Simultaneous nitrite and ammonium production in an autotrophic partial denitrification and ammonification of wastewaters containing thiocyanate

Jianxin Pan, Chaohai Wei*, Bingbing Fu, Jingde Ma, Sergei Preis, Haizhen Wu, Shuang Zhu

* School of Environment and Energy, South China University of Technology, Guangzhou Higher Education Mega Centre, Guangzhou 510006, PR China

Abstract

Various products are observed in biological oxidation and reduction of molecules containing elements of variable valence. The variability is caused by the diversity of microorganisms and their metabolic enzymes, which may develop into novel processes in wastewater treatment. The study aimed to develop a novel denitrification process forming nitrite and ammonium in wastewaters containing thiocyanate. High-efficiency nitrite and ammonium production was observed due to autotrophic partial denitrification and ammonification as a result of nitrate and thiocyanate removal. Nitrite, ammonium and sulfate were observed as the ultimate products. The increased NO₃⁻-N/SCN⁻-N ratio in the treated wastewater resulted in the decreased removal efficiency of nitrate, and the increased nitrate-to-nitrite transformation ratio and the ratio of NO₂⁻-N to NH₄⁺-N. Thiocyanate sulfur was oxidized to sulfate via intermediate elementary sulfur providing electron to nitrate or nitrite. The *Thiobacillus* genus dominated in the sludge providing ammonium and nitrite as substrate for the potentially anammox process.

1. Introduction

Thiocyanate is used in a number of industrial processes, such as dyeing, herbicide and insecticide production, photofinishing, manufacture of thiourea, acrylic fiber production, mining and electroplating. Thiocyanate is often found as a contaminant in industrial wastewaters, for example, coal gasification effluents (Felsoeldi et al., 2010). Although thiocyanate is reported to be less toxic than its parental compound cyanide, it still poses a threat to the aquatic ecosystem being toxic to aquatic species. Meanwhile, thiocyanate is also a common source of...
COD and nitrogen, theoretically providing about 1.1 mg COD/mg SCN⁻ and 0.24 mg N/mg SCN⁻. Governments and international organizations have instituted safe release limits/guidelines for thiocyanate disposed to the receiving environment (Gould et al., 2012). Chemical and biological oxidation methods have been developed to remove SCN⁻ from wastewaters (Bezsudnova et al., 2007; Budaev et al., 2015), having the last ones considered to be the most cost-effective (Felfoldi et al., 2010).

Two distinct metabolic pathways were established in degradation of thiocyanate: (1) carbonyl pathway, in which thiocyanate is hydrolyzed to ammonium and carbonyl sulfide (COS) by thiocyanate hydrolase with COS further breaking to H₂S and CO₂; and (2) cyanate pathway, in which thiocyanate is initially degraded to cyanate (CNO⁻) and dihydrogen sulfide (H₂S) with further CNO⁻ hydrolysis to ammonium and CO₂ by cyanase (Bezsudnova et al., 2007; Ebbs, 2004). In both pathways, H₂S is converted to sulfate through biological oxidation using oxygen and nitrate as electron acceptors. Hence, with regard to these two pathways, their ultimate products are ammonium, sulfate and carbon dioxide formed in aerobic and anoxic degradation of thiocyanate (Broman et al., 2017; Staib and Lant, 2007).

Biological denitrification presents the means for nitrogen removal from wastewaters (Lu et al., 2014). Nitrite is often observed as an intermediate in denitrification. However, accumulation of high efficiency of nitrite was reported when organic substances and sulfides were used as electron donors (Cao et al., 2017; Liu et al., 2017). Nitrite was subsequently used in the anaerobic ammonium oxidation (anammox) process, an efficient and cost-effective nitrogen removal for complete denitrification (Ma et al., 2017). However, the authors failed to find reports on simultaneous efficient production of nitrite and ammonium in denitrification process for thiocyanate wastewater treatment.

The objective of the present study was to establish regularities of simultaneous nitrite and ammonium production in autotrophic denitrification process of the thiocyanate wastewater. The acclimated municipal sludge composition applied for the process was studied with detailed characterization of microbial communities. Batch experiments were performed to determine the effects of NO₃⁻/SCN⁻ nitrogen on nitrate and thiocyanate removal, nitrite and ammonium production and their ratio, and the total nitrogen (TN) removal. Sulfur conversion was also studied in the process. The potential of the process combination with subsequent anammox process was discussed.

