Short Communication

Elemental sulfur formation and nitrogen removal from wastewaters by autotrophic denitrifiers and anammox bacteria

Chunshuang Liu, Dongfeng Zhao, Laihong Yan, Aijie Wang, Yingying Gu, Duu-Jong Lee

College of Chemical Engineering, China University of Petroleum, Qingdao 266580, China
State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology 150090, China
Department of Chemical Engineering, National Taiwan University, Taipei 10617, Taiwan
Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei 10607, Taiwan

Highlights

- Denitrifying ammonium oxidation (DEAMOX) reactor was tested.
- Elemental sulfur ($S_0$) was accumulated with nitrogen being removed as nitrogen gas.
- Autotrophic denitrifiers and anaerobic ammonium oxidation bacteria were grown.
- The sulfide to nitrate ratio of 1.31:1 could lead to excess accumulation of $S_0$.
- Alishewanella, Thauera and Candidatus Anammoximicrobium were the key strains.

Article Info

Article history:
Received 6 April 2015
Received in revised form 8 May 2015
Accepted 11 May 2015
Available online 19 May 2015

Keywords:
Autotrophic denitrifier
Anammox
Elemental sulfur
Sulfide

1. Introduction

Inorganic or limited-organic wastewaters with high levels of nitrogen and sulfur compounds are produced in mining industry, chemical industry and landfills (Matsuto et al., 2015). Nitrogen compounds contribute to eutrophication of water bodies, besides the risks associated with ammonia toxicity and offensive odors to humans. Reduced sulfur compounds, like sulfide, can be toxic to living being and corrosive to metals in acidic environments.

Biological processes are applied to remove nitrogen or sulfur pollutants from waters (Wang et al., 2009). Certain chemolithotrophic denitrifiers, such as Thiobacillus denitrificans, are capable of utilizing sulfide, thiosulfate or elemental sulfur ($S_0$) as electron donors and nitrate or nitrite as electron acceptors to drive denitrification reactions (Guo et al., 2013; Li et al., 2009; Xu et al., 2013; Yu et al., 2013). In particular, under specific conditions, the final products of denitrification reactions are $S_0$ and nitrite (Gevertz et al., 2000). At loading rates of 0.238 kg-S m$^{-3}$ d$^{-1}$ of $S_2$ and 0.093 kg-N m$^{-3}$ d$^{-1}$ of NO$_3$, Li et al. (2010) achieved complete conversions of sulfide to $S_0$ and nitrate to nitrite with activated sludge from biological filter:

$$S_2^+ + NO_3^- + H_2O \rightarrow S_0 + NO_2^- + 2OH^-$$ (1)

The denitrifying sulfide removal (DSR) process was proposed to incorporate Eq. (1) with heterotrophic denitrification (as follow) to achieve nitrite removal from wastewaters (Chen et al., 2008):

$$NO_3^- + COD \rightarrow N_2 + CO_2$$ (2)

Combining Eqs. (1) and (2) the end products are $S_0$ and $N_2$, ideal in view of wastewater treatment and resource recovery. Although quantity of carbon dioxide being produced is low, the DSR process acquires organic carbon for heterotrophic denitrification, not feasible for treating organics-deficient wastewaters.
Anaerobic ammonium oxidation (anammox) process reduces nitrite to nitrogen gas (N$_2$) via the following reaction:

$$\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + \text{H}_2\text{O} \quad (3)$$

Anammox has advantages over heterotrophic denitrification reactions by its high denitrification rate, low sludge yield, and no need of organics as carbon source (Ma et al., 2013). Kalyuzhnyi et al. (2006) and Kalyuzhnyi and Gladchenko (2009) achieved denitrifying ammonium oxidation (DEAMOX) by first reducing nitrate to nitrite with sulfide oxidation (to sulfate), and then remove nitrite via anammox reaction to $\text{N}_2$. The DEAMOX was proposed an effective process to remove nitrate and ammonia under sulfide-laden conditions. However, since the end product of sulfur compounds in DEAMOX was sulfate, which can be easily converted back to sulfide in the presence of organic substances, the DEAMOX process is not a comprehensive treatment for sulfide wastewaters.

