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RESEARCH ARTICLE

Autotrophic perchlorate reduction kinetics of a microbial consortium using elemental sulfur as an electron donor

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Abstract The perchlorate reduction kinetic parameters of a microbial consortium using elemental sulfur (S^0) as an electron donor were investigated in batch experiments. Standard Monod substrate utilization and biomass accumulation models were employed to fit the experimental data for microbial perchlorate reduction. The maximum observed yield coefficient for the microbial consortium was 0.19 mg dry weight (DW)mg^{-1} ClO_4^-, suggesting that the microbial consortium had a slow growth rate using S^0 as the electron donor. The maximum specific substrate utilization rate (q_{max}) and half saturation constant (K_s) for microbial perchlorate reduction were 0.14 mg $ClO_4^-mg^{-1}$ DW day^{-1} and 5.71 mg L^{-1}, respectively, which indicated that the microbial consortium could effectively utilize perchlorate as an electron acceptor. The variation of q_{max} with pH was described well by using a Gaussian peak equation, and the maximal value of q_{max} was obtained at pH 6.7. The presence of nitrate in perchlorate-contaminated water delayed the onset of sulfur autotrophic perchlorate reduction. The modified Gompertz equation could adequately describe the formation of Cl^- and SO_4^{2-} during the process of sulfur autotrophic perchlorate reduction. The SO_4^{2-} production exceeded the theoretical SO_4^{2-} production due to S^0 disproportionation. The kinetic parameters for microbial perchlorate reduction are essential to design biological treatment systems, as well as to predict and evaluate their performance.

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Introduction

Perchlorate (ClO_4^-) is widely used in rockets, missiles, fireworks, and explosives, as well as in many other industrial processes (Coates and Achenbach 2004; Logan 2001). Perchlorate in surface and in ground water has been recognized as a significant contaminant in recent years. Perchlorate can disrupt thyroid function by interfering with iodine uptake of thyroid gland, which can lead to a decrease in the production of thyroid hormones (Siglin et al. 2000). The current methods for perchlorate removal from perchlorate-contaminated water mainly include ion exchange (Gu et al. 2007), membrane separation (Roach and Tush 2008), and biological reduction (Srinivasan and Sorial 2009). Ion exchange is a cost-effective technology for treatment of perchlorate-contaminated water. However, a major disadvantage of ion exchange is that it results in generation of contaminated resin or perchlorate-laden brine that must be further treated or disposed (Tripp et al. 2003). Membrane separation can remove perchlorate from the perchlorate-contaminated water, especially reverse osmosis, whereas it suffers from membrane fouling issue and generates large volumes of perchlorate-laden brine. Biological reduction can completely transform perchlorate into innocuous chloride, and it has been recognized as a promising technology to treat perchlorate-contaminated water.

Perchlorate-reducing bacteria are known to be ubiquitous in pristine and contaminated environments (Coates and Achenbach 2004; Coates et al. 1999). The heterotrophic ClO_4^- reduction, utilizing simple organic substrates as electron donors, has been proven to have high treatment capacity and ClO_4^- reduction rate (Min et al. 2004;

Patel et al. 2008; Nor et al. 2011). However, heterotrophic ClO_4^- reduction depends on the continual addition of organic substrates as electron donors to sustain microbial activity. The residual labile organic substrate in the effluent can stimulate microbial growth in water distribution systems and contribute to the formation of potentially toxic trihalomethanes during disinfection by chlorination (Thrash et al. 2007). To overcome the abovementioned problems, autotrophic microbial ClO_4^- reduction has been carried out by utilizing inorganic donors, such as hydrogen gas (H_2), elemental iron (Fe^0), and elemental sulfur (S^0). Perchlorate reduction using H_2 as the electron donor has been successfully used in some bioreactors (Logan and Lapoint 2002; Nerenberg et al. 2002; Zhang et al. 2002; Van Ginkel et al. 2010). The deficiency of H_2 as the electron donor is difficult to handle in bulk quantities, and it is publicly perceived as a significant disaster threat due to its inherent explosive nature. Microbial perchlorate reduction with Fe^0 as the electron donor provided some conflicting results in previous studies. Shrout et al. (2005) reported that the addition of Fe^0 to the anaerobic culture slowed the perchlorate reduction rate, which was attributed to the increase in pH and the encapsulation of microorganisms by iron precipitates. Yu et al. (2006) illustrated that Fe^0 could serve as the electron donor for perchlorate reduction by providing H_2 to *Dechloromonas* sp. HZ. Ju et al. (2008) found that S^0 was suitable as the electron donor for perchlorate reduction by comparing with different inorganic donors. The S^0 pellets as the electron donor can eliminate the issues associated with organic residuals, provide a slowly released supply of electron on demand, offer lower expense cost as well as lower maintenance cost, and produce little excess biomass. Batch experiments and packed-bed reactors utilizing S^0 as the electron donor have been proven to be efficient for perchlorate reduction (Boles et al. 2012; Ju et al. 2007; Sahu et al. 2009). As we all know, the kinetic parameters of microbial perchlorate reduction are essential to design biological treatment systems, as well as to predict and evaluate their performance. However, little information has been found in the previously published literatures on evaluating the kinetic parameters for microbial perchlorate reduction using S^0 as the electron donor. In order to design cost-effective biological treatment systems, it is very necessary to determine the kinetic parameters of sulfur autotrophic perchlorate reduction.

The main objectives in the present study were (1) to acclimatize a sulfur-utilizing perchlorate-reducing consortium; (2) to determine the maximum observed microbial yield on perchlorate, the microbial perchlorate reduction kinetics, and the kinetics on product formation; (3) to investigate the effects of pH and nitrate on sulfur-utilizing perchlorate reduction; and (4) to analyze the relationship between perchlorate reduction and product formation.

