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Effect of environmental factors on the synergistic denitrification of *Alcaligenes faecalis* and ammonia oxidizing bacteria: a preliminary study

Linke Zheng[a,†], Ying Xu[a,†], Yu Hua[a], Xiaohu Dai[a,b,*]

[a]*School of Environmental Science and Engineering. Tongji University, Shanghai 200092, PR China, emails: daixiaohu@tongji.edu.cn (X. Dai), zhenglinke@tongji.edu.cn (L. Zheng), assassinxu@sina.cn (Y. Xu), 1351530@tongji.edu.cn (Y. Hua)*
[b]*Shanghai Institute of Pollution Control and Ecological Security, Shanghai 200092, PR China*

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ABSTRACT

In wastewater treatment, ammonia oxidizing bacteria (AOB) can directly use hydroxylamine and NH_3, but these bacteria grow slowly and have additional nutrient requirements. In contrast, *Alcaligenes faecalis*, a recently discovered functional bacterium, is able to conduct both heterotrophic nitrification and aerobic denitrification. Moreover, *A. faecalis* can produce hydroxylamine to support the growth of AOB. Therefore, it is expected that a co-culture system of these two bacteria may achieve better synergistic denitrification. In this study, the effects of environmental factors on the synergistic nitrogen-removal efficiency of *A. faecalis* No.4 and AOB were investigated. The experimental results showed that the nitrogen-removal rate decreased with an increase in the medium carbon-to-nitrogen (C/N) ratio (from 1:1 to 3:1), whereas it increased with an increase in the stirring speed, which indicated that a low medium C/N ratio and a high stir speed benefited the synergistic denitrification of *A. faecalis* and AOB. In addition, the heterotrophic nitrification of *A. faecalis* and the ammonia oxidation of AOB proceeded better at 25°C than at 15°C. Further study revealed that, to some extent, the synergistic denitrification of *A. faecalis* and AOB increased with increases in the ratio of *A. faecalis* to AOB, and that high concentrations of *A. faecalis* did not inhibit AOB. The findings of this study are expected to enable the development of a new method for the removal of nitrogen from sewage and provide a theoretical basis for applications to AOB engineering.

Keywords: Biological denitrification; *Alcaligenes faecalis*; Ammonia oxidizing bacteria; Synergistic effect

1. Introduction

Nitrogen is one of the most fundamental chemical elements in the natural cycles of the Earth, where it is always in a state of dynamic equilibrium [1]. However, as human society continues to advance, large amounts of nitrogen are being discharged into the environment from artificially synthesized nitrogen fertilizers, especially into hydrological systems. This ongoing discharge interferes with the normal progress of the Earth's nitrogen cycle and can exacerbate natural phenomena such as "red tide" algal blooms, thereby causing environmental problems [2].

The denitrification treatment of sewage is important for managing the hydrological environment in China [3]. Biological denitrification technology is the most effective method for removing nitrogen pollution from wastewater and has become a research hot-spot in the field of wastewater treatment [4,5]. Ammonia oxidation is the first reaction step of nitrification, the central link of the global nitrogen cycle, and is also the rate-limiting step. Therefore, ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) have become the focus of research on the nitrification process [6]. AOB can directly use hydroxylamine and NH_3, but it grows slowly and has special nutrient requirements.

* Corresponding author.

†These authors have contributed equally to this work and regarded as co-first authors.

Therefore, the number of bacteria in the natural environment is low, which is the main reason that ammonia oxidation is the rate-limiting step in the nitrification process. Recent studies have shown that some heterotrophic microorganisms, such as *Alcaligenes faecalis*, can also perform nitrification [7,8]. Compared with autotrophic nitrifying bacteria, heterotrophic nitrifying bacteria have the advantages of a fast growth rate, and tolerance of low dissolved oxygen concentration and a more acidic environment [9], although a high concentration of ammonia nitrogen in wastewater inhibits their growth. Thus far, most researches have focused on the relationships between AOB and NOB or ammonia oxidizing archaea (AOA) [10–12], but the synergistic effect of AOB and heterotrophic denitrifying bacteria has not been studied. It is expected that heterotrophic nitrifying bacteria, specifically via the co-culture of heterotrophic nitrifying bacteria (*A. faecalis*) and AOB, can achieve the goal of removing carbon sources while also removing ammonia nitrogen.

It is widely acknowledged that environmental factors, including the C/N ratio, temperature, dissolved oxygen (DO) concentration and the ratio of different bacteria, are of great importance for bacterial co-culture [13–16]. However, the ideal environmental conditions for AOB and *A. faecalis* are not the same. The growth of AOB was reported to be more pronounced at temperatures between 10°C and 25°C than at either 4°C or at 30°C–37°C [17]. Unlike AOB, *A. faecalis* has proved to be more efficient at achieving heterotrophic nitrification and aerobic denitrification at 30°C–37°C [13,14]. A series of studies have shown that AOB is more vulnerable to change in DO concentration [18,19], whereas *A. faecalis* performs well at both high and low DO concentration [15]. With respect to the C/N ratio, AOB cannot withstand a C/N ratio >5 [20], but *A. faecalis* can survive at very high C/N ratios (even at a C/N ratio of 14.5) [21]. Also, in view of the synergistic or competitive relationships between different species, the ratio of inoculation is a critical regulatory condition in bacterial co-culture [22]. Therefore, understanding the effects of main environmental factors on synergistic denitrification is very important for improving the performance of functional microbes.

