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Reduction of bromate by biogenic sulfide produced during microbial sulfur disproportionation

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Abstract Bromate (BrO_3^-) is a carcinogenic contaminant formed during ozonation of waters that contain trace amounts of bromide. Previous research shows that bromate can be microbially reduced to bromide using organic (i.e. acetate, glucose, ethanol) and inorganic (H_2) electron-donating substrates. In this study, the reduction of bromate by a mixed microbial culture was investigated using elemental sulfur (S^0) as an electron donor. In batch bioassays performed at 30°C, bromate (0.30 mM) was completely converted to bromide after 10 days and no accumulation of intermediates occurred. Bromate was also reduced in cultures supplemented with thiosulfate and hydrogen sulfide as electron donor. Our results demonstrated that S^0-disproportionating microorganisms were responsible for the reduction of bromate in cultures spiked with S^0 through an indirect mechanism involving microbial formation of sulfide and subsequent abiotic reduction of bromate by the biogenic sulfide. Confirmation of this mechanism is the fact that bromate was shown to undergo rapid chemical reduction by sulfide (but not S^0 or thiosulfate) in abiotic experiments. Bromate concentrations above 0.30 mM inhibited sulfide formation by S^0-disproportionating bacteria, leading to a decrease in the rate of bromate reduction. The results suggest that biological formation of sulfide from by S^0 disproportionation could support the chemical removal of bromate without having to directly use sulfide as a reagent.

Keywords Elemental sulfur · Sulfide · Bromate reduction · Bromide · Sulfur disproportionation

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Introduction

Bromate (BrO_3^-) is a man-made compound which is mainly produced as a disinfection byproduct during the treatment by ozonation of potable water containing bromide (Br^-) (Butler et al. 2005a). This highly oxidized contaminant has been detected in both surface water and groundwater at concentrations ranging from 3 to 200 μg l^{-1} (Davis et al. 2004; Flury and Papritz 1993). The occurrence of bromate in drinking and surface water is of concern because this anion has been identified as a possible human carcinogen (Keith et al. 2006a). Therefore, environmental and regulatory agencies recommend very low levels of bromate in drinking water. As an example, the US federal standard is 10 μg l^{-1}, and the World

Health Organization has proposed a limit of 25 μg l^{-1} (WHO 1993).

Bromate is a very stable anion characterized by a high solubility and low reactivity. This pollutant cannot be removed by traditional water treatment methods such as filtration, chlorination or lime softening and, consequently, new methods for its removal such as membrane processes, ultraviolet irradiation and photocatalytic decomposition are under consideration (Butler et al. 2005a). However, many of these treatment techniques suffer from important drawbacks such as high energy requirements or the generation of concentrated brines that require further treatment or disposal. Biological processes for the reduction of bromate have been applied in different reactor configurations, e.g., bioreactors packed with activated carbon and membrane bioreactors with satisfactory results (Asami et al. 1999; Nerenberg and Rittmann 2004). Reduction of bromate to the benign ion bromide (Br^-) can be performed by mixed- and pure cultures of denitrifying bacteria when supplemented with ethanol as the energy and carbon source (Hijnen et al. 1995, 1999). Other organic electron donors such as glucose and acetate have also been used for the biological reduction of bromate by indigenous groundwater microbial populations and anaerobic mixed microbial cultures, respectively (Butler et al. 2005b; van Ginkel et al. 2005a). However, the use of organic electron-donating substrates can potentially leave behind organic residuals that can contribute to the formation of toxic disinfection byproducts during chlorination (Ju et al. 2007). Inorganic compounds can potentially overcome the disadvantages of organic electron donating substrates. For instance, hydrogen gas has been used as electron donor for the reduction of bromate by chlorate-reducing microorganisms in laboratory gas-lift bioreactors (van Ginkel et al. 2005b), and by mixed cultures in denitrifying membrane-biofilm reactors (Downing and Nerenberg 2007). Alternative electron donors such as inorganic sulfur compounds [i.e., elemental sulfur (S^0), thiosulfate ($S_2O_3^{2-}$), and sulfide (S^{2-})] have been shown to be effective electron donors in the microbial reduction of other oxidized inorganic compounds such as perchlorate (Balk et al. 2008; Ju et al. 2007, 2008) and nitrate (Beristain et al. 2006; Sierra-Alvarez et al. 2007; Zhang and Lampe 1999). Consequently, these reduced sulfur compounds might also be suitable for supplying reducing equivalents to bromate-reducing microorganisms. The theoretical stoichiometries for the reduction of bromate with elemental sulfur, thiosulfate, and sulfide are described in Eqs. 1–3, respectively.