2. Materials and methods

2.1. Synthetic wastewater

A synthetic wastewater containing total nitrogen (TN) in amount of 1000 mg N/L composed of NO₃⁻-N and SCN⁻-N was prepared. Sufficient amounts of nutrients and minerals were provided for microbial growth in concentrations of NaHCO₃ 2.00 g/L, KH₂PO₄ 0.01 g/L, MgSO₄·7H₂O, 0.40 g/L of CuSO₄·5H₂O, 0.05 g/L of NaMoO₄·2H₂O, 1.37 g/L of CaCl₂·2H₂O, 1.25 g/L of FeCl₃·6H₂O and 44.40 g/L of K₂HPO₄. Distilled water was used to prepare the synthetic wastewater and its dilution.

2.2. Acclimation and cultivation of sludge

The activated sludge from secondary sedimentation tank of the Lijiao Municipal Wastewater Treatment Plant (Guangzhou, China) was used as an inoculum of the autotrophic denitrification process. The sludge was acclimated in a 5-L reactor stirred continuously with a mechanical stirrer (Fig. 1) using the synthetic wastewater containing nitrate and thiocyanate (see Section 2.1) under anoxic conditions. The reactor was operated according to the cycle model composed of feeding (volume 2.5 L), stirring (time 23.5 h), settling (time 0.5 h) and decanting (volume 2.5 L). The time of feeding and decanting was negligible compared to its reaction time. The reactor was mixed through mechanical stirrer prior to 0.5 h of decanting operation and immediately after feeding. The volumetric exchange ratio of the reactor was about 50%, with the hydraulic retention time (HRT) of 48 h. After feeding, the initial pH was adjusted at 8.0 ± 0.1 by addition of 1.0-N HCl solution and the reactor was purged with nitrogen gas to reduce the dissolved oxygen (DO) concentration below 0.5 mg/L. Total nitrogen concentration of feeding solution was steadily increased from 130.0 to 520.0 mg N/L at a NO₃⁻/SCN⁻ nitrogen ratio of 1.6 and temperature of 35 °C. The acclimation history of the denitrification sludge was shown in Table 1. After 48 days, the denitrification rate remained constant in constant feeding solution. Therefore, a steady state for the acclimated sludge was confirmed. The sludge was cultivated continuously and then used as the stock for further experiments.

2.3. Batch tests

The batch tests were carried out using 1000-mL flasks. The sludge for each batch test was collected from the cultivated sludge reactor: the sludge was washed with phosphate buffer (140 mg/L KH₂PO₄ and 750 mg/L K₂HPO₄) and suspended in 1000 mL of synthetic wastewater. The NO₃⁻/SCN⁻ nitrogen starting ratios were set at 1.0, 1.5, 2.0 and 3.0 with the TN concentration of 100 mg N/L, initial pH 8.0 and the sludge concentration of 500 ± 50 mg/L. The samples were collected from the reactor in time until thiocyanate or nitrate was completely removed. The temperature of the reactors was kept constant at 35 °C, and constant mixing was performed with a magnetic stirrer. For each batch test, the reactor was purged with nitrogen gas by 5 min after each sampling in order to remove oxygen from the flask.

*Table 1*  Influent and effluent concentrations of SCN⁻-N, NO₃⁻-N, NO₂⁻-N, NH₄⁺-N and TN at the sludge acclimation phases.

<table>
<thead>
<tr>
<th>Phase 1 (day 0–11), mg N/L</th>
<th>Phase 2 (day 11–21), mg N/L</th>
<th>Phase 3 (day 21–47), mg N/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>Effluent</td>
<td>Influent</td>
</tr>
<tr>
<td>(day 11)</td>
<td>(day 21)</td>
<td>(day 21)</td>
</tr>
<tr>
<td>SCN⁻-N</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>NO₃⁻-N</td>
<td>80</td>
<td>160</td>
</tr>
<tr>
<td>NO₂⁻-N</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NH₄⁺-N</td>
<td>0</td>
<td>66.3</td>
</tr>
<tr>
<td>TN</td>
<td>130</td>
<td>68.4</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic diagram of the denitrification reactor.
2.4. Calculations