The objective of this study is to explore the effects of the main environmental factors on the synergistic denitrification of *A. faecalis* and AOB. The main environmental factors, including the C/N ratio, temperature, DO concentration and inoculation ratio, were investigated in a series of batch experiments. Specifically, the concentrations of different forms of nitrogen (i.e., the NH_4–N, NO_3–N, NO_2–N, and hydroxylamine) were monitored and the corresponding denitrification efficiency was evaluated. Moreover, the correlations between these environmental factors, the concentration of NH_4–N and the concentration of NO_2–N were investigated. These findings will be of great significance for improving the efficiency of denitrification in wastewater treatment and will provide a theoretical basis for future applications of AOB engineering.

2. Materials and Methods

2.1. Enrichment of Alcaligenes faecalis

The heterotrophic nitrifying denitrifying bacteria used in this study was *A. faecalis* No. 4 provided by Professor Masahiro Masato of the Tokyo Institute of Technology. The strain was stored in 25% glycerol solution at –80°C. The medium was then injected into a 500 mL conical flask, and cultured at 30°C and 100 rpm for 2 d to obtain a pre-cultured bacterial solution for resuscitation and proliferation of the strain [16]. The culture conditions were based on those of Shoda et al. [23] and were listed in Table 1. The mixed-liquor volatile suspended solids (MLVSS) concentration *A. faecalis* No. 4 used in the subsequent experiment was 30.544 g L^{-1}.

2.2. Enrichment of the ammonia oxidizing bacteria

An inoculum of AOB sludge was taken from a sequencing batch reactor. The main characteristics of the inoculum were as follows: solids residence time = 40 d and MLVSS = 1.442 g L^{-1}, of which AOB accounted for 10% of the total number of microorganisms. Then, the inoculum sludge was suspended in 1.2 L of a selective medium in a 2 L Erlenmeyer flask. The temperature of the culture was maintained at 30°C with mild agitation in the dark for 4–5 d [24]. Table 2 lists the main constituents of the selective medium.

2.3. Co-culture of Alcaligenes faecalis and the ammonia oxidizing bacteria

The experiment required mixing the two bacteria into a 200 mL Erlenmeyer flask with 50 mL of basic medium, and the C/N ratio, DO concentration, temperature and ratio of *A. faecalis* to AOB to be varied under different experimental conditions. Table 3 lists the main constituents of the basic medium.

Table 1
Basic culture conditions for the *A. faecalis* No. 4

Ingredient	Concentration	Ingredient	Concentration
KH_2PO_4	6 g L^{-1}	$(NH_4)_2SO_4$	2 g L^{-1}
K_2HPO_4	14 g L^{-1}	$MgSO_4{\cdot}7H_2O$	0.2 g L^{-1}
$C_6H_5Na_3O_7{\cdot}2H_2O$	15 g L^{-1}	Trace element	2 mL L^{-1}
EDTA·2Na	57.1 g L^{-1}	$FeSO_4{\cdot}7H_2O$	5.0 g L^{-1}
$ZnSO{\cdot}7H_2O$	3.9 g L^{-1}	$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$	1.1 g L^{-1}
$CaCl_2{\cdot}2H_2O$	7 g L^{-1}	$CuSO_4{\cdot}5H_2O$	1.6 g L^{-1}
$MnCl_2{\cdot}4H_2O$	5.1 g L^{-1}	$CoCl_2{\cdot}6H_2O$	1.6 g L^{-1}

Table 2
Main constituents of selective medium

Ingredient	Concentration	Ingredient	Concentration
KH_2PO_4	0.7 g L^{-1}	$(NH_4)_2SO_4$	2.5 g L^{-1}
Na_2HPO_4	13.5 g L^{-1}	$MgSO_4{\cdot}7H_2O$	0.01 g L^{-1}
$NaHCO_3$	0.5 g L^{-1}	Fe-EDTA	0.001 g L^{-1}

2.4. Experiments of effects the main environmental factors

2.4.1. C/N ratio

For the culture experiment, the culture medium was injected into a 200 mL Erlenmeyer flask. The experiment was divided into two groups: one consisting of separately cultured *A. faecalis* No. 4 and the other a mixed culture of *A. faecalis* No. 4 and AOB. The composition of the medium was prepared at C/N ratios (TOC/NH_4–N) of 1:1, 2:1, and 3:1 (the concentration of NH_4–N was kept at 300 mg L^{-1}), respectively, and the flask was maintained 25°C and 120 rpm for 5 d. Samples were taken every 24 h.

2.4.2. DO concentration

For the mixed culture experiment of *A. faecalis* No. 4 and AOB, the medium was injected into a 200 mL Erlenmeyer flask. The medium composition was prepared at a C/N ratio (TOC/NH_4–N) of 1:1 (the NH_4–N concentration was maintained at 300 mg L^{-1}) in a thermostatic magnetic stirrer at 25°C. The medium was cultured for 5 d at 80 rpm (DO = 0.8 mg L^{-1}), 100 rpm (DO = 1.3 mg L^{-1}), and 120 rpm (DO = 2.0 mg L^{-1}). Samples were taken every 24 h.

