Front. Environ. Sci. Eng. 2022, 16(6): 69
https://doi.org/10.1007/s11783-021-1503-6

RESEARCH ARTICLE

A heterotrophic nitrification-aerobic denitrification bacterium *Halomonas venusta* TJPU05 suitable for nitrogen removal from high-salinity wastewater

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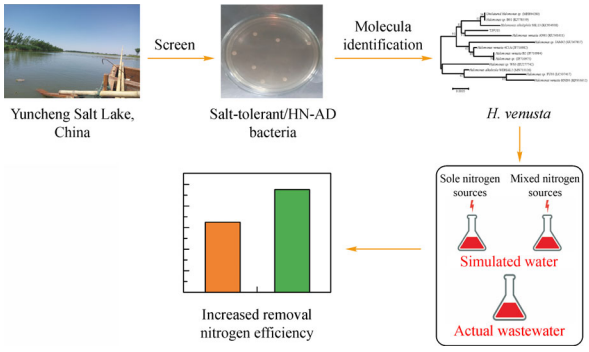
HIGHLIGHTS

- *H. venusta* TJPU05 showed excellent HN-AD ability at high salinity.
- Successful expression of AMO, HAO, NAR and NIR confirmed the HN-AD ability of TJPU05.
- *H. venusta* TJPU05 could tolerate high salt and high nitrogen environment.
- *H. venusta* TJPU05 is a promising candidate for the bio-treatment of AW.

GRAPHIC ABSTRACT



ARTICLE INFO

Article history:
Received 5 April 2021
Revised 9 August 2021
Accepted 16 August 2021
Available online 20 September 2021

Keywords:
Salt-tolerant bacteria
H. venusta TJPU05
Heterotrophic nitrification and aerobic denitrification
High-salinity wastewater

ABSTRACT

A novel salt-tolerant heterotrophic nitrification and aerobic denitrification (HN-AD) bacterium was isolated and identified as *Halomonas venusta* TJPU05 (*H. venusta* TJPU05). The nitrogen removal performance of *H. venusta* TJPU05 in simulated water (SW) with sole or mixed nitrogen sources and in actual wastewater (AW) with high concentration of salt and nitrogen was investigated. The results showed that 86.12% of NH_4^+-N, 95.68% of NO_3^--N, 100% of NO_2^--N and 84.57% of total nitrogen (TN) could be removed from SW with sole nitrogen sources within 24 h at the utmost. *H. venusta* TJPU05 could maximally remove 84.06% of NH_4^+-N, 92.33% of NO_3^--N, 92.9% of NO_2^--N and 77.73% of TN from SW with mixed nitrogen source when the salinity was above 8%. The application of *H. venusta* TJPU05 in treating AW with high salt and high ammonia nitrogen led to removal efficiencies of 50.96%, 47.28% and 43.19% for NH_4^+-N, NO_3^--N and TN respectively without any optimization. Furthermore, the activities of nitrogen removal–related enzymes of the strain were also investigated. The successful detection of high level activities of ammonia oxygenase (AMO), hydroxylamine oxidase (HAO), nitrate reductase (NAR) and nitrite reductase (NIR) enzymes under high salinity condition further proved the HN-AD and salt-tolerance capacity of *H. venusta* TJPU05. These results demonstrated that the *H. venusta* TJPU05 has great potential in treating high-salinity nitrogenous wastewater.

1 Introduction

Nitrogen in high salinity wastewater produced by various industries causes severe threat to the environment (Li et al., 2017). Discharge of high salinity wastewater increases the mineralization degree of river water, which brings serious pollution to soil, surface water and groundwater (Randall

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and Mulla, 2001). The accumulation of nitrogen, especially ammonia nitrogen, in aquatic environment often results in excessive multiplication of algae and leads to decrease of dissolved oxygen and the death of higher organisms (Duan et al., 2015). Nitrate in water has been reported to be able to induce methemoglobinemia of infants, and nitrosamine produced by nitrite hydrolysis has strong carcinogenicity (Rajta et al., 2020). Therefore, there is an urgent need to treat wastewater containing high concentration nitrogen and salt before discharging it into environment.

Biological treatment is a promising method due to its easy operation, free of secondary pollution and mild process condition (Qiu et al., 2021). Recently, the discovery of bacteria capable of HN-AD provides a much better solution to remove nitrogen from various wastewater (Joo et al., 2006; Pan et al., 2016). Compared with the traditional technique involving a combined process of autotrophic nitrification and heterotrophic denitrification, HN-AD bacteria possess the capability of simultaneous HN-AD and consequently saving floor space and operation costs of sewage treatment plant. However, current researches are mainly focused on the nitrogen removal capacity of HN-AD bacteria in a low salinity environment. For instance, some HN-AD bacteria such as *Bacillus cereus* (Yao et al., 2014), *B. subtilis* (Zhang et al., 2021) and *Pseudomonas mendocina* (He et al., 2019) exhibited excellent nitrogen removal performance when the salinity was lower than 2%. However, the HN-AD ability of these strains decreased dramatically in hypersaline environments. The reason was that the high salinity not only causes a sharp increase in osmotic pressure leading to the separation of cell wall, but also inhibits enzyme activity of these microorganisms (Abou-Elela et al., 2010; Yao et al., 2014).

