ~1 min than the mixed culture reactor (12 min). Acetate was used as the substrate for the culture, and the molar ratio (6.6, n = 156) of acetate-to-perchlorate for the pure culture was more than twice the ratio (2.9, n = 6) of the mixed culture.

Perchlorate degradation using granular-activated carbon (GAC) in a bioreactor was compared to reactor performance with sand media in fixed-bed reactors (Kim and Logan, 2000).

Table 2. Bench-scale bioreactors for treating perchlorate in water.

<table>
<thead>
<tr>
<th>Reactor type (media)</th>
<th>Inoculum</th>
<th>Substrates</th>
<th>Perchlorate (mg/L)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspended growth</td>
<td>Wolinella succinogenes</td>
<td>Protein nutrients</td>
<td>7750</td>
<td>Attaway and Smith (1993)</td>
</tr>
<tr>
<td>Fixed bed (diatomaceous earth pellets)</td>
<td>Wolinella succinogenes</td>
<td>BYF-100[^b^]</td>
<td>500, 1500</td>
<td>Wallace et al. (1998)</td>
</tr>
<tr>
<td>Fixed bed (sand or Celite)</td>
<td>Perclace</td>
<td>Acetate</td>
<td>0.13, 0.738</td>
<td>Herman and Frankenberger (1999); Giblin et al. (2000a)</td>
</tr>
<tr>
<td>Fixed bed (sand GAC)</td>
<td>Mixed culture</td>
<td>Acetate</td>
<td>20</td>
<td>Kim and Logan (2000)</td>
</tr>
<tr>
<td>Fixed bed (sand)</td>
<td>Dechlorosoma sp. KJ</td>
<td>Acetate</td>
<td>20</td>
<td>Kim and Logan (2001)</td>
</tr>
<tr>
<td>Fixed bed (cylindrical pall rings)</td>
<td>Primary sludge</td>
<td>Acetate</td>
<td>0.1, 1</td>
<td>Burns et al. (2001)</td>
</tr>
<tr>
<td>Fixed bed (Celite pellets)</td>
<td>Perclace</td>
<td>Acetate</td>
<td>0.8</td>
<td>Losi et al. (2002)</td>
</tr>
<tr>
<td>Fixed bed (Mixed culture)</td>
<td>Hydrogen</td>
<td></td>
<td>0.740</td>
<td>Miller and Logan (2000)</td>
</tr>
<tr>
<td>Fixed bed (Celite R-635)</td>
<td>Biosolids from the</td>
<td>Hydrogen</td>
<td>0.740</td>
<td>Giblin et al. (2000b)</td>
</tr>
<tr>
<td>Fixed bed (glass beads)</td>
<td>Mixed culture</td>
<td>Hydrogen</td>
<td>0.073</td>
<td>Logan and LaPoint, (2002)</td>
</tr>
<tr>
<td>Fluidized bed (sand or GAC)</td>
<td>Biological solids from an anaerobic digester</td>
<td>Ethanol, methanol, or a mixture of the two alcohols</td>
<td>25</td>
<td>Greene and Pitre, (2000); Hatzinger et al. (2000)</td>
</tr>
<tr>
<td>Fluidized bed (GAC)</td>
<td>Mixed culture</td>
<td>Acetic acid and ethanol</td>
<td>11–23</td>
<td>Togna et al. (2001)</td>
</tr>
<tr>
<td>Hollow-fiber membrane bioreactor</td>
<td>Ralstonia eutropha</td>
<td>Hydrogen</td>
<td>0.006–0.100</td>
<td>Nerenberg et al. (2002)</td>
</tr>
</tbody>
</table>

[^a^]Aged brewer’s yeast, cottonseed protein, or whey power;[^b^]Naturally occurring protein (54%), peptides, free amino nitrogen, vitamins, and trace elements.
Logan, 2000). The GAC media (12 x 40 mesh-sieved) had a bulk density of 0.44 g/cm^3 (NORIT Americas Inc., Atlanta, GA), while the sand had an average size of 0.425 mm (density of 2.67 g/cm^3). Both reactors were fed the same perchlorate-degrading enrichment. The GAC-packed reactor completely removed perchlorate for the first 4 days of operation, but after backwashing the media on day 6, the reactor performed erratically with complete perchlorate removal on some days and detectable perchlorate concentrations on others. The poor operation of the GAC reactor was attributed to desorption of perchlorate from the GAC in the lower parts of the reactor where biological activity was insufficient to degrade the perchlorate. Based on these results, the researchers recommended that GAC would not be suitable for fixed-bed bioreactors that would need backwashing to control biofilm formation and prevent clogging.

