Data Article

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

Jie Hu a,b, Jiabao Yan a,b, Ling Wu a,b, Yanzhou Bao a,b, Danqing Yu a,b, Jing Li a,b,*

a School of Chemistry and Chemical Engineering, Wuhan University of Science and Technology, Wuhan 430081, China
b Hubei Province Key Laboratory of Coal Conversion and New Carbon Materials, Wuhan University of Science and Technology, Wuhan 430081, China

**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 NH4+-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.

© 2021 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

DOI of original article: 10.1016/j.biortech.2021.125818

*Corresponding author.
E-mail address: jli82@wust.edu.cn (J. Li).

https://doi.org/10.1016/j.dib.2021.107519

2352-3409/© 2021 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)
### 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
through repeated-batch acclimation. The heterotrophic nitrification and aerobic denitrification performance of the eight isolates was evaluated 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 ≥ 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

### 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$_2$S  | -       |
| Starch hydrolysis     | -       | Citrate utilization   | +       |
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”.
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 NH₄⁺-N transforms to NH₂OH, NO₂⁻-N, and NO₃⁻-N and the produced nitrogen oxides are removed via biological catalysis (Fig. 10).

|                          | 0 min          | 15 min         | 30 min         | 60 min         |
|--------------------------|----------------|----------------|----------------|----------------|
| NH₄⁺-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     |
| NH₄⁺-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     |
| NH₄⁺-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      |
| NH₂OH 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     |
| NH₂OH 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     |
| NH₂OH 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      |
| NO₂⁻-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      |
| NO₂⁻-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     |
| NO₂⁻-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      |
| NO₃⁻-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     |
| NO₃⁻-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      |
| NO₃⁻-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      |

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) \(\text{NH}_4^+\)-N removal and bacterial growth and (C, D) \(\text{NO}_3^-\)-N removal and bacterial growth of *Halomonas salifodinae* at 15% salinity. The concentrations of \(\text{NH}_4^+\)-N and \(\text{NO}_3^-\)-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”.
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 $\text{NH}_4^+$-N and $\text{NO}_3^-$-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 $\text{NH}_4^+$-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 $\text{NH}_4^+$-N and $\text{NO}_3^-$-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

This work was supported by Science and Technology Innovation Special Major Project of Hubei Province (2017ACA179).

References

[1] J. Hu, J. Yan, L. Wu, Y. Bao, D. Yu, J. Li, Simultaneous nitrification and denitrification of hypersaline wastewater by a robust bacterium Halomonas salifodinae from a repeated-batch acclimation, Bioreour. Technol. 341 (2021) 125818, doi:10.1016/j.biortech.2021.125818.
[2] X.Z. Dong, M.Y. Cai, Manual of Systematic Identification for Common Bacteria, Science Press, Beijing, 2001.
[3] H. Chen, X. Zhao, Y. Cheng, M. Jiang, X. Li, G. Xue, Iron robustly stimulates simultaneous nitrification and denitrification under aerobic conditions, Environ. Sci. Technol. 52 (2018) 1404–1412, doi:10.1021/acs.est.7b04751.
[4] B. Zhao, Y.L. He, J. Hughes, X.F. Zhang, Heterotrophic nitrogen removal by a newly isolated Acinetobacter calcoaceticus HNR, Bioreour. Technol. 101 (2010) 5194–5200, doi:10.1016/j.biortech.2010.02.043.