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Metabolic divergence in simultaneous biological removal of nitrate and sulfide for elemental sulfur production under temperature stress

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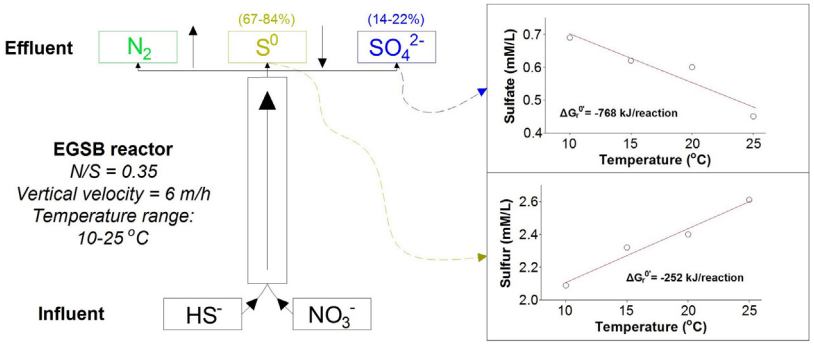
HIGHLIGHTS

- Simultaneous biological nitrate and sulfide removal at 25–10 °C was documented.
- Sulfide removal was from 98 (25 °C) to 89.2% (10 °C) with complete nitrate removal.
- Elemental sulfur yield ranged from 83.7% at 25 °C to 67% at 10 °C.
- Accumulation of elemental sulfur decreased with temperature.
- Metabolic shift at lower temperature was indicated by increased sulfate production.

GRAPHICAL ABSTRACT



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ABSTRACT

The simultaneous removal of NO_3^- and HS^- at temperature stress (25–10 °C) is evaluated here. An expanded granular sludge bed (EGSB) reactor was run over 120 days at N/S molar ratio of 0.35 (for S^0 production) under constant sulfur loading rate of 0.4 kg S/m^3 d. The simultaneous removal of NO_3^- and HS^-, was achieved at applied conditions. Average HS^--S removal varied from 98 (25 °C) to 89.2% at 10 °C, with almost complete NO_3^- removal. Average S^0 yield ranged from 83.7 at 25 °C to 67% at 10 °C. The temperature drop caused a decrease in granular sludge accumulated S^0 fraction by nearly 2.5 times. Decreased temperature caused metabolic pathway change observed as higher SO_4^{2-} production, apparently allowing the biomass to obtain more energy per HS^- consumed. It is hypothesized that the metabolic shift is a natural response to compensate for temperature-induced changes in energy requirements.

1. Introduction

Hydrogen sulfide (H_2S) is commonly present in biogas, septic tanks, domestic and industrial wastewaters. Wastewaters rich in organic matter and sulfate (SO_4^{2-}) are providing the ideal conditions for active growth of sulfate reducing bacteria (SRB), leading to H_2S production. Typically, domestic wastewater consists around 10 mg HS^-/L (Pikaar et al., 2011). H_2S is characterized by its adverse impact, even at low concentrations, such as corrosion, odor release and toxicity, thus, H_2S removal is deemed to be necessary (Reyes-Avila et al., 2004). Many methods have been employed for H_2S removal from both gas and liquid phase. Physicochemical methods are effective and widely used for this purpose, however at considerable energy and catalyst costs and great amount of produced sludge (Li et al., 2009).

Biological methods are attractive alternatives to physicochemical methods due to lower operational and environmental costs. Commercially applied biological methods for H_2S removal use mainly oxygen (O_2) as electron acceptor. Alternative electron acceptors for biological H_2S removal are nitrate (NO_3^-) and nitrite

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(NO_2^-) which can be easily added without system modifications. This property makes it easily applicable for sewer systems where NO_3^- or NO_2^- can be supplied at any place in the network (Auguet et al., 2016). NO_3^- and NO_2^- serve as electron acceptors for sulfide oxidizing bacteria (SOB) growth. Such impact of NO_2^- on H_2S production was highlighted already by Allen (1949) who found that the addition of nitrobenzene, dinitrobenzene or T.N.T. to sewage sludge inhibited H_2S production for prolonged periods. Autotrophic denitrification does not require organic substrates for bacterial growth so biomass production and operational costs are low (Sahinkaya and Dursun, 2012). Sulfide (HS^-) can be oxidized to elemental sulfur (S^0) and/or SO_4^{2-} depending on the relative presence of electron donor and acceptor as in Eqs. (1) and (2) (Kleerebezem and Mendez, 2002).

$$3HS^- + 3.9NO_3^- + 0.2NH_4^+ + HCO_3^- + 1.7H^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2} + 1.95N_2 + 3SO_4^{2-} + 2.3H_2O \quad (1)$$

$$14.5HS^- + 5NO_3^- + 0.2NH_4^+ + HCO_3^- + 20.3H^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2} + 2.5N_2 + 14.5S^0 + 17.4H_2O \quad (2)$$

Oxidation to S^0 can prevent the secondary pollution by SO_4^{2-}. NO_3^- and HS^- can be removed at their relative ratio corresponding to S^0 production according to Eq. (2). The combination of NO_3^- and O_2 as electron acceptors can enhance the S^0 production (Wang et al., 2015). S^0 obtained by such biological conversion is characterized by lower density and different crystalline structure than orthorhombic sulfur (Berg et al., 2014). Hydrophobic S^0 can be recovered by gravity sedimentation and its purity can be increased to above 99.9% (w/w) by applying a melting step (Janssen et al., 2001). Oxidation of HS^- to S^0 is convenient in terms of effluent quality and energy consumption since the lower amount of electron acceptor is used Eqs. (2) vs. (1). Biologically produced S^0 can be used as soil fertilizer or fungicide (Tan et al., 2016).