2.4.3. Temperature

For the mixed culture experiment of *A. faecalis* No. 4 and AOB, the medium was injected into a 200 mL Erlenmeyer flask. The medium composition was at a C/N ratio (TOC/NH_4–N) of 1:1 (the NH_4–N concentration was maintained at 300 mg L^{-1}), respectively at 15°C and 25°C, respectively, for 5 d at 120 rpm. Samples were taken every 24 h.

2.4.4. Ratio of A. faecalis to AOB

For the microorganisms cultured by synergistic denitrification, the results were influenced by different inoculation ratios. Therefore, experiments were conducted with a separate culture of *A. faecalis* No. 4 and a mixed culture of *A. faecalis* No. 4 and AOB. The medium was injected into a 200 mL Erlenmeyer flask for the culture experiments, which were divided into two groups: one consisting of a separate culture *A. faecalis* No. 4 and the other a mixed culture of *A. faecalis* and AOB. We used the MLVSS ratio of the two bacteria to characterize the inoculation ratio. The medium was at C/N ratio (TOC/NH_4–N) of 1:1 (the NH_4–N concentration was maintained at 300 mg/L) in a thermostatic magnetic stirrer with a rotation speed of 120 rpm at 25°C. The ratios of the two microorganisms in the bacteria of *A. faecalis* No. 4 and AOB were 60:3, 60:1.5, and 60:1 for 5 d. Samples were taken every 24 h.

2.5. Other analytical methods

The ammonia nitrogen concentration was measured using a Ness spectrophotometer with Biotek-EON. The nitrite nitrogen and nitrate nitrogen were determined using an ion chromatograph (883 Basic IC plus). The nitrite (NO_2–N) and nitrate (NO_2–N) nitrogen concentrations were measured according to standards established by the American Public Health Association [25]. The soluble total organic was measured using a total organic carbon (TOC) analyzer (SHIMADZU, TOC-L CPH/CPN, Japan). Hydroxylamine concentrations were measured by spectrophotometry [26]. The DO concentrations and pH values were measured using a JPBJ-608 portable dissolved oxygen meter. The alkalinity was measured using a G20 potentiometric titrator. The number of *A. faecalis* No. 4 and AOB was measured using a Supcre G6R. Statistical analyses of the environmental factors (i.e., DO concentration, C/N ratio, temperature, inoculation ratio, and time), NH_4–N concentration and NO_2–N concentration were performed using Statistical Package for the Social Sciences (SPSS) software (version 19).

3. Results and Discussion

3.1. Effect of the C/N ratio on synergistic nitrogen removal by the co-culture strains

Figs. 1 and 2 show the changes in the NO_2–N and NO_3–N concentrations in the co-culture system at different C/N ratios. As shown in Fig. 1, NO_2–N concentrations increased at all C/N ratios within the 5 d period, which suggested that the NH_4–N in the medium was smoothly converted into NO_2–N and NO_3–N by *A. faecalis* and AOB. Specifically, at a low C/N ratio (1:1), the NO_2–N concentration was 37.3% greater than at the high C/N ratio (2:1), which indicated that co-cultured microorganism performed better at the low C/N ratio. The same trends also can be seen in the changes in the NO_3–N content in Fig. 2. The NO_3–N concentration increased with the decreased C/N ratios, which suggested that NO_2–N was converted to NO_3–N by *A. faecalis*. This result is consistent with the findings of

Table 3
Main constituents of the basic medium

Ingredient	Concentration	Ingredient	Concentration
$C_6H_5Na_3O_7{\cdot}2H_2O$	0.3/0.6/0.9C-g L^{-1}	$(NH_4)_2SO_4$	0.3 N-g L^{-1}
KH_2PO_4	9.72 mg L^{-1}	EDTA	5 mg L^{-1}
$FeSO_4{\cdot}7H_2O$	9 mg L^{-1}	$NaHCO_3$	1.8 g L^{-1}

Ballinger et al. [20]. In addition, Fig. 3 shows the changes in the TOC content in different groups with different C/N ratios. In contrast to the trends observed for the NO_2–N and NO_3–N concentrations, the TOC content decreased with time. The higher the C/N ratio was, the more obvious was the decline in the TOC content. However, the magnitude of the decline in the TOC content was much smaller than that of the increases in the NO_2–N and NO_3–N concentrations, which suggested that carbon sources other than those based on deammonification and nitrification were used in other metabolic pathways. These results indicated that the co-culture system of *A. faecalis* and AOB was more adapted to a low C/N ratio and used the carbon source to more fully oxidize NH^{4+} more fully.

3.2. Effect of DO on synergistic nitrogen removal by the co-culture strains

The DO concentration has a significant effect on the growth of both microorganisms. Park et al. [27] found that the AOB community structure in the chemostat of activated sludge varied greatly at different DO concentrations. Previous experiments have revealed a very significant positive correlation between the DO concentration and the ammonia oxidation rate [28,29]. As the DO concentration is an important factor that directly affects the activity of ammonia oxidizing microorganisms [30,31], the effect of the DO concentration on the synergistic nitrogen removal efficiency of the strains was explored.

