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Reduction of bromate in a biological activated carbon filter under high bulk dissolved oxygen conditions and characterization of bromate-reducing isolates

Juan Liu, Jianwei Yu, Dong Li, Yu Zhang, Min Yang*

State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuang-qing Road, Beijing 100085, China

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ABSTRACT

A biological activated carbon (BAC) filter was constructed using BAC from a pilot system that exhibited the ability to reduce bromate (BrO_3^-) and two BrO_3^- -reducing bacteria were isolated and characterized. The BAC filter could almost completely reduce BrO_3^- ($60 \mu\text{gBr/L}$) to bromide (Br^-) at an influent dissolved oxygen (DO) level of approximately 8.0 mg/L and an empty bed contact time of $30 \pm 2 \text{ min}$ using acetate as the electron donor shortly after the start-up. Phylogenetic analysis of the 16S rRNA gene sequences of a biological sample from the BAC filter showed that among the six detected orders, *Rhodocyclales*- and *Burkholderiales*-related microorganisms were dominant and *Rhodocyclaceae*- and *Comamonadaceae*-related microorganisms may play a role in BrO_3^- reduction. Two isolated pure cultures, i.e. *Sphingomonas* sp. 4721 and *Deinococcus* sp. 4710, exhibited the ability to reduce BrO_3^- in the presence of NO_3^- . The result of this study clearly indicated that DO was a competitor of BrO_3^- as an electron acceptor while NO_3^- was not. Construction of a BAC filter which could restrict oxygen transfer within a biofilm still remains to be a challenge.

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1. Introduction

Bromate ion (BrO_3^-), which is one of the major ozonation by-products in drinking water containing bromide ion (Br^-) [1], has raised great concerns because of its carcinogenic potency in humans and the maximum contaminant level (MCL) in drinking water for BrO_3^- regulated by many countries is $10 \mu\text{g/L}$.

Two approaches to control BrO_3^- contamination in drinking water are recommended, including BrO_3^- minimization during ozonation and removal after formation. Several control technologies have been proposed for drinking water treatment to minimize the BrO_3^- formation during ozonation, such as pH depression, ammonia or hydrogen peroxide (H_2O_2) addition [2,3]. However, only a partial reduction of BrO_3^- could be expected using these methods under practical operating conditions. On the other hand, it has been reported that BrO_3^- could be effectively reduced to Br^- by anaerobic mixed microbial cultures [4] or denitrifying bacteria [5–7]. However, these methods require almost complete removal of dissolved oxygen (DO) and nitrate (NO_3^-) from water, leading to the significant increase of treatment costs. Recently, some studies have shown that biological removal of BrO_3^- by biological activated carbon (BAC) filters could be achieved in the presence of low

concentrations of DO and NO_3^- [8], whereas the removal of BrO_3^- decreased with the increase of DO and NO_3^- concentrations [9,10]. The NO_3^- levels in most surface source waters are relatively low. Therefore, a process sequence was proposed for biological BrO_3^- reduction in which a DO reducing step was added before the BAC filters [9]. However, the BAC filters in our previous pilot study showed significant BrO_3^- reduction without taking any special measures to control the influent DO ($10\text{--}11 \text{ mg/L}$) and NO_3^- ($1.5\text{--}4.4 \text{ mg/L}$) when the biofilm of the BAC filter had formed after continuous operation for 5 months [11]. This result suggested that it may be possible to construct a BAC filter to biologically remove BrO_3^- without reducing DO, which would be a significant advantage in O_3 -BAC process.

Furthermore, understanding the underlying mechanisms of BrO_3^- reduction would be very helpful for the removal of BrO_3^- using BAC filters. Recently, fifteen BrO_3^- -reducing bacteria from different phylogenetic groups were acquired from different sources [8]. Some denitrifying organisms were reported to be able to reduce BrO_3^- [5]. In addition, it has been speculated that NO_3^- reductase, which catalyzes NO_3^- reduction, may also catalyze microbial BrO_3^- reduction [10]. However, the mechanisms involved in biological BrO_3^- reduction are still not well understood.