$$BrO_3^- + S^0 + H_2O \rightarrow Br^- + SO_4^{2-} + 2H^+ \quad \Delta G^{0\prime} = -709.7\ \text{kJ/mol}\ BrO_3^- \tag{1}$$

$$BrO_3^- + 3/4S_2O_3^{2-} + 3/4H_2O \rightarrow Br^- + 3/2\,SO_4^{2-} + 3/2H^+ \quad \Delta G^{0\prime} = -736.2\ \text{kJ/mol}\ BrO_3^- \tag{2}$$

$$BrO_3^- + 3/4S^{2-} \rightarrow Br^- + 3/4SO_4^{2-} \quad \Delta G^{0\prime} = -720.0\,\text{kJ/mol}\,BrO_3^- \tag{3}$$

This study examined the capacity of a bromate-reducing enrichment culture to catalyze the reduction of bromate with several reduced sulfur compounds including elemental sulfur, thiosulfate, and sulfide. In addition to microbial assays, abiotic experiments were also performed to assist with the elucidation of the mechanisms involved in the reduction of bromate in these experiments.

Materials and methods

Microorganisms

The bromate-enrichment culture utilized in this study was developed using a perchlorate-reducing enrichment culture which was transferred (5%, v/v) every 2 weeks to a fresh medium containing bromate (0.30 mM) and elemental sulfur (10 mM) following bromate depletion. The procedure utilized to obtain and maintain the perchlorate-reducing enrichment culture is described elsewhere (Ju et al. 2008). The culture flasks were incubated in an orbital shaker (100 rpm) at 30°C under a N_2/CO_2 (80/20%, v/v) atmosphere. The basal mineral medium contained (g l^{-1}): NH_4HCO_3, 0.414; K_2HPO_4, 0.25; $MgSO_4{\cdot}7H_2O$, 0.1; $NaHCO_3$, 2.7; $Ca(OH)_2$, 0.005; yeast extract (YE), 0.02, and trace metal solution (1 ml l^{-1}). The trace metal solution contained (mg l^{-1}): H_3BO_3, 50; $FeSO_4{\cdot}7H_2O$, 2,800; $ZnSO_4{\cdot}7H_2O$, 106; $MnSO_4{\cdot}H_2O$, 415; $(NH_4)_6Mo7O_{24}{\cdot}4H_2O$, 200; $AlK(SO_4)_2{\cdot}12H_2O$, 175; $NiSO_4{\cdot}6H_2O$, 113; $CoSO_4{\cdot}7H_2O$, 2,360; $Na_2SeO_3{\cdot}5H_2O$, 100; $CuSO_4{\cdot}5H_2O$, 157; $Na_2WO_4{\cdot}2H_2O$, 500; EDTA, 1,000; and resazurin, 200.

Batch bioassays

All the experiments used the basal mineral medium described above. The assays were conducted in duplicate or triplicate in 160 ml serum bottles and inoculated with 5% (v/v) bromate enrichment culture. Flasks were supplied with 100 ml of basal medium spiked with bromate at a concentration ranging from 0.10 to 1.0 mM, depending on the experiment. The pH in the bioassays was between 7.0 and 7.2. The concentration of the different electron donors used was as follows: 10 mM of S^0, 1 mM H_2S and 0.64 mM $S_2O_3^{2-}$. All flasks were sealed with butyl rubber septa and aluminum caps and, subsequently, the headspace of the each flask was flushed with N_2/CO_2 (80/20%, v/v) for 5 min to exclude the presence of O_2 and create an anaerobic environment. Assays utilizing sulfide as electron donor were first flushed with N_2/CO_2 (80/20, v/v) and then spiked with sulfide to avoid losses of the compound by stripping. Controls inoculated with heat killed-cells and lacking inoculum were run in parallel to correct for abiotic losses of bromate. Heat-killed cells controls were sterilized by autoclaving twice. First the controls were autoclaved at 121°C for 1 h. After overnight incubation at room temperature, the flasks were sterilized one more time at 121°C for 20 min, allowed to cool and then sealed aseptically. Controls lacking bromate or added electron donor were also used to correct for losses of the donating substrate or bromate by microbial conversion of endogenous substrate in the inoculum. All the experiments were incubated at 30°C in an orbital shaker (150 rpm). Samples were collected periodically and they were analyzed for bromate, bromide, sulfate, thiosulfate, sulfide, and pH value.

