Bromate Reduction by Denitrifying Bacteria

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In the presence of bromide, ozonation as applied in water treatment results in the formation of bromate, an ion with carcinogenic properties. The reduction of bromate by mixed bacterial populations as well as pure cultures was studied under laboratory conditions. Bromate was reduced to bromide by a mixed bacterial population with and without a preceding nitrate reduction step in an anaerobically incubated medium with ethanol as the energy and carbon source at 20 and 25°C. The predominating bacteria isolated from the batches showing bromate reduction were identified as *Pseudomonas* spp. Strains of *Pseudomonas fluorescens* reduced BrO_3^- to Br^- but at a much lower rate than the mixed bacterial population did. Nitrate is a preferred electron acceptor for the bromate-reducing bacteria. Bromate reduction did not occur in the presence of NO_3^- , and the rate of bromate reduction was at least 100 times lower than the rate of nitrate reduction. Bromate was completely converted to Br^- , indicating that intermediates, e.g., BrO_2^- , did not accumulate during bromate reduction.

Recently, interest in the presence of bromate in drinking water arose because of its carcinogenic properties (15). The bromate concentration which would lead to less than 1 additional case of cancer in a population of 100,000 in a lifetime, is 3 $\mu\text{g/liter}$ (34). The World Health Organization has defined a provisional guideline value of 25 μg of bromate per liter for drinking water on the basis of analytical and treatment considerations. Bromate can be present in drinking water as a result of its formation from bromide during ozonation (4, 13) or chlorination (1). Recent full-scale plant investigations have shown bromate concentrations of 5 to 10 $\mu\text{g/liter}$ in ozone-treated water (12). Ozonation has become an important alternative to chlorination in water treatment because it avoids the production of chlorinated organic chemicals and is more effective against persistent pathogens like *Giardia* and *Cryptosporidium* species (2, 8). Furthermore, ozonation reduces the concentration of persistent micropollutants, e.g., pesticides in water. The growing interest in the application of ozonation initiated studies concerning factors affecting bromate formation by ozonation as well as bromate removal in water treatment.

The reduction of chlorate to chloride by denitrifying bacteria under anaerobic conditions has been studied extensively (5, 6, 18, 19, 21, 23, 24). A mixed bacterial population reduced ClO_3^- to chloride with acetate as the sole energy and carbon source (19). The same authors showed that 70 mg of chlorate per liter was removed completely in an anaerobic fixed-film process (18). Measurements of iodide produced from iodate added to soil have been used to determine nitrate reductase activity in soil (26). Bromate reduction was observed in both aerobically and anaerobically incubated soil by the same author (26), but the reduction was attributed to chemical rather than biological reduction. However, Hackenthal (5) suggested that bromate is a potential substrate for nitrate reductase. The objectives of this study were (i) to obtain evidence for the reduction of bromate by bacteria and (ii) to determine conditions influencing biological bromate reduction. This information is needed as a first step in assessing the application of biological processes for bromate removal in water treatment.

MATERIALS AND METHODS

Media and experimental conditions. For the growth experiments, a mineral salts (MS) medium was used. The MS medium contained 0.1 g of Na_2HPO_4 , 5 mg of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.005 mg of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.01 mg of H_3BO_3 , 5 mg of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.008 mg of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 0.3 mg of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.01 mg of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.3 mg of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 1 liter of demineralized water. For each experiment, this MS medium (pH 7.2) was supplemented with bromate, nitrate, and an excess amount of ethanol, as described in Table 1. All experiments, except the first one with a mixed bacterial population, were conducted with autoclaved media. Ethanol was filtered through a 0.22- μm -pore-size sterile filter (Millex-GS; Millipore) before being added to the autoclaved media. In the MS medium of experiments 3 and 5, 0.5 mg of $\text{NH}_4\text{Cl-N}$ per liter was added. In experiment 4 the MS medium contained 2 mg of $\text{NH}_4\text{Cl-N}$ per liter and 0.01 mg of molybdenum per liter. The latter compound was added to exclude Mo limitation of the denitrifying bacteria.

