

PERCHLORATE REDUCTION USING A HOLLOW-FIBER MEMBRANE BIOFILM REACTOR: KINETICS, MICROBIAL ECOLOGY, AND PILOT-SCALE STUDIES

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ABSTRACT: Perchlorate contamination of groundwaters and surface waters is a national concern, and biological treatment may be the most cost-effective option. A hydrogen-based, hollow-fiber membrane biofilm reactor (HFMBfR) is ideal for perchlorate reduction, as hydrogen gas is inexpensive, non-toxic, and leaves little or no residual in the treated water. We studied the HFMBfR at the bench and pilot scale for removal of low-level of perchlorate. At the pilot-scale, a 0.6-L/min HFMBfR with a 55-minute detention time reduced 55- $\mu\text{g/L}$ perchlorate to less than 4 $\mu\text{g/L}$, and it concurrently reduced 5.5-mg/L nitrate-N to less than 0.02 mgN/L. At the bench scale, *Dechloromonas* sp. PC1, a perchlorate-reducing autotroph, was found to have half maximum substrate utilization constant (K) for perchlorate of 0.15 mg/L, two orders of magnitude lower than reported for other perchlorate-reducing bacteria. The perchlorate growth threshold (S_{\min}) for autotrophic growth with hydrogen was 40- $\mu\text{g/L}$, suggesting that perchlorate must be reduced concurrently with another, primary acceptor to achieve the 4- $\mu\text{g/L}$ standard in a completely mixed system. For mixed cultures, when perchlorate was added to bench-scale HFMBfRs at steady state with nitrate or oxygen, initial perchlorate removals were less than 30%, but increased to over 90% after 4 to 12 days, suggesting that low levels of perchlorate can select for perchlorate-reducing bacteria, significantly improving removals.

INTRODUCTION

The Perchlorate Problem. Perchlorate (ClO_4^-) is an oxidizing anion that originates from ammonium, potassium, magnesium, or sodium salts. Ammonium perchlorate is a primary ingredient of solid rocket fuel. Due to rocket fuel's short shelf life, large amounts of perchlorate-containing wastes have been disposed of to the environment (EPA, 2002). At least 20 states have confirmed perchlorate contamination, and more sites may be found, as perchlorate has been used or manufactured in 40 states. The State of California recently lowered its drinking water action level from 18 to 4 $\mu\text{g/L}$. A recent EPA toxicological and risk characterization study suggests 1 $\mu\text{g/L}$ as a treatment goal for drinking water (EPA 2002).

Perchlorate is not removed by conventional physical-chemical water treatment, and advanced processes, such as ion exchange, electrodialysis, and reverse osmosis, are costly and concentrate perchlorate into waste streams that require disposal. Fortunately, perchlorate can be reduced to chloride by perchlorate-reducing bacteria, which use perchlorate as an electron acceptor for growth (Logan 1998; Coates, Michaelidou et al.

1999). Perchlorate-reducing bacteria have several traits that make them suitable for perchlorate treatment systems: they are common and therefore easily obtainable in the environment; and many have a wide range of metabolic capabilities, such as aerobic growth and denitrification, and therefore do not require highly specialized growth conditions.

Kinetics and Microbial Ecology. Researchers have shown that bioreactors can reduce perchlorate to below 4 $\mu\text{g/L}$ when the initial concentration is high or when the reactor has been previously operated at high perchlorate concentrations (Kim and Logan 2000; Logan 2002; Giblin, Herman et al. 2000). However, low initial perchlorate concentrations, in the $\mu\text{g/L}$ range, may preclude growth on perchlorate as the sole acceptor. Consider the biomass balance for batch growth:

$$\frac{dX}{dt} = q_{\max} \frac{S}{S + K} YX - bX$$

where S is the rate-limiting substrate concentration [$\text{M}_\text{S}/\text{L}^3$], q_{\max} is the maximum specific substrate utilization rate [$\text{M}_\text{X}/\text{M}_\text{S}\text{-T}$], K is the half-maximum-substrate-utilization constant [M/L^3], X is the biomass concentration [$\text{M}_\text{X}/\text{L}^3$], Y is the biomass true yield [$\text{M}_\text{X}/\text{M}_\text{S}$], and b [$1/\text{T}$] is the endogenous decay rate. When S is small with respect to K , it can render the positive term on the right side of the equation smaller than the negative term, providing a net decay in biomass for any value of X . Under such conditions, biomass cannot be produced. S_{\min} is the minimum concentration that can support steady-state biomass for a continuous suspended or biofilm system, and is calculated from

$$S_{\min} = \frac{Kb}{Yq_{\max} - b} \text{ (Rittmann and McCarty 2001)}$$

Fortunately, nitrate can serve as a primary electron-acceptor substrate, i.e., nitrate reduction can generate biomass that concurrently reduces perchlorate and nitrate. However, for a mixed-culture system, it is not clear whether nitrate reduction will result in perchlorate-reducing denitrifiers or common denitrifiers that cannot reduce perchlorate. In our bench-scale research, we studied kinetics of a novel, autotrophic isolate; the ability of nitrate and oxygen to serve as primary acceptors in mixed-culture HFMBfRs; and changes in microbial ecology of denitrifying/perchlorate reducing reactors.