Abiotic reduction of bromate

The abiotic reaction of bromate (0.30 mM) with hydrogen sulfide (0.11, 0.23, 0.45 and 0.68 mM) was investigated in shaken batch assays. The assays were conducted in duplicate at room temperature (23°C) using 160 ml serum bottles. The headspace in all the bottles was flushed with N_2/CO_2 (80/20%, v/v) for 5 min. Sulfide was added after the flushing with N_2/CO_2 to avoid losses of the compound by stripping. Liquid samples were obtained periodically and transferred immediately to vials containing zinc acetate (1.13 mM) to stop the redox reaction. Liquid samples were collected periodically and analyzed for bromate, bromide, sulfate, sulfide and thiosulfate.

Analytical methods

Bromate, bromide and sulfate were analyzed by ion chromatography with suppressed conductivity using a Dionex-500 system (with IP25 isocratic pump, EG40 eluent generation, CD20 conductivity detector, and LC20 chromatography enclosure, Sunnydale, CA) equipped with a Dionex IonPac AS16 column (4 × 250 mm), and a Dionex AG16 guard column (4 × 50 mm). The mobile phase was 3 mM KOH from 0 to 8 min, 3–30 mM from 8 to 10 min, 30 mM from 10 to 15 min, and 3 mM from 15 to 17 min. The injection volume was 75 µl and the detection limit for bromate was 0.002 mM. Hydrogen sulfide was measured immediately after sampling by the methylene blue method (Trüper and Schlegel 1964). Samples were not filtered or centrifuged to minimize losses of sulfide by volatilization. The pH was determined with a VWR symphony SB20 system (VWR International, West Chester, PA).

Chemicals

Sodium bromate (99.5%) and elemental sulfur (sublimed powder sulfur, >99.0%) were purchased from EM Science (EMD chemicals, Gibbstown, NJ, USA). Sodium sulfide nonahydrate (98%), sodium thiosulfate pentahydrate (99.5%), and sodium sulfate (99%) were supplied by Sigma–Aldrich (St. Louis, MO, USA). Zinc acetate dihydrate was obtained from J. T. Baker (99.9%).

Results

Microbial-mediated reduction of bromate with elemental sulfur

The ability of an enrichment culture to reduce bromate (0.30 mM) using elemental sulfur (10 mM) as an electron donor was evaluated in batch assays. Bromate was converted stoichiometrically to bromide in the inoculated bioassays spiked with S^0 (Fig. 1A, B). Intermediate products of bromate reduction such as bromite (BrO_2^-) were not detected. Interestingly,

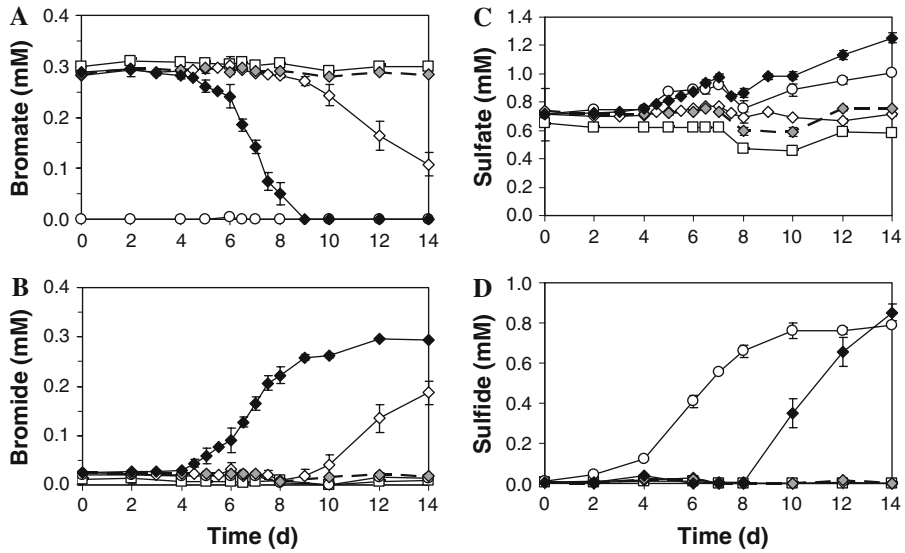

Fig. 1 Microbial mediated reduction of bromate (0.30 mM) in assays spiked with elemental sulfur (10 mM). Bromate removal (**A**), formation of bromide (**B**), sulfate (**C**), and sulfide (**D**) in assays inoculated with an enrichment culture (*filled diamond*), and in the control assays: No inoculum (*open square*); no S^0 (*open diamond*); no bromate (*open circles*); heat-killed cells (*shaded diamond*). The assays were incubated at 30°C

limited bromate reduction was also observed in assays lacking S^0, albeit at a considerably lower rate compared to the assays with added S^0. The reduction of bromate observed in the control was due to the utilization of endogenous substrate in the inoculum as electron donating substrate. The reductive transformation of bromate seemed to be microbial-mediated because bromate losses in assays with no inoculum and with heat-killed cells were negligible.

