



OPEN ACCESS

EDITED BY

Carmen Falagan,
University of Portsmouth, United Kingdom

REVIEWED BY

Kengo Inoue,
University of Miyazaki, Japan
Hai Pham,
VNU University of Science, Vietnam
Yoshihide Tokunou,
University of Tsukuba, Japan

*CORRESPONDENCE

Seigo Amachi
✉ amachi@faculty.chiba-u.jp

[†]These authors have contributed equally to this work

RECEIVED 09 June 2025

REVISED 12 December 2025

ACCEPTED 17 December 2025

PUBLISHED 20 January 2026

CITATION

Takahashi N, Fujiya H, Sato H, Yamamura S and Amachi S (2026) Bromate reduction by *Shewanella* species depends on both endogenous and exogenous iron. *Front. Microbiol.* 16:1643578. doi: 10.3389/fmicb.2025.1643578

COPYRIGHT

© 2026 Takahashi, Fujiya, Sato, Yamamura and Amachi. This is an open-access article distributed under the terms of the [Creative Commons Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Bromate reduction by *Shewanella* species depends on both endogenous and exogenous iron

Natsuki Takahashi^{1†}, Hiroko Fujiya^{1†}, Haruhiko Sato¹, Shigeki Yamamura² and Seigo Amachi^{1*}

¹Graduate School of Horticulture, Chiba University, Chiba, Japan, ²Regional Environment Conservation Division, National Institute for Environmental Studies, Tsukuba, Japan

In this study, we isolated *Shewanella* sp. strain M-Br, a bromate (BrO_3^-)-reducing bacterium, from seawater. In the presence of lactate as the electron donor, this strain reduced 250 μM bromate to bromide (Br^-) in 3 days. Interestingly, bromate reduction by M-Br was ferric iron [Fe(III)]-dependent, and its bromate reduction rate was proportional to the Fe(III) concentration in the medium. Subsequently, a washed cell suspension of M-Br was prepared to determine whether the effect of Fe(III) on bromate reduction by M-Br is due to the endogenous iron sources, such as c-type cytochromes, or exogenous iron sources, such as redox mediators. Notably, cells pre-grown in the absence of Fe(III) did not reduce bromate, regardless of the addition of exogenous Fe(III). In contrast, cells pre-grown with Fe(III) reduced bromate upon the addition of exogenous Fe(III) to the cell suspension. Such iron-dependent bromate reduction was also observed in other *Shewanella* species, such as *S. putrefaciens* CN-32 and *S. oneidensis* MR-1. Our results suggest that both endogenous and exogenous iron are essential for bromate reduction by *Shewanella* species. Based on these results, we proposed a model, in which outer membrane multi-heme cytochromes, such as MtrC and OmcA, reduce Fe(III) to Fe(II), which further chemically reduces bromate to bromide. Such bacteria are useful for the removal of bromate, a potential human carcinogen, from drinking water.

KEYWORDS

bromate (BrO_3^-), c-type cytochrome, iron, redox mediator, reduction, *Shewanella*

1 Introduction

Bromate (BrO_3^-) is an ozonation byproduct of drinking water containing bromide ion (Br^-) (Butler et al., 2005; Jahan et al., 2021). It is a potential human carcinogen, with a maximum contamination level of 10 $\mu\text{g L}^{-1}$ in drinking water, as regulated by the International Agency for Research on Cancer (WHO, 2011). Optimization of the ozone level (Wert et al., 2007; Grefte et al., 2013), decrease in pH (Legube et al., 2004), and addition of ammonia (Neemann et al., 2004; Ikehata et al., 2013) and hydrogen peroxide (Wu et al., 2020) are efficient methods to minimize bromate formation during ozonation. However, complete prevention of bromate formation remains challenging. Various physical, chemical, and biological methods have been extensively studied for bromate removal from treated water (Jahan et al., 2021). Granular (Kirisits et al., 2000; Zhang et al., 2015) and powdered (Wang et al., 2010) activated carbon remove bromate via reduction and/or adsorption and are superior to chemical methods, such as addition of coagulants and reducing agents (Siddiqui et al., 1994; Gordon et al., 2002). However, use of granular and powdered activated carbon is not cost-effective. Therefore, biological methods not requiring any chemicals or energy for bromate removal have recently attracted considerable attention.

Biologically activated carbon (BAC) filters effectively remove bromate from drinking water, particularly after ozonation (Liu et al., 2012). Specifically, microorganisms, particularly bacteria, in the BAC filters reduce bromate to innocuous bromide (Lv et al., 2019). Moreover, dissolved oxygen level in the reactor should be kept low, and an appropriate carbon source should be added as an electron donor (Kirisits et al., 2001; Kirisits et al., 2002). To date, many studies have analyzed the microbial community structures in BAC filters and other bromate-reducing reactors (Kirisits et al., 2002; Assunção et al., 2011; Davidson et al., 2011; Liu et al., 2012; Luo et al., 2017; Zhong et al., 2018) and revealed that bromate-reducing bacteria are phylogenetically diverse, suggesting the ubiquitous distribution of bromate-reducing ability among different bacteria. However, only a few studies have attempted to isolate such bacteria from the reactors and natural environments (Hijnen et al., 1995; Davidson et al., 2011; Wang et al., 2022c).

We previously isolated the bromate-reducing bacterium, *Rhodococcus* sp. Br-6, from the soil (Tamai et al., 2016). This strain completely reduced 250 μM bromate in 4 days using the redox mediators, 2,6-dichloroindophenol (DCIP) and ferric iron [Fe(III)]. It first enzymatically reduced DCIP to DCIPH₂, followed by the chemical reduction of Fe(III) to ferrous iron [Fe(II)], and finally Fe(II) chemically reduced bromate to bromide (Tamai et al., 2016). Although such hybrid biological and chemical bromate reduction mechanisms are interesting, the practical application of such systems is challenging because they require DCIP as a redox indicator. This difficulty motivated us to search for bacteria that can utilize iron alone as a redox mediator. In this study, we enriched bromate-reducing bacteria from seawater and isolated a new bromate-reducing bacterium, *Shewanella* sp. M-Br. Notably, bromate reduction by this strain was dependent on both the endogenous and exogenous iron. Additionally, our findings revealed the potential bromate reduction mechanisms of various *Shewanella* species, including the isolated strain.

