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Data Article

Descriptive data on simultaneous nitrification and denitrification of hypersaline wastewater by a robust bacterium *Halomonas salifodinae*

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ABSTRACT

This article aims to illustrate and expand the information published in the article "Simultaneous nitrification and denitrification of hypersaline wastewater by a robust bacterium *Halomonas salifodinae* from a repeated-batch acclimation" [1]. The data present the salt tolerance of strain Y5 at different salinities (0%, 5%, 10%, 15%, and 20%). The effect of salinity on the morphology of bacteria was observed by scanning electron microscope. The influence of culture conditions including carbon source, C/N ratio, initial pH value, temperature, and shaking speed on bacterial growth and NH_4^+-N removal capability of strain Y5 was investigated by single factor experiments. The enzymatic activities of ammonia monooxygenase (AMO), hydroxylamine oxidase (HAO), nitrite reductase (NIR), and periplasm nitrate reductase (NAP) were measured by extracting the cell-free crude enzymes from strain Y5.

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Specifications Table

Subject	Environmental science
Specific subject area	Wastewater treatment and biological nitrogen removal
Type of data	Image, figure, and table
How the data were acquired	SEM images of bacteria were obtained by using a field emission scanning electron microscope (Hitachi, SU8010). When the bacteria were cultivated at certain conditions, samples were taken periodically to measure the absorbance at 600 nm and the concentrations of nitrogen sources. Enzymatic activities related to the processes of simultaneous nitrification and denitrification (SND) were measured by extracting the cell-free crude enzymes from strain Y5.
Data format	Raw and analyzed
Parameters for data collection	SEM mode: 5 kV electron acceleration, 16 mA emission current. Unless otherwise stated, the culture conditions were sodium succinate as carbon source, C/N ratio of 15, nitrogen source concentration of 200 mg/L, initial pH value of 7.2, temperature of 30°C, shaking speed of 150 rpm, and 15% salinity. Cell-free crude enzymes were extracted by ultrasonication: work/pause 5/5 seconds, work for 70 cycles, power 400 W.
Description of data collection	Bacteria samples were concentrated by centrifugation and were fixed with 2.5% glutaraldehyde solution. Then, the samples were dehydrated with ethanol at different concentrations and were vacuum dried, sprayed with gold, and imaged by SEM. Photographs were captured with a mobile phone. Enzymatic reaction systems included buffer solution, electron receptor/electron donor, cell-free crude enzyme, and substrate.
Data source location	Institution: Wuhan University of Science and Technology City: Wuhan Country: China
Data accessibility	Hu, Jie; Li, Jing; Yan, Jiabao (2021), "Details on the Descriptive data on simultaneous nitrification and denitrification of hypersaline wastewater by a robust bacterium *Halomonas salifodinae*", Mendeley Data, V2, doi: 10.17632/8mjwzds5xf.2 http://dx.doi.org/10.17632/8mjwzds5xf.2
Related research article	Jie Hu, Jiabao Yan, Ling Wu, Yanzhou Bao, Danqing Yu, Jing Li, Simultaneous nitrification and denitrification of hypersaline wastewater by a robust bacterium *Halomonas salifodinae* from a repeated-batch acclimation, Bioresour. Technol. 341 (2021) 125818, https://doi.org/10.1016/j.biortech.2021.125818.

Value of the Data

- We isolate a robust SND bacterium *Halomonas salifodinae* strain Y5, which can effectively remove nitrogen at 15% salinity. In addition, the data illustrate the effect of environmental factors including carbon source, C/N ratio, initial pH value, culture temperature, and shaking speed on the bacterial growth and nitrogen removal of strain Y5 in detail. It is of great importance for the nitrogen removal conditions of hypersaline wastewater.
- The data is valuable to researchers interested in the biological nitrogen removal of hypersaline wastewater.
- The data can be used to compare the biological nitrogen removal performance of saline wastewater with other works in the future.
- These data might provide the technical guidance for biological nitrogen removal of actual saline wastewater.

1. Data Description

For biological nitrogen removal of hypersaline wastewater, we isolated eight distinct halotolerant SND bacteria from the activated sludge of pharmaceutical wastewater treatment system

Table 1
The SND capabilities of eight isolated bacteria.

	Removal of NH_4^+-N (%)	Removal of NO_3^--N (%)
Y1	85.90±1.11	87.28±0.93
Y2	37.85±2.97	33.53±1.62
Y3	83.41±1.78	81.33±1.50
Y4	81.18±1.85	82.10±1.88
Y5	91.88±1.07	89.34±0.81
Y6	80.70±1.80	78.06±1.54
Y7	76.57±1.57	69.16±1.24
Y8	89.07±0.98	82.42±1.32

Table 2
Physiological and biochemical characteristics of strain Y5.