Materials and methods

Acclimation of sulfur autotrophic perchlorate-reducing microbial consortium

The sludge for the acclimation of sulfur autotrophic perchlorate-reducing microbial consortium was obtained from the anaerobic section of an anaerobic-anoxic-oxic process in the sewage treatment plant of Licun River, Qingdao. A 500-mL anaerobic flask was used to acclimatize sulfur autotrophic perchlorate reducing microbial consortium, which was sealed with a Teflon-faced butyl rubber stopper and aluminum seal. The acclimation was performed in fill and draw modes. The sulfur pellets with a 1- to 2-mm particle diameter and bicarbonate were used as the electron donor and inorganic carbon source, respectively. The synthetic culture medium contained the following components (g L^{-1}): ClO_4^-, 0.12–0.36; NH_4HCO_3, 0.41; $NaHCO_3$, 2.7; $MgSO_4 \cdot 7H_2O$, 0.1; $Ca(OH)_2$, 0.005; and trace elements (1 mL L^{-1}). The trace elements consisted of the following components (g L^{-1}): H_3BO_3, 0.05; $FeSO_4 \cdot 7H_2O$, 1.4; $ZnSO_4 \cdot 7H_2O$, 0.106; $MnSO_4 \cdot H_2O$, 0.415; $(NH_4)_6Mo_7O_{24} \cdot H_2O$, 0.2; $KAl(SO_4)_2 \cdot 12H_2O$, 0.175; $NiSO_4 \cdot 6H_2O$, 0.113; $CoSO_4 \cdot 12H_2O$, 2.36; $Na_2SeO_3 \cdot 5H_2O$, 0.288; $CuSO_4 \cdot 5H_2O$, 0.157; $Na_2WO_4 \cdot 2H_2O$, 0.5; and EDTA, 1.0. Nitrogen gas was injected into the culture medium for 20 min to remove oxygen before use, and the headspace of anaerobic flask was flushed with N_2/CO_2 (4:1, *V*:*V*) mixture. The suspended sludge in the anaerobic flask was continuously stirred in a thermostatic culture box at 25±1 °C. When the removal efficiency of perchlorate was above 99 %, the acclimated mixed culture in the anaerobic flask was allowed to settle for 1 h. The supernatant was decanted and then replaced with the same volume of fresh culture medium. The entire acclimation process was continuously operated for 135 days.

Kinetic parameters of sulfur autotrophic perchlorate reduction

In order to determine the kinetic parameters of sulfur autotrophic perchlorate-reducing microbial consortia, the initial ClO_4^- concentrations in the anaerobic flask exceeded the range typically observed at most ClO_4^--contaminated sites. The anaerobic flask was used to determine kinetic parameters of autotrophic perchlorate reduction, which was filled with the acclimated sludge, sulfur pellets, and fresh culture medium containing ClO_4^-. Tables 1 and 2 illustrate the experimental conditions of kinetic parameter determination during autotrophic perchlorate reduction. Prior to the experiment, nitrogen gas was injected into the culture medium for

Table 1 Experimental conditions of kinetics parameter determination during autotrophic perchlorate reduction

Experimental purpose. Index	The maximum observed microbial yield on perchlorate[a]	Microbial perchlorate kinetics	pH effect on microbial perchlorate reduction kinetics[c]	Effect of nitrate addition on microbial perchlorate reduction[d]
S^0 m ass (g)	20	50	50	50
Particle size (mm)	1–2	1–2	1–2	1–2
Flask volume (mL)	250	500	500	500
Number of flasks	24	3	5	5
Volume of culture medium in each flask (mL)	100	200	200	200
Initial pH	7.2	7.2	5–9	7.2
Initial ClO_4^- concentration (mg L^{-1})	1200	250	250	250
Addition of NO_3^--N (mg L^{-1})	NA[b]	NA[b]	NA[b]	50–300

[a] The anaerobic flasks were divided into eight groups, and each group consisted of three anaerobic flasks as parallel experiments

[b] NA represented no addition of NO_3^--N

[c] The culture media in the five anaerobic flasks were adjusted to 5, 6, 7, 8, and 9, respectively

[d] The NO_3^--N concentrations in the five anaerobic flasks were 50, 100, 200, 250, and 300 mg L^{-1}, respectively

20 min to remove oxygen, and the headspace of the anaerobic flask was flushed with N_2/CO_2 (4:1, *V*:*V*) mixture. The anaerobic flask was sealed using the Teflon-faced butyl rubber stopper and aluminum seal. The anaerobic flask was shaken in an orbital incubator at a rotation speed of 180 rpm (revolutions per minute) during the experiment. Triplicate samples were analyzed to determine the concentrations of biomass, ClO_4^-, Cl^-, SO_4^{2-}, and NO_3^--N at different times.

Analytical methods

The water sample was filtered using a 0.22-μm filter membrane and then stored in a refrigerator at 4 °C for further analysis. The pH was monitored with a portable pH meter (Model6010, WTW, Germany). The concentrations of ClO_4^-, ClO_3^-, ClO_2^-, Cl^-, and SO_4^{2-} were determined by an ion chromatography instrument (ICS-3000, Dionex, USA). The biomass in the present study was expressed by mixed

Table 2 16S rDNA sequence analysis and species identification of selected dominant DGGE bands in Fig. 2a