Formation of sulfate was observed in the inoculated assays, both in the presence and absence of bromate (Fig. 1C). Furthermore, in the culture with bromate, sulfide accumulation was observed following bromate depletion (Fig. 1D). In contrast, in the culture lacking bromate, H_2S was detected from the inception of the test. The concentration of H_2S detected in those cultures at the end of the experiment was approximately 0.80 mM.

Microbial inhibition of bromate

The impact of bromate concentrations ranging from 0.10 to 1.0 mM on the microbial-catalyzed reduction of bromate with elemental sulfur (10 mM) was examined. The rate of bromate removal (Fig. 2A) decreased with increasing bromate concentration, and no removal was observed at the highest initial bromate concentration tested (1.0 mM). Similarly to the previous experiment, accumulation of sulfide was observed in all the cultures supplied with S^0, following bromate depletion (Fig. 2B). Sulfide accumulation was again detected earlier in the culture without added bromate.

Figure 3 shows the rate of bromate removal rate expressed as percentage of the rate determined for the reference bromate level (0.30 mM) as a function of the toxicant concentration. The estimated concentration of bromate that caused 50% inhibition of the bromate reducing activity (IC_{50}) was 0.52 mM.

Microbial-mediated reduction of bromate in the presence of different inorganic sulfur compounds

The ability of the enrichment culture to mediate the reduction of bromate with S^0 and with other inorganic sulfur compounds, i.e., thiosulfate and sulfide, was compared. Figure 4A shows that bromate (0.30 mM) was transformed in all the assays inoculated with the live culture. As observed in previous experiments, bromate was stoichiometrically converted to bromide (results not shown). No bromate reduction was observed in assays inoculated with heat-killed cells and supplemented with S^0 or thiosulfate. In contrast, complete conversion of bromate to bromide was

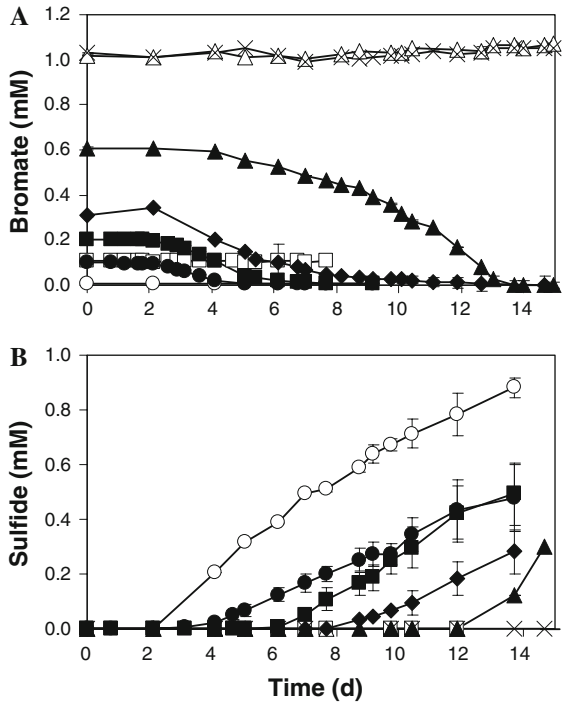

Fig. 2 Effect of the initial bromate concentration on the microbial mediated reduction of bromate in assays supplemented with elemental sulfur (10 mM). Bromate (**A**) and sulfide (**B**) concentrations in assays inoculated with the enrichment culture and containing the following bromate concentrations (mM): 0 (*open circle*); 0.1 (*filled circle*); 0.2 (*filled square*); 0.3; (*filled diamond*); 0.6 (*filled triangle*); 1.0 (*multi symbol*), and in the non-inoculated controls: 0.1 mM bromate (*open square*); 1.0 mM bromate (*open triangle*)

