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ENVIRONMENTAL BIOTECHNOLOGY

Kinetics of nitrate and sulfate removal using a mixed microbial culture with or without limited-oxygen fed

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Abstract The biological degradation of nitrate and sulfate was investigated using a mixed microbial culture and lactate as the carbon source, with or without limited-oxygen fed. It was found that sulfate reduction was slightly inhibited by nitrate, since after nitrate depletion the sulfate reduction rate increased from 0.37 mg SO_4^{2-}/mg VSS d to 0.71 mg SO_4^{2-}/mg VSS d, and the maximum rate of sulfate reduction in the presence of nitrate corresponded to 56 % of the non-inhibited sulfate reduction rate determined after nitrate depleted. However, simultaneous but not sequential reduction of both oxy-anions was observed in this study, unlike some literature reports in which sulfate reduction starts only after depletion of nitrate, and this case might be due to the fact that lactate was always kept above the limiting conditions. At limited oxygen, the inhibited effect on sulfate reduction by nitrate was relieved, and the sulfate reduction rate seemed relatively higher than that obtained without limited-oxygen fed, whereas kept almost constant (0.86–0.89 mg SO_4^{2-}/mg VSS d) cross the six R_{OS} states. In contrast, nitrate reduction rates decreased substantially with the increase in the initial limited-oxygen fed, showing an inhibited effect on nitrate reduction by oxygen. Kinetic parameters determined for the mixed microbial culture showed that the maximum specific sulfate utilization rate obtained (0.098±0.022 mg SO_4^{2-}/(mg VSS h)) was similar to the reported typical value (0.1 mg SO_4^{2-}/(mg VSS h)), also indicating a moderate inhibited effect by nitrate.

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Introduction

Nitrate (NO_3^-) contamination of surface and groundwater is a relevant problem due to its negative impact on human health, particularly for methemologlobinemia in infants. The main source of contamination is agricultural runoff, wastewater discharges, and leaching from septic tanks (Valencia et al. 2012). The US Environmental Protection Agency (EPA) recommends a maximum contaminant level of 45 mg/L for NO_3^- (USEPA 2010) and 50 mg/L by the European Union (European Community EC 1980).

Biological degradation of NO_3^- is a promising treatment for remediating water contaminated with the compounds, since NO_3^- can easily be metabolized to nitrogen gas (N_2) via denitrification by nitrate-reducing bacteria (NRB). These organisms, in anoxic conditions, use NO_3^- or nitrite (NO_2^-) as electron acceptors in a reductive pathway with four specialized enzymes: nitrate reductase, nitrite reductase, nitric oxide (NO) reductase, and nitrous oxide (N_2O) reductase. The suggested sequence pathway for denitrification is $NO_3^-\rightarrow NO_2^-\rightarrow NO\rightarrow N_2O\rightarrow N_2$ (Payne 1973; Knowles 1982; Rittmann and McCarty 2001).

Sulfate (SO_4^{2-}) is often found in water as a co-contaminant of nitrate since sulfur is the major element in the production of pesticide and pharmaceutical and imposes a health risk for diarrhea in humans when SO_4^{2-} is at concentrations higher than 250 mg/L (USEPA 2012). Even more important is that SO_4^{2-} reduction produces hydrogen sulfide (H_2S), a corrosive, odorous, and toxic substance (Reyes-Avila et al. 2004). Due to relatively high organic input in most waste streams, SO_4^{2-} reduction is inevitable, although it usually is an unwanted process. The use of hybrid technologies has shown

benefits, especially if biological sulfate reduction and sulfide oxidation are combined within one stage (Xu et al. 2012; Celis-Garcia et al. 2008; Okabe et al. 2005; van den Ende et al. 1997). In these technologies, a syntrophic interaction between sulfate-reducing bacteria (SRB) and sulfide-oxidizing bacteria (SOB) was a well developed subject to limited-oxygen fed. In particular, at limited-oxygen levels, sulfide oxidation process is believed to proceed via a limited-oxygen route (LOR), that is the SOB reduce NAD^+ without transfer electron directly to oxygen and favor the elemental sulfur (S^0) production (Klok et al. 2012). Moreover, the resulting water produces no secondary contamination with metabolic by-product (H_2S) by exploring the sulfide and oxygen balance and determining the predominate routes for the biological desulfurization process at S^0-forming process.

In order to optimize the process of simultaneous reduction of these oxy-anions, it is essential to elucidate the effect of nitrate on sulfate reduction kinetics. Simultaneous as well as sequential reduction, in which sulfate reduction starts only after depletion of nitrate, has been reported for mixed microbial cultures (Tang et al. 2010; Ziv-El and Rittmann 2009). In systems operated with mixed microbial cultures, the identification of the kinetic mechanism is more difficult (Ricardo et al. 2012), since multiple members of the microbial community can perform denitrification and/or sulfate reduction and sulfide oxidation. The first objective of this study is to elucidate the kinetics of nitrate and sulfate reduction and sulfide oxidation by a mixed microbial culture. Since the majority of nitrate-reducing bacteria can respire O_2 and denitrification process proceeds in anoxic condition, simultaneous reduction of these oxy-anions in a limited-oxygen fed bioreactor is a promising alternative, and the selectivity of S^0 formation from SO_4^{2-} substrate could be improved. Thus, the second objective is to investigate the kinetics of nitrate and sulfate co-reduction at limited-oxygen fed condition with varying pure oxygen input amount in biomedium. The second objective focuses especially on how limited-oxygen fed affects nitrate and sulfate reduction kinetics with a mixed microbial culture. The possibility of mathematical modeling of nitrate and sulfate co-reduction is an important step toward understanding the wastewater treatment systems, while at yet scarce effort has been dedicated to model the co-reduction dynamics, and thus the third objective of this study is to simulate the co-reduction process.