A slightly different GAC system was examined by Brown et al. (2000) for perchlorate degradation. They examined the use of biologically active carbon (BAC) filters for perchlorate removal at low influent perchlorate concentrations (Brown et al., 2000). The BAC filters were constructed of glass pipes 2.54 cm in diameter and Teflon™ endcaps. The filters were rendered biologically active by feeding 600 bed volumes (BV) of dechlorinated tapwater containing 50 µg/L perchlorate and 20 µg/L bromate. Deionized water containing 50–55 µg/L perchlorate was then fed to the column for 5,800 empty BV, with perchlorate removal occurring mainly by sorption to the carbon. Perchlorate removal was then stimulated by adding a mixture of acetate, lactate, and pyruvate (2 mg/L as carbon) to the water that also contained 2.5 mg/L of dissolved oxygen and nitrate. Perchlorate removal was found to be a function of the concentration of nitrate in the water. Perchlorate was removed from 52 µg/L to below the detection limit, for an influent nitrate concentration of 1.4 mg/L (0.07 mg/L in the effluent). However, perchlorate was not appreciably removed (from 51 to 50 µg/L) at a higher influent nitrate concentration of 4.5 mg/L (2.0 mg/L in the effluent). The effects of nitrate on perchlorate reduction are further discussed below.

Burns et al. (2001) reported that two fixed-bed bioreactors placed in series could remove perchlorate from an influent concentration in the range of 0.1 to 1 mg/L, to below the detection limit even in the presence of high concentrations of nitrate (50 mg/L). Both reactors (70 mm diameter; 460 mm in height) initially inoculated with primary sludge were fed a synthetic solution containing acetate (250 mg/L) and sodium sulfite (to remove dissolved oxygen in the reactor influent). HRTs of 1 h (0.1 mg/L) and 10 h (1 mg/L) were needed for complete removal of perchlorate.

Another bench-scale packed-bed biological reactor was examined in a 30-day study for its efficiency at removing low concentrations of perchlorate (<1 mg/L) from groundwater (Losi et al., 2002). The Plexiglas column reactor (total volume of 3,062 mL) was filled with Celite pellets and inoculated with the perchlorate-reducing bacterium perchlace (Herman and Frankenberger, 1999). Groundwater from a perchlorate-contaminated site in California was pumped (upflow mode) into the reactor along with nutrients (N and P) and acetate (approximately 500 mg/L). Influent perchlorate concentrations of about 800 µg/L were degraded to nondetectable levels (<4 µg/L) at residence times as low as 0.3 h. Acetate concentrations were less than 50 mg/L in the effluent. Nitrate (20 mg/L NO_3^-N) was also completely removed while sulfate reduction was not observed.

**Heterotrophic fluidized bed bench scale reactors**

Fluidized bed reactors (FBRs) have also been examined for perchlorate removal in bench scale studies (Greene and Pitre, 2000; Hatzinger et al., 2000). The FBRs contained either sand or GAC with particle sizes of 0.2 to 0.6 mm and 0.9 to 1.4 mm, respectively, as the media. The reactors were fed ethanol, methanol, or a mixture of the two alcohols as substrates for perchlorate biodegradation. Ethanol or the ethanol–methanol mixture produced better performance than just methanol. The FBR packed with GAC showed better performance than the sand reactor, and achieved consistent perchlorate removal from 25 mg/L to less than the detection limit of 4 µg/L (Greene and Pitre, 2000; Hatzinger et al., 2000).

A laboratory-scale FBR was used to treat perchlorate-contaminated groundwater from the Longhorn Army Ammunition Plant (Togna et al., 2001). The glass column reactor containing GAC as the fluidization medium was fed both acetic acid and ethanol as the electron donors. Effluent perchlorate concentrations were less than the detection limit except during a low substrate study conducted to determine the point of treatment failure.

**Autotrophic H₂ reactors**

Nitrate has been degraded in hydrogen-fed bioreactors for many years (Gayle et al., 1989; Kapoor and Viraraghavan 1997), but only very recently there have been reports of using autotrophic bioreactors for the degradation of perchlorate. Miller and Logan (2000) developed an unsaturated-flow, packed bed bioreactor for the degradation of perchlorate in drinking water. The bioreactor was inoculated with a hydrogen-oxidizing, autotrophic, and perchlorate degrading enrichment culture developed in a chemostat. The packed-bed reactor was fed with a gas mixture of hydrogen (5%) and carbon dioxide and a feed stream containing only perchlorate and in-
organic nutrients. The reactor was initially operated for a 10-day start-up period using a high concentration of perchlorate (50 mg/L) to build up the biofilm, and was then switched to a lower level of perchlorate (740 ± 110 µg/L) for the next 145 days of operation. During this operation period, perchlorate was reduced to 460 ± 80 µg/L at a constant hydraulic loading rate of 0.45 cm/min (1.1–1.3 min detention time).

