

## **Microbial Bromate Reduction in a Hydrogen-Based, Membrane Biofilm Reactor: Inhibitory Mechanisms**

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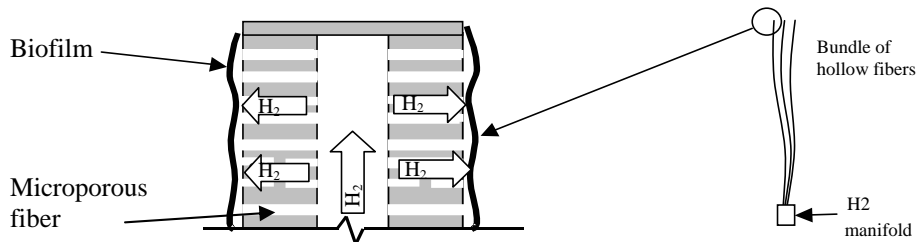
### **Abstract**

The biological reduction of bromate ( $\text{BrO}_3^-$ ) in a hydrogen based membrane biofilm reactor (MBfR) was investigated to determine (1) if bromate could be reduced below the MCL of 10  $\mu\text{g/L}$  and (2) the inhibitory impacts of nitrate and bromate concentration on bromate reduction. Reduction of bromate to below the 10  $\mu\text{g/L}$  MCL was achieved with an influent nitrate concentration of 5  $\text{mgN/L}$  and influent bromate concentrations ranging from 150  $\mu\text{g/L}$  to 1,500  $\mu\text{g/L}$ . Pseudo-steady state experiments were conducted to examine the influence of nitrate and bromate on bromate reduction. Nitrate was shown to inhibit bromate reduction at concentrations as low as 0.5  $\text{mgN/L}$ . Bromate reduction followed Monod-type kinetics, with increased bromate reduction being achieved until an effluent bromate of 15  $\text{mg/L}$  was reached. Over an extended period of exposure to bromate concentrations greater than 15  $\text{mg/L}$ , bromate appeared to exhibit an inhibitory effect on bromate reduction. The production of bromite, an intermediate of bromate reduction, may be responsible for this inhibition.

### **Introduction**

Bromate ( $\text{BrO}_3^-$ ) is produced from bromide ( $\text{Br}^-$ ) during ozonation or advanced oxidation of drinking water. Bromate is a suspected human carcinogen (Kurata 1992), and the maximum contaminant level (MCL) is 10  $\mu\text{g/l}$  (Clark 2001). Since bromate has been found at concentrations as high as 150  $\mu\text{g/l}$  following ozonation and advanced oxidation of drinking water (Krasner 1993), bromate management is key when such processes are used (Butler 2005). Treatment of bromate in the  $\text{mg/L}$  range may be required for reject waters from membrane filtration or regeneration brines from ion exchange systems. In both cases, high nitrate concentrations may also be present and may require treatment.

Biological reduction may be effective for treating low or high concentrations of bromate, with or without nitrate. A new reactor, the hollow-fiber membrane biofilm reactor (MBfR), may be an ideal vehicle for microbial bromate reduction in drinking water. The MBfR utilizes small-diameter hollow-fiber membranes to deliver hydrogen as a safe, non-toxic, sparsely-soluble, and inexpensive biological reductant. Hydrogen diffuses through the dry, hydrophobic pores of the membrane to biofilm naturally forming on the walls of the fiber (Figure 1). The MBfR has been studied for denitrification (Lee 2000; Lee and Rittmann 2002), perchlorate reduction (Nerenberg et al. 2002), selenate reduction (Chung et al. 2006), and chromate reduction (Chung et al. in press). Preliminary tests show it can be effective for bromate reduction to innocuous bromide as well, although these tests were carried out over short time periods, and with poor detection levels (Nerenberg and Rittmann 2004).



**Figure 1 Section of fiber (left) and schematic of hollow fiber membrane bundle (right)**

Two mechanisms may account for bromate reduction. First, denitrifying bacteria (Hijnen et al. 1995) and chlorate-reducing bacteria (van Ginkel et al. 2005a) reduce bromate co-metabolically under denitrifying or chlorate-reducing conditions. Co-metabolic reduction means that bromate reduction is carried out fortuitously, without providing energy for growth. Second, some bacteria grow on bromate as a primary electron acceptor (van Ginkel et al. 2005b). Both cometabolic and dissimilatory pathways may be responsible for bromate reduction in a mixed microbial biofilm treating nitrate and bromate. Understanding the interplay of these two reduction pathways is critical in designing an efficient reactor to treat waters containing bromate. Our research for the first time confirms the ability of an MBfR to reduce bromate to below the MCL, and examines the effects of the bromate and nitrate concentrations on bromate-reducing fluxes in an MBfR. This research provides both fundamental insight into the mechanisms of biological bromate reduction as well as practical insights for the application of a hydrogen based MBfR for treatment of bromate in drinking water.

