*Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) in press*
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

**Research Article**

https://doi.org/10.1631/jzus.B2400257

# A novel bacterial strain for the removal of ammonia nitrogen from wastewater: *Pseudomonas oleovorans* QS-7

Hao QIU[1,3], Min LIAO[1,3]✉, Xiaomei XIE[1,2], Xinyue LU[1,3], Feng YUAN[1,3], Zhe LUO[1,3], Chunlin FAN[4]

[1]*College of Environmental and Resource Science, Zhejiang University, Hangzhou 310058, China*
[2]*National Demonstration Center for Experimental Environmental and Resources Education (Zhejiang University), Hangzhou 310058, China*
[3]*Zhejiang Provincial Key Laboratory of Agricultural Resources and Environment, Hangzhou 310058, China*
[4]*Ningbo Ferno Biotechnology Co., Ltd., Ningbo 315502, China*

**Abstract:** Excessive ammonia nitrogen has been demonstrated to cause a serious hazard to water environments. Bacteria performing nitrification and denitrification (SND) simultaneously can be effective biological instruments to remove ammonia nitrogen completely from effluents. For the first time, *Pseudomonas oleovorans* QS-7 with SND function, isolated from the biogas treatment system of a pig farm, was found to efficiently remove ammonia nitrogen. Through the determination of key enzymes and functional genes related to the nitrogen metabolism of strain QS-7, combined with nitrogen balance measurements of the nitrogen metabolic process, it was speculated that the SND pathway of the novel strain is $NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$. QS-7 exhibited 98.6% ammonia nitrogen removal and a maximum ammonia degradation rate of 9.2 mg/(L·h) at 18 h in 100 mg/L ammonia nitrogen solution. This strain also has a certain capacity to remove nitrate and nitrite nitrogen; the maximum removal efficiencies were 54.22% and 73.93%, respectively, in systems with 100 mg/L of nitrate or nitrite nitrogen as the sole nitrogen source. Nitrogen metabolic balance analysis for QS-7, using ammonia (100 mg/L) as the sole nitrogen source, demonstrated that assimilation (56.1%) is the main mode of nitrogen removal, followed by conversion to $N_2$ (43.6%). Meanwhile, the presence of $NO_2^-$ was not detected eventually and almost no $NO_x$ was produced, which indicates that the nitrogen removal process of QS-7 is an environmentally friendly one. The optimal environmental conditions for QS-7 were found to be sodium citrate as carbon source, C/N=10, pH=7.0, 150 r/min, and 30 °C. The above results indicate that QS-7 may provide a material and conceptual basis for the advancement of SND technology.

**Key words:** Simultaneous nitrification and denitrification; Ammonia wastewater; Removal of ammonia nitrogen; *Pseudomonas oleovorans*; Metabolic pathway

unedited

## 1 Introduction

At present, a significant volume of ammonia nitrogen-polluted wastewater, such as farmland drainage, livestock and poultry effluent, is discharged into the environment with no or incomplete treatment, making ammonia nitrogen emissions a serious threat to the environment. Ammonia nitrogen has overtaken chemical oxygen demand (COD) to become the primary indicator of surface water quality, as well as the main cause of eutrophication in water (Dong et al., 2019). The accumulation of ammonia nitrogen in water can disrupt the ecosystem balance and affect human health through various pathways (Zhang Y et al., 2022; Ma et al., 2024). Therefore, some treatment measures for ammonia nitrogen-polluted water are necessary before discharge into the environment. The commonly used physicochemical method of ammonia stripping is based on Henry's law, which utilizes the dynamic equilibrium of $NH_3$ and $NH_4^+$ to remove ammonia nitrogen from water. However,

✉ Min LIAO, liaomin@zju.edu.cn
Min LIAO, https://orcid.org/0000-0001-9078-204X

Received May 21, 2024; Revision accepted Oct. 19, 2024;
Crosschecked xxx. xx, 20xx; Published online xxx. xx, 20xx

© Zhejiang University Press 2024

this method only converts ammonia nitrogen from dissolved form to gaseous form, ultimately causing atmospheric pollution. Moreover, the stripping process relies on high pH and temperature, which often proves costly (Provolo et al., 2017). Chemical precipitation uses magnesium and phosphate ions and is affected by the saturation of the reaction process, which has been mainly utilized to treat effluents with high concentrations of ammonia nitrogen. However, its application is limited by the need for large amounts of chemicals, the incomplete utilization of introduced phosphates, and a tendency to lead to secondary contamination (Dong et al., 2019). The traditional biological nitrogen removal technique usually utilizes microorganisms such as ammonia-oxidizing bacteria and nitrifying bacteria to transform $NH_4^+$-N to $NO_3^-$-N, subsequently converted to gas and released into the air by denitrifying bacteria. In the above process, the nutritional mode of nitrifying bacteria is chemolithoautotrophy, while denitrifying bacteria are heterotrophic organisms, with different nutrient conditions required for their growth. In addition, anaerobic ammonia-oxidizing bacteria and denitrifying bacteria often do not require oxygen for growth, while nitrifying bacteria are divided into two types: aerobic and facultative, indicating their different oxygen requirements. The conventional microbial ammonia nitrogen removal procedure has the disadvantages of system complexity, poor stability, high operation cost, etc. (Klontza, 2022; Xi et al., 2022). Therefore, there is an urgent need for new, cost-effective means to remove ammonia nitrogen from effluents.

Some heterotrophic bacteria can perform simultaneous nitrification and denitrification (SND) under aerobic conditions, simplifying conventional biological methods and reducing costs. This process addresses the shortcomings of anaerobic ammonia oxidation and short-range nitrification-denitrification, often yielding better deamination results (James and Vijayanandan, 2023). Nowadays, new types of SND bacteria are increasingly identified from activated sludge, industrial wastewater, or paddy soil. Examples include *Klebsiella sp.*(Ren et al., 2024), *Acinetobacter sp.*(Ke et al., 2024), and *Halomonas sp.* (Liu and Wu, 2021). However, the elimination of ammonia nitrogen by SND frequently leads to the buildup of nitrite (Ge et al., 2015), the presence of which might affect bacterial growth and metabolic processes, impairing the overall microbial ability to remove nitrogen (Chen et al., 2024). Therefore, it is crucial to search for new strains that can effectively remove ammonia nitrogen without accumulating inorganic nitrogen. Besides, the SND process is sensitive to environmental factors, such as changes in external conditions such as organic carbon sources, temperature, and pH. Moreover, in practical applications, nitrogenous wastewater often exhibits complex and diverse characteristics. To sustain effective treatment for nitrogen removal by a specific strain in real-world applications, it is crucial to understand that strain's ideal treatment conditions and metabolic processes. In this study, *Pseudomonas oleovorans* QS-7 (hereinafter referred to as QS-7), with the ability to efficiently remove ammonia nitrogen, was separated from a biogas treatment system, and its nitrogen removal ability and metabolic pathways were explored. This is the first report on the removal of ammonia nitrogen exclusively by *Pseudomonas oleovorans*. To better understand the metabolism of QS-7, nitrogen balance analysis, enzyme activity determination related to nitrogen conversion, and nitrogen metabolism-related gene profiling were conducted. Additionally, the environmental factors that may influence ammonia nitrogen removal performance by QS-7 were comprehensively explored.

## 2 Materials and methods

### 2.1 Figures

Nutrient broth (NB) medium was used for the isolation and enrichment of strains during the screening process, consisting of (g/L) 10 peptone, 5 NaCl, 3 beef extract, and 1 glucose.

Basic medium (BM-100mg/L $NH_4^+$-N) was utilized to characterize the ammonia nitrogen elimination efficiency, consisting of (g/L) 0.472 $(NH_4)_2SO_4$, 4.065 sodium citrate, 0.15 $K_2HPO_4$, 0.20 $MgSO_4$, and 2 mL elemental supplement. The elemental supplement was composed of (g/L) 5 $MnCl_2{\cdot}4H_2O$, 15 EDTA, 0.50 $CuSO_4{\cdot}5H_2O$, 0.20 $ZnSO_4$, 0.30 $CoCl_2{\cdot}6H_2O$, 0.50 $FeSO_4{\cdot}7H_2O$, 0.20 $Na_2MoO_4{\cdot}2H_2O$, and 0.10 $CaCl_2$.

Replacing 0.472 g $(NH_4)_2SO_4$ from BM with 0.722 g $KNO_3$ and 0.493 g $NaNO_2$, respectively, yielded

Unedited

denitrification medium (DM1-100 mg/L $NO_3^-$-N) and short-cut denitrification media (DM2-100 mg/L $NO_2^-$-N). Replacing the nitrogen source of DM1 and DM2 with 0.0722 g $KNO_3$ and 0.0493 g $NaNO_2$, respectively, yielded low concentrations of denitrification medium (DM3-10 mg/L $NO_3^-$-N) and short-cut denitrification media (DM4-10 mg/L $NO_2^-$-N). To prepare the corresponding solid media, 3%-4% agar was added to the above media.

Before being used, the media were sterilized for 30 min at 121 °C in an autoclave. The pH of the media was adjusted to neutral (pH=7) with phosphate buffer before inoculation.