A slightly modified version of the reactor used by Miller and Logan (2000) was tested to examine the removal of perchlorate from a perchlorate-contaminated groundwater (Logan and LaPoint, 2002). The reactor was also fed a gas mixture of hydrogen (5%) and carbon dioxide as the electron donor and carbon source, respectively. An average of 25 ± 5% of perchlorate was removed at a HRT of 1.5 min from a groundwater containing 73 ± 2 µg/L of perchlorate and 21 ± 5 mg/L of nitrate. It was estimated that a reactor with a detention time of 8 to 42 min (95% C.I.) a length of 0.65 to 3.4 m would be necessary to completely remove perchlorate in this type of unsaturated-flow system.

A 120-mL bioreactor packed with Celite R-635 (Celite Corporation, Lompoc, CA) was used to degrade perchlorate with hydrogen (Giblin et al., 2000b). The autotrophic consortium of bacteria was fed a pressurized water stream containing dissolved hydrogen and bicarbonate. Perchlorate (740 µg/L) was removed to below the detection limit at a 2-h detention time. Perchlorate removal was incomplete at shorter detention times, with perchlorate concentrations of 100 and 200 µg/L and detention times of 1 and 0.67 h, respectively. It was speculated that the perchlorate breakthroughs at the shorter detention times resulted from nonuniform distribution of biomass, pH variations of the groundwater, and limited hydrogen transport to the bacteria.

The degradation of perchlorate in a groundwater has also been demonstrated using a hollow-fiber membrane bioreactor (Nerenberg et al., 2002). The membrane module in the reactor contained 98 hollow fibers (93 cm long; 280 µm outside diameter) in a PVC pipe shell. Hydrogen gas fed through the fiber pores was used to support a biofilm growing on the membrane and in the liquid in the reactor. The groundwater contained both perchlorate (6–100 µg/L) and nitrate (2.5–3 mg/L). It was found that perchlorate could be removed to below the detection limit when the nitrate concentration from the effluent was less than 30 µg/L in the presence of at least 300 µg/L of dissolved hydrogen in the bulk solution.

Effects of nitrate on perchlorate removal

Both perchlorate and nitrate are common contaminants in surface and ground waters in the United States. As most of the PRB are also denitrifiers, some papers have discussed the simultaneous removal of perchlorate and nitrate from contaminated waters. Nitrate has been found to have different effects on perchlorate removal rates. Nitrate has inhibited the perchlorate reduction rate in some studies (Herman and Frankenberger, 1999; Brown et al., 2002) but not in others (Burns et al., 2001; Logan and LaPoint, 2002). A PRB isolate, perchlace, was able to completely remove perchlorate (130 µg/L) and to simultaneously remove more than 95% of the nitrate (20 mg/L) in a sand-packed bioreactor (Herman and Frankenberger, 1999). Coppola and McDonald (2000) reported that a mixed culture of W. succinogenes could completely degrade perchlorate (1.2–1.5 g/L), chlorate (3–3.5 g/L), and nitrate (0.2 g/L) in a 7-L continuously stirred tank reactor at a HRT of 16 h. Nerenberg et al. (2002) showed that perchlorate and nitrate reduction to nondetectable levels could be concurrently achieved using a hydrogen-fed, autotrophic membrane bioreactor. In pilot-scale tests, Min et al. (2003) found that perchlorate and nitrate were both completely removed in a fixed-bed bioreactor. Thus, it appears likely that perchlorate and nitrate can be simultaneously removed in bioreactors. However, the concentration of nitrate will increase the total amount of electron donor needed by the system for complete perchlorate removal.