Test Items	Results	Test Items	Results
Catalase	+	Nitrate reduction	+
Glycolysis	+	Indole	-
Methyl red	-	Gelatin liquefaction	-
Voges-Proskauer	-	Production of H_2S	-
Starch hydrolysis	-	Citrate utilization	+

through repeated-batch acclimation. The heterotrophic nitrification and aerobic denitrification peroformance of the eight isolates was evaluted in heterotrophic nitrification (HN) and aerobic denitrification (AD) media (Table 1). Strain Y5 showed the best SND capability at 15% salinity and was determined as the dominant bacterium. The physiological and biochemical properties of strain Y5 were accomplished according to the Manual of systematic identification for common bacteria [2] (Fig. 1A, 1B and Table 2). The morphology of strain Y5 was observed by using a field emission scanning electron microscope (Fig. 1C and D).

The halotolerance of strain Y5 was tested in the HN and AD media with different salinities (0%, 5%, 10%, 15%, and 20%). The data present that strain Y5 can effectively remove nitrogen at 15% salinity (Fig. 2). The effect of salinity on the morphology of bacteria was observed by using a scanning electron microscope. SEM images show that strain Y5 keeps its original shape after the hypersaline treatment (15% salinity) for 72 h and has high resistance to plasmolysis (Fig. 3C and D). In contrast, *Pseudomonas* sp. is wrinkled due to dehydration after being treated at 5% salinity for 3 h, leading to the apoptosis and aggregation of bacteria (Fig. 3A and B).

The effect of culture conditions (such as carbon source, C/N ratio, initial pH value, culture temperature, and shaking speed) on bacterial growth and nitrogen removal capability was investigated by single-factor experiments. The data declare that the optimal NH_4^+-N removal is achieved by using sodium succinate as carbon source (Fig. 4). When strain Y5 is cultivated with sodium succinate as carbon source for 60 h, the removal rate of NH_4^+-N is 94.34% and the bacterial growth reaches the maximum (OD_{600} = 1.7892). As C/N ratios are in the range of 5 to 20, the bacterial growth needs adequate carbon source (C/N $\geq$ 15) to efficiently remove NH_4^+-N (Fig. 5). Strain Y5 can grow well and effectively remove NH_4^+-N (above 80%) within a wide range of pH value (6.0-9.0), and the optimal initial pH value is 7.0-8.0 (Fig. 6). Temperature is also one of important factors for bacterial growth and nitrogen removal capability. When strain Y5 is cultivated at the optimal culture temperature (30°C) for 60 h, the removal efficiency of NH_4^+-N is as high as 96.31% (Fig. 7). The shaking speed mainly influences the dissolved oxygen in media and the contact between microorganisms and substrates. When the shaking speed is above 150 rpm, strain Y5 can grow well and effectively remove nitrogen (Fig. 8). Under the optimal culture conditions (sodium succinate as carbon source, C/N ratio of 15, initial pH value of 7.2, culture temperature of 30°C, and shaking speed of 150 rpm), strain Y5 was used to treat hypersaline wastewater (15% salinity) with different NH_4^+-N or NO_3^--N concentrations. The data