The nitrate-to-nitrite transformation ratio (NTR) and the TN removal efficiency (TNRE) were calculated using the Eqs. (1) and (2), respectively:

\[
\text{NTR} = \frac{C_{\text{initial}}(\text{NO}_3^- - \text{N}) - C_{\text{t}}(\text{NO}_3^- - \text{N})}{C_{\text{initial}}(\text{NO}_3^- - \text{N}) - C_{\text{initial}}(\text{NO}_2^- - \text{N})} \times 100\%
\]

(1)

where \(C_{\text{initial}}(\text{NO}_3^- - \text{N})\) and \(C_{\text{initial}}(\text{NO}_2^- - \text{N})\) are the initial \(\text{NO}_3^- - \text{N}\) and \(\text{NO}_2^- - \text{N}\) concentrations, respectively, and \(C_{\text{t}}(\text{NO}_3^- - \text{N})\) and \(C_{\text{t}}(\text{NO}_2^- - \text{N})\) the \(\text{NO}_3^- - \text{N}\) and \(\text{NO}_2^- - \text{N}\) concentrations at the sampling point, respectively, mg N/L.

\[
\text{TNRE} = \frac{C_{\text{initial}}(\text{TN}) - C_{\text{t}}(\text{TN})}{C_{\text{initial}}(\text{TN})} \times 100\%
\]

(2)

where \(C_{\text{initial}}(\text{TN})\) is the initial TN concentration, i.e. the sum of \(\text{SCN}^- - \text{N}\) and \(\text{NO}_3^- - \text{N}\) concentrations, \(C_{\text{t}}(\text{TN})\) the TN concentration at the sampling point, i.e. the sum of \(\text{SCN}^- - \text{N}, \text{NH}_4^+ - \text{N}, \text{NO}_2^- - \text{N}\) and \(\text{NO}_3^- - \text{N}\) concentrations, mg N/L.

2.5. DNA extraction, PCR and high-throughput sequencing

The sludge samples were collected from the acclimation reactor on day 0, 10, 20, and 48. The dynamics of microbial community structure was characterized using the Illumina high-throughput sequencing analysis. The sludge sample was freeze-dried in a lyophilizer (Beijing Songyuanhuaxing Technology Develop Co. Ltd., China). The DNA extraction was carried out from the dried sludge samples of 0.10 to 0.20 g with the MOBIO PowerSoil® DNA Isolation Kit (QBIOgene Inc., USA). The extracted DNA samples were stored at −4 °C. Concentrations of DNA were measured using a Nanodrop 2000 spectrophotometer (NanoDrop Technologies, USA).

Bacterial 16S rDNA were PCR-amplified with the forward primer 515F (5'-GTGCGCACGMCGCCGCGTAA-3') and the reverse primer 907R (5'-CGCTACATTCMTTTRAGTTT-3') for the V4 and V5 region (Zhong et al., 2017). The PCR reaction mixture containing 25 µL of 2x Premix Taq (Takara Biotechnology Dalian Co. Ltd., China), 1 µL of each primer (10 mM) and 60 ng of DNA template in a volume of 50 µL were amplified by thermo-cycling: 5 min at 94 °C for initialization, 30 cycles of 30 s denaturation at 94 °C, 30 s of annealing at 52 °C, and 30 s of extension at 72 °C, followed by 10 min of final elongation at 72 °C.

The DNA library was prepared using NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, USA) following manufacturer’s recommendations, and run on an IlluminaHiSeq2500 platform for sequencing (Sangon Tech, China). The read preprocessing, operational taxonomic unit (OTU) generation and identification, and statistical analyses were performed with QIIME. The taxonomic classification was assigned using the RDP Classifier. The OTUs were generated with an identity threshold of 97%. The major 16S rDNA sequences have been submitted to GenBank with the accession numbers: MF962816-MF962864.

2.6. Chemical analysis

The liquid samples collected during the experiments were filtered through a 0.45-µm membrane filter. Nitrate, nitrite, thiosulfate, sulfate and fluoride were measured using the ICS-900 ion chromatograph with a conductivity detector and an IC-AS16 analytical column (Dionex Co., USA). Thiocyanate was measured using a UV-vis spectrophotometer (UV-1800, Shimadzu, Japan) by the colorimetric method with ferric nitrate (Kim et al., 2011). Ammonium and the mixed liquor suspended solids (MLSS) were analyzed according to the Standard Methods (APHA, 2005). The solutions’ pH was determined with the PHS-3C pH-meter (Shanghai Precision & Scientific Instrument Co. Ltd., China). Dissolved oxygen was measured using the Oxi 3210 SET 3 m (WTW Company, Germany). The concentration of elementary sulfur was determined from the sulfur mass balance (Yang et al., 2016, Liu et al., 2017).