Unfortunately, industrial wastewater often contains a wide range of salt concentration that could lead to low or unstable nitrogen removal efficiency. At present, some researchers have studied the nitrogen removal performance of salt-tolerant bacteria in hypersaline environments. For instance, *Marinobacter hydrocarbonoclasticus* (Li et al., 2013), *Pannonibacter phragmitetus* (Wang et al., 2019b), and *Alishewanella sp.* (Cheng et al., 2020), have been isolated and used in the treatment of saline water. However, these strains only showed considerable nitrification ability but low denitrification ability under high salinity condition. Furthermore, these bacteria are not suitable for the treatment of wastewater with high nitrogen concentration. That is also a problem in the field of wastewater treatment. Hence, it is of great importance to explore more efficient HN-AD strains for the treatment of wastewater containing high salinity and nitrogen concentration.

In this study, a novel salt-tolerant HN-AD bacterium was isolated from the sediment of Salt Lake and identified as *H. venusta* TJPU05 based on 16S rDNA gene sequence analysis. The effect of salinity on the HN-AD performance and enzyme activity of *H. venusta* TJPU05 was investigated. Furthermore, the nitrogen removal capacity of *H. venusta* TJPU05 in AW containing high salinity and nitrogen concentration was also evaluated. The present work provides a very promising microorganism resource for the removal of nitrogen from hypersaline wastewater with high nitrogen.

2 Methods

2.1 Culture media

Water and sediment samples with high concentration of salt were collected from Yuncheng Salt Lake, located in Shanxi Province of China. The Enrichment Medium (EM) used for screening salt-tolerant HN-AD bacteria was similar to our previous report (He et al., 2019), except that the concentration of NaCl was increased to 2.5%. The Heterotrophic Nitrification Medium (HNM) used for evaluation of ammonium removal performance, the Aerobic Denitrification Medium (ADM-1&2) used for evaluation of nitrite and nitrate reduction and the Simultaneous HN-AD mixed medium (SND-1&2) used for the evaluation of HN-AD performance were also similar to our previous report, except that the media used in this study contained different salinity (2.5%, 5%, 8% and 10%) (He et al., 2019). All media were sterilized at 121°C for 20 min before inoculation. AW, i.e, landfill leachate, was provided by sewage treatment works of Xianyang Road, Tianjin, China.

2.2 Isolation and molecular identification of salt tolerant HN-AD bacterium

Sediment suspension (5 mL) was re-suspended in 15 mL sterile water with 10 glass beads (0.5 mm) and homogenized by drastically shaking for 2 min. The homogenized suspension of 5 mL was inoculated into EM (100 mL) and incubated at 30°C with rotation speed of 130 r/min for the first round of enrichment. After cultivation for 2 days, 10 mL of this cell suspension was transferred to 90 mL of sterile EM medium to carry out the second round of enrichment under the conditions described above. Then, the suspension was serially diluted and spread on the surface of EM agar and purified with several rounds of streaking. The HN-AD ability of these purified strains was tested separately according to our previous method (He et al., 2019).

The morphology of the selected HN-AD strain was observed by a scanning electron microscope (EVO18, ZEISS, Germany). The 16S rDNA gene sequence of the HN-AD strain was obtained by Shanghai Biozeron Co.,

Ltd., China. A phylogenetic tree was constructed using Clustal W program and MEGA 5.1 with the neighbor-joining method (Wei et al., 2021).

2.3 Evaluation of nitrogen removal performance from SW

To evaluate the nitrogen removal performance of the selected strain in SW with different salinity, single colony was inoculated into sterilized LB medium (with an initial salinity of 2.5%) and incubated at 30°C with shaking speed of 130 r/min for 12 h. After OD_{600} reached 0.667, 10 mL bacterial suspension was collected and centrifuged at 10000 r/min for 5 min. The collected bacterial cells were re-suspended in 10 mL sterile water with different salinity and inoculated into different SW (HNM, ADM-1&2 or SND-1&2) with salinity of 5%, 8% and 10% respectively by changing the concentration of NaCl. These SWs were incubated at 30°C with shaking speed of 120 r/min for 24 h. The concentration of NH_4^+-N, NO_3^--N, NO_2^--N and TN were monitored with previous method (He et al., 2019). All the experiments were carried out in three biological replicates.