Band no.	Closest relative	Accession number	Phylogeny	Similarity (%)
1	*Chitinophagaceae bacterium* L2-4	JX458466	Bacteroidetes	98
2	*Hydrotalea* sp. AF-51	JF739858	Bacteroidetes	99
3	*Brevundimonas vesicularis*	KC494336	α-Proteobacteria	97
4	*Desulfomicrobium thermophilum* (T)	AY464939	δ-Proteobacteria	92
5	*Methylobacterium fujisawaense*	HQ647259	α-Proteobacteria	99
6	*Litorilinea aerophila*	JQ733906	Chloroflexi	99
7	*Brevundimonas vesicularis*	FM955876	α-Proteobacteria	97
8	*Sulfuricella denitrificans* (T)	AB506456	β-Proteobacteria	97
9	Blackwater bioreactor bacterium BW23	AF394174	α-Proteobacteria	97
10	*Tistrella bauzanensis*	GQ240228	α-Proteobacteria	100
11	Bacterium TG159	AB308364	β-Proteobacteria	100
12	*Hoeflea alexandrii* (T)	AJ786600	α-Proteobacteria	100
13	*Aureispira maritima* (T)	AB278130	Bacteroidetes	100
14	*Georgfuchsia toluolica* (T)	EF219370	β-Proteobacteria	99
15	*Zoogloea caeni* (T)	DQ413148	β-Proteobacteria	99
16	*Bellilinea caldifistulae* (T)	AB243672	Chloroflexi	95
17	*Tetrasphaera australiensis*	AF125090	Actinobacteria	99
18	*Stenotrophomonas maltophilia*	FJ481929	γ-Proteobacteria	100
19	*Hyphomicrobium* sp. 16-60	HM124367	α-Proteobacteria	99
20	*Hydrogenophaga pseudoflava* (T)	AF078770	β-Proteobacteria	99

liquor volatile suspended solids (MLVSS). The MLVSS was separated from the mixture of sludge and S^0 by a centrifugal machine and was then measured according to the method described by Wei (2002).

Microbial community analysis

Denaturing gradient gel electrophoresis (DGGE) was used to analyze the microbial community of the acclimated microbial consortium. DNA extraction, DGGE, and sequencing were performed according to the method reported by Wang et al. (2014). Dendrogram relating to band pattern similarity was automatically calculated using the unweighted pair group method with the arithmetic average (UPGMA) clustering algorithm, which was included in the Quantity One software.

Bacteria serial numbers were obtained from the ribosomal database project (http://rdp.cme.msu.edu/). Sequence comparisons were conducted with the BLAST search option in the NCBI nucleotide sequence database (http://www.ncbi.nlm.nih.gov/).

Results and discussion

Acclimation of sulfur autotrophic perchlorate-reducing microbial consortium

The sulfur autotrophic perchlorate-reducing microbial consortium was acclimated in fill and draw modes in a 500-mL anaerobic flask. Figure 1 shows the variation of perchlorate concentration in the anaerobic flask during the acclimation. Stable ClO_4^- removal was observed in the anaerobic flask by repeatedly adding the fresh medium containing ClO_4^- for 135 days. The acclimation period to reach an eventual ClO_4^- concentration (less than 1 mg L^{-1}) gradually decreased with the increase of acclimation time. When the initial ClO_4^- concentrations were 240 and 360 mg L^{-1}, the shortest acclimation periods were 3 and 4 days, respectively. ClO_3^- and ClO_2^- in the anaerobic flask were not detected during the entire acclimation.

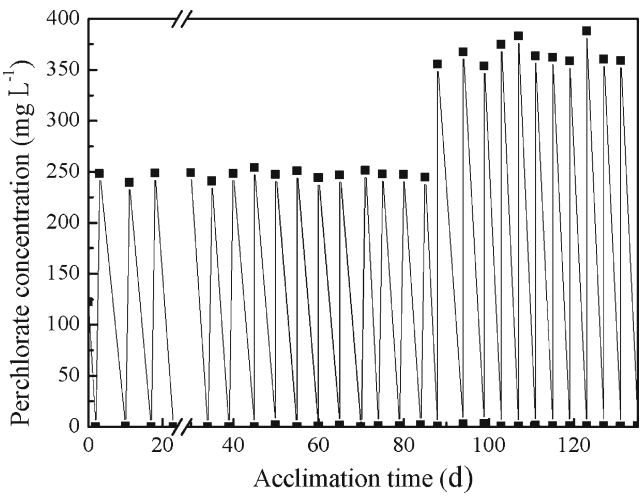

Fig. 1 Variation of perchlorate concentration during the acclimation

The PCR-DGGE was used to analyze the change of microbial community structure in the anaerobic flask with the increase of acclimation time (Fig. 2a). The quantity of discernable band was 11, 11, and 9 in the DGGE gel from the sludge samples at 0 (seed sludge), 31, and 135 days, respectively. The DGGE analysis showed that the band profile and band intensity changed with the increase of acclimation time. Only bands 1 and 2 were consistently present in the entire acclimation. Some bands (3, 4, 7, 9, 12, 13, 16, and 20) gradually disappeared or weakened, while others (5, 6, 15, 18, and 19) appeared at the end of acclimation.

A UPGMA clustering analysis was used to analyze the microbial community similarities among the sludge samples of 0 (seed sludge), 31, and 135 days. As shown in Fig. 2b, the microbial populations were categorized into two separate groups. The first group represented the seed sludge and the second group comprised the sludge samples of 31 and 135 days. The microbial community structure of sludge sample on 135 days had the least similarity to the other sludge samples, suggesting that the microbial community in the anaerobic flask was dramatically altered with the increase of the accumulation time.

To gain further insight into the microbial community structure, 20 discernable bands were excised from the DGGE gel and then sequenced. The detected sequences were compared with sequences deposited in the database using the BLAST program of GenBank. The most similar sequences of discernable DGGE bands and the phylogenetic tree are shown in Table 1 and Fig. 2c, respectively. Some bacteria in the seed sludge gradually disappeared or weakened with the increase of acclimation time, such as *Brevundimonas vesicularis* (band 3), *Brevundimonas vesicularis* (band 7), and *Hydrogenophaga pseudoflava* (T) (band 20). However, *Chitinophagaceae bacterium* L2-4 (band 1) and *Hydrotalea* sp. AF-51 (band 2) were present in the entire acclimation period, suggesting that these bacteria could utilize S^0 as the electron donor for microbial perchlorate reduction. Although some bacteria were not detected in the seed sludge, such as *Methylobacterium fujisawaense* (band 5), *Tistrella bauzanensis* (band 10), *Zoogloea caeni* (T) (band 15), *Stenotrophomonas maltophilia* (band 18), and *Hyphomicrobium* sp. 16-60 (T) (band 19), they gradually became predominant at the end of the acclimation, which might also have the ability to utilize S^0 as the electron donor for microbial perchlorate reduction.