This study tested the effects of sulfide to nitrate ratio on the end products of DEAMOX reaction, and proposed the optimal S/N ratio to maximize $S^0$ formation in the expense of nitrate and ammonium. The efforts reported herein are a step to practical applications of using DEAMOX for comprehensive treatment of limited-organic (S+N) wastewaters.

2. Methods

2.1. Reactor

The plexiglas expanded granular sludge bed (EGSB) reactor, a modified version of Chen et al. (2008), was 50 mm in diameter and 80 cm in height, giving a working volume of 1.57 L. Reactor was kept at $30 \pm 1^\circ C$. The influent was introduced at the column bottom by a peristaltic pump. A gas-washing device at column top collecting the $\text{H}_2\text{S}$ gas generated in experiments. Internal circulation from sedimentation region to column bottom gave reflux ratio of 6:1.

The reactor was seeded with 0.5 L mixed sludge, consisting of 0.25 L autotrophic denitrifying sludge collected from a UASB reactor and 0.25 L anammox sludge from a pilot EGSB reactor treating municipal wastewater (Ma et al., 2011). The autotrophic denitrifying sludge was acclimated at $30^\circ C$ for 30 days. Nitrite was added to the medium as sulfide source. The dissolved organics were used to prepare the sulfide solution was flushed by a molar ratio of 1:1 in the form of (NH$_4$)$_2$SO$_4$ and KNO$_3$. $333 \text{mg-N}_{\text{NO}_3^-}/\text{L}$ at $1 \text{ cm}^3$ min$^{-1}$ and sulfuric regeneration ($\text{H}_2\text{SO}_4 \text{25 mmol L}^{-1}$ at 5 cm$^3$ min$^{-1}$). The sulfide concentration was determined using the methylene blue method (APHA, 1998).

Sludge sample was collected from the EGSB reactor and immediately stored at $-80^\circ C$. Total genomic DNA of samples was extracted in duplicate using Power Soil DNA Isolation Kit (MoBio, Carlsbad, CA) according to the manufacturer’s instructions. The quality of the DNA extracted was examined by 1% (w/v) agarose gel electrophoresis and concentration measured with a UV–Vis spectrophotometer (NanoDrop 2000, USA). The V3–V4 region of the 16S rRNA gene was amplified using bacterial primers 338F (5’-ACT CCT ACG GGA GGC AGC AG-3’) and 806R (5’-GGA CTA CHV GGG TWT CTA AT-3’), with the reverse primer containing a 6 bp barcode used to tag each sample. PCR amplification was performed according to Chen et al. (2010). The purified amplicon was quantified using a QuantiFluor-STR Fluorometer (Promega, USA), and then a composite sequencing library was constructed by combining equimolar ratios of amplicons from all samples. The resulting library for pairedend sequencing (2 × 250 bp) was analyzed on an Illumina Miseq platform at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw sequence data of this study have been deposited to the NCBI Sequence Read Archive with accession No. SRP057217.

3. Results and discussion

3.1. Performance of the reactor

After inoculation, the synthetic wastewater fed at hydraulic retention time (HRT) of 12 h, pH 7.5 ± 0.2, and temperature of $30 \pm 1^\circ C$ for 22 days. Nitrate, sulfide and ammonium removals reached 99%, 99% and 97%, respectively, on day 10, 4 and 10 (Fig. 1a–c). Following the 23-day incubation, the concentrations of the influent were increased to $35 \text{ mg-NO}_3^-\text{N L}^{-1}$, $35 \text{ mg-NH}_4^-\text{N L}^{-1}$ and $105 \text{ mg-S}_2^2^-\text{L}^{-1}$ for another 23 days. At all feed concentrations were then increased (again) on day 47 to $45 \text{ mg-NO}_3^-\text{N L}^{-1}$, $45 \text{ mg-NH}_4^-\text{N L}^{-1}$ and $135 \text{ mg-S}_2^2^-\text{L}^{-1}$. On day 63, the HRT of the reactor was reduced from 12 h to 8 h to further increase the loading rates to 0.135 kg-N m$^{-2}$ d$^{-1}$, 0.135 kg-N m$^{-2}$ d$^{-1}$ and 0.405 kg-S m$^{-2}$ d$^{-1}$, respectively, for nitrate, ammonium and sulfide. The removal rates for sulfide and nitrate were >99%, and that for ammonium was >97%.

