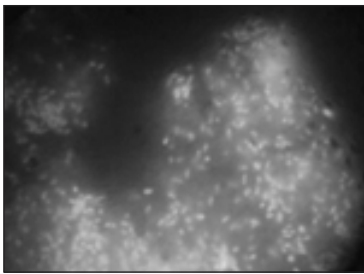


BY ROBERT NERENBERG,  
BRUCE E. RITTMANN,  
AND ISSAM NAJM

The perchlorate anion ( $\text{ClO}_4^-$ ) has been found in potentially harmful concentrations in numerous water sources. Because perchlorate is not removed by conventional water treatment processes, new treatment processes are needed. Biological perchlorate reduction is a promising alternative. The authors investigated a hydrogen-oxidizing hollow-fiber membrane–biofilm reactor system for perchlorate removal. Hydrogen is an ideal electron donor for biological drinking water treatment because it presents no toxicity, is inexpensive, and is unlikely to persist as a source of biological instability in distributions systems. The reactor delivers hydrogen in an efficient and safe manner. Results showed that biological perchlorate reduction takes place concurrently with nitrate reduction, no specialized inoculation is required, and perchlorate can be removed to below the preliminary regulatory standards with no chemical addition other than hydrogen gas. The optimal pH is 8, and the accumulation of intermediates is unlikely. Full denitrification and pH control may be required for excellent perchlorate removal.

# Perchlorate reduction

## in a HYDROGEN-BASED MEMBRANE–BIOFILM REACTOR



Following the development of a highly sensitive analytical method in 1997, perchlorate was detected in numerous water supplies in at least 14 states (USEPA, 2001; Gullick et al, 2001; Wang, 1999; USEPA, 1998). Perchlorate salts are widely used in the chemical industry (*Kirk–Othmer Encyclopedia*, 1991) and are naturally present in Chilean saltpeter, a mineral used in some chemical fertilizers (Urbansky et al, 2001; Ericksen, 1983). However, the most significant use of perchlorate and the most likely source of environmental contamination is as ammonium perchlorate used in rocket solid fuels (USEPA, 2001; USEPA, 1998; *Kirk–Othmer Encyclopedia*, 1991; Schilt, 1979). Since the 1950s, large amounts of perchlorate-containing wastes have been released to the environment from facilities where rockets were manufactured, tested, or stored (Urbansky, 1998; Wallace et al, 1998), and many perchlorate plumes in groundwater can be traced to such facilities (USEPA, 1998).

Perchlorate is an inhibitor of thyroid function (Clark, 2000; Wolff, 1998; Stanbury & Wyngaarden, 1952), and its carcinogenic, neurodevelopmental, developmental, reproductive, and immunotoxic effects are of concern (USEPA, 1998). There is no national standard for perchlorate in drinking water at this time, but perchlorate is currently on the US Environmental Protection Agency (USEPA) Contaminant Candidate List (Perciasepe, 1998). In 1997, the California Department of Health Services (CDHS) established a drinking water action level of 18  $\mu\text{g}/\text{L}$  (CDHS, 2001). This was based on a range of 4 to 18  $\mu\text{g}/\text{L}$  derived from a provisional USEPA reference dose (USEPA, 1995; USEPA, 1992). In 1998, USEPA issued a report suggesting a drinking water goal of 32  $\mu\text{g}/\text{L}$  (USEPA, 1998), and in January 2002, after completing fur-

**TABLE 1** Stoichiometry and yield for various electron donors\*

Electron Donor Substrate	Stoichiometric Reaction†	Y (g cells/g NO <sub>3</sub> <sup>-</sup> -N)‡
Ethanol§	0.69 C <sub>2</sub> H <sub>5</sub> OH + NO <sub>3</sub> <sup>-</sup> + H <sup>+</sup> → 0.14 C <sub>5</sub> H <sub>7</sub> O <sub>2</sub> N + 0.43 N <sub>2</sub> + 0.67 CO <sub>2</sub> + 2.07 H <sub>2</sub> O	0.689
Methanol§	1.08 CH <sub>3</sub> OH + NO <sub>3</sub> <sup>-</sup> + H <sup>+</sup> → 0.065 C <sub>5</sub> H <sub>7</sub> O <sub>2</sub> N + 0.47 N <sub>2</sub> + 0.76 CO <sub>2</sub> + 2.44 H <sub>2</sub> O	0.406
Acetate§	0.986 CH <sub>3</sub> COO <sup>-</sup> + NO <sub>3</sub> <sup>-</sup> + H <sup>+</sup> → 0.125 C <sub>5</sub> H <sub>7</sub> O <sub>2</sub> N + 0.44 N <sub>2</sub> + 0.36 CO <sub>2</sub> + 1.047 H <sub>2</sub> O + 0.99 HCO <sub>3</sub> <sup>-</sup>	0.643
Hydrogen**	3.03 H <sub>2</sub> + NO <sub>3</sub> <sup>-</sup> + H <sup>+</sup> + 0.229 CO <sub>2</sub> → 0.0458 C <sub>5</sub> H <sub>7</sub> O <sub>2</sub> N + 0.477 N <sub>2</sub> + 3.37 H <sub>2</sub> O	0.306
Sulfur**	0.98 S + NO <sub>3</sub> <sup>-</sup> + 0.609 H <sub>2</sub> O + 0.188 CO <sub>2</sub> → 0.0375 C <sub>5</sub> H <sub>7</sub> O <sub>2</sub> N + 0.48 N <sub>2</sub> + 0.98 SO <sub>4</sub> <sup>2-</sup> + 0.955 H <sup>+</sup>	0.194

\*Lee (1999)

†C<sub>2</sub>H<sub>5</sub>OH—ethanol, NO<sub>3</sub><sup>-</sup>—nitrate, H<sup>+</sup>—hydrogen ion, C<sub>5</sub>H<sub>7</sub>O<sub>2</sub>N—glutarimide cells, N<sub>2</sub>—nitrogen ion, CO<sub>2</sub>—carbon dioxide, CH<sub>3</sub>OH—methanol, CH<sub>3</sub>COO<sup>-</sup>—acetate, HCO<sub>3</sub><sup>-</sup>—hydrogen carbonate ion, SO<sub>4</sub><sup>2-</sup>—sulfate ion

‡Y—yield

§Heterotrophic donor

\*\*Autotrophic donor

**TABLE 2** Thermodynamics for perchlorate and selected environmental acceptors

Acceptor (Process)	Reaction With Hydrogen	Electrons Transferred	ΔG <sub>o</sub> '*—kJ/e <sup>-</sup>
Perchlorate (perchlorate reduction)	ClO <sub>4</sub> <sup>-</sup> + 4H <sub>2</sub> → Cl <sup>-</sup> + 4H <sub>2</sub> O	8	-112.1†
Oxygen (aerobic respiration)	O <sub>2</sub> + 4H <sub>2</sub> → 2H <sub>2</sub> O	8	-118.6
Nitrate (denitrification)	2NO <sub>3</sub> <sup>-</sup> + 2H <sup>+</sup> + 5H <sub>2</sub> → N <sub>2</sub> + 6H <sub>2</sub> O	10	-112.2
Sulfate (sulfate reduction)	2SO <sub>4</sub> <sup>2-</sup> + 9H <sub>2</sub> + H <sup>+</sup> → H <sub>2</sub> S + HS <sup>-</sup> + 8H <sub>2</sub> O	16	-19.2

\*ΔG<sub>o</sub>'—Gibb's free energy for standard conditions and pH = 2

†Excludes the free energy for the dismutation of chlorite ion (ClO<sub>2</sub><sup>-</sup> into Cl<sup>-</sup> + O<sub>2</sub>) but includes the energy for the reduction of O<sub>2</sub> to H<sub>2</sub>O

ther studies, USEPA issued a new report suggesting a drinking water goal of 1 µg/L (USEPA, 2002). On the basis of the most recent USEPA study, CDHS lowered its action level to 4 µg/L (CDHS, 2002).

