A Review of Chlorate- and Perchlorate-Respiring Microorganisms

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Abstract: Chlorate (ClO_3^-) and perchlorate (ClO_4^-) have been manufactured in large quantities, and therefore it is not surprising that they have been found at high concentrations (>50 mg/L and >1000 mg/L, respectively) in surface waters and groundwaters around the world. These compounds are chemically stable in water, and they are difficult to remove using typical physical-chemical water treatment technologies. Fortunately, chlorate and perchlorate can be removed by biodegradation to low levels in water. Both compounds are highly oxidized and can serve as electron acceptors for several strains of microorganisms under anoxic conditions. Although it has been known for more than 40 years that chlorate can be reduced by mixed cultures, several bacteria have been isolated recently that are able to respire using either chlorate or perchlorate. The purpose of this paper is to review the characteristics of these mixed cultures and isolates in order to assess their future potential for biological water and wastewater treatment processes.

Keywords: anaerobic processes, bioreactors, bioremediation, chlorate, perchlorate, halo-oxygenated, water treatment, wastewater treatment.

Introduction

The presence and persistence of several halo-oxygenated acids, such as chlorate and perchlorate, is becoming an important international water quality concern. Perchlorate recently has been measured in several surface waters and groundwaters in the United States at concentrations up to 3700 mg/L. The California Department of Health Services (CDHS), based on work the by U.S. Environmental Protection Agency (EPA), has established a provisional action level of 18 μg/L for drinking water due to perchlorate's interference with iodine in the production of hormones in the human thyroid. The presence of perchlorate at these high concentrations in the aqueous environment, coupled with a very low drinking water standard, has created a national water contamination crisis in the United States potentially affecting 12 million people. Perchlorate has been found in 30% of the wells sampled in California, and is above the state's action level in 9% of those wells. Perchlorate endangers the use of the Colorado River, which is a major water source for many Western states. For example, samples taken from the Las Vegas Wash, which feeds Lake Mead and the Colorado River, contained 1500 to 1680 μg/L, and samples from Lake Mead contained 8 μg/L; the Southern Nevada Water Authority has found 11 μg/L of perchlorate in its tap water (Urbansky, 1998).

Perchlorate has been manufactured for use as ammonium perchlorate (AP), a highly energetic compound used as an oxidizer in solid rocket propellent. AP is extremely soluble and chemically stable in water, even in highly reducing environments. Merely lowering the Eh of water to the range below –200 mV, for example, does not produce abiotic perchlorate reduction (Bliven, 1996). Because perchlorate is not easily removed from water, a team of experts who met at a special workshop on perchlorate agreed that there

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is no proven removal process to treat perchlorate-contaminated water to drinking water standards (AWWARF, 1997). Typical water treatment technologies, such as ion exchange, air stripping, carbon adsorption, and advanced oxidation, so far have not been proven to be cost-efficient at removing perchlorate from drinking water. Perchlorate removal in membrane processes, such as reverse osmosis, also has not yet been tested satisfactorily.

Chlorate has been produced industrially and disseminated widely into the environment for many years. Chlorate has been used for weed control in agriculture and as a defoliant; it is used for on-site generation of chlorine dioxide (ClO_2) by some pulp and paper industries. Chlorate can be released in large quantities when ClO_2 is used as a bleaching agent or for water disinfection (van Wijk and Hutchinson, 1995). Chlorate removal from bleached effluents is possible using sulfur dioxide, but removal is not always complete (Malmqvist and Welander, 1992). Surface water concentrations of chlorate in the Netherlands have been reported as 0.04 mg/L in the River Meuse and 0.02 mg/L in the Rhine and Ijssel rivers, and kraft mill effluents to marine waters have been reported to contain up to 53 mg/L of chlorate (Rosemarin et al., 1994; Versteegh et al., 1993). Chlorate can cause hemolytic anemia when fed to rats and mice in their drinking water, and the EPA recommends that the combined residuals of ClO_2, ClO_2^-, and ClO_3^- not exceed 1 mg/L in drinking water (Gonce and Voudrias, 1994).

Other halo-oxygenated compounds also have important environmental significance. For example, bromate can be formed during ozonation or chlorination of drinking water. Bromate is a carcinogenic compound with a lifetime risk of $<10^{-5}$ for a drinking water concentration of 3 μg/L. The World Health Organization (1993) established a provisional drinking water guideline for bromate of 25 μg/L (Hijnen et al., 1995).