2.4 Enzyme activity analysis

The activities of ammonia oxygenase (AMO), hydroxylamine oxidase (HAO), nitrate reductase (NAR) and nitrite reductase (NIR) were measured according to the methods reported in the literature (Wang et al., 2015; Zhang et al., 2019a). Specially, nitrogen removal–related enzymes were obtained from the cells cultured for 24 h in growth medium with different NaCl concentrations. A crude enzyme extract was prepared by sonification and centrifugation and the supernatant was used for enzyme activity assays in this study.

The HAO activity was assayed as follows: 1 mL crude enzyme extract was mixed with 9 mL reaction solution buffered with 0.01 mol/L Tris-HCl (pH = 7.1), containing 1 mmol/L hydroxylamine and 0.11 mmol/L cytochrome, and the reaction mixture was incubated at 30°C with shaking speed of 130 r/min for 1 h. The HAO activity was determined by the change of hydroxylamine before and after the reaction.

The AMO activity was assayed as follows: 4 mL crude enzyme extract was mixed with 6 mL reaction solution buffered with 0.01 mol/L Tris-HCl (pH = 8.0), containing 2 mmol/L $(NH_4)_2SO_4$ and 0.2 mmol/L NADH, and the reaction mixture was incubated at 30°C with shaking speed of 130 r/min for 1 h. The AMO activity was determined by the change of NH_4^+-N before and after the reaction.

The NAR activity was assayed as follows: 1 mL crude enzyme extract was mixed with 9 mL reaction solution buffered with 0.01 mmol/L PBS (pH = 7.4), containing 1 mmol/L $NaNO_3$ and 0.2 mmol/L NADH, the reaction mixture was incubated at 30°C with shaking speed of 130 r/min for 1 h. NAR activity was determined by the change of $NaNO_3$ before and after the reaction.

The NIR activity was assayed as follows: 1 mL crude enzyme extract was mixed with 9 mL reaction solution buffered with 0.01 mmol/L PBS (pH = 7.4), containing 1 mmol/L $NaNO_2$ and 0.2 mmol/L NADH, and the reaction mixture was incubated at 30°C with shaking speed of 130 r/min for 1 h. The NIR activity was determined by the change of $NaNO_2$ before and after the reaction.

All the above reagents were of analytical grade and purchased from Shanghai Aladdin Co., Ltd. (China). Bradford kit was used to determine the protein concentration. One unit of the enzymes is defined as the amount of enzyme that catalyzes the conversion of 1 mol of substrate per milligram of total protein per minute (Wang et al., 2015; Wei et al., 2021).

2.5 Estimation of nitrogen removal performance in AW

The main characteristics of AW were listed in Table 1. Total dissolved solids (TDS) of AW (1.93×10^4) corresponds to the salinity of 2.8% and the concentration of NH_4^+-N was also very high, therefore, this AW should belong to high salt and high nitrogen wastewater. The inoculation amount of 5% (v/v) was used in the treatment of AW in 500-mL flasks, and the cultures were incubated at 30°C with shaking speed of 170 r/min. No further nutrients were added. The concentration of different forms of nitrogen was monitored according to aforementioned method. AW without inoculation of HN-AD strain was used as a control.

Table 1 Characteristics of AW used in this study

Characteristics	Results
pH	8.30
COD (mg/L)	3878±0.3
TN (mg/L)	1615±57
TDS (mg/L)	1.93×10^4
NH_4^+-N (mg/L)	1572±5
NO_3^--N (mg/L)	45±1
NO_2^--N (mg/L)	nd

Notes: COD: Chemical Oxygen Demand; TDS: Total Dissolved Solids; nd: not detected.

2.6 Analytic methods

The methods used for NH_4^+-N, NO_3^--N, NO_2^--N and TN analysis were similar to our previous work (He et al., 2019). The removal efficiency and removal rate of different forms of nitrogen were analyzed by method described in our previous work (He et al., 2019). Results were presented as means±SD (standard deviation of means). Dates were analyzed by Hypothesis Testing with One-Sample t- Test ($P < 0.05$) using Origin 2019 (Wan et al., 2017).

3 Results and discussion

3.1 Identification of *H. venusta* TJPU05

Several salt-tolerant bacteria with HN-AD performance were obtained from sediment of Salt Lake and one of them (named as TJPU05) with the highest HN-AD performance was selected for further study. The colony of TJPU05 on Luria-Bertan (LB) plate was yellow and with a smooth surface (Fig. 1(A)). Single cell of TJPU05 showed short rod-shaped (Fig. 1(B)). The phylogenetic analysis indicated that 16S rDNA sequence of TJPU05 was closely related (97% similarity) to that of *H. venusta* (Fig. 1(C)). Therefore, TJPU05 was proposed as a member of *H. venusta* species and named as *H. venusta* TJPU05.