Perchlorate is highly soluble and extremely stable in aqueous solution (Urbansky, 1998; Wallace et al, 1998; Schilt, 1979), and it is not removed by conventional drinking water treatment processes (e.g., flocculation, coagulation, sedimentation, and filtration). Although advanced treatment processes such as reverse osmosis (RO), ion exchange, membrane filtration, and electrodialysis can remove perchlorate, these methods can be costly and generate perchlorate-containing wastes (Najm et al, 1999; Liang et al, 1998; Urbansky, 1998).

Biological perchlorate reduction may provide a cost-effective treatment solution for perchlorate-contaminated waters. Perchlorate can be biochemically reduced to innocuous chloride by perchlorate-reducing bacteria (PCRB), which are able to gain energy and grow through perchlorate reduction (Herman & Frankenberger, 1998; Logan, 1998). Additionally, perchlorate can be reduced by nitrate-reducing bacteria without providing energy for growth (Hackenthal, 1965).

Perchlorate reduction by PCRB is similar to biological denitrification, i.e., perchlorate serves as an electron acceptor for respiratory growth. In fact, many PCRB can reduce nitrate (Coates et al, 2000; Coates et al, 1999; Herman & Frankenberger, 1999; Rikken et al, 1996; Wal-

lace et al, 1996; Malmqvist et al, 1994; Korenkov et al, 1976). Given the links between perchlorate reduction and denitrification, a biological denitrification system may be effective for perchlorate treatment. In cases in which nitrate and perchlorate are present together, it may be possible to denitrify and reduce perchlorate concurrently. The following section provides background information on biological denitrification for drinking water, the new hydrogen-based reactor, and biological perchlorate reduction.

## BACKGROUND

**Drinking water denitrification.** There are two types of biological denitrification processes—heterotrophic denitrification and autotrophic denitrification.

**Heterotrophic denitrification.** Heterotrophic denitrification occurs when an organic compound is the electron donor that drives nitrate reduction. Typical heterotrophic donors added to stimulate denitrification include methanol, ethanol, and acetate (Gayle et al, 1989).

A drawback of heterotrophic denitrification in drinking water treatment is the potential for donor residuals in the effluent because of overdosing or fluctuations in the influent nitrate concentration. Because heterotrophic donors are readily biodegradable, donor residuals reaching the distribution system can promote serious microbiological growth. Methanol has special concerns because of its acute human toxicity (Keyvan-Larjarni & Tannenberg, 1974; Tephyl et al, 1974). As a result, heterotrophic

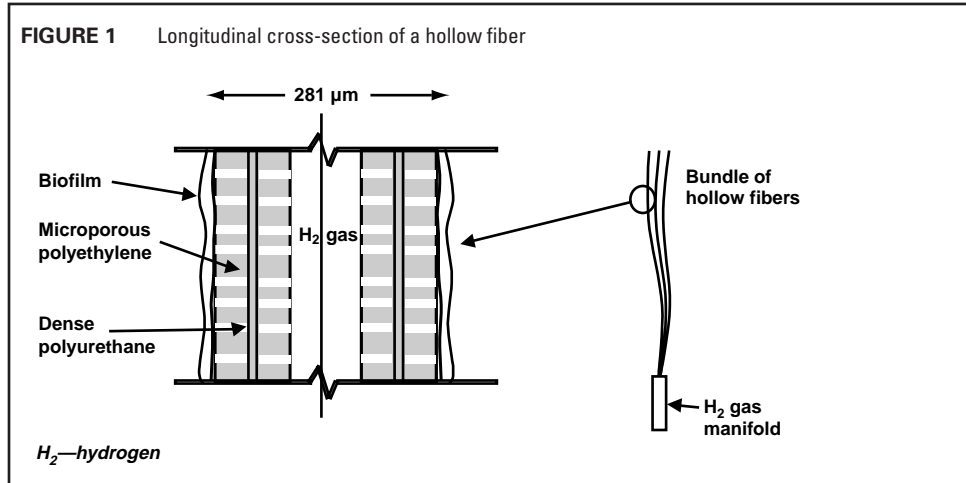
donors often require posttreatment in order to produce safe and biologically stable water (Liessens et al, 1993).

**Autotrophic denitrification.** In autotrophic denitrification, the electron donor is an inorganic compound. Typical donors include hydrogen and reduced sulfur compounds (Gayle et al, 1989). Researchers have compiled a significant body of information on autotrophic denitrification using hydrogen as the electron donor, a process called autohydrogenotrophic denitrification (Lee & Rittmann, 2002; Lee & Rittmann, 2000; Smith et al, 1994; Häring & Conrad, 1991; Dries et al, 1988; Gros et al, 1988; Kurt et al, 1987).

Autohydrogenotrophic denitrification offers two major advantages over heterotrophic denitrification. First, hydrogen evolves to the atmosphere once the water is exposed to an open surface, preventing microbial growth caused by excess donor. Second, hydrogen costs 3–15 times less than the common organic supplements to remove the same amount of nitrate (Lee, 1999). Also, autotrophic denitrification produces much lower yields than heterotrophic denitrification (Table 1), which reduces the potential for reactor fouling. Despite these advantages, hydrogen has not been widely used, because sparging has been required to supply hydrogen gas. Sparging wastes hydrogen gas because some must be vented. More important, the vented hydrogen can create an explosive atmosphere (Aragno & Schlegel, 1992).

**New hydrogen-based reactor.** In order to eliminate the drawbacks associated with hydrogen delivery, Lee and Rittmann (2002; 2000) developed a new reactor based on hollow-fiber membranes. The reactor delivers dissolved hydrogen directly to the biofilm by its diffusion through the substratum, avoiding the need to sparge the bulk liquid. The system, called a hollow-fiber membrane–biofilm reactor (HFMBfR), is distinct from most membrane bioreactors used for water denitrification. Other systems use either a membrane as a particle–filtration step following biological treatment (Barreiros et al, 1998; Urbain et al, 1996; Delanghe et al, 1994) or a membrane as a selective barrier separating contaminated water from the bacteria and substrates (Mansell & Schroeder, 1999; McClellan & Schroeder, 1995).

Figure 1 shows a schematic cross-section of a single fiber from the HFMBfR. The interior of the fiber is connected to a pressurized hydrogen supply at one end and sealed at the other end. Water circulates outside of the fiber, and hydrogen diffuses from the lumen of the fiber, through the wall, and toward the bulk liquid. A biofilm grows on the outside wall when an electron acceptor is present in the bulk liquid. The membrane pores remain dry because the membrane is made of hydrophobic polyeth-



ylene. A thin layer of dense polyurethane enclosed within the porous polyethylene fiber increases the bubbling pressure; thus, hydrogen molecules diffuse through the wall, making it a bubbleless transfer device.

