

Kinetics of Microbial Bromate Reduction in a Hydrogen-Oxidizing, Denitrifying Biofilm Reactor

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ABSTRACT: Bromate (BrO_3^-) is an oxidized contaminant produced from bromide (Br^-) during ozonation and advanced oxidation of drinking water. Previous research shows that denitrifying bioreactors can reduce bromate to innocuous bromide. We studied a hydrogen-based, denitrifying membrane-biofilm reactor (MBfR) for bromate reduction, and report the first kinetics for a hydrogen-based bromate reduction process. A mixed-culture MBfR reduced up to 1,500 $\mu\text{g/L}$ bromate to below 10 $\mu\text{g/L}$ with a 50-min hydraulic residence time. Kinetics were determined using short-term tests on a completely mixed MBfR at steady state with an influent of 5 mg N/L nitrate plus 100 $\mu\text{g/L}$ bromate. Short-term tests examined the impact of pH, nitrite, nitrate, and bromate on bromate reduction rates in the MBfR. Kinetic parameters for the process were estimated based on the short-term bromate tests. The q_{max} for bromate reduction was 0.12 mg $\text{BrO}_3^- \cdot \text{mg}_x^{-1} \cdot \text{day}^{-1}$, and the K was 1.2 mg BrO_3^-/L . This q_{max} is 2–3 times higher than reported for heterotrophic enrichments, and the K is the first reported in the literature. Nitrite and nitrate partially inhibited bromate reduction, with nitrite exerting a stronger inhibitory effect. Bromate was self-inhibitory at concentrations above 15 mg/L, but up to 50 mg/L of bromate had no inhibitory effect on denitrification. The optimum pH was approximately 7. We also examined the performance of an MBfR containing pure culture of the denitrifying bacterium *Ralstonia eutropha*. Under conditions similar to the mixed-culture tests, no bromate reduction was detected, showing that not all denitrifying bacteria are active in bromate reduction. Our results suggest the presence of specialized, dissimilatory bromate-reducing bacteria in the mixed-culture MBfR.

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Introduction

Bromate (BrO_3^-) is an oxidized contaminant produced from bromide (Br^-) during ozonation or advanced oxidation of drinking water. Both molecular ozone (O_3) and hydroxyl radicals (OH^\cdot) contribute to bromate formation (von Gunten and Hoigné, 1994). Bromide in water sources is typically of natural origin, such as from saltwater intrusion and sedimentary rock dissolution (Hutchinson et al., 1997), but also may be from anthropogenic sources, such as ethylene dibromide, a gasoline additive; agricultural chemicals; and burning of biomass from agricultural fields, forests, and savannahs (Davis et al., 2004). In a recent survey of water supplies, bromide was found to range from 3.2 to 58 $\mu\text{g/L}$ (Davis et al., 2004), which would result in 5–100 $\mu\text{g/L}$ bromate if bromide were fully oxidized during ozonation. In other studies, pilot and full-scale drinking water processes showed bromate formation at concentrations as high as 150 $\mu\text{g/L}$ (Kruithof and Meijers, 1995). Bromate is a suspected human carcinogen (Kurata et al., 1992) and has been shown to cause renal tumors in rats and male mice (DeAngelo et al., 1998). In the United States, bromate is regulated as a disinfection by-product at 10 $\mu\text{g/L}$ (Clark and Bouting, 2001).

A number of treatment methods are available for bromate in drinking water (Butler et al., 2005). However, traditional methods, such as coagulation–filtration (Siddiqui et al., 1993), granular activated carbon (Kirisits et al., 2000; Westerhoff, 2003), nanofiltration (Prados-Ramirez et al., 1995) or ultrafiltration (Marhaba and Medlar, 1993), either are not highly effective or produce bromate-containing concentrates requiring subsequent treatment and disposal.

Microbial bromate reduction may be an effective treatment strategy. Bromate is an energetic electron acceptor, with a redox potential exceeding that of oxygen (Butler et al., 2005; Kirisits and Snoeyink, 1999). Previous