3.2 Effect of salinity on nitrification performance of *H. venusta* TJPU05

The effect of salinity on heterotrophic nitrification of *H. venusta* TJPU05 was investigated by using NH_4^+-N as sole nitrogen source under different salinity. As shown in Fig. 2, with the increase of salinity, NH_4^+-N removal performance increased initially and then decreased after treatment for 24 h with an initial NH_4^+-N of 131.23 mg/L. The NH_4^+-N removal efficiencies were 72.49%, 86.12%, 82.63% and 77.4% for the salinity of 2.5%, 5%, 8% and 10%, respectively. These results revealed that the optimal salinity for the nitrification performance of *H. venusta* TJPU05 was 5%. Compared with other studies, the removal efficiency of NH_4^+-N by *H. venusta* TJPU05 after 24 h of incubation was much higher than that of *H. campisalis* ha3 (about 67%) (Guo et al., 2013). Additionally, *H. venusta* TJPU05 exhibited much faster nitrification rate than other strains at high salinity. The NH_4^+-N removal rates of *H. venusta* TJPU05 were 5.05 mg/L/h, 4.85 mg/L/h and 4.05 mg/L/h at the salinity of 5%, 8% and 10%, respectively. These results were higher than those of *V. diabolicus* SF16 (1.67 mg/L/h at the salinity of 5%) (Duan et al., 2015), *Serratia marcescens* CL1502 (2.27 mg/L/h at the salinity of 5% and 1.23 mg/L/h at the salinity of 8%) (Huang et al., 2017b), *B. cereus* X7