Chlorate, perchlorate, and bromate are biodegradable under certain conditions. Several bacterial isolates have been shown to be capable of not just reducing perchlorate, but completely reducing perchlorate to chloride for cell respiration (Table 1). The ability of microbes to respire on perchlorate is amazing given that perchlorate is not known to occur in appreciable concentrations except perhaps in Chile saltpeter and certain nitrate deposits in Chile (Schilt, 1979). Microbial strains capable of using perchlorate as an electron acceptor have been found to be capable also of chlorate respiration. Thus, although little is known about the biochemical pathways used by microorganisms for electron transport using chlorate and perchlorate, it seems likely that similar pathways are involved. Therefore, only the literature available on chlorate and perchlorate [(per)chlorate] respiration, and biological bromate reduction, is reviewed here to evaluate the potential for microbiological perchlorate reduction. A far larger set of literature on chlorate reduction by denitrifying bacteria is not reviewed. Although many denitrifying bacteria can reduce chlorate, reduction proceeds only to chlorite, which is toxic to these cells. Therefore, with a few exceptions, only the literature on strains capable of dissimilatory reduction is considered in this review.

Despite our current knowledge that perchlorate is biodegradable, no published reports in the refereed literature on water treatment studies have demonstrated that perchlorate can be removed from contaminated waters to the levels required for drinking water while maintaining water quality sufficient to meet other drinking water primary and secondary standards. However, there is sufficient evidence from published and ongoing research to suggest that such perchlorate-removing reactors may prove to be feasible for water treatment. Published data already exist on reactors capable of treating high concentrations of perchlorate, but there

Table 1. Bacterial isolates reported to reduce various oxygenated compounds.

Isolate	Electron Acceptors	Reference
Vibrio dechloraticans Cuznesove B-1168	ClO_3^-, ClO_4^-, NO_3^-,	Korenkov et al. (1976)
Acinetobacter thermotoleranticus	ClO_3^-, SO_4^{-2}	Stepanyuk et al. (1993)
Ideonella dechloratans	ClO_3^-, O_2, NO_3^-,	Malmqvist et al. (1994)
Pseudomonas sp. (various)	BrO_3^-,	Hijnen et al. (1995)
AB-1	ClO_3, NO_3	Bliven (1996)
GR-1	ClO_3^-, ClO_4^-, O_2, NO_3^-, Mn(IV)	Rikken et al. (1996)
Wolinella succinogenes HAP-1	ClO_3^-, ClO_4^-, NO_3^-,	Wallace et al. (1996)

is no proof of sustainable biological perchlorate treatment at lower (μg/L) concentrations.

Chlorate Degradation by Wastewater Cultures

Aslander (1928) may have been one of the first investigators to suspect the occurrence of biological chlorate reduction in the natural environment. He found that sodium chlorate decomposition took place in water-saturated soil, and that species of *Penicillium*, *Aspergillus*, and *Fusarium* grew on the surface of hay during the reduction of sodium chlorate solutions.

More than 40 years ago, Bryan and Rohlich (1954) demonstrated the biological reduction of chlorate, based on the suggestion of Zehnpfennig in 1952 that sodium chlorate might serve as a source of combined oxygen in the decomposition of organic matter in wastewater. Bryan (1966) developed a test to estimate the total concentration of biodegradable organic matter by measuring the increase in chloride ion concentration resulting from the microbial reduction of chlorate. Although the test originally was called the chlorate biochemical oxygen demand (CBOD) test, we refer to it here as a biochemical chlorate demand (BCD) test because oxygen consumption is not measured in the test, and because CBOD now is commonly used to designate carbonaceous biochemical oxygen demand (BOD). Samples tested using the conventional BOD technique usually require dilution to avoid depletion of dissolved oxygen in the sample because oxygen is only sparingly soluble (~8 to 10 mg/L at room temperature). The main advantage of the BCD test was that the concentration of biodegradable organic matter in a wastewater sample could be analyzed for full-strength samples, i.e., without the large dilutions required in a BOD test.

Findings by Bryan and Rohlich (1954) and Bryan (1966) that chloride release would occur in proportion to the concentration of organic matter in wastewater cultures were confirmed again as part of recent studies on microbial chlorate reduction in the author's laboratory. Measurements of BCD were examined using wastewater samples from two different wastewater treatment plants in Tucson, Arizona. In one set of tests using an unacclimated wastewater inoculum, Patnaik (1996) measured a BCD_{10} = 40 mg/L and a BOD_5 = 67 mg/L, or a ratio of 0.60 mg-BCD_{10}/mg-BOD_5, for a glucose-glutamic acid (50 mg/L^{-1} each) solution. These values were slightly lower that those for a 50 mg/L glucose solution measured by Bryan (1966) of BCD_{10} = 23.6 mg/L and BOD_5 = 33.4 mg/L, or a ratio of 0.71 mg-BCD_{10}/mg-BOD_5 using acclimated cultures. The lower-yield coefficient may reflect incomplete usage of the glutamic acid or the use of unacclimated mixed cultures compared to the acclimated cultures used in the earlier studies. These studies therefore provide two main findings: first, that microorganisms easily reduce chlorate; and second, that chloride production occurs in proportion to the concentration of biodegradable organic matter in the same way that oxygen is consumed in proportion to the concentration of biodegradable organic matter in a BOD test.