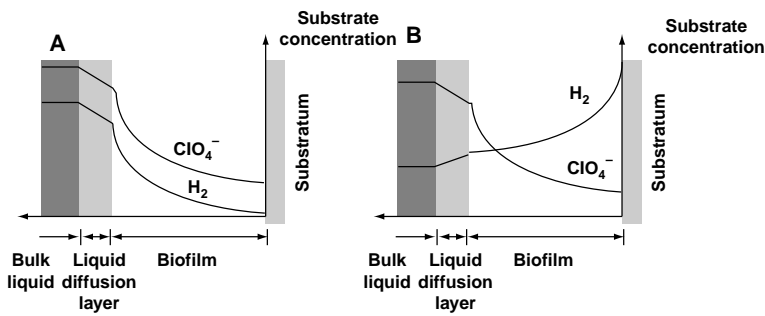
An important difference between the HFMBfR and conventional biofilm systems is that the electron donor and acceptor come into the biofilm from opposite sides, an effect called “counter-diffusion.” Figure 2 contrasts the counter-diffusion mode of hydrogen diffusion to the normal “co-diffusion.” With counter-diffusion, the hydrogen concentration is highest at the membrane–biofilm interface and lowest at the biofilm–water interface. The opposite is true for the electron acceptor, such as nitrate or perchlorate. This counter-diffusion allows the system to maintain high hydrogen concentrations within the biofilm while minimizing hydrogen release into the bulk liquid.

The HFMBfR is highly desirable for drinking water treatment because (1) it is clean, employing hydrogen as the only additive; (2) it is safe, because it does not create an explosive hydrogen atmosphere; (3) it is efficient, because it provides essentially 100% hydrogen utilization; and (4) it generates minimal regrowth potential because of low dissolved hydrogen in the treated water and hydrogen’s sparse solubility.

**Biological perchlorate reduction.** Perchlorate can be reduced biologically by two processes: dissimilatory reduction and co-metabolic reduction.

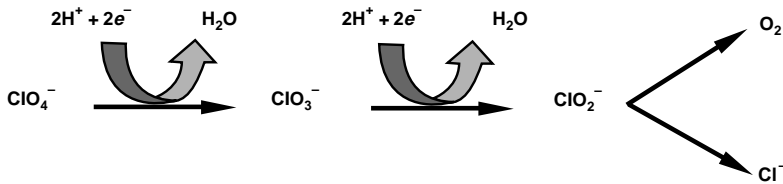
**Dissimilatory perchlorate reduction.** Dissimilatory perchlorate reduction is a respiratory process by which PCRB reduce perchlorate as a terminal electron acceptor and gain energy that supports growth. Perchlorate reduction proceeds in three steps (Rikken et al, 1996), shown in Figure 3. The first two steps are two-electron transfers: perchlorate to chlorate and chlorate to chlorite. The third step—the dismutation of chlorite into chloride and oxygen—does not consume electrons and therefore does not directly produce energy for the cells. However, produced oxygen may be used as an electron acceptor and yield energy (Rikken et al, 1996). Kinetic parameters for strain GR-1 for purified (per)chlorate

**FIGURE 2** Normal “co-diffusion” (A) and HFMBfR “counter-diffusion” (B)



HFMBfR—hollow-fiber membrane-biofilm reactor,  $\text{ClO}_4^-$ —perchlorate,  $\text{H}_2$ —hydrogen

**FIGURE 3** Perchlorate reduction pathway



$\text{O}_2$ —oxygen,  $\text{ClO}_4^-$ —perchlorate,  $\text{ClO}_3^-$ —chlorate,  $\text{ClO}_2^-$ —chlorite,  $\text{Cl}^-$ —chloride. The first two steps are catalyzed by (per)chlorate reductase in perchlorate-reducing bacteria (PCRB) and co-metabolically by nitrate reductase in other bacteria. The third step is catalyzed by chlorite dismutase in PCRB.

**TABLE 3** Characteristics of the hollow-fiber membrane biofilm reactor system

Item	Value
Pipe length—cm (in.)	120 (47)
Pipe internal diameter—cm (in.)	1.544 (0.608)
Pipe cross-sectional area— $\text{cm}^2$ (sq in.)	1.872 (0.29)
Fiber outside diameter— $\mu\text{m}$	280.0
Fiber cross-sectional area— $\text{cm}^2$ (sq in.)	0.00062 (0.0000961)
Number of fibers	98
Length of fibers—cm (in.)	92.7 (36.5)
Active length of fibers—cm (in.)	72.1 (28.4)
Area of single fiber— $\text{cm}^2$ (sq in.)	0.06 (0.0093)
Surface area of fibers— $\text{cm}^2$ (sq in.)	624 (96.7)
Feed flow rate—mL/min (gpd)	10 (3.8)
Detention time—min	44
Recycle flow—mL/min (gpd)	1,750 (666)
Recycle ratio	175

reductase (Kengen et al, 1999) and chlorite dismutase (van Ginkel et al, 1996) suggest that the perchlorate reduction is rate-limiting; thus, chlorate and chlorite should not accumulate.

Known PCRB include members of the  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ -, and  $\epsilon$ -subclasses of the *Proteobacteria* (Achenbach et al, 2001; Coates et al, 1999; Wallace et al, 1996). Many

known PCRB appear to be within two genera of the  $\beta$ -*Proteobacteria* named *Dechloromonas* and *Dechlorosoma* (Achenbach et al, 2001; Logan et al, 2001). In some cases, PCRB are phylogenetically similar to known species that do not reduce perchlorate (Wallace et al, 1996), whereas in others, isolates represent new genera and species. Morphologically, PCRB are mainly gram-negative, non-fermenting, motile rods.

PCRB can use a variety of organic and inorganic compounds as electron donors. Organic compounds include several carboxylic acids and alcohols (Logan, 1998); inorganic compounds include sulfur and iron (Bruce et al, 1999) and hydrogen gas (Giblin et al, 2000a; Miller & Logan, 2000; Wallace et al, 1996). Almost all PCRB are facultative anaerobes, which means they can use oxygen as an electron acceptor, if it is present.

For most PCRB, perchlorate reduction is inhibited by oxygen (Herman & Frankenberger, 1999; Rikken et al, 1996; Wallace et al, 1996) and slowed by nitrate (Herman & Frankenberger, 1999; van Ginkel et al, 1995). This suggests that perchlorate reduction is not possible under aerobic conditions and may not be efficient if nitrate concentrations are too high.