2 Materials and methods

2.1 Enrichment of bromate-reducing Bacteria

Surface seawater was collected from Shin-Maiko Beach (Futsu, Chiba, Japan). An enrichment culture was prepared by inoculating 1 mL of seawater into 18 mL of minimal medium containing NH₄Cl (0.535 g), KH₂PO₄ (0.136 g), MgCl₂·6H₂O (0.204 g), CaCl₂·2H₂O (0.147 g), NaCl (20 g), trace mineral element solution (5 mL), vitamin solution (10 mL), and NaHCO₃ (2.52 g) per liter. The trace mineral element solution was based on that of DSM 318 medium (DSMZ, 1993), and the vitamin solution was based on that of DSM 141 medium (DSMZ, 1993). The minimal medium used in this study contained 25 μM of Fe(III) as FeCl₃·6H₂O instead of FeCl₂·6H₂O in the original DSM 318 medium. For anaerobic incubation, the minimal medium was dispensed into a 60-mL serum bottle under an N₂/CO₂ (80:20) gas stream. The bottle was sealed with a thick butyl rubber stopper and aluminum cap. After autoclaving at 121 °C for 20 min, sodium acetate and sodium bromate were added separately from sterile anaerobic stock solutions to achieve final concentrations of 20 mM and 250 μM , respectively. The final pH of the medium was 6.8–7.0. The bottles were subsequently incubated at 30 °C without shaking. Under microaerobic conditions, incubation was performed similarly to that under anaerobic conditions in a sealed serum bottle,

but air substitution of the headspace and liquid phase with N₂/CO₂ gas was omitted. For microaerobic incubation, 0.174 g L⁻¹ of K₂HPO₄ was added to the minimal medium, but NaHCO₃ was excluded. The pH of the medium for microaerobic incubation was adjusted to 7.0 with NaOH. Under microaerobic conditions, oxygen in the medium is gradually consumed by bacteria, eventually resulting in anaerobic conditions (Tamai et al., 2016).

2.2 Isolation of bromate-reducing Bacteria

To isolate bromate-reducing bacteria, the enrichment culture sub-cultured five times under microaerobic conditions was serially diluted and spread on a solid minimal medium containing sodium acetate and 15 g L⁻¹ of agar. In some cases, Luria–Bertani (LB) agar medium was used instead of a solid minimal medium. After aerobic incubation at 30 °C, bacterial colonies were randomly selected and purified. Subsequently, their bromate-reducing capacity was evaluated using a liquid minimal medium under microaerobic conditions. Specifically, 25 isolates were cultured with 250 μM bromate for 3 to 30 days under microaerobic conditions and bromate concentration in the medium was determined.

2.3 Identification and phylogenetic analysis of bacterial isolates

Genomic DNA of the isolated bacteria was extracted as previously described (Hiraishi, 1992). The 16S rRNA gene was amplified via polymerase chain reaction (PCR) using the bacterial consensus primers, 10F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1500R (5'-GGTACCTTGTACGACTT-3'). PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and ABI Prism 3100 Genetic Analyzer (Applied Biosystems) using appropriate sequencing primers (Weisburg et al., 1991). The obtained 16S rRNA gene sequences were subjected to a Basic Local Alignment Search Tool search¹ to determine the sequence similarity. The retrieved sequences were aligned using ClustalX version 2.0 (Larkin et al., 2007). Finally, a phylogenetic tree was constructed using the neighbor-joining method with the MEGA11 software package (Kumar et al., 2016).

2.4 Bromate reduction by the strain M-Br growing culture

Strain M-Br was first cultured aerobically in cultured in LB liquid medium. Cells were collected and washed twice with 20 mM Tris–HCl buffer (pH 7.0). The washed cells were then inoculated into a minimal medium containing 250 μM bromate, and cultured under anaerobic, microaerobic, and aerobic growth conditions. Sodium lactate was added as the electron donor and carbon source at a final concentration of 20 mM. Aerobic incubation was performed similarly to microaerobic incubation, except that the medium (20 mL) was

¹ <https://www.ncbi.nlm.nih.gov/BLAST/>

dispensed into a 100-mL Erlenmeyer flask capped with a silicone plug, and the flask was incubated with shaking at 180 rpm.

To determine the effect of Fe(III) on bromate reduction, strain M-Br was grown in a minimal medium containing 0–100 μM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ under microaerobic conditions. To control the final Fe(III) concentration in the medium, Fe(III) in the trace mineral element solution was excluded from this experiment.

2.5 Bromate reduction by the washed cell suspensions of *Shewanella* species

S. putrefaciens CN-32 and *S. oneidensis* MR-1 were purchased from the American Type Culture Collection (ATCC BAA-453) and Japan Collection of Microorganisms (JCM 31522), respectively. Strain M-Br and these *Shewanella* spp. were grown in the minimum medium for 3 days under microaerobic conditions. The cells were collected, washed twice with 20 mM Tris-HCl buffer (pH 7.0), and resuspended in the same buffer to achieve an optical density at 600 nm of 0.5. Approximately 15 mL of the washed cell suspension was dispensed into a 100-mL Erlenmeyer flask or 60-mL serum bottle. Lactate, bromate, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and nitrilotriacetic acid (chelating reagent) were added to the suspension at final concentrations of 10 mM, 50 μM , 25 μM , and 335 μM , respectively. The flask was capped with a silicone plug and incubated with shaking at 180 rpm. In contrast, the serum bottle was degassed with N_2 gas, sealed with a butyl rubber stopper and an aluminum cap, and anaerobically incubated without shaking.

To determine the effects of endogenous and exogenous Fe(III) on bromate reduction, washed cell suspensions were prepared from the cells grown with or without Fe(III) and supplemented with lactate, bromate, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and nitrilotriacetic acid, as described above. In some cases, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was not added to the cell suspensions. Then, the cell suspensions were anaerobically incubated in serum bottles, as described above.

2.6 Bromate reduction by other Fe(III)-reducing Bacteria

To determine if other iron-reducing bacteria can reduce bromate, *Geobacter* sp. OR-1 (Ohtsuka et al., 2013) and *Anaeromyxobacter* sp. PSR-1 (Kudo et al., 2013) were used. They were grown anaerobically in the minimal medium containing 20 mM acetate and 20 mM Fe(III) as the electron donor and acceptor, respectively. Bromate was also added at a final concentration of 250 μM . In other cases, these strains were grown anaerobically with 20 mM acetate and 20 mM fumarate, which serves as an effective electron acceptor comparable to iron.

2.7 Analytical techniques

Bromate concentration was spectrophotometrically determined, as previously described (Tamai et al., 2016). Bromide was determined by the IC-2010 ion chromatography system (Tosoh, Tokyo, Japan) with the TSKgel SuperIC-Anion HR column (Tosoh) connected to the TSKgel SuperIC-A HS guard column (Tosoh). The mobile phase consisted of 2.2 mM NaHCO_3 and 2.7 mM Na_2CO_3 at a flow rate of 1.0 mL min^{-1} , and the column temperature was maintained at 40 °C.