### 2.2 Microbial screening and isolation

The samples were taken from the grain husk filter media of a pig farm's biogas treatment system. 3 mL of the original sample was aspirated and inoculated into 100 mL BM in a 250 mL conical flask, which was subsequently incubated at 30 °C and 150 r/min for 3 d. This process was repeated three times to screen for strains that survive high concentrations of ammonia nitrogen (Gao et al., 2023). Finally, the bacterial suspension obtained from the BM was inoculated in 100 mL NB and enriched for 24 h, then spread on solid BM at different concentrations and incubated in a thermostat at 35 ℃. until single colonies with different characteristics were formed, which were enriched by inoculating separately in 100 mL NB, while the enriched bacterial suspension was inoculated at 2% (volume fracton) into 100 mL BM. The denitrification capacity of each isolated strain was separately determined. Strains with higher removal efficiencies of total nitrogen (TN) and ammonia nitrogen were selected and later purified by inoculation onto solid BM and repeating the streaking step until the TN and ammonia nitrogen removal efficiency reached a stable level. These strains were then chosen for subsequent experiments.

### 2.3 Strain identification

The selected strains were enriched in NB, and an appropriate number of bacteria was extracted to obtain DNA. TransStart Fastpfu DNA Polymerase in a 50 μL reaction system was used for PCR amplification, and the product was sequenced by BIOZERON Biotech Co., Ltd. (Shanghai, China). The result was submitted to NCBI and compared with different bacteria by BLAST to determine the strain species (Liu et al., 2023). Multiple sequence comparison analysis was performed and a phylogenetic tree was created using the MEGA 11.0 program. After fixation, dehydration and drying an appropriate amount of enriched bacterial liquid, topographic characterization of the samples was performed with a scanning electron microscope (SU8010). The isolated strains were preserved in 25% (volume fraction) glycerol solution and frozen for storage in a commercial refrigerator at -20℃.

### 2.4 Ammonia nitrogen removal capacity and nitrogen (N) balance

The only nitrogen source used to assess the capacity of QS-7 for removing ammonia nitrogen was ammonia nitrogen at a concentration of around 100 mg/L. The bacterial suspension was inoculated at 2% (volume fraction) into 100 mL BM, which was then transferred to 250 mL conical flasks and covered with a breathable sealing film to ensure aerobic conditions. The strain was placed in a shaker at 30 °C and 150 r/min for 36 h. Samples were collected at three-hour intervals to determine the bacterial concentration ($OD_{600nm}$) and the content of TN, $NH_4^+$-N, $NO_3^-$-N, and $NO_2^-$-N, in order to evaluate the growth status of the strain and its ability to remove inorganic nitrogen. To clarify the transformation path of ammonia nitrogen in the process removal by QS-7, a nitrogen balance experiment was performed. A 100 mL serum bottle was prepared and filled with 40 mL BM, which was then inoculated with bacterial suspensions at the same ratio of 2% (v/v). To ensure an aerobic environment for QS-7, pure oxygen was continuously passed into the serum bottle for 1 minute. Afterwards, the samples were placed in a shaker under the same conditions. The gas was collected from the bottle after 36 h to quantify the amount of gaseous nitrogen produced. Liquid samples were collected and the amount of inorganic nitrogen in each form was determined as Organic Nitrogen = TN - Inorganic Nitrogen - Intracellular N (Chen et al., 2023). Samples were set up in three parallel groups with uninoculated medium serving as the control group.

unedited

All experimental manipulations were performed on an ultra-clean bench.

### 2.5 Investigation of optimal nitrogen removal conditions

Experiments were designed to determine the removal efficiency of strain QS-7 (hereinafter referred to as QS-7) for ammonia nitrogen in water under different environmental conditions, such as carbon source, carbon (C)/nitrogen (N) ratio, pH, temperature, and dissolved oxygen (DO). Glucose, sodium acetate, and sodium succinate were used separately to replace sodium citrate in BM, as a means of investigating the ideal carbon source for ammonia nitrogen removal. The ammonia nitrogen content of BM was kept constant, while the content of sodium citrate was varied, thereby adjusting the C/N ratios to be 3, 7, 10, 15, and 18. The initial pH of BM was changed by HCl and NaOH, with the setting of five values (5, 6, 7, 8, and 9) along a gradient. Different temperature conditions such as 25, 30, 35, and 40 °C were set up by changing the shaker temperature. The rotational speed was used to characterize dissolved oxygen changes, with the shaker speeds of 90, 120, 150, and 180 r/min corresponding to dissolved oxygen concentrations of 3.6, 4.3, 5.3, and 6.5 mg/L. The experiments were performed in 250 mL conical flasks containing 100 mL BM with 2 mL bacterial suspension added to each sample. The liquid samples were collected after 36 h to determine the relevant indexes of the growth status and the denitrification performance of the strain. The results were expressed as means ± standard deviation from three replicates.

### 2.6 Enzyme assays and PCR amplification of functional genes

The QS-7 strain was enriched in NB for 24 h at 30 °C and 150 r/min. The bacteria were gathered by centrifugation from the suspension (4 °C, 20 min, 8000 r/min) and then washed with PB buffer (pH=7.4) to remove impurities. Afterwards, they were lysed using ultrasound and centrifuged to obtain a crude enzyme extract (4 °C, 20 min, 10,000 r/min). BCA Protein Assay Kit (Labgic, Beijing, China) was employed to measure the protein concentration in the crude enzyme solution. The material composition of the reaction systems for different key enzyme activity assays was shown in Table 1. All reaction systems (20 mL) contained 4 mL of the crude enzyme solution (Xu et al., 2021). The concentrations of various types of inorganic nitrogen were detected 15 min after adding this solution. The amount of nitrogen (μmol) reduced by an enzyme in the system every minute was defined as one unit (U) of enzyme activity, while the U per mg of protein in the crude enzyme solution was defined as the specific activity of the enzyme (U/mg). The genomic DNA of QS-7 was extracted from the bacterial suspension as a template for PCR amplification to further validate the metabolic pathway of the strain for the removal of ammonia nitrogen. The amplified functional genes were ammonia monooxygenase (*amoA*), hydroxylamine oxidase (*hao*), periplasmic nitrate reductase *(napA*), nitrite reductase (*nirS*), nitric oxide reductase (*norB*), nitrous oxide reductase (*nosZ*), and copper-containing nitrite reductase (*nirK*). The corresponding primers and PCR amplification conditions were according to the literature (Xu et al., 2021).

### 2.7 Analytical methods

Bacterial concentration was measured at 600 nm ($OD_{600nm}$) by a UV-spectrophotometer (Shimadzu Co., Japan) to indicate the growth of the QS-7 strain. The determination of $NH_4^+$-N and $NO_3^-$-N was performed using salicylic acid-hypochlorite spectrophotometry and hydrochloric acid-spectrophotometry, respectively. Since alkaline potassium persulfate converts nitrogenous compounds to nitrate nitrogen, TN content was also determined using hypochlorite spectrophotometry. The concentrations of $NO_2^-$-N and $NH_2OH$ were measured by spectrophotometry and indirect spectrophotometry, respectively (Chen et al., 2024). The pH was measured by a precision pH meter (SG2, METTLER TOLEDO, China). $N_2O$ concentrations were determined by gas chromatography (GC-2010 Plus Shimadzu, Japan) and $N_2$ concentrations were determined by gas chromatography-mass spectrometry (GC/MS, Agilent, USA). Dissolved oxygen was measured by the electrochemical probe method. The following formula was used to calculate the removal rate: $R_1=(D_1-D_2)/D_1\times100\%$ (the nitrogen concentrations were initially represented by $D_1$ and finally by $D_2$); $R_2=(C_2-C_1)/\triangle t$ (where $C_2$-$C_1$ represents the change in nitrogen content over time $\triangle t$, $\triangle t=t_2-t_1$) (Wu et al., 2023). Origin 2023b and Excel were

Uncorrected

used to carry out the graphical and statistical work. Data were analyzed by one-way ANOVA (analysis of variance) with Tukey's HSD (Honestly Significant Difference) test ($P$<0.05) using SPSS 26.0 software. The results were expressed as means ± standard deviation from three replicates.

## 3 Results and discussion

### 3.1 Separation and identification

After three rounds of nitrogen removal efficiency measurements, the isolated strain showed a stable and efficient ability to remove ammonia nitrogen. Mature colonies formed by QS-7 on solid BM were characterized by off-white color, round shape, smooth surface, regular margins, and they were Gram-negative. The scanning electron microscopy results indicated that QS-7 was short rod-shaped (Approx. 1.2-1.6 μm×0.5 μm) (Fig. 1). According to the Blast results, QS-7 exhibited 99% similarity to *Pseudomonas oleovorans* JCM 13978, suggesting that it belonged to the genus *Pseudomonas*. This information was used to construct the neighbor-joining phylogenetic tree (Fig. 2). The 16S rRNA sequence (1421 bp) of QS-7 was held in the GenBank database with the accession number PP411421.