Materials and methods

Culture and medium

A mixed microbial biomass, taken from a simultaneous reduction of these oxy-anions bioreactor, was cultured in argon-sparged medium containing 500 mg/L nitrate, 1,000 mg/L sulfate, and 2,500 mg/L lactate, and an overview of the microbial communities present in the inoculums is given elsewhere in the literature (Chen et al. 2008). Cultures were grown in 250-mL serum bottles sealed with butyl rubber stoppers containing 150 mL of the prepared medium and were incubated at 30 °C with shaking. Furthermore, the medium contained 50 mg/L NH_4Cl, 50 mg/L K_2HPO_4, 1.5 g/L $NaHCO_3$, 50 mg/L $MgSO_4$, and 50 mg/L $CaCl_2 \cdot 2H_2O$ (all in demineralized water). Trace element solution was added as described by Xu et al. (2012). After addition of all compounds, the pH of the medium was 8.0–8.1.

Nitrate and sulfate co-bioreduction of enriched mixed microbial cultures

Five batch tests were performed using the enriched cultures as described in "Culture and medium". The batch tests were carried out in 250-mL serum bottles and inoculated with 200-mL inoculums with 8,000 mg L^{-1} volatile suspended solids (VSS). The initial sulfate and lactate concentrations were kept at 1,000 mg/L and 2.5 g/L, respectively, in all tests, whereas the initial nitrate concentration was varied from 0 to 1,000 mg/L. Before each batch test, the culture was centrifuged at 10,000 rpm for 20 min, and the pellets anaerobically collected were washed twice with demineralized water to remove residual substrates eliminating the disturbance of background. The biomass was then inoculated into serum bottles after which the medium was added. The bottles were flushed with argon gas for 5 min to remove oxygen from both the aqueous phase and headspace and sealed with butyl rubber stoppers and aluminum crimps. Anoxic conditions were maintained by argon in the serum bottle headspace. Samples were taken periodically for nitrate, nitrite, sulfate, sulfide, lactate, and biomass measurements. The concentration of biomass was determined according to standard methods (APHA 1995). In order to assess the steady state of the system, successive batch experiments under each tested condition were carried out until the residual substrate concentrations in serum bottle varied less than 10 %, and then the averaged results of the steady state were recorded and reported herein.

Effect of limited oxygen on nitrate, sulfate reduction, and sulfide oxidation

Six batch tests were performed using the enriched culture as described in "Culture and medium". The batch tests were carried out in 250-mL serum bottles and inoculated with 200-mL inoculums with 8,000 mg L^{-1} volatile suspended solids (VSS). The initial nitrate and sulfate concentrations were kept at 500 and 1,000 mg/L, respectively, in all tests, whereas the initial limited-oxygen addition was varied from 0 to 120 mL to generate the molar ratio of oxygen to sulfide (R_{OS}) to be 0.39, 0.77, 1.16, 1.55, 1.93, and 2.32. All other

operations were the same as that described in "Nitrate and sulfate co-bioreduction of enriched mixed microbial cultures". The oxygen was added according to Xu et al. (2013). Pure oxygen was added into the headspace of each bottle immediately after the bio-medium added. Using 42.1 mM as the concentration of gaseous oxygen at 23 °C and 1 atm, the volume (V) of pure oxygen to add was calculated as

$$V = (\text{mM } O_2 \text{ wanted in solution } 50\text{-mL headspace})/41.2 \text{ mM}$$
$$= (R_{OS} \text{ mM } SO_4^{2-}\text{-S in initial bio-medium } 50\text{-mL headspace})/41.2 \text{ mM}$$

Bottles were regularly sampled anaerobically. This involved syringe injection of argon to maintain pressure, prior to removing a liquid or gaseous sample (Johnston and Voordouw 2012).