Soil bioremediation feasibility tests

Important questions for soil bioremediation are whether the added substrate will be used for perchlorate degradation or lost to other biochemical routes (such as methanogenesis), and whether there are sufficient microorganisms in the soil to achieve perchlorate degradation using added substrate. The first indication that perchlorate bioremediation could be easily accomplished were studies that demonstrated perchlorate reduction could be achieved solely by adding substrate and nutrients to wastewater and soil samples. For example, it was shown that by adding acetate to headspace-free BOD bottles 50% of 20 mg/L perchlorate was degraded within 4 days, and 74% was removed within 9 days (Kim and Logan, 2000). The cultures obtained after 9 days of acclimation were transferred (5% by volume) to freshly prepared media containing acetate (200 mg/L) and either perchlorate (50 mg/L) or chlorate (50 mg/L), or same concentrations of both anions (100 mg/L). The perchlorate and chlorate degradation rates were similar (75 and 78%, respectively) within 5 days using the acclimated cultures (Kim and Logan, 2000).

Other researchers have shown that CRB are ubiquitous in soil samples, providing indirect evidence that bioremediation to remove perchlorate would not require bioaugmentation. For example, van Ginkel et al. (1995) found CRB in rivers, sediments, soils, and wastewater
treatment plants (van Ginkel et al., 1995) and Coates et al. (1999) found CRB at concentrations of $2.31 \times 10^3$ to $2.4 \times 10^6$ cells per gram in pristine and hydrocarbon-contaminated soils, aquatic sediments, paper mill waste sludges, and farm animal waste lagoons.

Direct evidence for the feasibility of perchlorate bioremediation with different substrates was provided by Wu et al. (2001). They examined perchlorate degradation using samples from soils, natural waters, and wastewaters. Complete degradation of 210 mg/L perchlorate was achieved within 4 to 7 days and 8 to 29 days for raw wastewater and creek water, respectively, using four different substrates (acetate, lactate, citric acid, or molasses). PRB were nonmeasurable using a five-tube MPN method in some soil samples, but were present at high concentrations at perchlorate-contaminated sites. For example, perchlorate reduction was not achieved with 10 g of “pristine” soil, but it was observed with 100 g of soil. Microorganisms in 10 g of a perchlorate-contaminated soil (from a site in Texas) completely removed perchlorate from 100 mg/L within only 7 days using polylactate or lactate.

Hunter (2001) demonstrated that injecting sand columns with a slowly degradeable substrate (vegetable oil) could remove perchlorate without the need for bioaugmentation with perchlorate-acclimated bacteria. Columns were seeded with bacteria from soil, and perchlorate (20 mg/L) was added to water and pumped into the columns at a rate of $\sim 25$ mL/day. After 14 days of operation, 0.47 mg of soybean oil was injected onto the treatment columns. Although perchlorate levels remained high in the control columns (no soybean oil), perchlorate in the effluent of the treatment columns decreased by $\sim 99\%$ over a 17-week operation in the columns containing the vegetable oil.

**MICROBIAL TREATMENT PROCESSES—PILOT AND FULL-SCALE PROCESSES**

**Fixed-bed reactors**

A pilot-scale bioreactor was operated as an ex situ treatment process to treat perchlorate-contaminated groundwater at the Naval Weapons Industrial Reserve Plant in McGregor, TX (Perlmutter et al., 2000). The reactor was constructed of steel (5-ft diameter, 18 ft in height) and fed water containing 7 to 20 mg/L of perchlorate. It was found that adding only acetate (acetate: ClO$_4^-$ ratio of 5:1) and nutrients (nitrogen and phosphorus) at a flow rate of 20 gpm, the perchlorate concentration could be completely removed (<20 µg/L, the detection limit at that site).

Two pilot-scale bioreactors were operated for about 7 months to treat perchlorate-contaminated groundwater (average 76 µg/L) at the Texas Street Well Facility in Redlands, CA (Logan et al., 2001a; Evans et al., 2002; Min et al., 2003). The 7-ft tall reactors contained two rectangular beds each $2 \times 1 \times 1$ ft. One reactor contained sand media (1 mm diameter) and the other one plastic media (3.76 cm diameter). The reactors were inoculated with a pure culture of *Dechlorosoma* sp. KJ. Acetic acid (47 ± 9 mg/L for the sand media reactor; 53 ± 10 mg/L for the plastic media reactor) and an ammonium phosphate solution (C:N ratio of 5:1) were added to the groundwater. The groundwater contained nitrate (4 mg/L), oxygen (7 mg/L), and sulfate (33 mg/L) as potential terminal electron acceptors in addition to perchlorate. Perchlorate was completely and consistently removed to nondetectable levels (4 µg/L) at flow rates of 1 gpm in the plastic media reactor, and 2 gpm in the sand reactor. Backwashing (weekly) was critical to maintain consistent perchlorate removal rates in the sand reactor. In the absence of regular backwashing, there was short circuiting in the sand reactor, resulting in insufficient groundwater detention time for consistent perchlorate removal.