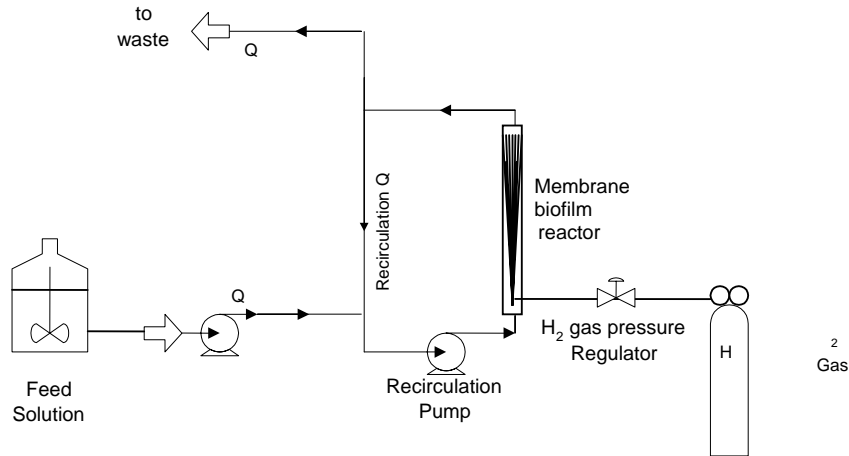
## **Methods**

### *Experimental Set up*

The hydrogen-based MBfR used in our research was similar to that used previously (Lee and Rittmann 2002; Nerenberg and Rittmann 2004; Nerenberg et al. 2002). It consisted of a bundle of 16 hollow-fiber membranes housed in glass tubing (Figure 2). The hollow-fiber membranes were made from a composite material consisting of microporous polyethylene encasing a dense, polyurethane core (HFM200TL, Mitsubishi Rayon, Japan). The membrane outside diameter was approximately 280  $\mu\text{m}$ , and the total membrane surface area was 30.5  $\text{cm}^2$ . Hydrogen gas was supplied to the inside of the hollow fiber membranes at a pressure of 5 psi, allowing it to diffuse through the membranes into a hydrogen-oxidizing biofilm that formed on the fibers' outside surface. The total volume of the reactor was 25  $\text{cm}^3$ . The reactor was inoculated with activated sludge from the local wastewater treatment facility (Mishawaka Wastewater Treatment Plant, Mishawaka, IN).

### *Synthetic Medium*

A synthetic medium was prepared from distilled water amended with 1.386 g  $\text{Na}_2\text{HPO}_4$ , 0.849 g  $\text{KH}_2\text{PO}_4$ , 0.05 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.025 g  $(\text{NH}_4)_2\text{SO}_4$  per liter, as well as a trace mineral solution (Nerenberg 2002). The medium was supplemented with  $\text{NaNO}_3$  and  $\text{NaBrO}_3$  to achieve the desired nitrate and bromate concentrations, respectively.



**Figure 2 Schematic of MBfR**

*Preliminary Testing*

Preliminary tests were conducted to determine if a denitrifying MBfR could reduce bromate to below the 10 µg/L MCL. Influent conditions are summarized in Table 1. The influent bottle was open to the atmosphere and contained an average of 6.5 mg/L of dissolved oxygen (O<sub>2</sub>). Influent flow rate was 0.5 mL/min, resulting in a hydraulic retention time of 50 minutes. The recirculation rate in the reactor was 150 mL/min, providing well-mixed condition in the reactor.