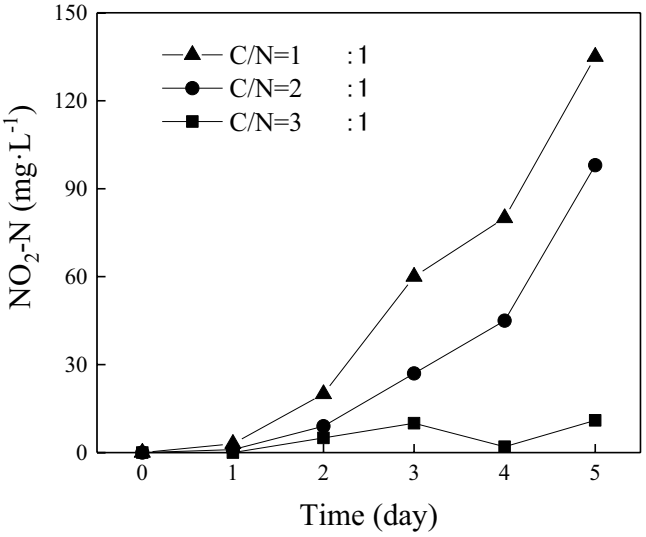

Fig. 1. Changes of NO_2–N concentrations from different groups with different C/N ratios.

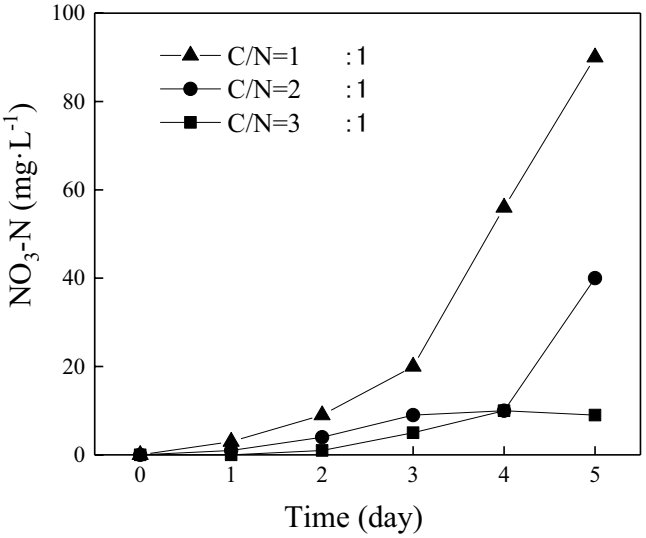

Fig. 2. Changes of NO_3–N concentrations from different groups with different C/N ratios.

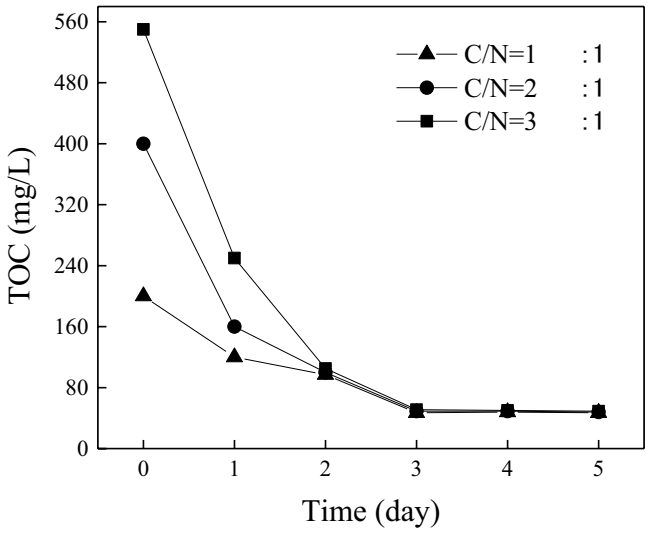

Fig. 3. Changes of TOC concentrations from different groups with different C/N ratios.

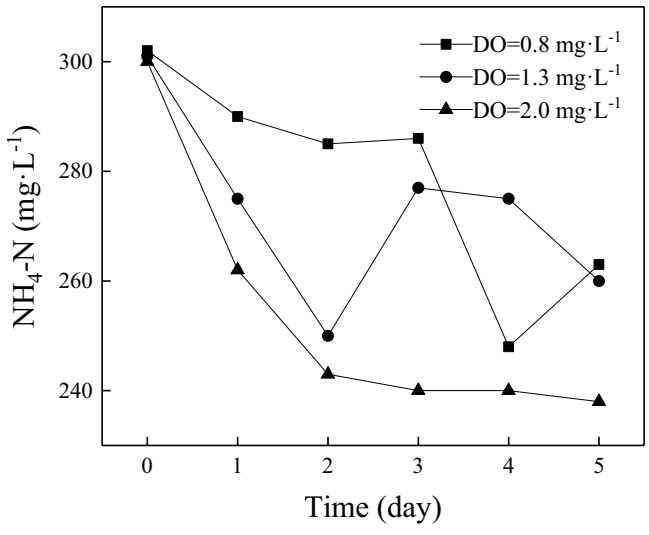

Fig. 4. Changes of NH_4–N concentrations from different groups with different DO contents.