Determination of kinetic parameters for nitrate and sulfate reduction

The reported reduction rates are the maximal reduction rates, determined by linear regression through the points with the maximal rate observed. The linear regression was estimated with a minimal of 5–6 experimental values. The inclusion of experimental values stopped when the reduction rate declined, i.e., when the slope of the regression curve of substrate concentration versus time decreased. Kinetic parameters for the mixed culture were calculated assuming a competitive inhibition model for nitrate and sulfate according to that proposed by Rittmann and McCarty (2001). The simple Monod expressions do not predict nitrate and sulfate's effect on specific reduction rates. Competitive inhibition can be incorporated through a modifier on the K term (half-maximum rate constant), which increases the effective K value for the substrate when the inhibitor concentrations are high relative to the inhibitor's K. For estimation of kinetic parameters for bioreduction of both oxy-anions, all experimental values were considered. A non-linear regression analysis by least squares was used to estimate the following parameters: maximum nitrate and sulfate reduction rates and the half-saturation constants for nitrate and sulfate. These parameters were estimated by fitting the results of five experiments performed at different initial nitrate concentrations while keeping constant the other substrate concentrations. Computation was performed on Matlab (2006) (The Mathworks, Inc., USA) using a non-linear least-squares function. The confidence intervals (95 % confidence level) for the estimated parameters were calculated assuming that the modeling errors are Gaussian distributed and that the variance is unknown.

Analytical methods

Prior to analysis, all samples were filtered through a 0.22-μm filter to remove cell debris. Using a Waters high-performance liquid chromatography (HPLC), lactate and volatile fatty acid (e.g. acetate and propionate) concentrations were determined with a Bio-RAD Carbohydrate analysis column (Aminex, HPX-87P, 300×7.8 mm) with deionized water eluent flowing at 0.6 mL/min and a Waters 2489 UV/Visible detector at 220 nm. Nitrate, nitrite, sulfate, and thiosulfate concentrations were determined by ion chromatography (ICS-3000, Dionex, Bannockbum, IL, USA). Aqueous sulfide was determined spectrophotometrically (UV759S, Shanghai, China) with N,N-dimethyl-*p*-phenylene diamine (Johnston and Voordouw 2012). Concentrations of nitrogenous species (NO, N_2O, N_2) were determined by gas chromatography (GC-6890, Agilent, Foster City, CA, USA).

Results

Influence of nitrate on sulfate reduction in mixed microbial culture

The influence of nitrate on the reduction rate of sulfate in mixed microbial culture was evaluated in batch experiments, and lactate was always kept above the limiting conditions. These experiments aimed at analyzing the effect of nitrate on sulfate reduction, when nitrate and sulfate were present in concentrations of the same order of magnitude. The initial concentration of nitrate varied from 0 to 1,000 mg/L, while sulfate was kept constant (at 1,000 mg/L) in all tests. A similar reduction pattern was observed in all the experiments: a slightly low sulfate reduction rate while nitrate was being reduced, followed by an increase in sulfate reduction after nitrate depletion. As an example, for an initial nitrate concentration of 500 mg/L a maximal nitrate reduction rate of 0.95 mg NO_3^-/mg VSS d was observed immediately after inoculation, whereas, during this phase, sulfate was reduced at 0.37 mg SO_4^{2-}/mg VSS d, as illustrated in Fig. 1. After depletion of nitrate, the sulfate reduction rate increased to 0.71 mg SO_4^{2-}/mg VSS d. Inhibition of the sulfate reduction rate by nitrate was observed in all of the tests. After exhaustion of nitrate, the sulfate reduction rate increased from 0.18 to 0.49 mg SO_4^{2-}/mg VSS d to values between 0.29 and 0.87 mg SO_4^{2-}/mg VSS d, depending on the initial concentration of nitrate tested (Fig. 2). In the presence of nitrate, the maximum rate of sulfate reduction was 0.49 mg SO_4^{2-}/mg VSS d, which corresponded only to 56 % of its maximal reduction rate (Fig. 2). The non-inhibited sulfate reduction rate (i.e., determined after nitrate was depleted) increased with the decrease in the initial nitrate concentration, reaching a maximal value of 0.87 mg SO_4^{2-}/mg VSS d (Fig. 2).

