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Bioreactor performance and functional gene analysis of microbial community in a limited-oxygen fed bioreactor for co-reduction of sulfate and nitrate with high organic input

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HIGHLIGHTS

- Co-removal of nitrate and sulfate from high organic-laden wastewater was achieved.
- Limited-oxygen fed enhanced sulfur recovery, up to 70%.
- Functional genes of microbial community were analyzed at limited-oxygen conditions.
- Limited oxygen hold strong impact on sulfide-oxidizing genes (fccA/B, sox).

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ABSTRACT

Limited-oxygen mediated synergistic relationships between sulfate-reducing bacteria (SRB), nitrate-reducing bacteria (NRB) and sulfide-oxidizing bacteria (SOB, including nitrate-reducing, sulfide-oxidizing bacteria NR-SOB) were predicted to simultaneously remove contaminants of nitrate, sulfate and high COD, and eliminate sulfide generation. A lab-scale experiment was conducted to examine the impact of limited oxygen on these oxy-anions degradation, sulfide oxidation and associated microbial functional responses. In all scenarios tested, the reduction of both nitrate and sulfate was almost complete. When limited-oxygen was fed into bioreactors, S^0 formation was significantly improved up to ~70%. GeoChip 4.0, a functional gene microarray, was used to determine the microbial gene diversity and functional potential for nitrate and sulfate reduction, and sulfide oxidation. The diversity of the microbial community in bioreactors was increased with the feeding of limited oxygen. Whereas the intensities of the functional genes involved in sulfate reduction did not show a significant difference, the abundance of the detected denitrification genes decreased in limited oxygen samples. More importantly, sulfide-oxidizing bacteria may alter their populations/genes in response to limited oxygen potentially to function more effectively in sulfide oxidation, especially to elemental sulfur. The genes fccA/fccB from nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB), such as *Paracoccus denitrificans*, *Thiobacillus denitrificans*, *Beggiatoa sp.*, *Thiomicrospira sp.*, and *Thioalkalivibrio sp.*, were more abundant under limited-oxygen condition.

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1. Introduction

Nitrate (NO_3^-) contamination of surface and ground water is a relevant problem due to its health risk for methemoglobinemia in infants and spur eutrophication of water bodies. Agricultural runoff, wastewater discharges, and septic tanks are common sources of these contaminants [1]. The most common technology for nitrate removal from wastewater streams is microbial reduction, denitrification. Denitrification, the microbial reduction of NO_3^- to nitrite (NO_2^-) to form nitrogen gas (N_2), involves the stepwise reduction driven by a series of enzymes [2–4]:

$$NO_3^- \xrightarrow{NO_3^- reductase} NO_2^- \xrightarrow{NO_2^- reductase} NO \xrightarrow{NO\ reductase} N_2O \xrightarrow{N_2O\ reductase} N_2$$

Sulfate (SO_4^{2-}) is another respiratory electron acceptor commonly found in water and wastewater as a coexistent content of nitrate. The most common two-stage biological process for SO_4^{2-} removal is microbial SO_4^{2-} reduction to sulfide (S^{2-}) by sulfate-reducing bacteria (SRB) and S^{2-} oxidation to sulfur (S^0) by sulfide-oxidizing bacteria (SOB) or nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB). Concomitant SO_4^{2-} reduction and biological S^{2-} oxidation with limited oxygen in a single reactor has proven to be a promising and cost-effective alternative for remediating water contaminated with the compound, and a low dissolved oxygen (DO) concentration has been demonstrated to play an important role in the coexistence of SRB and SOB [5–7].

In addition, during co-reduction of NO_3^- and SO_4^{2-}, NO_3^- availability also increases the potential that S^{2-} oxidation will occur because nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB) such as *Thiomicrospira denitrificans* and some strains of *Thiomicrospira sp.*, *Thiobacillus sp.*, and *Acrobacter sp.* can oxidize S^{2-} with NO_3^- as electron acceptor [8–11]. The NR-SOB mediated biooxidation of S^{2-} (also termed denitrifying sulfide removal, DSR) has been studied extensively over a range of reactor operation and performance, reactor configurations, mechanism and modeling or microbial community [12]. However, banking on the fact that the majority of NR-SOB is chemolithotroph that uses sulfide as an electron donor and nitrate as an electron acceptor, heterotrophic nitrate-reducing bacteria (h-NRB) may out-compete NR-SOB for the common electron acceptor in the presence of high organic input [12]. Thus it is important to understand how to maintain the balance between the h-NRB and NR-SOB during the co-reduction of NO_3^- and SO_4^{2-} with high organic input to reduce sulfide generation as much as possible.