It appears that chlorate-respiring microorganisms (CRMs) are widely distributed in the natural environment, although it is not known if chlorate respiration has ever occurred in any of the environments sampled. Bliven (1996) tested different inoculum sources for chloride production from chlorate (500 mg/L) in BOD bottles amended with a glucose and glutamic acid solution (1:1 ratio; final concentration of 300 mg/L). The concentrations of chloride (mg/L) obtained after 8 days of incubation by source were anaerobic digester, 156; pulp and paper mill wastewater, 63; primary clarifier effluent, 57; trickling filter effluent, 55; soil sample, 51.

Furthermore, van Ginkel et al. (1995) found chlorate reduction by Ijssel River samples, anoxic sediments from a ditch, surface soils from a public garden, and a wastewater treatment plant treating primarily domestic sewage. Cultures were enriched in BOD bottles with a defined mineral salts medium and sodium chlorate. Microbial reduction of chlorate was supported by different organic chemicals, including monocarboxylic and dicarboxylic acids, alcohols, and amino acids; and two inorganic compounds, H_2S and H_2 (Table 2). It was hypothesized that glucose utilization in batch cultures occurred first by fermentation, with subsequent utilization of fermentation products by CRMs. Other electron acceptors were found to affect chlorate reduction. Oxygen inhibited chlorate reduction, but chlorate was converted completely to chloride in the presence of sulfate, Fe(III), and Mn(IV). Under denitrifying conditions, gas formation but not chloride production was observed, implying that nitrate inhibited chlorate respiration (van Ginkel et al., 1995).

Chlorate- and Perchlorate-Respiring Isolates

Differing reports on cell size, morphology, and chemicals that serve as reductants make it apparent that the ability to reduce (per)chlorate is not limited to a single bacterial species. It is suspected, although not proven, that isolates capable of perchlorate respiration are ca-

Table 2. Compounds tested as growth substrates for bacterial isolates and mixed cultures during growth using chlorate and/or perchlorate.

Culture	Electron Acceptor	Growth substrates tested		Reference
		Positive	Negative	
V. dechloraticans	ClO_4^-	Acetate, ethanol, (glucose)[a]	Lactose, starch; salts of oxalic and citric acids.	Korenkov et al. (1976)
A. thermotoleranticus	ClO_3^-	Xylose, ethanol, n-alkanes (C_9–C_{18})	Benzoic or pyruvic acids, salicin, inositol, fructose, sucrose, lactose, raffinose, sorbose	Stepanyuk et al. (1993)
I. dechloratans	ClO_3^-	Acetate, alanine, asparagine, butyrate, fructose, glucose, lactate, propionate, pyruvate, succinate	Aminobenzoate, phenol, phenylalanine	Malmqvist et al. (1994)
GR-1	ClO_3^-	Acetate, propionate, caprionate, malate, succinate, lactate	Glucose, arabinose, mannose, mannitor, N-acetylglucosamine, maltose, gluconate, adipate, phenyl acetate	Rikken et al. (1996)
W. succinogenes HAP-1	ClO_4^-	H_2 and aspartate, fumarate, malate; mixture of H_2 and pyruvate, succinate, acetate, whey powder, peptone, yeast extract, brewers' yeast, casamino acids, cottonseed protein	Glucose, fructose, galactose, lactose, sucrose, butyrate, citrate, formate, propionate, benzoate, ethanol, methanol, 1-propanol, starch.	Wallace et al. (1996)
AB-1	ClO_3^-	Acetate	Phenol, benzene, toluene, xylenes	Bliven (1996)
Pseudomonas sp. (various)	BrO_3^-	Ethanol		Hijnen et al. (1995)
Mixed	ClO_4^-	Acetate, butyrate, citrate, lactate, propionate, pyruvate, succinate, glucose, fructose, lactose, sucrose, ethanol, methanol, nutrient broth, peptone, yeast extract, casamino acids		Attaway and Smith (1993)
Mixed	ClO_3^-	Acetate, glucose-glutamic acid, phenol		Logan et al. (1998)
Mixed	ClO_3^-	Acetate, propionate, buyrate, palmitate, lactate, citrate, fumarate, malate, benzoate, formate, ethanol, 1-propanol, 2-propanol, 1-butanol, ethanol, acetone, glycine, valine, proline, alanine, leucine, aspartate, H_2S, H_2		van Ginkel et al. (1995)

[a] Poor growth, and only in the presence of a small amount of acetate (Korenkov et al., 1976).