The media (400 ml) of experiment 1, in which a mixed bacterial population was used, were incubated in 500-ml flasks in a thermostated water bath at 25°C in the dark. During the first 6 days of incubation, these flasks were flushed with nitrogen gas. In experiment 2, the media (250 ml) were incubated at room temperature ($\pm 20^\circ\text{C}$) in 300-ml Erlenmeyer flasks and continuously aerated. The same 300-ml Erlenmeyer flasks were also used for experiments 3 and 5. In these experiments, the gas inlet was provided with a 0.22- μm -pore-size sterile filter (Millex-GS) to prevent infection of the media during incubation. Furthermore, the silicone tubing on both the inlet and outlet of the flasks was closed during incubation. The results of experiment 1 indicated that some oxygen was introduced under the experimental conditions. Therefore, the Erlenmeyer flasks used in experiments 3 and 5 were incubated in a vacuum desiccator at room temperature ($\pm 20^\circ\text{C}$) in the dark. This desiccator was continuously flushed with a small amount of nitrogen gas. The anaerobic conditions in the desiccator were tested with resazurin in buffered aqueous solution as the indicator (Oxoid Br55).

The media of experiment 4 with pure cultures were incubated in 100-ml flasks closed with a screw cap and a Teflon inlay. The flasks were filled completely to avoid oxygen introduction from the headspace and stored at 25°C in an incubator. Both media 4A and 4B were incubated in duplicate.

Inoculum and bacterial strains. The flasks used in experiment 1 were inoculated with 1 ml of the effluent of a denitrification reactor in which ethanol was used as a substrate (mixed culture). The media for experiments 2 and 3 were inoculated with the enrichment culture (EC) of medium 1B. In experiment 4 the flasks were inoculated with the EC of medium 3A and with pure cultures of isolates obtained from experiments 1A, 1B, 3A, and 3B. In addition, a fluorescent *Pseudomonas* isolated from drinking water, strain P17 (ATCC 49642) (33), was used.

Sampling and analytical procedures. Periodically, samples for chemical and microbiological analysis were collected from the flasks. The flasks from experiments 1, 2, 3, and 5 were sampled with a sterile syringe connected to a silicone tube (inner diameter, 4 mm) inserted into the flasks. After sampling, the media from experiments 1, 3, and 5 were flushed with nitrogen gas for a few minutes to remove as much introduced oxygen as possible. Removal of bromate and nitrate from the media used in experiment 4 was monitored by sampling flasks 1 and 2 after 32 and 75 days of incubation, respectively.

For the chemical analysis, 10 to 20 ml of the medium was filtered over a 0.22- μm -pore-size sterile filter (Millex GS) and stored at 4°C. The anions Br^- , NO_3^- , NO_2^- , and SO_4^{2-} were determined by ion chromatography (Dionex DX300) by Environmental Protection Agency method 300.0 (22) with an anion

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TABLE 1. Medium composition and other experimental conditions

Expt No.	Medium	Concn (mg/liter) of additive to MS medium			Inoculum ^a	Incubation temp (°C)
		BrO ₃ ⁻	NO ₃ ⁻ -N ^b	Ethanol		
1	A	(1) ^c	10 (2.5) ^c	50	MC	25
	B	1	10	50	MC	25
	C	1	— ^d	—	MC	25
	D	1	—	50	MC	25
2	A	A	—	100	EC	20
	B	1	10	100	EC	20
3	A	1	5	100	EC	20
	B	5	—	100	EC	20
	C	1	5	100	— ^e	20
	D	1	—	—	— ^e	20
4	A	0.2	10	100	PC/EC	25
	B	0.2	—	100	PC/EC	25
5	A	1	5	100	PC	20
	B	5	—	100	PC	20

^a MC, mixed bacterial population; EC, enrichment culture; PC, pure culture.