As shown in Fig. 4, when the DO concentrations were 0.8 and 1.3 mg L^{-1}, the NH_4–N concentration generally decreased but there was a large fluctuation, which suggested that AOB was not working well in the conditions with insufficient oxygen. The NH_4–N concentration in a co-culture DO concentration of 2.0 mg L^{-1} was always lower than that at DO concentrations of 0.8 and 1.3 mg L^{-1}, which indicated that a co-culture DO concentration of 2.0 mg L^{-1} was better for synergistic growth. As shown in Fig. 5, the maximum NO_2–N accumulations reached 42.1 and 60.4 mg L^{-1} at 3 d, at DO concentrations of 1.3 and 2.0 mg L^{-1} respectively, whereas the maximum NO_2–N accumulations of the group reached only 28.3 mg L^{-1} at 4 d, with a DO concentration of 0.8 mg L^{-1}. Overall, the accumulation of NO_2–N was greatest at a DO concentration of 2.0 mg L^{-1}, and the ammoxidation process proceeded most smoothly at this stirring speed (120 rpm). In Fig. 6, the hydroxylamine concentrations at different DO concentrations showed a significant decrease from 24–48 h and the accumulation of hydroxylamine was the smallest at a DO concentration of 2.0 mg L^{-1}, which indicated the rapid rate of hydroxylamine utilization by AOB at a DO concentration of 2.0 mg L^{-1}. The efficiency of nitrogen removal in the co-culture of *A. faecalis* and AOB was higher at a DO concentration of 2.0 mg L^{-1} than at other DO concentrations, exhibiting an improvement of 13.1%. This result seems inconsistent with the finding of Yang et al. [32], which was that nitrogen removal was most efficient for AOB at a condition of DO concentrations greater than 4.5 mg L^{-1}. One possible explanation is that a high DO concentration provided more electron acceptors (such as O_2), which would be beneficial to the growth and reproduction of AOB, thereby increasing community diversity [33].

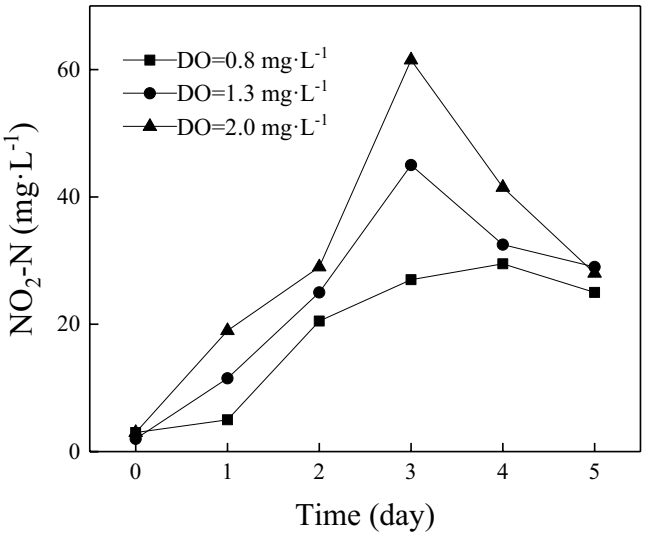

Fig. 5. Changes of NO_2–N concentrations from different groups with different DO contents.

However, a high DO concentration is not always beneficial to the co-culture activity of *A. faecalis* and AOB. The results showed that compared with DO = 0.8 mg L^{-1} and DO = 1.3 mg L^{-1}, DO = 2.0 mg L^{-1} provided a relatively suitable condition for the co-culture of *A. faecalis* and AOB, whereas nitrogen removal efficiency was generally not promoted in conditions with a low DO concentration.

3.3. The effect of temperature on synergistic nitrogen removal by the co-culture strains

To explore the nitrogen removal efficiency of the co-culture system at low temperature, the co-culture of *A. faecalis* and AOB was tested at 25°C and 15°C, respectively. As shown in Fig. 7, the NH_4–N concentration at 25°C was lower, but at 15°C more than 240 mg L^{-1} of NH_4–N remained after 5 d, which indicated that the denitrification effects of those two temperatures were not ideal.

As indicated by Fig. 8, the NO_2–N concentration accumulation increased both at 15°C and 25°C. Specifically, the NO_2–N concentration of 62.1 mg L^{-1} at 25°C was nearly twice

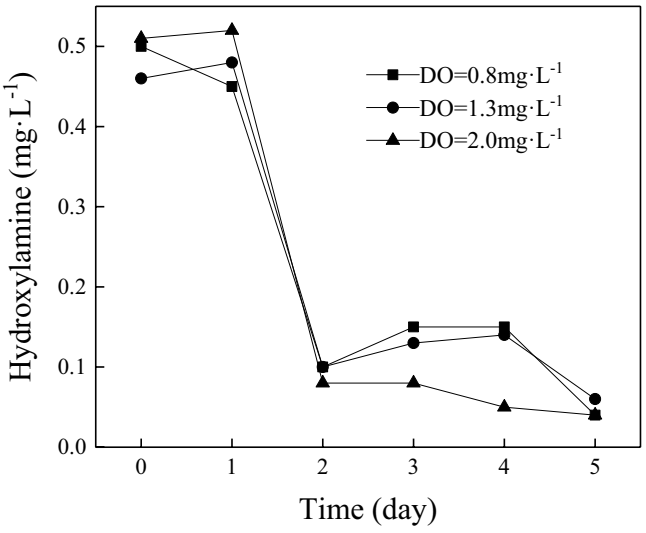

Fig. 6. Changes of hydroxylamine concentrations from different groups with different DO contents.