3 Results

3.1 Enrichment and isolation of bromate-reducing Bacteria

Seawater was inoculated into a minimal medium and incubated with 250 μM bromate under anaerobic and microaerobic conditions (Supplementary Figure 1). Under anaerobic conditions, few bromate was reduced, even after incubation for 50 d. However, bromate was completely reduced in 14 d under microaerobic conditions. After five sub-cultures, bromate reduction was completed in 5 days. From this enrichment culture, multiple bacteria were randomly isolated and evaluated for their bromate-reducing capacity. Most isolated strains reduced bromate; however, their bromate reduction rates were very slow (data not shown). Among the tested strains, one exhibited the fastest bromate reduction rate, completely reducing 250 μM bromate in 4 days. This strain was selected as a novel bromate-reducing bacterium and designated as the strain M-Br.

Phylogenetic analysis via 16S rRNA gene sequencing revealed that the strain M-Br (NCBI accession number: LC900506) was closely related to the *S. putrefaciens* W3-18-1 and *S. putrefaciens* CN-32, with a sequence similarity of over 99% (Supplementary Figure 2). Other related bacteria included *S. oneidensis* MR-1, a representative dissimilatory Fe(III)-reducing bacterium.

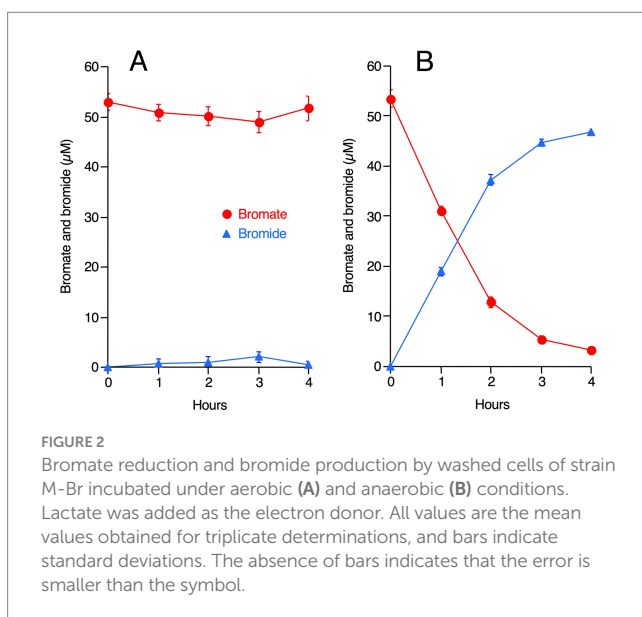
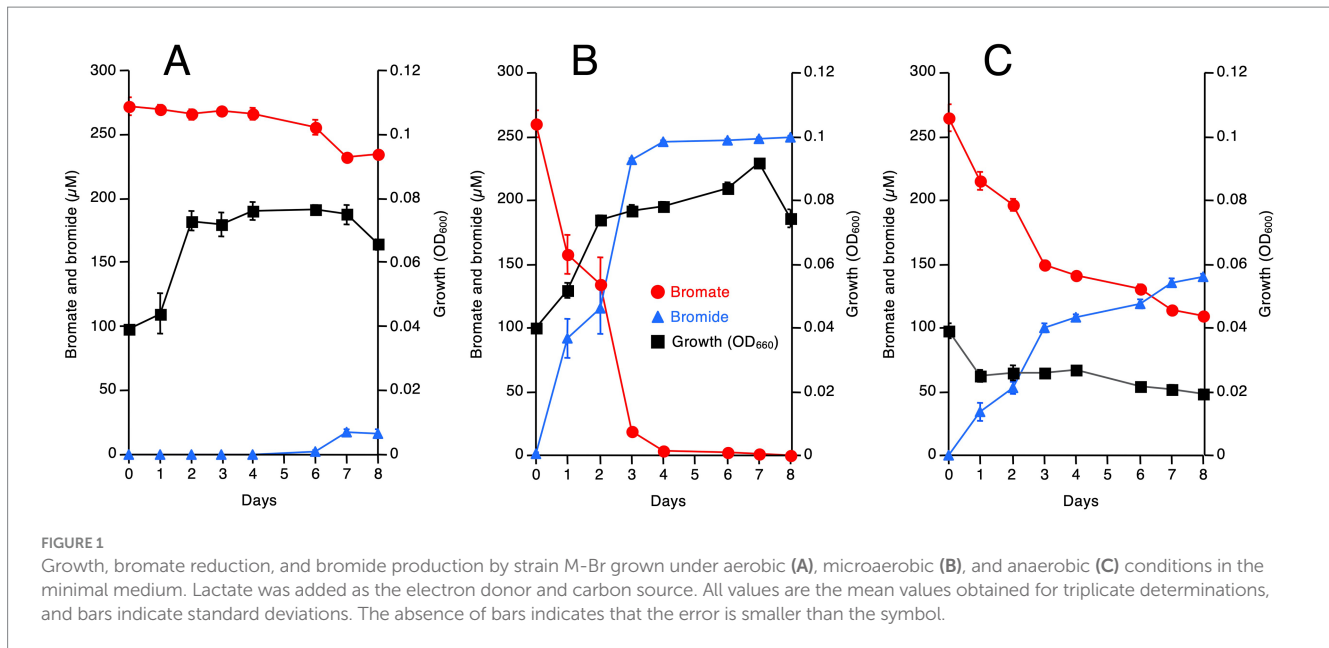
3.2 Bromate reduction by strain M-Br under various growth conditions

M-Br was grown with bromate under aerobic, microaerobic, and anaerobic conditions, and its growth and bromate reduction rate were analyzed (Figure 1). Lactate was used instead of acetate as the electron donor and carbon source because it is generally the preferred substrate for the growth of *Shewanella* species under aerobic and anaerobic conditions (Zhang et al., 2021). As shown in Figure 1A, M-Br grew well but only reduced a small amount of bromate under aerobic conditions. Notably, M-Br grew better under microaerobic conditions than under aerobic conditions, completely reducing 250 μM bromate in 4 days (Figure 1B). Interestingly, the strain did not grow under anaerobic conditions, but it reduced approximately half of the bromate in 8 days (Figure 1C).