The objectives of the current study focused on (i) confirming the possibility of biological BrO_3^- reduction using a BAC filter under high bulk DO conditions; (ii) identifying the major microbes responsible for BrO_3^- removal by constructing clone libraries for

* Corresponding author. Tel.: +86 10 62923475; fax: +86 10 62923541.
E-mail address: yangmin@rcees.ac.cn (M. Yang).

biological samples; (iii) isolating BrO_3^- -reducing bacteria from different BAC samples, comparing and investigating their BrO_3^- -reducing behaviors. The result of this study will be helpful for constructing and operating a BAC filter to remove BrO_3^- more effectively.

2. Materials and methods

2.1. Chemicals and BrO_3^- -containing water preparation

Potassium bromate (KBrO_3) and potassium bromide (KBr) were guaranteed reagents and other chemicals used in the current study were analytical grade. Tap water (pH 7.5–7.7, $\text{NO}_3^- = 1.5\text{--}2.4\text{ mg/L}$, $\text{DO} = 7.7\text{--}8.2\text{ mg/L}$) containing approximately $20\text{ }\mu\text{g/L}$ of Br^- , spiked with KBrO_3 ($60\text{ }\mu\text{g/L}$ as Br) was used as influent for the bench-scale experiment. Furthermore, 1 mg/L sodium acetate (CH_3COONa) was added to the influent as exogenous carbon source.

2.2. Experimental apparatus set-up

The BAC sample (columnar type with a diameter of $1.5\text{--}2.0\text{ mm}$ and length of $3\text{--}5\text{ mm}$; Shanxi Xinhua Activated Carbon Company) used for the experiment was obtained from our previous O_3 -BAC pilot system [11]. The operating result of the BAC pilot system is shown in Fig. S1. Two glass columns (inner diameter = 4.0 cm) with a working volume of 450 mL were filled with the BAC samples above, and one of them was used as a control by sterilizing the BAC before filling ($121\text{ }^\circ\text{C}$, 30 min , 102.9 kPa). Peristaltic pumps (Longer-Pump, YZ1515x Bt00-300M) were used to maintain the empty bed contact time (EBCT) at $30 \pm 2\text{ min}$. The filters were maintained at room temperature ($25\text{ }^\circ\text{C}$) and backwashed every 15 days, and the experiment was operated for 45 days.

2.3. Phylogenetic analysis of biological samples of the BAC filter

2.3.1. DNA extraction of biological samples

In order to investigate the change of microbes before and after the operation of bench-scale BAC system in the current study, two biological samples were used for DNA extraction. Sample A was the original BAC taken from the pilot-scale system, and sample B was from the backwashed water after the BAC filter had been operated for 45 days in the lab. 5 g BAC from sample A was collected into a 10-mL of centrifuge tube and for sample B, the bacteria harvested by membrane filtration with $0.22\text{-}\mu\text{m}$ filters (Millipore GSWP, Millipore, USA) were suspended in 10 mL physiological saline for DNA extraction. DNA from the two samples was extracted using a FastDNA SPIN kit for soil (MP Biomedicals, France) facilitated with a FastPrep-24 bead beater system (FastPrep-24, MP, USA), and then quantified using a Nanodrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) for denaturing gradient gel electrophoresis (DGGE) analysis and bacterial gene clone library construction.

2.3.2. DGGE analysis and construction of bacterial gene clone libraries

For DGGE analysis [12], the V3 region of the 16S rRNA gene was amplified with primers GC341f and 534r using touchdown PCR methods. In addition, almost the full-length of the 16S rRNA gene sequences were amplified to construct bacterial gene clone libraries using universal primers 27f and 1492r [13]. All of the primers used in the current study are listed in Table S1.

The procedures for DGGE analysis and construction of the bacterial clone libraries are detailed in the supporting information. The DNA sequences used for the construction of clone libraries were searched against the Ribosomal Database Project II (RDP II) release 9.49 and the GenBank database using the BLAST

program [14]. Phylogenetic trees were constructed using MEGA version 4.0 by the neighbor-joining algorithm and the Jukes–Cantor distance estimation method with bootstrap analyses for 1000 replicates [15]. Sequences sharing 97% or greater identity were grouped into the same operational taxonomic unit (OTU) using software MOTHUR [16]. Sixty 16S rRNA gene sequences were submitted to the GenBank database under the accession numbers HQ697371–HQ697431.

