Sulfur-based denitrification: Effect of biofilm development on denitrification fluxes

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ABSTRACT

Elemental sulfur (S^o) can serve as an electron donor for denitrification. However, the mechanisms and rates of S^o-based denitrification, which depend on a biofilm development on a solid S^o surface, are not well understood. We used completely-mixed reactors packed with S^o chips to systematically explore the behavior of S^o-based denitrification as a function of the bulk nitrate (NO_3^-) concentration and biofilm development. High-purity (99.5%) and agricultural-grade (90% purity) S^o chips were tested to explore differences in performance. NO_3^- fluxes followed a Monod-type relationship with the bulk NO_3^- concentration. For high-purity S^o, the maximum NO_3^- flux increased from 0.4 gN/m²-d at 21 days to 0.9 g N/m²-d at around 100 days, but then decreased to 0.65 gN/m²-d at 161 days. The apparent (extant) half-saturation constant for NO_3^- K_{Sapp}, based on the bulk NO_3^- concentration and NO_3^- fluxes into the biofilm, increased from 0.1 mgN/L at 21 days to 0.8 mgN/L at 161 days, reflecting the increasing mass transfer resistance as the biofilm thickness increased. Nitrite (NO_2^-) accumulation became significant at bulk NO_3^- concentration above 0.2 mgN/L. The behavior of the agricultural-grade S^o was very similar to the high-purity S^o. The kinetic behavior of S^o-based denitrification was consistent with substrate counter-diffusion, where the soluble sulfur species diffuse from the S^o particle into the base of the biofilm, while NO_3^- diffuses into the biofilm from the bulk. Initially, the fluxes were low due to biomass limitation (thin biofilms). As the biofilm thickness increased with time, the fluxes first increased, stabilized, and then decreased. The decrease was probably due to increasing diffusional resistance in the thick biofilm. Results suggest that fluxes comparable to heterotrophic biofilm processes can be achieved, but careful management of biofilm accumulation is important to maintain high fluxes.

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1. Introduction

Nitrate (NO_3^-) is a widespread environmental contaminant. It is commonly found in surface and ground waters, and municipal wastewater treatment can constitute major point sources of NO_3^- discharges (Howarth et al., 2002). An effective approach to remove NO_3^- is biological denitrification, where NO_3^- serves as an electron acceptor and is sequentially reduced to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O) and di-nitrogen gas (N_2) (Knowles, 1982; Zumft, 1992, 1997).

In many denitrification processes, an electron donor must be added (Ashok and Hait, 2015; Cherchi et al., 2009; Kapoor and Viraraghavan, 1998; Rivett et al., 2008; Zhu and Getting, 2012). Organic electron donors, such as methanol, ethanol and acetate, are commonly used in the wastewater field, but often are expensive, have handling concerns, may form disinfection by-products, and have high biomass yields (Zhu and Getting, 2012).

Inorganic electron donors, such as hydrogen gas, reduced sulfur species, and reduced iron species, can be advantageous (Ashok and Hait, 2015; Di Capua et al., 2015; Nerenberg et al., 2002; Shao et al., 2010; Till et al., 1998). Among inorganic donors, S^o has gained significant attention in recent years (Qambrani et al., 2015; Sahinkaya and Kilic, 2014; Simard et al., 2015; Zhang et al., 2015). S^o is a waste product from petroleum refineries, gas processors, metal smelters and coal and oil burning electric power plants (Kirk-Othmer, 2007). As such, it can be significantly less expensive, on a per-electron basis, than carbon sources such as methanol and acetate, and may be considered more sustainable (Park and Yoo, 2009). Additional benefits of S^o include the low biomass yield,

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safe handling, non-toxicity, and "on demand" availability without concerns of overdosing. In wastewater treatment, the most likely application of S^o is in tertiary denitrification filters, although it possibly also could be used in a fluidized bed reactor. Other applications, aside from wastewater treatment, include denitrification of potable water, permeable reactive barriers for groundwater treatment, and treatment of nitrate in headwater streams (Moon et al., 2004; Read-Daily et al., 2011; Sahinkaya and Dursun, 2012; Sierra-Alvarez et al., 2007; Soares, 2002).

The stoichiometry of S^o-based denitrification has been reported as follows (Batchelor and Lawrence, 1978):

$$NO_3^- + 1.1S^o + 0.4CO_2 + 0.76H_2 + 0.08NH_4^+ \rightarrow 0.5N_2 + 1.1SO_4^{2-}$$
$$+ 1.28H^+ + 0.08C_5H_7O_2N$$
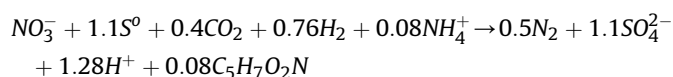
From the above, it can be seen that the process requires 2.5 gS^o/gNO$_3^-$ -N, has a relatively low yield (0.24 gCOD/gCOD), and consumes alkalinity.

S^o-based denitrification relies on specialized S^o-oxidizing bacteria (Baalsrud and Baalsrud, 1954; Fernandez et al., 2006; Sanchez et al., 2008; Shao et al., 2010; Soares, 2002), and S^o-based denitrification systems tend to be dominated by *Thiobacillus* species (Koenig et al., 2005; Moon et al., 2010; Shao et al., 2010; Zhang et al., 2015).

