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Original Research

Changing the Nutrient Source from Ammonia to Nitrate: Effects on Heterotrophic Bacterial Growth in Wastewater

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Abstract

Bacteria can use nitrate as a nutrient for growth, but the underlying mechanisms of this pathway have not yet been identified. We investigated the effects of changing the nitrogen source from ammonia to nitrate on the properties of heterotrophic bacterial growth in anoxic and anoxic/oxic (A/O) SBRs. Both SBR types were seeded with activated sludge cultivated with ammonia and were then fed with 1,400 $mg \cdot L^{-1}$ chemical oxygen demand (COD) and 250 $mg \cdot L^{-1}$ of nitrate nitrogen. Heterotrophic bacteria had a lag period of 8-9 d and 13-14 d in terms of growth and COD and nitrogen removal, respectively, in both reactors with nitrate as nutrient. Of the influent nitrate, 15% were converted to biomass nitrogen. Compared with ammonia or organic nitrogen as a nutrient source, with the use of nitrate more energy was needed for proteins synthesis, which resulted in a lower sludge yield (0.32-0.35) and lower amounts of proteins and phosphorus compounds. Furthermore, fewer extracellular polymer substances (EPS) and more soluble microbial products (SMP) were produced, both of which also had low proteins and high polysaccharide contents. The proteins in the cells were synthesized via dissimilatory nitrate reduction to ammonia (DNRA).

Keywords: denitrification, nitrogen source, dissimilatory nitrate reduction to ammonia, nitrate assimilation, sludge yield

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Introduction

In municipal wastewater treatment plants (WWTP), denitrification is the main pathway for nitrogen removal. However, it is still unclear whether nitrate is used as a nitrogen source as well as a terminal electron acceptor.

Nitrate as the sole nitrogen source for bacterial growth has been reported in *sulfur-oxidizing bacteria* [1-3], *Methanococcus thermolithotrophicus* [4], and activated sludge [5]. The common pathway is the dissimilatory nitrate reduction to ammonia (DNRA). DNRA bacteria reduce nitrate to ammonia in a dissimilar way, the produced ammonia is then used by other bacterial species for growth. Activated sludge with nitrate as a nutrient source can perform as well as sludge with ammonia as a nutrient source in organic and nitrogen removal [5]. The use of nitrate also results in lower biomass yield compared to the use of ammonia [5-7], thereby reducing the costs of subsequent sludge handling and disposal. In this context, it is crucial to know the characteristics of activated sludge with nitrate as a nutrient source and the influence of DNRA on overall nitrate reduction.

In soil, DNRA is a widespread process [8], but this process has also been reported in freshwater [9, 10], marine systems [11], anammox bacteria [12-14], human feces [15], digested sludge [16, 17], and constructed wetlands [18]. Bacteria carrying out this process may also occur in activated sludge, potentially contributing to nitrate reduction. High C/N ratio and nitrate limitation favours nitrate reduction to ammonia via DNRA [6]. Will DNRA occur when the environment is full of nitrates and organics without ammonia?

In this context, this study was designed (1) to investigate the properties of heterotrophic bacteria from anoxic and anoxic/oxic (A/O) SBRs, using nitrate as the sole nitrogen source for growth; (2) to investigate whether these bacteria reduce nitrate via the DNRA pathway; and (3) to compare the differences between bacterial growth in the anoxic/oxic reactor and in the anoxic reactor.

Materials and Methods

Experimental System and Operation

Two parallel SBRs with a working volume of 5 L each were used for the cultivation of denitrifying activated sludge. The A/O SBR was operated at an anoxic/oxic cycle, and the anoxic SBR was a complete anoxic system. Both reactors were inoculated with activated sludge from a wastewater treatment plant in Xi'an. Synthetic wastewater, which was used as an influent for both reactors, had the following composition: 1,400 mg COD·L^{-1} (glucose), 250 mg NO_3^-–N·L^{-1} (sodium nitrate), 12 mg PO_4^{3-}–P·L^{-1} (KH_2PO_4), 90 mg·L^{-1} of $MgSO_4.7H_2O$, 14 mg·L^{-1} of $CaCl_2 \cdot 5H_2O$, and 0.3 mL of the trace element solution (per liter): 0.12 mg·L^{-1} of $ZnSO_4.7H_2O$, 0.12 g of $MnCl_2 \cdot H_2O$, 7.5 g of $FeSO_4 \cdot 7H_2O$, 0.15 g of H_3BO_3, 0.03 g of $CuSO_4 \cdot 5H_2O$, 0.18 g of KI, 0.06 g of $Na_2MoO_4 \cdot 2H_2O$, 0.15 g of $CoCl_2$. The pH was kept between 7.6 and 8.0 by adding H_2CO_3/HCO_3^- buffer solution, which was prepared by mixing HCL with $NaHCO_3$. The temperature in the SBRs was maintained at 25±2ºC. A cycle time of 6 h was operated for the A/O SBR in a sequential mode: 2 min influent feeding without mixing, 178 min mixing under anoxic conditions, 60 min of mixing under aerobic conditions, 20 min of settling, 2 min of discharging, and 98 min of idling. The anoxic SBR was operated at 2 min of feeding without mixing, 238 min of mixing under anoxic conditions, 20 min of settling, 2 min of discharging, and 98 min of idling. The pH probe (METTLER InPro 4010, Germany) and the DO probe (METTLER Inpro 6050, Germany) were monitored online. The exchange ratio was controlled at 0.5, which corresponded to 12 h of hydraulic retention time (HRT); sludge retention time (SRT) was 10 d through discharging 500 ml liquid mixture daily.