**Fluidized bed bioreactor**

A pilot-scale FBR was tested in 1996 at the Aerojet facility in Sacramento, CA, using water containing 7,000 to 8,000 µg/L of perchlorate and 1.5 mg/L of nitrate-nitrogen (Catts and McCullough, 1998). There are few details available on this system, but it was reported that effluent concentrations of <400 µg/L were achieved for perchlorate, and <50 µg/L for nitrate-nitrogen. Ethanol was added in proportion to perchlorate at a molar ratio of 4:1.

In 1998, a second FB bioreactor was tested at the Baldwin Park site in the San Gabriel Basin in California. The reactor (20 in high, 15 ft long) contained activated carbon (10 × 30 mesh), and was similar to one used at the Aerojet site, except this reactor was inoculated with sludge from a food processing industry (vs. a wastewater treatment plant). The reactor was fed groundwater (30 gpm, 114 L/min) amended with ethanol (40 to 70 mg/L) and nutrients. Effluent ethanol concentrations ranged from <10 mg/L to nondetectable concentrations. Perchlorate (50 to 100 µg/L) removal was generally greater than 90% when dissolved oxygen was low (near 1 mg/L). At other times, removal varied from 23 to 45%. Nitrate (5 to 6 mg/L NO$_3^-$-N) removal was generally greater than 99%. The denatured ethanol used for the study contained low concentrations of methanol, methyl isobutyl ketone and isopropyl alcohol (Catts and McCullough, 1998). These chemicals can be toxic to humans, and therefore the addition of these chemicals to drinking water sources should be avoided. Nitrosodimethylamine
present in the water at 70 to 80 μg/L was not affected by the bioreactor treatment.

A full-scale FBR was operated to treat perchlorate in groundwater at a site in Rancho Cordova, CA (www.envirogen.com/perchlorate.htm; Green and Pitre, 2000; Hatzinger et al., 2000). The system consisted of four FBRs each 14-ft in diameter treating 4,000 gpm of groundwater. The reactors were filled with GAC, inoculated with a perchlorate-degrading enrichment, and fed ethanol as the electron donor. Following a startup period of 3 weeks, the effluent concentration of perchlorate was removed from 6 to 8 mg/L to less than the detection limit (4 μg/L) for 8 weeks of operation during periods of excess ethanol addition. Periods of incomplete perchlorate removal occurred at lower ethanol doses (Green and Pitre, 2000; Hatzinger et al., 2000).

FBRs have recently been approved for treatment of perchlorate contaminated drinking water by the California Department of Health Service (DHS, 2002a). However, the presence of methanol and other impurities in ethanol may prohibit the use of ethanol as a substrate in these bioreactors if they are to be used for drinking water treatment.

In situ bioremediation

Pilot-scale tests for perchlorate remediation have shown that perchlorate reduction can be achieved by adding various electron donors to create anoxic conditions in the subsurface. An excess of an electron donor can be used for the degradation of perchlorate, nitrate, and other pollutants. In situ bioremediation field tests of perchlorate were conducted at the Naval Weapons Industrial Reserve Plant in McGregor, TX (Perlmutter et al., 2000). Trenches were dug at the site to collect groundwater and direct the flow of this water through mounds of material containing various organic sources such as compost (from an edible mushroom production facility) and cottonseed meal. Perchlorate in the water was removed from 16–27 mg/L to less than 100 μg/L (detection limit in this study) after only 2 weeks of operation. Nitrate was also reduced from 15 mg/L to nondetectable concentrations.

Cox et al. (2001) reported that perchlorate in situ biodegradation was successfully achieved in a deep aquifer at the Aerojet Superfund site in California. Acetate was added into the aquifer to support perchlorate reduction. Perchlorate concentrations in the groundwater decreased from 12 mg/L to less than the detection limit (4 μg/L) within 15 feet of the electron donor delivery well. In addition to perchlorate, trichloroethylene, another common contaminant of groundwater, was completely dechlorinated to ethane.