pable of also reducing several other halo-oxygenated compounds such as chlorate and bromate. Microbes known to respire both chlorate and perchlorate include *Vibrio dechloraticans* Cuznesove B-1168 (Korenkov et al., 1976); *Acinetobacter thermotoleranticus* (Stepanyuk et al., 1993); *Ideonella dechloratans* (Malmqvist et al., 1994); GR-1, a strain identified to belong to the β subgroup of Proteobacteria (Rikken et al., 1996); and *Wolinella succinogenes* HAP-1, originally classified as an obligate anaerobe (Wallace et al., 1996) but later reported to be microaerophilic (Hurley, 1997; Wallace et al., 1998). Strains of *Pseudomonas fluorescens* have been found to reduce bromate (Hijnen et al., 1995) (Table 1). In all cases tested, perchlorate reducers also reduced chlorate. All of these CRMs except HAP-1 are facultative anaerobes and are thought to be related to denitrifying organisms; it has been reported, however, that chlorate-respiring cultures may lose the ability to reduce nitrate when cultivated on chlorate for long periods (Hackenthal et al., 1964; Malmqvist et al., 1994).

The first suggestion that CRMs might be spore formers was provided by Bryan and Rohlich (1954). They found that pasteurization of sewage at 85°C for 10 min did not remove the ability of a chlorate-acclimated culture to reduce chlorate, and they hypothesized that the microorganisms were spore formers. However, isolates reported to date have not indicated any species that are spore formers.

Vibrio dechloraticans Cuznesove B-1168 was identified as a non-spore-forming, slightly bent 0.8 to 1 × 0.4 to 0.5 μm motile (single flagellum), Gram-negative bacterium (Korenkov et al., 1976). It was found to be well colored with fuchsione but noncolored with methylene blue. *V. dechloraticans* was isolated though liquid transfers and found to grow on liquid media containing acetate and ethanol; it did not grow on meat-peptone agar or agarized medium. It grew poorly on glucose, and only in the presence of a small amount of an acetate (Table 1).

Malmqvist and Welander (1992) obtained four chlorate-reducing isolates using acetate/chlorate agar plates. All isolates were Gram-negative, catalase- and oxidase-positive, motile rods. None of the isolates grew on glucose, and all isolates could use oxygen and nitrate as electron acceptors.

Malmqvist et al. (1994) isolated a new chlorate-respiring species, *Ideonella dechloratans,* from laboratory wastewater treatment reactors inoculated with municipal wastewater treatment plant sludge. One of the laboratory reactors was a suspended-growth chemostat, and the other two were biofilm reactors with support structures made of reticulated polyurethane. *I. dechloratans* was found to be Gram-negative, motile by two or several polar or subpolar flagella, rod-shaped (straight or slightly curved, sometimes growing in filaments), and 0.7 to 1.0 μm by 2.5 to 5 μm in size, but it did not produce prosthecae. It was cytochrome C-oxidase positive, catalase weakly positive, chemoorganotrophic, and capable of growth using oxygen or nitrate. *I. dechloratans* grew on acetate and several other sole carbon sources, but did not grow on aminobenzoate, phenol, or phenylalanine (Table 2). Identification with Biolog microplates was not reproducible, but based on 16S rDNA sequences, *I. dechloratans* was found to be most similar to *Comamonas testosteroni* (90% sequence similarity), *Alcaligenes eutrophus* (90%), and *Burkholderia cepacia* (89%); 17 other species of the β subgroup of the Proteobacteria were indicated to fit within an 81 to 89% sequence similarity. The DNA base composition of *I. dechloratans* was 68 mol% G+C. Growth occurred between 12 and 42°C, with no growth at 10 or 46°C.

Stepanyuk et al. (1993) isolated 20 phenotypically close strains of bacteria from wastewaters from a match factory. Most, but not all, of the isolates respired using chlorate. Based on the ability of these isolates to grow at elevated temperatures (4 to 47°C, with an optimum of 36 to 37°C), they suggested a new name for these bacteria, *Acinetobacter thermotoleranticus.* This species consists primarily of coccoid cells 0.7 to 1.2 μm in diameter, but cells could grow as rods or filaments up to 60 μm in length. Flagella were absent, fimbrae were found only in certain strains, and the cells were Gram-negative although staining appears slightly positive due to the presence of a microcapsule containing polysaccharides. These were oxidase-negative and catalase-positive facultative anaerobes. They did not reduce nitrates, but at least one chlorate-reducing isolate (S-1) was able to reduce sulfate. Cells could assimilate xylose, ethanol, and n-alkanes (C_9–C_{18}), but not lactose and other compounds (Table 2). The optimum pH was 6.8 to 7.2 (range 6.0 to 7.5), and strains required sodium chloride for growth (optimum concentration of 3%). Their generation time was measured as 3 to 4 h, and the G+C content in DNA was 43 to 46 mol%.