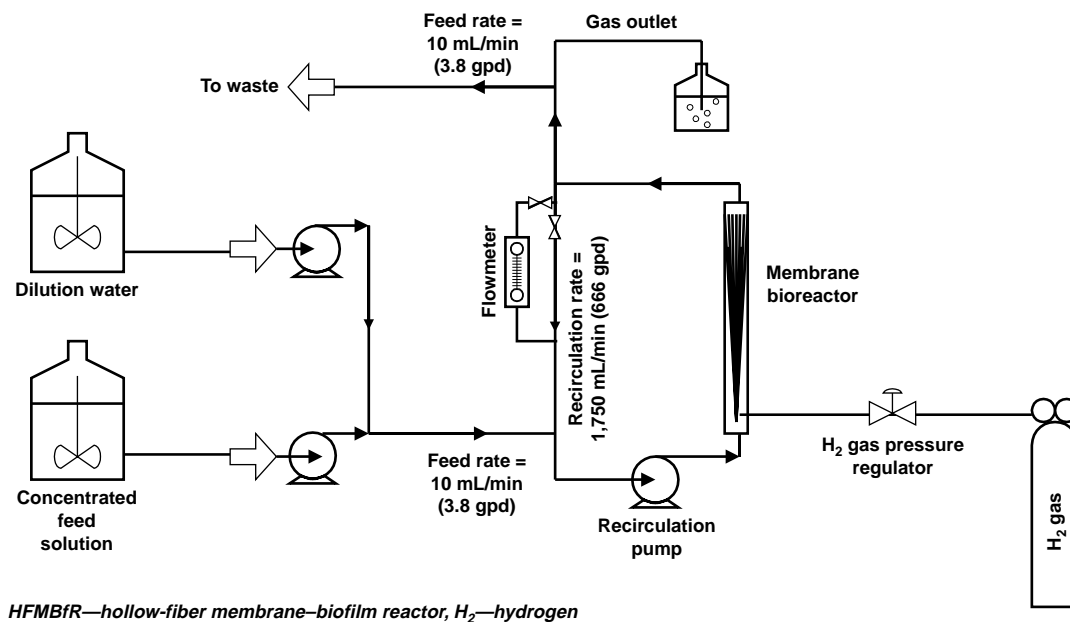
PCRB are ubiquitous in the environment. They have been found in pristine environments (Coates et al, 1999; van Ginkel et al, 1995) and sites with various types of municipal or industrial wastes (Coates et al, 1999; Stepanyuk et al, 1992). PCRB probably exist in environments lacking perchlorate because many are also facultative anaerobes and denitrifiers.

Table 2 compares the thermodynamic energy yield of perchlorate with other common electron acceptors when hydrogen is used as the

electron donor. Perchlorate’s energy yield as an electron acceptor is only slightly less than that for oxygen (aerobic respiration) and similar to that of nitrate (denitrification). It is much higher than that for sulfate reduction.

Given the ubiquity of PCRB, the favorable energy yield of perchlorate reduction, and the ability of many PCRB to respire on oxygen and nitrate, it should be “easy” to

FIGURE 4 Schematic of hydrogen-based HFMBfR



use PCRB in a biological treatment process. In fact, several researchers demonstrated perchlorate reduction in reactor systems using organic donors (Giblin et al, 2000b; Kim & Logan, 2000a; Kim & Logan, 2000b; Herman & Frankenberger, 1999; Wallace et al, 1998) or hydrogen (Giblin et al, 2000c; Miller & Logan, 2000). In all of these studies, however, high influent perchlorate concentrations and no nitrate were used, or the reactors were preloaded with high perchlorate concentrations. When the perchlorate concentration is low, as in most contaminated water supplies, the PCRB may not be able to compete for the electron donor with nonperchlorate-reducing bacteria if other acceptors, such as oxygen or nitrate, are present in much higher concentrations.

**Co-metabolic perchlorate reduction.** Denitrifying bacteria and other bacteria with membrane-bound nitrate reductase potentially can reduce perchlorate and chlorate, apparently without obtaining energy for growth. The authors call this “co-metabolic” perchlorate reduction, although the term is more commonly applied to electron donors (Sáez & Rittmann, 1991). Co-metabolism refers to a reaction that is incidentally catalyzed by an enzyme that has another biochemical purpose and that does not generate energy for the cells.

Co-metabolic chlorate reduction by nitrate-reducing bacteria has been known to exist since the 1920s (Quastel et al, 1925). The immediate product of co-metabolic chlorate reduction is chlorite (Pichinoty et al, 1969; Goksøyr, 1952). Chlorite is toxic to cells if it builds up to a high enough concentration (Anderson et al, 2000; Karki & Kaiser, 1979; Pichinoty et al, 1969; Stouthamer, 1967; Goksøyr, 1952). However, chlorite may react with reduced

cellular constituents or components of the medium to form chloride (Attaway & Smith, 1993; Hynes & Knowles, 1983; Karki & Kaiser, 1979; Pichinoty et al, 1969). Although information about perchlorate reduction by nitrate-reducing bacteria is limited, perchlorate can be reduced to chloride by whole cells of several nitrate-reducing bacteria, although the reduction rates vary significantly among species (Hackenthal et al, 1964).

Because nitrate and perchlorate are reduced by the same nitrate-reductase enzyme (Hackenthal, 1965; Hackenthal et al, 1964), they probably are competitive inhibitors of each other’s reduction. This suggests that co-metabolic perchlorate reduction may be significant only in denitrifying systems in which the nitrate concentration is low. Even though co-metabolic perchlorate reduction is rapid at high perchlorate concentrations (e.g., hundreds of milligrams per litre) (Hackenthal et al, 1964), trace concentrations of perchlorate (e.g., micrograms per litre) may allow slow reduction kinetics.

## EXPERIMENTAL MATERIALS AND METHODS

A schematic of the HFMBfR used in this study is shown in Figure 4, and reactor characteristics are provided in Table 3. The reactor consisted of a membrane module connected to a recirculation loop. The system behaved as a completely mixed biofilm reactor because of the high recirculation rate.

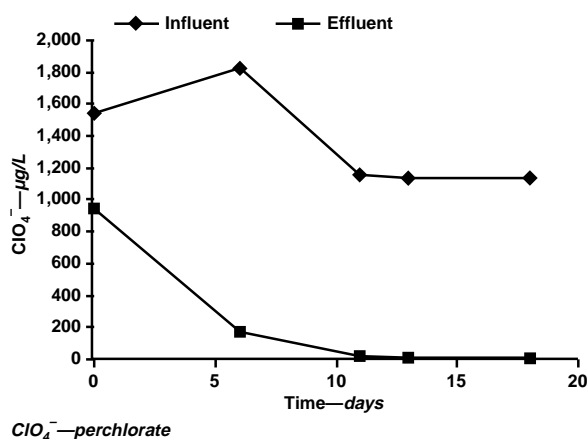
The membrane module<sup>1</sup> consisted of a bundle of 98 hydrophobic hollow-fiber membranes inside a polyvinyl chloride pipe shell. The free end of each fiber was sealed so that 100% of the supplied hydrogen was transferred through the fiber walls to the surrounding biofilm. Each fiber was 93

**TABLE 4** Typical tap water quality parameters

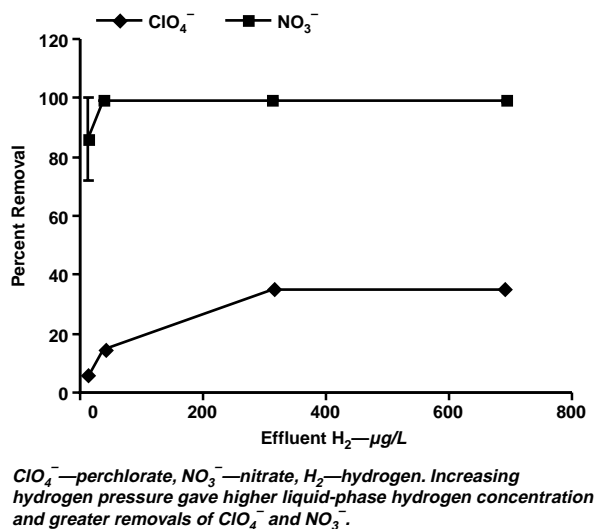
Parameter*	Typical Concentration—mg/L
Cl <sup>-</sup>	12.4
NO <sub>3</sub> <sup>-</sup> (as N)	0.4
NO <sub>2</sub> <sup>-</sup> (as N)	<0.1
SO <sub>4</sub> <sup>2-</sup>	23.2
pH	7.6
Hardness (as CaCO <sub>3</sub> )	138
Alkalinity (as CaCO <sub>3</sub> )	103