3. Results and discussion

3.1. Acclimation and performance of sludge

The performance of the denitrification reactor sludge during its acclimation was shown in Fig. 2. The acclimation operation was divided into three phases (phases 1, 2 and 3). Thiocyanate and nitrate were removed during all phases, which indicated a satisfactory work of the properly acclimated municipal wastewater treatment plant sludge.
Beneficially, TN was removed from aqueous solution without additional organic carbon supplement, which realized advantages of the autotrophic denitrification. After twenty-three days of acclimation, effluent concentration of TN reached a stable value of 310 ± 15.0 mg N/L (number of data points \( n = 24 \); mean ± SD).

The nitrite accumulation was observed at incomplete removal of nitrate in phases 2 and 3 showing the denitrification divided to two stages, the nitrate conversion to nitrite and the nitrite removal. The reduction rate of nitrate was higher than the one of nitrite, making the latter accumulating, thus witnessing the insufficiency of an electron donor for denitrification during the acclimation (Ge et al., 2012). Similar phenomenon was reported for sulfide and thiosulfate used as electron donors (Chung et al., 2014; Fajardo et al., 2012). After twenty-three days of sludge acclimation, concentrations of nitrate and nitrite in the effluent remained at 17 ± 3.0 (\( n = 24 \)) and 110 ± 10.0 mg N/L (\( n = 24 \)), respectively.

Formation of ammonium was detected in all acclimation phases. Formation of ammonium in stoichiometric quantities was also detected in aerobic degradation of thiocyanate (Lay-Son and Drakides, 2008). In anoxic reactor, ammonium concentration also increased with thiocyanate degradation (Broman et al., 2017). In the present research, however, the ammonium formation in the initial phase exceeded the quantities stoichiometrically expected from the thiocyanate transformation. \( \text{NH}_4^+ \)-N concentration of 75.2 ± 10.9 mg/L (\( n = 11 \)) in the effluent was higher than that of SCN\(^-\)-N, 50.0 mg N/L, in the influent. The excess ammonium in amount of 25.2 ± 10.9 mg N/L (\( n = 11 \)) might come from partial lysis of the sludge in the phase 1 (Qin et al., 2017). In the phase 3, ammonium production of 200 ± 5.0 mg N/L (\( n = 24 \)) became equimolar to the thiocyanate degradation of 200 mg SCN\(^-\)-N/L after twenty-three days of sludge acclimation.

The removal of thiocyanate, nitrate and TN and production of nitrite and ammonium kept constant levels after twenty-three days of sludge acclimation in constant feeding solution. These results indicated which the denitrification sludge forming nitrite and ammonium was acclimated successfully and the denitrification process achieved a constant level in wastewaters containing thiocyanate. The influent and effluent concentrations of thiocyanate, nitrate, nitrite, ammonium and TN were shown in Table 1.

### 3.2. Effect of \( \text{NO}_3^-\)-N/SCN\(^-\)-N ratio on nitrogen compounds conversion

Earlier publications reported the degree of nitrate reduction dependent on the initial ratio of an electron donor and nitrate as an electron acceptor, resulting in different nitrate removal path and electron donor utilization patterns (Huang et al., 2013; Mohan et al., 2016). Fig. 3 showed the effect of \( \text{NO}_3^-\)-N/SCN\(^-\)-N ratio on nitrogen compounds conversion. The \( \text{NO}_3^-\)-N/SCN\(^-\)-N ratios were varied to study the thiocyanate, nitrate and TN removal, ammonium and nitrite production, and the resultant ratios of nitrite to ammonium concentrations. The initial lag time of about 2 h was observed for the nitrate concentration remaining constant. Simultaneously, however, the decrease in thiocyanate concentration of about 5.0 mg N/L was observed. This might be explained by incomplete removal of oxygen with nitrogen gas stripping prior to the experiment inhibiting the reduction of nitrate: sludge utilized residual oxygen to oxidize thiocyanate at the initial stage as shown earlier (Annachhatre and Suktrakoolvait, 2001; Li et al., 2016). Concentrations of residual nitrate increased in the experiments with the increasing \( \text{NO}_3^-\)-N/SCN\(^-\)-N ratio, making 0, 2.8, 11.6 and 30.9 mg N/L at the ratios of 1.0, 1.5, 2.0 and 3.0, respectively. Thiocyanate was removed completely in all experiments. Although the complete removal of both nitrate and thiocyanate was observed at their ratio of 1.0, more nitrate (43%) was removed using thiocyanate and residual rate of nitrate was only 4.7% at ratio of 1.5. This result demonstrated that the ratio of 1.5 might be better suited than others for subsequent process.