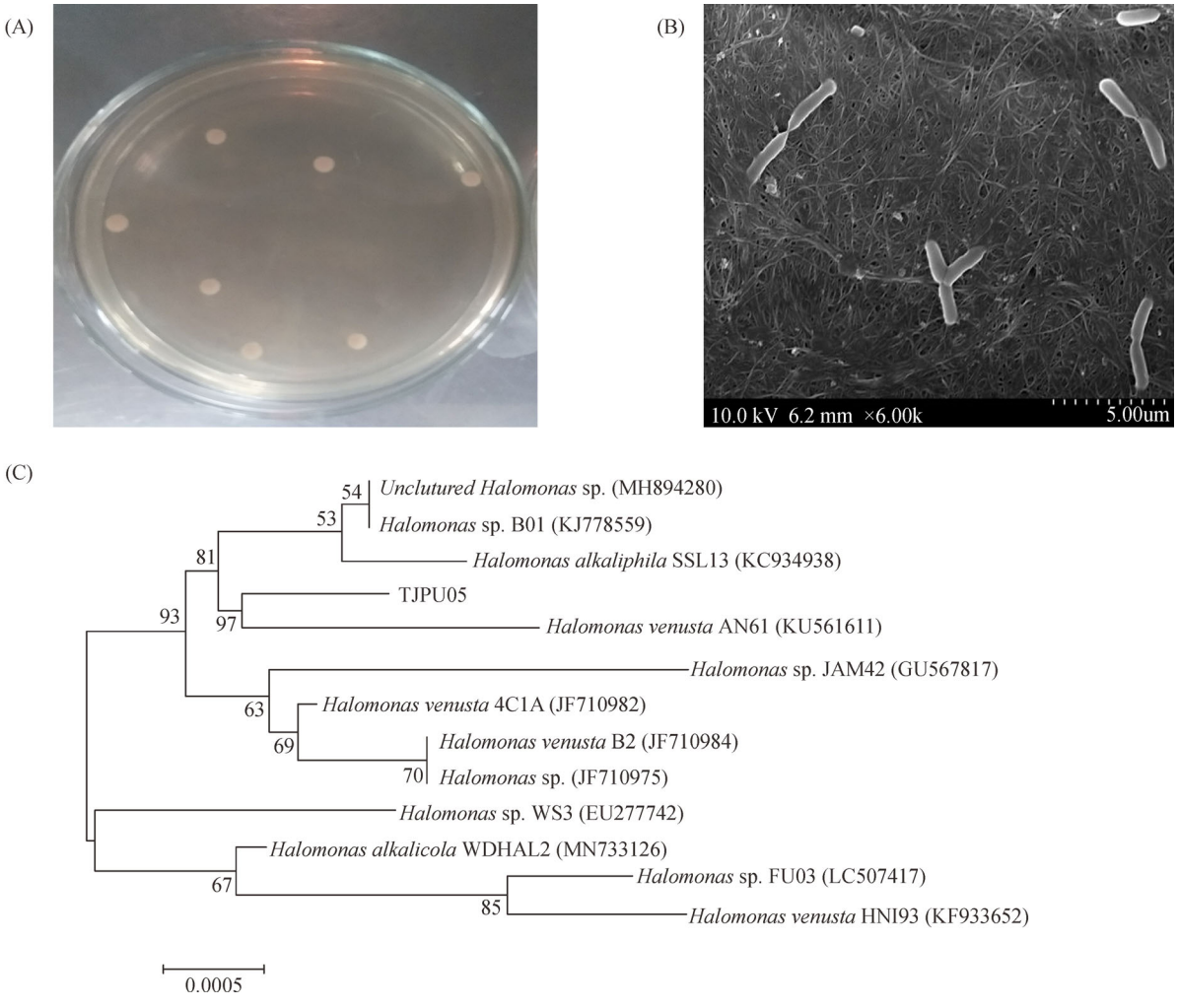

Fig. 1 Colony morphology on LB medium plate (A) and corresponding single cell morphology observed by SEM (B) of *H. venusta* TJPU05. Phylogenetic tree based on the neighboring joining method of 16S rDNA gene sequences (C).

(0.015 mg/L/h at the salinity of 2%) and *Bacillus strain* N31 (0.017 mg/L/h at the salinity of 3%) (Yao et al., 2014; Huang et al., 2017a).

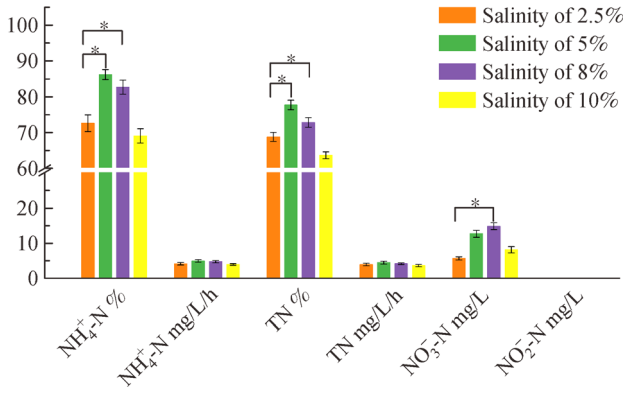

Fig. 2 Nitrogen removal characteristics of *H. venusta* TJPU05 in HNM medium under different salinity after incubation for 24 h. Statistical significance of data at 5%, 8% and 10% salinity compared with those at 2.5% salinity was evaluated, respectively; * $P < 0.05$.

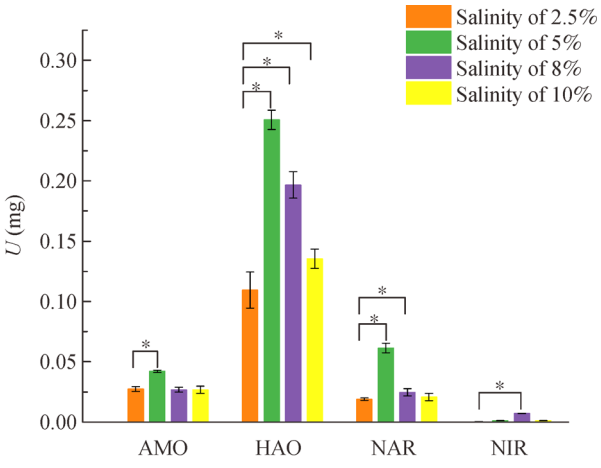

Fig. 3 Effects of salt concentration in the medium on the N removal-related enzyme activity of *H. venusta* TJPU05. Statistical significance of data at 5%, 8% and 10% salinity compared with those at 2.5% salinity was evaluated, respectively; * $P < 0.05$.

To further study the nitrogen removal performance of *H. venusta* TJPU05, the activities of AMO, HAO, NIR and NAR in *H. venusta* TJPU05 were also determined in SW with different salinity and the results were shown in Fig. 3. NH_4^+-N is metabolized by AMO into hydroxylamine, and then converted into NO_2^--N by HAO (Wei et al., 2021). The AMO and HAO exhibited the highest enzyme activity at the salinity of 5%, which further supported the above results.

The effect of salinity on TN removal efficiency of *H. venusta* TJPU05 also exhibited a first increasing and then decreasing trend. At the initial salinity of 2.5%, the removal efficiency of TN was 68.5% and increased to 77.6% at the salinity of 5%. When the salinity was increased to 8% and 10%, the TN removal efficiencies were decreased slightly to 72.2% and 70.7%, respectively. This result was similar to that of *Halomonas sp.* strain B01 that possessed 75.4% TN removal efficiency at the salinity of 6% after 60 h of incubation (Wang et al., 2017). Based on these results, we could conclude that *H. venusta* TJPU05 could efficiently remove NH_4^+-N and TN over a wide range of salinity from 2.5% to 10% and the optimal salinity was up to 5%. However, most previous works reported that the optimal salinity of HN-AD bacteria was less than 3% (Guo et al., 2013).