Pilot-scale Tests. The HFMBfR has been studied at the bench scale (Lee and Rittmann 2002; Nerenberg, Rittmann et al. 2002), but this is the first time a HFMBfR was tested at the pilot scale. The main goals were to determine performance of a pilot-scale HFMBfR for perchlorate removal, evaluate and determine system operational and design parameters that affect the biodegradation of perchlorate, and evaluate reactor design.

MATERIALS AND METHODS

Hollow-Fiber Membrane Biofilm Reactor. Hydrogen is an ideal electron donor for bioreactors reducing oxidized anions, such as perchlorate, because (1) it is much less expensive, per electron equivalent, than organic donors, such as acetate or methanol; (2) it is non-toxic, increasing public acceptance for its use for water treatment; (3) it is sparsely soluble, so it is not possible to “overdose” the system and cause re-growth; and (4) it can be generated on-site. The historic disadvantage is that hydrogen is difficult to deliver without sparging, which is wasteful and potentially dangerous. A novel hydrogen-based bioreactor, the hollow-fiber membrane biofilm reactor (HFMBfR), delivers hydrogen safely and efficiently without sparging.

The HFMBfR consists of a bundle of composite, hydrophobic hollow-fiber membranes collected into a hydrogen-supplying manifold at one end and sealed at the other (Figure 1). Pressurized hydrogen is supplied to the interior of the fibers and diffuses through the wall to a biofilm growing on the fiber surface. The biofilm consumes hydrogen as it reduces oxidized contaminants present in the water. No hydrogen bubbles are produced, and the bulk liquid may have a negligible hydrogen concentration, providing utilization efficiencies approaching 100%. The HFMBfR is different from “membrane bioreactors” used in wastewater treatment. Membrane bioreactors use porous, hydrophilic membranes to separate particles from permeating water, while the HFMBfR uses composite microporous/non-porous membranes to deliver hydrogen gas to bacteria.

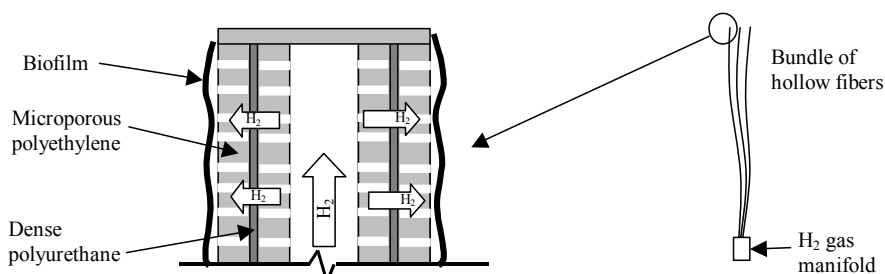


FIGURE 1. (a) Schematic of hollow fiber membrane bundle; (b) section of fiber.

Hollow fiber membranes are 280 μm in diameter with a 40- μm wall. They are made of two materials: a 1- μm layer of dense polyurethane encased within microporous polyethylene (Figure 1). Because the fiber material is hydrophobic, the pores remain dry and do not foul. The dense polyurethane layer prevents bubbling at higher gas pressures, allowing a wide range of gas pressures that offer a high degree of control over the hydrogen-delivery rate. A scanning electron micrograph (SEM) image of the fiber wall is shown in Figure 2a, and a confocal laser scanning micrograph (CLSM) image of biofilm on a hollow-fiber membrane is shown in Figure 2b.