### 3.2 Ammonia nitrogen removal capacity

Regardless of whether QS-7 was inoculated into 100 mL BM with sodium citrate removed or in BM with sodium carbonate substituted for sodium citrate, there was no increase in $OD_{600nm}$ and no decrease in TN content during the experiment (data not shown), demonstrating the heterotrophic nature of QS-7. When ammonia nitrogen was the only nitrogen source, as shown in Fig. 3, the $OD_{600nm}$ value of the strain was 0.013 at the beginning of inoculation. From 6 to 12 h, QS-7 was in the logarithmic period of growth with the fastest increase rate of $OD_{600nm}$, and reached the stabilization phase at 18 h with a peak $OD_{600nm}$ of 1.314. The $NH_4^+$-N content of BM began to quickly decrease at the early stage of inoculation and reached a maximum removal rate of 9.2 mg/L/h at 9-12 h. In comparison, the maximum removal rate of *Exiguobacterium mexicanum* SND-01 (Cui et al., 2021) and *Lysinibacillus fusiformis* B301 (Wu et al., 2023) were significantly lower at 2.24 mg/L/h and 2.11 mg/L/h, respectively, than that of QS-7. The maximum ammonia nitrogen eliminate efficiency of 98.6% was achieved at 18 h and the decreasing trend of TN content in the medium was basically consistent with that of $NH_4^+$-N. After 18 h, the $OD_{600nm}$ of QS-7 showed a decrease, likely due to the diminishing supply of organic carbon and nitrogen sources in the medium as the reaction progressed. Correspondingly, the content of $NH_4^+$-N in the medium showed a minor rebound during this time, which some reports attribute to the release from decomposition by microorganisms at a competitive disadvantage (Lei et al., 2016). Throughout the degrading process, $NO_2^-$-N was not found when ammonia nitrogen was the only nitrogen supply, which is inconsistent with past reports, suggesting that $NO_2^-$-N accumulates during the process (Zhang et al., 2014). Trace amounts of $NO_3^-$-N were present in the early stage but gradually disappeared as the reaction progressed. The degradation of ammonia nitrogen by QS-7 was carried out in a single reactor, which demonstrated that QS-7 can be of great value to building an economic technology for the removal of ammonia nitrogen in effluents (Xie et al., 2021).

### 3.3 Verification of denitrification capacity of strain QS-7

A small amount of $NO_3^-$-N accumulated at the beginning of the strain's growth but it gradually disappeared over time (Fig. 3), which result was similar to the degradation process of strain LJ81 (Lei et al., 2019) and *Acinetobacter sp.* ND7 (Xia et al., 2020). The reason might be the oxidation of nitrite at the preliminary stage, proving that QS-7 has denitrification ability during the nitrification process. To further investigate the denitrification capacity of QS-7, it was inoculated into DM1 and DM2 under aerobic conditions. Unlike strain NR which is unable to take advantage of single nitrate and nitrite (Zhao et al., 2012), QS-7 grew in both DM1 and DM2. When QS-7 was inoculated into DM2 (Fig. 4b), it entered into a logarithmic growth phase at 12 h, and its growth reached the peak at 24 h with an $OD_{600nm}$ value of 1.05. The maximum degradation rate of

Uncorrected

$NO_2^-$-N was 8.33 mg/L/h at 18-24 h, and the maximum removal efficiency of $NO_2^-$-N was 73.93%. When the strain was inoculated into DM1 (Fig. 4a), the reduction of nitrate content was detected; however, the growth rate of QS-7 was slower in this state, the maximum degradation rate of nitrate appeared after 24 h at 5.64 mg/(L·h), and the maximum removal efficiency of $NO_3^-$-N was 54.22%. In comparison to QS-7 in ammonia nitrogen, it exhibited a lag in the treatment of a single identical concentration of nitrate nitrogen. Because nitrite has less oxidized nitrogen, thus is more easily absorbed by bacterial growth, QS-7 grown in DM2 showed faster rates of nitrogen removal and bacterial growth than QS-7 grown in DM1 (Yang et al., 2019). Under the same conditions, QS-7 could not completely remove nitrate and nitrite nitrogen at a concentration of about 100 mg/L as it did for ammonia nitrogen (Figs. 4a and 4b). It was assumed that the concentration of oxidized nitrogen in DM1 and DM2 was too high, and the carbon source was not sufficient to help QS-7 completely remove the nitrogen source. When adjusting the initial nitrogen concentration to about 10 mg/L, QS-7 achieved the complete removal of nitrate and nitrite nitrogen (Figs. 4c and 4d), which was in line with the dynamic characteristics of nitrate nitrogen in Fig.3 and confirmed denitrification in the process of ammonia nitrogen removal by QS-7. The increase in pH (Fig. 5) with the biochemical reaction time extended in three different nitrogen source systems also demonstrated the presence of denitrification by QS-7, due to this process consuming protons (Ren et al., 2014). In addition, compared with DM1 (Fig. 5), the pH was relatively low in BM at the beginning of the biochemical reaction (0-15 h), which is because $NH_4^+$-N needs to be converted to $NO_3$-N before the denitrification reaction could be carried out, thus the rise of pH at the beginning of the reaction was slower. Subsequently, the ammonia oxidation and denitrification in BM occurred rapidly and simultaneously (Fig. 3), and the depletion rate of TN in BM (Fig. 3) was significantly higher than DM1 (Fig. 4a), which implied more proton consumption, leading to the growth rate of pH being faster (Fig. 5). In contrast, the pH in DM2 was the slowest to rise at the beginning of the biochemical reaction, because nitrite nitrogen was toxic to the strain (Chen et al., 2024), which needed time to adapt. When the biochemical reaction had been carried out for 36 h, the consumption rate of TN in DM2 was higher than DM1 (Figs. 4a and 4b), but lower than BM (Fig. 3). Overall, the pH growth rate of the whole biochemical reaction process in DM2 was faster than DM1 but slower than BM (Fig. 5). Some heterotrophic nitrifying bacteria could neither utilize nitrate nor nitrite (Zhao et al., 2010), but QS-7 could carry out aerobic denitrification to remove $NO_3^-$-N and $NO_2^-$-N, which proves its application value.

### 3.4 Nitrogen balance analysis

In order to understand the transformation direction and mechanism of the ammonium nitrogen removal process, the nitrogen content of different forms in the conversion system was analyzed at the maximum removal rate of ammonium nitrogen. A nitrogen balance analysis of the $NH_4^+$-N conversion was carried out, and the results were shown in Table 2. The content of nitrogen-containing intermediates was detected after 36 h. It was noted that neither $NO_2$-N nor $NO_3$-N could be found in the medium, and $NH_4^+$-N was consumed up to 93.2%. Based on the calculation of N balance, (47.8±3.2) mg/L of the original $NH_4^+$-N became a source of intracellular nitrogen (N). QS-7 utilizes ammonia nitrogen in BM mainly through assimilation, which is similar to the effect of strain DK-6 (Liao et al., 2022). Gas chromatography was used to investigate the gaseous nitrogen components, and (41.2 ± 2.3) mg/L of $N_2$ and negligible quantities of $N_2O$ were found, which can be considered as having a low environmental impact. Combined with the experimental results in Sections 3.2 and 3.3, it can be seen that QS-7 had the best effect on ammonia nitrogen removal at 18 h, but at this time, there was residual $NO_3$-N. Due to the lag in the strain's treatment of nitrate nitrogen, it was not until after 36 h that QS-7 achieved the complete removal of nitrate nitrogen, and at this time, the death of microorganisms led to the increase of ammonia nitrogen content in the environment. Considering this result, the appropriate treatment time can be selected for practical application.

### 3.5 Analysis of metabolic pathways

The mechanism of ammonia degradation by QS-7 were established based on the expression status of the relevant enzymes such as AMO, HAO, NR, and NIR (Liao et al., 2022). Among them, AMO and HAO are

Unedited

expressed during ammonia oxidation, as they oxidize $NH_4^+$-N to $NH_2OH$ and $NH_2OH$ to $NO_2^-$-N, respectively (Xu et al., 2021). NR and NIR, on the other hand, are expressed during denitrification (He et al., 2020). To understand the denitrification pathway of QS-7 during heterotrophic denitrification under aerobic circumstances, the activities of the above-related enzymes were detected, and the outcomes demonstrated that each of them could be effectively produced in cell extracts (Table 3). AMO exhibited a higher specific enzyme activity (0.921 U/mg), comparable to *Pseudomonas taiwanensis* EN-F2 (Zhang et al., 2022). The specific activity of HAO (0.729 U/mg) in QS-7 was significantly higher than in *Pseudomonas taiwanensis* J488 (0.049 U/mg) (He et al., 2021) and *Pseudomonas malodora* ZN1 (0.158 U/mg) (Zhang et al., 2019). When NADH was used as the electron donor, NR (0.135 U/mg) exhibited a higher specific enzyme activity than NIR (0.120 U/mg) in QS-7, the former being slightly lower than that of *Glutamicibacter arilaitensis* EM-H8 (0.314 U/mg) (Chen et al., 2023), but much higher than that of *Pseudomonas putida* Y-9 (0.0018 U/mg) (Huang et al., 2019). Compared to NR and NIR, QS-7's specific activity of AMO was significantly higher, which explains that QS-7 is more adept at utilizing $NH_4^+$-N as a nitrogen source. The expression of NIR in QS-7 was lower than that of NR, further explaining why QS-7 showed $NO_3^-$-N accumulation but not $NO_2^-$-N accumulation early in the degradation process. The ability of QS-7 to perform the SND process was proved by the expression of all relevant enzymes. In the analysis of genes in QS-7 related to nitrogen metabolism, the following were successfully amplified: the genes of *amoA* and *hao* characterizing nitrification (Xia et al., 2020), *napA* playing a role in the conversion of $NO_3^-$ to $NO_2^-$ under aerobic conditions (Ouyang et al., 2020), *nirS* participating in catalyzing the conversion of $NO_2^-$ to NO (Philippot et al., 2011), *norB* gene participating in catalyzing the conversion of NO to $N_2O$ (Gui et al., 2017), and *nosZ* gene participating in catalyzing the conversion of $N_2O$ to $N_2$ (Gui et al., 2017) (Fig. 6). Meanwhile, the *nirk* gene with the same function as *nirS* was not found in QS-7. Therefore, after combining the analysis results of functional enzymes, genes related to nitrogen metabolism, and N metabolic balance, it was inferred that the SND pathway in QS-7 was $NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ (Fig. 7).