MICROBIAL TREATMENT PROCESSES—SYSTEMS USED IN CONJUNCTION WITH OTHER PROCESSES

Processes such as anion exchange (Venkatesh et al., 2000; Gu et al., 2001), membrane filtration (Urbansky and Schock, 1999), and activated carbon (Brown et al., 2002) have been studied for the removal of perchlorate, especially for treating waters contaminated with low (<100 μg/L) concentrations of perchlorate. For example, anion exchange resins have been shown to be highly effective for perchlorate removal (Urbansky, 2000a). A field experiment has shown that one bed volume of a unique bifunctional resin can treat greater than 100,000 BV of groundwater with an initial perchlorate concentration of 50 μg/L (Gu et al., 2000). Because these physical/chemical techniques do not degrade perchlorate, further treatment using chemical or biological processes is necessary. Perchlorate in the brine wastes can be reduced to chloride by chemical methods using catalytic air-sensitive metal cations such as titanium or ruthenium (Abu-Omar et al., 2000; Espenson, 2000), or by electrochemical reduction (Urbansky, 1998). One company (Calgon Carbon) has patented a treatment process (Venkatesh et al., 2000) for perchlorate removal that uses ion exchange to remove perchlorate and a bioreactor or catalytic reactor for complete perchlorate destruction. This treatment process has been approved for treatment of perchlorate contaminated water by the California Department of Health Service (http://www.calgoncarbon.com/industry/productdata.php?id=11).

Biological treatment of concentrated waste streams from ion exchange processes can be difficult to treat using biological systems due the high salt content of the brine. In the case of an anion exchange process, the regeneration of the resin typically generates a 7–12% NaCl brine solution enriched in perchlorate. Gingras and Batista (2002) were unable to adapt a PRB culture to degrade perchlorate in an ion exchange brine. As little as 1% NaCl reduced perchlorate reduction rates by their perchlorate-degrading culture by half (Gingras and Batista, 2002). High NaCl systems may not be required for certain types of specialized ion exchange resins. In one system recently developed by Gu et al. (2001), the resin was regenerated using a tetrachloroferrate displacement technique. Using this method, only 5 BV of regenerant solution were needed to achieve nearly 100% recovery of the resin. Large-scale field application of this technique is reported to be under way (Gu et al., 2001).

Although biological degradation of perchlorate in ion exchange brines can be difficult, some bacteria can grow and degrade perchlorate even in highly saline solutions. A culture containing primarily W. succinogenes HAP-1
was reported to degrade perchlorate in brines derived from the regeneration of ion exchange resins (7% NaCl, 180 mg/L perchlorate) (Coppola and McDonald, 2000). However, the 7% brine solution had to be diluted to 3% with water to allow biodegradation of the perchlorate. This dilution is undesirable, as it increases the total volume of salt solution requiring disposal. Logan et al. (2001b) demonstrated that the biological reduction of perchlorate was possible at salinities of up to 11% NaCl. The perchlorate-degrading enrichment was developed using samples from The Great Salt Lake. Although perchlorate was degraded in these highly saline solutions, the degradation rates were slow. Okeke et al. (2002) isolated a salt tolerant PRB strain, Citrobacter sp. IsoCock1, that was able to achieve a 32% reduction of perchlorate in a 7.5% solids solution in 1 week at an initial perchlorate concentration of 0.5 g/L. A 46% reduction of perchlorate was achieved by a mixture of this bacterium and the strain perchlace (Okeke et al., 2002).

Reverse osmosis (RO) can also be used to remove perchlorate from drinking water. The waste stream containing perchlorate that was produced from an RO process contained much lower amount of salts (<1%) than the NaCl brine generated using an ion exchange process. A packed bed bioreactor, inoculated with the pure culture perchlace, was tested for its ability to remove perchlorate from a simulated RO rejectate (Giblin et al., 2002). It was found that this system removed 98% of perchlorate from a twice-concentrated rejectate (total dissolved solids of 0.4%) with an influent perchlorate concentration of 8 mg/L and a residence time of 2 h. Nitrate was removed simultaneously with perchlorate from an initial concentration as high as 900 mg/L to below 4 mg/L. Despite the efficiency of perchlorate removal, the system suffered from clogging due to the high total dissolved solids of the twice-concentrated rejectate.

It is possible to simultaneously remove and degrade perchlorate using BAC. BAC has been used in drinking water treatment to remove trace amounts of halo-acetic acids in drinking water (Xie and Zhou, 2002). Kim and Logan (2000) reported that GAC could be used to treat water containing 10 mg/L of perchlorate. They found that perchlorate was completely removed by the reactor at the beginning of operation, but that after backwashing reactor efficiency was substantially reduced due to perchlorate desorption from the carbon in regions of low biological activity near the end of the column. At much lower influent perchlorate concentrations of 50 μg/L, Brown et al. (2002) found, over a 103-day study, that it was possible to remove perchlorate to below the detection limit in a BAC operated at a 25-min empty bed contact time. Although the BAC was not bioaugmented with PRB, they found that Dechloromonas and Dechlorosoma were present in the carbon bed during the period when perchlorate removal was successful (Lin et al., 2002).