Bromate reduction was much faster under microaerobic conditions than under aerobic and anaerobic conditions, suggesting that the strain M-Br reduces bromate preferably under oxygen-limited conditions but grows well under oxygen-rich conditions. To verify this hypothesis, a washed cell suspension of M-Br was prepared, and bromate reduction was examined under aerobic and anaerobic conditions (Figure 2). Indeed, no bromate was reduced under aerobic conditions, whereas 50 μM bromate was almost completely reduced in 3 h under anaerobic conditions.

3.3 Effect of Fe(III) on bromate reduction by strain M-Br

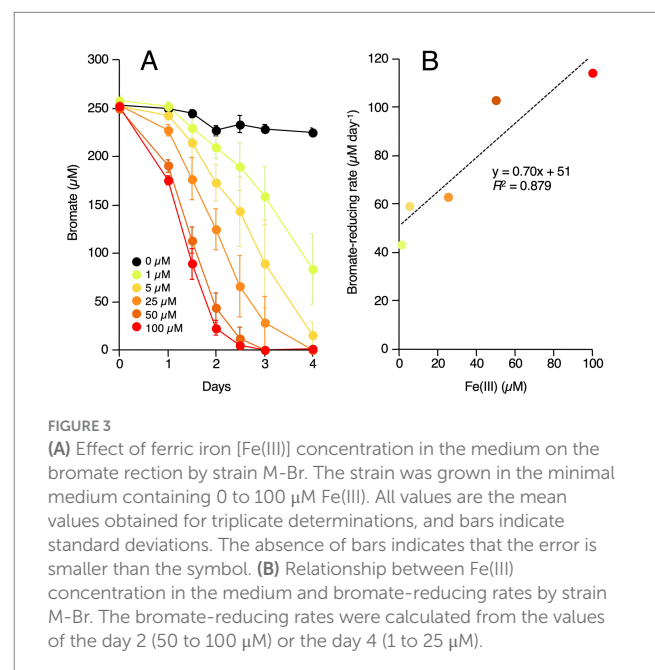
We previously reported that *Rhodococcus* sp. Br-6 uses Fe(III) as a redox mediator for bromate reduction (Tamai et al., 2016). As *Shewanella* species are well-known dissimilatory Fe(III)-reducing bacteria, strain M-Br possibly also uses Fe(III) as a redox mediator for



bromate reduction. To verify this, a minimal medium lacking Fe(III) was prepared, and M-Br was grown with or without 1–100 μM Fe(III) under microaerobic conditions. As shown in Figure 3A, M-Br did not reduce bromate in the absence of Fe(III). In contrast, Fe(III) addition accelerated bromate reduction in a dose-dependent manner. As shown in Figure 3B, bromate reduction rate ($\mu\text{M day}^{-1}$) of M-Br was positively correlated with the Fe(III) concentration in the medium.

3.4 Effect of Fe(III) on bromate reduction by washed cells of strain M-Br

Accelerated bromate reduction by Fe(III) is due to two possible reasons. First, Fe(III) functions as a redox mediator for bromate reduction, as in *Rhodococcus* sp. Br-6 (Tamai et al., 2016). Second, iron-associated proteins, such as *c*-type cytochromes, are involved in



bromate reduction. To determine the specific mechanism, M-Br was pre-grown with or without Fe(III), and washed cells were prepared. The cells were incubated with bromate with or without exogenous Fe(III). As shown in Figure 4, bromate was reduced only when M-Br was pre-grown in the presence of Fe(III) and its washed cells were incubated with exogenous Fe(III). However, no significant bromate reduction was observed when M-Br was pre-grown in the absence of Fe(III) and its cells were incubated without exogenous Fe(III).

3.5 Effects of Fe(III) on bromate reduction by other *Shewanella* species

To determine whether Fe(III)-dependent bromate reduction is a general feature of *Shewanella* species, similar experiments were

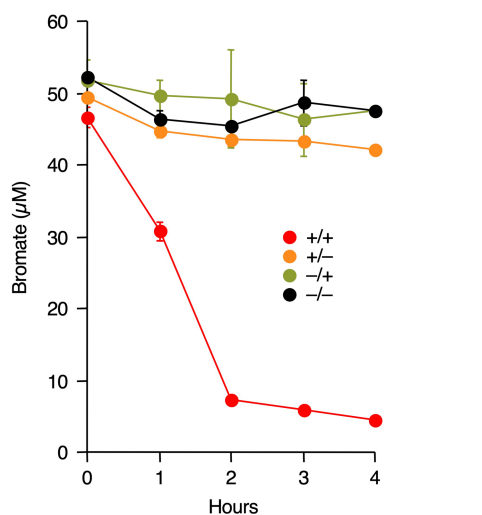


FIGURE 4
Effect of endogenous Fe(III) and exogenous Fe(III) on bromate reduction by washed cells of strain M-Br. The strain was pre-grown in the presence (red and orange circles) or absence (olive and black circles) of Fe(III). After washed cells were prepared, they were incubated with bromate in the presence (red and olive circles) or absence (orange and black circles) of exogenous Fe(III). All values are the mean values obtained for duplicate determinations, and bars indicate standard error of the mean. The absence of bars indicates that the error is smaller than the symbol.

performed using *S. putrefaciens* CN-32 and *S. oneidensis* MR-1. As shown in Figure 5, bromate reduction by both bacteria was also Fe(III)-dependent, i.e., bromate reduction occurred only when the strains were pre-grown with Fe(III) and their washed cells were incubated with exogenous Fe(III).

3.6 Bromate reduction by other iron-reducing bacteria

To determine if other iron-reducing bacteria can reduce bromate, *Geobacter* sp. OR-1 (Ohtsuka et al., 2013) and *Anaeromyxobacter* sp. PSR-1 (Kudo et al., 2013) were grown anaerobically in the minimum medium containing 20 mM acetate and 20 mM Fe(III). Bromate was also added at a final concentration of 250 µM. However, due to the yellow color of the iron-containing culture supernatants, colorimetric quantification of bromate was difficult. In contrast, ion chromatographic analysis revealed that almost no bromide was produced (only up to 3 µM), indicating that bromate reduction had not proceeded. We next cultivated the strains anaerobically with 20 mM acetate and 20 mM fumarate [plus 25 µM Fe(III)], which serves as an effective electron acceptor comparable to iron. In the absence of bromate, both strains exhibited good growth, with OD₆₀₀ values ranging from 0.11 to 0.16 (Figures 6A,B). However, in the presence of 250 µM bromate, growth increased only up to a maximum OD₆₀₀ of 0.02. Quantification of bromate during this incubation showed that although a slight reduction of bromate occurred at the early stage of cultivation, no further reduction was observed thereafter (Figure 6C).