Nitrate Conversion Pathway Experiments

We evaluated the influent nitrate conversion pathway on days 1, 14, 35, and 60. The experiment was carried out in an airtight reactor. The flow of argon to the reactor was maintained at 50 mL·min^{-1} for 5 min using a mass flow controller to evacuate all the air and nitrogen in the reactors prior to the experiment. Subsequently, the reactors were sealed with silicone rubber stoppers. The concentration of volatile suspended solids (VSS) was used for biomass determination. Samples for N_2O and N_2 determination were collected directly from the headspace gas. The culture sample was harvested via centrifuge at 10,000 g for 10 min, and the sludge was washed with pure water and further dried to constant weight at 102±2ºC for 6 h in order to analyze SS, VSS, total Kjeldahl nitrogen (TKN), total phosphorus, and lipids, according to the standard methods. The supernatant was filtered through 0.22-μm disposable Sartorius filters to separate any residual biomass for the analysis of soluble chemical oxygen demand (SCOD), NO_3^-–N, NH_4^+–N, and NO_2^-–N, according to the standard methods for wastewater analysis [19].

To determine the concentrations of denitrification end products (N_2O and N_2), N_2O mole fractions, denitrification rate (N_2 and N_2O), DNRA rate, total NO_3^- consumption, denitrification/total NO_3^- consumption ratio, and DNRA/total NO_3^-consumption ratio were examined, along with their interactions.

Gas Analysis

We sampled 20 mL of headspace gas with a syringe and injected them into re-evacuated 20 mL-glass vials. The concentration of N_2O was measured using a gas

chromatograph (PE Clause 600) equipped with an electron capture detector (ECD) and packed columns (Porapak Q). The N_2 concentration of the gas sample was analyzed via a gas chromatograph (Agilent 6890N) equipped with a thermal conductivity detector (TCD) and stainless steel packed columns of TDX-01.

Analysis of Extracellular Polymeric Substance (EPS) and Soluble Microbial Products (SMP)

Soluble microbial products are defined as the pool of organic compounds from metabolism processes and biomass decay and are released into the solution. Total SMP was determined as follows:

SMP = soluble polysaccharide (as glucose) + soluble nucleic (as CT DNRA)

The EPS of the sludge samples were extracted according to the cation-exchange resin technique (Dowex Marathon C, 20-50 mesh, sodium form, Fluka 91973) proposed by FRØLUND and WANG [20, 21]. The major components of EPS and SMP, including proteins, polysaccharides, and humic compounds, were quantified separately by colorimetric methods. The protein concentration was determined according to the method described by the modified Lowry method [22], bovine serum albumin (V900933, Vetec™, Sigma-Aldrich) was used as standard, and the results are expressed in mg equivalent of BSA per gram of adsorbent. Polysaccharide concentration was measured by the phenol sulfuric acid method described by [23] Dubois et al., glucose (V900392, Vetec, Sigma-Aldrich) was used as standard, and the results are expressed in mg equivalent of glucose per gram of adsorbent. The nucleic acid content was measured via the diphenylamine colorimetric method using calf thymus DNA (D4522, Vetec, Sigma-Aldrich) as standard [24]. Total EPS was determined as follows:

EPS = proteins (as BSA) + polysaccharides (as glucose) + nucleic acids (as CT DNRA)

Nitrate Conversion Pathway

On day 60, activated sludge was sampled from both reactors and washed with pure water to remove other nitrogen compounds in the supernatant. If the sole nitrogen source of the influent is nitrate, the isotopic method is not required for the analysis of the nitrate conversion pathway. Subsequently, the sludge samples were placed into two 100-mL serum bottles. In this experiment, the influent C/N was increased to 7, facilitating DNRA. The detection of ammonia indicated that the bacteria reduced nitrate via the DNRA pathway; if ammonia was absent, the assimilatory pathway was used.