Batch tests. Batch tests were carried out to determine the kinetic parameters q_{max} , Y , and K for *Dechloromonas* sp. PC1 (GenBank accession number AY126452), a novel, autotrophic, perchlorate-reducing bacterium isolated from a previous bench-scale HFMBfR (Nerenberg, Rittmann et al. 2002). The kinetic parameters were determined for autotrophic growth using hydrogen as an electron donor. The Y and q_{max} were

determined using batch experiments with high initial acceptor and low initial biomass concentrations. The K was determined using batch non-growth tests with low initial biomass and acceptor concentrations. The experiments used 1-L bottles filled with 200 mL of media or 160 mL serum bottles filled with 25 mL of media, capped with butyl

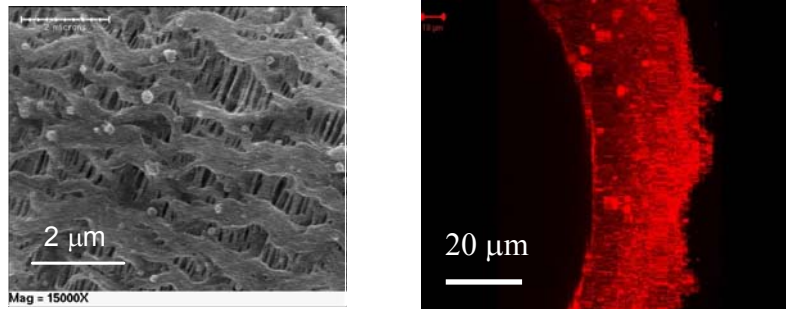


FIGURE 2. (a) SEM image of hollow fiber membrane surface, and (b) CLSM image of biofilm growing on hollow-fiber membrane.

rubber stoppers, vacuum degassed, and filled with a gas mixture of 95% hydrogen and 5% CO_2 (for q_{\max} and Y) or with pure hydrogen (for K). The bottles were shaken on their side at 200 rpm. The experiments were carried out at least in triplicate. The growth medium contained, per liter: 1.386 g Na_2HPO_4 , 0.849 g KH_2PO_4 , 0.1 g $(\text{NH}_4)_2\text{SO}_4$, 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 1 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The trace mineral solution is described in (Nerenberg, Rittmann et al. 2002). The K experiments were carried out in a 12-mM phosphate buffer at pH of 7 with no nutrients or trace minerals. The pH was adjusted using 1 M NaOH for a final pH of 7.0. Curve fitting was used to estimate kinetic parameters q_{\max} and K for PC1 using a finite-differences solution of the substrate-utilization and biomass-growth equations:

$$\frac{dS}{dt} = -\frac{q_{\max} S}{S + K} X, \text{ and}$$

$$\frac{dX}{dt} = \frac{Yq_{\max} S}{S + K} X - bX.$$

These equations neglect competitive inhibition from chlorate during perchlorate reduction, so the q_{\max} for perchlorate is an “apparent” value, valid only for the perchlorate range for which it was determined.

Mixed-culture tests. Two bench-scale HFMBfRs were seeded with a mixed culture from the pilot-scale reactor. The primary acceptor for one reactor was 8-mg/L oxygen, 5 mgN/L nitrate for the other. The reactor configurations followed the schematic in Figure 3. The physical characteristics are summarized in Table 1. A high recirculation rate provided completely mixed conditions. After reaching effluent steady state with the primary acceptors, perchlorate was added to the influent: 1,000 $\mu\text{g/L}$ perchlorate for the oxygen reactor, and 100 $\mu\text{g/L}$ for the nitrate reactor. The media was the same as in the batch tests, except the phosphate buffer was reduced to 4 mM, the pH was 7.5, and no ammonium was added to the nitrate reactor medium.

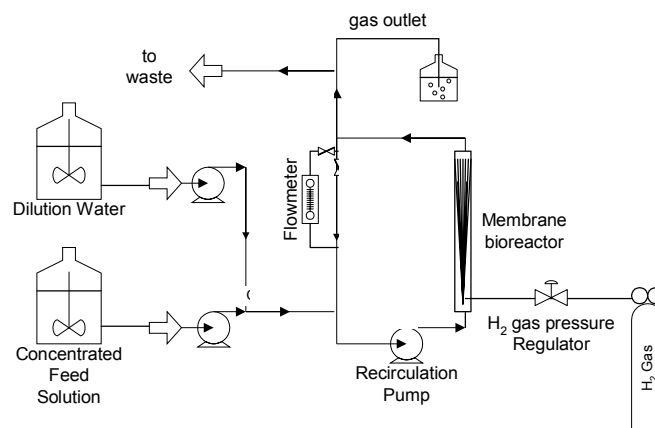


FIGURE 3. Schematic of the HFMBfR.