A chlorate-respiring isolate, AB-1, was obtained by Bliven (1996) from settled primary effluent on agar plates amended with acetate and chlorate incubated under anaerobic conditions in GasPack jars. Strain AB-1 cells were slightly curved, 0.5 × 1.5 μm in size, and possess a single polar flagellum. AB-1 grew aerobically on acetate or anaerobically on acetate and chlo-

rate, but not anaerobically on phenol, benzene, toluene, or xylenes. Classification of AB-1 using Biolog microplates (aerobic growth) indicated closest similarity to *Comamonas testosteroni.*

A perchlorate-respiring isolate, strain GR-1, isolated by Rikken et al. (1996) was identified as a gram-negative, oxidase-positive, motile rod. GR-1 was isolated from activated sludge by enrichment in liquid media in BOD bottles, plating onto agar containing acetate and sodium perchlorate, and incubation under anaerobic conditions. Examination using a 16S rDNA analysis indicated that the strain belongs to the β subdivision of Proteobacteria. GR-1 grew on acetate and lactate, but was unable to grow on glucose and several other compounds with perchlorate as an electron acceptor (Table 2). It did not have β-glucosidase, β-galactosidase, or protease. GR-1 grew aerobically and anoxically on nitrate, chlorate, Mn(IV), and perchlorate, but the presence of nitrate during growth on perchlorate increased the measured doubling times from 3 to 9 h. GR-1 was unable to grow using sulfate, iodate, bromate, chlorite, selenate, or Fe(III) as electron acceptors.

Although bromate was not reduced by GR-1, Hijnen et al. (1995) demonstrated that bromate could be completely reduced to bromide by mixed suspended bacterial populations. Bromate reduction occurred either with or without a preceding denitrification step under anaerobic conditions when cultures were fed ethanol as an energy and carbon source. Only bromate reduction, and not cell growth supported by dissimilatory bromate reduction, was demonstrated. Bromate reduction was completely inhibited by nitrate, and even in the absence of nitrate, proceeded at a rate that was 100 times lower than the denitrification rate. Enrichment cultures, prepared using the effluent of a denitrification reactor, were incubated in 500-mL Erlenmeyer flasks either flushed with nitrogen or placed in a vacuum desiccator. Of the 25 isolates obtained from colonies grown on R2A agar (a medium developed for enumeration of oligotrophic bacteria from potable water), 6 isolates were identified as denitrifying *Pseudomonas* spp., and 6 were denitrifying fluorescent pseudomonads.

An anaerobic Gram-negative, non-spore-forming rod (0.5 wide by 2 to 8 μm long), designated as *Wolinella succinogenes* HAP-1, was isolated by Hubert Attaway (Wallace et al., 1996) from an anaerobic sewage enrichment culture using agar plates. The strain was capable of perchlorate respiration at concentrations of 7000 mg/L of perchlorate with an optimum growth temperature of 40°C (range 20 to 45°C). Based on physiological data and sequencing, the organism was placed in the δ-ε subdivision of the Proteobacteria. Strain HAP-1 was separated from *W. succinogenes* (ATCC 29543) by a combined branch length of only two changes; a sequence divergence of 0.75% from *W. succinogenes* was found compared to others in the single most parsimonious tree of >15%. Colonies of HAP-1 were circular, pale yellow, and mucoid. HAP-1 grew in suspension on H_2 and a variety of different substrates, including aspartate, peptone, yeast extract, and brewers' yeast; it did not grow on glucose, lactose, or methanol (Table 2). HAP-1 used chlorate and perchlorate, reduced nitrate to nitrite, but did not use nitrite, sulfate, sulfite, thiosulfate, iron oxide, manganese dioxide, or magnesium oxide as electron acceptors. Earlier work reported by Attaway and Smith (1993) was conducted using suspended-growth reactors that presumably were highly enriched with HAP-1. Aeration was found to inhibit perchlorate reduction and to completely inactivate mixed cultures after a 12-h exposure (Attaway and Smith, 1993). The mixed cultures reduced chlorate, chlorite, nitrate, nitrite, and sulfate. Neither nitrate nor sulfate affected perchlorate reduction; however, chlorate (10 mM) reduced the rate of perchlorate reduction, and nitrite and chlorite (10 mM) completely inhibited perchlorate reduction.

These (per)chlorate-respiring strains appear to have many common characteristics. All strains tested reduced both chlorate and perchlorate. Except for *W. succinogenes* (HAP-1) and *A. thermotoleranticus*, (per)chlorate reduction was partially or completely inhibited by high concentrations of either nitrate and oxygen. Only *A. thermotoleranticus* was reported to use sulfate as a terminal electron acceptor. Chlorate reductase has been isolated from microorganisms that also possess nitrate reductase (DeGroot and Stouthamer, 1969), implying that some chlorate-respiring strains may share many of the attributes of denitrifiers. Although most (per)chlorate strains may be denitrifying facultative anaerobes, not all denitrifiers are chlorate reducers (Hackenthal et al., 1964). Nitrate has an interesting variable effect on (per)chlorate reduction.