Nitrite accumulation was observed in all tests. At the nitrate-to-thiocyanate ratio of 1.0, nitrite was not produced for the first 2 h, its concentration increased from 2 to 6 h reaching 37.7 mg N/L, and finally decreased to 22.1 mg N/L at times from 6 to 10 h. At the nitrate to thiocyanate ratios of 1.5, 2.0 and 3.0, the nitrite concentration increased from zero to a stable value with time, i.e. no nitrite reduction was observed. In all tests, the maximum nitrite concentrations were...
observed when the concentration of nitrate approached its minimum. These results demonstrated that nitrate was reduced prior to nitrite also observed earlier (Fajardo et al., 2012). The NTR value increased from 44.6 to 86.1% with the increasing nitrate-to-thiocyanate ratio (Fig. 4a). These results indicated that high efficiency production of nitrate could be achieved, which suggested the occurrence of partial autotrophic denitrification (Cao et al., 2017; Liu et al., 2017). The nitrite concentration increased from 22.1 to 46.1 mg N/L with the nitrate to thiocyanate ratio from 1.0 to 2.0, further decreasing to 37.4 mg N/L with the nitrate-to-thiocyanate ratio growth from 2.0 to 3.0 (Fig. 3). This result might be due to the combined effect of increasing NTR and decreasing initial electron donor concentration.

Ammonium was observed to be the ultimate product in all tests with its concentration increasing with time. Finally, ammonium concentrations of 47.4, 40.9, 33.2 and 23.6 mg N/L were achieved in initial thiocyanate concentrations of 49.5, 41.0, 33.9 and 24.0 mg N/L at the nitrate-to-thiocyanate ratios of 1.0, 1.5, 2.0 and 3.0, respectively. These concentrations were close to the amounts of thiocyanate degradation, which proved ammonium was originated from hydrolysis and ammonification of the thiocyanate without further ammonium removal in the experiments. This indicated that thiocyanate nitrogen did not provide electron to nitrate and nitrite reduction. These observations correlated with findings by Broman et al. (2017).

The TN removal efficiency decreased with the increased nitrate-to-thiocyanate ratio comprising 30.9, 11.1, 9.1 and 8.0%, respectively (Fig. 4b). The inefficiency was observed for the nitrate conversion to nitrite, whereas ammonium produced by thiocyanate was not removed. The ratio of NO$_3^-$-N to NH$_4^+$-N increased with the nitrate-to-thiocyanate ratio, comprising 0.47, 1.10, 1.39 and 1.58, respectively (Fig. 4c). This observation was attributed to the increasing NTR and reduced ammonium production.

Nitrite and ammonium were produced as the ultimate products thus making the process ineffective in TN removal. Ammonium and nitrite, however, present a substrate for the anammox process mentioned in Introduction (Nozhevnikova et al., 2012). Simultaneous thiocyanate, nitrate and TN removal in the process combined with the anammox process may be feasible in further ammonium and nitrite, and, thus, TN removal. For example, at the nitrate-to-thiocyanate ratio of 1.5, the resultant NO$_3^-$-N/NH$_4^+$-N ratio in the effluent was 1.10, which was close to the substrate composition in the anammox process (Tang et al., 2010) suggesting nitrite and ammonium accumulation being a prerequisite of an extended treatment alternative for thiocyanate containing wastewaters. Therefore, compared with nitrification-denitrification process, the process combining anammox and nitrification could remove more nitrogen with thiocyanate in wastewater, which might save organic carbon sources and energy. Moreover, compared with shortcut nitrification-anammox process, the process could achieve the simple control and stable production of NO$_2^-$-N and NH$_4^+$-N in wastewater, which avoided aerobic degradation of thiocyanate and shortcut nitrification for anammox, thus reducing operational complexity.