^b The nitrate concentration in MS medium was < 0.2 mg of NO₃⁻-N per liter, except for experiment 1.

^c The value in parentheses indicates the addition on day 8.

^d —, no addition.

^e Media not inoculated: medium of experiments 3C and 3D were autoclaved and not autoclaved, respectively, before incubation.

micromembrane suppressor, and AS-9 anion separator column (Dionex), a conductivity detector, and a Na₂CO₃-NaHCO₃ eluent solution. BrO₃⁻ was determined by a similar ion chromatography method after removal of excess chloride by passing the sample over a silver filter cartridge. The identity of bromate was confirmed by a second ion chromatography method which included an AS-10 anion separator column (Dionex) with a UV detector and a Na₂CO₃-NaHCO₃-NaCl eluent solution (32). The detection limit for bromate was 5 µg/liter, and the error was 5 to 10%. An improved method for bromate analysis, with a detection limit of 1 µg/liter, was used to determine BrO₃⁻ removal in experiment 4 (20). Ethanol was determined by direct sample injection in a gas chromatograph (Carlo-Erba 4130) with a Porapak-Q column (Chrompack).

The bacterial density in the media was assessed by determining the heterotrophic colony counts on R₂A medium plates (Difco) after aerobic incubation for 10 days at 25°C (25). R₂A medium, which was developed for the enumeration of oligotrophic bacteria from potable water, contains yeast extract (0.5 g), peptone (0.5 g), Casamino Acids (0.5 g), glucose (0.5 g), starch (0.5 g), pyruvate (0.3 g), potassium phosphate (0.3 g), magnesium sulfate (0.05 g), and Bacto Agar (15 g) per liter of medium.

Characterization procedures. Isolates were characterized by the KOH test as an alternative for Gram staining (3), the oxidase test (11), oxidation-fermentation with glucose (7), the arginine deiminase test (30), production of NO₂⁻ or N₂ from NO₃⁻ (28) with ethanol (10 g/liter) instead of glycerol, and production of a fluorescent pigment on King B medium (10).

RESULTS

Bromate removal by a mixed bacterial population. In the first two experiments, bromate reduction by a mixed population of denitrifying bacteria was tested under anaerobic (experiment 1) and aerobic (experiment 2) conditions. In all media used in experiment 1, nitrate reduction started after 2 days of incubation (Fig. 1a) and was complete after 8 days. In medium 1B, the nitrate concentration on day 6 was 1.8 mg of NO₃⁻-N per liter and nitrite was observed (2.3 mg of NO₂⁻-N per liter). The pH increased from 7.2 to 8.3 in media 1A and 1B but did not change in media 1C and 1D. On day 6, the supply of nitrogen gas to all flasks was stopped and the pH of media 1A and 1B was adjusted to 7.2 by adding HCl from a sterile solution. More ethanol (50 mg/liter) was added to prevent ethanol limitation. Subsequently, the sampling tubes were

FIG. 1. Nitrate (○, experiments 1A and 1B), bromate (●, 1A and 1C; ○, 1B and 1D), and bromide (■, 1A and 1C; □, 1B and 1D) concentrations in the media used in experiment 1. Vertical dashed lines: 1, stop nitrogen supply, pH adjustment, ethanol addition; 2, addition of 5 mg of NO₃⁻-N per liter and 1,000 µg of BrO₃⁻ per liter (a); 3, inoculation from medium 1B (b).

closed to prevent oxygen introduction. The sulfate concentration in the media did not decrease during the experimental period.

Bromate removal in experiment 1 was observed for the first time in medium 1B after 8 days of incubation (Fig. 1a). The bromate concentration decreased linearly with time (86 µg liter⁻¹ · day⁻¹) and was < 5 µg/liter after 23 days. Medium 1A was supplemented with 2.5 mg of NO₃⁻-N per liter and 1,000 µg of BrO₃⁻ per liter on day 8. On day 15, nitrate was completely removed from this medium and bromate removal was observed (Fig. 1a). The rate of bromate removal initially was of the same order of magnitude as monitored in medium 1B but stopped after 27 days of incubation at a concentration of about 200 µg of BrO₃⁻ per liter. After 37 days, this experiment was terminated because of depletion of the medium.