2.4. Isolation of pure cultures

Two BAC samples, i.e. one taken from a full-scale waterworks (the waterworks located in Zhejiang Province, and the ozone dose was between 1.0 and 2.5 mg/L with Br^- ranging from 85 to $130\text{ }\mu\text{g/L}$ in source water) and the other from the original BAC taken from the pilot-scale system, were used to isolate BrO_3^- -reducing bacteria. The BAC samples were put into glass beakers containing 50 mL phosphate buffer (pH 7.2) and oscillated with glass beads (diameter = 5.0 mm) for 2 h . The buffered solutions were then transferred into liquid mineral salts (MS) medium (0.1 g/L Na_2HPO_4 , 5 mg/L CaCl_2 , 0.005 mg/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.01 mg/L H_3BO_3 , 5 mg/L $\text{MgCl}_2 \cdot 7\text{H}_2\text{O}$, 0.3 mg/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.01 mg/L $\text{ZnCl}_2 \cdot \text{H}_2\text{O}$, and 0.3 mg/L $\text{FeCl}_2 \cdot 7\text{H}_2\text{O}$; pH 7.2) [5] for enrichment. After being autoclaved, the MS medium was poured into 300-mL serum bottles. KBrO_3 , CH_3COONa and ammonia chloride solutions were then added after filtration through a $0.22\text{-}\mu\text{m}$ sterile filter. Thus, the MS medium for enrichment was obtained with BrO_3^- , acetate and ammonia at final concentrations of 1.0 , 20 and 4.0 mg/L , respectively. In addition, resazurin (0.5 mg/L) was added as an indicator to show the changes of the DO level.

After inoculation, the serum bottles were flushed with filtered nitrogen gas using a $0.22\text{-}\mu\text{m}$ sterile filter to reduce the DO level in the medium, capped with a rubber stopper and then incubated at $25\text{ }^\circ\text{C}$ in the dark. Periodically, samples were collected from the bottles for the measurement of BrO_3^- and Br^- concentrations. The cultures that exhibited BrO_3^- removal were transferred to fresh medium after being cultivated for 20 days. The enrichment cultures were transferred twice during the study.

At the end of the third generation of enrichment, bacterial purification was performed on R_2A medium plates after aerobic incubation for 3 days at $35\text{ }^\circ\text{C}$. The R_2A medium was developed for the enumeration of oligotrophic bacteria from potable water [17]. Two strains were obtained, of which strain Y (deposited in China General Microbiological Culture Collection Center, CGMCC, No. 4721) was from our BAC filter and strain R (CGMCC No. 4710) from the full-scale waterworks. The BrO_3^- -reducing behaviors of the isolates were evaluated using the MS medium with and without addition of NO_3^- and SO_4^{2-} under an initial DO level below 1 mg/L .

2.5. Characterization of the isolates

Micrographs of the isolates (named strains Y and R, respectively) were taken using a scanning electron microscope (SEM) (S-3000N, Hitachi, Japan). The 16S rRNA nucleotide sequence data of the two strains were deposited in the GenBank database under the accession numbers JF710625 for strain Y and JF710626 for strain R.

2.6. Analytical methods

A Dionex ICS-1500 ion chromatography (IC) equipped with an IonPac analytical column (AS19, $4\text{ mm} \times 250\text{ mm}$) was used to determine the concentrations of BrO_3^- , Br^- , NO_3^- and sulfate ion (SO_4^{2-}) in the BAC filter samples and in the batch BrO_3^- reduction studies. A HQ Model 30d DO meter (HACH, USA) and a Delta Model 320 pH meter (METTLER, Switzerland) was used to measure DO and pH, respectively. Samples for DOC analysis were filtered

Fig. 1. Variations of BrO_3^- and Br^- concentrations in the BAC filter. Data shown are means and the data standard deviation of all data points was below 5%.

through 0.45- μm nylon membranes and were analyzed by a total organic carbon (TOC) analyzer (Phoenix 8000, Tekmar Dohrmann Co., USA). All of the experiments were sampled and determined in duplicate. The data standard deviation of all data points was below 5%.