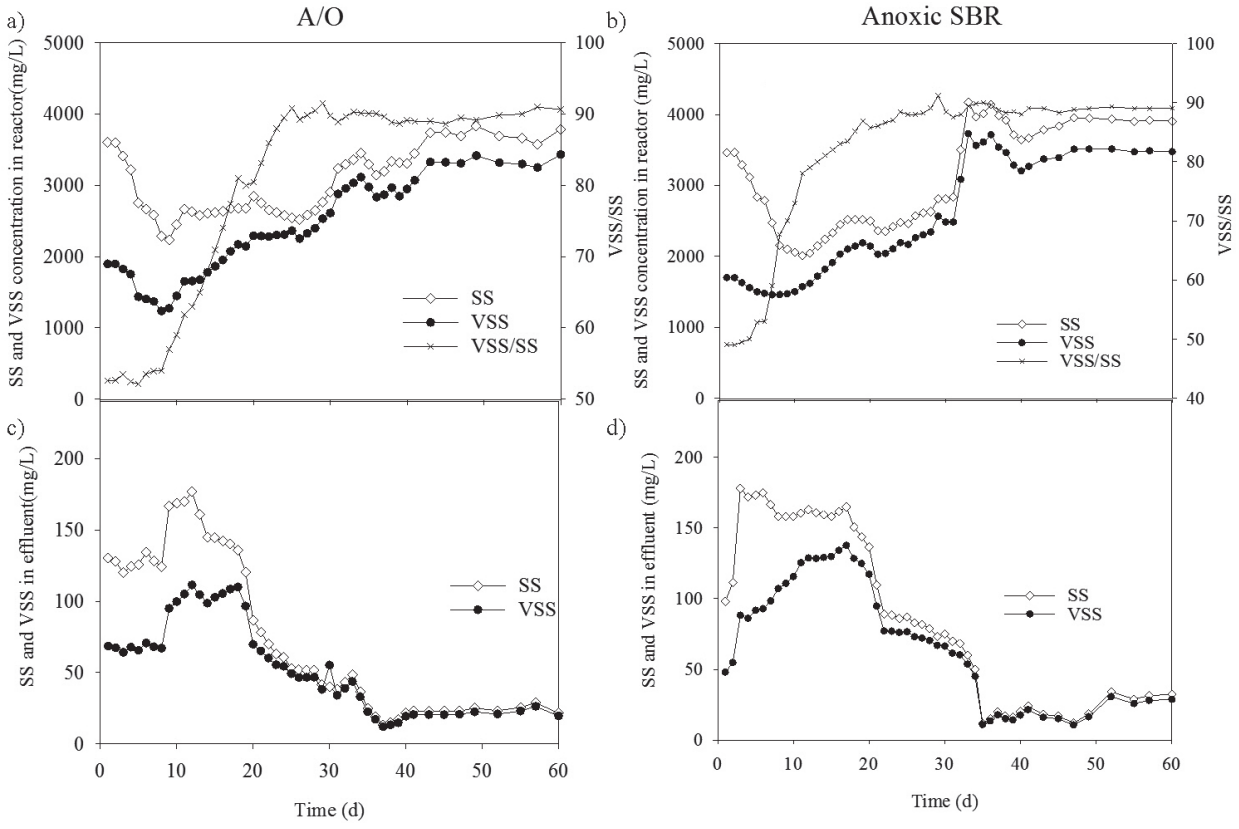

Fig. 1. Variation of concentrations in SS, VSS, and VSS/SS: a) and b) SS, VSS, and VSS/SS in reactors; c) and d) SS and VSS in the effluent.

Results and Discussion

Effects of Changing Nutrients on Bacterial Growth

The experiment lasted for 60 d, with an influent COD of 1,400 mg·L^{-1} (glucose) and 250 mg·L^{-1} nitrate nitrogen (sodium nitrate). The nitrogen source of the seed sludge was organic or ammonia nitrogen. Fig. 1(a-b) shows the similar trends of SS and VSS concentrations with changing nitrogen sources from ammonia to nitrate. Bacterial growth showed a lag period of 8-9 days in both reactors due to the changes in the nitrogen source. However, Boopathy and Kulpa [7] reported long lag periods in the growth rates of sulfate-reducing bacteria when TNT or nitrite was used as the nitrogen source, but no lag phase was observed when nitrate was the sole nitrogen source. It is well known that lag stages have also been observed in pure cultures. Microorganisms need time to adapt to a new matrix environment. At the beginning of the experiment, bacteria that used nitrate as a nutrient did not dominate the seed sludge, and the SS level decreased quickly. In both reactors, SS levels started to increase after 9 d, regardless of the dramatic sludge washing out (Fig. 1c, d). Results indicated that the microorganisms adapted to nitrate as the sole nitrogen source, with rapid growth rates.

Experimental work supports how the substrate types and operation systems affect sludge yield. In this study, the observed heterotrophic yields were 0.35 g COD·g COD^{-1} for A/O SBR and 0.30 g COD·g COD^{-1} for the anoxic SBR. Belay et al. [4] and Boopathy and Kulpa [7] also reported that nitrate as the sole nitrogen source resulted in 20-25% lower sludge yield than ammonia for sulfate-reducing bacteria and *Methanococcus*. However, Van den Berg et al. [6] reported the DNRA had little effect on the biomass yields (0.47 and 0.45 for denitrification and DNRA periods, respectively). The observed biomass yield factor is a function of the maximum biomass yield, the sludge decay rate and the sludge retention time [25]. In principle, bacteria need more energy to reduce NO_3^- to ammonia and, subsequently, to synthesize new cells than they would need for cell synthesis from ammonia or organic nitrogen. Consequently, nitrate as the sole nitrogen source results in lower sludge yield than ammonia as the nitrogen source. In addition, the A/O sludge yield was higher than that of the anoxic sludge using nitrate. The low yield of heterotrophic bacteria in this study resulted from low ammonia concentration for bacterial growth due to low DNRA rate and more COD needed for bacterial growth with nitrate.

