



Evaluating simultaneous chromate and nitrate reduction during microbial denitrification processes

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ABSTRACT

Sulfur-based autotrophic denitrification and heterotrophic denitrification have been demonstrated to be promising technological processes for simultaneous removal of nitrate (NO₃⁻) and chromate (Cr (VI)), two common contaminants in surface and ground waters. In this work, a mathematical model was developed to describe and evaluate the microbial and substrate interactions among sulfur oxidizing denitrifying organism, methanol-based heterotrophic denitrifiers and chromate reducing bacteria in the biofilm systems for simultaneous nitrate and chromate removal. The concomitant multiple chromate reduction pathways by these microbes were taken into account in this model. The validity of the model was tested using experimental data from three independent biofilm reactors under autotrophic, heterotrophic and mixotrophic conditions. The model sufficiently described the nitrate, chromate, methanol, and sulfate dynamics under varying conditions. The modeling results demonstrated the coexistence of sulfur-oxidizing denitrifying bacteria and heterotrophic denitrifying bacteria in the biofilm under mixotrophic conditions, with chromate reducing bacteria being outcompeted. The sulfur-oxidizing denitrifying bacteria substantially contributed to both nitrate and chromate reductions although heterotrophic denitrifying bacteria dominated in the biofilm. The mixotrophic denitrification could improve the tolerance of autotrophic denitrifying bacteria to Cr (VI) toxicity. Furthermore, HRT would play an important role in affecting the microbial distribution and system performance, with HRT of higher than 0.15 day being critical for a high level removal of nitrate and chromate (over 90%).

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1. Introduction

Nitrate (NO₃⁻) has become the most ubiquitous chemical contaminant in groundwater due to excessive utilization of nitrogenous fertilizer in agricultural activity and inappropriate disposal of untreated industrial waste (Spalding and Exner, 1993; Almasri and Kaluarachchi, 2004). The ingestion of nitrate contaminant can cause acute health problem involving methemoglobinemia in infants after nitrate transformation into nitrite and the formation of carcinogenic nitrosamines after reacting with secondary or tertiary amines (Della Rocca et al., 2007). The US Environmental Protection Agency's standard has set the maximum contaminant level of nitrate at 10 mg/L and the acute toxicity level at concentrations above 50 mg/L (Spalding and Exner, 1993).

Microbial denitrification, including heterotrophic denitrification

and autotrophic denitrification, serves to be one of the most promising and efficient technological processes for nitrate removal. Although heterotrophic denitrification has higher denitrification rate, it would generate excessive biomass and soluble microbial products that require subsequent treatment prior to water utilization (Shin and Cha, 2008). Another disadvantage of heterotrophic denitrification is the requirement of external organic compounds. Insufficient dosage of the organic matters would potentially cause nitrite accumulation, whilst excess addition may lead to residual organic matters in effluent (Nuhoglu et al., 2002; Jianping et al., 2003). Alternatively, autotrophic denitrification with elemental sulfur (Hashimoto et al., 1987; Kimura et al., 2002; Sierra-Alvarez et al., 2007; Sahinkaya and Dursun, 2015) and hydrogen gas (Häring and Conrad, 1991; Smith et al., 1994; Mansell and Schroeder, 2002) as the electron donor has been demonstrated to be able to remove nitrate contaminant with minimal biomass yield. Sahinkaya et al. (2014) successfully applied the sulfur-based autotrophic denitrifying technology for treatment of nitrifying activated sludge effluent in two pilot-scale reactors. For full-scale application,

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the sulfur-based autotrophic denitrification process can be implemented for post denitrification of nitrified domestic wastewater in small-scale wastewater treatment plants, with sulfur being present as solid particles in the system (Sahinkaya et al., 2014). However, the major disadvantages of this process are the produced sulfate and acid (Sahinkaya and Dursun, 2012). External alkalinity needs to be provided in order to maintain a neutralized pH condition. Liu et al. (2009) proposed a process coupling heterotrophic denitrification to sulfur-based autotrophic denitrification in a fluidized reactor for the nitrate removal, where the alkalinity generated by heterotrophic denitrifying bacteria met the alkalinity need of autotrophic denitrifying organisms.

The industrial activities of electroplating, wood preservation, and leather tanning lead to chromium contamination in soil and groundwater (Wielinga et al., 2001). Hexavalent chromium (Cr (VI)) and its trivalent chromium (Cr (III)) are the most dominant ions in environment among the wide range of valency states of chromium (from -4 to $+6$) (Cheung and Gu, 2007). Cr (VI) is highly soluble and mobile and of acute toxicity, mutagenicity and carcinogenicity in ecosystem, while Cr (III) has limited solubility at neutral pH and less toxicity (Somasundaram et al., 2009). Consequently, the effective detoxification of chromate lies largely in the reduction of chromate to less mobile trivalent form. Microbial reduction of chromate has been demonstrated using chromate reducing bacteria, iron reducing bacteria, sulphate reducing bacteria and sulfur reducing bacteria in previous studies (Fude et al., 1994; Wang and Shen, 1995; Smith and Gadd, 2000; Wielinga et al., 2001; Somasundaram et al., 2009; Sahinkaya et al., 2012).

