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Sulfur-based mixotrophic denitrification corresponding to different electron donors and microbial profiling in anoxic fluidized-bed membrane bioreactors

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ABSTRACT

Sulfur-based mixotrophic denitrifying anoxic fluidized bed membrane bioreactors (AnFB-MBR) were developed for the treatment of nitrate-contaminated groundwater with minimized sulfate production. The nitrate removal rates obtained in the methanol- and ethanol-fed mixotrophic denitrifying AnFB-MBRs reached 1.44–3.84 g NO_3^-–N/$L_{reactor}$ d at a hydraulic retention time of 0.5 h, which were significantly superior to those reported in packed bed reactors. Compared to methanol, ethanol was found to be a more effective external carbon source for sulfur-based mixotrophic denitrification due to lower sulfate and total organic carbon concentrations in the effluent. Using pyrosequencing, the phylotypes of primary microbial groups in the reactor, including sulfur-oxidizing autotrophic denitrifiers, methanol- or ethanol-supported heterotrophic denitrifiers, were investigated in response to changes in electron donors. Principal component and heatmap analyses indicated that selection of electron donating substrates largely determined the microbial community structure. The abundance of *Thiobacillus* decreased from 45.1% in the sulfur-oxidizing autotrophic denitrifying reactor to 12.0% and 14.2% in sulfur-based methanol- and ethanol-fed mixotrophic denitrifying bioreactors, respectively. Heterotrophic *Methyloversatilis* and *Thauera* bacteria became more dominant in the mixotrophic denitrifying bioreactors, which were possibly responsible for the observed methanol- and ethanol-associated denitrification.

1. Introduction

Nitrate (NO_3^-) is a prevalent contaminant in groundwater. Pollution of drinking water with nitrate presents a serious health hazard because at concentrations higher than 10 mg N/L, nitrite formed may lead to methemoglobinemia or blue baby syndrome in infants and gastrointestinal cancer in adults (Fonseca et al., 2000; Ward et al., 2005). Groundwater polluted by nitrates typically contains almost no organic matter, thus sulfur-based autotrophic denitrification as illustrated in Eq. (1) has been reported to be an alternative for the removal of nitrate in contaminated drinking water (Sierra-Alvarez et al., 2007; Sahinkaya et al., 2011; Sahinkaya and Kilic, 2014).

$$55S^0 + 20CO_2 + 50NO_3^- + 38H_2O + 4NH_4^+ \rightarrow 4C_5H_7O_2N + 55SO_4^{2-} + 25N_2 + 64H^+ \quad (1)$$

To date, packed bed reactors have been predominantly employed for sulfur-based autotrophic denitrification (Sierra-Alvarez et al., 2007; Sahinkaya et al., 2011; Sahinkaya and Kilic, 2014; Sun and Nemati, 2012; Zhang and Zeng, 2006). However, such systems often suffer from mass transfer limitation and are hampered by low nitrate loading rates in full-scale applications. Moreover, post-treatment is generally required to remove sloughed biomass from the product water, resulting in additional treatment cost.

Fluidized bed bioreactor is considered to be a high-rate reactor configuration because it has good mass transfer characteristics (Kim et al., 2011). The current challenge for application of anaerobic fluidized bed bioreactors lies in effective solid liquid separation in

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order to control biomass retention as well as to improve effluent quality. In fact, this problem can be easily overcome by adopting membrane bioreactor (MBR). Recently, AnFB-MBRs have been considered as a suitable technology for handling dilute wastewater (Gao et al., 2014; McCurry et al., 2014; Sahinkaya et al., 2015). For example, lab-scale extractive and diffusive MBRs have been applied for the heterotrophic/autotrophic denitrification of drinking water (McAdam and Judd, 2007; Zhao et al., 2013), whereas a sulfur-oxidizing autotrophic denitrifying AnFB-MBR had also been developed for the treatment of nitrate-contaminated drinking water (Zhang et al., 2015).

