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High-rate autotrophic denitrification in a fluidized-bed reactor at psychrophilic temperatures

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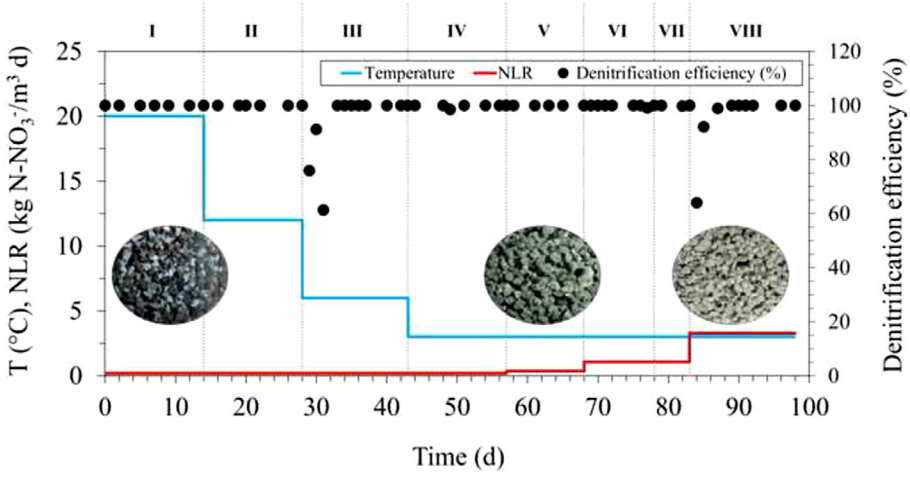
HIGHLIGHTS

- Sulfur-based denitrification was investigated in FBR at psychrophilic temperatures.
- Complete denitrification was maintained at 3 °C and NLR of 3.3 kg N-NO_3^-/m^3 d.
- Low temperature operation increased the effluent DOC and promoted bed expansion.
- *Thiobacillus* species dominated the FBR biofilm at all temperatures investigated.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, high-rate autotrophic denitrification driven by thiosulfate ($S_2O_3^{2-}$) was maintained under psychrophilic conditions in a lab-scale fluidized-bed reactor (FBR) with a *Thiobacillus*-dominated biofilm. The temperature effects on the denitrifying performance of the FBR were monitored by gradually decreasing the temperature from 20 to 3 °C. The potential of the FBR biofilm to maintain thiosulfate-driven denitrification at 3 °C was further investigated at different HRTs (5.4, 3 and 1 h) and influent NO_3^- concentrations (200, 600 and 1078 mg/L), resulting in a gradual increase of the nitrogen loading rate (NLR) from 0.20 to 3.3 kg N-NO_3^-/m^3 d. Complete thiosulfate-driven denitrification could be maintained at all temperatures, HRTs and influent NO_3^- concentrations tested. PCR-DGGE analysis revealed the dominance of the sulfur-oxidizing chemolithotrophs *T. denitrificans* and *T. thioparus* at all temperatures investigated. The FBR operation at a temperature as low as 3 °C promoted bed expansion and increased the dissolved organic carbon (DOC) concentration in the effluent, but had no significant effects on the denitrification efficiency. The findings of this study are highly significant for the treatment of cold nitrogen-contaminated waters poor in organics and confirm the FBR as a robust and powerful bioreactor system for autotrophic denitrification.

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1. Introduction

Autotrophic denitrification is a promising, clean and cost-effective treatment method for nitrogen removal from drinking water [1] and industrial effluents [2]. Up to date, most of the lab-, pilot- and full-scale biological systems performing autotrophic denitrification have been operated in the 20–30 °C temperature range with reduced sulfur compounds or H_2 as electron donor [3]. Operational temperatures are usually chosen to enhance bacterial growth and denitrification kinetics. The operation of high-efficiency biological systems at psychrophilic temperatures (<20 °C) is of major importance to minimize the energy consumption and reduce capital and operational costs, as many waters have naturally low temperatures.

Temperature is a key parameter affecting the performance of denitrifying systems at different scales. A significant decrease (50–70%) in the efficiency of sulfur-based denitrification was observed when temperature decreased from 20–25 °C to 5–10 °C both in lab [4] and pilot scale [5] applications. In cold and temperate regions, the temperature of groundwater and certain nitrate-contaminated wastewaters, e.g. mine waters, can be lower than 5 °C [6], resulting in extreme conditions for denitrifiers. Performing high-efficiency autotrophic denitrification at temperatures below 5 °C is a challenge that requires a robust engineered system. Biofilm reactors demonstrated to be reliable and high-performance systems for autotrophic denitrification with various electron donors [3]. In particular, the denitrification rates obtained with fluidized-bed reactors (FBRs) are among the highest observed for sulfur-driven denitrification [7–9].