In flasks 1A and 1B, bromide production was observed as soon as bromate removal occurred. The observed average ratio of bromide to removed bromate in media 1A and 1B was 0.69 (standard deviation, 0.12), which is close to the stoichiometric bromide/bromate ratio of 0.63 µg of Br⁻ per µg of BrO₃⁻.

TABLE 2. Main characteristics of the predominant strains isolated from experiments 1 and 3 with R₂A medium

Characteristic	Result for isolates categorized after denitrification test		
	Denitrifying bacteria, group I ^a	Nitrite-producing bacteria, group II ^b	Nondenitrifying bacteria, group III ^c
Origin ^d	1B, 3A	3A, 3B	1A, 1B, 3A, 3B
Cell morphology	Rod	Rod	Coccus/rod
KOH test (Gram stain) ^e	Slime (-)	Slime (-)	(+/-)
Fluorescent pigments	+/-	+/-	-
N ₂ production from NO ₃ ⁻	+	-	-
Nitrite production ^f	(+)	+	-/(-)
Oxidase	+	+	+/-
Arginine dihydrolase	+/-	+/-	+/-
Acid from glucose O-F ^g	+/-	(+ -)/-	-/-

^a This group included isolates Br1, Br4, Br5, Br14, Br16, Br22, and Br23.

^b This group included isolates Br15, Br17, Br18, Br19, Br21, Br24, and Br25.

^c This group included isolates Br2, Br3, Br6 to Br13, and Br20.

^d Experimental media from which the isolates were obtained.

^e Gram-negative bacteria produce slime in the KOH test.

^f (+), nitrite as intermediate; (-), nitrite production and no growth.

^g O-F, oxidation-fermentation.

In experiment 1 the MS medium initially contained a small amount of NO₃⁻ (0.5 mg of NO₃⁻-N per liter), which was removed from the control flasks 1C and 1D within the first few days of incubation. During the entire incubation period of 71 days, the bromate concentration in medium 1C remained at the initial concentration of 900 µg/liter (standard deviation, 28) added to all media used in experiment 1 (Fig. 1b). The bromide concentration in this medium remained below the detection limit of 20 µg of Br⁻ per liter. Also, in flask 1D containing BrO₃⁻ and ethanol, no bromate removal occurred until day 30. On day 24 this flask was inoculated with 5 ml of medium 1B. A small decrease in the BrO₃⁻ concentration was observed after inoculation and coincided with a small increase in the Br⁻ concentration (Fig. 1b). After 37 days of incubation, the ethanol concentration in flask 1D had decreased to < 0.1 mg/liter despite the excess amount of ethanol initially added. High biomass densities observed in the media suggested that some oxygen was introduced during sampling and/or incubation.

In experiment 2 the MS medium with 100 mg of ethanol per liter was supplemented either with bromate and nitrate (medium 2A) or with bromate alone (medium 2B) (Table 1). The media were inoculated with the EC of medium 1B and incubated aerobically at room temperature (±20°C). Under these conditions, the bromate concentrations in flasks 2A and 2B after 1 month of incubation were 930 and 970 µg/liter, respectively, and no bromide production or nitrate reduction was observed.

Effect of denitrification on bromate removal. In medium 1D of experiment 1, some bromate reduction has been observed under anaerobic conditions without significant denitrification. In experiment 3 the EC of medium 1B was used to determine the effect of a preceding nitrate reduction on bromate removal.