### 3.6 Optimal conditions of ammonia nitrogen removal

3.6.1 Carbon sources

The primary nutrients required for the growth of heterotrophic bacteria comprise organic materials; therefore, extra carbon compounds are needed as a source of energy and electrons for heterotrophic bacteria. There was a significant difference between the ammonia nitrogen removal capacities of carbon source species (Table 4). Among the selected carbon sources, QS-7 showed an inability to utilize glucose and sodium acetate. As shown by the $OD_{600nm}$ value change, the strain's growth potential was limited when utilizing glucose. The reason for this was likely that due to the metabolic process, the pH of the medium gradually decreases and eventually generates acidic conditions, hindering the growth of QS-7. Meanwhile, QS-7 was completely unable to grow when utilizing sodium acetate, and at the same time, there was no change in ammonia nitrogen content, indicating that sodium acetate inhibits the activity of QS-7. However, when QS-7 extracted from NB was inoculated into the new medium, QS-7 still had certain activity, resulting in a small amount of $NH_4^+$-N being assimilated and converted into oxidized nitrogen through nitrification, which indicated that a small amount of ammonia nitrogen was removed (6.85%) (Table 4).

QS-7 exhibited effective ammonium removal and development when utilizing sodium citrate; at the end of the reaction, the removal efficiencies of ammonia and TN were measured to be 94.31% and 93.07%, respectively. When utilizing sodium succinate, its degradation of $NH_4^+$-N (84.09%) and TN (83.28%) were slightly worse compared to sodium citrate under the same environmental conditions. The latter two carbon sources are organic acids with simple molecular structures, which could be added to the metabolic process directly and without modification, possibly explaining the situation mentioned above (Gao et al., 2023). The remaining carbon sources more easily enter the tricarboxylic acid cycle (TCA) than glucose, facilitating QS-7's development and improving its degradation efficiency. Sodium citrate as the best choice for QS-7 had higher $NH_4^+$-N and TN removal efficiency than other media, similar to that of *Glutamicibacter arilaitensis* EM-H8 (Chen et al.,

Unedited

2023).

3.6.2 C/N ratios

Table 4 shows how the removal effectiveness of QS-7 on $NH_4^+$-N varied under different C/N ratio circumstances. At lower C/N ratios, the QS-7 strain demonstrated a reduced capacity for $NH_4^+$-N degradation. Whether the ratio was 3 or 7, it was not beneficial for QS-7 to function; 32.72% and 17.86% of $NH_4^+$-N and TN, respectively, remained in BM after 36 h of reaction. When the C/N ratio > 10, QS-7 showed significant differences in its ability to eliminate ammonia nitrogen compared to lower C/N ratio conditions (Table 4); as the C/N ratio was increased to 15 or 18, its $NH_4^+$-N removal reached 96.3% and 97.1%, respectively, at the end of reaction. However, the removal efficiency for TN and cell growth condition did not increase significantly compared to the C/N ratio of 10 (Table 4). Considering the economic benefits, the ideal C/N ratio for QS-7 is 10. QS-7 also showed lower $OD_{600nm}$ for a lower C/N ratio compared to environmental conditions with a higher C/N ratio; the biomass increased with increasing C/N ratio. The removal of ammonia nitrogen increased with rising C/N ratio in a certain a range, a phenomenon that was also observed for *Stutzerimonas frequens* TF18 (Liu et al., 2023), but different from that exhibited by *Acinetobacter sp.* Y16 (Huang et al., 2013). The latter has an ideal C/N ratio of 2 and the removal efficiency decreases with both increasing and decreasing C/N ratio. The above results indicate that QS-7 could remove a lot of ammonia nitrogen with high contents of organic loading. Therefore, QS-7 can be more advantageous for application on effluents containing high organic carbon, such as livestock and poultry wastewater or municipal waste leachate.

3.6.3 Environmental pH

One of the key elements influencing the effectiveness of N removal is the initial pH. Table 4 displays QS-7's cell growth and heterotrophic nitrification capacity under various pH values. After incubating in BM for 36 h, the removal rates for $NH_4^+$-N (8.33%) and TN (2.74%) at pH=5 showed significant differences compared to other pH conditions ($p$<0.05), because QS-7 could not sustain growth under acidic conditions. At a starting pH of 7, the $NH_4^+$-N removal efficiency was 92.6%, and the final $OD_{600nm}$ was 0.921. Then, as pH rose, QS-7's growth conditions and denitrification efficiency improved. Therefore, it was considered that alkaline conditions were more favorable for QS-7 to perform nitrogen removal. This is consistent with the preferences of strains SND-01 (Cui et al., 2021) and *Bacillus* N31 (Huang et al., 2017). The reason is that during heterotrophic nitrification, the substrate for the AMO enzyme that catalyzes ammonium oxidation is $NH_3$-N rather than $NH_4^+$-N. Under alkaline conditions, the medium contains more free $NH_3$-N, providing optimal conditions for AMO (Li et al., 2024). When the initial pH was 6, its pH also increased to 9.35 after 36 h, presumably due to the alkaline metabolites and denitrification process during the growth of the strain (Cui et al., 2021). In our study, QS-7 grew in a broad range of pH values, indicating its versatility in practical scenarios.

3.6.4 Dissolved oxygen

Dissolved oxygen (DO) concentration has an essential effect on the SND process of strains (Table 4). Generally, an ideal DO level is required for the SND process to be carried out, attributed to the fact that DO can both suppress the expression of $N_2O$ reductase and function as an acceptor of electrons during the nitrogen removal process (Hu et al., 2023). The amount of DO in the culture solution was regulated by the shaker's spinning speed, with the amount of DO in the conical flask increasing with growing shaking rate, owing to the increased rotation rate quickening the mass transfer of oxygen. The $OD_{600nm}$ value at different rotational speeds showed significant differences (Table 4), as the rotational speed was increased. Meanwhile, the $OD_{600nm}$ values reflecting the growth status of QS-7 also increased, enhancing the removal efficiency of $NH_4^+$-N and TN significantly ($P$<0.05) (Zhang et al., 2019). At shaking rates of 90, 120, 150, and 180 rpm, the approximate DO concentrations in the culture solution were 3.6, 4.3, 5.3, and 6.5 mg/L, respectively. The effectiveness of $NH_4^+$-N removal improved steadily as the DO content rose from 3.6 mg/L to 6.5 mg/L, which demonstrated QS-7’s tolerance to high concentrations of oxygen.

Unedited

3.6.5 Temperature

For most of the SND strains, the optimal temperature range is 30-37 ℃ (Carneiro Fidélis Silva et al., 2019). As shown in Table 4, higher temperatures improved the capacity of QS-7 to remove N, which might be attributed to its rapid growth under this condition and the enhanced expression of relevant enzymes. When the temperature exceeded 30℃, there was no significant difference ($P$>0.05) in the removal rates of $NH_4^+$-N and TN by QS-7, whereas the removal rate of $NH_4^+$-N and TN by QS-7 under 40 °C was slightly lower than that at 35 °C. Unlike *Acinetobacter sp*. Y16 (Huang et al., 2013), which showed a rapid decrease in $NH_4^+$-N removal at temperatures greater than 20 °C, QS-7 still had a strong ability to remove $NH_4^+$-N and TN at 40 °C. Moreover, compared with *Acinetobacter tandoii* MZ-5 (optimal temperature 25-30 ℃) (Ouyang et al., 2020) and *Pseudomonas stutzeri* T13 (optimal temperature 30-37 ℃) (Ma et al., 2015), QS-7 had a stronger ability to remove $NH_4^+$-N at 25-40 ℃. It was proved that QS-7 was more adaptable to a high-temperature environment and could tolerate a wider range of temperature conditions.

## 4 Conclusions

A new *Pseudomonas oleovorans* strain QS-7 was isolated from grain husk filter media in a pig farm's biogas treatment system, which could remove ammonia nitrogen from wastewater through SND, with a maximum $NH_4^+$-N removal efficiency of 98.6% in a short time. The SND pathway of QS-7 was determined as $NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$. QS-7 was also found to have a certain removal effect on a single nitrogen source of nitrate or nitrite, while the corresponding removal efficiencies were significantly lower than that of ammonia nitrogen. When the nitrogen source was ammonia or nitrate, $NO_2^-$-N did not build up during the entire procedure, and almost no $N_2O$ was produced. QS-7 seems to be well-adapted to various external environmental conditions, while the ideal ambient conditions for QS-7 to remove ammonia nitrogen were found to be sodium citrate as carbon source, C/N=10, pH=7.0, 150 r/min, and 30 °C. Overall, strain QS-7 was demonstrated to be effective in purifying ammonia nitrogen-polluted wastewater, making it an excellent choice for this task.

unedited

### Data Availability Statement

The authors declare that the data supporting the findings of this study were available within the article.