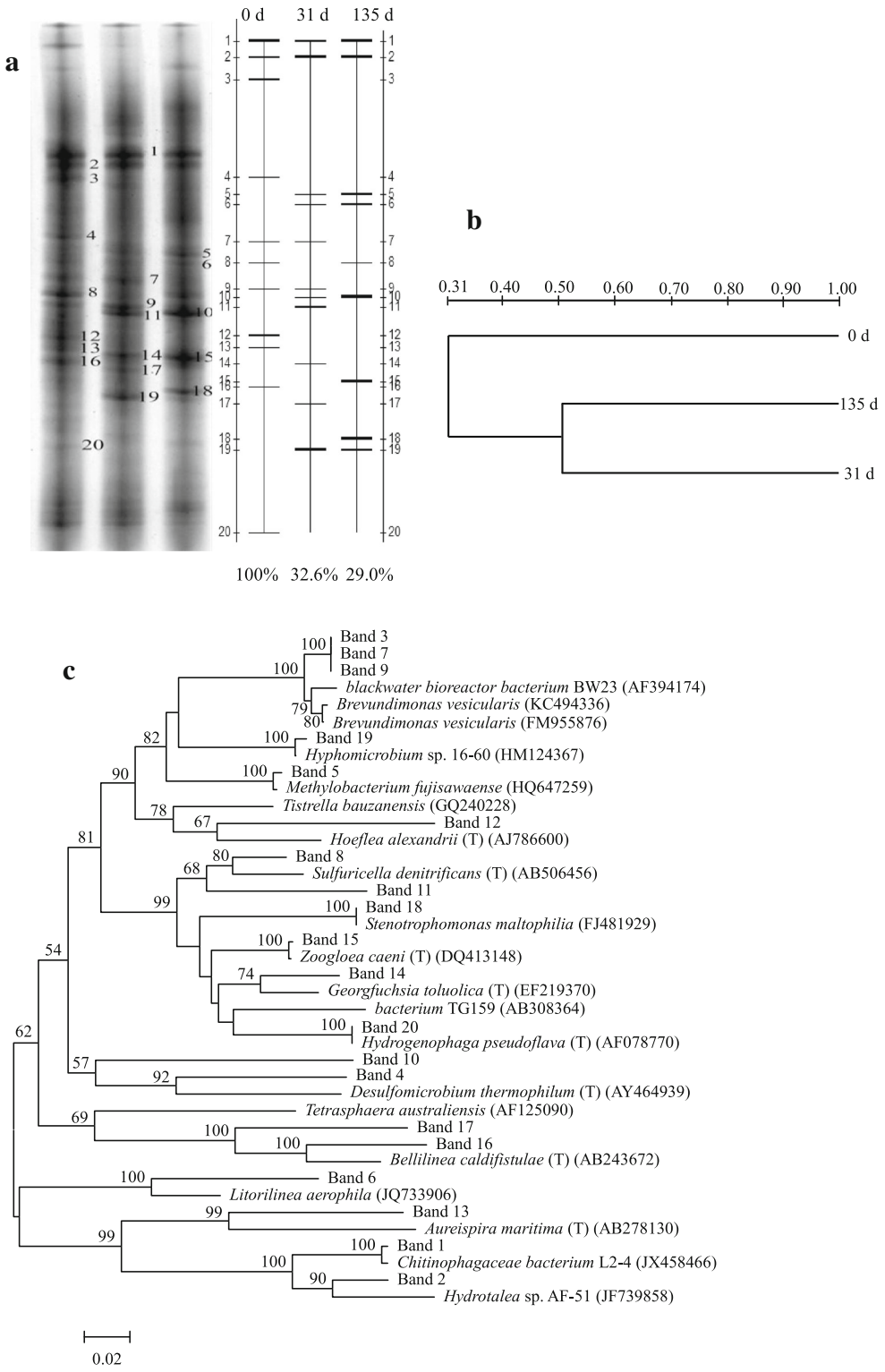

Fig. 2 Variation in microbial community with the increase of acclimation time in the anaerobic flask. **a** DGGE gel banding profiles, **b** cluster analysis based on "UPGMA" method, and **c** phylogenetic tree of bacteria based on the results of BLAST

The maximum observed microbial yield on perchlorate

Batch experiments were used to determine the kinetic parameters of microbial perchlorate reduction under anaerobic conditions using S^0 as the electron donor. The perchlorate reduction occurred concurrently with the growth of microbial consortium. When the contents of S^0 and inorganic nutrients in the anaerobic flask are sufficient, the kinetics of microbial growth and perchlorate reduction can be determined in separate experiments using the standard Monod substrate

utilization and biomass accumulation equations as follows (Nerenberg et al. 2006; Wang et al. 2008):

Monod substrate utilization equation

$$\frac{ds}{dt} = -\frac{q_{\max} S\max}{S + K_s} X \tag{1}$$

Biomass accumulation equation

$$\frac{dX}{dt} = \mu_{\max}\left[\frac{S}{S + K_s}\right] X - bX \tag{2}$$

where S is the perchlorate concentration (mg L^{-1}), t is the time (h), $q_{\max}$ is the maximum specific substrate utilization rate for perchlorate (h^{-1}), K_s is the half saturation constant (μg L^{-1}), X is the microbial concentration (mg L^{-1}), $\mu_{\max}$ is the maximum specific growth rate (h^{-1}), and b is the endogenous decay rate (h^{-1}).