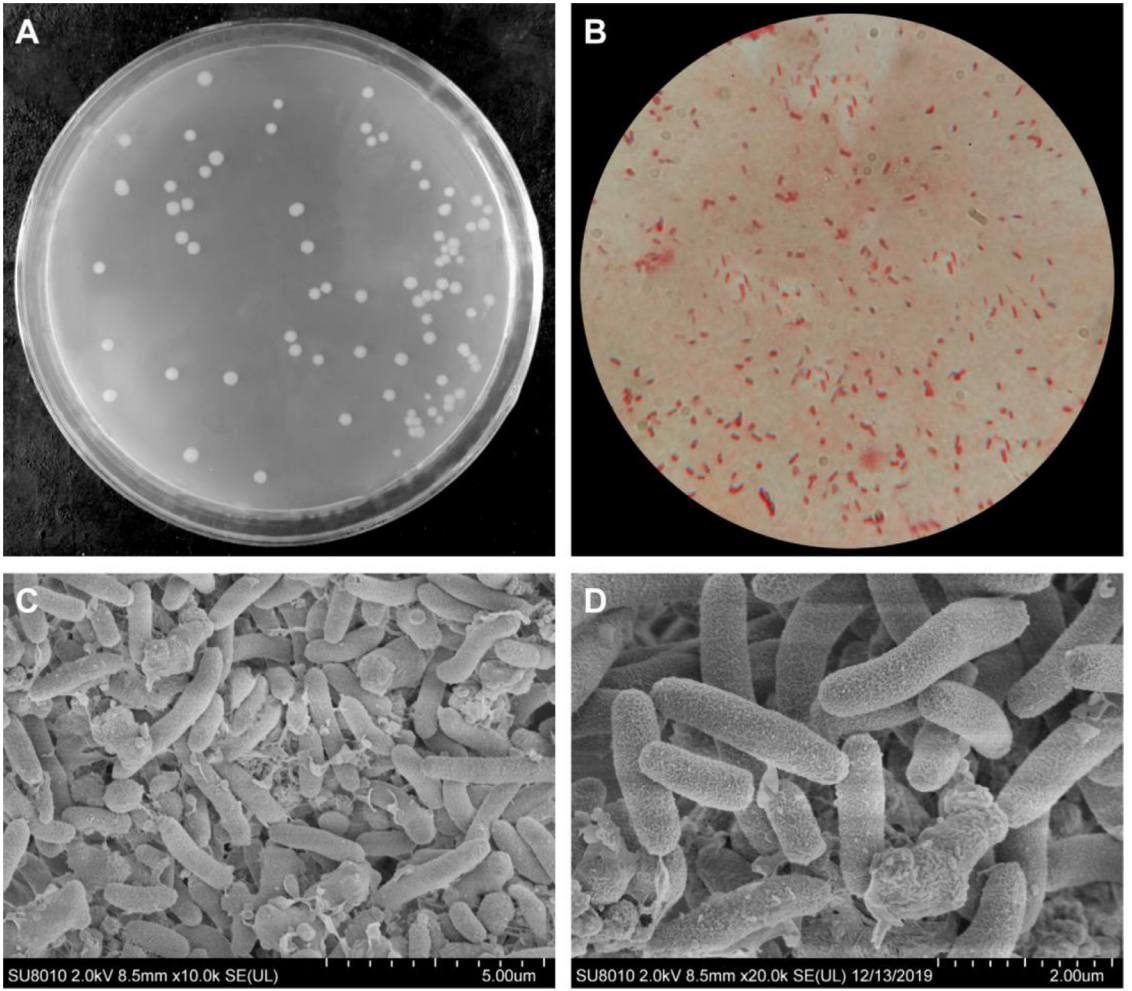

Fig. 1. (A) Bacterial colony, (B) gram stain, and (C, D) SEM images of strain Y5.

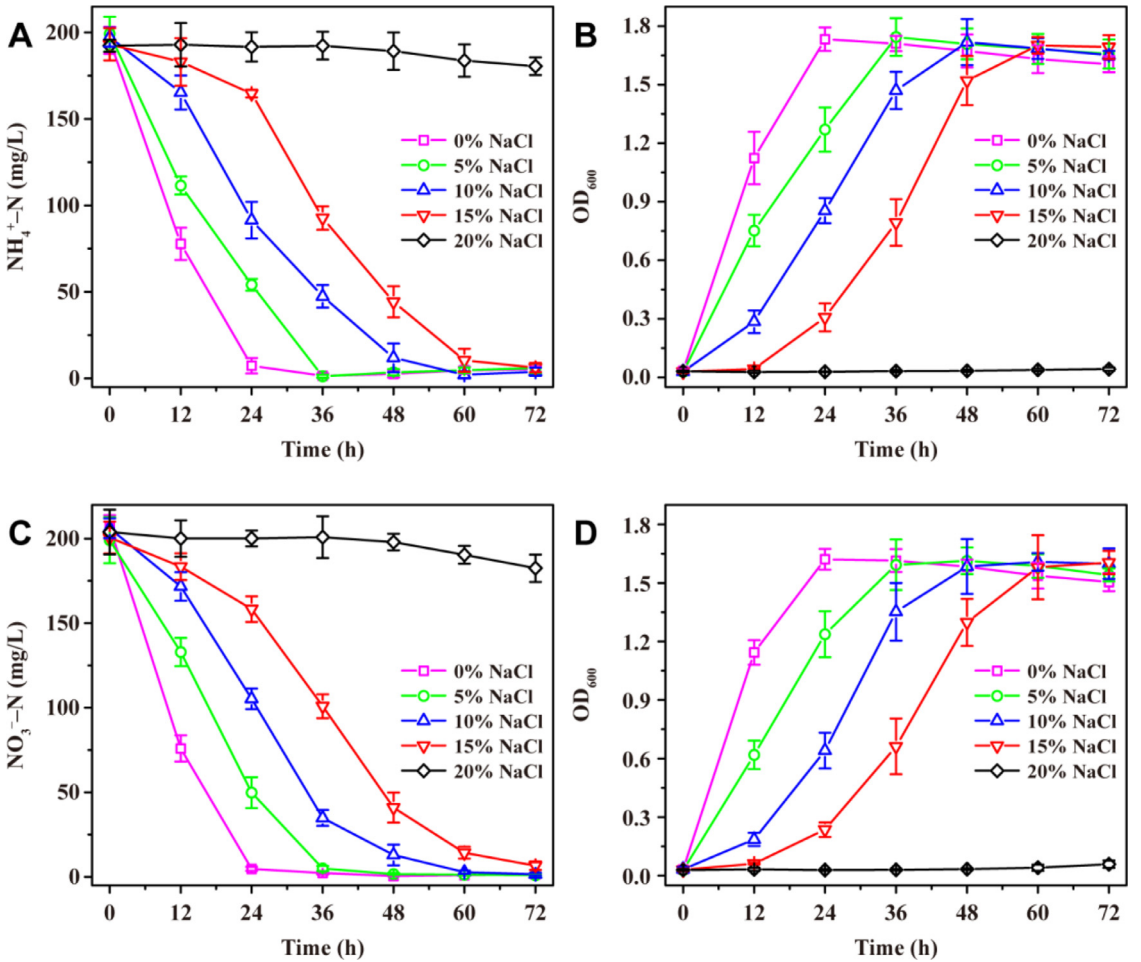

Fig. 2. (A, B) NH_4^+-N removal and bacterial growth and (C, D) NO_3^--N removal and bacterial growth of *Halomonas salifodinae* at different salinities. The raw data are shown in Mendeley Data with the title "Raw data of the salt tolerance of strain Y5 at different salinities".