Under ambient conditions, S^o exists as a ring formed by eight sulfur atoms (S_8), forming a crystalline solid (Meyer, 1964). Many S_8 allotropes exist, but the most common is α-S_8, which is essentially insoluble in water (Meyer, 1964). Given its low solubility, α-S_8 is unlikely to sustain the observed microbial growth rates on S^o (Schauder and Kroger, 1993; Schauder and Muller, 1993). Some S^o-oxidizing bacteria are believed to produce extracellular enzymes that convert α-S_8 into soluble polysulfides, S_x^{2-}, which may diffuse into a biofilm (Boyd and Druschel, 2013; Franz et al., 2007; Kamyshny, 2009). Nano-crystalline S_8 could also be an intermediate formed from S_x^{2-} (Boyd and Druschel, 2013). Other S^o-oxidizing bacteria are known to have membrane-bound enzymes that obtain electrons via direct contact with the S^o surface, incorporating them directly into the cell (Rohwerder and Sand, 2003; Suzuki, 1999).

The mechanism of electron transfer is important, as it can impact the biofilm development and kinetic behavior. If electron transfer is limited to bacteria that are in direct contact with S^o, increasing the biofilm thickness will not increase denitrification fluxes (NO$_3^-$ removal rates per unit area of S^o). However, if soluble sulfur species diffuse into the biofilm, increasing the biofilm thickness would provide more active biomass. This would allow greater NO$_3^-$ fluxes.

Biofilms growing on S^o are "counter-diffusional," where the donor and acceptor diffuse into the biofilms from opposite sides (Fig. 1). The kinetic behavior of counter-diffusional biofilms is different from conventional, co-diffusional biofilms (Martin and Nerenberg, 2012). For example, for a given bulk substrate concentration, substrate fluxes are initially biomass limited, due to low initial biofilm thicknesses. Fluxes increase as the biofilm thickness increases, but eventually decline when the thickness becomes excessive. The decline occurs due to diffusional limitation, as the donor and acceptor penetrate the biofilm from opposite sides, and therefore have longer diffusion distances and therefore lower concentrations when they meet (Essila et al., 2000; Martin and Nerenberg, 2012).

As mentioned above, it is not clear what form of sulfur (e.g., α-S_8, polysulfides, nano-crystalline S_8) is the actual electron donor. It also is not clear whether soluble sulfur is only available to bacteria at the S^o surface, or if it diffuses into the biofilm. This uncertainty, and the complexity of counter-diffusion, explains why, despite decades of research, the kinetics of S^o-based denitrification remain poorly characterized compared to other electron donors.

Most previous studies on the kinetics of S^o-oxidizing bacteria provide specific rates of NO$_3^-$ reduction without considering biofilm development or potential donor limitation (Koenig and Liu, 2001; Sierra-Alvarez et al., 2007; Zeng and Zhang, 2005). Also, only a few studies explicitly addressed S^o denitrification as a biofilm process, mostly using empirical half-order rate expression to link NO$_3^-$ concentrations to denitrification rates, without considering biofilm thickness (Darbi and Viraraghavan, 2003; Koenig and Liu, 1996, 2001; Qambrani et al., 2015). One study identified NO$_3^-$ and S^o counter diffusion, but without identifying the actual soluble sulfur species or its degree of rate limitation (Batchelor and Lawrence, 1978).

Given that the mechanisms of electron transfer from S^o are not well established, the best approach may be to characterize denitrification rates as a function of biofilm development and NO$_3^-$ concentration. As a biofilm process, the S^o-based denitrification rate should be linked to the S^o surface area rather than S^o concentration (Sierra-Alvarez et al., 2007). It should vary with both NO$_3^-$ concentration and biofilm thickness. By assessing denitrification fluxes, i.e., removal rates per unit biofilm attachment area, rather than volumetric removal rates, it is easier to compare results from different studies. Relating fluxes to the NO$_3^-$ concentration is important for assessing bioreactors (e.g., plug-flow reactors or sequencing batch reactors) where the NO$_3^-$ concentration varies with position, time, or both.

In this study, we used reactors packed with S^o-chips to explore NO$_3^-$ reduction fluxes as a function of bulk NO$_3^-$ concentrations for different stages of biofilm development, for both high-purity and agricultural-grade S^o. Effluent recirculation was used to ensure the entire packed bed experienced similar NO$_3^-$ concentrations (Rittmann et al., 1986).

2. Material and methods

2.1. Reactor set-up

Two packed-bed, completely mixed column reactors were used in this study (Fig. 2). Each had an inside diameter of 2.5 cm and a total volume of 90 ml. Each was packed with 43 g of S^o chips. The chips were 1–2 mm thick and 3–5 mm in maximum length. The S^o surface area in the reactors was determined by measuring the dimensions of around 200 sulfur chips, measuring their aggregate weight, and relating this area to total weight of S^o in each reactor. The calculated S^o surface area per reactor was 0.05 m², and the specific surface area was 1051 m²/m³. Reactor 1 used industrial-grade S^o with 99.5%-purity (Georgia Gulf Sulfur Corporation, Valdosta, GA), while Reactor 2 used agricultural-grade S^o with 90%-purity, which was purchased from a local agricultural supply store. The reactors were operated in upflow mode and supplied with a NO$_3^-$-amended minimal medium. For long-term operation, the feed rate was 1.5 ml/min and was supplied using a peristaltic pump (Manostat Carter, Barnant Company, Barrington, IL). The steady-state hydraulic retention time (HRT) was 60 min, based on the S^o pore volume and free reactor volume (tubing volume and reactor volumes without S^o). Recirculation was provided at 60 ml/min by a peristaltic pump (Masterflex Cole-Palmer, Vernon Hills, IL). This provided a recirculation ratio (recirculation flow/influent flow) of 40, resulting in completely mixed conditions within the reactors (Rittmann et al., 1986). The recirculation flow provided a contact time and linear velocity that were somewhat higher than for a real application. The contact time (total flow/S^o volume) was around 3 min, and the linear flow velocity (total flow rate/net cross-sectional area for flow) was approximately 150 m/h. The flow-