As the S^0 content in the anaerobic flask was in excess for microbial perchlorate reduction, the mass transfer did not limit the reduction rate in the present study. According to Wang et al. (2008), the observed yield coefficient (Y) is defined as follows:

$$Y = \frac{dX}{-dS} = \frac{dX/dt}{-(dS/dt)} = \frac{\mu_{\max}[S/(S + K_s)]X - bX}{q_{\max} XS/(S + K_s)}$$
$$= \frac{\mu_{\max}}{q_{\max}} - \frac{b(S + K_s)}{q_{\max} S} = \left(\frac{\mu_{\max}}{q_{\max}}\right)\left[1 - \frac{b(S + K_s)}{\mu_{\max} S}\right] \tag{3}$$

Because the initial perchlorate concentration at 1200 mg L^{-1} in the anaerobic flask was much higher than K_s ($S >> K_s$), the Y_{max} could be calculated as follows:

$$Y = \left(\frac{\mu_{\max}}{q_{\max}}\right)\left(1 - \frac{b}{\mu_{\max}}\right) \tag{4}$$

When S in the aerobic flask is much higher than K_s, the microbial yield for perchlorate is considered to be constant. Wang et al. (2008) reported a linear relationship between X and S by integrating $Y = \frac{dX}{-dS}$ in Eq. (3) as follows:

$$X = X_0 + S_0 Y_{\max} - Y_{\max} S \tag{5}$$

where X_0 is the initial biomass concentration (mg L^{-1}), S_0 is the initial perchlorate concentration (mg L^{-1}), and $Y_{\max}$ is the maximum observed microbial yield on perchlorate.

Figure 3 shows the maximum observed yield on perchlorate for sulfur autotrophic perchlorate reducing microbial consortium. The residual perchlorate concentration in the aerobic flask was controlled at a level higher than 200 mg L^{-1}, which is much larger than the known K_s values for mixed or pure perchlorate-reducing bacteria (Rikken et al. 1996; Logan et al. 2001; Waller et al. 2004). As shown in Fig. 3, the maximum observed yield coefficient for the sulfur autotrophic perchlorate-reducing microbial consortium in the present study was 0.19 mg DW mg^{-1} ClO_4^-. Waller et al. (2004) reported that the maximum observed yield coefficients for heterotrophic perchlorate reduction bacteria were in the range of 0.34 and 0.36 mg DW mg^{-1} ClO_4^-. Compared to heterotrophic perchlorate reduction bacteria, autotrophic perchlorate-reducing bacteria using S^0 as the electron donor had a lower growth rate.

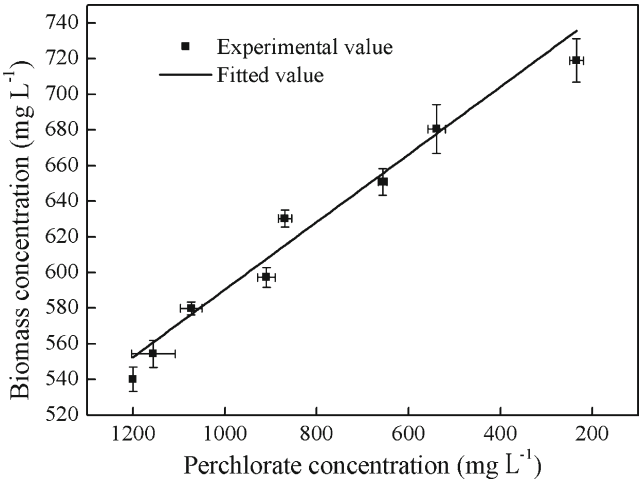

Fig. 3 The maximum observed yield on perchlorate for sulfur autotrophic perchlorate-reducing microbial consortium

Microbial perchlorate reduction kinetics

To determine the microbial perchlorate reduction kinetics, the reciprocal style of Eq. (1) is shown as follows:

$$\frac{dt}{dS} = -\frac{1}{q_{\max} X} - \frac{K}{q_{\max} XS} \tag{6}$$

The integral of Eq. (6) is defined as follows:

$$t = -\frac{1}{Xq_{\max}}(S - S_0) - \frac{K_s}{Xq_{\max}}\ln\frac{S}{S_0} = A(S - S_0) + B\ln\frac{S}{S_0} \tag{7}$$

where S_0 is the initial perchlorate concentration (mg L^{-1}), $A = -\frac{1}{Xq_{\max}}$, and $B = -\frac{K_s}{Xq_{\max}}$.

The parameters A and B are obtained by fitting the numerically calculated data with least square method, and K_s and q_{max} are determined in turn. Figure 4 shows the curve fitting for the microbial perchlorate reduction kinetics. S_0 and X in the present study were 250 and 421.7 mg L^{-1}, respectively. The parameters K_s and q_{max} were determined to be 5.71 and 0.14 mg ClO_4^- mg^{-1} DW day^{-1}, respectively, which indicated that the microbial consortium could effectively utilize perchlorate as an electron acceptor.

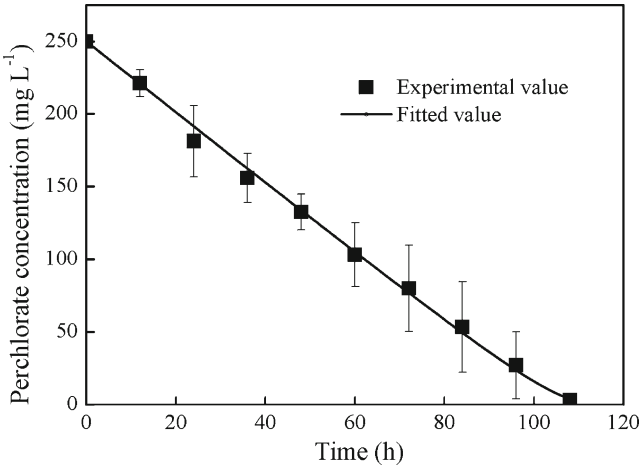

Fig. 4 Kinetics of microbial perchlorate reduction by sulfur autotrophic perchlorate-reducing microbial consortium