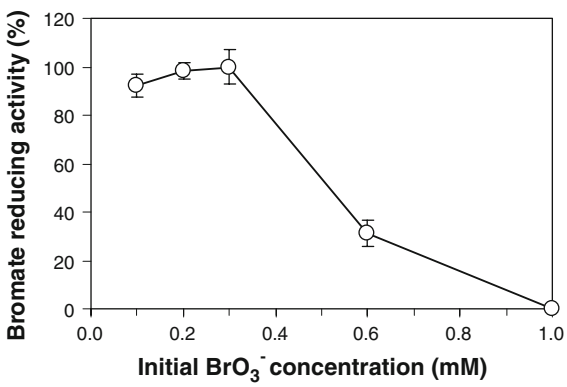

Fig. 3 Impact of the initial bromate concentration on the bromate reducing activity determined during the microbial mediated reduction of bromate in the presence of S^0. Bromide generation rates are expressed as percentage of the rate determined for the reference bromate level (0.30 mM), which was 0.048 mM Br^- day^{-1}

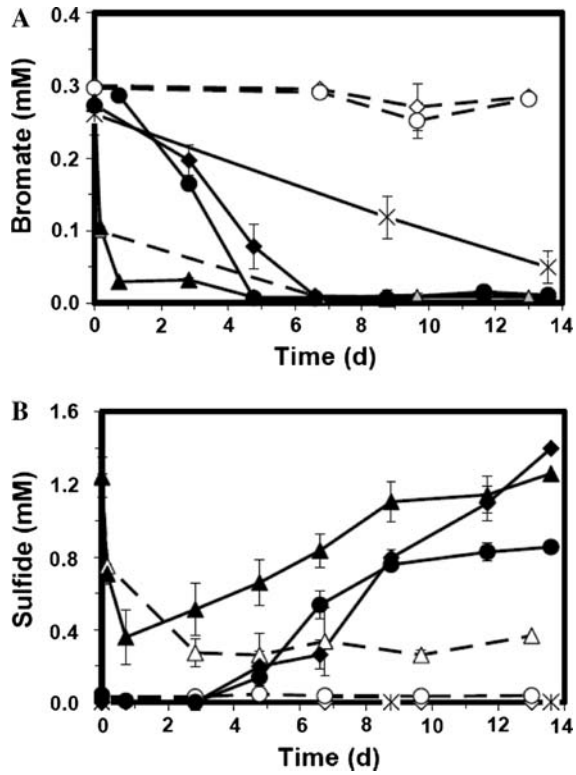

Fig. 4 Microbial mediated transformation of bromate in the presence of various inorganic sulfur compounds: Bromate (**A**) and sulfide (**B**) concentrations in assays inoculated with the enrichment culture and spiked with: 10 mM elemental sulfur (*filled diamond*); 0.64 mM thiosulfate (*filled circle*); 1 mM sulfide (*filled triangle*); no electron donor added (*multi symbol*); and in assays inoculated with heat-killed cells and spiked with 10 mM elemental sulfur (*open diamond*); 0.64 mM thiosulfate (*open circle*); 1 mM sulfide (*open triangle*)

observed in the heat-killed inoculum control spiked with H_2S, indicating chemical reduction.

Accumulation of sulfide at concentrations of 0.89 and 1.52 mM was detected after 13.6 days in the inoculated bioassays spiked with thiosulfate and S^0, respectively (Fig. 4B). In the live culture supplemented with sulfide, the sulfide concentration decreased during the first day due to oxidation by bromate, and then increased gradually to 1.30 mM after 13.6 days. The reversion in sulfide concentration was most likely due to biological reduction of the oxidized sulfur compound (formed from bromate oxidation) by microorganisms capable of using the endogenous substrate in the inoculum as electron donor. An increase in the concentration of sulfate with time was observed in all the assays inoculated

with live cells (results not shown). The sulfate concentration determined at the end of the experiment in the assays supplied with S^0, thiosulfate and sulfide was 0.63, 0.97 and 0.23 mM SO_4^{2-}, respectively.

Chemical reduction of bromate using hydrogen sulfide

The ability of sulfide to reduce bromate (0.30 mM) in the absence of microorganisms was examined in abiotic batch experiments utilizing varying concentrations of H_2S (0.11, 0.23, 0.45 and 0.68 mM). Sulfide was found to be an effective bromate-reducing agent (Fig. 5). As an example, a near complete removal of bromate was attained after 17 h in the assays supplemented with the highest sulfide concentration (0.68 mM). During the same period, 24.4, 65.4 and 88.7% of the bromate was removed in the assays spiked with 0.11, 0.23, and 0.45 mM H_2S, respectively.