### Acknowledgments

A Project Supported the National Key Research and Development Project of China (2023YFD1702003)

### Author contributions

Hao QIU performed the experimental research and data analysis, wrote and edited the manuscript. Xinyue LU, Feng YUAN and Zhe LUO participated in experimental research and data analysis. Min LIAO and Xiaomei XIE contributed to the study design, data analysis, writing and editing of the manuscript. Chunlin FAN provided the original sample. All authors read and approved the final manuscript and, therefore, had full access to all the data in the study and take responsibility for the integrity and security of the data.

### Compliance with ethics guidelines

Hao QIU, Min LIAO, Xiaomei XIE, Xinyue LU, Feng YUAN, Zhe LUO, Chunlin FAN declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

## References

Carneiro Fidélis Silva L, Santiago Lima H, Antônio de Oliveira Mendes T, et al., 2019. Heterotrophic nitrifying/aerobic denitrifying bacteria: Ammonium removal under different physical-chemical conditions and molecular characterization. *J Environ Manage*, 248:109294.
https://doi.org/10.1016/j.jenvman.2019.109294

Chen M, He T, Wu Q, et al., 2023. Enhanced heterotrophic nitrification and aerobic denitrification performance of *Glutamicibact -er arilaitensis* EM-H8 with different carbon sources. *Chemosphere*, 323:138266.
https://doi.org/10.1016/j.chemosphere.2023.138266

Chen X, Li Shuangfei, Zhang W, et al., 2024. A Newly Isolated *Rhodococcus* sp. S2 from Landfill Leachate Capable of Heterotrophic Nitrification and Aerobic Denitrification. *Water-Sui*, 16:431.
https://doi.org/10.3390/w16030431

Cui Y, Cui Y-W, Huang J-L, 2021. A novel halophilic Exiguobacterium mexicanum strain removes nitrogen from saline wastewat -er via heterotrophic nitrification and aerobic denitrification. *Bioresour. Technol*, 333:125189.
https://doi.org/10.1016/j.biortech.2021.125189

Dong Y, Yuan H, Zhang R, et al., 2019. Removal of Ammonia Nitrogen from Wastewater: A Review. *T Asabe* 62, 1767–1778.
https://doi.org/10.13031/trans.13671

Gao Y, Zhu J, Wang K, et al., 2023. Discovery of a heterotrophic aerobic denitrification *Pseudomonas* sp. G16 and its unconventional nitrogen metabolic pathway. *Bioresour. Technol*, 387:129670.
https://doi.org/10.1016/j.biortech.2023.129670

Ge Q, Yue X, Wang G, 2015. Simultaneous heterotrophic nitrification and aerobic denitrification at high initial phenol concentration by isolated bacterium *Diaphorobacter* sp. PD-7. *Chin. J. Chem. Eng*, 23:835–841.
https://doi.org/10.1016/j.cjche.2015.02.001

Gui M, Chen Q, Ni J, 2017. Effect of sulfamethoxazole on aerobic denitrification by strain *Pseudomonas stutzeri* PCN-1. *Bioresour. Technol*, 235:325–331
https://doi.org/10.1016/j.biortech.2017.03.131

He T, Chen M, Ding C, et al., 2021. Hypothermia *Pseudomonas taiwanensis* J488 exhibited strong tolerance capacity to high dosages of divalent metal ions during nitrogen removal process. *Bioresour. Technol*, 341:125785.
https://doi.org/10.1016/j.biortech.2021.125785

He T, Xie D, Ni J, et al., 2020. Nitrous oxide produced directly from ammonium, nitrate and nitrite during nitrification and denitrification. *J Hazard Mater*, 388:122114.
https://doi.org/10.1016/j.jhazmat.2020.122114

Hu B, Lu J, Qin Y, et al., 2023. A critical review of heterotrophic nitrification and aerobic denitrification process: Influencing factors and mechanisms. *J. Water Process. Eng*, 54:103995.
https://doi.org/10.1016/j.jwpe.2023.103995

Huang F, Pan L, Lv N, et al., 2017. Characterization of novel *Bacillus* strain N31 from mariculture water capable of halophilic heterotrophic nitrification–aerobic denitrification. *J. Biosci*, 124:564–571.
https://doi.org/10.1016/j.jbiosc.2017.06.008

Huang X, Li W, Zhang D, et al., 2013. Ammonium removal by a novel oligotrophic *Acinetobacter sp*. Y16 capable of heterotrophic nitrification–aerobic denitrification at low temperature. *Bioresour. Technol*, 146:44–50.
https://doi.org/10.1016/j.biortech.2013.07.046

Huang X, Xu Y, He T, et al., 2019. Ammonium transformed into nitrous oxide via nitric oxide by *Pseudomonas putida* Y-9 under aerobic conditions without hydroxylamine as intermediate. *Bioresour. Technol*, 277:87–93.
https://doi.org/10.1016/j.biortech.2019.01.040

James S.N.N, Vijayanandan A, 2023. Recent advances in simultaneous nitrification and denitrification for nitrogen and micropollutant removal: a review. *Biodegradation*, 34:103–123.
https://doi.org/10.1007/s10532-023-10015-8

Ke X, Liu C, Yu H, et al., 2024. Coculture of the Heterotrophic Nitrification–Aerobic Denitrification Strains *Acinetobacter sp*. A12 and *Paracoccus sp*. T8 Enables Effective Biological Nitrogen Removal from Landfill Leachate. *ACS EST Water*, 4:1786–1797.
https://doi.org/10.1021/acsestwater.3c00789

Klontza D.E, 2022. A review on application of external carbon sources for denitrification for wastewater treatment [WWW Document]. URL
https://journal.gnest.org/publication/gnest_04248 (accessed 2.25.24).

Lei X, Jia Y, Chen Y, et al., 2019. Simultaneous nitrification and denitrification without nitrite accumulation by a novel isolated *Ochrobactrum anthropic* LJ81. *Bioresour. Technol*, 272:442–450.
https://doi.org/10.1016/j.biortech.2018.10.060

Unedited

Lei Y, Wang Y, Liu H, et al., 2016. A novel heterotrophic nitrifying and aerobic denitrifying bacterium, *Zobellella taiwanensis* DN-7, can remove high-strength ammonium. *Appl Microbiol Biotechnol*, 100:4219–4229. https://doi.org/10.1007/s00253-016-7290-5

Li S, He Z, Li C, et al., 2024. Nitrogen removal by heterotrophic nitrification-aerobic denitrification bacteria: A review. *Desalin. Water Treat*, 317:100227. https://doi.org/10.1016/j.dwt.2024.100227

Liao M, Luo Y, Xu N, et al., 2022. Nitrogen removal and metabolic pathway of *Enterobacter cloacae* DK-6. *ENVIRON TECHNOL INNO*, 28:102630. https://doi.org/10.1016/j.eti.2022.102630

Liu T, Wang B, Liu M, et al., 2023. *Stutzerimonas frequens* strain TF18 with superior heterotrophic nitrification-aerobic denitrification ability for the treatment of aquaculture effluent. *Process Biochem*, 130:156–165. https://doi.org/10.1016/j.procbio.2023.04.018

Liu W, Wu Y, 2021. Simultaneous nitrification, denitrification and electricity recovery of *Halomonas* strains in single chamber microbial fuel cells for seawater sewage treatment. *J. Environ*, 9:106761. https://doi.org/10.1016/j.jece.2021.106761

Ma F, Sun Y, Li A, et al., 2015. Activation of accumulated nitrite reduction by immobilized *Pseudomonas stutzeri* T13 during aerobic denitrification. *Bioresour. Technol*, 187:30–36. https://doi.org/10.1016/j.biortech.2015.03.060

Ma M, Gui Q, Zheng W, et al., 2024. Nitrogen Removal Mechanism and Microbial Community Changes of the MBR Bioaugmented with Two Novel Fungi *Pichia kudriavzevii* N7 and *Candida tropicalis* N9. *Water-Sui*, 16:757. https://doi.org/10.3390/w16050757

Ouyang L, Wang K, Liu X, et al., 2020. A study on the nitrogen removal efficacy of bacterium *Acinetobacter tandoii* MZ-5 from a contaminated river of Shenzhen, Guangdong Province, China. *Bioresour. Technol*, 315:123888. https://doi.org/10.1016/j.biortech.2020.123888

Philippot L, Andert J, Jones C.M, et al., 2011. Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for $N_2O$ emissions from soil. *Glob. Chang. Biol*, 17 (3):1497–1504. https://doi.org/10.1111/j.1365-2486.2010.02334.x

Provolo G, Perazzolo F, Mattachini G, et al., 2017. Nitrogen removal from digested slurries using a simplified ammonia stripping technique. *Waste Manage*, 69:154–161. https://doi.org/10.1016/j.wasman.2017.07.047