3. Results

3.1. BrO_3^- removal performance of the BAC filter

The changes of BrO_3^- and Br^- concentrations in the influent and effluent of the BAC filter are shown in Fig. 1. The effluent BrO_3^- decreased quickly after the start-up of the filter, and was almost not detected in steady state (from day 11) except for occasional slight rebounds. Corresponding increase of Br^- was observed simultaneously, of which the formed Br^- in the effluent accounted for 92–100% of the added BrO_3^- (as 60 $\mu\text{g/L}$ Br^- in the influent during the continuous treatment. This indicates that almost all of the added BrO_3^- was reduced to Br^- . In contrast, no BrO_3^- removal was observed in the control system (data not shown) in which the BAC had been sterilized before the start-up. This means that BrO_3^- was biologically reduced to Br^- by the BAC filter.

In addition, the variations of DOC, DO and co-existing NO_3^- and SO_4^{2-} during BrO_3^- reduction were also investigated. An average of 1.4 mg/L DOC in the influent decreased to 1.0 mg/L in the effluent (Fig. S2). No apparent removal of NO_3^- or SO_4^{2-} was observed (Fig. S3), whereas DO was reduced from approximately 8.0 mg/L in the influent to 5.5–6.0 mg/L in the effluent (Fig. S2). Thus, carbon source supplied in the influent was utilized as the electron donor for both the reduction of oxygen and BrO_3^- while the reduction of NO_3^- and SO_4^{2-} did not occur.

3.2. Characterization of bacterial community structures

The comparison of the bacterial DGGE phylotype of samples A and B is demonstrated in Fig. 2. The two samples exhibited a low similarity (42.8%). Bright DGGE bands were retrieved for sequence analysis, and only 5 sequences were acquired (Table S2). Samples A and B shared some common DGGE bands, such as bands 4 (*Bradyrhizobium elkanii* strain) and 5 (uncultured *Acidobacteria*). However, bands 1 (*Janthinobacterium* sp. 4-62) and 2 (uncultured *Chloroflexi bacterium*) were unique to sample A, and band 3 was (uncultured *Rhodocyclaceae bacterium*) unique to sample B.

Fig. 2. DGGE profiles of partial 16S rRNA fragments for samples A and B (sample A was from the initial bench-scale BAC filter in this study and sample B was from the backwashed water after the filter had been operating for 45 days).

Two 16S rRNA gene clone libraries were also constructed and the blast results are summarized in Table 1. A total of 72 and 58 sequences were obtained and grouped into 37 and 23 OTUs for samples A and B, respectively. Based on the blast results, phylogenetic trees for samples A and B were constructed as shown in Fig. S4.

Consistent with the DGGE results, the clone libraries of samples A and B were quite different from each other (Fig. S4). In clone library A, the majority (84.7%) were affiliated with the phylum *Proteobacteria*, dominated by the classes *Beta* (45.8%) and *Alpha* (36.1%) (Table 1). The order *Burkholderiales* (37.5% of all sequences) belonging to *Betaproteobacteria* and the order *Rhizobiales* (33.3% of all sequences) belonging to *Alphaproteobacteria* were the two dominant bacterial groups. The clones belonging to the orders *Burkholderiales* and *Rhizobiales* were mainly grouped into the families *Oxalobacteraceae* (34.7% of all sequences) and *Bradyrhizobiaceae* (9.7% of all sequences), respectively. There were several clones with very high identity to *Janthinobacterium* sp. (GenBank accession no. D84590.2) and *Variovorax* sp. (GenBank accession no. D84645.2), which belong to the families *Oxalobacteraceae* and *Comamonadaceae*, respectively, in the order *Burkholderiales*.

In contrast, clone library B was more dominated by the phylum *Proteobacteria* (96.3% of all sequences), particularly *Betaproteobacteria* (67.2% of all sequences). Most of the clones in the class *Betaproteobacteria* fell into the orders *Rhodocyclales* (34.5% of all sequences) and *Burkholderiales* (25.9% of all sequences). Several sequences that are aligned with the genus *Dechloromonas* in the family *Rhodocyclaceae* were detected. Moreover, there were 14 sequences in sample B belonging to the family *Rhodocyclaceae* that showed very high identity (97–100%) to the *Betaproteobacteria* that was able to reduce NO_3^- with p-xylene [18]. The major clones in the order *Burkholderiales* fell into the families *Oxalobacteraceae* (19.0% of all sequences) and *Comamonadaceae* (6.9% of all sequences), which were also found in clone library A. On the other hand, the clones belonging to the family *Sphingomonadaceae* (5.2% of all sequences) in the order *Sphingomonadales*, which have