Effect of Changing Nutrients on Sludge Performance in Terms of COD and Nitrogen Removal

The activated sludge in both reactors displayed a lag period of 13-15 days in terms of COD and nitrate removal (Fig. 2). The COD removal efficiency decreased from 99 and 96% at the start of the experiment to 71 and 70% on day 13 in the A/O SBR and the anoxic SBR, respectively (Fig. 2a). We also observed a significant decrease in TN removal (from 70 to 39% for A/O and to 51% for the anoxic SBR) due to the changes in the nitrogen source, and the accumulated nitrite was as high as 64 and 45 mg·L^{-1}, respectively, on day 13. Afterward, COD and nitrogen removal improved gradually in both reactors. At the end of the experiment, COD and TN removal efficiencies were as high as 99 and 84% for the A/O SBR and 97 and 87% for the anoxic SBR, respectively. This shows that the sludge with nitrate as nutrient performed as well as the conventional activated sludge (seed sludge) with ammonia. Fig. 2c shows that the A/O process results in higher nitrite accumulation than the anoxic process. The substrate was degraded by aerobic organisms, so less nitrate was reduced to N_2 or NH_4^+, and nitrite levels were higher in the A/O SBR.

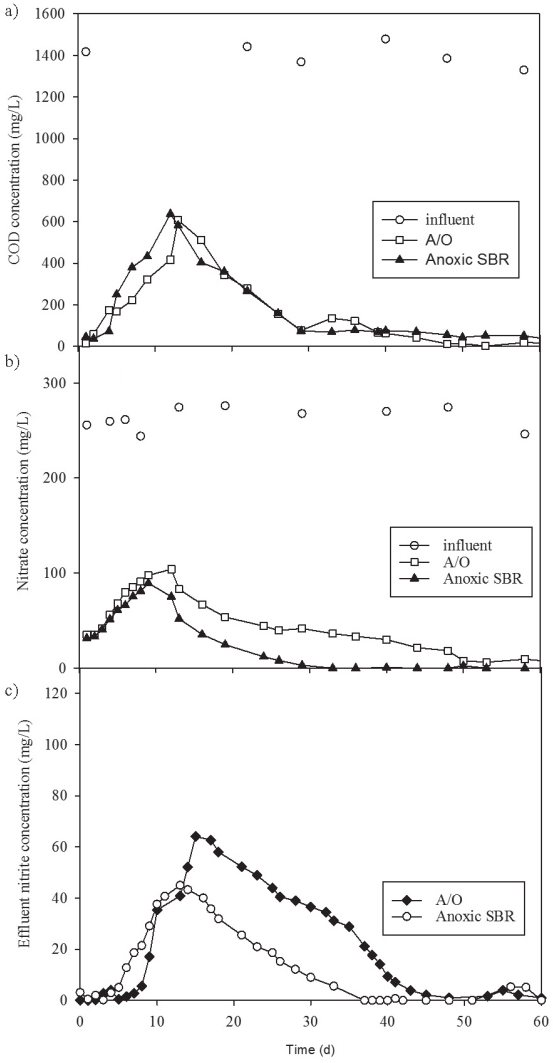

Fig. 2. Performance of reactors over time a) Concentration profiles of COD; b) Concentration profiles of nitrate; c) Concentration profiles of nitrite.

Endogenous respiration results in some biomass decay as well as in ammonification and nitrification.

Effect of Changing Nutrients on Sludge Components

To compare the properties of sludge cultivated in different nitrogen media and at different operation processes, we determined the components of seed sludge and experimental sludge on day 60. The results showed that protein synthesis was not affected by the operation process, but the synthesis of polysaccharides, phosphorus, and lipid substances was significantly affected (Fig. 3a). Higher polysaccharides and phosphorus compounds were synthesized in A/O sludge compared to anoxic sludge, while the amounts of lipids were relatively low. It should be noted that the proteins content was more significantly affected by the nitrogen source. Nitrate as nitrogen source had a negative effect on the synthesis of cell proteins and phosphorus compounds, but a positive effect on polysaccharides and lipids synthesis. Compared with the anoxic process, the A/O process had a positive effect on polysaccharides synthesis and a negative effect on lipid synthesis.

The sludge proteins contents were significantly affected by the nitrogen source, and the proteins contents in the experimental sludge were determined in terms of nitrogen content. The nitrogen content of the sludge markedly decreased from 10-11% to 4.64 and 5.10% within 7 days, which was induced by the change of the nitrogen source (Fig. 3b). As a result, cell growth was highly inhibited. After 8 days of cultivation, the nitrogen content gradually increased and stabilized at 8.6% in VSS until the end of the experiment. At this time, the nitrogen content was significantly lower than that of the activated sludge from WWTP (11-13%), if considering that the formula $C_5H_7NO_2$ is representative for cell mass. It was also lower than that of the anoxic/aerobic activated sludge (10.5-12.5%) [26] and the seed sludge from WWTP(10.3%). A previous study [6] reported that the nitrogen content of biomass is 12.3% in a continuous culture-enriching of DNRA bacteria. Under the condition of nitate as sole nitrogen source and low DNRA rate, there was not enough ammonia for growth. Microbes had to use endogenous organics such as some proteins or other nitrogen-containing organic from activated sludge and EPS, and these nitrogen-containing groups were released into the system in the