**Table 1 Sustained bromate reduction in the presence and absence of nitrate was tested under the following loading conditions during preliminary testing**

Day	Nitrate (mgN/L)	Bromate (mg/L)
1-21	5	0.1
22-50	5	1.5
51-95	5	5.0

*Inhibition Mechanisms*

An identical MBfR was used to determine (1) the effect of nitrate on bromate reduction and (2) the effect of bromate on bromate reduction. For these tests, the influent flow rate was doubled to 1.0 mL/min, resulting in a hydraulic retention time (HRT) of 25 minutes. The shorter HRT was needed to allow the reactor to equilibrate more quickly, shortening the duration of each test. The influent nitrate concentration was maintained at 5 mgN/L, while the bromate concentration was 100 µg/L. After steady-state removal of nitrate and bromate were achieved, short-term tests were conducted. The tests were conducted over short periods of time, usually less than two hours, to avoid changes in the biomass quantity and community structure (Lee and Rittmann

2002). Short term tests were conducted over 5 HRTs. Pseudo steady-state conditions were achieved after 3 HRTs, and samples were taken at 3, 4, and 5 HRTs. The MBfR was returned to steady-state conditions (5 mgN/L nitrate and 100 µg/L) for a period of at least 24 hours between each short-term test. Influent nitrate concentration were varied from 0 to 30 mgN/L while the influent bromate was maintained at 100 µg/L. For bromate tests, the influent nitrate was 0 mgN/L and bromate ranged from 50 µg/L to 50 mg/L. The impact of high bromate concentration on denitrification was also tested with 50 mg/L bromate and 5 mgN/L nitrate in the influent.

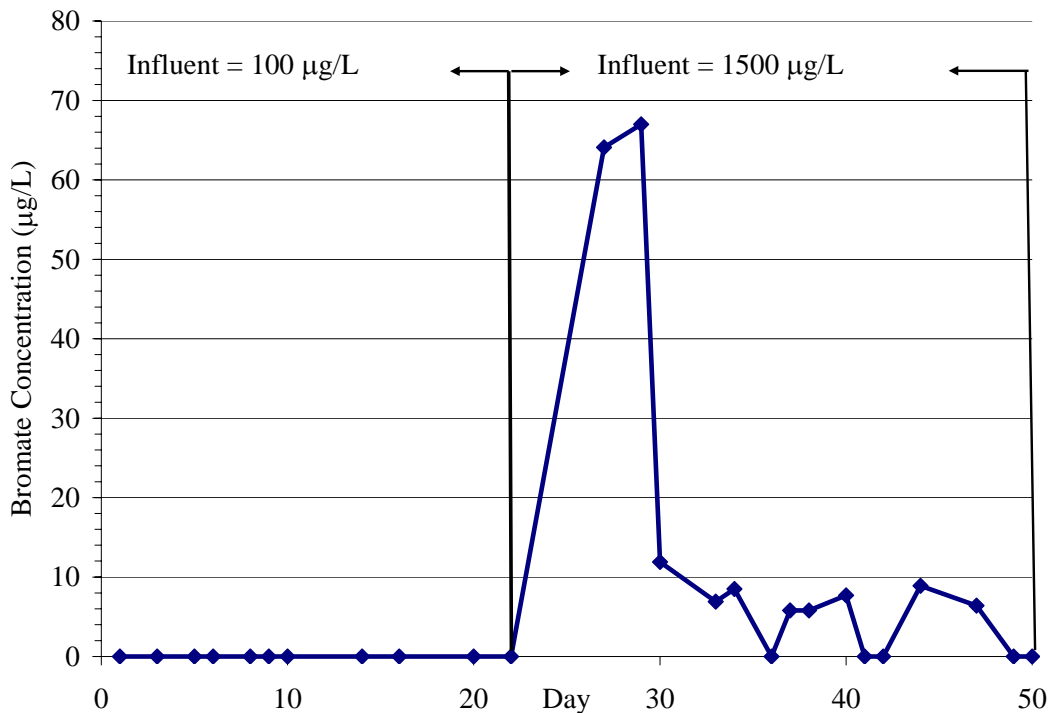
### *Analytical Techniques*

Bromate, bromide, nitrate, and nitrite were monitored by ion chromatography (IC2500 with AS19/AG19 column, method detection limit of 5 µg/L; Dionex Corp, Sunnyvale CA.) with a sodium hydroxide eluant.