research shows that bromate is reduced to innocuous bromide by denitrifying (Hijnen et al., 1995) and chlorate-reducing enrichments (van Ginkel et al., 2005a; Wolterink et al., 2003), possibly via co-metabolic action of nitrate reductase and chlorate reductase enzymes. However, research also showed that continuously exposing an enrichment to bromate increased its specific reduction rates (Hijnen et al., 1999), suggesting dissimilatory bromate reduction, that is, bromate reduction coupled to growth. More recently, researchers have found a heterotrophic enrichment that grows on bromate as the sole electron acceptor (van Ginkel et al., 2005b). For both co-metabolic and dissimilatory growth, nitrate or chlorate may play an important role, serving as a primary electron acceptor that supports growth of bromate-reducing bacteria. Given that nitrate concentrations in drinking water are typically orders of magnitude higher than bromate, nitrate is likely to serve as a primary acceptor in a bioreactor treating water. While nitrate may serve as a primary electron acceptor, it also inhibits bromate reduction (Hijnen et al., 1995; Kirisits and Snoeyink, 1999; Kirisits et al., 2001), so high nitrate concentrations may be detrimental to bromate-reducing processes. Also, high bromate concentrations, in excess of 190 mg/L, were shown to be self-inhibitory (van Ginkel et al., 2005b). Slow bromate reduction rates (Hijnen et al., 1995), despite the favorable energetics, and inhibition at high bromate concentrations may result from the formation of a toxic intermediate, such as bromite (van Ginkel et al., 2005b). Bromite is highly unstable and reactive at neutral pH (van Ginkel et al., 2005b) and would not be expected to accumulate.

In recent years, a number of hydrogen-based bioreactors have been developed for contaminants such as perchlorate (Giblin et al., 2000; Lee and Rittmann, 2002; Miller and Logan, 2000; Nerenberg et al., 2002), chromate (Chung et al., 2006b), and selenate (Chung et al., 2006a). Our

previous research showed that hydrogen-based bromate reduction occurs under denitrifying conditions (Nerenberg and Rittmann, 2004). The objectives of this research were to (1) determine whether a hydrogen-based, denitrifying membrane biofilm reactor (MBfR) can reduce bromate to below the 10- $\mu\text{g/L}$ treatment objective; (2) assess the effects of pH, nitrate, nitrite, and bromate on bromate reduction rates; (3) determine the kinetics of hydrogen-based bromate reduction under denitrifying conditions; (4) explore bromate reduction in a pure-culture, denitrifying MBfR.

Materials and Methods

Experimental Setup

Bromate reduction was explored using a completely mixed, hydrogen-based, membrane-biofilm reactor (MBfR), as described previously (Chung et al., 2006a,b; Lee and Rittmann, 2002; Nerenberg and Rittmann, 2004; Nerenberg et al., 2002). Our experimental MBfR consisted of a bundle of 16 hollow-fiber membranes housed in a 6-mm internal diameter glass tube (Fig. 1). The hollow-fiber membranes were made from microporous polyethylene with a thin, dense polyurethane core (HFM200TL, Mitsubishi Rayon, Tokyo, Japan). The hollow-fiber membrane outside diameter was approximately 280 μm , and the MBfR's total membrane surface area was 35.2 cm^2 . The fibers were connected to a hydrogen-supplying manifold supplied at the bottom end and were sealed individually at the top end. Hydrogen gas was supplied at 34 kPa, unless otherwise noted. The glass tube with the fibers was mounted in a hydraulic circuit, with recirculation via a peristaltic pump (MasterFlex L/S 15, Cole-Palmer, Chicago, IL) at 150 mL/min. The high recirculation rate provided completely mixed conditions and helped avoid excessive biofilm accumulation

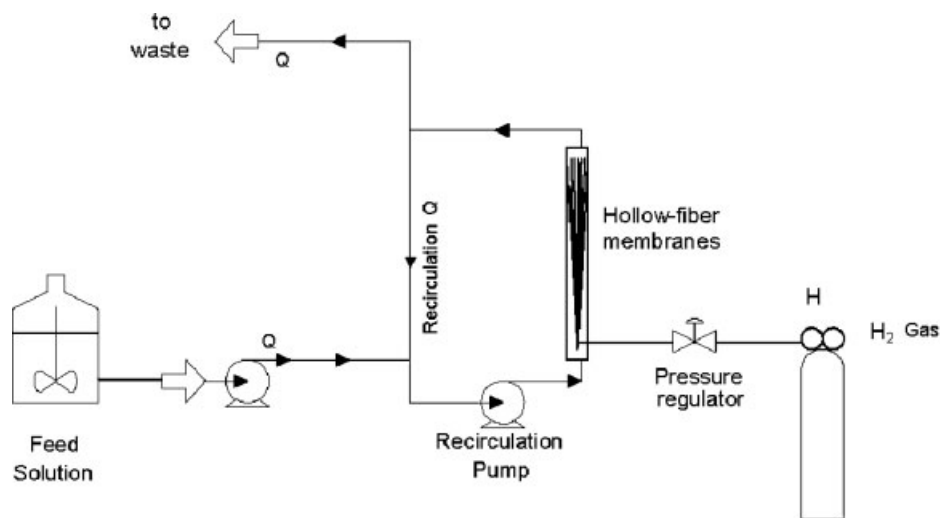


Figure 1. Schematic of MBfR.