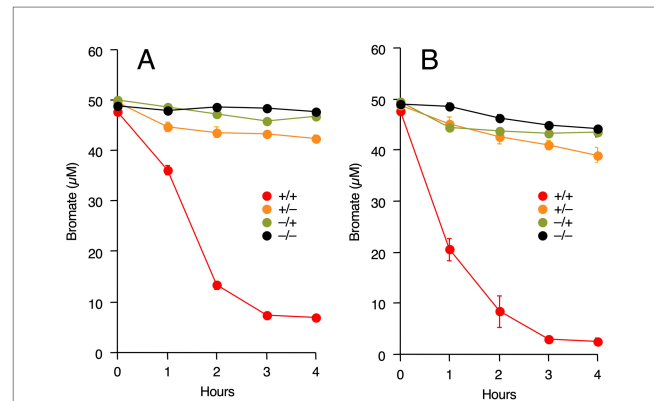


FIGURE 5
Effect of endogenous Fe(III) and exogenous Fe(III) on bromate reduction by washed cells of *Shewanella putrefaciens* CN-32 (A) and *S. oneidensis* MR-1 (B). The strains were pre-grown in the presence (red and orange circles) or absence (olive and black circles) of Fe(III). After washed cells were prepared, they were incubated with bromate in the presence (red and olive circles) or absence (orange and black circles) of exogenous Fe(III). All values are the mean values obtained for duplicate determinations, and bars indicate standard error of the mean. The absence of bars indicates that the error is smaller than the symbol.

4 Discussion

In this study, we first attempted to isolate respiratory bromate-reducing bacteria using bromate as a terminal electron acceptor for growth. However, such bacteria were not enriched under anaerobic conditions (Supplementary Figure 1A). This is consistent with the results of our previous study, in which soil was incubated with bromate under anaerobic conditions (Tamai et al., 2016).

After enrichment under microaerobic conditions, we successfully isolated the bromate-reducing bacterium, *Shewanella* sp. M-Br. This strain grew well under aerobic and microaerobic conditions but reduced bromate only under anaerobic conditions (Figures 1, 2). Notably, M-Br did not grow anaerobically with bromate as the sole electron acceptor, indicating that bromate reduction by this strain is not a respiratory process (Figure 1C). The physiological reason why M-Br reduces bromate remains unclear. As bromate significantly inhibits the growth of specific bacteria, probably because of its strong oxidative power (Wang et al., 2022a), bromate reduction by M-Br and other bacteria is possibly a detoxification process.

Our results strongly suggest that bromate reduction by M-Br is dependent on both endogenous and exogenous iron (Figures 3, 4). M-Br cells grown without Fe(III) appeared white, whereas those grown with Fe(III) appeared pink to pale orange (data not shown). The latter color suggests the presence of heme in the cells, suggesting that endogenous iron is essential for cytochrome biosynthesis. In *S. oneidensis* MR-1, multi-heme *c*-type cytochromes, such as CymA, MtrA, MtrB, MtrC, and OmcA, are involved in the extracellular reduction of Fe(III) (Shi et al., 2012). Among these, CymA, MtrC, and OmcA catalyze the reduction of soluble Fe(III), such as chelated Fe(III) (Gescher et al., 2008; Shi et al., 2012). The draft genome sequence of M-Br (genome assembly number: ASM3624592v1) revealed the presence of orthologous genes encoding these proteins (Supplementary Figure 3). These findings suggest that M-Br reduces

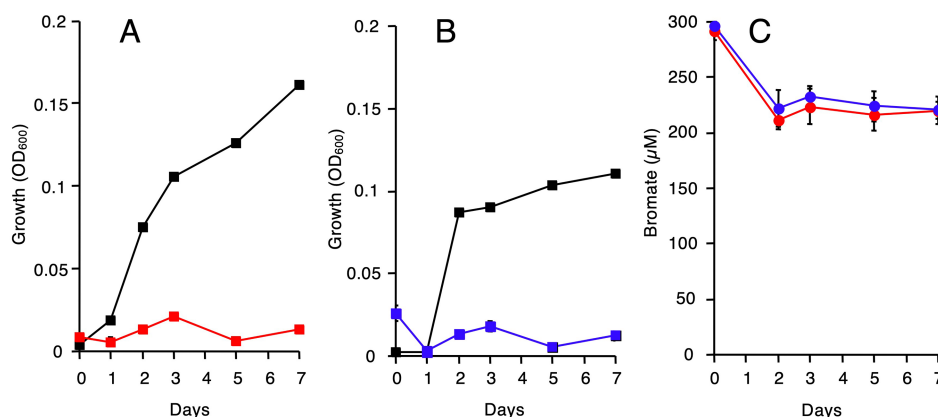
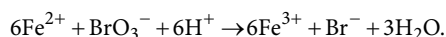


FIGURE 6

Growth and bromate reduction by other iron-reducing bacteria, *Geobacter* sp. OR-1 and *Anaeromyxobacter* sp. PSR-1. (A) *Geobacter* sp. OR-1 was grown with 20 mM fumarate as the electron acceptor in the absence (black squares) or presence (red squares) of 250 μM bromate. (B) *Anaeromyxobacter* sp. PSR-1 was grown with 20 mM fumarate as the electron acceptor in the absence (black squares) or presence (blue squares) of 250 μM bromate. (C) Bromate reduction by *Geobacter* sp. OR-1 (red circles) and *Anaeromyxobacter* sp. PSR-1 (blue circles). Growth conditions were same as (A,B). All values are the mean values obtained for triplicate determinations, and bars indicate standard deviations. The absence of bars indicates that the error is smaller than the symbol.

exogenous Fe(III) using multi-heme *c*-type cytochromes to form Fe(II), which chemically reduces bromate to bromide according to the following equation (Xie and Shang, 2005; Xie et al., 2008):