Ren J, Tang J, Min H, et al., 2024. Nitrogen removal characteristics of novel bacterium *Klebsiella* sp. TSH15 by assimilatory/dissimilatory nitrate reduction and ammonia assimilation. *Bioresour. Technol*, 394:130184. https://doi.org/10.1016/j.biortech.2023.130184

Ren Y-X, Yang L, Liang X, 2014. The characteristics of a novel heterotrophic nitrifying and aerobic denitrifying bacterium, *Acinetobacter junii* YB. *Bioresour. Technol*, 171:1–9. https://doi.org/10.1016/j.biortech.2014.08.058

Wu S, Lv N, Zhou Y, et al., 2023. Simultaneous nitrogen removal via heterotrophic nitrification and aerobic denitrification by a novel *Lysinibacillus fusiformis* B301. *Water Environ. Res*, 95:e10850. https://doi.org/10.1002/wer.10850

Xi H, Zhou X, Arslan M, et al., 2022. Heterotrophic nitrification and aerobic denitrification process: Promising but a long way to go in the wastewater treatment. *Sci. Total Environ*, 805:150212. https://doi.org/10.1016/j.scitotenv.2021.150212

Xia L, Li X, Fan W, et al., 2020. Heterotrophic nitrification and aerobic denitrification by a novel *Acinetobacter* sp. ND7 isolated from municipal activated sludge. *Bioresour. Technol*, 301:122749. https://doi.org/10.1016/j.biortech.2020.122749

Xie F, Thiri M, Wang H, 2021. Simultaneous heterotrophic nitrification and aerobic denitrification by a novel isolated *Pseudomonas mendocina* X49. *Bioresour. Technol*,319:124198. https://doi.org/10.1016/j.biortech.2020.124198

Xu N, Liao M, Liang Y, et al., 2021. Biological nitrogen removal capability and pathways analysis of a novel low C/N ratio heterotrophic nitrifying and aerobic denitrifying bacterium (*Bacillus thuringiensis* strain WXN-23). Environ. Res,195:110797. https://doi.org/10.1016/j.envres.2021.110797

Yang L, Wang, X-H, Cui S, et al., 2019. Simultaneous removal of nitrogen and phosphorous by heterotrophic nitrification-aerobic denitrification of a metal resistant bacterium *Pseudomonas putida* strain NP5. *Bioresour. Technol*, 285:121360. https://doi.org/10.1016/j.biortech.2019.121360

Zhang M, He T, Chen M, et al., 2022. Ammonium and hydroxylamine can be preferentially removed during simultaneous nitrification and denitrification by *Pseudomonas taiwanensis* EN-F2. *Bioresour. Technol*, 350:126912.

Unedited

https://doi.org/10.1016/j.biortech.2022.126912

Zhang N, Chen H, Lyu, Y, et al., 2019. Nitrogen removal by a metal-resistant bacterium, *Pseudomonas putida* ZN1, capable of heterotrophic nitrification–aerobic denitrification. *J CHEM TECHNOL BIOT*, 94:1165–1175. https://doi.org/10.1002/jctb.5863

Zhang WW, Andong ZY, Zhang M, et al., 2014. Isolation and characterization of a heterotrophic nitrifier *Proteus mirabilis* strain V7 and its potential application in $NH_4^+$-N removal. *Ann Microbiol*, 64:1231–1238. https://doi.org/10.1007/s13213-013-0764-0

Zhang Y, Yin S, Li H, et al., 2022. Treatment of ammonia-nitrogen wastewater by the ultrasonic strengthened break point chlorination method. *J. Water Process Eng*, 45:102501. https://doi.org/10.1016/j.jwpe.2021.102501

Zhao B, An Q, He YL, et al., 2012. N2O and N2 production during heterotrophic nitrification by *Alcaligenes faecalis* strain NR. *Bioresour. Technol*, 116:379–385. https://doi.org/10.1016/j.biortech.2012.03.113

Zhao B, He YL, Hughes J, et al., 2010. Heterotrophic nitrogen removal by a newly isolated *Acinetobacter calcoaceticus* HNR. *Bioresour. Technol*, 101:5194–5200. https://doi.org/10.1016/j.biortech.2010.02.043