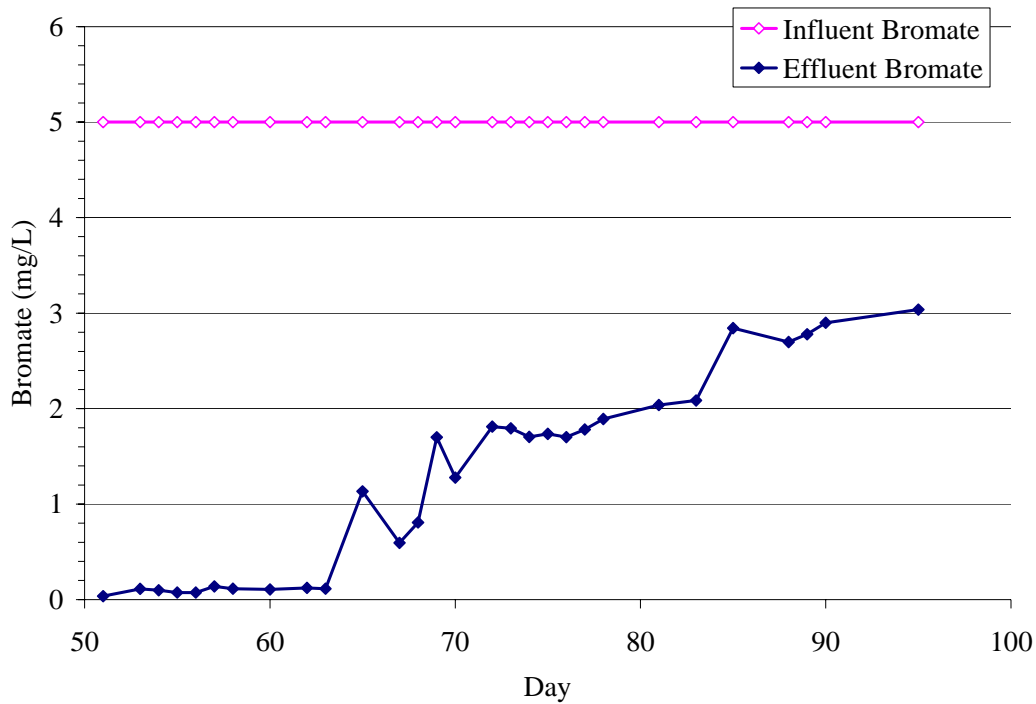
## **Results**

### *Preliminary Testing*

Bromate was reduced concurrently with 5 mgN/L nitrate to below the 10 µg/L MCL with influent bromate concentrations of 100 and 1,500 µg/L (Figure 3). However, when the influent bromate concentration was increased to 5 mg/L, effluent bromate initially increased to 0.1 mg/L, and progressively increased over 30 days to over 3 mg/L (Figure 4). In all cases, nitrate was reduced to below 20 µgN/L.



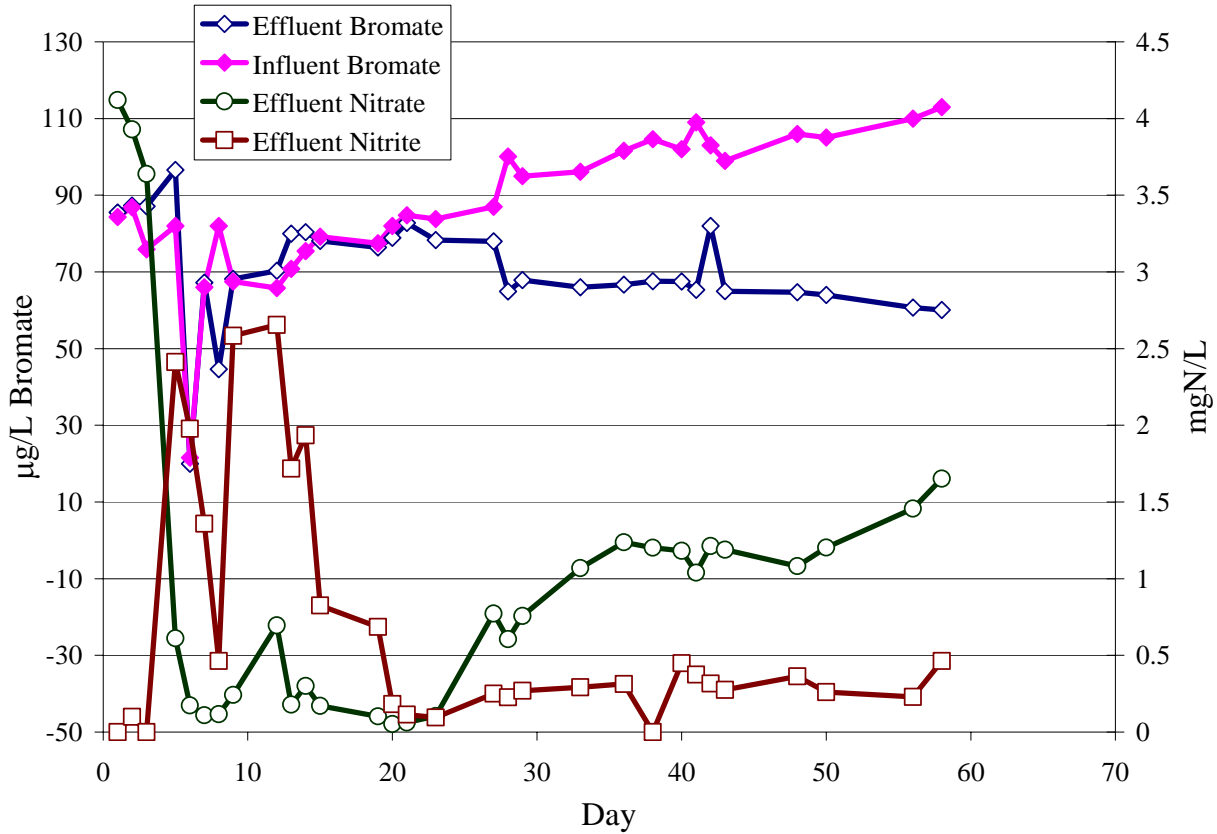
**Figure 3 Typical bromate concentration are reduced to below the 10 µg/L MCL**