(Chang et al., 1991). As a completely mixed reactor, the concentrations in the bulk liquid were approximately equal to the effluent concentrations. A manifold pump (Rainin Dynamax, Oakland, CA) was used to supply the influent medium. The liquid holdup was 25 cm³.

Synthetic Medium

The medium consisted of distilled water amended with 1.386 g Na₂HPO₄, 0.849 g KH₂PO₄, 0.05 g MgSO₄·7H₂O, and 0.025 g (NH₄)₂SO₄ per liter, and aliquots of trace mineral and calcium-iron stock solutions (Nerenberg et al., 2002). NaNO₃ and NaBrO₃ were added to achieve the concentrations shown in Table I. pH adjustments were made with either 1-M NaOH or 1-M HCl. The medium was stored in a 10-L polypropylene carboy with air in the headspace. The influent dissolved oxygen (DO) concentration was approximately 4.5 mg/L.

Bromate Reduction to Below 10 µg/L

Tests were carried out to evaluate the MBfR's ability to reduce bromate to below the 10-µg/L standard. An MBfR was inoculated with 0.1 mL of denitrifying biomass from a previous MBfR that had run for approximately 3 months with 5 mg N/L nitrate plus 1 to 5 mg/L bromate. The influent flow rate was 0.5 mL/min, resulting in a 50-min hydraulic retention time (HRT). Two influent conditions were tested: 5 mg N/L nitrate plus 100 µg/L bromate and 5 mg N/L nitrate plus 1.5 mg/L bromate (low-level bromate tests, Table I). Each influent condition was maintained for at least 21 days.

Short-Term Tests

Short-term, "pseudo-steady-state" tests were used to determine the effects of hydrogen pressure, pH, nitrate, nitrite, and bromate concentrations on bromate reduction rates. The tests were "pseudo-steady-state" because they

were long enough to allow a stable response in effluent concentrations, but not long enough to allow significant changes in the microbial community (Lee and Rittmann, 2002). The MBfR used for these tests was inoculated as described above. The influent flow rate was 1.0 mL/min, resulting in a 25-min HRT. An influent of 5 mg N/L nitrate plus 100 µg/L bromate was maintained as a baseline condition before and in-between short-term tests. Unless noted otherwise, each test lasted 125 min, equivalent to 5 HRTs. Replicate samples were collected at 3, 4, and 5 HRTs. Prior to the next experiment, the MBfR was returned to the baseline influent conditions for at least 24 h. Influent conditions are listed in Table I (short-term tests) in the order in which they were carried out. Bromate removal rates were calculated as the mass of bromate removed per membrane surface area per time (mg m⁻² day⁻¹).

The mean biofilm thickness and total biomass were determined after the last experiment. Biofilm thicknesses were measured for three membranes using a stereomicroscope (Model 48920, Cole-Parmer, Chicago, IL). For each membrane, measurements were taken at the end closest to the hydrogen influent manifold, at the end opposite the manifold, and at a location approximately half way in between. The mean biofilm thickness was used in the kinetic parameter estimation. Total biomass, expressed as volatile solids (VS), was determined by physically removing biofilm from the fibers by scraping with padded forceps and sonicating the fibers for 5 min. The chemical oxygen demand (COD) of the removed biofilm was measured using a colorimetric test (Hach Company, Loveland, CO) and converted to VS using an average ratio of 0.79 g VS:g COD. The biofilm density (g VS/m³) was calculated by dividing the total volatile solids by the biofilm volume, which in turn was determined by the biofilm's average cross-sectional area and the total length of fibers.

Kinetic Parameter Estimation

Kinetic parameters for bromate reduction were estimated using data from the short-term tests with bromate as a

Table I. Influent conditions used in this study.

Experiment	Condition	Flow (mL/min)	BrO ₃ ⁻ (µg/L)	NO ₃ ⁻ (mg N/L)	NO ₂ ⁻ (mg N/L)	pH	H ₂ (kPa)
Low-level bromate tests	Steady state 1	0.5	0	5	0	7	34
	Steady state 2	0.5	100	5	0	7	34
	Steady state 3	0.5	1,500	5	0	7	34
Short-term tests	Baseline	1.0	100	5	0	7	34
	Short term 1	1.0	100	5	0	7	34–69
	Short term 2	1.0	100	0–30	0	7	34
	Short term 3	1.0	50–50,000	0	0	7	34
	Short term 4	1.0	30,000–50,000	5	0	7	34
	Short term 5	1.0	100	0	0–10	7	34
	Short term 6	1.0	100	0	0	4–9	34
Pure culture tests	Steady state 1	1.0	0	5	0	7	34
	Steady state 2	1.0	100	5	0	7	34
	Short term 3	1.0	100	0	0	7	34