Granular and filamentous precipitates in yellow were observed in the reactor liquor and effluent (figure not shown). Then the sample was air dried, soaked in acids and then in alkali solutions, and then dissolved in carbon disulfide. Hexahydropyridine reacted with the dissolved solids to generate red products confirmed that the precipitate was $S^0$. Mass balance calculations based on quantities of sulfide, sulfate, and thiosulfate in the influent and effluent streams estimated the generation quantities of $S^0$. The calculation results revealed that conversion rates of $S^0$ were all >99% (Fig. 1c).

3.2. Effects of sulfide to nitrate ratio

At fixed ammonium to nitrate ratio of 1:1 and HRT of 12 h, four sulfide to nitrate ratios – 1.06:1, 1.12:1, 1.31:1, and 1.68:1 – were applied to the EGSB reactor. Restated, with both the nitrate loading...
rate and ammonium loading rate at 0.07 kg-N m$^{-3}$ d$^{-1}$, sulfide was loaded at 0.17, 0.18, 0.21 and 0.27 kg-S m$^{-3}$ d$^{-1}$ for stage I–IV, respectively, to the EGSB reactor.

At sulfide to nitrate ratios of 1.06:1 and 1.12:1, nitrate and ammonium removals were all >97% (Fig. 2a and b). The corresponding sulfide removals were both >99%; however, the S$^0$ conversions were only 92% and 95%, respectively (Fig. 2c). This occurrence may be attributable to the excess nitrate for converting sulfide to sulfate. At sulfide to nitrate ratio of 1.31:1, nitrate, sulfide and ammonium removal efficiencies were all >99%, with complete conversion to S$^0$.

At sulfide to nitrate ratio of 1.68:1, nitrate and ammonium removals remained at >99%. However, the sulfide removal efficiency was low (80.5%), leading to 80.0% of S$^0$ conversion. This occurrence should be attributable to insufficient nitrate supply for complete sulfide removal.

3.3. Microbial community

The phylogenetic classification of bacterial 16S rRNA sequence from the sludge sample on day 71 was illustrated based on phylum (Fig. 3a) and on genus (Fig. 3b). The bacterial sequences affiliated with Proteobacteria (60.2%) were the most abundant, followed by Planctomycetes (17.4%), Firmicutes (7.21%) and Chloroflexi (8.3%). The identified dominant genera in the reactor under steady-state operation were Alishewanella (34.2%), Thauera (22.0%) and Candidatus Anammoximicrobium (12.9%), which were common denitrifiers and anammox bacteria, respectively.

Alishewanella could use multiple electron acceptors for reducing nitrate to nitrite, which is likely one of the autotrophic denitrifier for the present system. Thauera were autotrophic denitrifiers in denitrifying sulfide removal process which could oxidize sulfide to S$^0$ and reduce nitrate to N$_2$ (Chen et al., 2009). Candidatus Anammoximicrobium is a typical anammox bacterium.

Co-working of Alishewanella, Thauera and Candidatus Anammoximicrobium are proposed to be the main functional strains for the studied DEAMOX process.