In the process of nitrification, NO_3^--N was detected and its production was also affected by the salinity of SW. The production of NO_3^--N was 5.76 mg/L, 12.73 mg/L, 14.87 mg/L, 8.2 mg/L at the salinity of 2.5%, 5%, 8% and 10%, respectively. These results indicated that some of the NH_4^+-N was converted to NO_3^--N. Similar result was also reported for *Bacillus* N31 in HNM medium (Huang et al., 2017a). In addition, it was interesting to find that no nitrite was detected during the whole process of NH_4^+-N removal at all salinity, indicating that *H. venusta* TJPU05 did not produce nitrite or the produced nitrite was quickly converted to other substance (He et al., 2017). This property is beneficial to the wastewater treatment since nitrite can poison other microbes in the system, leading to low and unstable operation performance (Ruan et al., 2020).

3.3 Effect of salinity on denitrification performance of *H. venusta* TJPU05

The aerobic denitrification capability of *H. venusta* TJPU05 in ADM-1&2 medium under different salinity was also evaluated, and the results were shown in Fig. 4. It can be seen from Fig. 4(A) that the NO_3^--N removal efficiencies were significantly increased from 89.39% to 95.21%, 95.68% and 92.27% after treatment for 24 h with the increase of salinity from 2.5% to 5%, 8% and 10%, respectively. Moreover, the corresponding removal rates of NO_3^--N were also increased from 5.59 to 5.96, 5.99 and 5.78 mg/L/h, responsively. *H. venusta* TJPU05 exhibited the maximal NO_3^--N removal performance under the salinity of 5% and 8%. However, the maximal salinity for nitrogen removal of *B. subtilis* GHSP10 was only 3% (Zhang et al., 2021). Furthermore, the removal efficiency of NO_3^--N by *H. venusta* TJPU05 within 24 h was much higher than that of *Pannonibacter phragmitetus* F1 at the same salinity (Wang et al., 2019b).

The TN removal was also affected by the salinity of SW. When the salinity was gradually increased from 2.5% to 5%, 8% and 10%, the TN removal efficiency was increased from 42.1% to 59.79% and then decreased to 53.06% and 45.13%, respectively. The corresponding removal rate of

TN was also increased from 2.69 to 3.9 and then decreased to 3.46 and 2.94 mg/L/h, respectively. These results indicated that the optimal salinity for TN removal of *H. venusta* TJPU05 was 5%.

NO_2^--N was produced in the process of denitrification and its amount was also affected by the salinity of SW. The concentration of NO_2^--N was the lowest at the optimal salinity of 5%. Similar result was also reported for *A. arilaitensis* Y-10 (He et al., 2017). This result was very interesting because the production of nitrite is harmful to microorganism in the process of wastewater treatment. *H. venusta* TJPU05 could reduce the accumulation of NO_2^--N during the denitrification under proper salinity (5%), which suggested that this strain might be helpful in solving the problem of excessive nitrite accumulation in hypersaline wastewater.

NO_2^--N was also used as sole nitrogen source to evaluate the effect of salinity on the aerobic denitrification capability of *H. venusta* TJPU05. Considering the reported deleterious effect of NO_2^--N on the growth of bacteria, a lower initial concentration of NO_2^--N (50 mg/L) was used in this section. As shown in Fig. 4(B), when the salinity was increased from 2.5% to 10%, the NO_2^--N removal efficiency was up to 100% and the removal rate was also up to 2.08 mg/L/h. Our results indicated that the higher salinity improved NO_2^--N removal performance of *H. venusta* TJPU05. However, it is reported that the denitrification efficiency and rate of most other denitrifiers decreased dramatically when the salinity of medium was higher than 3% (Yao et al., 2014; Ren et al., 2019; Cheng et al., 2020; Zhang et al., 2021). Moreover, most denitrifiers exhibited lag stage in ADM-2 medium due to the fact that the growth of bacteria was inhibited by the presence of NO_2^-, which reduced the denitrification performance of bacteria (Zhang et al., 2012; Ruan et al., 2020). However, *H. venusta* TJPU05 did not showed lag stage in ADM-2 medium and the nitrite nitrogen was quickly and completely removed within 24 h, which was beneficial for wastewater treatment with high salinity.