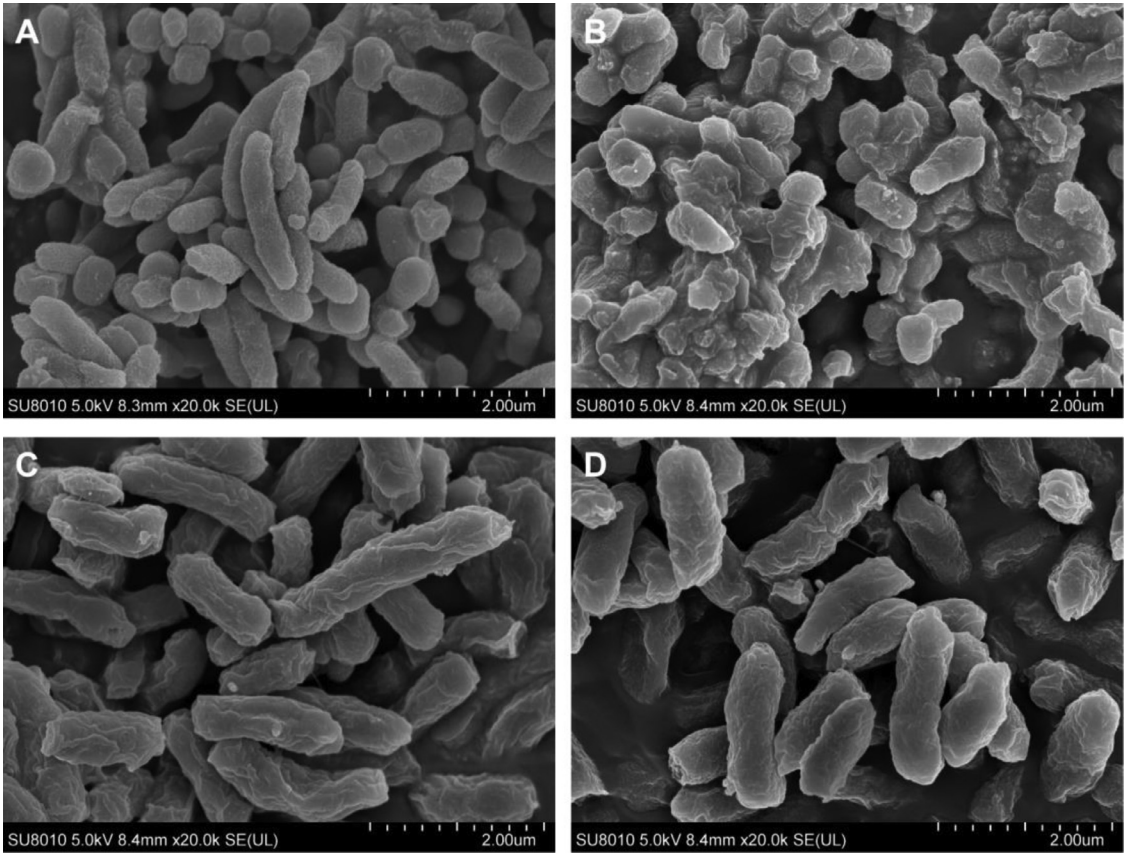

Fig. 3. SEM images of *Pseudomonas* sp. (A, B) and *Halomonas salifodinae* (C, D) before (A, C) and after (B, D) adding 5% and 15% NaCl for 3 h and 72 h, respectively.

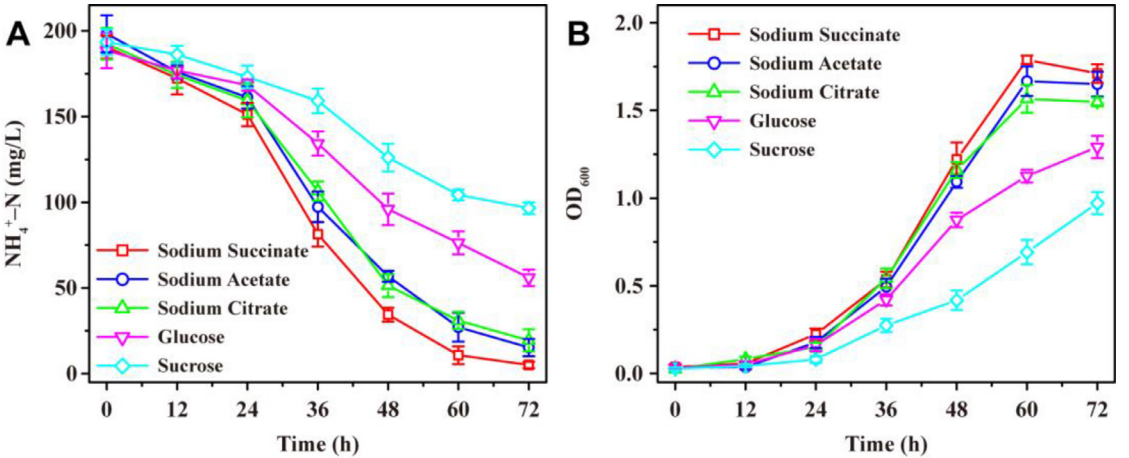

Fig. 4. Effect of carbon source on NH_4^+-N removal (A) and bacterial growth (B) of strain Y5. The raw data are shown in Mendeley Data with the title "Raw data of the effect of carbon source on NH_4^+-N removal and bacterial growth of strain Y5".