The nitrate reduction was complete in medium 3A within 14 days of incubation (Fig. 2). Significant BrO₃⁻ removal with Br⁻ production was observed after 21 days of incubation, both with and without preceding NO₃⁻ reduction. In medium 3A with an initial bromate concentration of 1,000 µg/liter, the removal rate was about 16 µg of BrO₃⁻ per liter per day. The removal rate in medium 3B, without preceding nitrate reduction and with a bromate concentration of 5,000 µg/liter was about 34 µg of BrO₃⁻ per liter per day. The bromide/bromate ratio was 0.62 (standard deviation, 0.15). Bromate removal or bromide production was not observed in the autoclaved con-

trol with ethanol (experiment 3C) or in the nonautoclaved control without ethanol (experiment 3D).

After 100 days of incubation, the ethanol concentration in media 3A and 3B was 4 and 3.1 mg/liter, respectively. These results suggest that some oxygen was introduced during the experiment despite the incubation in a vacuum desiccator with a continuous nitrogen supply. The removal of BrO₃⁻ in medium 3B was not complete, and therefore 100 mg of ethanol per liter was added to this medium at day 111 to achieve complete bromate removal.

Isolation and characterization of bacteria. The biomass production in the media used in experiments 1 and 3 was monitored by the colony count on R₂A agar plates incubated aerobically. The maximum colony count in the media with nitrate and bromate removal (1A, 1B, 3A, and 3B) varied between 1.4 × 10⁷ and 4.3 × 10⁷ CFU/ml.

Isolation of the predominant colonies cultivated on R₂A medium inoculated with samples from media 1A, 1B, 3A, and 3B yielded 25 bacterial strains. Seven isolates (group I) were

FIG. 2. Nitrate (●, experiment 3A) and bromate (●, 3A; ○, 3B) reduction and bromide (■, 3A; □, 3B) production in the media used in experiment 3 inoculated with the enrichment culture.

FIG. 3. Bromate and bromide concentrations in the media used in experiment 4 inoculated with EC from medium 3A and pure cultures isolated from media 1A, 1B, 3A, and 3B (Table 4). Solid bar, flask 1; hatched bar, flask 2 analyzed after 32 and 75 days, respectively.

identified as denitrifying *Pseudomonas* spp. (Table 2), six were denitrifying fluorescent pseudomonads (strains Br4, Br5, Br14, Br16, Br22, and Br23). Seven strains (group II) showed significant growth and nitrite production but no nitrogen production in the denitrification test with ethanol. Two of these strains, Br17 and Br25, were fluorescent pseudomonads. Eleven microorganisms (group III) showed no growth in the denitrification test with ethanol. In this group, strains Br3, Br6, Br7, Br8, Br9, Br10, and Br12 produced nitrite in the denitrification test. None of these strains produced fluorescent pigments (Table 2).

Bromate removal by pure cultures. Eight strains from the 25 pure cultures were selected for an anaerobic-growth experiment in the MS medium containing 200 µg of BrO_3^- per liter, either with NO_3^- (medium 4A) or without NO_3^- (medium 4B). Molybdenum (0.01 mg/liter) was added to ensure that this element did not limit denitrifying activity. Also, the EC of medium 3A and a fluorescent pseudomonad, strain P17, an organism isolated from drinking water, were tested in these media. The media were incubated in duplicate, and concentrations of NO_3^- , BrO_3^- , and Br^- were determined on day 32 in flask 1 and on day 75 in flask 2 (Fig. 3).

Nitrate was completely removed from medium 4A inoculated with the EC, strain P17, and the strains identified as denitrifying *Pseudomonas* spp. (group I, e.g., Br4, Br5, and Br14) (Table 3). Almost complete removal of BrO_3^- was observed at day 32 in media 4A and 4B inoculated with EC, and

at day 75 the bromate concentration in these media was <1 µg/liter. The pure cultures removed bromate in medium 4A to about 140 and 30 µg/liter after 32 and 75 days of incubation, respectively. BrO_3^- removal was also observed in medium 4B inoculated with these bacteria; however, at day 75 the bromate concentration still was about 130 µg/liter. Bromide production was observed in all media inoculated with EC, P17, Br4, Br5, and Br14. The $\text{Br}^-/\text{BrO}_3^-$ ratio was 0.59 (standard deviation, 0.1), indicating that all BrO_3^- was converted to Br^- .