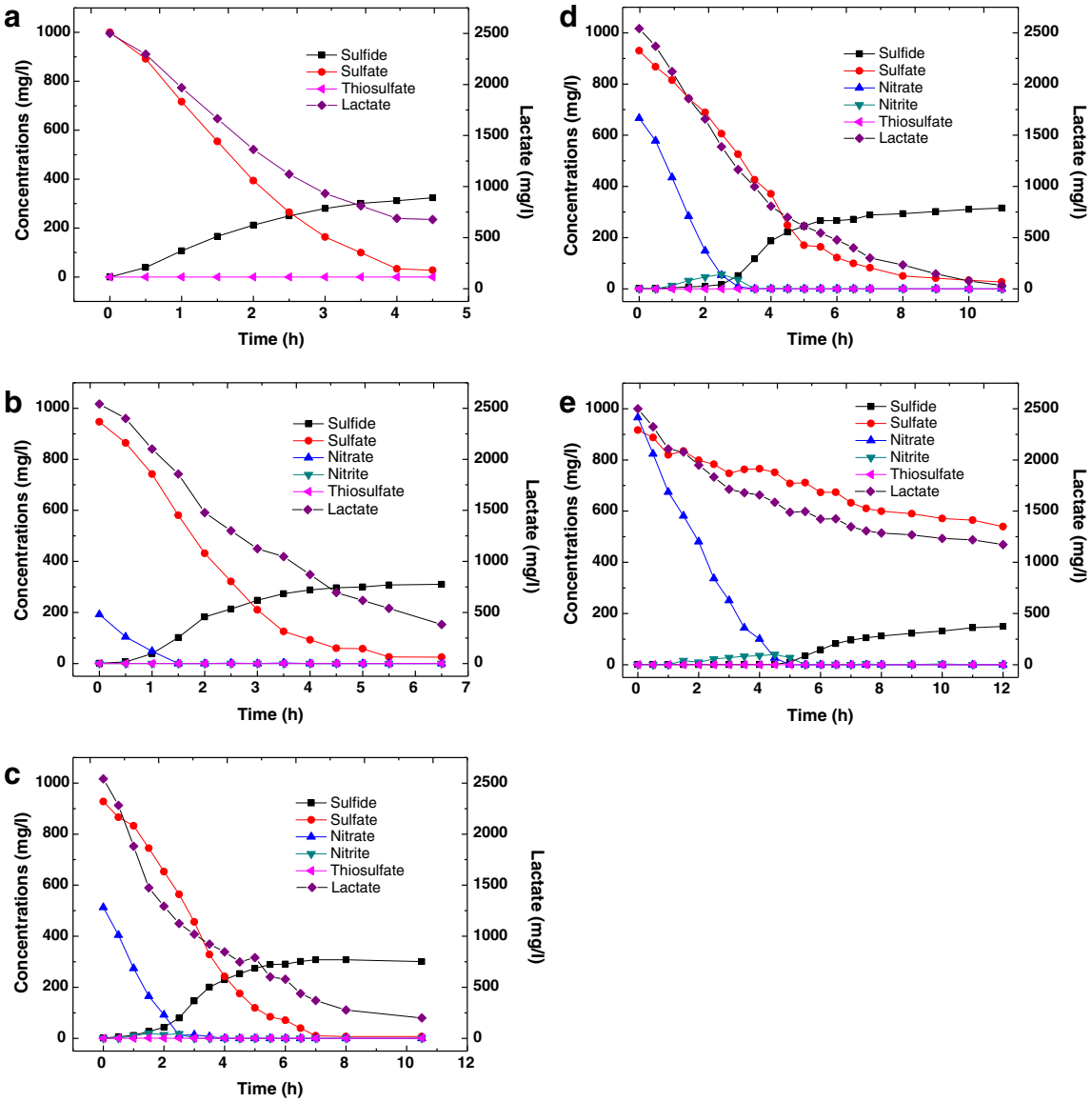

Fig. 1 Profiles of nitrate, sulfate, nitrite, sulfide, thiosulfate, and lactate concentrations in the batch tests with 1,000 mg/L sulfate and various initial nitrate concentrations: **a** 0, **b** 200, **c** 500, **d** 700, and **e** 1,000 mg/L

The increase in the initial nitrate concentration led to a longer phase in sulfate reduction. With 200, 500, and 700 mg/L nitrate (Fig. 1), the sulfate concentration reached to an undetectable value in less than 10 h, and reduction of sulfate led to production of sulfide. However, with 1,000 mg/L nitrate, only 40 % of sulfate was reduced, indicating a significant inhibition effect by nitrate. Interestingly, during nitrate reduction period, a gap between sulfide concentration we measured and theoretical production from sulfate reduction was observed, indicating a potential of sulfide oxidation by nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB). And this observance was in agreement with those reported by Kelly and Wood (2000), Gevertz et al. (2000), Greene et al. (2003), Okabe et al. (2005), and Garcia de Lomas et al. (2006, 2007). The increase in initial nitrate concentration led to a higher level in sulfide oxidation (except for 1,000 mg/L nitrate).

Influence of limited oxygen on nitrate, sulfate reduction, and sulfide oxidation

The influence of limited oxygen on the reduction of nitrate and sulfate and oxidation of sulfide in mixed microbial cultures was investigated in batch tests. The initial concentration of limited-oxygen fed varied from 20 to 120 mL, while nitrate, sulfate, and lactate were kept constant in all tests. The results of bio-reduction of nitrate and sulfate and biooxidation of sulfide at initial limited-oxygen fed were shown in Fig. 3.

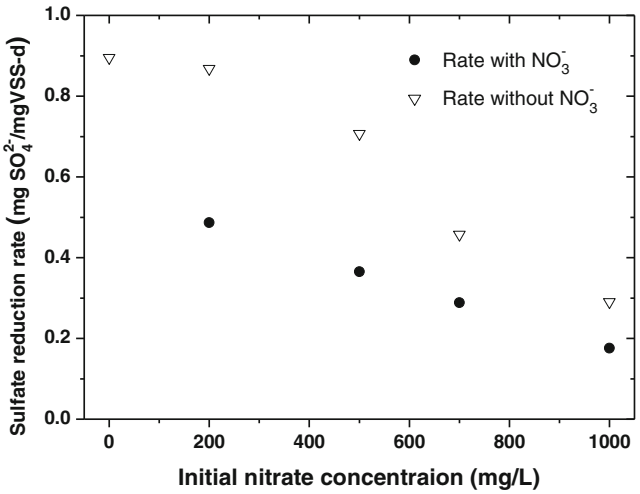

Fig. 2 Specific sulfate reduction rate when nitrate is present (*full circles*) and after nitrate exhaustion (*open triangles*), determined in batch tests with the mixed microbial culture