In this study, we evaluated S^{2-} elimination and S^0 production under co-reduction of nitrate and sulfate conditions in a bioreactor fed with limited oxygen. We applied a fixed O_2 supply rate to each bioreactor which was selected based on our previous batch results with which S^0 formation was significantly improved [13]. We hypothesized that effectively synergistic communities among SRB, h-NRB, and (NR-)SOB could be developed by controlling the O_2 supply rates to suppress sulfide generation. The performance of the limited O_2-fed bioreactor was compared to that of a control, keeping anaerobic condition during the experiments. In addition, the mass balance for SO_4^{2-}, S^0, and S^{2-} in reactors was examined to evaluate the development of the internal sulfur cycle. Here, we evaluated the interplay among O_2 fed, SO_4^{2-} reduction, NO_3^- reduction and S^{2-} oxidation. We also focused on how these performances factors are linked to the structure of the microbial community.

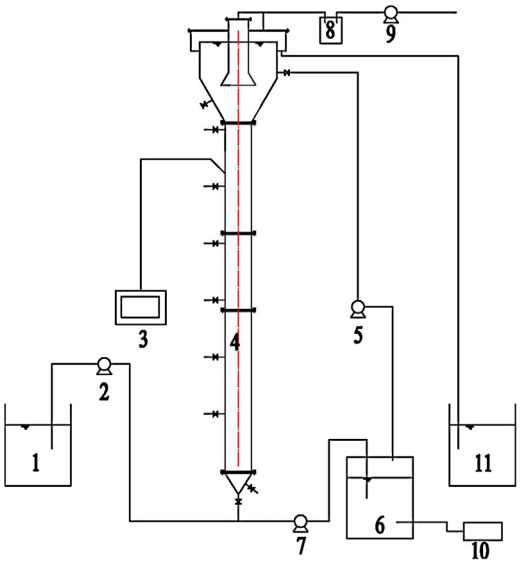

Fig. 1. Scheme of the expanded granule sludge bed (EGSB) reactor used in the experiments.

2. Materials and methods

2.1. Reactor setup and operational conditions

Experiments were conducted using expanded granular sludge bed (EGSB) reactors made of Perspex. The total volume was 4 L, with a working volume of 1 L. The reactors were insulated and the temperature was maintained at 30 °C via electric resistance heating. A gas-washing device collected the H_2S gas generated at the column top. Limited-oxygen condition was maintained using the regulated flow of air with a mass flow controller from an air cylinder, and air was injected into a separated aeration tank as previously described [7] (Fig. 1). Since gas–liquid mass transfer resistance exists, moderate stirring was required in the aeration tank to avoid air stripping as much as possible.

Two EGSB reactors were set up and inoculated with granule sludge from EGSB reactor operated by [14] for more than six months. The EGSB reactors were both operated in a continuous mode with an influent flow rate of 5.4 L/day and a recirculation rate of 54 L/day in each reactor for complete mixing of the liquid. For Reactor A, the substrates were sulfate and organic carbon (Table 1) and once the concentration of SO_4^{2-} and COD in the effluent reached a steady state (the variations of COD and SO_4^{2-} effluent concentrations were less than 10% over a minimum of three hydraulic retention times (HRT) and each steady state had a duration of a minimum of 20 days [1]), limited oxygen was fed into the reactor with a fixed O_2 supply rate of 0.5 ml min^{-1} $L_{reactor}{}^{-1}$. For Reactor B, all operating conditions were the same as Reactor A except for nitrate added to the influent and the oxygen was fed at 1.0 ml min^{-1} $L_{reactor}{}^{-1}$. In both reactors, organic carbon (lactate) was supplied in excess since relatively high organic input in wastewater. Recently, in certain environment nitrate has been shown to enhance sulfide bio-oxidation by NR-SOB [9]. Thus to clarify whether nitrate or limited-oxygen improved biological sulfide oxidation to S^0, in this study we also operated Reactor A.

The feed medium contained (g/L): Na_2SO_4 as S, 1.478; KNO_3 as N, 0.815; lactate as C, 5 ml/L; NH_4Cl, 0.575; $CaCl_2$, 0.070; $MgSO_4{\cdot}7H_2O$, 0.100; K_2HPO_4, 0.22; and 1 ml of trace solution [15]. The pH was adjusted to 8.0 ± 0.3 with bicarbonate. Before pumped into reactors, the feed medium was sparged with N_2 for 10 min to remove oxygen from aqueous phase.

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