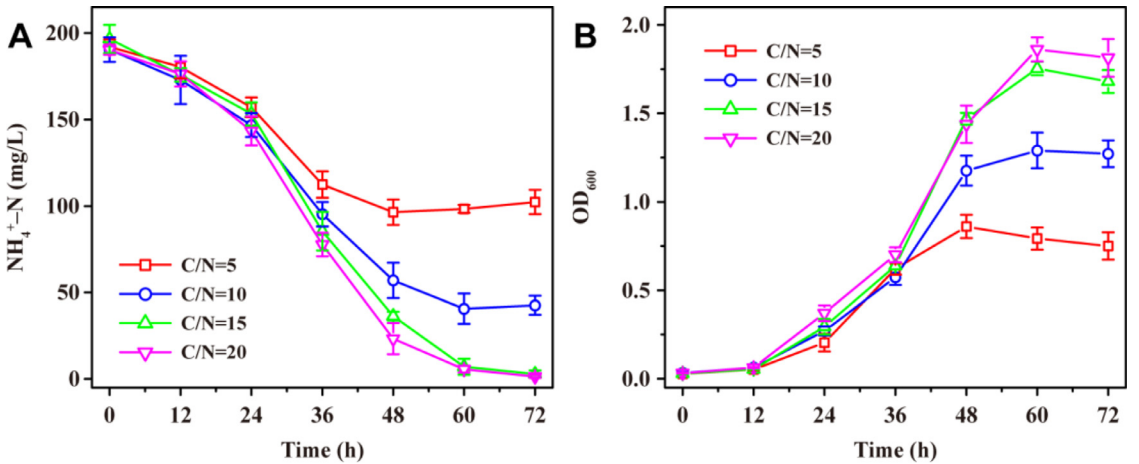

Fig. 5. Effect of C/N ratio on NH_4^+-N removal (A) and bacterial growth (B) of strain Y5. The raw data are shown in Mendeley Data with the title "Raw data of the effect of C/N ratio on NH_4^+-N removal and bacterial growth of strain Y5".

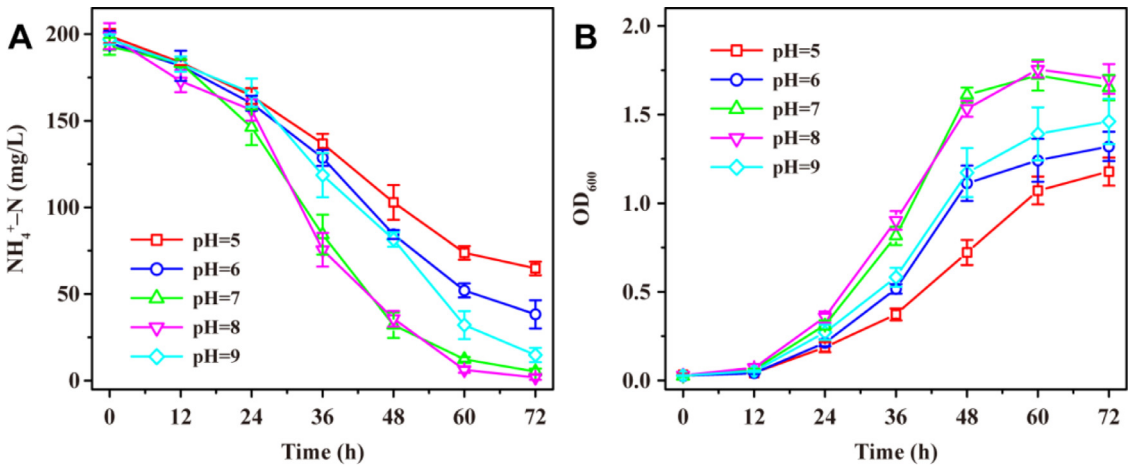

Fig. 6. Effect of initial pH value on NH_4^+-N removal (A) and bacterial growth (B) of strain Y5. The raw data are shown in Mendeley Data with the title "Raw data of the effect of initial pH value on NH_4^+-N removal and bacterial growth of strain Y5".