Complete reduction of nitrate and sulfate was achieved in all tests except 120 mL of initial oxygen fed (the sulfate rebound may be due to sulfide oxidation). Different from the observed reduction pattern in 3.1, the inhibition of sulfate reduction by nitrate was relieved due to the presence of oxygen, and no sequential reduction of nitrate followed by sulfate was found. A high sulfate reduction rate was observed at all tests even while nitrate was being reduced. And with various initial oxygen fed, the sulfate reduction rate almost kept constant (0.86–0.89 mg SO_4^{2-}/mg VSS d, Fig. 4) but higher than that (0.71 mg SO_4^{2-}/mg VSS d) in anaerobic condition, suggesting that the sulfate reduction rate was not interfered by the oxygen addition amount. However, a decline in nitrate reduction rate was observed in all of the tests. For an initial oxygen fed of 20 mL a maximal nitrate reduction rate of 0.95 mg NO_3^-/mg VSS d was observed immediately after inoculation similar to the value obtained at anaerobic condition; whereas for an initial oxygen fed of 100 mL the maximal rate of nitrate reduction was 0.59 mg NO_3^-/mg VSS d, which corresponded only to 62 % of its maximal reduction

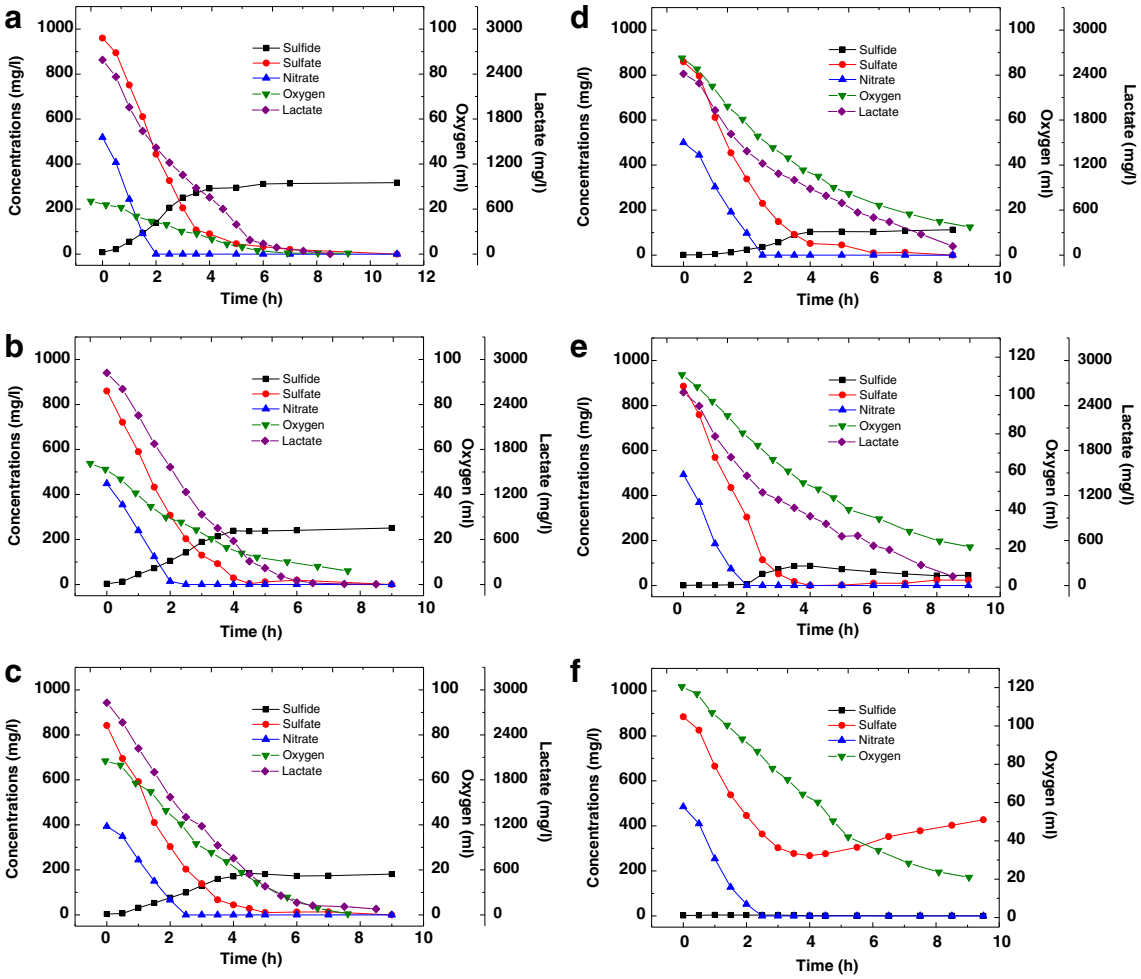

Fig. 3 Profiles of nitrate, sulfate, and sulfide concentrations in the batch tests with 500 mg/L, 1,000 mg/L sulfate, and various initial oxygen concentrations: **a** 20, **b** 40, **c** 60, **d** 80, **e** 100, and **f** 120 mL