\*Cl<sup>-</sup>—chloride, NO<sub>3</sub><sup>-</sup>—nitrate, NO<sub>2</sub><sup>-</sup>—nitrite, SO<sub>4</sub><sup>2-</sup>—sulfate, CaCO<sub>3</sub>—calcium carbonate

**FIGURE 5** Fate of perchlorate in the screening experiment



**FIGURE 6** Mechanisms experiment 1—hydrogen variable



ClO<sub>4</sub><sup>-</sup>—perchlorate, NO<sub>3</sub><sup>-</sup>—nitrate, H<sub>2</sub>—hydrogen. Increasing hydrogen pressure gave higher liquid-phase hydrogen concentration and greater removals of ClO<sub>4</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>.

cm (37 in.) long. The top 10 cm (4 in.) of each fiber was treated with a wetting agent to allow moisture condensate to exit to the bulk liquid. This portion of the membrane was much less active for hydrogen transfer than were the untreated portions. Also, the bottom 10 cm (4 in.) of each

fiber was deadened to minimize gas transfer and avoid excessive growth of biofilm that could cause the fibers to stick together. Pure hydrogen was supplied to the inside of the hollow fibers through a manifold at the base. The fibers were attached at the manifold end and free at the opposite end. Water was pumped through the pipe from the manifold end of the fibers toward the free end, causing the fibers to fluidize or move independently. The water temperature in the reactor was 20 ± 1°C during the experiments.

**Types of experiments.** Three groups of experiments were carried out: screening, mechanisms, and groundwater experiments. In the screening and mechanisms experiments, the reactor was supplied with a concentrated feed solution that was diluted with water that was continuously sparged with helium or argon to minimize dissolved oxygen in the influent. Tap water was used for dilution in the screening experiments, and RO-treated water was used for dilution in the mechanisms experiments. In the groundwater experiments, no dilution water was required, and oxygen was not removed from the influent.

In all experiments, the feed rate was 10 mL/min (3.8 gpd), the hydraulic detention time was 44 min, and water was recirculated within the reactor at a rate of 1,750 mL/min (666 gpd). The high recycle flow rate helped promote completely mixed conditions and helped control biomass accumulation on the fibers.

For the screening experiments, tap water was supplemented to provide 1 mg/L perchlorate, 5 mg/L NO<sub>3</sub><sup>-</sup> as N, and a pH of 7.0 controlled with a 2-mM phosphate buffer. Initially, excess hydrogen was supplied by maintaining a 7.0 psi (0.48 atm) hydrogen supply pressure to the hollow-fiber membrane. Subsequently, the pressure was dropped to 3.0 psi (0.20 atm), which was adequate for nitrate and perchlorate reduction.

Table 4 shows the typical tap water characteristics, prior to supplementation. The mechanism experiments used a minimal medium (shown in Table 5) that Aragno & Schlegel (1992) employed for autotrophic hydrogen-oxidizing, denitrifying bacteria. The influent pH was 6.9 for the mechanisms experiments, and the effluent pH was 7.2. The influent contained 1 mg/L perchlorate and 5 mg/L NO<sub>3</sub><sup>-</sup> as N. The hydrogen pressure applied to the fiber was 2.5 psi (0.17 atm).

Groundwater for the groundwater experiment was collected from a well located in the Main San Gabriel Basin, Calif., and was periodically shipped in coolers. Once received, the water was stored in a cold room at 4°C and brought to room temperature (20°C) before it was fed into the reactor. No chemical additions were made during the groundwater experiments other than perchlorate spikes and hydrogen delivered by the HFMBfR. The quality of the groundwater sample is shown in Table 6.

The groundwater tests were performed with the hydrogen reactor previously at steady state with 1 mg/L perchlorate and 5 mg/L NO<sub>3</sub><sup>-</sup> as N. Three phases of testing were used over a period of 28 days. First, the ground-

water was applied for seven days with its natural perchlorate concentration of 6 µg/L to represent a low-perchlorate scenario. Second, the feedwater was spiked with 100 µg/L perchlorate for 15 days to represent a high range of perchlorate in most contaminated groundwaters in Southern California (CDHS, 2001). Finally, the reactor feed was spiked with 50 µg/L perchlorate for five days, representing a midrange of perchlorate contamination. The reactor operating conditions were similar to those used in the previous experiments, except the applied hydrogen pressure was 5 psi (0.34 atm).

The hydrogen reactor was originally inoculated in early 1998 using a pure culture of *Ralstonia eutropha* and was then used in a denitrification study (Lee & Rittmann, 2002; Lee & Rittmann, 2000). During the denitrification study, the reactor was fed with nonsterile tap water and presumably developed a mixed culture including hydrogen-utilizing autotrophic, denitrifying bacteria (such as *R. eutropha*).

For this study, a new hollow-fiber membrane was inoculated by connecting it in parallel with the old membrane for three weeks. The new reactor was then fed 5 mg/L NO<sub>3</sub><sup>-</sup> as N for two days. Screening studies began with an influent containing 1 mg/L perchlorate and 5 mg/L NO<sub>3</sub><sup>-</sup> as N.

Table 7 summarizes the analytical methods used in this study. The method for perchlorate was a modification of USEPA method 300.1 (1997). Most of the parameters were measured following *Standard Methods* (1995) or USEPA method 300.1 (1997). Analyses following *Standard Methods* included those for chloride, nitrate, nitrite, total organic carbon/dissolved organic carbon, dissolved oxygen, pH, and alkalinity. Analyses following USEPA method 300.1 (1997) included those for chlorate ion and chlorite ion. Low-level perchlorate was analyzed using an ion chromatograph (IC)<sup>2</sup> with conductivity detection and a column,<sup>3</sup> a 500-mL loop, and an autosampler. Based on repeated injections of a 10-µg/L standard, the method detection limit was 2 µg/L. The IC was used with a second column<sup>4</sup> to analyze for nitrate, nitrite, chlorate, chlorite, and chloride. This column was also used to determine perchlorate in some experiments with high perchlorate concentrations.

A headspace analysis method was used for dissolved hydrogen (Schmidt & Ahring, 1993). A 1-mL liquid sample was transferred from the reactor to a 160-mL serum vial with a thick butyl-rubber stopper previously outgassed with nitrogen. The vial was shaken vigorously to liberate the dissolved hydrogen. A gas-tight syringe was used to sample the headspace (1 mL) and test for hydrogen by reduction gas analysis.<sup>5</sup> In this method, hydrogen is directed through a mercuric oxide (HgO) bed and produces mercury gas [Hg(g)], which is measured by an ultraviolet photometer. Once the hydrogen concentration was known, Henry's law and mass balance were used to determine the dissolved hydrogen concentration.