In the past, sulfur-driven denitrification has been studied mainly in packed-bed reactors (PBRs) with granular elemental sulfur (S^0) both as electron donor and biofilm carrier and limestone ($CaCO_3$) as carbon source [3,10]. The so-called SLAD (sulfur-limestone autotrophic denitrification) process is an easy and cheap solution for denitrification, but results in several flaws, e.g. N_2 entrapment, clogging and channeling in the packed bed mainly due to biofouling [3]. Bed fluidization reduces biofouling and enhances the contact between biomass and substrate. FBRs have been used in previous studies on heterotrophic denitrification under harsh conditions such as low temperatures (7–8 °C), acidic influents (pH 2.5) and high heavy metal concentrations (5–500 mg Ni/L in the feed), resulting in high denitrification efficiencies [11–13]. In contrast, the number of studies on autotrophic denitrification in FBRs is exiguous. Recently, Zou et al. [8] studied thiosulfate-driven denitrification in two FBRs with *T. denitrificans*-dominated biofilms at 20 and 30 °C, respectively. The performance of the two FBRs was similar, resulting in high-efficiency and high-rate denitrification with the following stoichiometry [14]:

$$S_2O_3^{2-} + 1.16\ NO_3^- + 0.035\ CO_2 + 0.124\ H_2O + 0.519\ HCO_3^- + 0.110\ NH_4^+ \rightarrow 0.110\ C_5H_7O_2N + 0.578\ N_2 + 0.435\ H^+ + 2SO_4^{2-} \quad (1)$$

Moreover, in temperature gradient incubations performed in a range of 1–46 °C, denitrifying activity was observed even at 1 °C [8]. Up to date, sulfur-driven denitrification has never been investigated at temperatures below 5 °C in continuous reactors. The impact of low temperature on fluidized-bed sulfur-driven denitrification is, therefore, a topic of interest, given the lack of information and the potential benefits in terms of cost and energy savings. Investigating the effects of various nitrogen loadings and influent NO_3^- concentrations is also important to test the FBR reliability to sustain low-temperature denitrification and the response to short-term fluctuations of the operational parameters.

The present work aimed to study the effects of decreasing the operational temperature (from 20 to 3 °C) on continuous thiosulfate-driven denitrification in terms of NO_3^- and NO_2^- removal efficiency in a FBR with a *Thiobacillus*-dominated biofilm growing on granular activated carbon (GAC). In order to investigate the potential of the FBR to maintain high-efficiency denitrification at 3 °C, three different HRTs (5.4, 3 and 1 h) and influent NO_3^- concentrations (200, 600 and 1078 mg/L) were also tested, resulting in a stepwise increase of the nitrogen loading rate (NLR). The evolution of the microbial community during the FBR operation was monitored through polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE).

2. Materials and methods

2.1. FBR set-up

A lab-scale glass upflow FBR (580 mL) as described by Papirio et al. [11] was used to study the effects of psychrophilic temperatures on continuous thiosulfate-driven denitrification. GAC (Calgon Carbon Corporation, USA) with a particle size in the range of 0.5–1 mm was used as biomass carrier. The bed fluidization was adjusted to 25% by using a recycle flow rate of 800 mL/min.

2.2. Inoculum

The FBR was initially filled with a N_2-purged mineral medium [8] amended with $S_2O_3^{2-}$ and NO_3^- and inoculated with 58 mL (10% v/v) of the *T. denitrificans* culture previously cultivated in the batch flasks. During the first 57 operational days, the FBR was operated at 20 (±2)°C in fed-batch mode to allow the GAC colonization by *T. denitrificans* [8]. Continuous thiosulfate-driven denitrification was studied in the FBR for 488 days at 20 (±2)°C under different feed NLRs, HRTs [8] and pH values [9], maintaining the feed sulfur-to-nitrogen (S/N) ratio at 2.0. Subsequently, steady denitrification at 20 (±2)°C was maintained for 17 days prior to this study using a feed NO_3^- concentration of 200 mg/L, S/N ratio of 2.0, pH of 7.0 and an HRT of 5.4 h.

2.3. Synthetic influent composition

The synthetic influent (pH 7.0 ± 0.1) fed to the denitrifying FBR contained 200 mg/L of NO_3^- (as KNO_3), 368 mg/L of $S_2O_3^{2-}$ (as $Na_2S_2O_3\ 5H_2O$), 1 g/L of $NaHCO_3$ and nutrients in the following concentrations (g/L): KH_2PO_4 (0.20), NH_4Cl (0.10), $MgSO_4{\cdot}7H_2O$ (0.08) and micronutrients as reported by Zou et al. [8]. The influent was maintained in a refrigerator at 4 °C prior to feeding.

2.4. Experimental design

The denitrifying FBR was operated for 98 days at stepwise decreasing temperatures from 20 to 3 °C (Table 1). At 3 °C, the impacts of several HRTs, influent NO_3^- concentrations and NLRs on thiosulfate-driven denitrification were investigated (Table 1). The feed S/N ratio was maintained at 2.0 until the end of the study (day 98). During period I (days 0–14), the FBR was operated at 20 (±2)°C and HRT of 5.4 h to ensure that denitrification was stable. On day 14, the FBR was placed into a refrigerator with a controlled temperature (±1 °C) of 12 °C. The temperature was then stepwise decreased from 12 to 3 °C (days 28–43, periods II-IV) maintaining the same HRT and feed NO_3^- concentration (Table 1).

The temperature was maintained at 3 °C from day 43 until the end of the study (day 98). On days 57 and 68, the HRT was decreased to 3 and 1 h, respectively. On day 78, the feed NO_3^- concentration and the HRT were increased to 1078 mg/L and 5.4 h, respectively, in order to investigate the response of the FBR to sudden changes in the operational conditions (period VII). On day 83,