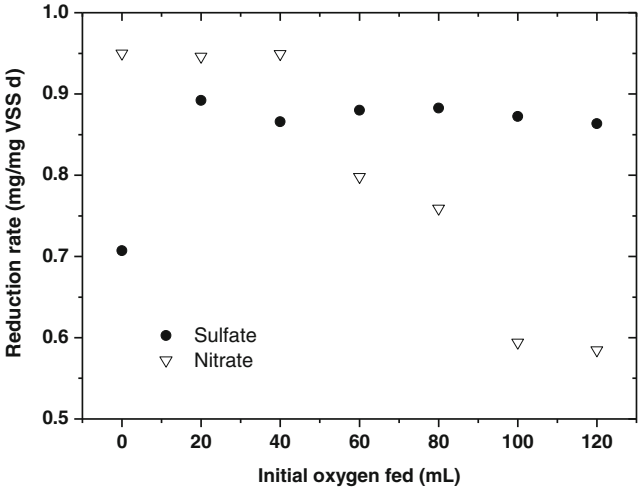

Fig. 4 Specific nitrate (*open triangles*) and sulfate (*full circles*) reduction rate determined in mixed-culture batch tests with the limited-oxygen fed

rate (Fig. 4). We would address this interpretation in the discussion section.

More importantly, the initial oxygen concentration held a strong impact on sulfide oxidation (Fig. 3). The level of sulfide oxidation increased with the increase in the initial oxygen concentration, reaching a maximal level of ~100 % with 100 mL of oxygen. During the sulfide oxidation phase, turbidity of the medium increased, and suspended particles were observed in the liquid. The concentration of thiosulfate, however, did not change significantly, an indication that sulfur (S^0) was the main metabolic product. The syntrophic relationship between SRB and SOB subject to limited oxygen observed in this study was in accordance with the findings reported by Xu et al. (2012), Celis-Garcia et al. (2008), Okabe et al. (2005), and van den Ende et al. (1997). The equation $2H_2S+O_2 \rightarrow 2S^0+2H_2O$ indicated that all sulfide could be converted to sulfur at oxygen fed of 20 mL or higher. The fact that only complete sulfide conversion to sulfur was observed at 100-mL oxygen suggested that besides sulfide oxidation, part of oxygen consumption was spent on lactate oxidation (Xu et al. 2013; Johnston and Voordouw 2012).

Kinetic parameters for nitrate and sulfate bio-reduction

Kinetic parameters for nitrate and sulfate bio-reduction were estimated based on Monod kinetic expressions incorporated with a competitive inhibition modifier to predict nitrate and sulfate's effect on specific reduction rates. It was assumed that the majority of the microbial population was responsible for nitrate and sulfate reduction and sulfide-oxidizing bacterial was few as described in "Influence of nitrate on sulfate reduction in mixed microbial culture". Since a mixed microbial culture was used, the estimated parameters represent average values, lumping the intrinsic kinetic constants for the individual pure strains composing the microbial community. The kinetic equations for nitrate, sulfate, and biomass, considering the inhibition model, can be defined by:

$$\frac{dS_s}{dt} = -q_{s,max}\frac{S_s}{S_s + K_s(1 + (S_n/K_n))}X \quad (1)$$

$$\frac{dS_n}{dt} = -q_{n,max}\frac{S_n}{S_n + K_n(1 + (S_s/K_s))}X \quad (2)$$

$$\frac{dX}{dt} = Y_s q_{s,max}\frac{S_s}{S_s + K_s(1 + (S_n/K_n))}X + Y_n q_{n,max}\frac{S_n}{S_n + K_n(1 + (S_s/K_s))}X - bX \quad (3)$$

where S is the substrate concentration (mg/L), t is the time (h), q_{max} is the maximum specific substrate utilization rate (mg S/mg VSS h), K is the half-saturation constant (mg/L), and X is the biomass concentration measured as mg VSS/L. Y is the yield coefficient (mg VSS/mg S), and b is the endogenous biomass decay rate (d^{-1}). The subscripts represent nitrate (n) and sulfate (s), respectively. In the present work, the cultures were well developed and adapted granular sludge, and the whole experiments lasted for a short period, and thus the biomass concentration was specially assumed to keep constant, proven to be reasonable by our measurements (Fig. 5).

The competitive inhibition model describes accurately both the nitrate and sulfate concentration decay profiles in all experiments (performed at five different initial nitrate concentrations). As an example, Fig. 5 illustrates the estimated concentration profiles by the model (full lines), with squared correlation coefficients (R^2) of 0.99 for both nitrate and sulfate for an experiment with initial concentrations of 1,000 mg/L of sulfate and 500 mg/L of nitrate.