**Figure 4 Prolonged exposure to 5 mg/L bromate results in reduced bromate reduction capabilities**

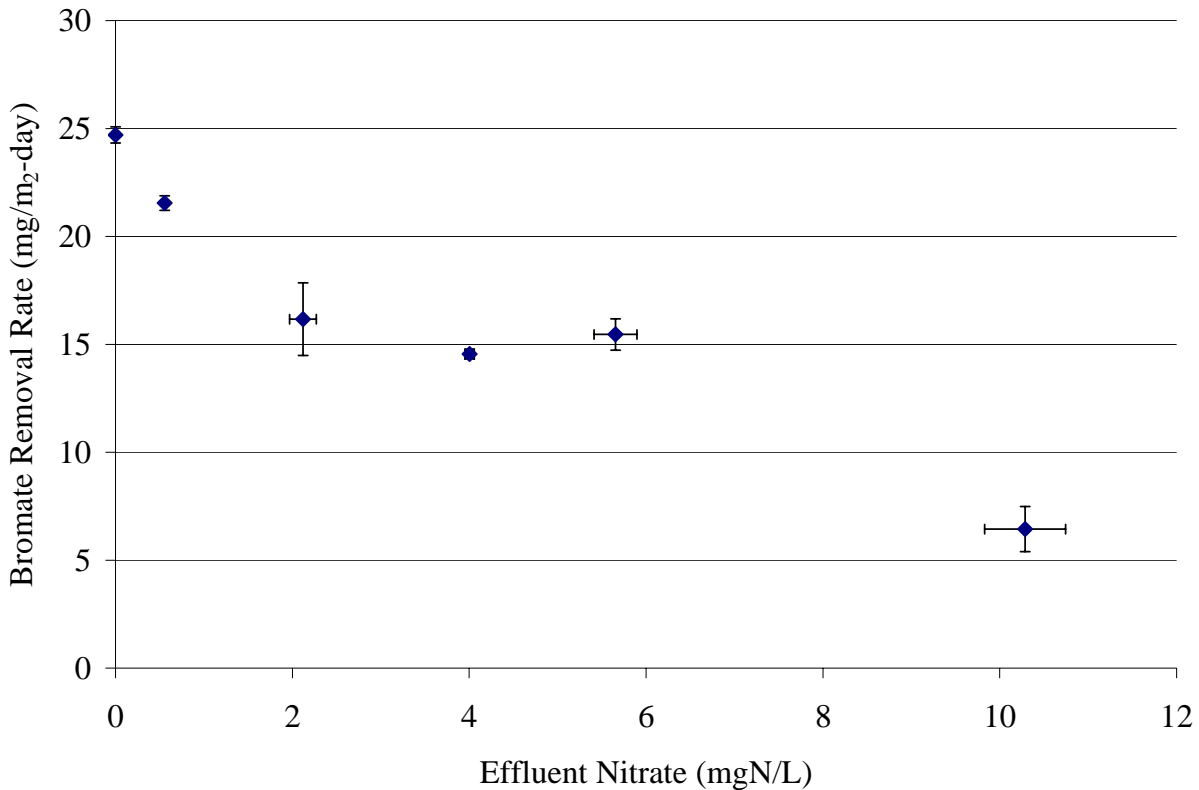
*Inhibition Mechanism*

A new MBfR initially was supplied with 5 mgN/L nitrate and 100 µg/L bromate for 60 days. Steady-state effluent concentrations of bromate, nitrate, and nitrite were achieved after 35 days. Full bromate and nitrate removal was not achieved, presumably because the HRT was half that used in the preliminary tests. Results of the start-up period are shown in Figure 5. These results show that bromate reduction did not occur until the sum of nitrate and nitrite in the effluent was below 1.5 mgN/L.