Concentrations listed are the targeted concentrations.

variable. A biofilm model that included diffusion and reaction was developed and utilized for parameter fitting. The model assumed a constant biofilm thickness of 340 μm , which corresponded to the experimental value. The model was solved by a finite differences method, where the biofilm was divided into 340 one- μm -thick sections. For a given biofilm section, i , the bromate flux contributed by that section was determined from the following equation:

$$J_i = q_{\max} \frac{S_i}{K + S_i} X_f \Delta L_f \quad (1)$$

where J_i is the bromate flux provided by biofilm section i ($\text{g BrO}_3^- \cdot \text{m}^{-2} \cdot \text{day}^{-1}$), q_{\max} is the maximum specific rate of substrate utilization ($\text{mg BrO}_3^- \cdot \text{mg}_x^{-1} \cdot \text{day}^{-1}$), S_i is bromate concentration at the interior of section i ($\text{mg BrO}_3^-/\text{L}$), K is the half-maximum rate constant ($\text{mg BrO}_3^-/\text{L}$), ΔL_f is the thickness of biofilm section i (1 μm), and X_f is the biofilm density expressed in terms of volatile solids ($\text{g VS}/\text{m}^3$). The cumulative flux passing through section i was determined by adding the fluxes from all sections deeper in the biofilm, that is,

$$J_{\text{cum},i} = \sum_{k=1}^i J_k \quad (2)$$

where $J_{\text{cum},i}$ is the cumulative bromate flux through section i , and J_k is the flux contribution from section k , where $k = 1$ corresponds to the biofilm attachment surface. Once the total flux through section i was known, the bromate concentration at section $i + 1$ was determined from Fick's law, assuming linear diffusion through the section:

$$S_{i+1} = S_i + J_{\text{cum},i} \frac{\Delta L_f}{D_f} \quad (3)$$

where D_f is the diffusivity of bromate through the biofilm ($0.5 \text{ cm}^2/\text{day}$) and S_i is the bromate concentration at section i ($\text{mg BrO}_3^-/\text{L}$). D_f was estimated from nitrate's diffusivity (Rittmann and McCarty, 2001) using a molecular weight correction (Thibodeaux, 1979). Substrate changes through the liquid diffusion layer (L) also were estimated using Fick's law (Eq. 3). In this case, the diffusivity of bromate through water ($D = 0.59 \text{ cm}^2/\text{day}$) was used instead of D_f , and L was used instead of ΔL_f . The L was estimated as 7 μm , based on the mass transfer coefficient, k_m , where $k_m = D/L$ (Rittmann and McCarty, 2001). k_m was estimated using a modified Sherwood number (Horn and Hempel, 1995) as shown by Thibodeaux (1979). S_1 , the concentration at the attachment surface, was not measured. However, for an arbitrary set of kinetic parameters q_{\max} and K , a unique S_1 exists that, when input into the biofilm model, provides the experimentally measured bulk liquid concentration S . The model-predicted total flux can be calculated from Eq. (2), where $i = 340$, that is, the cumulative flux at the outermost section of the biofilm. When the best-fit q_{\max} and K values are used, the S_1

value that provides the experimental S also provides a total flux that matches the experimental value. This step-wise calculation of bromate concentration through the biofilm and local flux values was performed for each pair of flux and bulk-liquid bromate concentration from the short-term experiments with bromate. The best-fit q_{\max} and K values were determined using relative least-squares minimization (Sáez and Rittmann, 1992) for the entire data set. An iterative process of fitting the bulk-liquid bromate concentration and adjusting the q_{\max} and K values to reduce the error associated with the overall flux was performed.

Pure-Culture Experiment

A third MBfR was constructed as described above to test the ability of a pure-culture denitrifying biofilm to reduced bromate. If the dissimilatory nitrate reductase is responsible for bromate reduction, all denitrifying organisms should be capable of reducing bromate. *Ralstonia eutropha* (ATCC No. 17697) was used to quantify co-metabolic bromate reduction. *R. eutropha* is a hydrogen-oxidizing autotroph and expresses both periplasmic (NAP) and membrane bound (NAR) nitrate reductase enzymes (Siddiqui et al., 1993). The synthetic medium was as described above. Prior to inoculation, the MBfR was sterilized with 6% hydrogen peroxide for 8 h. The influent was filter-sterilized into a sterile feed carboy using an in-line filter (Pall Corporation, Ann Arbor, MI). MBfR effluent was plated weekly on aerobic agar plates (R2A agar, Becton, Dickinson and Company, Sparks, MD) to confirm purity. The influent flow rate was 1 mL/min, and an influent medium containing 5 mg N/L nitrate was used for the first 21 days (Pure-culture tests, Table I). Bromate was added to the influent on day 21, and operated for an additional 21 days. One short-term experiment was conducted with 100 $\mu\text{g}/\text{L}$ bromate and no nitrate in the influent. The short-term experiment was conducted as described previously.