**TABLE 5** Minimal media

Component*	Concentration—mg/L
NaNO <sub>3</sub>	30.36
Na <sub>2</sub> HPO <sub>4</sub>	142
KH <sub>2</sub> PO <sub>4</sub>	136
NaHCO <sub>3</sub>	5.1
NaClO <sub>4</sub>	1.225
MgSO <sub>4</sub> ·7H <sub>2</sub> O	200
CaCl <sub>2</sub> ·2H <sub>2</sub> O	1
FeSO <sub>4</sub> ·7H <sub>2</sub> O	1
EDTA	3
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	1
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.3
H <sub>3</sub> BO <sub>3</sub>	3
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.2
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.1
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.2
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.3

\*NaNO<sub>3</sub>—sodium nitrate, Na<sub>2</sub>HPO<sub>4</sub>—sodium hydrogen phosphate, KH<sub>2</sub>PO<sub>4</sub>—potassium diphosphate, NaHCO<sub>3</sub>—sodium bicarbonate, NaClO<sub>4</sub>—sodium perchlorate, MgSO<sub>4</sub>·7H<sub>2</sub>O—magnesium sulfate heptahydrate, CaCl<sub>2</sub>·2H<sub>2</sub>O—calcium chloride dihydrate, FeSO<sub>4</sub>·7H<sub>2</sub>O—hydrated ferrous sulfate, EDTA—ethylenediaminetetraacetic acid, ZnSO<sub>4</sub>·7H<sub>2</sub>O—zinc sulfate heptahydrate, MnCl<sub>2</sub>·4H<sub>2</sub>O—manganese chloride tetrahydrate, H<sub>3</sub>BO<sub>3</sub>—boric acid, CoCl<sub>2</sub>·6H<sub>2</sub>O—cobalt(II) chloride hexahydrate, CuCl<sub>2</sub>·2H<sub>2</sub>O—copper chloride dihydrate, NiCl<sub>2</sub>·6H<sub>2</sub>O—nickel(II) chloride hexahydrate, Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O—sodium molybdate dihydrate

**TABLE 6** Quality of California groundwater sample

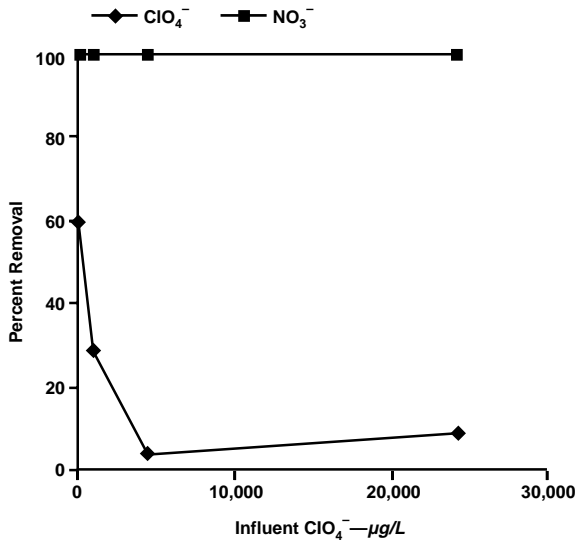
Parameter	Value
Perchlorate—µg/L	6
Nitrate—mg/L NO <sub>3</sub> <sup>-</sup> as N	2.5–3
pH	7.7
Alkalinity—mg/L as calcium carbonate	202
Total organic carbon—mg/L	1.4

## RESULTS AND DISCUSSION

**Screening experiment.** The purpose of the screening experiment was to determine whether hydrogen was an effective electron donor for perchlorate reduction in the HFMBfR. The experiment commenced when the influent was changed from 5 mg/L NO<sub>3</sub><sup>-</sup> as N to 1 mg/L perchlorate plus 5 mg/L NO<sub>3</sub><sup>-</sup> as N. Nitrate removal (not shown) was 96% after one day, increasing to 99% after the 18th day. Perchlorate removal (Figure 5) was 39% after one day, 90% after one week, and 99% after 18 days. No accumulation of intermediates (i.e., chlorate, chlorite) was detected. The chloride concentration increased in approximately stoichiometric amounts, although exact measurements were not possible because of the relatively high background chloride concentration in the tap water. The lowest effluent perchlorate concentration was 13 µg/L.

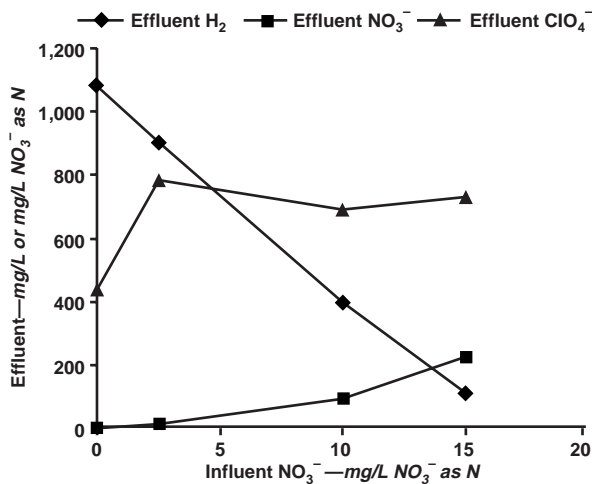
The screening experiments documented that hydrogen was an effective donor and that no special inoculation

**FIGURE 7** Mechanisms experiment 2—perchlorate variable



$\text{ClO}_4^-$ —perchlorate,  $\text{NO}_3^-$ —nitrate. Increasing influent loading of  $\text{ClO}_4^-$  had no effect on  $\text{NO}_3^-$  removal, but the percent of  $\text{ClO}_4^-$  declined.

**FIGURE 8** Mechanisms experiment 3—nitrate variable



$\text{H}_2$ —hydrogen,  $\text{NO}_3^-$ —nitrate,  $\text{ClO}_4^-$ —perchlorate.  $\text{ClO}_4^-$  removal increased when no  $\text{NO}_3^-$  was in the influent but was at 25–30% when effluent  $\text{NO}_3^-$  was 0.014  $\text{mg/L NO}_3^- \text{ as N}$  or greater.

was required. The HFMBfR initially gave approximately 30% perchlorate removal, even though the biofilm had not been previously exposed to perchlorate and no PCRB had been introduced. Some form of adaptation occurred so that the perchlorate removal increased to 99% over the three-week screening experiment. The most likely cause for the adaptation to perchlorate was an enrichment of PCRB. A PCRB species capable of using nitrate and perchlorate could have had a metabolic advantage over common denitrifiers, particularly when the nitrate concentration was low. Because the perchlorate loading was less

than 5% of that of nitrate on a hydrogen-accepting basis, the number of PCRB probably was small compared with the common denitrifiers. Another feasible explanation for the improved perchlorate removal is that the biomass increased for all bacteria, perhaps including denitrifiers responsible for co-metabolic perchlorate reduction. The increase in nitrate removal is consistent with this mechanism but does not prove it.

**Mechanism experiments.** Mechanism experiments were conducted to explore whether and how the hydrogen concentration, perchlorate concentration, nitrate concentration, and pH affected perchlorate reduction. Once the biofilm in the HFMBfR reached steady state, pseudo-steady-state conditions were achieved by varying a single parameter during a time frame long enough to reach hydraulic steady state but short enough to prevent appreciable changes in biomass. Prior to the experiments, the reactor was cleaned and allowed to return to biological steady state with an influent with 1  $\text{mg/L}$  perchlorate, 5  $\text{mg/L}$   $\text{NO}_3^-$  as N, and a 2.5 psi (0.17 atm) hydrogen pressure. A minimal medium based on RO water was used instead of the tap-water-based medium.