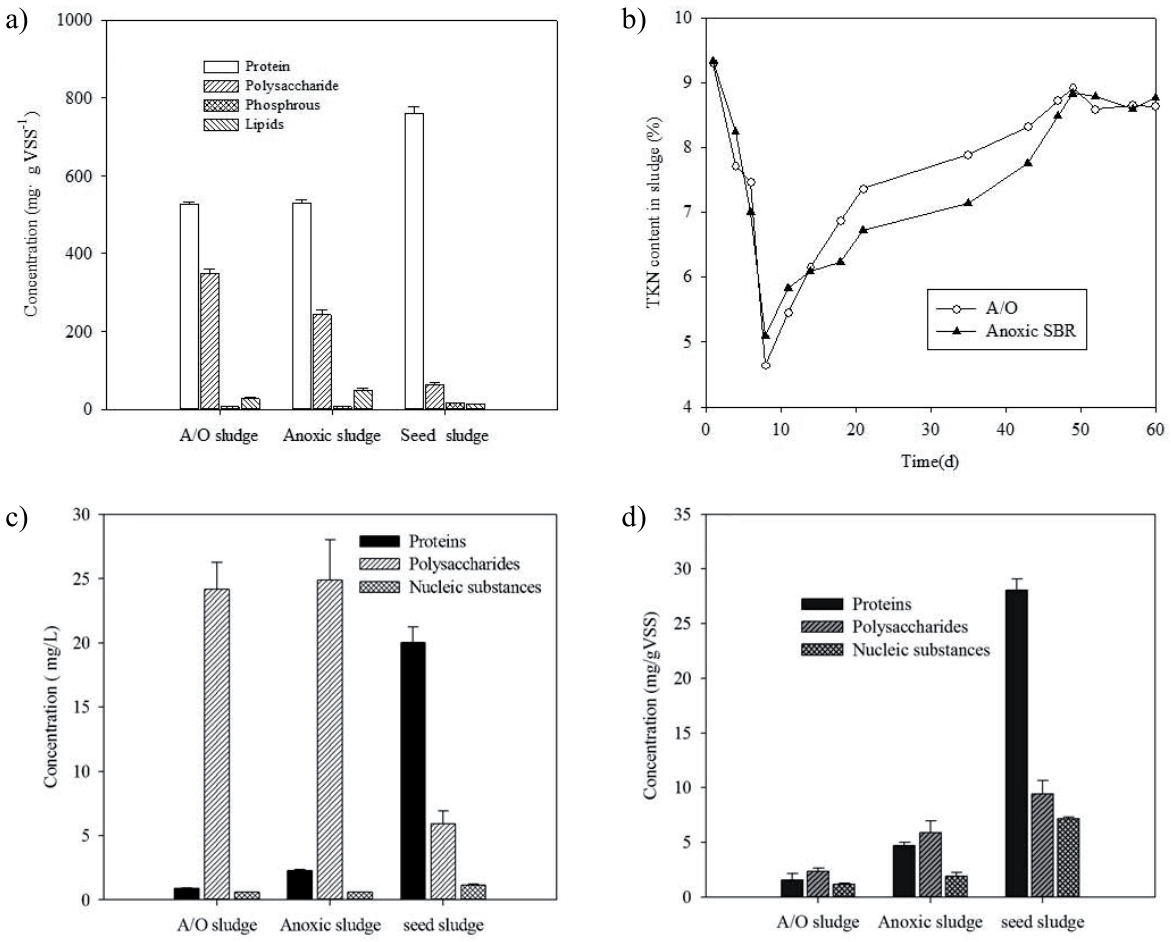

Fig. 3. a) Components and contents of A/O sludge, anoxic sludge and seed sludge; b) Variation of protein content in the experimental sludge during change of nitrogen source; c) EPS and d) SMP produced in experimental sludge and seed sludge.

utilization process, thus the nitrogen in activated sludge was low.

Effect of Changing Nutrients on the Production of EPS and SMP

EPS and SMP are secreted by cells, which are important for the performance of the sludge and can be affected by insufficient essential nutrients [27]. The proteins content was well known as being much higher than the polysaccharides content. The EPS and SMP of the seed sludge, the A/O sludge, and the anoxic sludge under steady state were evaluated to investigate the effect of changing the nitrogen source. As shown in Fig. 3, the proteins contents in the sludge, in EPS, and in SMP were lower than that in the seed sludge; for the polysaccharides content, an opposite trend was observed. The amount of SMP was significantly higher in A/O and anoxic SBRs with nitrate as the sole nitrogen source, compared to the seed sludge with ammonia as the nutrient. This indicates that the use of nitrate as nutrient results in a higher SMP production, especially in terms of polysaccharide production; while the production of proteins and nucleic substances was significantly lower compared to the medium with ammonia as nutrient. The total contents of EPS in both reactors were considerably lower than those in the seed sludge, although EPS had similar components than SMP; polysaccharides, in contrast to proteins, constituted a larger fraction of EPS in the experimental sludge. The proteins/polysaccharides (PN/PS) ratios were as low as 0.67 and 0.79 in A/O and anoxic sludge, respectively, which were lower than DEAMOX-UASB sludge (4-9) [28], anammox dominated in mix culture (2.92) [29] and conventional activated sludge (2.2-2.9) [30]. It was speculated that the increase of PS component in the EPS and SMP was due to long-term lack of ammonia as a nitrogen source for bacterial growth. The influent C/N ratio of SBRs was low, which is a benefit for denitrification over DNRA. So the ammonia produced via DNRA was deficient for bacterial growth. Microorganisms used the proteins in EPS and SMP as nitrogen source for growth. Although the biomass growth exhibited a lag period, the bacterial growth rate and the denitrification capacity were hardly affected in the treatment with nitrate as nutrient. However, the cell components and the microbial products were considerably affected.