Kinetics of Cl^- and SO_4^{2-} formation

Sahu et al. (2009) reported a stoichiometric equation for sulfur autotrophic perchlorate reduction as follows:

$$2.9\,S^0 + 3.3\,H_2O + ClO_4^- + 1.8\,CO_2 + 0.46\,HCO_3^- + 0.46\,NH_4^+ \rightarrow 5.7\,H^+ + 2.9\,SO_4^{2-} + Cl^- + 0.46\,C_5H_7O_2N \quad (8)$$

As sulfur autotrophic perchlorate reduction could lead to the presence of Cl^- and SO_4^{2-}, their formation kinetic parameters were evaluated in the present study. A modified Gompertz equation was employed to model the formation of Cl^- and SO_4^{2-} as follows (van Ginkel et al. 2001; Lin and Lay 2004):

$$P_i(t) = P_{\max,i}\exp\left\{-\exp\left[\frac{R_{\max,i}e}{P_{\max,i}}(\lambda_i - t) + 1\right]\right\} \quad (9)$$

where i represents Cl^- or SO_4^{2-}, $P_i(t)$ is the cumulative amount of product i at perchlorate reduction time t, $P_{\max,i}$ is the maximal amount of product i produced, $R_{\max,i}$ is the maximum formation rate of product i, and λ_i is the lag time before the exponential product is formed.

The cumulative amounts of Cl^- and SO_4^{2-} were fitted as a function of the reaction time by using the modified Gompertz equation (Fig. 5), and the kinetic parameters for the formation of Cl^- and SO_4^{2-} were summarized in Table 3. The Cl^- and SO_4^{2-} correlation coefficients (R^2) of the nonlinear analysis using Eq. (9) were 0.995 and 0.981, respectively. The results suggested that the modified Gompertz equation could adequately describe the formation of Cl^- and SO_4^{2-} during the process of sulfur autotrophic perchlorate reduction. According to Eq. (8), the theoretical SO_4^{2-} production is 699.5 when the initial ClO_4^- concentration is 250 mg L^{-1}. However, the practical SO_4^{2-} production was 892.6 mg L^{-1} (Fig. 5), which was higher than the theoretical SO_4^{2-} production. The excess SO_4^{2-} production might come from S^0 disproportionation that was a competing microbial reaction in the anaerobic flask. Ju et al. (2007) also reported that S^0 disproportionation could lead to the higher SO_4^{2-} production for perchlorate reduction than the theoretical SO_4^{2-} production.

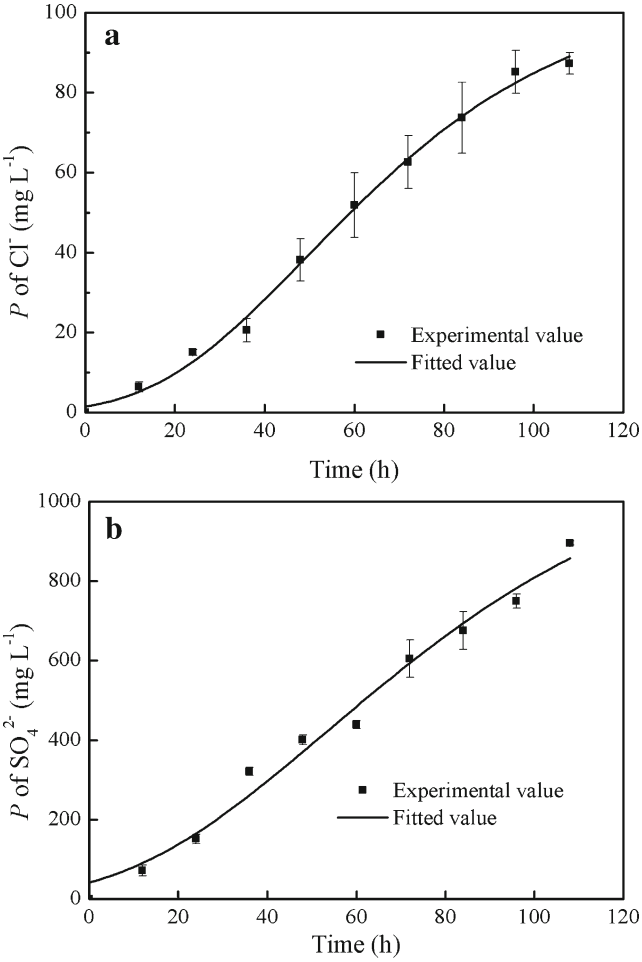

Fig. 5 Modified Gompertz equation plot for the formation of Cl^- and SO_4^{2-}. **a** Cl^-. **b** SO_4^{2}

Relationship between perchlorate reduction and product formation

As the initial ClO_4^- concentration was 250 mg L^{-1} ClO_4^-, the S^0 concentration at 250 g L^{-1} in the anaerobic flask was much greater than the theoretical required S^0 content for microbial perchlorate reduction. Therefore, the initial ClO_4^- concentration was a key factor for evaluating the relationship between

Table 3 Calculated results using the modified Gompertz equation for the formation of Cl^- and SO_4^{2-}

Product	$P_{\max}$ (mg L^{-1})	$R_{\max}$ (mg mg^{-1} day^{-1})	λ (h)	R^2
Cl^-	106.56	0.07	15.73	0.995
SO_4^{2-}	1152	0.54	9.05	0.981

the perchlorate reduction and product formation in the present study. The correlation between the product and substrate could be expressed as follows (Yang et al. 1988):

$$\frac{dP_i}{dt} = -Y_{P_i}\frac{dS}{dt} \tag{10}$$

$$dP_i = -Y_{P_i}dS \tag{11}$$

$$\int_0^{P_i} dP_i = -Y_{P_i}\int_{S_0}^{S} dS \tag{12}$$

$$P_i = Y_{P_i}(S_0 - S) \tag{13}$$

where Y_{P_i} is the yield of product i, t is the time, S_0 is the initial ClO_4^- concentration, and S is the ClO_4^- concentration at time t.