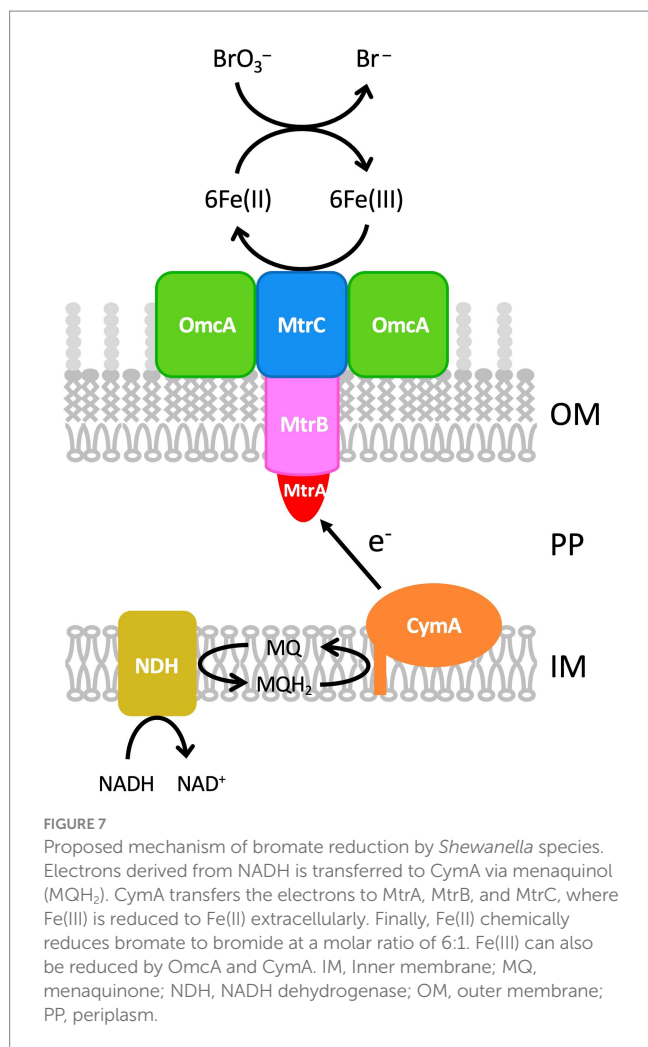
Thus, exogenous iron may act as a redox mediator in bromate reduction, as previously reported in *Rhodococcus* sp. Br-6 (Tamai et al., 2016).

In our previous study, we observed the chemical reduction of bromate by Fe²⁺ (Tamai et al., 2016). Specifically, in the presence of six times the molar amount of Fe²⁺ relative to bromate, bromate was oxidized at a rate of 144 μM day⁻¹. In the present study, the bromate reduction rate by *Shewanella* sp. M-Br was 114 μM day⁻¹ (Figure 3), which agrees well with the above chemical reduction rate. In addition, M-Br reduced 250 μM bromate efficiently even in the presence of a stoichiometrically very low concentration of Fe²⁺ (Figure 3A). This observation indicates that the Fe²⁺ oxidized during bromate reduction was quickly re-reduced to Fe²⁺, suggesting that iron was functioning as a redox mediator in this process. Therefore, it seems reasonable to propose that iron functions not only in the biosynthesis of *c*-type cytochromes but also as a redox mediator.

Our results demonstrated that bromate reduction by *S. putrefaciens* CN-32 and *S. oneidensis* MR-1 was dependent on both endogenous and exogenous iron (Figure 5). Therefore, it is possible that bromate reduction by *Shewanella* species is generally iron-dependent. Recently, Wang et al. (2022a, 2022b) demonstrated bromate reduction by *S. decolorationis* Ni1-3 and *S. oneidensis* MR-1. The culture medium they used contained approximately 3 μM iron as FeSO₄·7H₂O, suggesting the involvement of iron in bromate reduction by the tested strains. Interestingly, Wang et al. (2022b) constructed gene deletion mutants of *S. oneidensis* MR-1 and found that CymA, MtrB, and MtrC are involved in bromate reduction. They proposed that these multi-heme cytochromes are necessary for the degradation of H₂O₂, which is possibly formed as a byproduct during bromate reduction. However, our results suggest that exogenous iron is required as a redox mediator

for bromate reduction and that multi-heme cytochromes function as ferric [Fe(III)] reductases for the continuous supply of Fe(II) during bromate reduction. Additionally, Wang et al. (2022b) proposed that dimethyl sulfoxide reductase encoded by *dmsA* is a bromate reductase, as the Δ*dmsA* mutant showed significantly decreased bromate reduction. However, M-Br, *S. putrefaciens* CN-32, and *S. decolorationis* Ni1-3 do not harbor *dmsA* in their genomes, suggesting that this gene is not always necessary for bromate reduction.

Figure 7 shows a hypothetical model of bromate reduction by *Shewanella* species, including the strain M-Br. Electrons derived from NADH are transferred to menaquinone by NADH dehydrogenase, forming menaquinol in the inner membrane. CymA, a tetraheme *c*-type cytochrome (Myers and Myers, 1997), oxidizes menaquinol in the inner membrane and transfers the released electrons to MtrA in the outer membrane. MtrA is a decaheme *c*-type cytochrome embedded in MtrB, a porin-like protein (Shi et al., 2012). MtrAB facilitates electron transfer across the outer membrane to MtrC and OmcA on the cell surface. Both MtrC and OmcA are decaheme *c*-type cytochromes extracellularly reducing soluble Fe(III) to Fe(II) (Shi et al., 2006). Finally, Fe(II) chemically reduces bromate to bromide at a molar ratio of 6:1. Thus, bromate reduction by *Shewanella* species seems to be a hybrid process of biological and chemical reactions. However, this model is simpler than that of *Rhodococcus* sp. Br-6, in which DCIP and iron function as redox mediators (Tamai et al., 2016). According to this hypothetical model, one might suppose that all of iron-reducing bacteria can reduce bromate. We determined if iron-reducing bacteria maintained in our laboratory (*Geobacter* sp. OR-1 and *Anaeromyxobacter* sp. PSR-1) can reduce bromate in the presence of Fe(III), but neither growth nor bromate reduction was observed (Figure 6). This was probably because those strains were less tolerant to bromate. In other words, two key characteristics appear to be essential for a bacterium to function as a bromate-reducing organism: (1) tolerance to bromate, and (2) the ability to rapidly reduce small amounts of Fe³⁺. It is still unclear how bromate-reducing bacteria isolated to date reduce bromate, i.e., biologically, chemically, or through the hybrid process (Hijnen et al., 1995; Davidson et al., 2011; Wang et al., 2022a, 2022c).