Nitrogen Mass Balance Analysis

The nitrogen mass balance is important for understanding the conversion of the influent nitrate during the change of the nitrogen source. Samples were taken from the experimental reactors on days 1, 13, 35, and 60 to determine the nitrogen mass balance, which was measured by evaluating the input and output nitrogen fluxes in a typical period. Typical relative distributions of nitrogen are shown in Fig. 4. The stoichiometry equations calculated from the N mass balance based on the change of different N in the reactor were as follows.

For the bacteria of seed sludge in A/O SBR with ammonia as nutrient and nitrate as terminal electron acceptor:

$$NO_3^- \rightarrow 0.137NO_3^- + 0.0035N_2O + 0.408N_2 + 0.039N_{cell} \quad (1)$$

For the bacteria in A/O SBR using nitrate as sole nitrogen source for growth and denitrification:

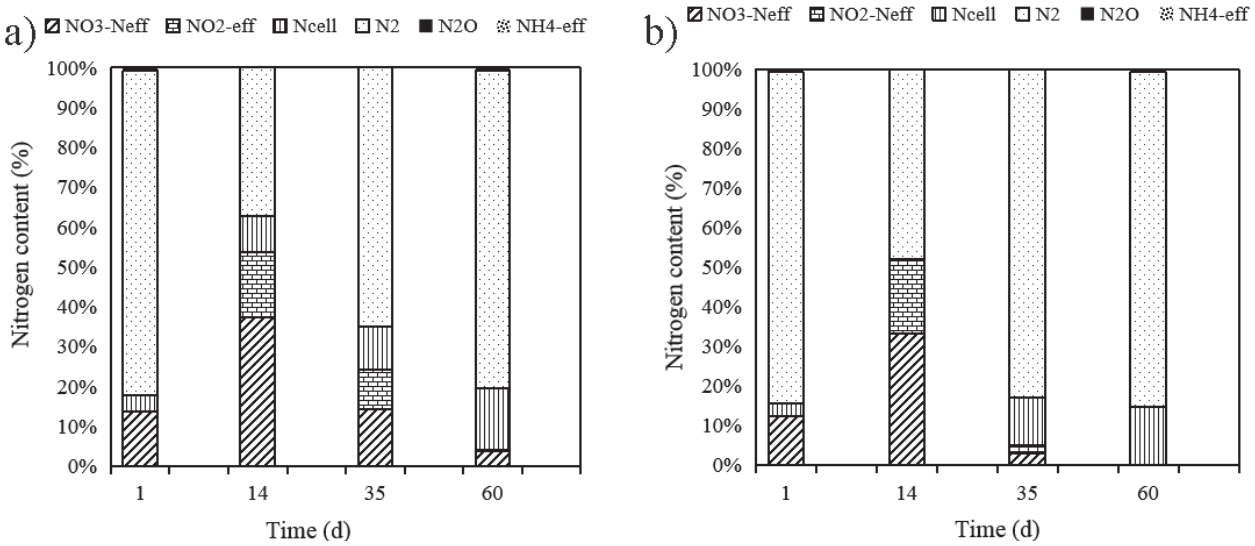

Fig. 4. Mass balance for nitrogen in the A/O SBR a) and the anoxic SBR b) on days 1, 14, 35, and 60. The heights of the bars indicate the NO_3^- concentration converted in the reactor. Heights of different parts of bars represent fractions of concentrations of specific compounds relative to the amount of converted NO_3^-. Where NO_3^- Neff is effluent nitrate concentration, NO_2-Neff is effluent nitrite concentration, Ncell is nitrogen content in bacterial cell, N_2 and N_2O represent the nitrogen in gas emitted by denitrification or DNRA.

$$NO_3^- \rightarrow 0.036NO_3^- + 0.004NO_2^- - +0.0035N_2O + 0.398N_2 + 0.156N_{cell} \quad (2)$$

For the bacteria of seed sludge in anoxic SBR with ammonia as nutrient and nitrate as terminal electron acceptor:

$$NO_3^- \rightarrow 0.122NO_3^- + 0.002NO_2^- + 0.0035N_2O + 0.419N_2 + 0.032N_{cell} \quad (3)$$