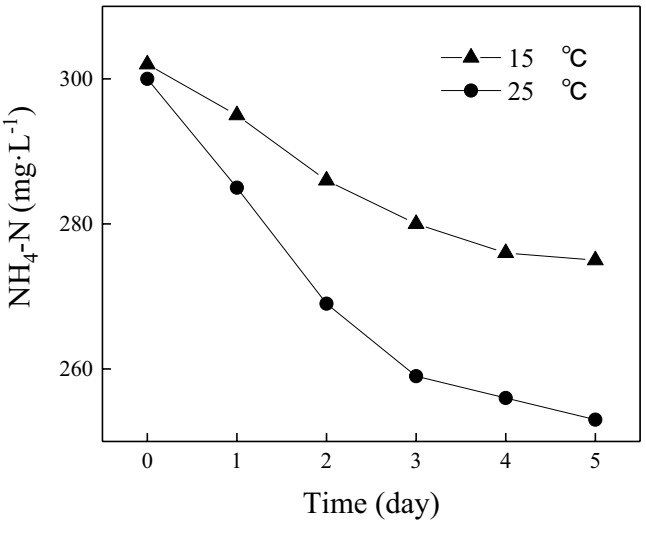

Fig. 7. Changes of NH_4–N concentrations from different groups with different temperatures.

that at 15°C after 5 d. As shown in Fig. 9, the concentrations of hydroxylamine at 15°C and 25°C showed an increasing trend within 24 h. After a significant decrease from 48–72 h, the initial hydroxylamine concentration at 15°C increased, and then continued to decrease. Correspondingly, the hydroxylamine concentration kept decreasing at 25°C and was always lower than that at 15°C, which indicated that hydroxylamine was utilized more by AOB at 25°C and that the synergistic effect of *Alcaligenes* and AOB was better at 25°C.

Overall, the mixed culture of *A. faecalis* and AOB did not perform well at 15°C or 25°C. However, when the reaction temperature was raised from 15°C to 25°C, the denitrification greatly improved, which will be of great use for improving the synergistic nitrogen removal performance of *A. faecalis* and AOB in low-temperature conditions.

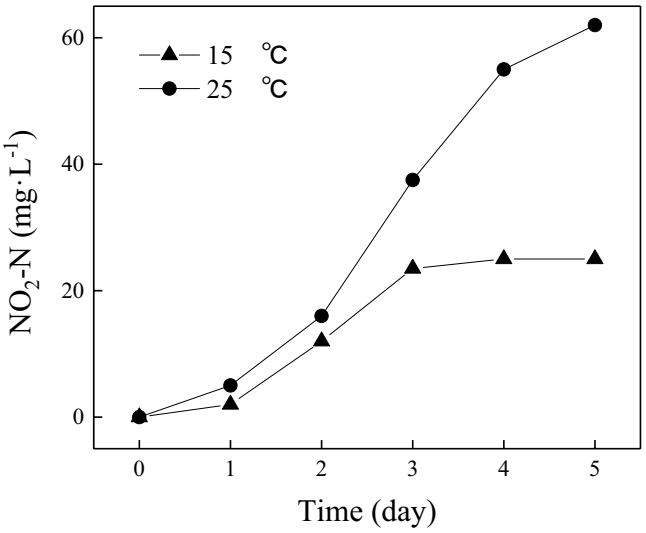

Fig. 8. Changes of NO_2–N concentrations from different groups with different temperatures.

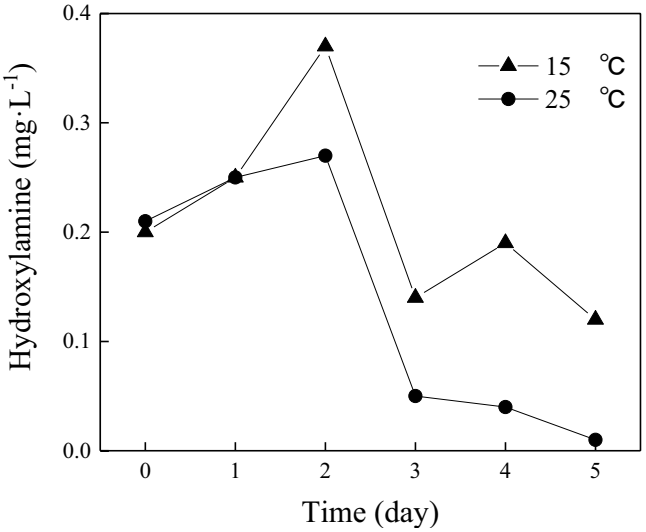

Fig. 9. Changes of hydroxylamine concentrations from different groups with different temperatures.