Factors evaluated in previous studies are C/N/S ratios, hydraulic retention time, load, pH, reactor configuration and stimulation of process startup (Guo et al., 2016; Huang et al., 2016; Mahmood et al., 2007; Montalvo et al., 2016; Reyes-Avila et al., 2004). However, temperature impact can be of utmost importance especially in cold climates. Sulfur-based denitrification under different temperatures has been evaluated in batch assays, where it showed that at 15 °C the denitrification efficiency ranged from 36 to 59% (Fajardo et al., 2014). Another study showed that a process operated at 15 °C can completely remove NO_3^- (Xu et al., 2016). Autotrophic denitrification driven by thiosulfate ($S_2O_3^{2-}$) even at 3 °C in a *Thiobacillus denitrificans* inoculated fluidized bed reactor has been reported (Di Capua et al., 2017). This report is a continuation of a preliminary study of temperature impact (Sposob et al., 2016), now with a longer experimental investigation and a thermodynamic evaluation of observations. Sposob et al. (2016) observed simultaneous NO_3^- and HS^- removal at 10 °C in a fluidized bed reactor.

In the present work, a laboratory-scale expanded granular sludge bed (EGSB) reactor was used to measure temperature effects on sulfide oxidation products distribution. The objective of this study was to evaluate the temperature impact (25–10 °C) on simultaneous NO_3^- and HS^- removal at N/S = 0.35 according to Eq. (2), constant sulfur loading rate of 0.4 kg S/m^3 d and continuous flow with focus on S^0 recovery.

2. Materials and methods

2.1. Inoculum and enrichment

The inoculum was taken from an up-flow anaerobic sludge blanket (UASB) methane generating reactor treating pulp and paper industry wastewater at Norske Skog Saugbrugs, Halden, Norway. The EGSB reactor was inoculated with 0.25 L of sludge, with a total solid content of 59.9 g/L and 86% organic fraction. The reactor was fed continuously with the same influent composition for one month at 25 ± 0.1 °C in order to condition and enrich the sludge prior to the experimental period reported here. The imposed lithoautotrophic conditions caused no methane production and the presence of sulfur components was observed during the conditioning stage.

2.2. Synthetic wastewater

The EGSB reactor synthetic feed contained $Na_2S{\cdot}9H_2O$ (3.12 mM S/L) with $NaHCO_3$ at concentration equivalent to Eq. (2). Potassium phosphate was used as buffer. Nitric acid (HNO_3) was used as a source of electron acceptor at N/S ratio of 0.35 according to Eq. (2). Electron acceptor feed contained the following stock solutions: (A) NH_4Cl (10 g/L), $MgCl_2{\cdot}6H_2O$ (10 g/L), $CaCl_2{\cdot}2H_2O$ (10 g/L); (B) K_2HPO_4 (300 g/L); (C) $MnSO_4{\cdot}H_2O$ (0.04 g/L), $FeSO_4{\cdot}7H_2O$ (2.7 g/L), $CuSO_4{\cdot}5H_2O$ (0.055 g/L), $NiCl_2{\cdot}6H_2O$ (0.1 g/L), $ZnSO_4{\cdot}7H_2O$ (0.088 g/L), $CoCl_2{\cdot}6H_2O$ (0.05 g/L), H_3BO_3 (0.05 g/L); (D) vitamin solution (Wolin et al., 1963), 10 times concentrated. HNO_3, stock solutions A (10 ml/L), B (2 ml/L), C (2 ml/L) and D (1 ml/L) were dissolved in distilled water. Electron donor ($Na_2S{\cdot}9H_2O$) and acceptor (HNO_3) were fed from separate bottles to prevent contamination and reactions in the feed bottles (Fig. 1).

2.3. Experimental setup

The laboratory-scale EGSB reactor was made of polycarbonate tube with an inner diameter of 32 mm and an effective height of 620 mm, giving a working volume of 0.5 L (Fig. 1). The reactor was equipped with a measurement tape (mm) for sludge bed height monitoring. Reactor temperature was maintained on the recirculation loop by cold plate cooler (TE Technology, Inc.). Four different temperatures (25, 20, 15 and 10 ± 0.1 °C) were tested under invariable influent composition. Temperature change was imposed when effluent composition reached pseudo-steady state.

Synthetic influent was introduced from 2 L influent vessels under nitrogen gas to avoid influent aging. Influent was pumped to reactor bottom at 2 L/d, equivalent to 6 h hydraulic retention time (HRT). Recycling pumping was set to reach 6 m/h vertical velocity necessary to expand the sludge bed. Reactor pH was maintained in the range 8.0–9.0 by a constant supply of potassium phosphate buffer and monitored by electrode (Hanna Instruments) on the recirculation loop (Fig. 1).

2.4. Analytical procedure

Effluent samples were collected daily and analysed immediately. Nitrate (NO_3^-), sulfate (SO_4^{2-}), sulfide (HS^-) and thiosulfate ($S_2O_3^{2-}$) in collected liquid samples (following 0.45 μm filtration) were measured by ion chromatography (Dionex ICS-5000). The concentration of HS^- (as HS^--S) was determined indirectly by potassium permanganate oxidation ($KMnO_4$). Sample separation and elution was performed using an IonPac AS11-HC 2 mm analytical column with potassium hydroxide (KOH) as eluent. Analysis started at 22 mM KOH, gradient started at 6 min, ramped up in 3 min to 45 mM and kept at this concentration for another 4 min. The data acquisition time is 13 min. The injection volume was 10 μl and the flow rate 0.3 ml/min.

2.5. Thiosulfate measurements

Measured effluent concentration of $S_2O_3^{2-}$ and SO_4^{2-} constituted a substantial fraction of the influent sulfur concentration. Applied