Strains Br17, Br21, and Br25 of group II, which had been identified as nitrite-producing bacteria (Table 2), differed from each other with respect to nitrate and/or bromate reduction in media 4A and 4B (Fig. 3). Strain Br17 did not reduce nitrate or bromate in these media, whereas strain Br21 removed nitrate from medium 4A and bromate from both media. Strain Br25 reduced nitrate and bromate in medium 4A. However, the colony count revealed that medium 4A of strain Br25 had been infected with other organisms. Production of NO_2^- was not detected in any of the media. Strains Br9 and Br12 were tested as representatives of group III, i.e., isolates identified as non-denitrifying bacteria. These strains did not reduce nitrate and/or bromate in the media tested.

The results obtained in experiment 4 revealed that the removal rate of BrO_3^- observed in the media with the pure cultures was lower than the removal rate observed for the EC (Fig. 3). To determine the effect of bromate concentration on

TABLE 3. Characteristics of the bacterial strains tested for bromate removal

Characteristic	Result for strain:						
	<i>P. fluorescens</i> P17 ^a	Denitrifying bacteria, group I (Br4, Br5, Br14)	Nitrite-producing bacteria, group II			Nondenitrifying bacteria, group III	
			Br17	Br21	Br25	Br9	Br12
Fluorescent pigments	+	+	+	—	+	—	+
Oxidase	+	+	+	+	+	+	+
Arginine dihydrolase	+	+	+	—	+	—	—
Acid from glucose O-F ^b	+/-	+/-	+/-	+/-	+/-	+/-	+/-
NO ₃ ⁻ /NO ₂ ^{-c} in medium 4A	+/-	+/-	-/-	+/-	+/- ^d	-/-	-/-
Bromate removal in medium 4A	+	+	—	+	+i	—	—
Bromate removal in medium 4B	+	+	—	+	—	+i	—

^a Strain isolated from drinking water.^b O-F, oxidation-fermentation.^c NO₃⁻ removal/NO₂⁻ production.^d +i, experimental medium infected.

the bromate removal rate in a pure culture, strain Br5, a fluorescent pseudomonad (Table 3), was tested at 1,000 and 5,000 µg of BrO₃⁻ per liter under conditions identical to those of experiment 3 (Fig. 4). Nitrate was removed from medium 5A within the first few days of incubation, and BrO₃⁻ removal and Br⁻ production were observed after 21 and 28 days of incubation in media 5A and 5B, respectively. The BrO₃⁻ removal rate in the medium with a BrO₃⁻ concentration of 1,000 and 5,000 µg/liter was 1.6 and 3.5 µg liter⁻¹ · day⁻¹, respectively. After 150 days of incubation, the BrO₃⁻ removal and Br⁻ production stopped. The concentration of ethanol was less than 0.1 mg/liter on day 167.

DISCUSSION

The results presented above show that bromate was reduced to bromide after complete nitrate reduction by a mixed denitrifying bacterial population with ethanol as an energy source under anaerobic conditions at 20 and 25°C. Denitrifying *Pseudomonas* spp., including representatives of the species *Pseudomonas fluorescens*, predominated in the aerobic colony counts on R₂A medium. Pure cultures of denitrifying fluorescent pseudomonads showed the ability to reduce bromate to bromide with ethanol as the sole energy and carbon source. The group of nitrite-producing *Pseudomonas* spp. was more