**PHYTOREMEDIATION**

Perchlorate contamination of the environment may affect agricultural plants as well as naturally occurring flora (U.S. EPA, 2002). Chlorate has been used as a defoliant (van Wijck and Hutchinson, 1995), and therefore, it is not surprising that perchlorate can also be taken up by plants. The accumulation of perchlorate in plants is of concern for several reasons. Perchlorate can be toxic to some plants, if the perchlorate accumulates and is not degraded, and the death of the plant may release perchlorate back into the environment that could be toxic to other plants or wildlife (Urbansky et al., 2000). Perchlorate accumulation in food plants could present another route of human exposure to perchlorate. Perchlorate contaminated water, such as Lake Mead or the Colorado River, is presently used for irrigating food crops (Susarla et al., 1999; Urbansky et al., 2000). Hutchinson et al. (2000) are currently studying the accumulation of perchlorate in lettuce irrigated with perchlorate-tainted water.

Because perchlorate can accumulate in plants, phytoremediation has been suggested as a potential mechanism for degrading perchlorate in soil systems. Phytoremediation may occur by phytoextraction (accumulation in the branches and leaves), phytodegradation, or rhizotransformation (degradation in the root sphere primarily due to microbial activity). Although many plants have shown the ability to accumulate perchlorate, some plants can drive perchlorate degradation completely to chloride (Nzengung et al., 1999; Susarla et al., 1999; Hutchinson et al., 2000; Nzengung and Wang, 2000). Nzengung and Wang (2000) found that willow trees could degrade 100 mg/L of perchlorate in 53 days, and that minced spinach and tarragon leaves could degrade 7 mg/L of perchlorate in 30 days. There were no lag times for perchlorate degradation in either experiment. Perchlorate degradation by plants was found to occur in two stages (Nzengung and Wang, 2000). The first stage consisted of an initial uptake of perchlorate proportional to the water uptake by the plant, and a slow transformation of perchlorate to chloride in the plant tissues. The second stage was characterized by a rapid removal of perchlorate by degradation in the root zone with little perchlorate taken up by the plant. This second stage was assumed to arise from the stimulation of growth of PRB in the rhizosphere (Nzengung et al., 1999; Nzengung and Wang, 2000).

Several phytoremediation studies have been conducted to study the rate of perchlorate uptake and degradation, and the effects of other chemicals on degradation rates.
Nzengung et al. (1999) examined removal and degradation of perchlorate in water amended with acetate and differing amounts of nitrate using sand or hydroponic systems cultivated with willow (Salix nigra). Both system types were capable of removing perchlorate from the water. Degradation was found to occur due to transformation in the leaves and stems, and degradation by the microbial population in the plant rhizosphere. Multiple dosing of sand systems with 100 mg/L perchlorate, and less than 100 mg/L of nitrate, achieved a maximum degradation rate of perchlorate of 2.35 mg/L-h (Nzengung et al., 1999). Krauter (2001) constructed a wetland system to degrade both nitrate and perchlorate in groundwater. The system consisted of four tanks containing gravel and planted with a variety of indigenous wetland plants. Influent water contained nitrate (68 mg/L), perchlorate (4.5 µg/L), and trichloroethene (54 µg/L), but it was further amended with sodium perchlorate (0.1 mg/L). It was discovered that although the young wetland system was capable of supplying sufficient carbon to support denitrification, perchlorate reduction was carbon-limited. When acetate (260 mg/L) was added to the influent, both nitrate and perchlorate were completely degraded within 52 h. The maturity of the plants in the bioreactor and the time of year were found to greatly influence the nitrate and perchlorate degradation rates in the bioreactor (Krauter, 2001).

CONCLUSIONS

Many heterotrophic biological treatment systems have been tested to degrade perchlorate including suspended, fixed-bed, and fluidized-bed reactors (Attaway and Smith, 1993; Wallace et al., 1998; Attaway et al., 1999; Green and Pitre, 2000; Coppola and McDonald, 2000; Hatzinger et al., 2000; Perlmutter et al., 2000; Logan et al., 2001a; Evans et al., 2002). Organic electron donors that have been used include simple compounds such as acetate and ethanol, as well as more complex organic substrates such as those found in compost piles. Perchlorate degradation has also been achieved in bioreactors using only inorganic amendments. These reactors are sustained by hydrogen gas delivered by pressurization, gas transfer across liquid films, or synthetic membranes (Giblin et al., 2000b; Miller and Logan, 2000; Nerenberg et al., 2002). These hydrogen-based technologies are promising technologies for water treatment because less biomass is produced by autotrophic processes than heterotrophic processes. Large-scale tests are needed to evaluate process efficiency and the economics of these different hydrogen-based systems.