For the bacteria in anoxic SBR using nitrate as sole nitrogen source for growth and denitrification:

$$NO_3^- \rightarrow 0.0035N_2O + 0.422N_2 + 0.147N_{cell} \quad (4)$$

No ammonia was detected during the entire study period. It is speculated that the ammonia nitrogen produced by microorganisms through DNRA was removed by biomass growth. At the beginning of the experiment, only 3.2-3.9% of the influent nitrate was converted to cell proteins, but the ratio increased up to 15.5 and 14.7% for the A/O SBR and the anoxic SBR, respectively, after 60 days of operation. At this time, 81 and 84% of the influent nitrate were reduced to N_2 by denitrification. Using the stoichiometry equation, it was predicated that 18% of the influent nitrate would be converted into organic nitrogen, while the rest would be converted into nitrogen gas [31]. Obviously, the theoretical value from the stoichiometry equation, for this case, is higher than the experimental value. The process DNRA is favored in environments with a high C/N ratio [10]; it is therefore more competitive than denitrification in environments rich in organic matter and poor in nitrate. The study of Roland et al. [32] focused on the co-occurrence of denitrification, anammox, and DNRA in a tropical freshwater lake and on the influence of H_2S on these three processes. At a C/N ratio of 7.7, 90% of nitrate was recovered in ammonia and biomass [6]. A high C/N ratio and nitrogen efficiency [6], temperature [33], pH [10] nitrogen source, and redox potential [34], a low DO and cathode potentials [35], the presence of sulfide and Fe^{2+}, and high salinity [36] are potentially important environmental controls in the competition of denitrifying and DNRA bacteria. The impacts on the C/N ratio, redox potential, nitrogen efficiency, cathode potentials, sulfide, and Fe^{2+}, as well as the low DO on this competition result in an increased ratio of electron donors to acceptors, thereby decreasing the bottleneck in electron supply to the nitrite reductases of the DNRA pathway. Friedl et al. also reported that high labile C availability drives heterotrophic soil respiration, ultimately shifting NO_3^- consumption from denitrification to DNRA [37]. The electron donors to acceptors is the primary factor influencing DNRA, and could be changed by adjusting operational parameters when DNRA is inhibited or promoted.

Furthermore, N_2O is an important carbon dioxide-equivalent greenhouse gas which is produced in nitrification and denitrification processes. This study was driven by the question of whether it is produced during bacterial growth with nitrate as the sole nitrogen source. Results show that 0.7% of influent nitrate were converted to N_2O for A/O sludge and anoxic sludge with nitrate as a nitrogen source, which was lower than the value of constructed wetland (1.44% -5.08%) [38] and in anoxic tank of full-scale WWTP(3.5 %) [39, 40]. DNRA was not a source of N_2O [41]. N_2O load emitted was mainly related to the variability of the influent C/N ratio [38, 42]. It is more than the system dominant in DNRA (0.035%) [18]. The results showed that using nitrate as the nutrient for bacterial growth and the DNRA process leads to a lower N_2O production than the denitrification process. The fate of N_2O was higher in low redox potentials [38], low pH [18] and low C/N ratio [38, 42].

Nitrogen Reduction Pathways

To investigate the *nitrate reduction pathways* in activated sludge with nitrate as nutrient, the C/N ratio of the influent was increased to 7 on day 60, with 92 mg/L of nitrate and 42 mg/L of ammonia in the batch test. The reactors were cultivated with nitrate as the nitrogen source. The ammonia concentration decreased from 42 to 38 mg/L in the first 60 min and then increased up to 49 mg/L (Fig. 5). Results showed that the bacteria reduced a part of nitrate in the DNRA pathway. Compared to nitrate, bacteria primarily use ammonia for growth. The rate of nitrate reduction to ammonia via DNRA bacteria was lower than the consumption rate, which resulted in a decrease of ammonia in the first 60 min. This also explains why ammonia could not be detected in both reactors in the first 60 d. The DNRA rate was higher than the consumption rate in the following stage, which led to ammonia accumulation. This result also demonstrates that DNRA is not inhibited by the presence of ammonia. Numerous studies confirmed that DNRA is a common process in bacteria [18, 43, 44]. Based on our results, nitrate can be reduced via DNRA in both reactors, and the process

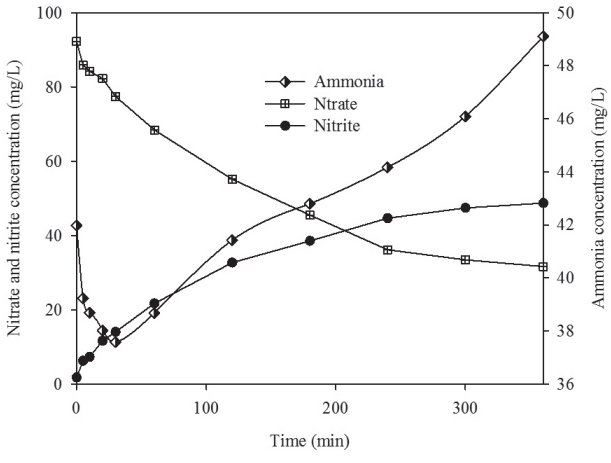

Fig. 5. Variation of nitrogen in the sludge slurry in the study of nitrate transformation pathway.

itself was probably stimulated by the change of the nitrogen source and ageing of the sludge.