Recently, Sahinkaya et al. (2013) found that the sulfur-based mixotrophic denitrification process with autotrophs growing on elemental sulfur and heterotrophs growing on methanol was able to remove nitrate and chromate simultaneously. The electron donors (methanol or ethanol) were found to affect the microbial community in sulfur-based mixotrophic denitrifying anoxic fluidized bed membrane bioreactors (Zhang et al., 2015). Sahinkaya and Kilic (2014) further characterized the effect of chromate loading on the sulfur-based autotrophic denitrification and heterotrophic denitrification in two separated column reactors, respectively and found that heterotrophs were more tolerant to chromate toxicity. As nitrate is a common co-contaminant in aquifer, nitrate and chromate may co-exist in drinking water resources (Chung et al., 2006). Therefore, the sulfur-based mixotrophic denitrification process for simultaneous removal of nitrate and chromate is of great significance towards groundwater remediation.

Mathematical modeling has been applied to predict contaminant removal during water treatment. A mathematical model was developed by Peng et al. (2015b) to provide significant insight into the chemical and microbial pathways for chromate reduction in a stirred-flow reactor containing iron reducing bacteria. The kinetics for autotrophic denitrification were evaluated in different systems with varying electron donors (i.e. sulfur or hydrogen gas) (Zeng and Zhang, 2005; Peng et al., 2015a), while heterotrophic denitrification was well-established in the widely applied IWA (International Water Association) Activated Sludge Models (ASMs) (Henze et al., 2000). Since the contaminants (i.e. nitrate and chromate) are usually coexisted in water environment, the findings on simultaneous nitrate and chromate removal in sulfur-based mixotrophic denitrifying biofilm are of great significance. However, none of the existing models are able to describe this process, which may hinder the future application of the sulfur-based mixotrophic denitrification in groundwater contaminant remediation. More efforts should be dedicated to mathematical modeling, which provides a powerful tool for gaining an in-depth understanding of the processes containing complex microbe and substrate interactions and also supports the design and optimization of such biological treatment

systems. Therefore, the aim of this work is to develop a comprehensive multi-species model to describe and evaluate the simultaneous removal of nitrate and chromate under autotrophic, heterotrophic and mixotrophic conditions, and provide insights into the dynamics of microbial distribution and chromate reduction pathways in the denitrification system. The validity of the model was tested using experimental data from literature from three independent biofilm systems under different conditions.

2. Materials and methods

2.1. Model development

A mathematical model is developed to describe all relevant biological reactions of sulfur oxidizing autotrophic denitrifiers (AD), heterotrophic denitrifiers (HD) and chromate reducing organisms (CR), in terms of the removal of nitrate and chemical oxygen demand (COD) and reduction of Cr (VI) as well as sulfate production. The definitions of model components are listed in Table S1 in Supplementary Material. The kinetics and stoichiometry of the developed model are summarized in Tables S2 and S3 in the Supplementary Material. Both growth and decay processes are considered for each microbial species. Kinetic control of all the enzymatic reaction rates is described by the Michaelis–Menten equation. The rate of each reaction is modeled by an explicit function of the concentrations of all substrates involved in the biological reaction. A multispecies and multisubstrate one-dimension biofilm model is then constructed through employing the software AQUASIM 2.1d (Reichert, 1998) to simulate these bioconversion processes and microbial community structure for simultaneous nitrate and chromate reduction in biofilm reactors.

The inhibition of chromate on the activities of sulfur oxidizing denitrifying bacteria, heterotrophic denitrifying bacteria and Cr (VI) reducing bacteria is also included in the model through incorporating non-competitive inhibition functions of chromate ($\frac{K_i}{K_i + S_{Cr(VI)}}$) into the corresponding kinetic rate expressions (Table S3 in the Supplementary Material) (Somasundaram et al., 2009; Sahinkaya and Kilic, 2014). Nitrate removal is considered through two biological processes (Tables S2 and S3), namely, anoxic growth of AD (Process 1 in Tables S2 and S3) and anoxic growth of HD (Process 5 in Tables S2 and S3) with sulfur and COD as the electron donor, respectively. Each process is modeled as one-step denitrification process from NO_3^- to N_2 with individual reaction-specific kinetics. The kinetic equations of AD and HD growth on nitrate reduction are provided in Eq. (1) and Eq. (2) as below. The definitions and values for equation components can be found in Tables S1 and S4.

$$r_{A,NO3} = u_{A,1} \frac{S_{NO3}}{S_{NO3} + K_{NO3}^{AD}} \frac{X_S}{X_S + K_S^{AD}} \frac{K_{I,1}}{K_{I,1} + S_{Cr(VI)}} X_{AD} \quad (1)$$

$$r_{H,NO3} = u_{H,1} \frac{S_{NO3}}{S_{NO3} + K_{NO3}^{HD}} \frac{S_{COD}}{S_{COD} + K_{COD}^{HD}} \frac{K_{I,2}}{K_{I,2} + S_{Cr(VI)}} X_{HD} \quad (2)$$

Sahinkaya and Kilic (2014) clearly demonstrated that both sulfur oxidizing denitrifying bacteria and heterotrophic denitrifying bacteria contribute to Cr (VI) reduction, which has been incorporated into the model matrix as Process 2 and Process 6 in Table S2. Since chromate reduction provides much less free energy per electron than nitrate and stimulate negligible biomass growth (Chung et al., 2006), biomass yield during the sulfur-based Cr (VI) reduction (Process 2 in Table S2) and the COD-based Cr (VI) reduction (Process 6 in Table S2) are not considered. Cr (VI) reducing bacteria have been detected in sulfur-based mixotrophic denitrifying culture (Sahinkaya et al., 2013). Thus, the developed model also integrates

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