3.3. Effect of NO$_3^-$-N/SCN$^-$-N ratio on sulfur conversion

Sulfur of the thiocyanate molecule was considered as an electron donor for nitrate and nitrite reduction (Broman et al., 2017). Previous studies demonstrated sulfur, thiosulfate and sulfate being the intermediate products of sulfide oxidation (Xu et al., 2016; Yang et al., 2016). Intermediary products, however, were insufficiently studied in thiocyanate sulfur conversion through denitrification processes. Fig. 5 showed the effect of NO$_3^-$-N/SCN$^-$-N ratio on the sulfur-containing compounds conversion. The thiosulfate and sulfate intermediates were not detected. However, elemental sulfur intermediate was observed in all experiments: elemental sulfur concentration increased from 0 to 9.0 mg S/L within the first 3 h, disappearing within the next 3 h at the NO$_3^-$-N/SCN$^-$-N ratio of 2.0. Elemental sulfur accumulation might result from the difference in the rates of thiocyanate oxidation and production of sulfate. Compared to elemental sulfur, thiocyanate was readily used in the nitrate reduction. Depletion of thiocyanate was consistent with further transformation of elemental sulfur to sulfate. These results were similar to the ones reported by Xu et al. (2016). In the present research, the peak concentration of elemental sulfur decreased from 15.0 to 4.1 mg S/L with the nitrate-to-thiocyanate ratio increasing from 1.0 to 3.0, respectively, and the peak appeared earlier with the growing ratio. This might be explained by the reduced thiocyanate starting concentration with the increasing NO$_3^-$-N/SCN$^-$-N ratio resulting in the diminishing elemental sulfur accumulation. The accumulated elemental sulfur was completely oxidized to sulfate in nitrate or nitrite reduction. Ultimately, the SO$_4^{2-}$-S effluent concentrations were consistent with the influent SCN$^-$-S concentrations in all experiments. These results demonstrated that thiocyanate was oxidized to sulfate via elemental sulfur intermediate in the denitrification which resulted in nitrite and ammonium production.

These observations were similar with findings in sulfide oxidation through denitrification process (Xu et al., 2016). In short, the elemental sulfur was only intermediate and could not be recovered from the wastewater through denitrification process which devoted to production of nitrite and ammonium.

3.4. Microbial communities in the sludge

The sludge samples were collected from the acclimation reactor on day 0, 10, 20, and 48 for the analysis of the microbial community. We obtained approximately 19,149–37,852 effective sequences (Table 2) for each sample after removing low quality sequences and chimeras. Operation taxonomic units (OTU) were generated in amount of 808–1046 (Table 2) following normalizing the sequence number of each sample. The parameters related to the alpha diversity of microbial community in the samples are shown in Table 3. As shown in Table 3, the values of Ace, Chao and observed species presented decreasing trends as the acclimation time increased from 0 to 48 days, which demonstrated that the microbial community richness were weakened with acclimation time in the reactor. The decreasing Shannon index and PD whole tree numbers also illustrated the alpha-diversity decreasing as acclimation time going. The reduced diversity was further validated by Simpson index. However, Dominance index increased obviously from
0.014 to 0.408 with acclimation time from 0 to 48 days, which demonstrated that the key bacteria was selected and enriched over time. Overall, these results suggested that decreasing of richness and diversity and the selection and enrichment of the key bacteria in microbial communities under stress of thiocyanate and nitrate over time (Cao et al., 2017).