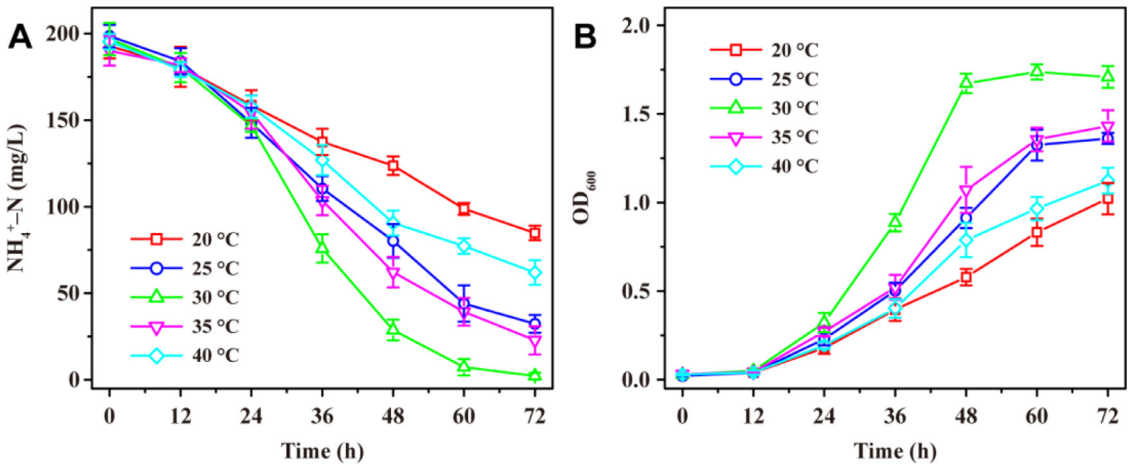

Fig. 7. Effect of temperature on NH_4^+-N removal (A) and bacterial growth (B) of strain Y5. The raw data are shown in Mendeley Data with the title "Raw data of the effect of culture temperature on NH_4^+-N removal and bacterial growth of strain Y5".

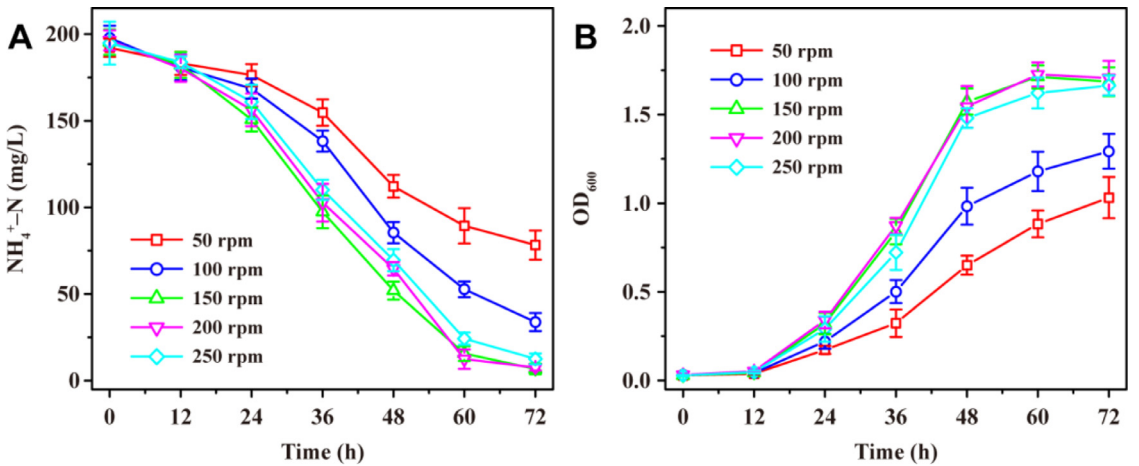

Fig. 8. Effect of shaking speed on NH_4^+-N removal (A) and bacterial growth (B) of strain Y5. The raw data are shown in Mendeley Data with the title "Raw data of the effect of shaking speed on NH_4^+-N removal and bacterial growth of strain Y5".

Table 3
The enzyme activities of AMO, HAO, NIR, and NAP in the crude enzyme extracts.

	0 min	15 min	30 min	60 min
NH_4^+-N removal with Cyt *c* in the enzymatic reaction systems (mg/L)	10.58±0.16	10.47±0.24	10.39±0.32	10.22±0.26
NH_4^+-N removal with crude enzyme in the enzymatic reaction systems (mg/L)	10.33±0.38	10.37±0.44	10.36±0.24	10.37±0.18
NH_4^+-N removal with Cyt *c* + crude enzyme in the enzymatic reaction systems (mg/L)	10.58±0.23	8.40±0.25	6.88±0.33	6.10±0.12
NH_2OH removal with Cyt *c* in the enzymatic reaction systems (mg/L)	10.84±0.30	10.55±0.17	10.35±0.28	10.28±0.39
NH_2OH removal with crude enzyme in the enzymatic reaction systems (mg/L)	10.89±0.43	10.81±0.20	10.73±0.28	10.72±0.24
NH_2OH removal with Cyt *c* + crude enzyme in the enzymatic reaction systems (mg/L)	11.24±0.37	9.17±0.28	7.56±0.41	6.37±0.37
NO_2^--N removal with NADH in the enzymatic reaction systems (mg/L)	10.19±0.25	10.17±0.19	10.03±0.36	9.95±0.28
NO_2^--N removal with crude enzyme in the enzymatic reaction systems (mg/L)	10.47±0.30	10.36±0.22	10.33±0.21	10.33±0.11
NO_2^--N removal with NADH + crude enzyme in the enzymatic reaction systems (mg/L)	10.42±0.11	9.47±0.19	8.69±0.16	8.35±0.28
NO_3^--N removal with NADH in the enzymatic reaction systems (mg/L)	10.62±0.19	10.41±0.36	10.19±0.24	10.09±0.37
NO_3^--N removal with crude enzyme in the enzymatic reaction systems (mg/L)	10.50±0.20	10.45±0.28	10.40±0.39	1.35±0.26
NO_3^--N removal with NADH + crude enzyme in the enzymatic reaction systems (mg/L)	10.74±0.29	8.45±0.23	6.75±0.11	6.01±0.22

show that all the SND efficiencies can achieve above 95% at the concentration range of 50-400 mg/L (Fig. 9).