Sampling and Analytical Techniques

Influent and effluent samples were collected daily and filtered with 0.45 μm syringe filters (IC Acrodisc, Pall Corporation). Samples were stored at 4°C until analyzed. Bromate, bromide, nitrate, and nitrite were monitored by ion chromatography (IC2500 with AS19/AG19 column, bromate detection limit of 5 $\mu\text{g}/\text{L}$; Dionex Corp, Sunnyvale, CA) with a sodium hydroxide eluent. The IC program used for analysis was: 5 min equilibration with 4 mM NaOH; injection of sample; 10 min at 10 mM NaOH; ramp from 10 mM NaOH to 45 mM NaOH over 15 min; and 25 min at 45 mM NaOH. The eluent flow rate was 1 mL/min, and the suppressor current was 115 mA. The injection volume was 200 μL . Each sample from the short-term tests was analyzed in triplicate. pH was measured with a standard glass electrode (Fisher Scientific International).

Results

Bromate Reduction to Below 10 $\mu\text{g/L}$

After operating the MBfR with a 5 mg N/L nitrate influent for 21 days, 100 $\mu\text{g/L}$ bromate was added to the nitrate medium. Bromate was immediately reduced to below the 5- $\mu\text{g/L}$ detection limit. After operating under these conditions for 21 days, the influent bromate was increased to 1.5 mg/L. Initially, effluent bromate increased to approximately 60 $\mu\text{g/L}$. However, during the next 9 days, the effluent bromate gradually decreased to below 10 $\mu\text{g/L}$ and remained there for an additional 20 days. Nitrate was reduced to below the detection limit throughout the experiment. Bromide accumulation from bromate was approximately stoichiometric.

Short-Term Tests

A second MBfR was used for short-term tests. After 25 days of operation, nitrate was reduced from 5 mg N/L (influent) to 1–2.5 mg N/L (effluent), with 0.3–1.5 mg N/L nitrite accumulation, and bromate was reduced from 100 to 40–90 $\mu\text{g/L}$. The average effluent bromate concentration was 65 ± 10 $\mu\text{g/L}$ over 120 days of operation. Effluent nitrate was below 2 mg N/L and nitrite below 1 mg N/L for nearly the entire period of operation. Effluent bromate and nitrate concentrations returned to these baseline values following each short-term test. Hydrogen gas pressures ranging from 34 to 69 kPa showed no effect on nitrate or bromate reduction rates, indicating that hydrogen was not rate limiting (data not shown). Bromate reduction rates significantly decreased with increasing nitrate concentrations (Fig. 2). For 20 mg N/L effluent nitrate, the bromate

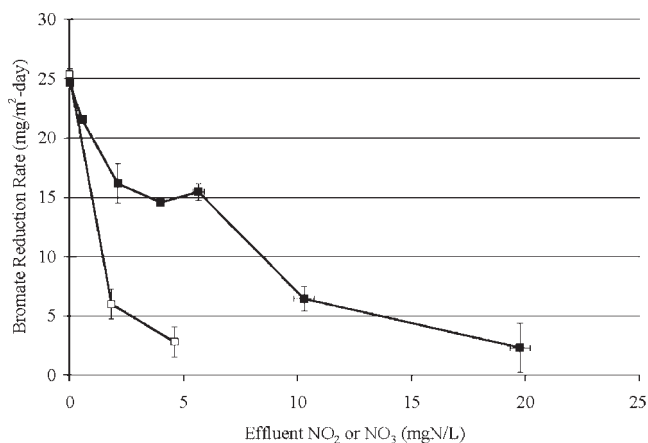


Figure 2. Effect of nitrate (■) and nitrite (□) on bromate reduction rates. Error bars show one standard deviation for three samples analyzed in triplicate ($n=9$). The influent bromate was 100 $\mu\text{g/L}$.