The phylogenetic structure of the sludge community was characterized at the phylum and genus levels. The relative abundances of the main phyla and genera with a sequence percentage more than 0.1% were shown in Fig. 6. At the phylum level, the sludge was dominated by Bacteroidetes, Chlorobi, Chloroflexi, Firmicutes, Nitrospirae, OD1, Planctomycetes, Proteobacteria and TM7; the relative abundance of Proteobacteria in sludge samples increased from 27.2% to 57.6% during acclimation from the day 0 to 48. At the genus level, thirteen genera were dominant at relative abundances exceeding 0.1%, including WCHB1-05, Thiobacillus, Thauera, 178, SHD-231, Rubrivivax, Planctomyces, Nitrospira, Niabella, Longilinea, Hydrogenophaga, Flavobacterium and Dechloromonas. Relative abundance of other species including unclassified, unidentified and taxa showing relative abundance below 0.1% decreased from 87.1% to 27.7% within acclimation time, which might indicate the decreased diversity and richness of sludge samples. The relative abundance of *Thiobacillus* in sludge samples increased from 0.1% to 64.8% within the acclimation time. According to previous reports, *Thiobacillus* is an obligate autotrophic bacteria gaining energy through oxidizing sulfide, thiosulfate and sulfur using oxygen, nitrate and nitrite as electron acceptors (Di Capua et al., 2016; Xing et al., 2017). Furthermore, *Thiobacillus* is reported to dominate in aerobic and anoxic degradation of thiocyanate (Kantor et al., 2017, 2015; Lee et al., 2008). The *Thiobacillus* OTUs from the 16S rDNA gene data in this study were most closely related to *T. denitrificans* (Accession number NR_025358.1) from NCBI GenBank. These results indicated that *Thiobacillus* might play the main role in the autotrophic denitrification. These observations are supported by the findings of Broman et al. (2017).

The dominance of *Thiobacillus*, however, may not explain the efficient production of nitrite observed in the considered process. According to the previous studies, *Thauera* genera played a key role in high nitrite accumulation using organic carbon source as an electron donor (Du et al., 2017). The low relative abundances of *Thauera* genera, however, comprising only 0.16% in microbial communities, might not explain accumulation of nitrite. As well known, a cooperative work of nitrate and nitrite reductase is necessary to reduce nitrate to inert nitrogen in denitrification. The experimental temperature was kept at 35 °C in this study, which might increase the activity of nitrate reductase compared with nitrite reductase, resulting in nitrite reductase.

---

### Table 2
Sequences and operation taxonomic units (OTUs) number in the samples.

<table>
<thead>
<tr>
<th>Sampling day</th>
<th>Sequences</th>
<th>OTUs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>19,149</td>
<td>1046</td>
</tr>
<tr>
<td>10</td>
<td>20,950</td>
<td>869</td>
</tr>
<tr>
<td>20</td>
<td>25,872</td>
<td>868</td>
</tr>
<tr>
<td>48</td>
<td>37,852</td>
<td>808</td>
</tr>
</tbody>
</table>

---

### Table 3
Richness and diversity of the four samples at 0.03 distance limit.

<table>
<thead>
<tr>
<th>Sampling day</th>
<th>PD whole tree</th>
<th>ACE</th>
<th>Chao1</th>
<th>Dominance</th>
<th>Observed species</th>
<th>Shannon</th>
<th>Simpson</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>92</td>
<td>1207</td>
<td>1166</td>
<td>0.014</td>
<td>1046</td>
<td>7.607</td>
<td>0.985</td>
</tr>
<tr>
<td>10</td>
<td>73</td>
<td>1155</td>
<td>1121</td>
<td>0.033</td>
<td>845</td>
<td>6.489</td>
<td>0.966</td>
</tr>
<tr>
<td>20</td>
<td>69</td>
<td>1174</td>
<td>1091</td>
<td>0.044</td>
<td>778</td>
<td>6.180</td>
<td>0.955</td>
</tr>
<tr>
<td>48</td>
<td>58</td>
<td>988</td>
<td>968</td>
<td>0.408</td>
<td>614</td>
<td>3.433</td>
<td>0.591</td>
</tr>
</tbody>
</table>

---

Fig. 5. The effect of NO$_3^-$-N/SCN$^-\$-N ratio of 1.0 (a), 1.5 (b), 2.0 (c) and 3.0 (d) on the thiocyanate sulfur compounds conversion.
accumulation (Chen et al., 2017). Meanwhile, the pH value was increased gradually from 8.0 ± 0.1 to 8.7 ± 0.3 during denitrification in the acclimation reactor, which might inhibit the activity of copper nitrite reductase (Tavares et al., 2006). Therefore, the observed efficient nitrite production might thus be caused by the higher activity of nitrate reductase and insufficient electron donor (thiocyanate) (Fajardo et al., 2012; Ge et al., 2012).