The only exception to the inhibitory effect of nitrate on perchlorate reduction was reported in conjunction with mixed cultures, presumably containing *W. succinogenes* HAP-1, which reduced perchlorate in the presence of nitrate (Attaway and Smith, 1993). Although the facultative anaerobe (GR-1) isolated by Rikken et al. (1996) also reduced perchlorate in the presence of nitrate, the decrease in cell-doubling times in the presence of nitrate implies an inhibitory effect of nitrate on perchlorate respiration.

Little is known about the biochemical pathways involved in bacterial utilization of chlorate or perchlo-

rate as an electron acceptor. Until very recently, chlorate reductases had been isolated only from denitrifying strains known to reduce, but not necessarily shown to respire, chlorate. Electron transport to oxygen by *Proteus mirabilis* represses formation of nitrate reductase A (NR-A), but in the absence of oxygen and the presence of nitrate, NR-A was de-repressed (Oltmann et al., 1976). Although the presence of nitrate has been found to repress the expression of chlorate reductase-C (CR-C) in *P. mirabilis*, CR-C is otherwise constitutive even in the presence of oxygen, although it is present at lower per cell activities (DeGroot and Stouthamer, 1969). If these chlorate reductases were used for chlorate respiration, both nitrate and oxygen would have to be absent for cells to respire chlorate. This observation is true only for some chlorate-respiring strains. In batch cultures, the presence of oxygen may not be detrimental to cell growth for all species examined (except HAP-1), because these chlorate-reducing isolates have been shown to be facultative anaerobes; dissolved oxygen would be removed by cell growth prior to chlorate respiration.

The conversion of chlorine in perchlorate to chloride requires the overall transfer of eight electrons. Chlorite dismutase, a novel enzyme isolated from strain GR-1 (van Ginkel et al., 1996), was capable of converting chlorite (ClO_2^-) to oxygen and chloride. Recently, van Ginkel et al. (1998) reported that they isolated a (per)chlorate reductase capable of reducing both perchlorate and chlorate. This establishes for strain GR-1 a sequence of intermediates involved in perchlorate reduction of $ClO_4^- \rightarrow ClO_3^- \rightarrow ClO_2^- \rightarrow O_2 + Cl^-$. Oxygen did not accumulate in cell suspension solutions fed perchlorate or chlorate and therefore oxygen is used as an electron acceptor, but oxygen can accumulate in cultures amended with elevated concentrations of chlorite (van Ginkel et al., 1996). It is not yet known if chlorite dismutase and the (per)chlorate reductases found in strain GR-1 are common to other strains of chlorate- and perchlorate-respiring microorganisms. GR-1 is the only facultative anaerobe that can continue to reduce chlorate in the presence of oxygen, suggesting that other strains may not necessarily contain chlorite dismutase.

Engineered Reactors

Reactors have two important purposes: wastewater treatment and drinking water treatment. To date, microbiological degradation of chlorate and perchlorate to low levels has been achieved only in engineered bioreactors suitable for wastewater treatment. The distinction between wastewater and water reactors is that for drinking water treatment, all toxic chemicals and most organic matter must be removed. For wastewater treatment, microbes and organic matter may remain in the water (subject to regulations) if it is to be discharged to a receiving water body or a publicly owned treatment works (POTW). Examples of these reactors, including both small and large continuous reactors, are wastewater treatment lagoons to remove chlorate produced during bleaching operations (Malmqvist et al., 1991); suspended-growth and fixed-film reactors to remove perchlorate from rocket propellent wash waters (Attaway and Smith, 1993; Wallace et al., 1996; Wallace et al., 1998); and sand columns to treat chlorate- and perchlorate-contaminated waters (Logan and Kim, 1998). The development of biological reactors for water treatment requires new design strategies. No large-scale biological drinking water treatment systems currently exist in the United States. Thus, there is no established design basis for developing (per)chlorate drinking water treatment systems.

To specify the design characteristics of a new biological treatment process in terms of reactor detention time or loading rate, kinetic constants and growth yields for mixed or pure cultures would need to be known, or pilot- or full-scale operation data would need to be available. Important kinetic constants include μ_m, the maximum growth rate; K_s, the half-saturation constant; Y, the yield of cell biomass produced per substrate consumed; and b, the endogenous decay coefficient (Logan, 1998a). An oxidizable substrate must to be added to a bioreactor to microbiologically reduce (per)chlorate, but any remaining substrate must be removed to limit subsequent biological growth in the water distribution system. Due to cell maintenance and energy requirements, there is a minimum achievable substrate concentration that is a function of the reactor configuration and microbial kinetic constants. For example, the minimum substrate concentration achievable in a completely mixed, constant-flow, suspended-growth bioreactor is $S_{min} = b\ K_s/(\mu_m - b)$ (Rittmann and McCarty, 1980). The values of these constants must be known to calculate this minimum substrate concentration.