NAR and NIR are involved in the aerobic conversion of nitrate to nitrite and the aerobic conversion of nitrite to nitrogen-containing gas, respectively (Zhang et al., 2019a). From the data in Fig. 3, the highest enzyme activities of NAR and NIR were observed at the salinity of 5% and 8%, respectively. As discussed above, *H. venusta* TJPU05 exhibited the optimal NO_3^--N and NO_2^--N removal performance under the salinity of 5% and 8%. The results indicated that the nitrogen removal capacity of *H. venusta* TJPU05 was closely related to the salt-tolerance of corresponding enzymes. This is consistent with previous results (Wang et al., 2019a).

TN removal efficiency reached the highest level of 84.57%, with a fast removal rate of 2.6 mg/L/h at the salinity of 5%. However, the TN removal efficiency and rate both decreased when the salinity was higher than 5%. This phenomenon was possibly related to the growing production of NO_3^--N and NH_4^+-N resulting from the increasing salinity. As we can see that the level of NO_3^--N increased significantly when the salinity was higher than 5%. A small amount of NH_4^+-N was detected in SW at the salinity of 5%. However, the increase of salinity led to a higher production of NH_4^+-N. Similar result was also reported for *P. mendocina* LYX when NO_2^--N was used as the sole nitrogen resource (Li et al., 2021). The production of NH_4^+-N might come from microbial cells in the decomposition stationary or death phase suffering from the high salinity stress (He et al., 2019). These results implied that the 5% salinity was the most suitable concentration for sub-denitrification by *H. venusta* TJPU05.

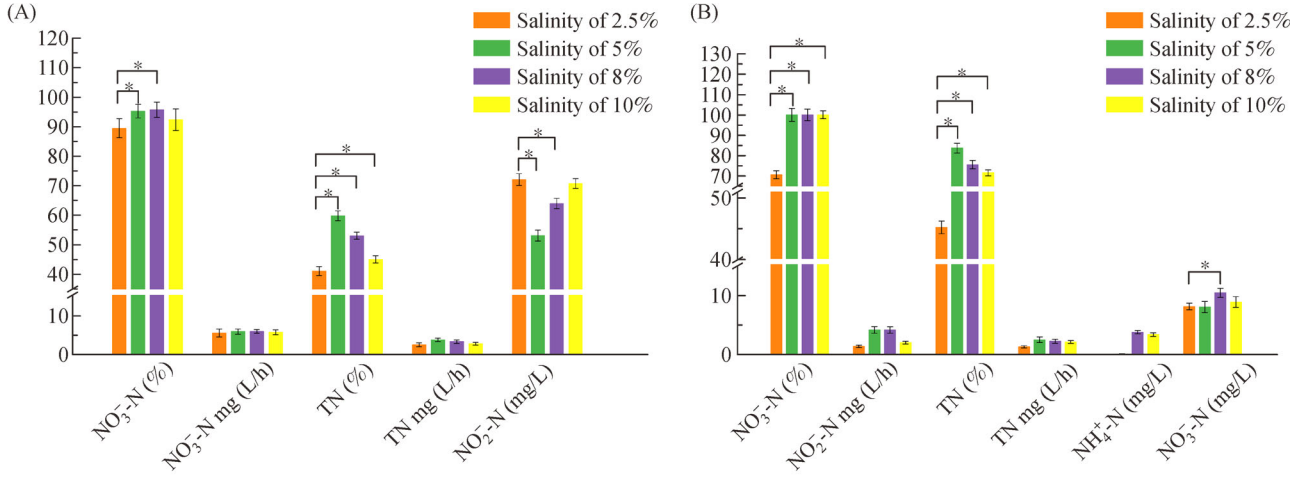

Fig. 4 Nitrogen removal performance of *H. venusta* TJPU05 in ADM-1 (A) and ADM-2 (B) medium under different salinity after incubation for 24 h. Statistical significance of data at 5%, 8% and 10% salinity compared with those at 2.5% salinity was evaluated, respectively; * $P < 0.05$.

3.4 Effect of salinity on simultaneous HN-AD performance

To investigate the simultaneous HN-AD performance of *H. venusta* TJPU05, NH_4^+-N and NO_3^--N were used as the mixed nitrogen sources in SW (i.e. SND-1 medium) with different salinity. As shown in Fig. 5(A), at the initial salinity of 2.5%, the removal efficiency of NH_4^+-N and the corresponding removal rate were 63.26% and 3.05 mg/L/h, respectively. And 83.96% of NO_3^--N was removed with a removal rate of 3.5 mg/L/h. Under the salinity of 5%, the removal efficiencies of NH_4^+-N and NO_3^--N reached 64.15% and 95.76%, and the corresponding removal rates were 3.49 and 7.29 mg/L/h, respectively. With salinity increasing to 8%, approximately 72.47% of NH_4^+-N and 90.76% of NO_3^--N were removed, and the corresponding removal rates were 3.02 and 3.78 mg/L/h, respectively. When the salinity was increased to 10%, approximately 84.06% of NH_4^+-N and 92.33% of NO_3^--N were removed with the corresponding removal rates of 3.50 and 3.85 mg/L/h, respectively. These results manifested that the NH_4^+-N and NO_3^--N could be simultaneously removed by *H. venusta* TJPU05. Furthermore, the simultaneous HN-AD performance was improved with the increase of salinity. In addition, the denitrification capacity of *H. venusta* TJPU05 was stronger than its nitrification capacity due to the higher removal rate of NO_3^--N than that of NH_4^+-N.