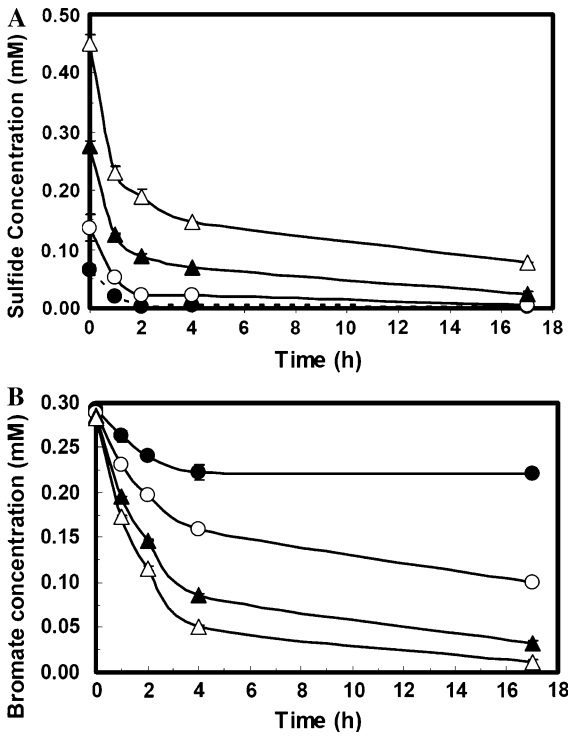

Fig. 5 Abiotic reduction of bromate (0.30 mM) by different concentrations of sulfide at 23°C. Time course of sulfide (**A**) and bromate (**B**) in the assays spiked with the following concentrations of sulfide (mM): 0.11 (*filled circles*); 0.23 (*open circles*); 0.45 (*filled triangles*); and 0.68 (*open triangles*)

The bromate removed was transformed stoichiometrically to bromide in all the assays (results not shown). In contrast, the concentrations of sulfate determined after 17 h were considerable lower than those expected based on the stoichiometric of sulfide conversion to sulfate, and the recovery of sulfate decreased with increasing sulfide dosage. Only 45.0, 60.9, 2.3 and 11.6% of the sulfide-S was recovered as sulfate-S in the assays supplied with initial sulfide concentrations of 0.11, 0.23, 0.45 and 0.68 mM, respectively. Thiosulfate was detected in the aforementioned assays at concentrations of 0.004, 0.005, 0.043 and 0.075 mM, respectively, indicating that at least part of the sulfur was transformed into other inorganic compounds.

Discussion

Abiotic reduction of bromate by sulfide

Abiotic experiments performed in our study confirmed that sulfide can readily reduce bromate chemically (Fig. 5). Bromate was reduced stoichiometrically to bromide. In contrast, the oxidation of sulfide to sulfate appeared to be incomplete when the molar bromate to sulfide ratio was low ($\leq$0.67). Intermediate oxidation products could include thiosulfate, which was detected in our experiments, and possibly also elemental sulfur.

The rate of bromate reduction was found to be strongly affected by the initial concentrations of bromate and sulfide. The reaction was determined to be first-order with respect to bromate and with respect to sulfide (Fig. 6). The rate of bromate reduction can be described by Eq. 4, where the rate constant, k, determined at 23°C is 0.636 mM^{-1} h^{-1}.

$$\frac{d[BrO_3^-]}{dt} = -k \cdot [BrO_3^-] \cdot [S^{2-}] \quad (4)$$

Evidence for the chemical reduction of bromate by H_2S has been documented earlier in studies with real and synthetic gastric juice (Keith et al. 2006a, b). The observation that bromate is chemically reduced by sulfide provides clues for the development of a chemical method for the removal of bromate from contaminated water. A biological method based on S^0 disproportionation could be attractive since it would deliver biogenic sulfide as needed without any

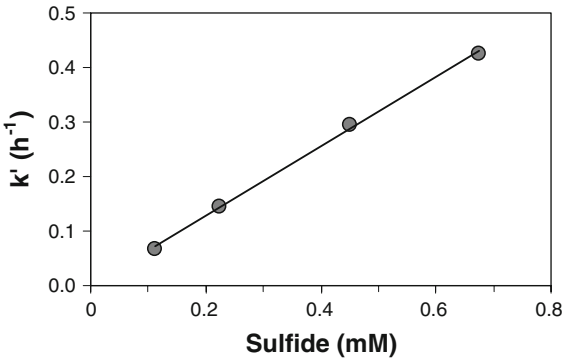

Fig. 6 First-order rate parameter, $k' = k \cdot [S^{2-}]$, versus sulfide concentration for the chemical reaction between bromate and sulfide. k' was calculated from the slope of the plot of ln $[BrO_3^-]$ as a function of time for times <4 h as calculated in the time course of the chemical reaction between bromate (0.30 mM) and different concentrations of sulfide (0.11, 0.23, 0.45 and 0.68 mM) at 23°C