Stoichiometry and Bacterial Reaction

It is important to discuss the bacterial metabolisms with different nitrogen sources. Microorganisms prefer to use ammonia or organic nitrogen for biomass synthesis if available, because the status of organic nitrogen within the cells is the same as that of ammonia (-III), which is also demonstrated in Fig. 5. When an oxidized form of nitrogen is used, the microorganisms must reduce it to the (-III) oxidation state of ammonia. This process requires electrons and energy, thus reducing their availability for biomass synthesis [31]. For anoxic bacteria with ammonia as nutrient and nitrate as terminal electron acceptor in the activated sludge model 1 (ASM1), the heterotroph anoxic yield is 0.53 g COD·g COD^{-1} for ordinary heterotrophic organisms (OHOs) [45]; the stoichiometric equations are as follows:

Catabolism $$0.8NO_3^- + CH_2O \rightarrow 0.4N_2 + 0.6H_2O + CO_2 + 0.8OH^- \quad (5)$$

Anabolism $$0.2NH_3 + CH_2O \rightarrow 0.2C_5H_7NO_2 + 0.6H_2O \quad (6)$$

Total metabolism:

$$0.106NH_3 + CH_2O + 0.376NO_3^- \rightarrow 0.106C_5H_7NO_2 + 0.6H_2O + 0.188N_2 + 0.47CO_2 + 0.376OH^- \quad (7)$$

When nitrate is the sole nitrogen source, the nitrogen should be reduced from the +V state to the -III state prior to assimilation. For the anoxic sludge used in this research, with nitrate as nutrient, the heterotroph anoxic yield was 0.30 g COD·g COD^{-1}, with the following metabolism:

Catabolism $$0.8NO_3^- + CH_2O \rightarrow 0.4N_2 + 0.6H_2O + CO_2 + 0.8OH^- \quad (8)$$

Anabolism $$0.1429NO_3^- + CH_2O \rightarrow 0.1429C_5H_7NO_2 + 0.7143H_2O + 0.1429OH^- + 0.2858CO_2 \quad (9)$$

Total metabolism reaction

$$0.524NO_3^- + CH_2O \rightarrow 0.232N_2 + 0.06C_5H_7NO_2 + 0.648H_2O + 0.524OH^- + 0.700CO_2 \quad (10)$$

Subsequently, 11.45% of the nitrate is converted to biomass, while the remaining biomass is reduced to nitrogen gas. This result is approximately in line with the nitrogen mass balance analysis.

For A/O sludge with nitrate as nutrient, the heterotroph yield was 0.35 g COD·g COD^{-1}. The catabolism and anabolism of sludge from A/O sludge are the same as in the anoxic SBR (Eq. 8, Eq. 9), but the total stoichiometric equation is as follows:

Total metabolism reaction

$$0.486NO_3^- + CH_2O \rightarrow 0.208N_2 + 0.07C_5H_7NO_2 + 0.662H_2O + 0.486OH^- + 0.660CO_2 \quad (11)$$

Microorganisms obtain their energy from oxidation-reduction reactions for growth and maintenance. Bacterial growth involves two basic reactions: one for energy production and one for cellular synthesis. Our results showed that of all the COD removed from the reactors, 30–35% was incorporated into the newly synthesized biomass, while the rest was fully oxidized to CO_2 for energy and denitrification.

Fig. 2 shows that when 1 g of nitrate was removed, 5.55 g and 5.41 g of COD were consumed by the sludge in the A/O and anoxic SBRs by day 60, respectively. These values were 5.31 g and 5.30 g when the nitrogen source was changed to ammonia, indicating the different metabolic pathways in the systems with nitrate or ammonia as nitrogen source. Based on the anabolism equations (Eqs. 6 and 8) for the production of 1 g of cells, 1.97 g COD and 0.12 g nitrate nitrogen are needed when nitrate is the sole nitrogen source. When the heterotrophic bacteria used ammonia as nitrogen source, 1.40 g COD were needed for the synthesis of 1 g of cells, while 0.34 g COD were needed for denitrifying 0.12 g nitrate nitrogen. Consequently, an additional 0.57 g COD is needed for the synthesis of 1 g of cells when using nitrate as compared to ammonia. The bacteria need an additional 1.14 g COD/g NO_3^-in DNRA than denitrification compared with denitrification. Thus, the amounts of COD used to reduce 1 gram of nitrate in both reactors were higher than those used in the same systems with ammonia as nutrient source.

Conclusions

This work set out to study the properties of heterotrophic growth at different nitrogen sources (ammonia and nitrate) in anoxic and A/O SBRs. Based on our results, heterotrophic bacteria performed similarly in both reactors, with a lag period of 8-9 d in growth and of 13-15 d in sludge performance in terms of COD and nitrogen removal due to the change of the nitrogen source from ammonia to nitrate. Nitrate as nitrogen source had a negative effect on the synthesis of cell proteins and phosphorus, but a positive effect on polysaccharides and lipid synthesis. A similar pattern was observed in EPS and SMP production. The nitrate as nutrient for cell growth was used via the DNRA pathway. The results of this study contribute to understanding the effect of nitrogen source on sludge properties and help uncover the utilization mechanism of nitrogen source in the wastewater treatment process without ammonia as a nitrogen source.

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Conflict of Interest

The authors declare no conflict of interest.

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