The fitted plots are shown in Fig. 6. The correlation coefficients (R^2) for Cl^- and SO_4^{2-} were 0.987 and 0.994, respectively, suggesting that Eq. (13) could successfully simulate the relationship between the products and substrate. The yield of Cl^- was estimated at 0.35 mg mg^{-1} ClO_4^-, which was close to the theoretical Cl^- production according to Eq. (8). However, the yield of SO_4^{2-} was estimated at 3.41 mg mg^{-1} ClO_4^-, which exceeded the theoretical SO_4^{2-} production due to S^0 disproportionation.

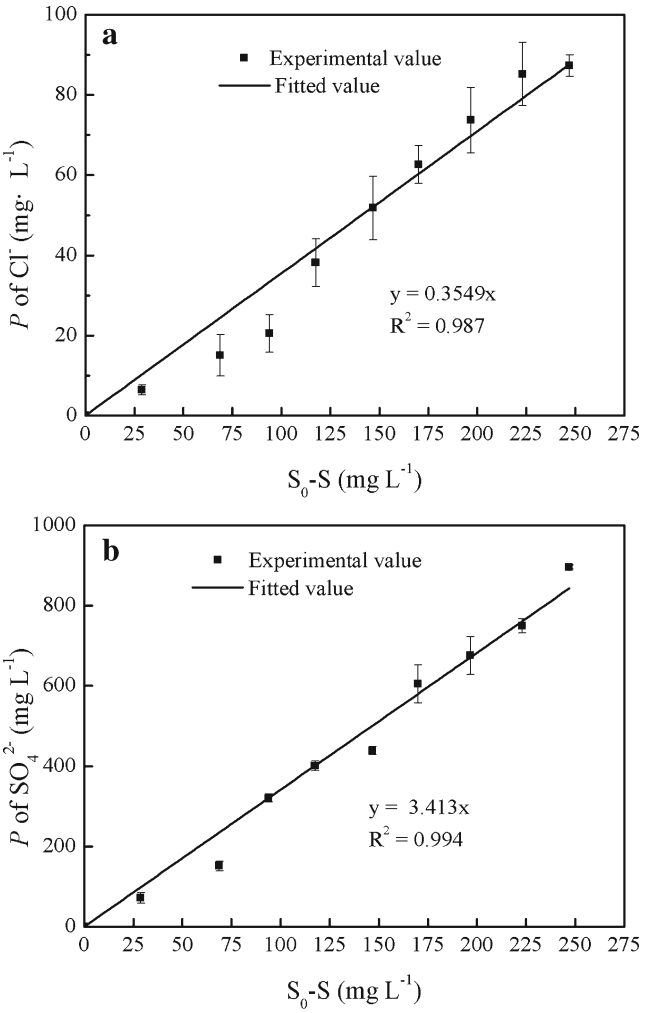

Fig. 6 Plot for the relationship between the products and perchlorate. **a** Cl^-. **b** SO_4^{2-}

pH effect on microbial perchlorate reduction

The anaerobic flasks with 200 mL culture medium were employed to investigate the effect of pH on sulfur autotrophic perchlorate reduction, which contained 250 mg L^{-1} ClO_4^-, 426.76 mg L^{-1} MLVSS, and 250 g L^{-1} S^0 with a 1- to 2-mm particle diameter. Low concentration of phosphate in the culture medium was used to buffer the pH. Figure 7a shows the effect of the initial pH on the microbial perchlorate reduction. The microbial consortium could hardly reduce perchlorate at pH 5. When the initial pH of the culture medium was 6, 7, 8, and 9, the ClO_4^- removal efficiency was 65.2, 99.6, 58.0,

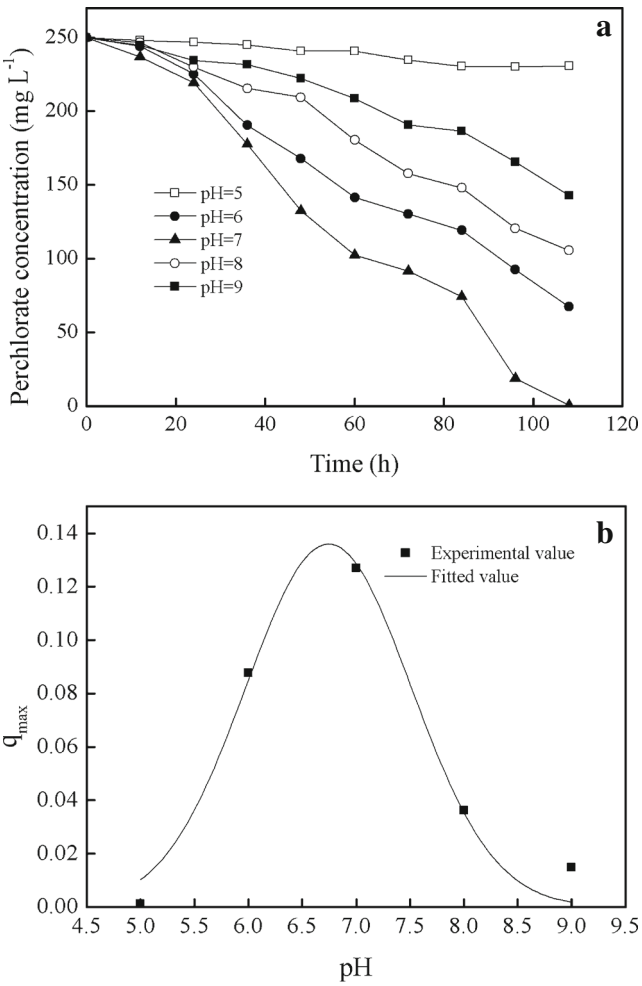

Fig. 7 pH effect on microbial perchlorate reduction (**a**) and Gaussian peak fit of q_{max} against pH for the sulfur autotrophic perchlorate reducing microbial consortium (**b**)

and 43.2 % after 108 h, respectively. Based on the experimental results, the optimal pH range for perchlorate reduction was near the neutral environment for the acclimated microbial consortium in the present study. Wang et al. (2008) also reported that the maximum perchlorate removal efficiency for heterotrophic and mixed perchlorate-reducing bacteria occurred at pH 7.0.