requirement for handling H_2S as a reagent. Malodor problems typical associated with sulfide are unlikely to pose a limitation due to the very low reductant dosage required to eliminate bromate when present at the concentrations expected in drinking water. A recent survey of potable groundwater in the United States showed that the bromide concentration ranged from 3 to 58 μg l^{-1} (Davis et al. 2004), which would result in 5–100 μg l^{-1} bromate if bromide was fully oxidized.

Microbial-mediated reduction of bromate with inorganic sulfur compounds

Limited reduction of bromate was carried out by the enrichment culture in the absence of added S^0 or thiosulfate (Figs. 1, 4). The observed microbial reduction was most likely catalyzed by microorganisms capable of utilizing the endogenous organic substrate in the inoculum as electron donor. Microbial reduction of bromate with electron-donating substrates such as acetate (van Ginkel et al. 2005a), glucose (Butler et al. 2005b), and with H_2 gas (Nerenberg and Rittmann 2004; van Ginkel et al. 2005b) has been reported in recent studies. The assay lacking S^0 had a longer lag phase as well as a lower bromate removal rate than the treatment with added S^0, indicating that the S^0-dependent reduction of bromate was more effective compared to the microbial reduction utilizing the low levels of complex endogenous organic matter in the inoculum.

Rapid bromate reduction was observed in bioassays inoculated with live cells and spiked with sulfide (Fig. 4A). Nevertheless, bromate was removed at a comparable rate in the control with sulfide and heat-killed cells, suggesting that bromate was abiotically reduced by sulfide. Reduction by sulfide also seems to be responsible for the removal of bromate in the bioassays supplied with S^0 and thiosulfate. Sulfide was not added in those experiments but can be formed by microbial disproportionation of S^0 or thiosulfate. Simultaneous production of sulfide and sulfate was observed in the cultures with S^0 but not added bromate, confirming that S^0 disproportionation had occurred (Fig. 1C, D). Furthermore, the levels of sulfate detected in the bioassays with bromate can not be explained only by bromate oxidation of S^0 or thiosulfate. For example, the concentrations of sulfate determined at the end of the experiments with S^0 and thiosulfate, 0.63 and 0.97 mM, respectively, were two to threefold higher than expected from the stoichiometry of direct oxidation of these reduced sulfur compounds by bromate (Eqs. 1, 2). Also, the sulfide concentration in the same bioassays was 1.40 and 0.82 mM, respectively, whereas sulfide is not expected as a product from the reaction of S^0 or thiosulfate with bromate.

Microbial disproportionation of S^0 and $S_2O_3^{2-}$ is well documented (Finster et al. 1998; Jackson and McInerney 2000; Janssen et al. 1996). S^0 disproportionation is an inorganic fermentation in which S^0 is simultaneously oxidized to sulfate and reduced to hydrogen sulfide (Eq. 5).

$$S^0 + 4H_2O \rightarrow 3H_2S + SO_4^{2-} + 2H^+ \quad \Delta G^{0'} = +10.2 \text{ kJ/mol } S^0 \tag{5}$$

Under standard conditions the disproportionation of S^0 is endergonic (Janssen et al. 1996), but the reaction can be exergonic under the prevailing conditions during the biological reactions. Ju et al. (2007) reported that the Gibb's free energy change for this reaction at pH 7 and concentrations of H_2S and SO_4^{2-} of 1.5 and 12.0 mM, respectively, is −4.8 kJ/mol S^0. It is interesting to note that bacterial disproportionation of S^0 has been observed in experiments with perchlorate and nitrate reducing cultures under conditions where alternative electron acceptors are not available (Ju et al. 2007, 2008).