In all sulfur-oxidizing autotrophic denitrification processes, sulfate formation leads to high sulfate content in treated effluent (Sahinkaya and Kilic, 2014). The allowable limit of sulfate for drinking water set by the US EPA is 250 mg/L (Oh et al., 2001). Theoretically, around 33 mg/L NO_3^-–N could be denitrified without exceeding the above limit if water does not contain background sulfate (Sahinkaya et al., 2011). So, for higher nitrate concentrations, the control of excess sulfate production is a serious challenge. The mixotrophic process combining autotrophic and heterotrophic denitrification is an effective strategy to control sulfate formation since heterotrophic denitrifiers share fractions of autotrophic denitrification. Liu et al. (2009) utilized separate reactors for heterotrophic and sulfur-based autotrophic processes, which might increase process costs. Sahinkaya and Kilic (2014) reported that simultaneous sulfur-based autotrophic and heterotrophic denitrification could be achieved in a packed bed reactor to decrease sulfate production for drinking water treatment. Although methanol has been most commonly used in the previous studies (Sahinkaya et al., 2011; Sahinkaya and Kilic, 2014; Liu et al., 2009), mixotrophic processes have adopted alternative external carbon sources due to the increasing costs of methanol. Ethanol, though also expensive, can foster significantly higher denitrification rates (Lu and Chandran, 2010). However, to our knowledge sulfur-based mixotrophic denitrification established in AnFB-MBRs has not been reported so far, and the impact of specific carbon source on sulfate generation and denitrifying performance has received limited attention. For novel sulfur-based mixotrophic denitrifying processes, examining the microbial community structures is necessary to understand the complex interactions occurring in mixotrophic denitrification and find ways to improve the design and operation of scale-up systems. However, the co-existence of both heterotrophs and autotrophs in the microbial community makes the optimization and operation of such a process much more difficult. A mechanistic understanding of denitrifying microbial fractions in the sulfur-based mixotrophic denitrification is lacking.

Here, we studied sulfur-based mixotrophic denitrification with different electron donating substrates in a novel AnFB-MBR system to achieve complete nitrate removal and reduce sulfate generation. The objectives of this study were to: (1) compare the performance of methanol- and ethanol-fed sulfur-based mixotrophic denitrification and sulfate production, and demonstrate the feasibility of the sulfur-based mixotrophic denitrifying AnFB-MBR system; and (2) provide insight into the microbial community composition to better predict how communities assemble in these ecosystems.

2. Materials and methods

2.1. AnFB-MBR setup and operation

The experimental setup was presented in Fig. S1 which is the same as reported by Zhang et al. (2015). In this study, two identical AnFB-MBR systems were operated under the same conditions except for external carbon. One reactor (R1) was operated with methanol supplement, and the other (R2) with ethanol addition. The enriched sulfur-oxidizing autotrophic denitrifying mixed culture was employed as the inoculum. Initially about 200 g of sulfur particles (50–200 μm) were added to AnFB-MBR as an electron donor. A top-up 50 g of sulfur particles was supplemented after 60 days of operation to maintain a relatively stable sulfur content in the AnFB-MBR. From day 31 onwards, methanol and ethanol as external carbon sources were introduced into R1 and R2, respectively. Detailed operational conditions of R1 and R2 are summarized in Table 1. Methanol and ethanol were supplemented according to about 40–200% of theoretical requirements of heterotrophic denitrification.

An overhead mixer with a rotating speed of 450 rpm was used to maintain fluidization of the sulfur particles. A liquid level indicator was connected to a peristaltic pump to maintain a constant water level at the top of the AnFB-MBR. Changes in the transmembrane pressure (TMP) were monitored with a vacuum pressure meter. The AnFB-MBRs were operated at 28 ± 3 °C. The composition of the feed solution can be found elsewhere (Zhang et al., 2015), which was tap water amended with the NO_3^- concentration as N, the PO_4^- concentration as P, the total alkalinity of the feed and trace element solution.

2.2. Continuous and batch experiments

The conversion of substrates was monitored periodically by measuring the influent concentrations of nitrate, and the effluent concentrations of nitrate, nitrite, sulfate, and total organic carbon (TOC). When the AnFB-MBR was continuously operated until TMP increased by 0.3 bar, the membrane module was taken out of the bioreactor and replaced with a clean membrane. The fouled membrane was soaked in 0.5% NaOCl solution overnight, followed by thorough flushing with deionized water.

The conversion of NO_3^-–N and N-balance of the sulfur-based mixotrophic denitrifying process were elucidated with a ^{15}N-tracer technique. The bath assays were conducted in glass serum flasks (500 mL) supplemented with 200 mL of medium free of NH_4^+–N. ^{15}N–NO_3^- expresses the nitrogen isotope ^{15}N of the nitrate (NO_3^-) as a sole nitrogen substrate. The mineral medium (pH 7.5) contained variable concentrations of ^{15}N-labeling potassium nitrate ($K^{15}NO_3$) with 99.7 atom% ^{15}N. The experiments were inoculated with 0.2 g SS/L of the denitrifying culture and elemental

Table 1
Operational conditions of the R1 and R2.

Operating periods	I			II	III	IV
Days	0–12	13–20	21–30	31–80	81–118	119–180
NO^{3-}–N (mg/L)	30	50	80	30	50	80
R1 Methanol (mg/L)[a]	0	0	0	32.0–124.0 (12.4–48.3)	120.9–129.1 (47.1–50.3)	123.2–419.6 (48.0–163.5)
R2 Ethanol (mg/L)[a]	0	0	0	23.7–92.1 (12.7–48.0)	90.1–100.5 (48.2–52.4)	97.7–316.0 (50.7–164.0)

[a] Values in parenthesis shows the methanol or ethanol concentrations as TOC.