Enzymatic activities related to the SND processes were measured by extracting the cell-free crude enzymes from strain Y5 grown at 15% salinity, including AMO, HAO, NIR, and NAP. The enzymatic reaction systems contain buffer solution, electron receptor/electron donor, cell-free crude enzyme, and substrate. After the enzymatic reaction for a certain time, samples were taken periodically to determine the concentration of substrates to evaluate the enzymatic activity (Table 3). The data present that the enzymatic activities of AMO, HAO, and NAP are significantly higher than that of NIR. According to the enzymatic activities related to the SND processes, the proposed SND pathway of strain Y5 is that NH_4^+-N transforms to NH_2OH, NO_2^--N, and NO_3^--N and the produced nitrogen oxides are removed via biological catalysis (Fig. 10).

2. Experimental Design, Materials and Methods

2.1. Culture media

The culture media used in the experiment includes Luria-Bertani (LB) medium, heterotrophic nitrification (HN) medium, and aerobic denitrification (AD) medium, whose compositions are the same as the previous research [1].

2.2. Identification of bacteria

Three milliliters of activated sludge were inoculated into 100 mL LB medium to activate and enrich bacteria twice. Afterward, the repeated-batch acclimation was carried out according to the previous work [1]. After the repeated-batch acclimation, the bacterial suspensions were diluted in gradient and spread on LB agar plates. The LB plates were incubated at 30°C for 48 h