4. Conclusions

Efficient production of nitrite and ammonium was observed in autotrophic partial denitrification and ammonification of thiocyanate wastewater, respectively. The nitrate-to-nitrite transformation ratio and the ratio of NO$_3^-$-N to NH$_4^+$-N were subjected to the NO$_3^-$-N/SCN$^-$-N ratio adjustment. Providing electron to nitrate or nitrite reduction, thiocyanate sulfur and its intermediate elementary sulfur were oxidized completely to sulfate.

Autotrophic partial denitrification and ammonification process resulting in ammonium and nitrite production was developed for the treatment of wastewater containing thiocyanate, in which Thiobacillus genus dominated. The process showed a potential in nitrogen removal in combination with subsequent anammox process.

Acknowledgements

This work was supported by the State Program of the National Natural Science Foundation of China (No. 21377040), the State Key Program of the National Natural Science Foundation of China (No. 21037001), and the Program for Science and Technology of Guangdong Province, China (No. 2015B020235005, No. 2015A020215008 and No. 2016A020221037).

References


trification accompanied with nitrite accumulation at the sediment-water inter-

Chung, J., Amin, K., Kim, S., Yoon, S., Kwon, K., Bae, W., 2014. Autotrophic deni-

source and temperature on sulfur-driven denitrification by pure and mixed cultures of

community analysis of a novel DEAMOX based on partial-denitrification and ana-


Fajardo, C., Mosquera-Corral, A., Campos, J.L., Mendez, R., 2012. Autotrophic deni-
trification with sulphide in a sequencing batch reactor. J. Environ. Manage. 113, 552–556.

Felfoeldi, T., Szekely, A.J., Goral, R., Barkacs, K., Scheirich, G., Andras, J., Racz, A.,
Fajardo, C., Mosquera-Corral, A., Campos, J.L., Mendez, R., 2012. Autotrophic deni-
trification process: The e

critical review on destruction of thiocyanate in mining e

Huang, B., Feng, H., Wang, M., Li, N., Cong, Y., Shen, D., 2013. The e

critical review on destruction of thiocyanate in mining e


Lay-Son, M., Drakides, C., 2008. New approach to optimize operational conditions for the
biological treatment of a high-strength thiocyanate and ammonium waste: pH as key

community in relation to changes in process performance in mixed culture systems

Li, R., Morrison, L., Collins, G., Li, A., Zhan, X., 2016. Simultaneous nitrate and phosphate
removal from wastewater lacking organic matter through microbial oxidation of
pyrroline coupled to nitrate reduction. Water Res. 96, 32–41.

Liu, C., Li, W., Li, X., Zhao, D., Ma, B., Wang, Y., Liu, F., Lee, D.J., 2017. Nitrite accu-
mulation in continuous-flow partial autotrophic denitrification reactor using sulfide

Lu, H., Chandran, K., Stensel, D., 2014. Microbial ecology of denitrification in biological

Ma, B., Qian, W., Yuan, C., Yuan, Z., Peng, Y., 2017. Achieving Mainstream Nitrogen

Mohan, T.V.K., Nanchanahia, Y.V., Venugopalan, V.P., Sai, P.M.S., 2016. Effect of C/N
ratio on denitrification of high-strength nitrate wastewater in anoxic granular sludge

process of anaerobic ammonium oxidation (ANAMMOX) in biotechnological waste-

Qin, Y., Han, B., Cao, Y., Wang, T., 2017. Impact of substrate concentration on anammax

Staib, C., Lant, P., 2007. Thiocyanate degradation during activated sludge treatment of

oxidizers under high organic content in high-rate Anammox UASB reactor. Biosour.
Technol. 101, 1762–1768.

Tavares, P., Pereira, A.S., Moura, J.J.G., Moura, I., 2006. Metalloenzymes of the deni-

Xing, W., Li, J., Gong, Y., Gao, W., Jia, Z., Li, B., 2017. Identification of the autotrophic
denitrifying community in nitrate removal reactors by DNA-stable isotope probing.

denitrification (AD) process with sulphide as electron donor. Water Res. 91, 225–234.

Yang, W., Zhao, Q., Lu, H., Ding, Z., Meng, L., Chen, G., 2016. Sulfa-driven autotrophic

Water Res. 115, 130–137.