In this study, the apparent bromate reduction rate of M-Br in the presence of 100 μM Fe(III) was 114 $\mu\text{M day}^{-1}$ (Figure 3), which is the highest rate reported among bromate-reducing bacteria to date. For example, bromate reduction rate of the denitrifying bacterium, *Pseudomonas fluorescens* Br5, is 0.013–0.027 $\mu\text{M day}^{-1}$ (Hijnen et al., 1995). Four bromate-reducing bacteria previously isolated from BAC filters or urban watershed exhibited bromate reduction rates < 0.04 $\mu\text{M day}^{-1}$ (Davidson et al., 2011). *Rhodococcus* sp. Br-6 exhibits a high bromate reduction rate of 60 $\mu\text{M day}^{-1}$, which is only approximately half of that of the strain M-Br (Tamai et al., 2016). Its superior bromate-reducing capacity over other bacteria highlights M-Br as a good candidate for the practical removal of bromate from treated waters. Although the addition of iron as a redox mediator is undesirable for water treatment, the amount of iron required for environmental levels of bromate reduction would be negligible. Future studies should explore nanomolar levels of bromate reduction by various *Shewanella* species for more valuable insights.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repository and accession number(s) can be found in the article/Supplementary material.

Author contributions

NT: Writing – original draft, Investigation, Writing – review & editing, Methodology, Visualization, Data curation, Validation. HF: Methodology, Writing – original draft, Data curation, Validation, Investigation, Visualization, Writing – review & editing. HS: Methodology, Investigation, Visualization, Writing – review & editing. SY: Validation, Supervision, Visualization, Methodology, Conceptualization, Writing – review & editing, Investigation, Writing – original draft. SA: Visualization, Writing – review & editing, Project administration, Data curation, Writing – original draft, Validation, Supervision, Funding acquisition, Conceptualization.

Funding

The author(s) declared that financial support was not received for this work and/or its publication.

Acknowledgments

The authors would like to thank Ms. Chisato Imura for her experimental supports.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that Generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2025.1643578/full#supplementary-material>