Table 1Distribution of phylogenetic groups among bacterial 16S rRNA gene clone libraries for the two samples in the current study.^a

Phylum	Class	Order	Family	Genus	No. of clones (no. of OTUs) in sample ^b	
					A	B
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	7(2)	
				Afipia		1(1)
			Hyphomicrobiaceae	Hyphomicrobium	1(1)	
				Rhodoplanes	1(1)	
			Unclassified		15(4)	
		Sphingomonadales	Sphingomonadaceae	Blastomonas		3(1)
			Rhodobacteraceae	Rhodobacter		2(1)
			Unclassified		2(2)	11(3)
	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Unclassified	25(6)	11(3)
			Comamonadaceae	Unclassified	2(2)	4(1)
			Rhodocyclaceae	Dechloromonas		3(1)
		Rhodocyclales		Zoogloea		1(1)
				Unclassified		14(5)
			Unclassified			2(2)
		Unclassified			6(6)	4(2)
	Deltaproteobacteria	Syntrophobacteriales	Syntrophaceae	Unclassified	1(1)	
	Gammaproteobacteria	Pseudomonadales;	Pseudomonadaceae	Pseudomonas		1(1)
		Unclassified			1(1)	
	Unclassified				1(1)	
Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Pirellula	2(2)	
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Unclassified	2(2)	
		Unclassified			1(1)	1(1)
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Unclassified		1(1)	
Chloroflexi	Unclassified				1(1)	
Unclassified					3(3)	
Total					72(37)	58(23)

^a Classification was based on match results of RDP and the GenBank database.^b A blank indicates that no related clones were obtained.

been found in the treated water of several drinking water treatment plants using GAC filters [19], were not found in clone library A.

3.3. Isolation and phylogenetic classification of the BrO_3^- -reducing bacteria

Two BrO_3^- -reducing strains, i.e. Y (rod-shaped) and R (round), were obtained (Fig. S5). The 16S rRNA gene sequencing results showed that strain Y (GenBank accession no. JF710625) shared 98% 16S rRNA gene sequence identity over 1466 bp with *Sphingomonas* sp. BBCT20 (GenBank accession no. DQ337548.1) affiliated with the family *Sphingomonadaceae* in the order *Sphingomonadales*, while strain R (GenBank accession no. JF710626) shared 95% identity over 1476 bp with *Deinococcus murrayi* DSM 11303 (GenBank accession no. NR_026416.1) affiliated with the family *Deinococcaceae* in the order *Deinococcales*.

Both strains could reduce BrO_3^- to Br^- in the MS medium with the removal rates of approximately 20 $\mu\text{g}/(\text{Ld})$ and 8 $\mu\text{g}/(\text{Ld})$ for strains Y and R, respectively, as shown in Fig. 3. Similarly, the formation rates of Br^- were 13.5 $\mu\text{g}/(\text{Ld})$ and 5.8 $\mu\text{g}/(\text{Ld})$, respectively. The presence of NO_3^- (5 mg/L) and SO_4^{2-} (5 mg/L) did not inhibit BrO_3^- (1000 $\mu\text{g}/\text{L}$) removal perceptibly (data not shown), and both ions were not removed by the strains.

To further elucidate the impacts of DO and NO_3^- on BrO_3^- reduction, an experiment with higher levels of initial DO (5.0 mg/L) and NO_3^- (22.1 mg/L) was conducted with approximately 1000 $\mu\text{g}/\text{L}$ BrO_3^- and 200 mg/L CH_3COONa (Fig. 4). The two isolates began to reduce BrO_3^- when DO was exhausted with the removal rates of 20 $\mu\text{g}/(\text{Ld})$ and 13 $\mu\text{g}/(\text{Ld})$ for strains Y and R, respectively, as shown in Fig. 4. The formation rates of Br^- were 12.5 $\mu\text{g}/(\text{Ld})$ and 9.0 $\mu\text{g}/(\text{Ld})$. However, no obvious removal of NO_3^- was observed for the duration of 40 days. This suggests that DO may be a competitor of BrO_3^- as an electron acceptor while NO_3^- was not.