3.4. Effect of Alcaligenes faecalis/AOB ratio on synergistic nitrogen removal by the co-culture strains

For the microorganisms cultured by synergistic denitrification, the ratio of the number of different microorganisms has an important influence on the denitrification performance [22]. Therefore, experiments were conducted with a separate culture of *A. faecalis* and a mixed culture of *A. faecalis* and AOB. Figs. 10 and 11 show the synergistic denitrification efficiency of *A. faecalis* and AOB strains under different microbial biomass conditions.

As shown in Fig. 10, the reduction rate of the NH_4–N concentrations in the group with a 60:3 *A. faecalis*/AOB ratio was higher than that in the other groups, which indicated that a low *A. faecalis*/AOB ratio is beneficial for the removal of NH_4–N. This result is also confirmed by the changes in the NO_2–N concentrations of different groups with different *A. faecalis* /AOB ratios, which are shown in Fig. 11. Thus, it can be concluded that the synergistic denitrification efficiency was increased with a decrease in the *A. faecalis*/AOB ratios in their co-culture systems. Fig. 12 shows the changes in the hydroxylamine concentration in the co-culture system for different ratios. *A. faecalis*, cultured separately, produced a certain amount of hydroxylamine accumulation, whereas under the same conditions, the hydroxylamine concentration of the co-cultured system showed a continuous decreasing trend, which indicated that the hydroxylamine produced by *A. faecalis* was utilised by AOB. The concentration of hydroxylamine in the mixed groups decreased significantly from the start of the reaction during the 24–48 h period, and then showed a continuous decreasing trend, which proved that the hydroxylamine produced by *A. faecalis* can also be consumed by AOB. It can be seen that AOB and a higher concentration of *A. faecalis* can also achieve synergistic denitrification. The hydroxylamine of the three co-cultured groups increased from 96 and 120 h. Simultaneously, the hydroxylamine of the separate *A. faecalis* group decreased, due to the lack of a sufficient carbon source in the later

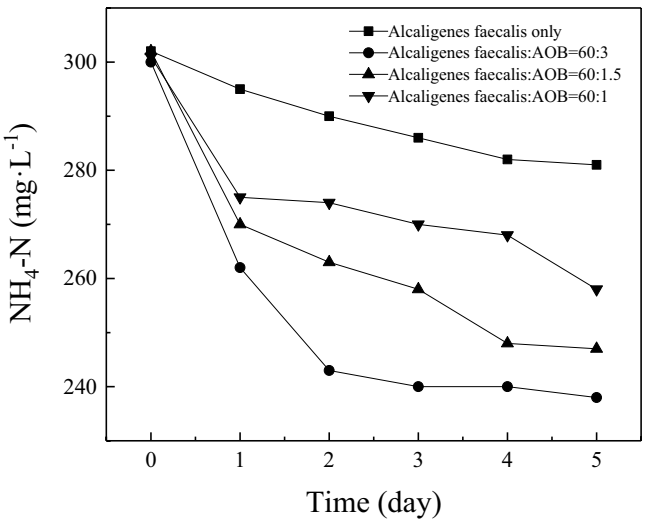

Fig. 10. Changes of NH_4–N concentrations from different groups with different *A. faecalis*/AOB ratios.

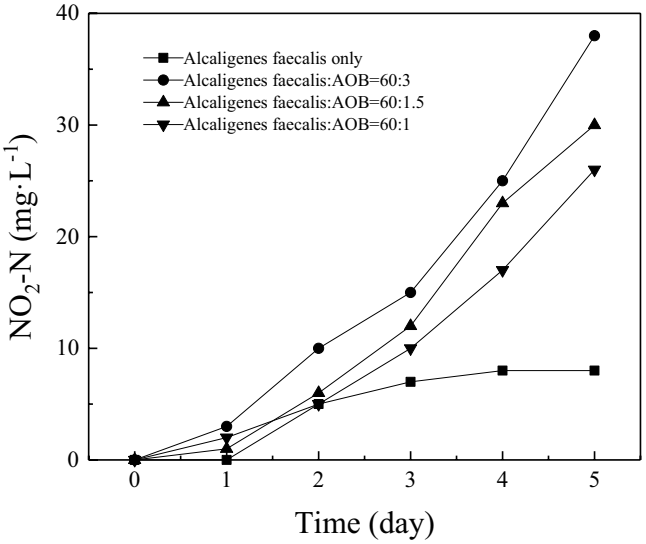

Fig. 11. Changes of NO_2–N concentrations from different groups with different *A. faecalis*/AOB ratios.