**Figure 5** When nitrate and bromate are included at start-up, bromate reduction lags behind nitrate reduction

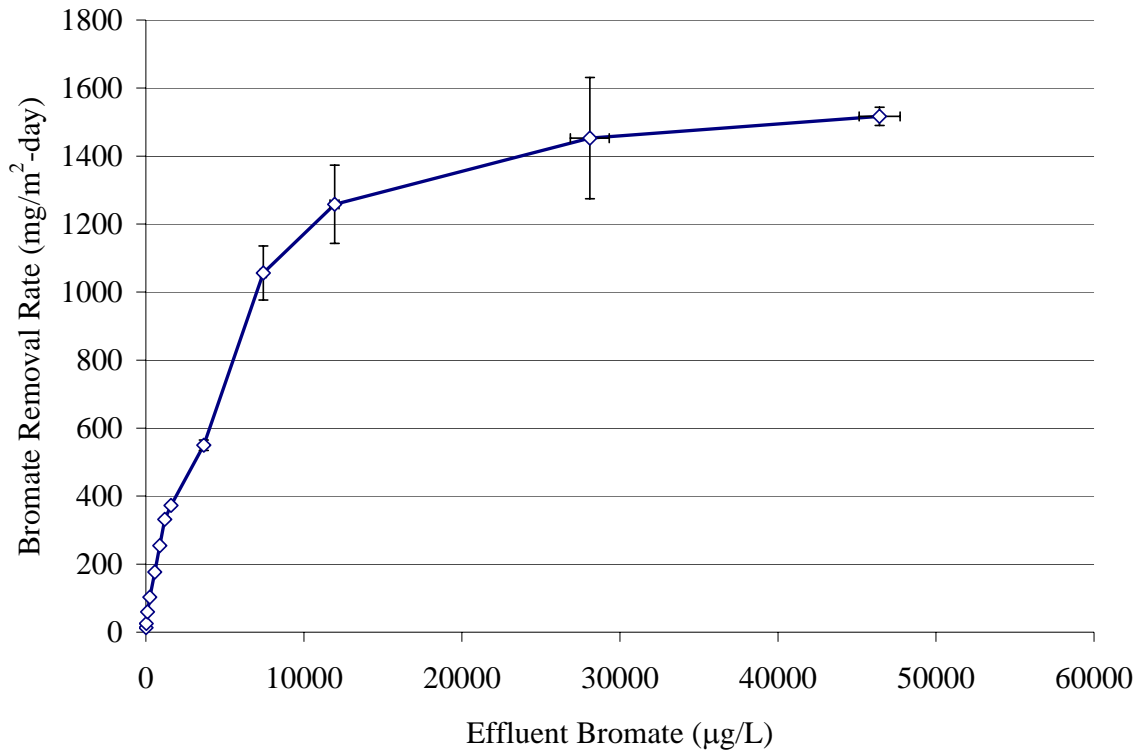
The impact of the nitrate concentration on bromate reduction was explored with short-term tests. Influent nitrate concentrations ranged from 0 to 15 mgN/L, while bromate was maintained at 100 µg/L. A bromate removal flux of 25 mg/m<sup>2</sup>-day was achieved with 0 mgN/L nitrate in the effluent. The bromate removal flux was 15 mg/m<sup>2</sup>-day for effluent nitrate between 2 and 5 mgN/L, and decreased to 5 mg/m<sup>2</sup>-day when the effluent nitrate was 7.5 mg/L (Figure 6).