References

- Assunção, A., Martins, M., Silva, G., Lucas, H., Coelho, M. R., and Costa, M. C. (2011). Bromate removal by anaerobic bacterial community: mechanism and phylogenetic characterization. *J. Hazard. Mater.* 197, 237–243. doi: 10.1016/j.jhazmat.2011.09.076
- Butler, R. A. Y., Godley, A., Lytton, L., and Cartmell, E. (2005). Bromate environmental contamination: review of impact and possible treatment. *Crit. Rev. Environ. Sci. Technol.* 35, 193–217. doi: 10.1080/10643380590917888
- Davidson, A. N., Chee-Sanford, J., Lai, H. Y. M., Ho, C. H., Klenzendorf, J. B., and Kirisits, M. J. (2011). Characterization of bromate-reducing bacterial isolates and their potential for drinking water treatment. *Water Res.* 45, 6051–6062. doi: 10.1016/j.watres.2011.09.001
- DSMZ (1993). Catalogue of strains. 5th Edn. Braunschweig: Gesellschaft für Biotechnologische Forschung.
- Gescher, J. S., Cordova, C. D., and Spormann, A. M. (2008). Dissimilatory iron reduction in *Escherichia coli*: identification of CymA of *Shewanella oneidensis* and NapC of *E. coli* as ferric reductases. *Mol. Microbiol.* 68, 706–719. doi: 10.1111/j.1365-2958.2008.06183.x
- Gordon, G., Gauw, R. D., Emmert, G. L., Walters, B. D., and Bubnis, B. (2002). Chemical reduction methods for bromate ion removal. *J. AWWA* 94, 91–98. doi: 10.1002/j.1551-8833.2002.tb09410.x
- Grefte, A., Ross, P. S., Dignum, M., Cornelissen, E. R., and Rietveld, L. C. (2013). The influence of the removal of specific NOM compounds by anion exchange on ozone demand, disinfection capacity, and bromate formation. *Ozone Sci. Eng.* 35, 283–294. doi: 10.1080/01919512.2013.793581
- Hijnen, W. A. M., Voogt, R., Veenendaal, H. R., van der Jagt, H., and van der Kooij, D. (1995). Bromate reduction by denitrifying bacteria. *Appl. Environ. Microbiol.* 61, 239–244. doi: 10.1128/aem.61.1.239-244.1995
- Hiraishi, A. (1992). Direct automated sequencing of 16S rDNA amplified by polymerase chain reaction from bacterial cultures without DNA purification. *Lett. Appl. Microbiol.* 15, 210–213. doi: 10.1111/j.1472-765x.1992.tb00765.x
- Ikehata, K., Wang, L., Nessel, M. B., Komor, A. T., Cooper, W. J., and McVicker, R. R. (2013). Effect of ammonia and chloramine pretreatment during the ozonation of a colored groundwater with elevated bromide. *Ozone Sci. Eng.* 35, 438–447. doi: 10.1080/01919512.2013.815105
- Jahan, B. N., Li, L., and Pagilla, K. R. (2021). Fate and reduction of bromate formed in advanced water treatment ozonation systems: a critical review. *Chemosphere* 266:128964. doi: 10.1016/j.chemosphere.2020.128964
- Kirisits, M. J., Snoeyink, V. L., Chee-Sanford, J. C., Daugherty, B. J., Brown, J. C., and Raskin, L. (2002). Effect of operating conditions on bromate removal efficiency in BAC filters. *J. Am. Water Works Assoc.* 94, 182–193. doi: 10.1002/j.1551-8833.2002.tb09462.x
- Kirisits, M. J., Snoeyink, V. L., Inan, H., Chee-Sanford, J. C., Raskin, L., and Brown, J. C. (2001). Water quality factors affecting bromate reduction in biologically active carbon filters. *Water Res.* 35, 891–900. doi: 10.1016/s0043-1354(00)00334-1
- Kirisits, M. J., Snoeyink, V. L., and Kruihof, J. C. (2000). The reduction of bromate by granular activated carbon. *Water Res.* 34, 4250–4260. doi: 10.1016/S0043-1354(00)00189-5
- Kudo, K., Yamaguchi, N., Makino, T., Ohtsuka, T., Kimura, K., Dong, D. T., et al. (2013). Arsenic release from soil by a novel arsenate-reducing bacterium *Anaeromyxobacter* sp. PSR-1. *Appl. Environ. Microbiol.* 79, 4635–4642. doi: 10.1128/AEM.00693-13
- Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874. doi: 10.1093/molbev/msw054
- Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., et al. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* 23, 2947–2948. doi: 10.1093/bioinformatics/btm404
- Legube, B., Parinet, B., Gelinet, K., Berne, F., and Croue, J. P. (2004). Modeling of bromate formation by ozonation of surface waters in drinking water treatment. *Water Res.* 38, 2185–2195. doi: 10.1016/j.watres.2004.01.028
- Liu, J., Yu, J., Li, D., Zhang, Y., and Yang, M. (2012). Reduction of bromate in a biological activated carbon filter under high bulk dissolved oxygen conditions and characterization of bromate-reducing isolates. *Biochem. Eng. J.* 65, 44–50. doi: 10.1016/j.bej.2012.04.004
- Luo, J.-H., Wu, M., Yuan, Z., and Guo, J. (2017). Biological bromate reduction driven by methane in a membrane biofilm reactor. *Environ. Sci. Technol. Lett.* 4, 562–566. doi: 10.1021/acs.estlett.7b00488
- Lv, X., Wang, D., Iqbal, W., Yang, B., and Mao, Y. (2019). Microbial reduction of bromate: current status and prospects. *Biodegradation* 30, 365–374. doi: 10.1007/s10532-019-09882-x
- Myers, C. R., and Myers, J. M. (1997). Cloning and sequence of *cymA*, a gene encoding a tetraheme cytochrome c required for reduction of iron(III), fumarate, and nitrate by *Shewanella putrefaciens* MR-1. *J. Bacteriol.* 179, 1143–1152. doi: 10.1128/jb.179.4.1143-1152.1997
- Neemann, J., Hulsey, R., Rexing, D., and Wert, E. (2004). Controlling bromate formation: during ozonation with chlorine and ammonia. *J. Am. Water Works Assoc.* 96, 26–28. doi: 10.1002/j.1551-8833.2004.tb10542.x
- Ohtsuka, T., Yamaguchi, N., Makino, T., Sakurai, K., Kimura, K., Kudo, K., et al. (2013). Arsenic dissolution from Japanese paddy soil by a dissimilatory arsenate-reducing bacterium *Geobacter* sp. OR-1. *Environ. Sci. Technol.* 47, 6263–6271. doi: 10.1021/es400231x
- Shi, L., Chen, B., Wang, Z., Elias, D. A., Mayer, M. U., Gorby, Y. A., et al. (2006). Isolation of a high-affinity functional protein complex between OmcA and MtrC: two outer membrane decaheme c-type cytochromes of *Shewanella oneidensis* MR-1. *J. Bacteriol.* 188, 4705–4714. doi: 10.1128/JB.01966-05
- Shi, L., Rosso, K. M., Clarke, T. A., Richardson, D. J., Zachara, J. M., and Fredrickson, J. K. (2012). Molecular underpinnings of Fe(III) oxide reduction by *Shewanella oneidensis* MR-1. *Front. Microbiol.* 3:50. doi: 10.3389/fmicb.2012.00050
- Siddiqui, M., Amy, G., Ozekin, K., Zhai, W., and Westerhoff, P. (1994). Alternative strategies for removing bromate. *J. Am. Water Works Assoc.* 86, 81–96. doi: 10.1002/j.1551-8833.1994.tb06263.x
- Tamai, N., Ishii, T., Sato, Y., Fujiya, H., Muramatsu, Y., Okabe, N., et al. (2016). Bromate reduction by *Rhodococcus* sp. Br-6 in the presence of multiple redox mediators. *Environ. Sci. Technol.* 50, 10527–10534. doi: 10.1021/acs.est.6b02261
- Wang, Y., Cai, X., Fan, J., Wang, D., and Mao, Y. (2022a). Transcriptome analysis provides new insights into the tolerance and aerobic reduction of *Shewanella decolorationis* Ni1-3 to bromate. *Appl. Microbiol. Biotechnol.* 106, 4749–4761. doi: 10.1007/s00253-022-12006-w
- Wang, Y., Fan, J., Shen, Y., Ye, F., Feng, Z., Yang, Q., et al. (2022b). Bromate reduction by *Shewanella oneidensis* MR-1 is mediated by dimethylsulfoxide reductase. *Front. Microbiol.* 13:955249. doi: 10.3389/fmicb.2022.955249
- Wang, D., Wang, Y., Lv, X., Cai, X., Iqbal, W., Yang, B., et al. (2022c). Isolation of anaerobic bromate-reducing bacteria using different carbon sources and transcriptomic insights from *Klebsiella variicola* Glu3. *Front. Microbiol.* 13:851844. doi: 10.3389/fmicb.2022.851844
- Wang, L., Zhang, J., Liu, J., He, H., Yang, M., Yu, J., et al. (2010). Removal of bromate ion using powdered activated carbon. *J. Environ. Sci. (China)* 22, 1846–1853. doi: 10.1016/s1001-0742(09)60330-2
- Weisburg, W. G., Barns, S. M., Pelletier, D. A., and Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* 173, 697–703. doi: 10.1128/jb.173.2.697-703.1991
- Wert, E. C., Rosario-Ortiz, F. L., Drury, D. D., and Snyder, S. A. (2007). Formation of oxidation byproducts from ozonation of wastewater. *Water Res.* 41, 1481–1490. doi: 10.1016/j.watres.2007.01.020
- WHO (2011). Guidelines for Drinking-Water Quality. 4th Edn. Geneva: World Health Organization, 324–325.
- Wu, Q. Y., Yang, L. L., Zhang, X. Y., Wang, W. L., Lu, Y., Du, Y., et al. (2020). Ammonia-mediated bromate inhibition during ozonation promotes the toxicity due to organic byproduct transformation. *Environ. Sci. Technol.* 54, 8926–8937. doi: 10.1021/acs.est.0c02984
- Xie, L., and Shang, C. (2005). Role of humic acid and quinone model compounds in bromate reduction by zerovalent iron. *Environ. Sci. Technol.* 39, 1092–1100. doi: 10.1021/es049027z
- Xie, L., Shang, C., and Zhou, Q. (2008). Effect of Fe(III) on the bromate reduction by humic substances in aqueous solution. *J. Environ. Sci. (China)* 20, 257–261. doi: 10.1016/s1001-0742(08)60040-6
- Zhang, J., Chen, Z., Liu, C., Li, J., An, X., Wu, D., et al. (2021). Construction of an acetate metabolic pathway to enhance electron generation of engineered *Shewanella oneidensis*. *Front. Bioeng. Biotechnol.* 9:757953. doi: 10.3389/fbioe.2021.757953
- Zhang, Y. Q., Wu, Q. P., Zhang, J. M., and Yang, X. H. (2015). Removal of bromide and bromate from drinking water using granular activated carbon. *J. Water Health* 13, 73–78. doi: 10.2166/wh.2014.084
- Zhong, Y., Yang, Q., Fu, G., Xu, Y., Cheng, Y., Chen, C., et al. (2018). Denitrifying microbial community with the ability to bromate reduction in a rotating biofilm-electrode reactor. *J. Hazard. Mater.* 342, 150–157. doi: 10.1016/j.jhazmat.2017.08.019