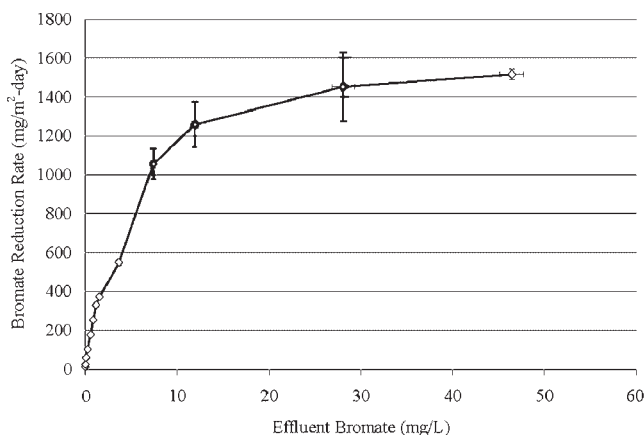


Figure 3. Effect of effluent bromate concentrations on bromate reduction rates. Error bars show one standard deviation for three samples analyzed in triplicate ($n=9$).

reduction rate decreased to 10% of the rate observed with no nitrate. For nitrite, reduction rates decreased to approximately 10% of their initial value at only 4 mg N/L nitrite. The effect of the bromate concentration on bromate reduction rates is shown in Figure 3. The bromate reduction rates increased linearly with effluent bromate concentrations up to 2 mg/L and reached a plateau at around 30 mg/L. For short-term tests with effluent bromate concentrations above 5 mg/L, the bromate reduction rates decreased between the first sample (75 min) and the second and third samples (100 and 125 min) of the short-term tests. The effect was small for effluent concentrations below 12 mg/L, but significant when the influent bromate was 30 and 50 mg/L, where the bromate removal rates decreased by 83% between the first and third samples (Fig. 4). For the two highest influent

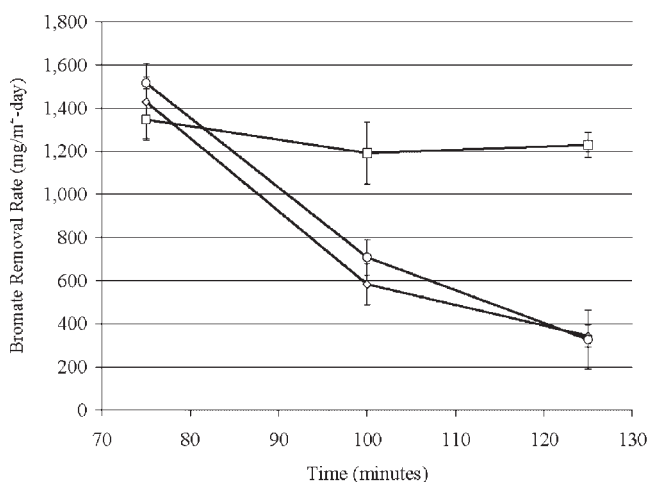


Figure 4. Bromate reduction rates decrease over time when high concentrations of bromate are present in the reactor; effluent bromate concentrations at three HRTs of 12 mg/L (□), 24 mg/L (◇), and 45 mg/L (○). Error bars show one standard deviation for one sample analyzed in triplicate ($n=3$).

concentrations, only the first sample was included in Figure 3, which assumes that the first sample was not significantly affected by inhibition. The error bars for those two points are for the triplicate analyses carried out on the first sample. Denitrification rates were unaffected by bromate concentrations as high as 50 mg/L (data not shown). However, bromate self-inhibition was evident from the significant decrease in bromate reduction rates over the course of each short-term experiment, similar to the effect shown in Figure 4. The effect of pH on bromate reduction rates also was assessed via short-term tests with 100 $\mu\text{g/L}$ bromate in the influent. A maximum removal rate of 25 mg $\text{BrO}_3^- \text{ m}^{-2} \text{ day}^{-1}$ occurred at pH of 7, with decreased bromate reduction at higher and lower pH. Bromate reduction rates were higher than expected at low pH values, with reduction rates of 15 mg $\text{BrO}_3^- \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ at pH 4, while reduction rates decreased to 5 mg $\text{BrO}_3^- \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ at pH greater than 8.

Bromate Reduction Kinetics

Kinetic parameters were estimated based short-term test results (Fig. 5). The average biofilm thickness was 340 μm and total biomass was 104 mg VS, resulting in a biofilm density of 39,000 mg VS/L. The best-fit parameters were $q_{\text{max}} = 0.12 \text{ mg BrO}_3^- \cdot \text{mg}_x^{-1} \cdot \text{day}^{-1}$ (or $0.0064 \text{ mg H}_2 \cdot \text{mg}_x^{-1} \cdot \text{day}^{-1}$) and $K = 1.3 \text{ mg BrO}_3^-/\text{L}$. Although the bromate reduction rates may be affected by bromate self inhibition at higher concentrations, the parameters determined from only the data points with effluent bromate concentrations below 1.5 mg/L, which had no inhibition over time, were essentially identical to the above.