heterogeneous with respect to the reduction of bromate. A fluorescent pseudomonad, strain P17, isolated from drinking water not enriched with bromate (33), also reduced bromate to bromide. Hence, bromate reduction may be a common characteristic of denitrifying *Pseudomonas* spp. *Pseudomonas* spp. are also able to reduce chlorate (ClO₃⁻) (18) and selenate (SeO₄²⁻) (16, 17). Chlorate is an effective electron acceptor for bacteria (19), and it is likely that bromate is used in the same way. This hypothesis is supported by the observation that bromate removal stagnated in the absence of ethanol. Furthermore, the ratios of produced Br⁻ to removed BrO₃⁻ indicated that bromate was completely converted to bromide without accumulation of intermediates, e.g., BrO₂⁻ and BrO⁻. Bromate was not reduced in the presence of nitrate. Nitrate was also found to inhibit the reduction of chlorate and selenate (6, 23, 27, 29, 31). Chlorate is a substrate for nitrate reductase (5, 14, 23), and the same enzyme may be responsible for the reduction of bromate. A specific pathway in bacteria for the reduction of bromate seems unlikely, since there is no evidence that bromate is an ion of natural origin. However, chlorate reductase has been observed in microorganisms (21, 24), and a preceding nitrate reduction was not necessary to initiate bromate removal (see above). Therefore, elucidation of the biochemical pathway of bacterial bromate reduction requires further investigations.

The reduction rate of bromate in a medium with a mixed bacterial population of denitrifying bacteria was higher than in a medium inoculated with denitrifying pure cultures obtained from this population (Fig. 2 and 4; Table 4). Obviously, the organisms with the highest affinity (which is defined as the

FIG. 4. Nitrate (●, experiment 5A) and bromate (●, 5A; ○, 5B) reduction and bromide (■, 5A; □, 5B) production in the media used in experiment 5 with isolate Br5 (*P. fluorescens*).

TABLE 4. Nitrate and bromate removal rates as observed in media with different bromate concentrations inoculated with EC and *P. fluorescens* Br5

Culture	BrO ₃ ⁻ concn (µg/liter)	Removal rate (µg liter ⁻¹ · day ⁻¹) of:			
		NO ₃ ^{-a}		BrO ₃ ⁻	
		20°C	25°C	20°C	25°C
EC	1,000	>1,570	>7,300	16	86
	5,000	ND ^b	ND	34	ND
Br5	1,000	>2,260	ND	1.6	ND
	5,000	ND	ND	3.5	ND

^a Concentrations in media, ≥ 5 mg of NO₃⁻ per liter.^b ND, not determined.

quotient of the maximum activity and the half-saturation constant K_s) for bromate were not isolated. On the other hand, a consortium of microorganisms may be more effective than a pure culture in bromate reduction (synergism). Since no evidence was obtained for accumulation of intermediates, e.g., BrO_2^- or BrO^- , it is unlikely that these compounds hampered bromate reduction rate by the mixed bacterial or pure cultures under the test conditions. Further research is needed to determine (i) the presence of the bacteria in the environment with a higher affinity for bromate than the strains used in this study and (ii) the kinetics of the reduction of bromate to bromide by individual isolates as well as a mixed population of denitrifying bacteria.

Under the experimental conditions used, the BrO_3^- removal rate was at least a 100 times lower than the NO_3^- removal rate (Table 4). Raising the initial BrO_3^- concentration in the medium from 1,000 to 5,000 $\mu\text{g/liter}$ resulted in a doubling of the reduction rate. The optimum temperature for the bacterial bromate reduction is most probably above 20°C (Table 4). Malmqvist et al. (19) found a reduction of 110 mg of ClO_3^- per liter (1.31 mM) within 9 days of incubation at 37°C in a batch experiment with activated sludge supplemented with acetate. The BrO_3^- removal rate most probably is greater at higher biomass concentrations, e.g., those present in anaerobic fixed-biofilm reactors. However, the results of the present study indicate that biological bromate removal in water treatment processes may not be very promising because (i) complete removal of both oxygen and nitrate is required and (ii) bromate removal is much slower than nitrate removal. Investigations on bromate reduction in fixed-film reactors are required to assess the bromate removal capacity of these processes. Such studies are also useful to predict bromate removal during anaerobic soil passage.

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