Although at least one biological treatment process has been approved for use in the state of California for drinking water treatment (DHS, 2002a), little has been done to study the removal of PRB from the treated water. Membrane bioreactors can be used to keep the bacteria separated from the contaminated water (Batista and Liu, 2001), but these systems are at a less advanced stage of development than other biological perchlorate treatment systems. It has been suggested that reactors based on enzymes to reduce perchlorate could avoid the potential health problems associated with biological treatment (Betts, 1999). Perchlorate reductase, which can reduce both perchlorate and chloride (Kengen et al., 1999), and chlorite dismutase (van Ginkel et al., 1996; Coates et al., 1999; Stenklo et al., 2001) have both been purified. However, no such enzyme-based systems have been reported in the literature for treatment of perchlorate contaminated water.

The presence of alternate electron acceptors in perchlorate contaminated water will be an issue for all types of biological reactors. Oxygen is an important intermediate in the perchlorate degradation pathway (Rikken et al., 1996). It is well known that for PRB oxygen is a preferential electron acceptor to perchlorate, and that high concentrations of dissolved oxygen inhibit perchlorate reduction (Kengen et al., 1999; Song and Logan, 2002). It is not clear what concentration of dissolved oxygen will completely inhibit perchlorate reduction, how long bacteria can withstand exposure to high concentrations of oxygen before losing the ability to reduce perchlorate, or how long it would take oxygen-exposed bacteria to regain the ability to reduce perchlorate. However, the presence of oxygen, nitrate, or sulfate in bioreactor feed streams does not appear to be a problem for the steady operation of such systems. In a pilot-scale test for ex situ groundwater treatment, it was found that oxygen, nitrate, and perchlorate were all completely reduced but that sulfate was not measurably degraded (Logan et al., 2001a; Evans et al., 2002). Thus, it is likely that the main impact of oxygen and nitrate on a treatment system will be to increase the requirement of substrate (such as acetate or hydrogen) that is oxidized by the bacteria.

One of the most important issues for designing a perchlorate treatment system will be the regulatory requirement for perchlorate removal. It now appears likely that the removal of perchlorate to very low levels will be necessary. The U.S. EPA is expected in the near future to finalize a draft of its final assessment of its toxicological effects of perchlorate, which could lead to recommendations for perchlorate removal to <1 µg/L for drinking water (Renner, 2002). The action level of perchlorate for drinking water in several states has been set at levels of 1–4 µg/L based on the release of the draft report (www.clu-in.org/studio/perchlorate-060402). If enacted,
these regulatory levels could require perchlorate treatment systems for users of large water sources such as Lake Mead and the Colorado River that serve major cities in California, Utah, and Arizona (Motzer, 2001). Both biological and chemical treatment systems can be used to treat perchlorate contaminated water to low levels. The most appropriate system will likely be site and case-specific, with economic, social, and political factors playing a role in the selection of each treatment system.

ACKNOWLEDGMENTS

The authors thank Husen Zhang for constructing the phylogenetic tree, and support from the National Science Foundation (Grants BES9714575 and BES0001900), and support via a grant from the United States Environmental Protection Agency, the East Valley Water District, and the American Water Works Association Research Foundation (AwwaRF Grant No. 2557).

REFERENCES


BENDER, K.S., CHAKRABORTY, R., LACK, J.G., COATES, J.D., and ACHENBACH, L.A. (2002). Isolation and characterization of genes involved in (per)chlorate reduction and the utility of these genes as metabolic probes. In The 102nd general meeting of American Society for Microbiology, Salt Lake City, Utah, USA.


CRUMP, C., MICHAUD, P., TELLEZ, R., REYES, C., GONZALEZ, G., MONTGOMERY, E.L., CRUMP, K.S., LOBO,


SONG, Y., and LOGAN, B.E. (2002). Effect of O$_2$ on perchlorate reduction and recovery of perchlorate degradation following O$_2$ exposure. In The 102nd general meeting of American Society for Microbiology. Salt Lake City, UT.