Pure Culture Experiment

The MBfR with *R. eutropha* exhibited almost complete denitrification after 21 days of operation. When 100 $\mu\text{g/L}$

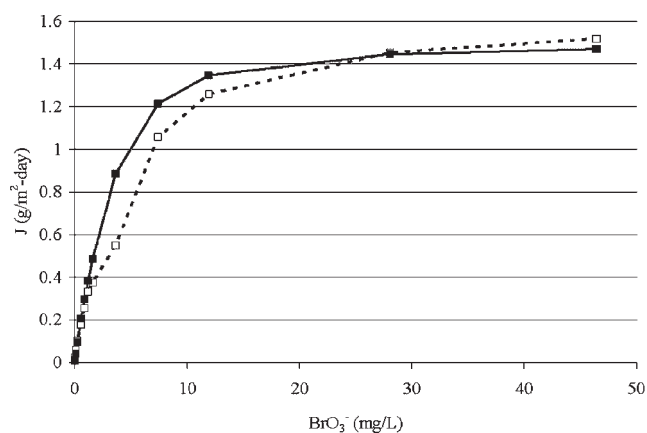


Figure 5. Curve-fitting for kinetic parameter estimation. Experimental flux (□) and calculated flux (■). The best-fit parameters are $q_{\text{max}} = 0.12 \text{ mg BrO}_3^- \text{ mg}_x^{-1} \text{ day}^{-1}$ and $K = 1.3 \text{ mg BrO}_3^-/\text{L}$.

bromate was added to the influent, no bromate reduction was detected during an additional 21 days of operation (data not shown.) A short-term experiment with 100 $\mu\text{g/L}$ bromate and no nitrate also was conducted, and no bromate reduction was detected.

Discussion

Bromate Reduction to Below 10 $\mu\text{g/L}$

This experiment confirmed the MBfR's ability to reduce 100–1,500 $\mu\text{g/L}$ bromate to below the 10- $\mu\text{g/L}$ standard, showing its promise for treating low-level bromate in drinking water. When the influent was increased from 100 to 1,500 $\mu\text{g/L}$, the effluent initially spiked to above 60 $\mu\text{g/L}$, but then gradually decreased over 10 days. The gradual response suggests a selection for bacteria that grow on bromate. This result is similar to perchlorate reduction in a denitrifying MBfR, where adding perchlorate in a mixed-culture denitrifying bioreactor also resulted in a gradual decrease in the effluent perchlorate concentrations (Nerenberg et al., 2002).

Short-Term Experiments

Nitrate inhibition of bromate reduction has been noted previously (Hijnen et al., 1995, 1999; Kirisits et al., 2001); however, this is the first report of nitrite inhibition. If bromate is reduced co-metabolically via the nitrate reductase enzyme, then nitrate should inhibit bromate reduction competitively (Levenspiel, 1998). However, since nitrite is not a substrate for the nitrate reductase, its inhibition is likely to be non-competitive. If bromate is reduced via a specialized enzymatic pathway, nitrate and nitrite could be competitors for the active enzyme, or could inhibit bromate at a regulatory level. The situation is similar to nitrate inhibition of perchlorate reduction in perchlorate-reducing bacteria, where nitrate inhibits perchlorate reduction despite separate reduction pathways for perchlorate and nitrate (Kengen et al., 1999; Xu et al., 2003).

Bromate reduction rates increased with the effluent bromate concentrations up to approximately 30 mg/L, where it began to plateau. The maximum bromate removal rate was approximately $1.5 \text{ g BrO}_3^- \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ (equivalent to $0.05 \text{ mg H}_2 \cdot \text{mg}_x^{-1} \cdot \text{day}^{-1}$), which occurred with an effluent bromate concentration of approximately 46 mg/L. These results are consistent with Monod kinetics in a biofilm system, where the high substrate concentrations lead to kinetic saturation throughout the biofilm. At higher bromate concentrations, bromate reduction decreased over time, suggesting self-inhibition. van Ginkel et al. (2005b) reported self-inhibition of suspended cultures for initial bromate concentrations of 190 mg/L. This is the first evidence of bromate self inhibition at much lower concentrations. Bromate self-inhibition probably is due to the formation of bromite or other toxic intermediates.