3.4. S$^0$ accumulation in DEAMOX process

The DEAMOX process tested in literature converted most sulfide to sulfate. van de Graaf et al. (1996) added sulfide to simulate anammox activity using nitrate and ammonium as substrates. Jin et al. (2013a) claimed that the activities of anammox bacteria were easily inhibited in the presence of 32 mg-S L$^{-1}$. The present study successfully operated the DEAMOX process to simultaneously convert 0.315 kg-S m$^{-3}$ d$^{-1}$ of S$^2_2$, 0.125 kg-N m$^{-3}$ d$^{-1}$ of nitrate, and 0.125 kg-N m$^{-3}$ d$^{-1}$ of ammonium to S$^0$ and N$_2$ without significant sulfide inhibition effects. Likely since the inoculated autotrophic denitrifiers could quickly convert sulfide to S$^0$, the sulfide inhibition effects on anammox bacteria were minimized.

The sulfide to nitrate ratio presented a key process parameter for the distributions of end products of sulfur. As sulfide to nitrate was increased from 1.06:1 to 1.31:1, the conversion of S$^0$ was increased to nearly complete. At higher sulfide to nitrate ratio, the conversion of S$^0$ was declined. The stoichiometry (S$^2_2$ + NO$_3$ $\rightarrow$ S$^0$ + NO$_2$ + NH$_4$ + N$_2$) said S$^2_2$ : NO$_3$ : NH$_4$ : N$_2$ = 1:1:1:1; the present study revealed that the optimal ratio was 1.31:1:1:1. Very likely since that the optimal nitrite to ammonium ratio was 1.30:1 in anammox reaction and 0.26 mol nitrate was produced when consuming 1 mol ammonium (Tang et al., 2011). To produce more nitrite needed in anammox reaction, excess sulfide was required in this study.

In DEAMOX test, Kalyuzhnyi et al. (2006) achieved preferable ammonium removal at influent S-H$_2$S/N-NO$_3$ $>$ 0.25 (>0.57 mgS/mgN) with most sulfide being converted to sulfate. In this test, at S-H$_2$S/N-NO$_3$ = 1.31, greater than 99% sulfide was converted to S$^0$. This occurrence can be interpreted by the following
The rate of oxidation of sulfide to S\textsuperscript{0} and reduction of nitrate to nitrite (r1) is higher than the oxidation rate of ammonium. The oxidation rate of S\textsuperscript{0} (r2) in order to reduce nitrite to N\textsubscript{2} is slower than the oxidation rate of ammonium (r3) for reducing nitrite to N\textsubscript{2} (see Fig. 4).

To minimize reaction (r2) to preventing excess oxidation of sulfide to sulfate and to have r2 < r3 are the essence to reach S\textsuperscript{0} formation in DEAMOX. The sulfide to nitrate ratio is shown a feasible way of manipulation of reaction rates r2 and r3 for preferred production of S\textsuperscript{0} by DEAMOX process. Further studies on possible inhibition by sulfide ions on anammox bacteria when handling high-strength sulfide-containing, organics-deficient wastewaters are recommended.

4. Conclusions

Elemental sulfur (S\textsuperscript{0}) formation and nitrogen removal were realized by DEAMOX process. The present reactor simultaneously convert 0.405 kg-S m\textsuperscript{-3} d\textsuperscript{-1} of S\textsuperscript{2-}, 0.135 kg-N m\textsuperscript{-3} d\textsuperscript{-1} of nitrate and 0.135 kg-N m\textsuperscript{-3} d\textsuperscript{-1} of ammonium to S\textsuperscript{0} and N\textsubscript{2} with insignificant
sulfide inhibition. The sludge acclimated in the reactor has *Alishewanella, Thauera* and *Candidatus Anammoximicrobium* respectively representing the autotrophic denitrifiers and anammox bacteria for the reactor. The sulfide to nitrate ratio was proposed to be a key process parameter for the distributions of end products of sulfur. A ratio of 1.31:1 was necessary for achieving high $S_0^+$ accumulation.

**Acknowledgements**

This research was supported by National Natural Science Foundation of China (Nos. 21307160 and 41201303), Natural Science Foundation of Shandong Province (ZR2013EEQ030) and Fundamental Research Funds for the Central Universities (R1404005A).

**References**


学霸图书馆
www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。
图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：
图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具