4. Discussion

Although microbial reduction of BrO_3^- was identified nearly 20 years ago [5], BrO_3^- -reducing conditions in these studies were very strict. In most cases, almost complete removal of DO and NO_3^- was required, making it too expensive and complex to apply the process to drinking water treatment.

Kirisits and Snoeyink [9] reported that considerable BrO_3^- reduction occurred in BAC filters even when 2 mg/L of DO and 5.1 mg/L of NO_3^- were present. However, the removal of BrO_3^- in BAC filters decreased with the increase of DO and NO_3^- concentrations [9,10]. The two isolates acquired in the current study and some of the isolates acquired in the previous study [8] have exhibited BrO_3^- -removal capacity in the presence of NO_3^- (Fig. 4). Furthermore, in comparison with DO, the NO_3^- levels are normally much lower in surface water. Hence, it is assumed that the presence of NO_3^- may not be a practical problem for BrO_3^- reduction.

In order to cancel the adverse effect of DO, removal of DO before BAC filtration was recommended to ensure sufficient BrO_3^- removal [9]. The result in the current study, however, shows that almost complete BrO_3^- removal was achieved for an influent containing BrO_3^- (as 60 $\mu\text{g}/\text{L}$ Br) under high bulk DO condition (8.0 mg/L) when acetate was supplied as the electron acceptor (Figs. 1 and S2). The removal of BrO_3^- under saturated DO condition, which was formed during ozonation, was also observed in our previous pilot O_3 -BAC system (Fig. S1). An average BrO_3^- removal of 55% or 90% was achieved in BAC filters packed with granular or columnar activated carbon, respectively, even at a DO level of 10.0–11.0 mg/L. This is the first report showing that remarkable biological BrO_3^- reduction could occur in a BAC filter in the presence of high bulk DO concentrations, which is very meaningful and important for the practical application of this process for BrO_3^- removal.

BrO_3^- reduction tests using the pure cultures showed that the removal of BrO_3^- did not start until DO was depleted, indicating

Fig. 3. Variations of BrO_3^- and Br^- during the cultivation of the two strains (a: strain Y; b: strain R). Error bars indicate standard deviation (all below 5%).

that DO was an inhibitor of BrO_3^- reduction. However, both the current study and our previous pilot study showed that the BAC filters could effectively remove BrO_3^- under the high bulk DO conditions. Hence, the formation of a DO gradient within the biofilm in the BAC filters may have made it possible for bacteria to reduce BrO_3^- [9]. As shown in Fig. S1, it was almost 5 months until the BAC filter was able to reduce BrO_3^- significantly, the time of which might be needed for the formation of biofilm. The quick start-up of the bench-scale BAC filter in the current study may be attributed to the direct use of the BAC from the pilot system. The formation of a DO gradient could be affected by many factors including biofilm thickness, micropore distribution of activated carbon, abundance of biodegradable organic compounds, etc., which will require further studies. Construction of a BAC filter which could restrict oxygen transfer within biofilm still remains to be a challenge.

Clone library analysis showed that the orders *Burkholderiales* and *Rhizobiales* constitute the two largest bacterial groups in sample A (the original BAC sample). Large numbers of bacteria affiliated to the orders *Rhizobiales* and *Burkholderiales* have previously been isolated from BAC filters [20]. *Burkholderiales* are believed to be important in the mineralization of dissolved organic matter in BAC, preferably using oxygen as an electron acceptor. *Burkholderiales* were also the second largest group in the clone library of sample B (the backwashed sample), which is understandable considering the fact that this group of bacteria has been found to be ubiquitous in BAC filters. It should be noticed that some clones affiliated into the family *Comamonadaceae* were detected in both samples, and it had been proved that some members of the family *Comamonadaceae*

Fig. 4. Influence of NO_3^- added into the medium on BrO_3^- reduction performance by strain Y and R (a: strain Y; b: strain R). Error bars indicate standard deviation (all below 5%).

are primary denitrifiers under anoxic conditions in activated sludge [21,22]. Therefore, the *Comamonadaceae*-related bacteria might be related with BrO_3^- reduction since it has been speculated that NO_3^- reductase may also catalyze microbial BrO_3^- reduction [10]. Although the *Oxalobacteraceae*-related bacteria were found abundant in both samples, and could be also detected in different aquatic environments [23,24], their functions in BrO_3^- reduction process was still not clear.