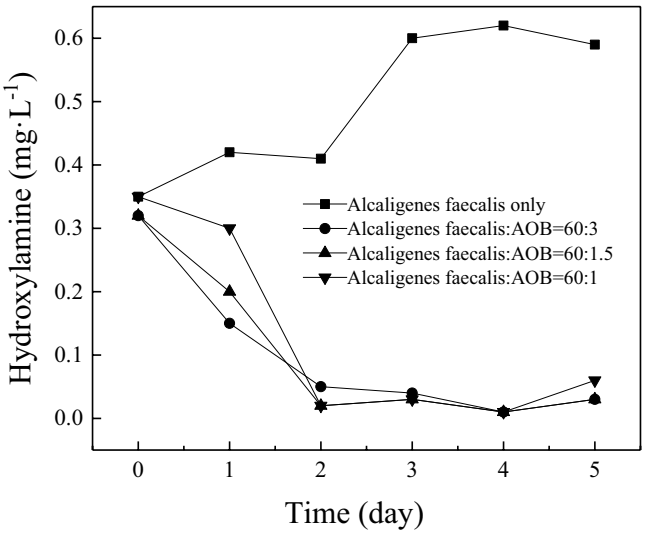

Fig. 12. Changes of hydroxylamine concentrations from different groups with different *A. faecalis*/AOB ratios.

stage of the experiment [16]. After 5 d, the concentration of hydroxylamine in the co-cultured groups remained stable at ~0.10 mg L^{-1}.

These results indicated that high concentrations of *A. faecalis* did not inhibit AOB and the synergistic denitrification rate could be improved by increasing the AOB biomass to a certain extent.

3.5. Principal component analysis

To further reveal the effects of environmental factors on the synergistic denitrification of *A. faecalis* and AOB, the correlations between the environmental factors (i.e., DO, C/N ratio, temperature, inoculation ratio, and time), NH_4–N and NO_2–N were investigated using Principal component analysis (PCA). Table 4 shows a summary of the eigenvalues and percentages of the variance of the main components. Fig. 13 shows the distribution and correlation between studied variances.

Table 4
Eigenvalues and the percentages of the variances for the seven components

Component	Eigenvalue	Percentage of variance	Cumulative
1	2.49569	35.65%	35.65%
2	1.73385	24.77%	60.42%
3	1.22798	17.54%	77.96%
4	0.70549	10.08%	88.04%
5	0.50582	7.23%	95.27%
6	0.2509	3.58%	98.85%
7	0.08027	1.15%	100.00%

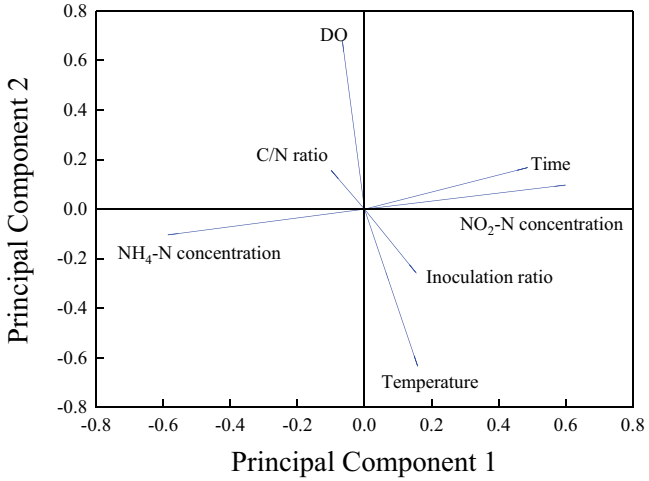

Fig. 13. Distribution and correlation between studied variances.

As evident in Table 4, of the seven components, the first, second and third components with percentages of 35.65%, 24.77%, 17.54%, respectively, had the highest percentages of variance. Fig. 13 shows that there is a significant negative correlation between the NH_4–N and NO_2–N concentrations, which is mainly attributed to the one-way transformation relationship (i.e., nitration). Temperature and DO concentration are also negatively correlated, presumably because high temperature can promote the metabolism of microorganisms and thereby consume more DO. As shown in Fig. 13, among all the environmental factors, the C/N ratio has the most significant positive correlation to the NH_4–N concentration, which indicated that a low C/N ratio would usually lead to a low NH_4–N concentration. This suggested that the C/N ratio has a significant effect on the synergistic denitrification of *A. faecalis* and AOB. This finding was also consistent with previous studies [34–36], which have found that a lower C/N ratio was more favorable for the conversion of NH_4–N. In addition, it can be seen in Fig. 13, that a longer the reaction

time, is correlated with a lower NH_4–N concentration and higher NO_2–N concentration. This result implied that the reaction time maybe mainly affected the nitration process of the co-culture system of *A. faecalis* and AOB.

4. Conclusions

In this study, the effects of different environmental factors on the synergistic denitrification of *A. faecalis* and AOB were explored, and, based on the results, yielding the following conclusions can be drawn:

- The optimal C/N ratio of *A. faecalis* to AOB was 1:1, and the higher the C/N ratio, the poorer the synergistic denitrification.
- The co-culture of *A. faecalis* and AOB had a good denitrification effect at a DO concentration of 2.0 mg·L^{-1}.
- Under low-temperature condition (15°C, 25°C), the mixed culture of *A. faecalis* and AOB did not perform well, but with an increase in temperature, there was an increase in synergistic denitrification by *A. faecalis* and AOB.
- High concentrations of *A. faecalis* did not inhibit AOB; a low ratio of *A. faecalis* to AOB yield a high rate of synergistic denitrification.

In general, under the environmental conditions of C/N ratio = 1:1, DO = 2.0 mg L^{-1}, temperature = 25°C, and a ratio of *A. faecalis* to AOB = 60:3, *A. faecalis* and AOB can achieve a high level of synergistic denitrification.

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