Logan and co-workers (Bliven, 1996; Logan et al., 1998; Olsen, 1997; Patnaik, 1996) determined growth rate constants for chlorate-respiring mixed cultures by conducting laboratory-scale chemostat experiments using nitrogen-air purged reactors fed acetate, glucose-glutamic acid, or phenol as substrates. The small-capacity reactors (0.5 to 1 L working volume) were run at room temperature with no recycle. Chlorate was added in excess in order to measure kinetic parameters for growth on the oxidizable sub-

strates. Maximum growth rates using acetate, glucose-glutamic acid, and phenol were 0.56, 0.12, and 0.040 h^{-1}, with cell yields of 0.12, 0.41, and 0.12 g-cell/g-substrate, respectively (Logan et al., 1998). These results indicate that growth rates of CRMs are quite high, that CRMs might be able to outcompete other anaerobic microorganisms for oxidizable substrates in water treatment bioreactors. However, some minimum concentration of substrate will remain in solution. Assuming an endogenous decay coefficient of b = 0.005 d^{-1}, acetate would be reduced to only S_{min} = 0.26 mg/L based on the kinetic constants given above for acetate.

A few other reports have been published on reactors suitable for treating (per)chlorate, but much of the information is incomplete with respect to engineering design information such as microbial growth constants, cell yields, and reactor configuration details. For example, Malmqvist and Welander (1992) reported that fixed-film reactors could be used to eliminate chlorate from paper mill wastewaters, but they did not specify the type of media packing and other reactor configuration details. In laboratory tests, chlorate (50 mg/L) in a kraft mill wastewater (COD = 1600 mg/L) was 100% removed (no detection limit noted) at detention times larger than 0.6 h under conditions of 37°C and at a wastewater pH of 7. In pilot tests in a 20-m^3 reactor operating at a pH range of 6.2 to 7, a temperature range of 36° to 38°C, and influent chlorate concentrations of 40 to 75 mg/L, removals were complete at a detention time of 12.8 h, with removals of >90% at detention times of 1.6 h.

Malmqvist et al. (1991) conducted laboratory-scale chemostat experiments at 37°C at pH = 7, using a 0.36-L working volume in a 0.4-L reactor with no recycle. The reactor (but not the feed) was sparged with nitrogen to maintain anoxic conditions, and only one detention time of 25 h was examined. Chlorate removal at concentrations of 83 to 1250 mg/L (1 to 15 mM) was complete with stoichiometric conversion to chloride. Cell yields were high, ranging from 1.9 to 3.8 g of volatile suspended solids (VSS) per g-equivalent electrons.

Researchers at the Tyndall Air Force Base have developed a suspended-growth reactor to remove perchlorate from high concentrations (3000 mg/L) down to relatively lower concentrations (<0.5 mg/L). Some information on their system was reported by Attaway and Smith (1993), and Wallace et al. (1996). Further information is available though Internet postings (www.brooks.af.mil, and www.afcesa.af.mil). Their skid-mounted system consisted of two reactors, a 1300-L anaerobic reactor and a 2700-L aerobic reactor, complete with automatic pH control, clarifier, and feed- and product-holding tanks. The anaerobic reactor used primarily cultures of *W. succinogenes* HAP-1 with brewers' yeast as a nutrient source. In a pilot test that began in May 1995 and ran for >600 h, they examined the effect of reactor detention times of 8 to 36 h on perchlorate reduction; perchlorate was reduced to <0.5 mg/L even at the lowest detention times. Although this system successfully treated perchlorate-contaminated wastewater, it is unlikely that this system could be used to treat drinking water to acceptable levels (<18 μg/L) due to the high concentrations of organic matter used in the system.

An upflow fixed-bed reactor inoculated with a microbial consortium containing primarily HAP-1 (28 to 47% of the population) was tested for treatment of a perchlorate wastewater from a Minuteman III rocket motor washout (Wallace et al., 1998). The reactor was packed with diatomaceous earth pellets (mean pore diameter of 20 μm), fed a solution containing perchlorate (1.5 g/L or 0.5 g/L) and brewers' yeast extract (BYF-100; 3 g/L or 1.5 g/L to maintain a 2:1 ratio), and operated at detention times of 1.17 and 0.46 h. Reduction rates of perchlorate to chloride were maintained at 1 g/L-h in the fixed-bed reactor versus 0.5 to 0.7 g/h with the same bacterial consortium in a suspended-growth reactor. Effluent perchlorate concentrations from the fixed-bed reactor were below 0.3 g/L during a test over a 228-d period, and below 0.1 g/L 95% of the time.

AWWARF (1997) reports that Aerojet has developed an anoxic fluidized-bed methanol-fed reactor capable of treating perchlorate down to 100 μg/L, but details of this process are not available in the published literature, likely because of the proprietary nature of this process. A recent presentation by Catts (1998) indicated that the fluidized-bed reactor uses granular activated carbon (GAC) as a support medium for a consortium of microbes obtained from a food-processing wastewater. In tests using 14-ft (4.3-m) reactors, perchlorate concentrations of 20 to 60 μg/L were reduced to <10 μg/L using a methanol-ethanol feed solution that was below the detection level (5 mg/L) in the reactor effluent. Although it is not yet known whether this process would be suitable for treatment of water to drinking water standards, fluidized-bed reactors generally are expensive compared to fixed-bed reactors.