On the other hand, the clones belonging to the order *Rhodocyclales*, which was not detected in sample A, became the largest bacterial group in sample B. Bacteria belonging to the order *Rhodocyclales* prefer oligotrophic conditions [25] and *Rhodocyclus* spp. are able to use (per)chlorate as the final electron acceptor [26,27]. Several sample B clones shared high identity with *Rhodocyclaceae* microorganisms that are highly enriched cultures of freshwater denitrifying bacteria [18]. With 95% 16S rRNA gene sequence identity to *Denitratisoma oestradiolicum* [28], these denitrifying *Rhodocyclaceae* microorganisms could use (per)chlorate as electron acceptors with acetate as the electron donor. Some clones in sample B were similar to the highly enriched *Betaproteobacteria*, which is able to reduce NO_3^- with p-xylene [18]. It is known that p-xylene can be removed by microorganisms thriving under NO_3^- - or SO_4^{2-} -reducing conditions. As shown in Table 1, there were several sequences that are aligned with the genus *Dechloromonas*

in the family *Rhodocyclaceae*. *Dechloromonas* sp. (GenBank accession no. AF170356.1) have been found to be associated with (per)chlorate reduction in previous studies [26,29,30]. In addition, two BrO_3^- -reducing isolated in a previous study were close neighbors with *Dechloromonas* spp. [8]. In general, the clones belonging to the order *Rhodocyclales* in general exhibit the common ability to utilize chlorate, perchlorate, NO_3^- or BrO_3^- as the electron acceptors. Therefore, the appearance of abundant *Rhodocyclaceae*- and *Comamonadaceae*-related bacteria in sample B suggests that these bacterial groups may have the ability to reduce BrO_3^- , considering the fact that BrO_3^- was one of the most abundant electron acceptor in the BAC filter.

However, the two BrO_3^- -reducing strains isolated in the current study, *Sphingomonas* sp. 4721 and *Deinococcus* sp. 4710, belong to the orders *Sphingomonadales* and *Deinococcales*, respectively. Both genera were different from the BrO_3^- -reducing bacteria reported in the previous studies [5,8,31]. One sequence in sample B had high identity to the order *Sphingomonadales*, but no *Deinococcales*-related sequence was detected in either sample. In addition, *Sphingomonas* spp. are found ubiquitously in the environment [32], possessing unique abilities to degrade refractory contaminants including a broad range of aromatic compounds [33,34], whereas reports on the genus *Deinococcus* have been very limited [35]. Based on the fact that various BrO_3^- -reducing bacteria have been isolated [5,8,31], it is possible that diverse phylogenetic groups revealed by the clone libraries were able to utilize BrO_3^- as an electron acceptor.

5. Conclusions

In this study, a bench-scale BAC filter was constructed using BAC from a previous pilot plant showing biological BrO_3^- reduction capacity. Almost complete BrO_3^- removal was achieved for an influent containing BrO_3^- (as $60\text{ }\mu\text{g/L Br}$) under high bulk DO (8.0 mg/L) condition with sodium acetate used as the electron donor. For the first time, this study showed that almost complete biological BrO_3^- reduction could happen in a BAC filter without reducing the DO level, which is very important to apply the BAC process for the biological BrO_3^- removal. The *Rhodocyclaceae*- and *Comamonadaceae*-related microorganisms, some of which are close relatives of (per)chlorate-reducing or nitrate-reducing bacteria, might have played an important role in BrO_3^- reduction. The two BrO_3^- -reducing strains isolated in this study were *Sphingomonas* sp. 4721 belonging to the order *Sphingomonadales* and *Deinococcus* sp. 4710 belonging to the order *Deinococcales*, respectively. The two strains could selectively use BrO_3^- as the final electron acceptor even under the presence of NO_3^- . The result of this study clearly indicated that DO was a competitor of BrO_3^- as an electron acceptor while NO_3^- was not. Construction of a BAC filter which could restrict oxygen transfer within a biofilm still remains to be a challenge.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bej.2012.04.004>.

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