unedited

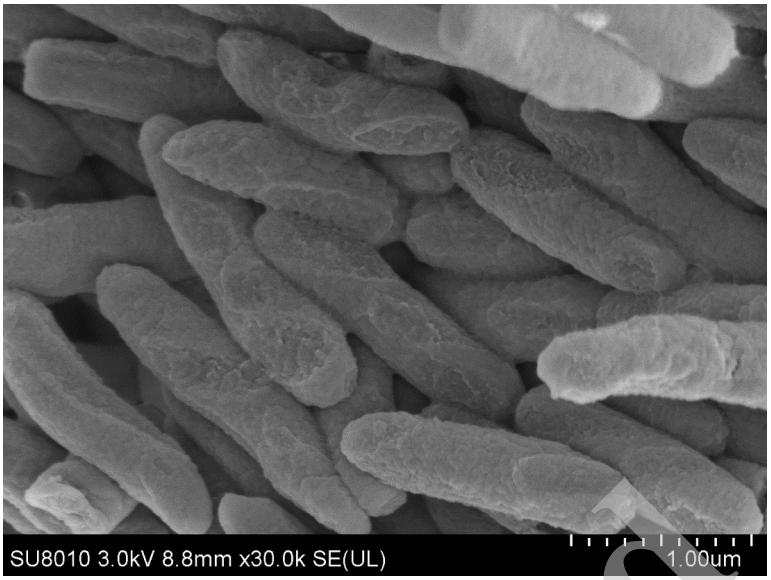



**Fig.1** Scanning electron microscope image of *Pesudomonas oleovorans* strain QS-7

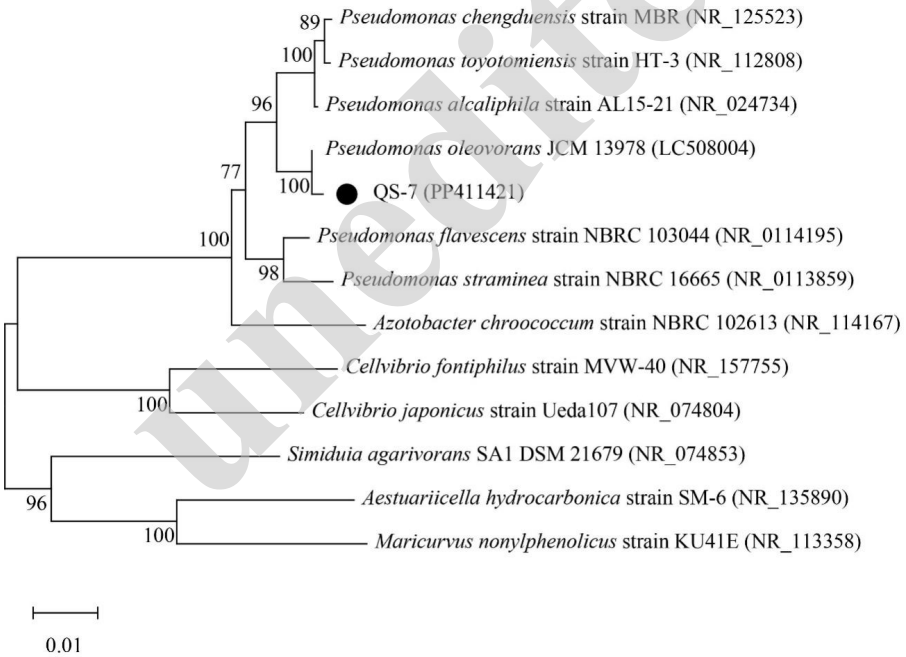



**Fig.2** Phylogenetic tree constructed based on 16S rRNA sequences of QS-7 from Neighbor-Joining analysis.

unedited

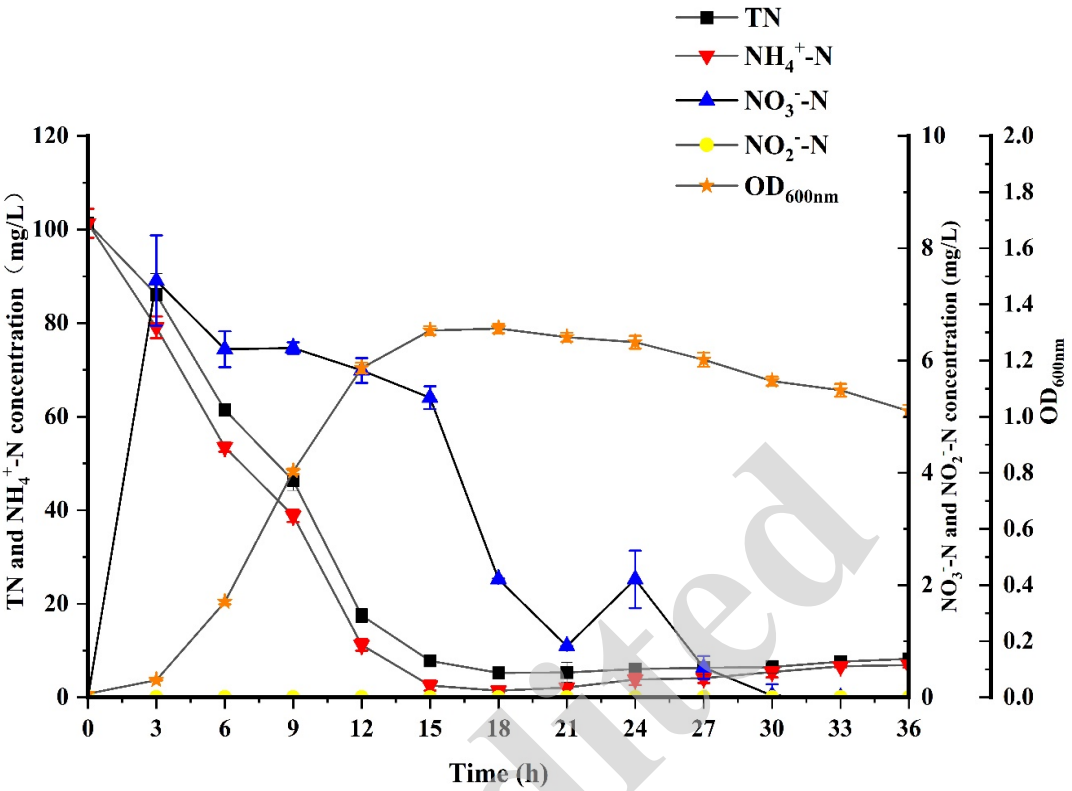
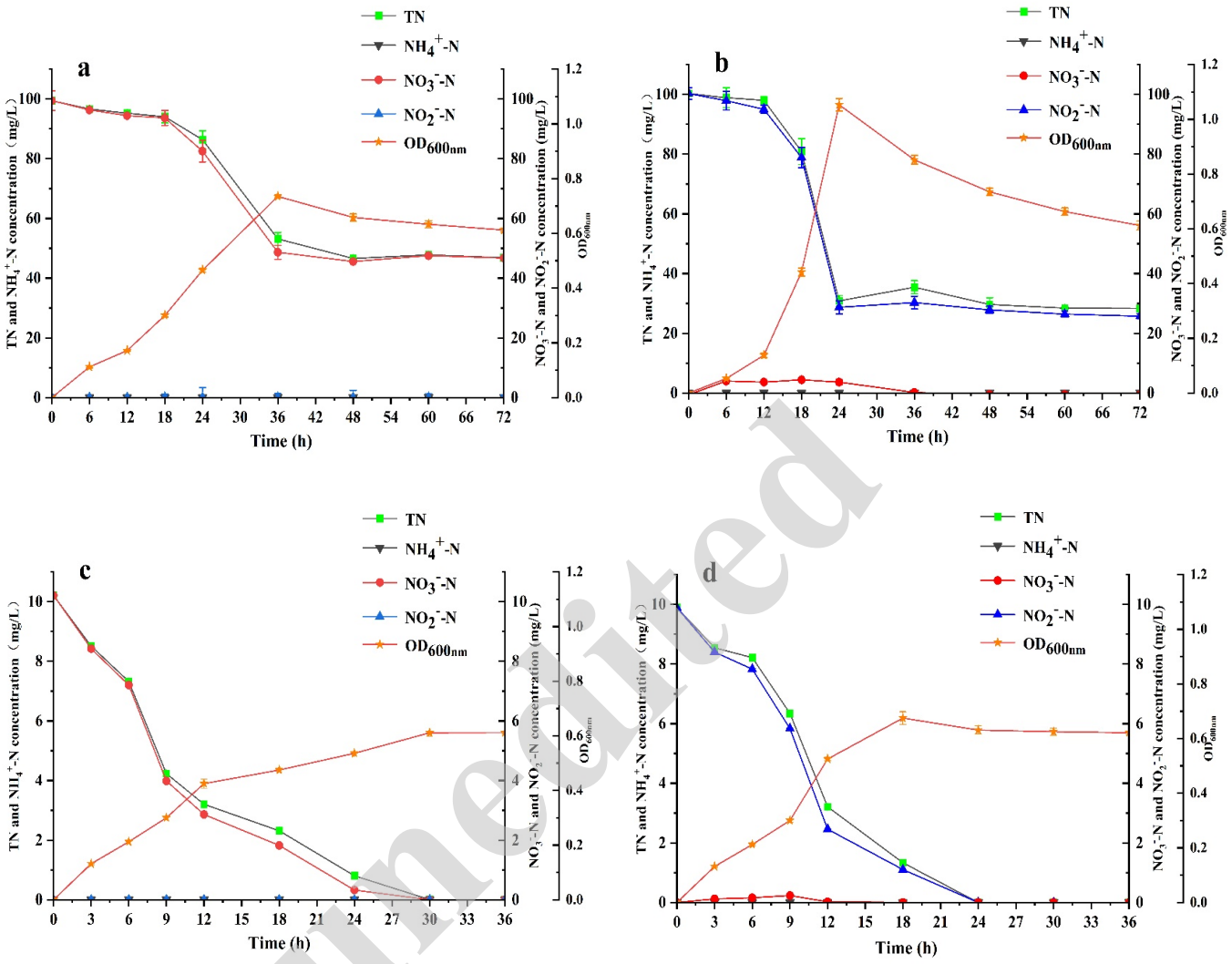



**Fig. 3** Dynamic characteristics of various nitrogen forms and growth of QS-7 in a system (BM-100 mg/L $NH_4^+$-N) with ammonia as the sole nitrogen source. Data are expressed as mean±standard drviation (SD), *n*=3. TN = Total Nitrogen, $OD_{600nm}$ = Optical Density in 600 nm.

unedited




unedited

**Fig.4.** Dynamic characteristics of various nitrogen forms and growth of QS-7 in a system with nitrate or nitrite nitrogen as the sole nitrogen source at different concentrations: DM1-100 mg/L $NO_3^-$-N (a); DM2-100 mg/L $NO_2^-$-N (b); DM3-10 mg/L $NO_3^-$-N (c); DM4-10 mg/L $NO_2^-$-N (d). Data are expressed as mean±standard drviation (SD), *n*=3.

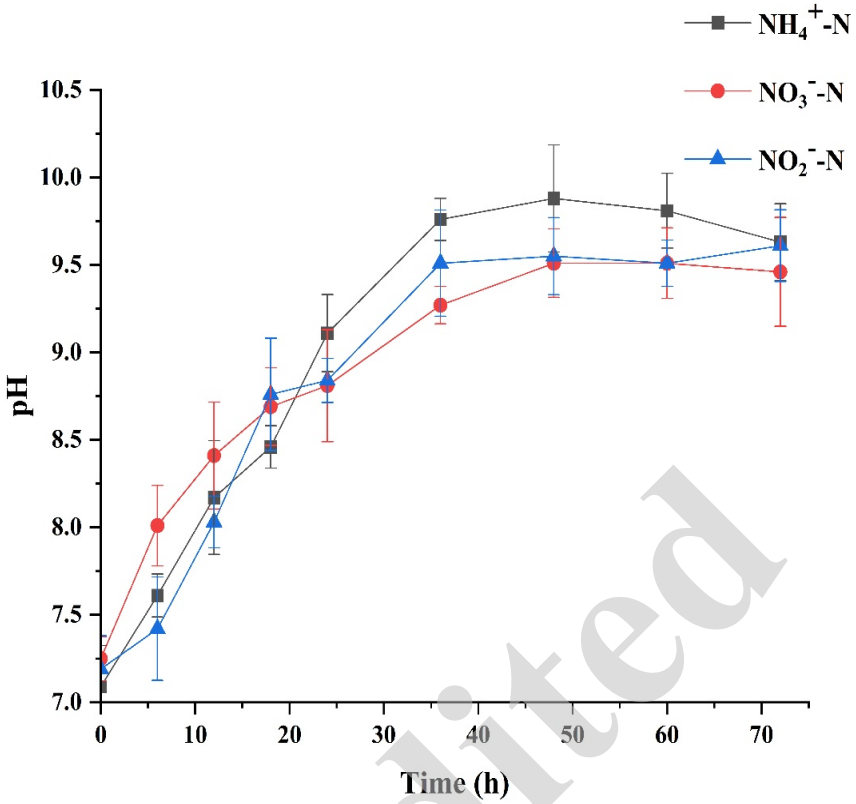



**Fig.5** Dynamic characteristics of pH in media with different nitrogen sources and QS-7. Data are expressed as mean±standard drviation (SD), *n*=3.