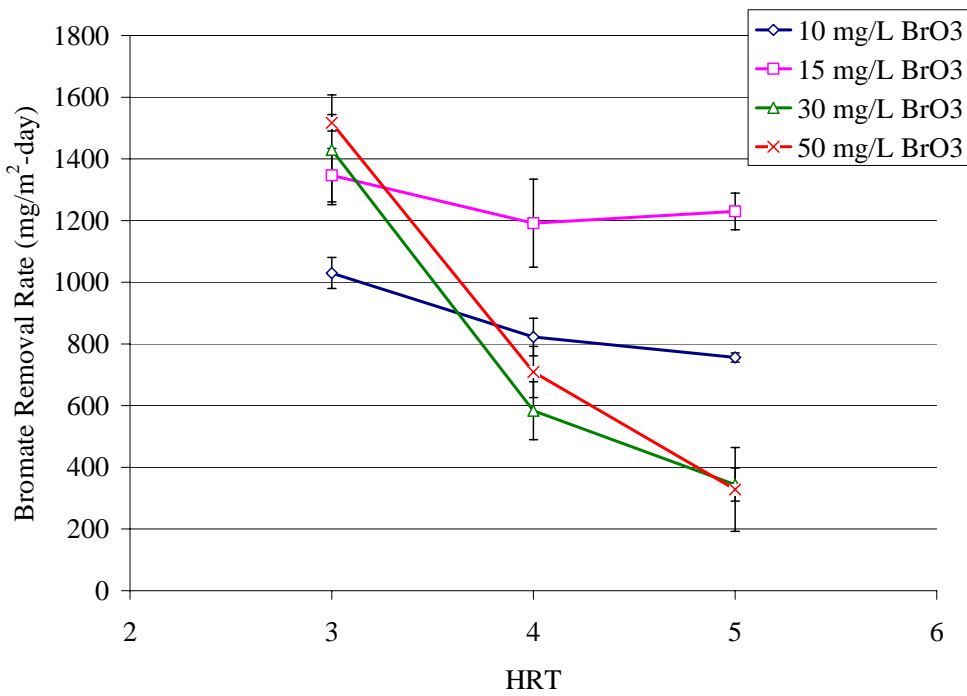
**Figure 6 Effect of nitrate on bromate reduction**

Bromate concentrations ranging from 50  $\mu\text{g/L}$  to 50  $\text{mg/L}$  were tested in short-term tests with no influent nitrate. The results are shown in Figure 7. As expected, higher effluent bromate concentrations led to higher fluxes. This presumably resulted from higher bromate concentrations within the biofilm and less Monod-type kinetic limitations from bromate (Rittmann and McCarty, 2001). Bromate removal fluxes increased until reaching a maximum of approximately 1500  $\text{mg/m}^2\text{-day}$  with effluent concentration of around 48  $\text{mg/L}$  bromate. The last two data points shown in Figure 7 are for the first sample collected at 3 HRTs of short-term testing. The other samples are excluded due to the significant decreases in bromate flux.

Figure 8 shows that the initial fluxes for influent bromate concentrations of 15, 30, and 50  $\text{mg/L}$  bromate are approximately the same, consistent with the results shown in Figure 7. However, after operation for 4 and 5 HRTs the bromate reduction flux was greatly reduced, especially for influent bromate concentrations of 30 and 50  $\text{mg/L}$  bromate.



**Figure 7** Bromate reduction continually increases with increased bromate concentration until a plateau is reached at approximately 30,000 µg/L bromate in the effluent, indicating Monod-type reduction kinetics



**Figure 8** Over a period of 5 HRT (approximately 2 hours), bromate reduction significantly decreases with increased influent bromate

After observing the drastic decrease in bromate reduction over 5 HRTs with 30 and 50 mg/L bromate in the influent, the impact of this high bromate concentration on denitrification was tested. Even with 5 mgN/L nitrate in the influent, the response of bromate removal with 50 mg/L bromate in the influent was almost identical as with 0 mgN/L nitrate in the influent. As bromate removal decreased over the 5 HRT time period, the nitrate removal rate remained the same as it was with 100 µg/L of bromate in the influent Figure 9.