Elemental sulfur is not the only inorganic sulfur compound that can undergo disproportionation. Several organisms such as *Desulfobulbus propionicus* (Lovley and Phillips 1994), *Desulfocapsa thiozymogenes* (Janssen et al. 1996), and *Desulfocapsa sulfoexigens* (Finster et al. 1998) can grow by the disproportionation of thiosulfate or sulfite. Similarly to the conversion of S^0, microbial disproportionation of thiosulfate occurs via an intramolecular redox change at each of the sulfur atoms and produces 1 mol of sulfate and 1 mol of sulfide (Eq. 6) (Jackson and McInerney 2000). Although the Gibb's free energy change for this reaction is low, microorganisms capable of this conversion are common in both freshwater and marine environments (Bak and Cypionka 1987; Jorgensen 1994).

$$S_2O_3^{2-} + H_2O \rightarrow H_2S + SO_4^{2-} \quad \Delta G^{0'} = -22.0 \text{ kJ/mol } S_2O_3^{2-} \tag{6}$$

A balance for sulfate in the experiment illustrated in Fig. 1 was performed assuming that sulfate was produced by disproportionation of S^0 (Eq. 5) or thiosulfate (Eq. 6) and, also as a result of the chemical reaction between sulfide (produced by disproportionation) and bromate (Eq. 3). A balance for sulfide was also conducted using the same assumptions. The results showed a good correlation between the theoretical and the actual amount of sulfate produced (Table 1). Also, the molar ratio of sulfide (sum of the concentration in the gas and liquid phase) to sulfate estimated to be produced by microbial activity in cultures lacking bromate was 2.87, which is close to the theoretical stoichiometric ratio of 3 corresponding to S^0 disproportionation. A good fit between the theoretical and experimental stoichiometric ratios was also obtained for the rest of the experiments (results not shown).

Taken as a whole, these findings indicate that bromate was reduced by the sulfide produced as a result of the sulfur disproportionation reaction. The presence of an active sulfur disproportionating population in the enrichment culture suggests that sulfide was produced during the initial phase of the bioassays. However, sulfide could only be detected after bromate was depleted (Figs. 1, 2 and 4).

Microbial inhibition by bromate

Our experiments demonstrate that bromate is a microbial inhibitor. Bromate concentrations exceeding 0.30 mM inhibited the indirect microbial reduction of bromate by the enrichment culture utilized in this study, and no bromate reduction was observed for the treatment spiked with 1.0 mM bromate

Table 1 Sulfate balances determined in bioassays inoculated with an enrichment culture and spiked with 0.3 mM bromate and 10 mM sulfur (biological treatments) or with only 10 mM sulfur (controls) after 14 days of incubation

Parameter	Concentration (mM)	
	Control	Biological treatment
Actual sulfide produced (liquid phase)	0.778	0.848
Actual sulfide produced (gas phase)[a]	0.069	0.076
Sulfide consumed by bromate reduction[b]	0	0.216
Total sulfide[c]	0.847	1.140
Sulfate produced by disproportionation[d]	0.282	0.380
Sulfate produced by sulfide oxidation with bromate[b]	0	0.216
Total theoretical sulfate[e]	0.282	0.596
Actual sulfate measured	0.295	0.539

The experimental results for this experiment are shown in Fig. 1

[a] The amount of sulfide in the gas was calculated using Henry's law (dimensionless H factor = 0.4)

[b] Based on stoichiometry of bromate reduction by sulfide (Eq. 3)

[c] Sum of the actual sulfide produced (liquid and gas phase) and the sulfide consumed by bromate reduction (Eq. 3)

[d] Based on stoichiometry of sulfur disproportionation (Eq. 5)

[e] Sum of the sulfate produced by sulfur disproportionation (Eq. 5) and the sulfate produced by sulfide oxidation with bromate (Eq. 3)

(Fig. 3). The initial concentration causing 50% inhibition of the bromate reduction rate was 0.52 mM. The observed decrease in the rate of bromate reduction might be due to a decrease in the concentration of the reductant, H_2S, due inhibition of sulfur disproportionating microorganisms by bromate. Microbial inhibition by bromate has been reported in a few other studies. Van Ginkel et al. (2005a) demonstrated that the reduction of bromate by an anaerobic mixed culture was inhibited at bromate concentrations of 1.5–3.0 mM. An unidentified metabolite of bromate was hypothesized to be responsible for the inhibition observed in the latter study. Bromate (1.1–3.1 mM) has also been reported to inhibit the growth and metabolic activity of the thermophilic lactic-acid degraders, *Streptococcus cremoris* and *Thermobacterium helveticum* (Hietaranta 1949).

Conclusions

The results of this study indicated that bromate can be reduced by sulfur and thiosulfate disproportionating enrichment cultures. The mechanism involves the chemical reduction of bromate by the biogenic sulfide produced by these cultures. Bromate was not directly utilized as an electron acceptor. Our results also revealed that bromate concentrations above 0.30 mM were inhibitory to sulfur disproportionating microorganisms.

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