Pilot-scale Reactor. A pilot-scale reactor was tested with perchlorate-contaminated groundwater at a site owned and operated by La Puente Valley County Water District in Southern California. The pilot plant included two HFMBfRs in series, followed by an aeration basin and a granular media filter. The modules (Mitsubishi, Japan) were conceptually similar to Figure 3, except the fibers were potted at both ends. The upstream potted end was connected to a hydrogen supply, and the downstream end was sealed. Periodic air scouring and backwashing were used to control biomass accumulation. The influent characteristics were: temperature, 18°C; pH, 8.0; alkalinity, 176 mg/L as CaCO₃; perchlorate, 60 µg/L; nitrate, 5.4 mgN/L; and sulfate, 31 mg/L. Table 1 provides the reactor’s main characteristics.

TABLE 1. Bench- and pilot-scale reactor characteristics.

PARAMETER	BENCH-SCALE	PILOT-SCALE
Feed rate	1 mL/min	1,100 mL/min
Recirculation ratio	150	27
Detention time	24 min	55 min
Number of modules	1	2
Module length	25 cm	120 cm
Module diameter	0.6 cm	14 cm
Number of fibers per module	33	7040
Total fiber surface area	72.3 cm ²	14.6x10 ⁴ cm ²

Analytical Methods. Perchlorate was analyzed by ion chromatography (IC) using a Dionex DX-320 (pilot) or 4000i (bench) with conductivity detection. An AS-16 or AS-11 column was used followed EPA Method 314.0. The lowest standard used during calibration was 2 µg/L. All anions other than perchlorate (i.e., chloride, chlorate, chlorite, nitrate, nitrite, and sulfate) were analyzed on the same systems based on EPA Method 300.1 modified for a hydroxide-selective column. Dissolved H₂ at the pilot-scale

system was analyzed using an Orbisphere Model 3654 Portable Micro Logger configured for dissolved hydrogen. At the bench-scale, hydrogen gas was analyzed with a reduction gas analyzer (Trace Analytical RGA3) using a headspace analysis (Nerenberg, Rittmann et al. 2002).

RESULTS AND DISCUSSION

Kinetic Parameters for Strain PC1. Kinetics parameters were determined for *Dechloromonas* sp. PC1. Figure 4a shows a typical growth curve for perchlorate, and 4b shows a typical K experiment. Similar plots were obtained for nitrate.

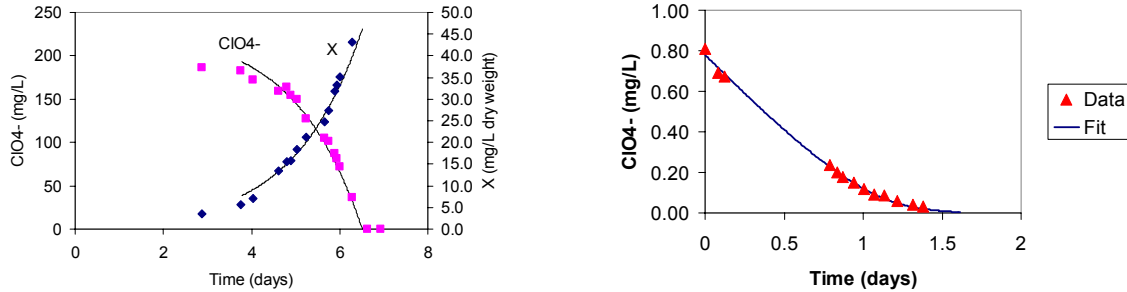


FIGURE 4. Perchlorate kinetics tests for estimating (a) q and Y , (b) K .

As shown in Table 2, the yields for perchlorate were very similar to those for nitrate. This is consistent with the similar Gibb's free energy at pH 7 ($\Delta G_o'$) for perchlorate and nitrate reduction with hydrogen (118 and 112 kJ/eq e⁻ H₂, respectively). The q_{max} for nitrate reduction was around 6 times higher than for perchlorate, on an electron-equivalent (or hydrogen-accepting) basis. This makes growth on nitrate much faster than on perchlorate. The K value for perchlorate was 0.15 mg/L, two orders of magnitude lower than values from the literature for other perchlorate-reducing bacteria (Logan, Zhang et al. 2001).

Based on the kinetic parameters, the S_{min} for perchlorate is 40 μ g/L. This is an approximate value, since q_{max} does not include competitive inhibition with chlorate. It is unlikely that the actual S_{min} would be much less than this value, therefore it is unlikely that perchlorate can be reduced to 4 μ g/L with perchlorate as the sole electron acceptor.

TABLE 2. Kinetic parameters for *Dechloromonas* sp. PC1.