The relation between the q_{max} and the initial pH is shown in Fig. 7b. According to Wang et al. (2008), a transformed Gaussian peak equation was used to fit the calculated q_{max} by changing σ in Excel Solver to minimize SSE as follows:

$$q_{\max,pH} = q_{\max,pH_{6.7}} \exp\left[-\frac{(pH-6.7)^2}{2\sigma^2}\right] \quad (14)$$

where $q_{max,\,pH}$ is q_{max} at a specific pH, $q_{max,\,pH\ 6.7}$ is q_{max} at pH 6.7, and σ is the standard deviation.

As shown in Fig. 7b, the maximum microbial perchlorate reduction rate was obtained at pH 6.7 by analyzing the variation of q_{max} in the pH range from 5 to 9.

The optimum pH in the present study was consistent with the previous researches for both pure and mixed perchlorate-reducing bacteria (Attaway and Smith 1993; Herman and Fankenberger 1999; Wallace et al. 1996).

Effect of nitrate addition on microbial perchlorate reduction

The effect of nitrate addition on microbial perchlorate reduction was evaluated in a culture medium containing 250 mg L^{-1} ClO_4^-, 564.2 mg L^{-1} MLSS, and 250 g L^{-1} S^0 pellet with a 1- to 2-mm particle diameter. As shown in Fig. 8, the required time for complete microbial perchlorate reduction gradually increased from 156 to 204 h with the increase of initial nitrate concentration from 50 to 300 mg L^{-1}, suggesting that the presence of nitrate could delay the process of sulfur autotrophic perchlorate reduction. Although the addition of nitrate delayed the onset of sulfur autotrophic perchlorate reduction, the simultaneous reductions of perchlorate and nitrate were found by investigating the variation of Cl^- production (data not shown). These phenomenon could be explained as follows: (1) sulfur autotrophic perchlorate reduction bacteria and denitrifiers might coexist in the acclimated microbial consortium; (2) some bacterial strains in the acclimated microbial consortium could use nitrate as an electron acceptor, whereas they could not use perchlorate as an electron acceptor; (3) some bacterial strains in the acclimated microbial consortium could simultaneously utilize nitrate and perchlorate as electron acceptors; and (4) some sulfur autotrophic perchlorate reduction bacteria might not utilize nitrate as a electron acceptor. The results in the present study showed the presence of nitrate in the anaerobic flask delayed the onset of sulfur autotrophic perchlorate reduction. Ju et al. (2007) also reported that the addition of nitrate to sulfur-oxidizing perchlorate reduction bacteria delayed the onset of ClO_4^- reduction until nitrate was partially consumed.

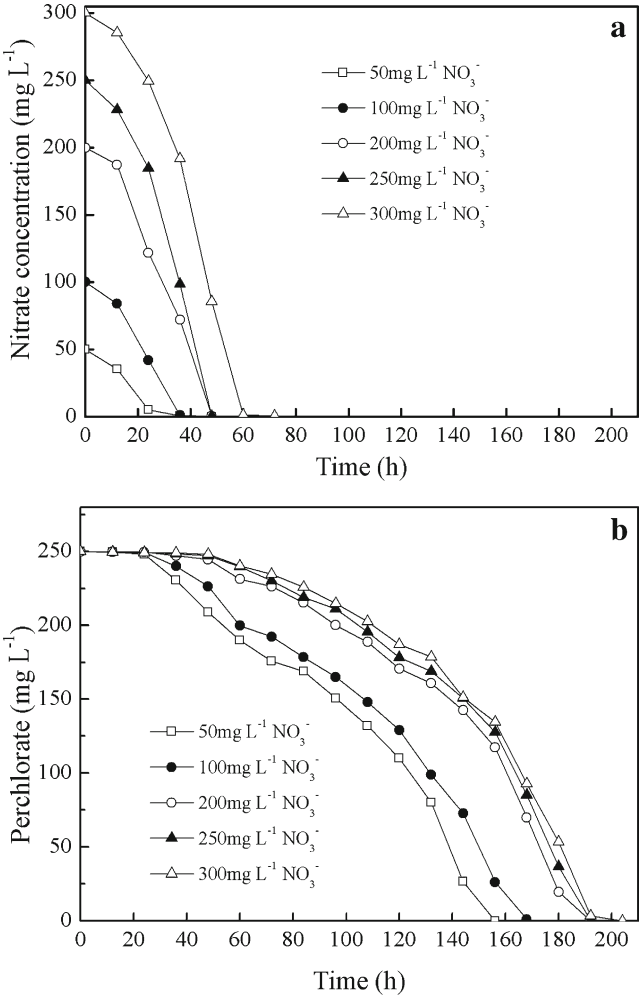

Fig. 8 Effect of nitrate addition on microbial perchlorate reduction

Conclusions

The kinetic parameters for sulfur autotrophic perchlorate reduction were determined in batch experiments. The maximum observed yield coefficient indicated that the microbial consortium using S^0 as the electron donor had a slower growth rate than heterotrophic perchlorate reduction bacteria, which could produce little excess biomass in practical applications. The maximum specific substrate utilization rate and half saturation constant for microbial perchlorate reduction showed that the microbial consortium could effectively utilize perchlorate as the electron acceptor. The maximum microbial perchlorate reduction rate was obtained at pH 6.7 using a Gaussian peak equation. The presence of nitrate in perchlorate-contaminated water could delay the onset of sulfur autotrophic perchlorate reduction. The modified Gompertz equation could adequately describe the formation of Cl^- and SO_4^{2-} during the process of

sulfur autotrophic perchlorate reduction. The fitted SO_4^{2-} production exceeded the theoretical SO_4^{2-} production due to S^0 disproportionation. The perchlorate reduction kinetic parameters in the present study can be used to design biological treatment systems in practical applications.

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