Bromate's lack of inhibition on denitrification may result from weak competition for the nitrate reductase, which is consistent with previous work (Morpeth and Boxer, 1985). However, a lack of inhibition also is consistent with bromate reduction by a small population of dissimilatory bromate-reducing bacteria, possibly also denitrifiers, where the bromate-reducers alone are subject to the toxic effect from bromate reduction. As discussed previously, bromite is a likely toxic intermediate that would not be expected to accumulate, due to its high reactivity.

Bromate Reduction Kinetics

Our q_{\max} of $0.12 \text{ mg BrO}_3^- \cdot \text{mg}_x^{-1} \cdot \text{day}^{-1}$ is 2–3 times greater than reported in the literature for heterotrophic bromate-reducing enrichments. Our K value of $1.3 \text{ mg BrO}_3^-/\text{L}$ is the first reported K value for bromate reduction. For hydrogen oxidizing, autotrophic denitrifying bacteria, previous studies showed a $q_{\max} = 0.38\text{--}0.74 \text{ mg N} \cdot \text{mg}_x^{-1} \cdot \text{day}^{-1}$ (equivalent to $0.14\text{--}0.26 \text{ mg H}_2 \cdot \text{mg}_x^{-1} \cdot \text{day}^{-1}$) (Rezania et al., 2005) and $K = 0.18\text{--}0.16 \text{ mg N/L}$ (Kurt et al. 1987). For hydrogen oxidizing, autotrophic perchlorate-reducing bacteria, the parameters were $q_{\max} = 3.1 \text{ mg ClO}_4^- \cdot \text{mg}_x^{-1} \cdot \text{day}^{-1}$ (equivalent to $0.25 \text{ mg H}_2 \cdot \text{mg}_x^{-1} \cdot \text{day}^{-1}$) and $K = 0.14 \text{ mg ClO}_4^-/\text{L}$ (Nerenberg et al., 2006). Our bromate reduction kinetics are clearly slower than kinetics for autotrophic denitrification and perchlorate reduction.

Our q_{\max} was determined assuming the all biofilm biomass was capable of bromate reduction. However, results from the pure-culture MBfR tests and short-term tests suggest that bromate-reducing bacteria may be a sub-population of the denitrifying community. If this were true, the q_{\max} specific to bromate-reducing bacteria alone could be much higher. However, our parameters are applicable to a denitrifying, bromate-reducing bioreactor under drinking water treatment conditions.

Pure Culture Experiment

The pure-culture results showed that not all denitrifying bacteria can reduce bromate at high rates. A similar situation exists for perchlorate and chlorate-reducing bacteria, most of which are denitrifiers but possess a specialized pathway for perchlorate reduction (Kengen et al., 1999; Xu et al., 2003). While chlorate, and presumably perchlorate, are substrates for the dissimilatory nitrate reductase (De Groot and Stouhamer, 1969; Hackenthal et al., 1964), the reduction rates on the specialized reduction pathway are much greater (Logan et al., 2001; Nerenberg et al., 2006).

Conclusions

Our research demonstrates that a hydrogen-based MBfR can reduce bromate to below $10 \text{ }\mu\text{g/L}$ under denitrifying

conditions. Bromate, nitrate, and nitrite inhibited bromate reduction, with nitrite as a stronger inhibitor, but bromate did not inhibit denitrification. Bromate reduction followed Monod kinetics when bromate was the rate-limiting substrate. Kinetic parameters for bromate reduction were $q_{\max} = 0.12 \text{ mg BrO}_3^- \cdot \text{mg}_x^{-1} \cdot \text{day}^{-1}$ and $K = 1.3 \text{ mg}_s \text{ L}^{-1}$. Results from continuous and short-term experiments with mixed culture reactors, and the lack of bromate reduction by a pure culture biofilm of *R. eutropha* in the MBfR, suggest that specialized bromate-reducing bacteria were present in our MBfR.

Nomenclature

D	diffusivity of bromate through water ($0.59 \text{ cm}^2/\text{day}$)
D_f	diffusivity of bromate through biofilm ($0.5 \text{ cm}^2/\text{day}$)
HRT	hydraulic retention time
J	bromate flux through biofilm ($\text{g BrO}_3^- \cdot \text{m}^{-2} \cdot \text{day}^{-1}$)
K	half maximum rate constant ($\text{mg BrO}_3^-/\text{L}$)
L	liquid diffusion layer thickness
ΔL_f	thickness of the generic biofilm section used in the finite-differences biofilm model
MBfR	hollow-fiber membrane biofilm reactor
q_{\max}	maximum specific growth rate ($\text{mg BrO}_3^- \cdot \text{mg}_x^{-1} \cdot \text{day}^{-1}$)
S	bromate concentration (mg/L)
VS	volatile solids
X_f	biofilm density ($\text{g VS}/\text{m}^3$)

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