Fixed-bed systems hold great promise for water treatment. Recently, perchlorate has been removed to below detectable levels in fixed-bed sand filters inoculated with perchlorate-degrading enriched cultures. Logan and Kim (1998) developed a perchlorate-

degrading biofilm in 14.2-cm-long sand columns by injecting enriched cultures into the column. After incubating them overnight (30°C), they switched the column to continuous flow and fed an artificial groundwater amended with acetate, trace minerals, and nutrients (ammonia and phosphorus), and 20 mg/L of perchlorate. They did not remove oxygen from the feed water, but instead allowed it to be removed within the column by the microbial consortium, resulting in anoxic conditions. Perchlorate concentrations in the column effluent were below detectable levels (<4 µg/L) at loading rates of <4.48 L/min · m^2 (<0.11 gpm/ft^2). Higher loading rates, such as >4.89 L/min · m^2 (>0.12 gpm/ft^2), resulted in perchlorate breakthough producing high effluent concentrations (>150 µg/L) that rapidly increased with hydraulic loading.

A critical factor in the design of reactors for water treatment will be the choice of oxidizable substrate added to the reactor. Acetate has been used extensively in laboratory work, but acetate may not be cost-effective for larger systems. Likely alternative candidates as oxidizable substrates include ethanol, methanol, and hydrogen gas. Of these, hydrogen is particularly appealing and is the subject of a recent patent application (Logan, 1998b). Hydrogen gas is sparingly soluble, and therefore would not be likely to persist in water at appreciable concentrations. Chlorate- and perchlorate-respiring microorganisms are known to oxidize hydrogen (van Ginkel et al., 1995; Wallace et al., 1996). Recent tests using hydrogen gas have demonstrated that chlorate can be removed continuously in gas-lift reactors containing pumice particles inoculated with activated sludge (van Ginkel et al., 1998). Chlorate removal rates of >95% were measured in gas-lift bioreactor tests at detention times of up to 6 h. Columns packed with biofilm-coated pumice particles from the gas-lift bioreactor were fed water saturated with hydrogen gas. At hydraulic detention times of 0.2, 8, and 120 min, there was nearly complete removal of chlorite, chlorate, and perchlorate, respectively (van Ginkel et al., 1998). At this time, however, there are no data on the sustainability of these removal rates or the specific details of the packed-bed reactor.

Many groundwaters contaminated with perchlorate also contain volatile organic compounds such as trichloroethylene (TCE). A side benefit of using hydrogen gas to biologically treat water may also be that hydrogen has been shown to support biological dehalogenation of several chlorinated aliphatic compounds including tetrachloroethylene, TCE, and vinyl chloride (Ballapragada et al., 1997). Methanol and ethanol may prove useful for water treatment, but the cost-effectiveness of these and other substrates has not been determined. To evaluate a substrate, bench and pilot studies will be necessary to determine optimum feedrates, reactor detention times, and other parameters.

It is not yet known if water treatment systems will require the inclusion of other unit processes. For example, water polishing by conventional filtration or further treatment in additional biological reactors may be necessary to remove any residual substrate in the water. For systems treating low concentrations of perchlorate in water (<100 µg/L), it may be necessary to include a system to regenerate the perchlorate-degrading capacity of the biofilm by taking the system off line and feeding the reactor high concentrations of chlorate and oxidizable substrate (Logan, 1998b). The fact that (per)chlorate respiration proceeds in the absence of substrate in the source water (i.e., the microbes can use stored substrates) may make it possible to develop reactors based solely on endogenous decay. Laboratory- and pilot-scale testing will be necessary to determine which of these approaches ultimately will prove to be the most chemically stable and economical.

Conclusions

A number of bacterial isolates have been shown to be capable of dissimilatory reduction of chlorate, perchlorate, and other halo-oxygenated compounds. These microbes appear to be sufficiently abundant in domestic wastewater to be cultured using standard dilution and streak plate methods. One significant obstacle with the microbiological removal of perchlorate from drinking water is that biological treatment of potable water has never been used in the United States to remove a single organic compound. Essentially all drinking water treatment processes currently are designed as physical-chemical treatment systems. Although biological treatment of chlorate- and perchlorate-contaminated waters is proven for wastewater treatment, it has not been proven (beyond the scale of laboratory experiments) for drinking water treatment, and detailed design information for both wastewater and water treatment reactors is not yet available. Thus, much work still remains to be done on the optimization of bioreactors for wastewater treatment and the design of (per)chlorate bioreactors for drinking water treatment.

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