The steady-state nitrate removal was 99%, the effluent hydrogen was 0.07  $\text{mg/L}$ , and the perchlorate removal was approximately 30%. The steady-state perchlorate removals after cleaning and with the minimal medium were much lower than in the screening experiment, although nitrate removals were approximately the same. The biofilm community did not adapt to higher perchlorate removals, as in the screening experiment. It appears likely that PCRB did not compete as well with the common denitrifiers when the minimal medium was used instead of the tap water. Although not desirable in a treatment setting, the lower perchlorate reduction was advantageous for determining mechanisms because if 99% perchlorate removal had been present at steady state, faster removal rates would not have been observable.

In the first short-term experiment, the applied hydrogen pressure was set at 1.5, 2.5, 4, and 5.5 psi (0.10, 0.17, 0.27, and 0.37 atm). Results are shown in Figure 6. At 1.5 psi (0.10 atm), the effluent residual hydrogen was 11  $\mu\text{g/L}$ , providing partial reduction of nitrate to 0.03  $\text{mg/L NO}_3^-$  as N, or 86% removal. Under these conditions, the perchlorate reduction was only 5%. At 2.5 psi (0.17 atm) applied pressure, the effluent residual hydrogen was 37  $\mu\text{g/L}$ , and denitrification reached its maximum removal of 99%. Nitrate removals did not increase further at higher hydrogen pressures. At 2.5 psi (0.17 atm) perchlorate reduction increased to 15%. At an applied pressure of 4 psi (0.27 atm) with effluent residual hydrogen of 310  $\mu\text{g/L}$ , perchlorate reached its maximum removal of 35%.

In the second short-term experiment, the influent perchlorate concentration was set at 0, 0.2, 1, 5, or 25  $\text{mg/L}$ , with applied hydrogen and influent nitrate at the steady-state values of 2.5 psi (0.17 atm) and 5  $\text{mg/L NO}_3^-$  as N,

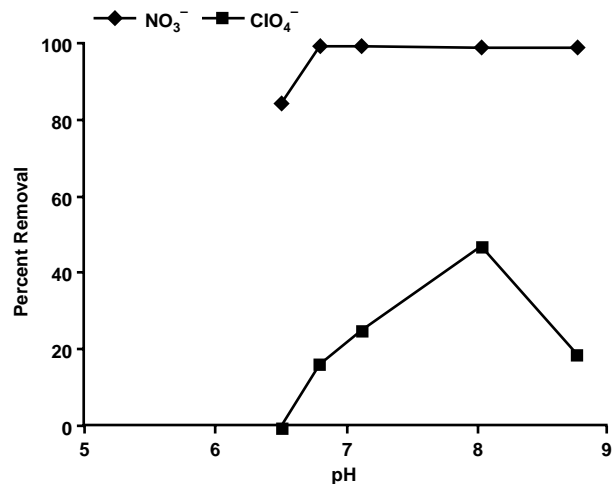
respectively. Results are shown in Figure 7. Although nitrate reduction was not affected by the influent perchlorate load, perchlorate removal decreased significantly with the increased perchlorate load. Chlorite was not detected in any of the experiments. A trace amount of chlorate (0.33 mg/L) was detected during the experiment with 25 mg/L perchlorate (data not shown). The effluent hydrogen concentration varied from 30 to 50  $\mu\text{g/L}$  during these experiments (data not shown).

In the third short-term experiment, influent nitrate concentrations were varied from 0 to 15 mg/L  $\text{NO}_3^-$  as N, with hydrogen and perchlorate at the steady-state concentrations of 2.5 psi (0.17 atm) and 1 mg/L, respectively. Results are shown in Figure 8. With zero nitrate in the influent, no nitrate was found in the effluent, and perchlorate reduction increased to 57%. However, with influent nitrate  $\geq 2.5$  mg/L  $\text{NO}_3^-$  as N and the effluent nitrate concentrations  $\geq 0.014$  mg/L  $\text{NO}_3^-$  as N, perchlorate reduction decreased to 25–30%. The hydrogen concentration in the effluent was inversely proportional to the nitrate loading.

In the fourth short-term experiment, the influent pH was adjusted to achieve effluent pH values of 6.5, 6.8, 8.0, and 8.8, with applied hydrogen and influent nitrate at their steady-state values of 2.5 psi (0.17 atm) and 5 mg/L  $\text{NO}_3^-$  as N, respectively. Results are shown in Figure 9. Although nitrate reduction was hardly sensitive to pH, perchlorate reduction was pH-sensitive, and the best removal occurred at a pH of 8.

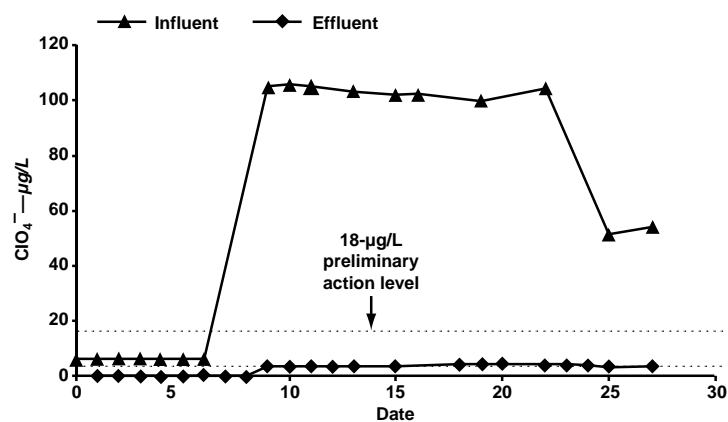
The mechanisms experiments showed that the maximum perchlorate reduction required a minimum hydrogen residual of about 300  $\mu\text{g/L}$  in the bulk liquid, whereas maximum nitrate reduction required only 0.050 mg/L. Furthermore, perchlorate reduction was very sensitive to the nitrate concentration: reduction was 57% with zero nitrate but dropped to about 30% with nitrate at levels as low as 0.014 mg/L  $\text{NO}_3^-$  as N. However, nitrate inhibition of perchlorate reduction did not increase as the effluent nitrate concentration increased from 0.014 to 0.23 mg/L. The observation of inhibition may indicate that perchlorate reduction is incompatible with partial denitrification (Lee & Rittmann, 2000). On the other hand, partial inhibition of perchlorate reduction by nitrate suggests either that some PCBs within the biofilm were not subject to nitrate inhibition, such as HAP-1 (Wallace et al, 1996), or that nitrate did not penetrate into the deeper portions of biofilm, where perchlorate reduction occurred free of nitrate inhibition.

**FIGURE 9** Mechanisms experiment 4—effluent pH variable



$\text{NO}_3^-$ —nitrate,  $\text{ClO}_4^-$ —perchlorate.  $\text{ClO}_4^-$  reduction was sensitive to pH, with a maximum removal at pH = 8.

**FIGURE 10** Perchlorate concentrations in the groundwater experiment



$\text{ClO}_4^-$ —perchlorate, MRL—minimum reporting level.  $\text{ClO}_4^-$  concentration was well below the 18- $\mu\text{g/L}$  preliminary action level and was near or below the 4- $\mu\text{g/L}$  MRL at all times.