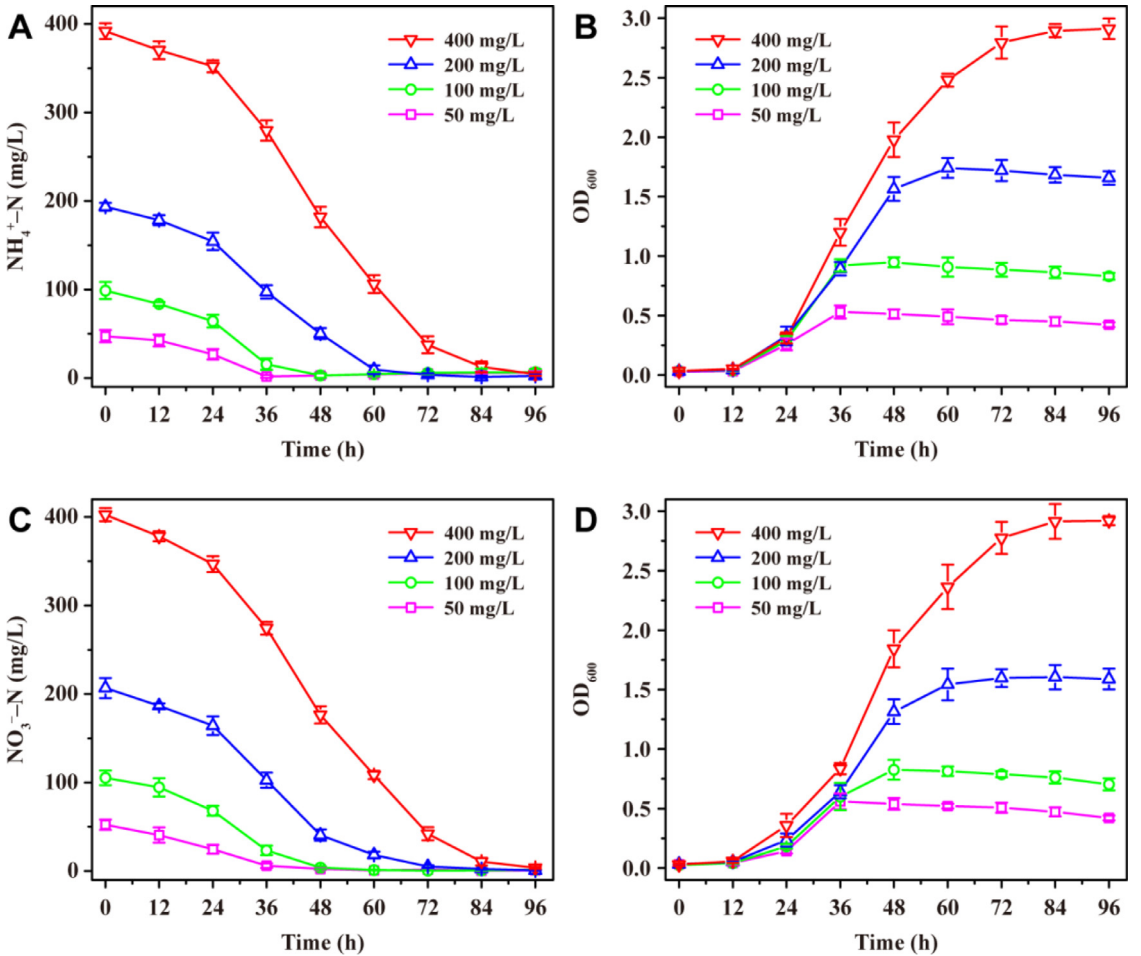

Fig. 9. (A, B) NH_4^+-N removal and bacterial growth and (C, D) NO_3^--N removal and bacterial growth of *Halomonas salifodinae* at 15% salinity. The concentrations of NH_4^+-N and NO_3^--N are 50, 100, 200, and 400 mg/L. The raw data are shown in Mendeley Data with the title "Raw data of the SND performance of strain Y5 at different nitrogen source concentrations".

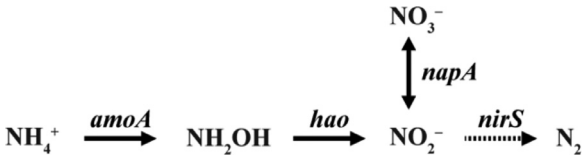

Fig. 10. Proposed SND pathway of strain Y5.

and eight unique isolates were acquired for further use. The SND capability of the eight isolated strains was measured in the HN and AD media with 15% salinity. Strain Y5 showed the highest SND efficiency and was determined as the dominant bacterium for further study. The bacterial colony, gram stain, physiological and biochemical properties of strain Y5 were measured according to the Manual of systematic identification for common bacteria [2]. The morphology of strain Y5 was observed by using a field emission scanning electron microscope (Hitachi, SU8010).

2.3. Salt tolerance of strain Y5

The salt tolerance of strain Y5 was evaluated by investigating the effect of different salinities on the bacterial growth and nitrogen removal performance. The bacterial suspension (3 mL) of strain Y5 was inoculated into the HN and AD media with different salinities (0%, 5%, 10%, 15%, and 20%) and was cultivated at 30°C, 150 rpm for 72 h. The samples were taken at intervals to measure the bacterial growth and the removal of NH_4^+-N and NO_3^--N. To investigate the effect of salinity on the morphology of bacteria, *Pseudomonas* sp. and strains Y5 were cultivated in the HN medium with 0% salinity for 24 h. After a large number of microorganisms grow, 5% and 15% NaCl were added directly into the HN medium. After *Pseudomonas* sp. and strains Y5 were treated by 5% and 15% NaCl for 3 h and 72 h, respectively, the morphology of the treated bacteria was observed by using the field emission scanning electron microscope.

2.4. Effect of culture conditions on nitrogen removal

The bacterial growth and nitrogen removal capability of strain Y5 were investigated under different culture conditions, including carbon source, C/N ratio, initial pH value, culture temperature, and shaking speed. For all of the single-factor experiments, the bacterial suspension (3 mL) of strain Y5 was inoculated into HN medium with sodium succinate as carbon source, initial nitrogen source concentration of 200 mg/L, C/N ratio of 15, initial pH value of 7.2, salinity 15% and was cultivated at 30°C, 150 rpm for 72 h. The samples were taken at intervals to measure the bacterial growth and the removal of NH_4^+-N.

2.5. SND performance of strain Y5

To investigate the SND performance of strain Y5, the bacterial suspension (3 mL) of strain Y5 was inoculated into the HN and AD media containing different nitrogen source concentrations (50 mg/L, 100 mg/L, 200 mg/L, and 400 mg/L) and was cultivated at 30°C, 150 rpm for 96 h. The samples were taken at intervals to measure the bacterial growth and the removal of NH_4^+-N and NO_3^--N.

2.6. Enzyme assay

Cell-free crude enzymes were extracted according to the reported work [3,4]. AMO and HAO activities were measured in the enzymatic reaction systems containing 10 mM Tris-HCl buffer (pH 7.1), cell-free crude enzymes, 0.11 mM cytochrome *c*, and target substrates. In addition, NIR and NAP activities were measured in the enzymatic reaction systems containing 10 mM phosphate buffer (pH 7.4), cell-free crude enzymes, 0.2 mM NADH, and target substrates. In addition, the enzymatic reaction systems without cell-free crude enzymes, cytochrome *c* or NADH were served as control. The enzymatic activities related to the SND process were evaluated by measuring the reduction of target substrates.

Ethics Statement

This work did not involve use of human subject, animal experiments or social media data.

CRediT Author Statement

Jie Hu: Methodology, Investigation, Formal analysis, Writing - original draft. **Jiabao Yan:** Conceptualization, Supervision, Funding acquisition. **Ling Wu:** Investigation, Formal analysis. **Yanzhou Bao:** Investigation, Formal analysis. **Danqing Yu:** Methodology, Investigation. **Jing Li:** Conceptualization, Supervision, Formal analysis, Visualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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