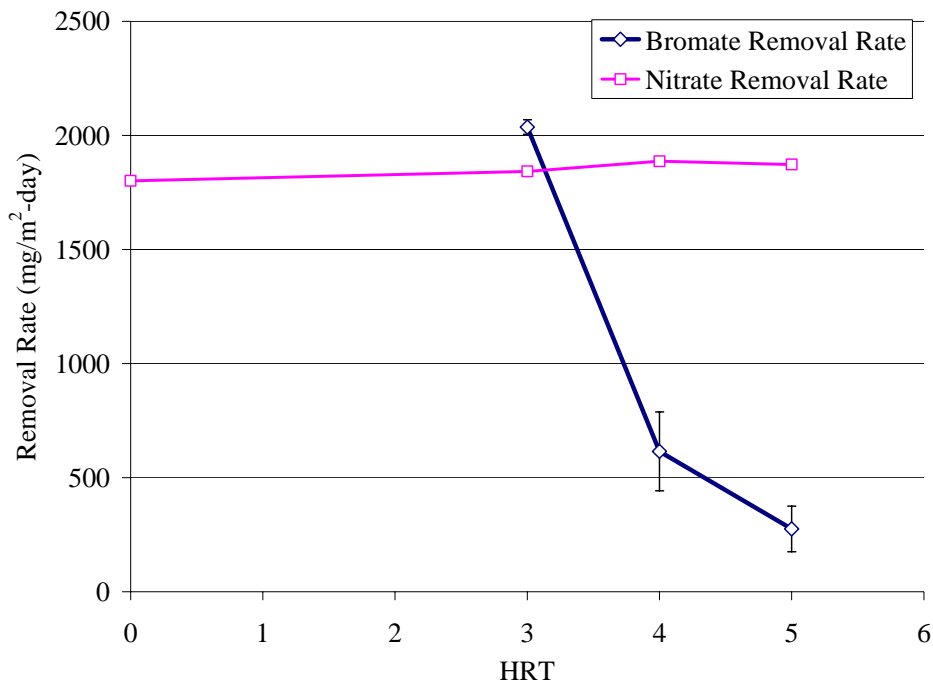


Figure 9 The toxicity effect of bromate on bromate reduction is not observed by nitrate removal rate

## Discussion

The preliminary tests demonstrate that a denitrifying MBfR can reduce bromate concentrations ranging from 100 to 1,500 to below the 10 µg/L MCL. A short increase in the effluent bromate concentration occurred when the influent suddenly increased to 1,500 µg/L, but then rapidly fell below the MCL.

When nitrate and bromate are both included in the influent at start-up of an MBfR, bromate reduction lags behind nitrate reduction. Bromate reduction did not occur until day 21, after combined nitrate and nitrite were below 1.5 mgN/L. This could be explained by a sub population of bromate-reducing bacteria that are inhibited by nitrate, but that can grow on bromate once the nitrate levels are sufficiently low.

Mechanism testing with nitrate confirmed that nitrate has an inhibitory effect on bromate reduction. The data also suggests that two mechanisms may be occurring to reduce bromate reduction. With no influent nitrate, bromate reduction was at its maximum. As the effluent nitrate concentration increased to 2 mgN/L, bromate reduction steadily decreased. This indicates

that the nitrate is inhibiting bromate reduction in some way, possible denitrifying bacteria out competing bromate reducing bacteria for hydrogen.

When the reactor was operated with 0 mgN/L nitrate in the influent, bromate reduction continually increased as bromate concentration increased, until a plateau was reached at approximately 15 mg/L of bromate in the effluent. This suggests a half saturation constant (K) for bromate of around 10 mg/L (Rittmann and McCarty 2001).

Prolonged exposure to bromate may have an inhibitory effect on bromate reducing bacteria. During preliminary testing, when the influent bromate concentration was higher than 4 mg/L, the effluent bromate concentrations increased with time. Further investigation of this effect was conducted during the mechanism testing. Influent bromate concentrations higher than 10 mg/L resulted in decreasing bromate reduction fluxes over 5 HRT. Bromate reduction was almost lost by 5 HRT at concentrations of bromate over 30 mg/L. However, after 3 HRT, the removal rates at 30 and 50 mg/L of influent bromate followed the Monod pattern for substrate reduction established during the entire mechanism testing with bromate concentrations.

Bromate did not have an inhibitory effect on denitrification. At pseudo steady-state conditions, denitrification remained constant over 5 HRT with 50 mg/L of bromate in the influent, while bromate reduction followed the same decreasing pattern observed previously. The production of a toxic intermediate, bromite, may explain this response. The reduction of bromate to bromide has an intermediate step, the production of bromite, a powerful oxidant. If high levels of bromite are produced during bromate reduction, it could be toxic to the bacteria reducing it. If this is the case, bacteria not oxidizing bromate would be less likely to be affected. The fact that denitrification was maintained despite the drastic drop in bromate reduction capabilities suggests that two distinct populations are present in the biofilm, those reducing nitrate and those reducing bromate.

## **Conclusions**

The results indicate that a hydrogen-oxidizing bacteria capable of growth using bromate as an electron acceptor may exist. Most importantly, tests show a denitrifying, hydrogen-based MBfR is capable of reducing up to 1,500 µg/L bromate to below the 10 µg/L MCL. This suggests the MBfR may be a useful technology for concurrently removing bromate and nitrate from drinking water.

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