The optimal pH for perchlorate reduction was  $\sim 8$ , and the range for perchlorate reduction appeared to be from 6.8 to  $\sim 9$ . The lack of perchlorate reduction at 6.5 may have been exacerbated by nitrate inhibition, because nitrate reduction was partially inhibited at this pH. The sensitive pH effects on perchlorate reduction are significant, because denitrification adds base, which can cause a pH increase that might slow perchlorate reduction and limit the accumulation of PCBs.

An additional mechanism experiment was performed to determine the fate of chlorite in the reactor. With an influent containing 5 mg/L  $\text{NO}_3^-$  as N, 10 mg/L chlorite, and no perchlorate, more than 95% of the chlorite was removed, with more than 80% transformed to chloride. A trace amount of chlorate (0.17 mg/L) was detected. With

**TABLE 7** Summary of analytical procedures

Parameter*	Method Number	Method Title	Reference or Source
ClO <sub>4</sub> <sup>-</sup>	Modified USEPA† method 300.1	Analysis of Low Concentrations of Perchlorate in Drinking Water and Groundwater by Ion Chromatography	Dionex Application Note 121, Dionex, Sunnyvale, Calif.
ClO <sub>3</sub> <sup>-</sup>	USEPA method 300.1		USEPA (1997)
ClO <sub>2</sub> <sup>-</sup>	USEPA method 300.1		USEPA (1997)
Cl <sup>-</sup>	4500-Cl <sup>-</sup> F	Chloride-Ion Chromatography Method	<i>Standard Methods</i> (1995)
H <sub>2(g)</sub>		Headspace	Schmidt and Ahring (1993)
NO <sub>3</sub> <sup>-</sup>	4500-NO <sub>3</sub> <sup>-</sup> C	Ion Chromatographic Method	<i>Standard Methods</i> (1995)
NO <sub>2</sub> <sup>-</sup>	4500-NO <sub>2</sub> <sup>-</sup> C	Ion Chromatographic Method	<i>Standard Methods</i> (1995)
TOC/DOC	5310 B	Combustion Infrared	<i>Standard Methods</i> (1995)
DO	4500-O G	Membrane Electrode Method	<i>Standard Methods</i> (1995)
pH	4500-H <sup>+</sup>	Electrometric Method	<i>Standard Methods</i> (1995)
Conductivity	2510	Electrical Conductivity	<i>Standard Methods</i> (1995)
Alkalinity	2320 B	Titration Method	<i>Standard Methods</i> (1995)

\*ClO<sub>4</sub><sup>-</sup>—perchlorate, ClO<sub>3</sub><sup>-</sup>—chlorate, ClO<sub>2</sub><sup>-</sup>—chlorite, Cl<sup>-</sup>—chloride, H<sub>2(g)</sub>—hydrogen gas, NO<sub>3</sub><sup>-</sup>—nitrate, NO<sub>2</sub><sup>-</sup>—nitrite, TOC/DOC—total organic carbon/dissolved organic carbon, DO—dissolved oxygen  
 †USEPA—US Environmental Protection Agency

an influent containing 5 mg/L NO<sub>3</sub><sup>-</sup> as N, 1 mg/L chlorite, and no perchlorate, neither chlorite nor chlorate was detected. Because chlorate accumulation has not been observed in pure cultures of PCRB, it may have been a product of co-metabolic perchlorate reduction, either accu-

mulating directly or through abiotic disproportionation of chlorite into chlorate and chloride (3ClO<sub>2</sub><sup>-</sup> → 2ClO<sub>3</sub><sup>-</sup> + Cl<sup>-</sup>) (Gordon et al, 1972). The chlorite-addition tests performed at the end of the mechanisms experiments confirmed that chlorate can be produced from chlorite in the

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reactor. More important, these experiments confirmed that chlorite dismutation to chloride and oxygen is faster than perchlorate reduction, which means that intermediate accumulations normally do not occur.

**Groundwater experiment.** Because the previous tests were all conducted with amended tap water or minimal media, an additional experiment was performed to test the reactor with a groundwater from a contaminated site and with no chemical amendments. The test included phases with different concentrations of perchlorate in the influent: 6, 100, and 50 µg/L. The influent nitrate concentration was 2.6–3.0 mg/L NO<sub>3</sub><sup>-</sup> as N. The influent and effluent perchlorate results are shown in Figure 10. For all three phases of the test, the effluent perchlorate varied from nondetects (<2 µg/L) to 4.5 µg/L, and the effluent nitrate varied from 13 to 32 µg/L NO<sub>3</sub><sup>-</sup> as N, which corresponds to at least 99% removals of perchlorate and nitrate.

The groundwater experiments clearly demonstrated the feasibility of using the HFMBfR to remove perchlorate from groundwater. Over a four-week period, the reactor consistently achieved removals at or below the minimum reporting level (MRL) of 4 µg/L and well below the 18-µg/L preliminary action level. The reactor also responded well to sudden changes in perchlorate concentrations. For example, when the influent concentration was suddenly increased from 6 to 100 µg/L,

the effluent perchlorate concentrations did not increase above the 4-µg/L MRL.

## CONCLUSIONS

The experimental work clearly demonstrated that biological reduction of perchlorate to chloride is technically feasible in the drinking water setting using hydrogen as an electron donor. For example, the hydrogen-based HFMBfR successfully reduced perchlorate concentrations to below the 4-µg/L MRL when the influent perchlorate concentration was as high as 100 µg/L in an actual contaminated groundwater. Perchlorate reduction in the HFMBfR occurred immediately upon exposure to perchlorate and without inoculation with specialized PCRB.

The mechanisms studies showed that excellent perchlorate removal was possible when only a small residual concentration of the electron donor was present in the effluent, e.g., ~ 300 µg/L of hydrogen. However, nitrate in the water slowed perchlorate reduction, which suggests that partial nitrate removal may not be feasible when perchlorate reduction is a treatment goal. On the other hand, the presence of perchlorate had no effect on denitrification. Results indicated that pH control may be important, because the optimal pH for perchlorate reduction was ~ 8, whereas denitrification adds base to the water.

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## ABOUT THE AUTHORS:

**Robert Nerenberg**<sup>6</sup> is a doctoral candidate in the Department of Civil Engineering, Northwestern University, 2145 Sheridan Rd., Evanston, IL 60208-3109, e-mail [r-nerenberg@northwestern.edu](mailto:r-nerenberg@northwestern.edu). He has a BS degree in civil engineering from the University of Buenos Aires, Argentina, and an MS degree in environmental engineering from Wayne State University in Detroit, Mich. He was also the Abel Wolman doctoral fellow for 2001. Bruce E. Rittmann is the John Evans



Professor of Environmental Engineering in the Department of Civil Engineering at Northwestern University. Issam Najm is president of Water Quality & Treatment Solutions, Chatsworth, Calif. At the time of this study, Najm was manager of the Applied Research Group at MWH (formerly Montgomery Watson).

## FOOTNOTES

- <sup>1</sup>Porous Media Inc., Minneapolis, Minn.
- <sup>2</sup>Dionex 4000i, Dionex, Sunnyvale, Calif.
- <sup>3</sup>AS16, Dionex, Sunnyvale, Calif.
- <sup>4</sup>AS11, Dionex, Sunnyvale, Calif.
- <sup>5</sup>RGA3, Trace Analytical, Menlo Park, Calif.
- <sup>6</sup>To whom correspondence should be addressed

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