The q_{max} value obtained for the mixed microbial cultures was 0.042±0.002 mg NO_3^-/(mg VSS h) for nitrate and 0.098 ±0.022 mg SO_4^{2-}/(mg VSS h) for sulfate due to a higher concentration of sulfate and non-limiting lactate in the biomedium both at all tests and microbial enrichment period. Furthermore, the $q_{s,max}$ values obtained were similar to values reported in the literature, which are typically of 0.1 mg SO_4^{2-}/(mg VSS h) (Moosa et al. 2002; Xu et al. 2013). This finding further proved that no obvious inhibition effect on sulfate reduction by nitrate was observed when lactate was always kept above the limiting conditions. The K value obtained was tenfold lower for nitrate (76.3±4.65 mg NO_3^-/L) than for sulfate (939.1±75.07 mg SO_4^{2-}/L), and the lower value of K_n compared to that of K_s indicated a higher affinity of the culture for nitrate. In a hydrogen-fed biofilms, Valencia et al.

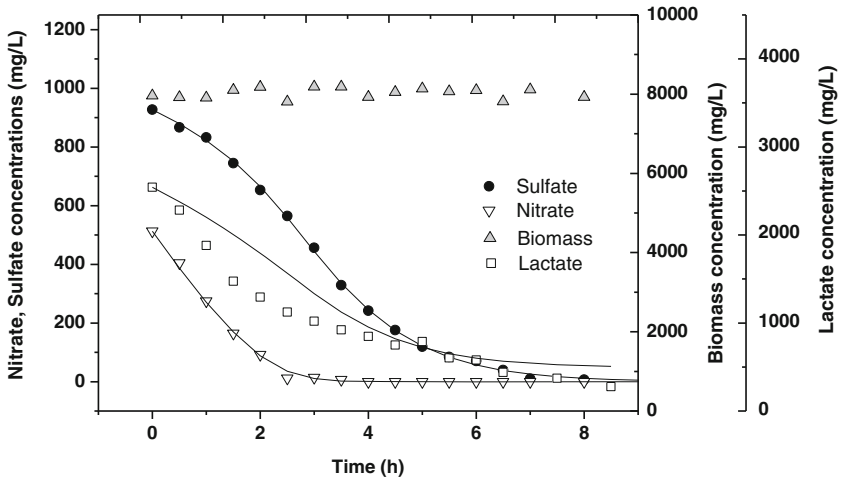

Fig. 5 A mixed-culture batch test for reduction of 500 nitrate and 1,000 mg/L sulfate. *Solid lines* represent estimated values using the competitive inhibition model

(2012) and Zhao et al. (2013) also observed that nitrate-reducing bacteria outcompeted for the electrons than sulfate reducing bacteria, suggesting a slight preference for this electron acceptor. This was explained by higher energy yield of denitrification over sulfate reduction and moreover slower grow rate by SRB. The value obtained for K_n by the mixed culture, 76.3 mg NO_3^-/L, is within the values reported in the literature: 1.12–155.43 mg NO_3^-/L (von Schulthess and Gujer 1996; An et al. 2011), and this deviation was probably caused by the different microbial communities and cultivation conditions, such as substrate type, nutrient concentration, operating conditions, and many factors. The estimated value of K_s (939.1 mg SO_4^{2-}/L) was relatively above the reported value in the literature. Studying the production of methane with livestock waste as the carbon source, Chen and Hashimoto (1980) found K_s value for bioreduction of sulfate to be varied between 18 and 33 mg SO_4^{2-}/L. Studying the reduction of sulfate by a mixed SRB culture in a continuous UASB, Visser (1995) found K_s to be 33 mg SO_4^{2-}/L for a granular biomass system and 18 mg SO_4^{2-}/L for a freely suspended cell system. Studying a pure culture of Desulfobacter postgatei in a continuous stirred tank reactor under sulfate limitation, Ingvorsen et al. (1984) found K_s to be 24 mg SO_4^{2-}/L. Studying a mixed culture of anaerobic bacteria containing acidogenic bacteria, methanogenic bacteria, and SRB, Moosa et al. (2002) found K_s to be 27–125 mg SO_4^{2-}/L. The obtained K_s value here indicated a lower sulfate affinity and a suppressed SRB activity by nitrate. The estimated $q_{n,max}$ value of 0.042 mg NO_3^-/(mg VSS h) is lower than the values obtained by Ni et al. (2011), An et al. (2011), and Vasiliadou et al. (2006), and this average value suggested that the VSS measured comprised not only nitrate-reducing bacteria but also other bacteria present in the mixed population. During the period of both oxy-anions reduction, a strong deviation from the nitrate and sulfate/lactate stoichiometric ratio was observed (Fig. 5). This suggested that other organisms, such as, e.g., fermentative bacteria, may be consuming lactate. Under anoxic conditions, fermentative bacteria may use lactate as a carbon source and electron acceptor to produce acetic acid via homoacetogenic reactions. In fact, acetic acid could be detected during the batch experiments, and a pH decrease could also be observed. As a consequence, the value of $q_{n,max}$ was underestimated.