S	q_{max} (eq e ⁻ H ₂ /g X-day)	Y (gX/eq e ⁻ H ₂)	K (mg/L)	S_{min} (μ g/L)
ClO ₄ ⁻	0.25	2.88	0.15	40
NO ₃ ⁻	1.43	2.46	<0.05	<2

Notes: (1) "eq e⁻ H₂" = equivalent of electrons from hydrogen; (2) 1 eq e⁻ H₂ = 1 g H₂; (3) b=0.1 1/day

Mixed-Culture Experiments. Two bench-scale HFMBfRs were operated for 20 days with 5-mgN/L nitrate or 8-mg/L oxygen. In the nitrate reactor, the effluent nitrate reached 0.01 mgN/L after around 10 days. In the HFMBfR with oxygen and no nitrate, the DO levels were below 0.1 mg/L after 4 days of operation. Therefore, the oxygen reactor approached an anoxic condition. After 20 days, 100- μ g/L and 1,000- μ g/L

perchlorate was added to the nitrate and oxygen reactors, respectively. In the nitrate reactor, the initial removal was 30%, but it increased to more than 90% after 4 days. In the oxygen reactor, the initial removal was 5%, but it increased to more than 99% after 12 days. These results support the hypothesis that even low levels of perchlorate can provide a selective pressure for perchlorate-reducing bacteria, dramatically improving removals.

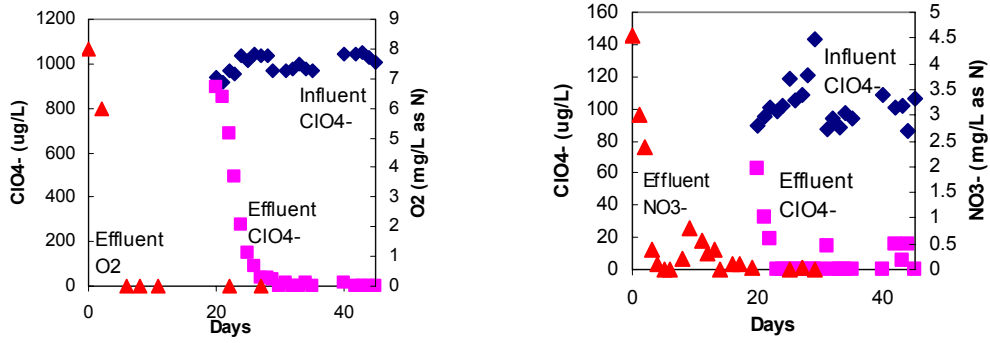


FIGURE 5. Perchlorate addition to reactors at steady-state with (a) nitrate, and (b) oxygen. Influent nitrate is 5 mgN/L and influent oxygen is 8 mg/L.

Pilot-scale tests. A 1.1 L/min pilot-scale HFMBfR plant was operated using a perchlorate-contaminated groundwater. With a 55-minute detention time, the pilot-scale results reduced 55-µg/L perchlorate to less than 4 µg/L concurrently with total reduction of 5.5-mgN/L nitrate and 8-mg/L oxygen (Table 3).

TABLE 3. Reduction of Oxygen, Nitrate, and Perchlorate.

Parameter	Influent Concentration	Effluent Concentration	Removal Efficiency	Substrate Flux
Perchlorate	55 µg/L	2 µg/L	96%	5500 µg/m ² -day
Nitrate	5.5 mg-N/L	<0.02 mg-N/L	>97%	2700 mg/m ² -day
DO	8 mg/L	<0.10 mg/L	>99%	860 mg/m ² -day

Two operating strategies proved useful for improving performance in the pilot studies. The first strategy was effluent recycling, which increased the water velocity past the membrane surface. The second strategy was air scour with backwashing. Together, these strategies minimized the formation of loose, fluffy biofilm; short-circuiting; and mass-transport resistance of acceptor to the biofilm. Future designs of the HFMBfR will maximize these benefits.

Although no attempts were made to optimize the hydrogen utilization efficiency, over 90% of the delivered hydrogen was used by the bacteria. On the average, the effluent contained 0.5-mg/L hydrogen. Future designs will have lower H₂ concentrations so that the hydrogen utilization efficiency approaches 100%.

CONCLUSIONS

The results at the pilot scale show that the HFMBfR is effective in reducing low-level perchlorate contamination to below 4 µg/L. At the bench scale, the K for

perchlorate for PC1 was much lower than for other species, but kinetics suggest that perchlorate cannot be removed to 4 µg/L without another, primary acceptor. Nitrate or oxygen can serve as primary acceptors that allow concurrent perchlorate removal. Even if the perchlorate concentration is low compared to nitrate or oxygen, perchlorate helps select for a perchlorate-reducing population. Since most groundwaters and surface waters contain nitrate, oxygen, or both, the HFMBfR is likely to be effective for a wide range of field applications.

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