unedited

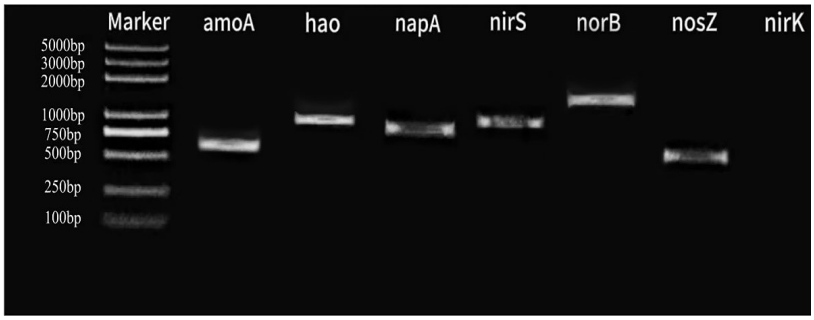



**Fig.6** PCR amplification results of genes relevant to nitrogen metabolism

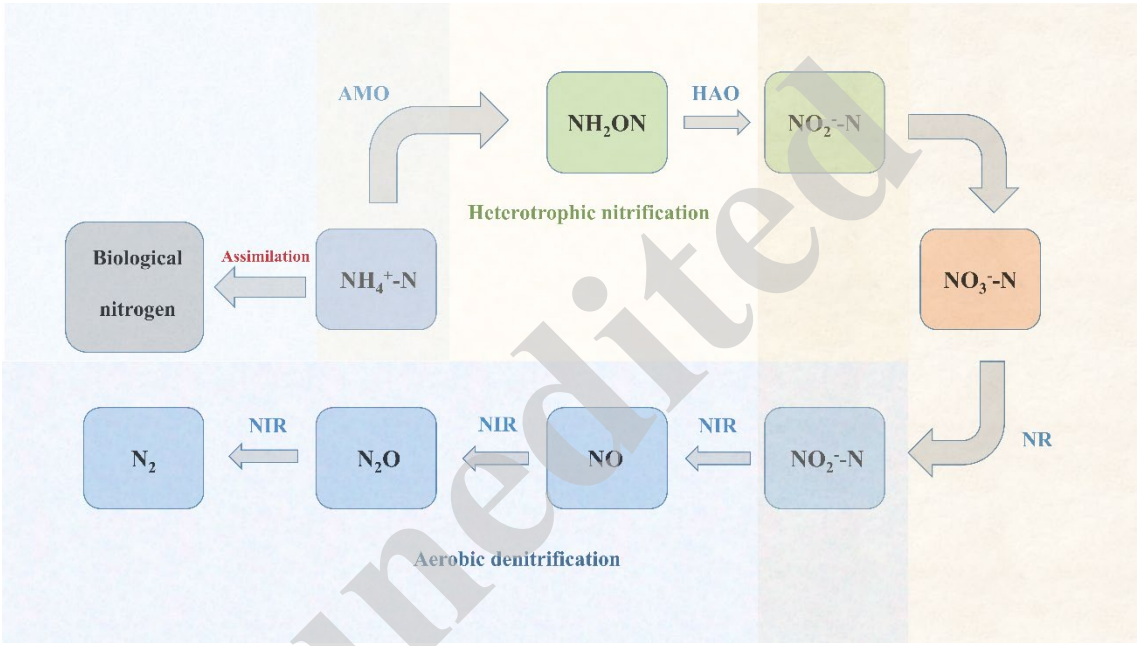



**Fig.7.** Metabolic pathways of ammonia nitrogen removal by QS-7

unedited

**Table 1** Composition of reaction system for determination of key enzyme activity

| Key enzyme | Composition and content | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Tris-HCl (pH=7.4) (mmol/L) | NADH (mmol/ L) | EDTA (mmol/L) | $NH_4Cl$ (mg/L) | $NH_2OH$ (mg/L) | $KNO_3$ (mg/L) | $NaNO_2$ (mg/L) | $K_3[Fe(CN)_6]$ (mmol/L) |
| AMO (20 mL) | 10 | 0.2 | — | 15 | — | — | — | — |
| HAO (20 mL) | 10 | — | 0.04 | — | 15 | — | — | 0.01 |
| NR (20 mL) | 10 | 0.2 | — | — | — | 15 | — | — |
| NIR (20 mL) | 10 | 0.2 | — | — | — | — | 15 | — |

AMO: Ammonia Monooxygenase, HAO：Hydroxylamine Oxidoreductase, NR：Nitrate Reductase, NIR：Nitrite Reductase, EDTA: Ethylene Diamine Tetraacetic Acid.

unedited

**Table 2** Nitrogen balance of strain QS-7 in BM after 36 h. Values are means ± standard deviation of triplicates.

| Substrate | Initial N (mg/L) | Final N (mg/L) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $NH_4^+$-N | $NO_3$-N | $NO_2$-N | Intracellular-N | Organic N | $N_2O$ | $N_2$ |
| Ammonia-N | 101.3±3.12 | 6.9±0.3 | -- | -- | 47.8±3.2 | 5.1±0.3 | 0.29±0.002 | 41.2±2.3 |

--: undetectable. Data are expressed as mean±standard drviation (SD), *n*=3.

**Table 3** Specific activities of Key enzyme

| Key enzyme | Specific activity/U·(mg protein)$^{-1}$ |
|---|---|
| AMO | 0.921±0.025 |
| HAO | 0.729±0.023 |
| NR | 0.135±0.012 |
| NIR | 0.120±0.013 |

Data are expressed as mean±standard drviation (SD), *n*=3.

unedited

**Table 4** Effects of different factors on nitrogen removal performance by strain QS-7: carbon source, C/N ratio, pH, shaking speed (r/ min), temperature (℃).

| different factors | $NH_4^+$-N removal Efficiency (%) | TN removal Efficiency (%) | Final pH | $OD_{600nm}$ |
|---|---|---|---|---|
| **Carbon source** | | | | |
| **Glucose** | 5.24±0.86$^{c}$ | 2.69±0.40$^{c}$ | 5.61±0.01$^{b}$ | 0.133±0.012$^{b}$ |
| **Sodium acetate** | 6.85±0.29$^{c}$ | 0.78±0.15$^{c}$ | 6.80±0.14$^{c}$ | 0.011±0.001$^{c}$ |
| **Sodium citrate** | 94.31±1.51$^{a}$ | 93.07±2.75$^{a}$ | 9.51±0.15$^{a}$ | 1.046±0.088$^{a}$ |
| **Sodium succinate** | 84.09±1.04$^{b}$ | 83.28±2.05$^{b}$ | 9.42±0.10$^{a}$ | 0.961±0.026$^{a}$ |
| **C/N ratio** | | | | |
| **3** | 67.61±3.19$^{c}$ | 65.01±1.65$^{c}$ | 9.21 ±0.95$^{b}$ | 0.745±0.119$^{c}$ |
| **7** | 82.44±3.59$^{b}$ | 78.32±2.50$^{b}$ | 9.51±0.11$^{a}$ | 1.020±0.099$^{b}$ |
| **10** | 93.79±2.41$^{a}$ | 91.52±2.20$^{a}$ | 9.62±0.11$^{a}$ | 1.049±0.025$^{b}$ |
| **15** | 96.33±2.33$^{a}$ | 93.35±2.01$^{a}$ | 9.52±0.95$^{a}$ | 1.166±0.024$^{ab}$ |
| **18** | 97.11±1.99$^{a}$ | 93.85±1.75$^{a}$ | 9.58±0.95$^{a}$ | 1.253±0.051$^{a}$ |
| **Initial pH** | | | | |
| **5** | 8.33±0.91$^{b}$ | 2.74±0.13$^{b}$ | 5.21±0.14$^{d}$ | 0.004±0.001$^{d}$ |
| **6** | 91.76±2.16$^{a}$ | 90.12±2.06$^{a}$ | 9.35±0.18$^{c}$ | 0.850±0.002$^{c}$ |
| **7** | 92.60±2.11$^{a}$ | 90.85±1.38$^{a}$ | 9.42±0.08$^{bc}$ | 0.921±0.03$^{b}$ |
| **8** | 93.45±1.89$^{a}$ | 91.71±2.46$^{a}$ | 9.62±0.03$^{ab}$ | 0.943±0.03$^{a}$ |
| **9** | 94.31±2.11$^{a}$ | 92.06±2.95$^{a}$ | 9.71±0.02$^{a}$ | 0.922±0.38$^{b}$ |
| **Shaking speed (r/ min)** | | | | |
| **90** | 71.16±1.11$^{c}$ | 67.39±0.37$^{c}$ | 8.59±0.07$^{c}$ | 0.627±0.025$^{d}$ |
| **120** | 75.95±1.43$^{b}$ | 71.11±1.56$^{b}$ | 9.20±0.04$^{b}$ | 0.741±0.018$^{c}$ |
| **150** | 93.48±1.54$^{a}$ | 91.78±1.80$^{a}$ | 9.52±0.07$^{a}$ | 0.951±0.026$^{b}$ |
| **180** | 94.66±0.71$^{a}$ | 93.38±0.87$^{a}$ | 9.58±0.06$^{a}$ | 1.034±0.047$^{a}$ |
| **Temperature(℃)** | | | | |
| **25** | 87.79±3.35$^{b}$ | 81.19±2.35$^{c}$ | 9.13±0.17$^{c}$ | 0.736±0.017$^{c}$ |
| **30** | 94.34±1.26$^{a}$ | 93.68±0.56$^{ab}$ | 9.48±0.21$^{a}$ | 0.913±0.061$^{b}$ |
| **35** | 97.48±1.07$^{a}$ | 95.95±0.76$^{a}$ | 9.42±0.12$^{ab}$ | 1.052±0.044$^{a}$ |
| **40** | 95.52±1.11$^{a}$ | 92.14±1.18$^{b}$ | 9.63±0.11$^{a}$ | 0.942±0.041$^{b}$ |

Data are expressed as mean±standard drviation (SD), *n*=3.

Means with the same letter are not significantly different in a single-factor treatment. (Tukey's HSD test, following one-way ANOVA: P > 0.05).

Unedited