Discussion

The inhibition of sulfate reduction by nitrate observed in this study is in agreement with the findings of other studies performed with pure and mixed microbial cultures (Zhao et al. 2013; Valencia et al. 2012; Tang et al. 2010; Ziv-El and Rittmann 2009; Kaster et al. 2007; Hubert and Voordouw 2007; Greene et al. 2003). Studying a mixed microbial culture, Valencia et al. (2012) observed complete inhibition of sulfate reduction by nitrate (in concentrations from 44 to 110 mg/L) since sulfate (with an initial concentration of 46 mg/L) reduction initiated only after complete nitrate reduction. Also, a few studies were conducted to investigate the onset of sulfate reduction in a membrane biofilm reactor (MBfR) for denitrification and concluded that sulfate reduction occurred when nitrate was almost completely reduced (Ziv-El and Rittmann 2009; Tang et al. 2010) and the same reduction pattern was also observed when bio-reduction of both nitrate and perchlorate (Gal et al. 2008; Ricardo et al. 2012); whereas in this study, we found that during the nitrate reduction phase, sulfate reduction also occurred at a relatively moderate rate which greatly differed from those reported by Ziv-El and Rittmann (2009) and Tang et al. (2010). No strictly sequential reduction but a slight decrease in the sulfate reduction rate due to the presence of nitrate was observed, and this case might be due to the non-limiting carbon source (lactate) and different microbial communities. The mechanism on the inhibition of sulfate reduction by nitrate was complex. Since sulfate reduction yields ~16 % of the energy of denitrification and SRB grow proportionally slower than do nitrate-reducing bacteria

(Thauer et al. 1977; Zumft 1992), nitrate reduction is thermodynamically more exergonic than sulfate reduction, and nitrate inhibiting sulfate reduction might be due to electron donor competition when limited (Zhang et al. 2008); whereas other studies showed that inhibition of sulfate reduction by nitrate was attributed to accumulation of denitrification intermediates, nitrite (Greene et al. 2003). On the other hand, Garcia-de-Lomas et al. (2007) observed a simultaneous reduction of both nitrate and sulfate and sulfide elimination in the water phase with nitrate stimulation of the indigenous NR-SOB rather than inhibition of the sulfate reduction activity. In the mixed cultures, this relationship is even more complex, since they may be composed of specialized NRB, SRB, and NR-SOB. Thus, NRB and SRB may compete for the common electron donor (lactate); meanwhile, NRB and NR-SOB may compete for the common electron acceptor (nitrate). Following this hypothesis, in the presence of both oxyanions and non-limiting lactate, a reduced number of SRB might have been responsible for the lower initial sulfate reduction rate, while after complete nitrate reduction, the majority of SRB started to use sulfate; thus, a maximal sulfate reduction rate was achieved. Nevertheless, during the whole process, NR-SOB was always not actively responsible for low sulfide oxidation.

During the last decades extensive literature on SRB indicates that the anaerobic sulfate-reducing bacteria are able to survive or even take advantage of the presence of molecular oxygen, which can be classified into three categories (Dannenberg et al. 1992): (i) Sulfate-reducing bacteria are to some extent O_2 tolerant, and they remain viable for hours or even days when exposed to O_2; (ii) sulfate-reducing bacteria are able to reduce sulfate in the presence of O_2, and sulfate reducers are found in high numbers near or within the anoxic/oxic interfaces in sediments; and (iii) sulfate-reducing bacteria can respire with O_2 coupled with utilization H_2, various organic compounds, and even sulfur compounds as electron donor, with rates comparable to those of aerobic bacteria, e.g., *Desulfovibrio desulfuricans*. Furthermore, anaerobic environment created by oxygen-mediated sulfide oxidation could favor SRB to function well in this study (Celis-Garcia et al. 2008). The inhibition of nitrate reduction by oxygen in this study was also reported by many other researchers (Plosz et al. 2003; Oh and Silverstein 1999; Kornaros and Lyberatos 1998; Stouthamer 1988; Tiedje 1988). Studying the factors that influence the deterioration in open anoxic reactor, Plosz et al. (2003) showed that oxygen entering an anoxic reactor through the surface may not just affect denitrification metabolically, but also kinetically, due to increased dissolved oxygen (DO) concentration exerting an inhibitory effect on the denitrification rate. In the work reported by Oh and Silverstein (1999), it was shown that mixed liquor DO as low as 0.09 mg/L was found to significantly inhibit denitrification, resulting in a rate decrease of 35 %. While some researchers have reported that pure strains of denitrifying bacteria grow simultaneously using both oxygen and nitrate electron acceptors (Robertson and Kuenen 1984; Robertson et al. 1988; Bell et al. 1990; Hooijmans et al. 1990; Patureau et al. 1994), oxygen appears to be available as an alternate and energetically preferable electron acceptor for facultative denitrifying bacteria and regulates synthesis of nitrate reductase enzyme and inhibits denitrification in pure cultures of facultative denitrifying bacteria so that substrate electrons flow to oxygen cytochromes (Stouthamer 1988; Wu et al. 1994). Therefore, oxygen may compete with nitrate for the same enzymes, resulting in a lower nitrate reduction rate.

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