On the other hand, the highest removal efficiency of TN

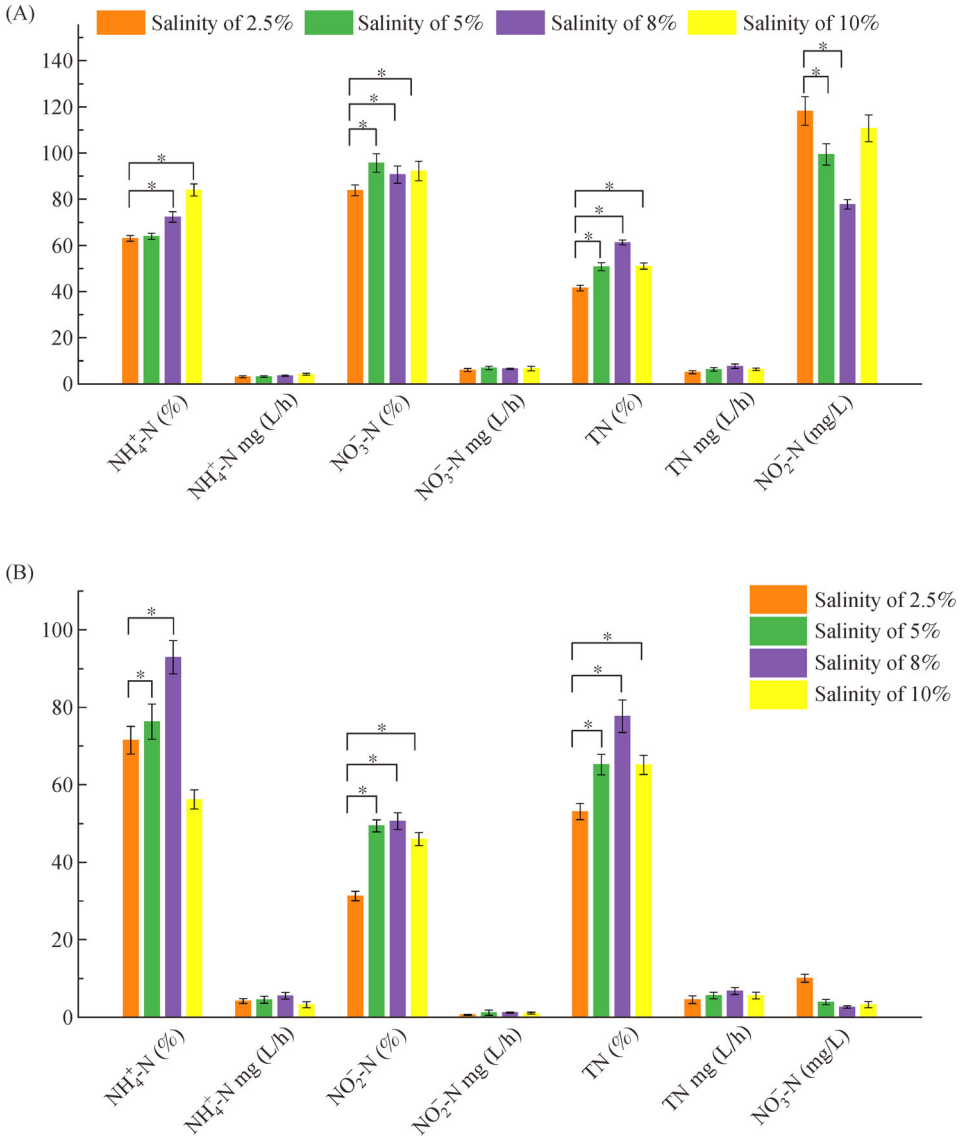

Fig. 5 Nitrogen removal performance of *H. venusta* TJPU05 in SND-1 (A) and SND-2 (B) medium under different salinity after incubation for 24 h. Statistical significance of data at 5%, 8% and 10% salinity compared with those at 2.5% salinity was evaluated, respectively; * $P < 0.05$.

was obtained at the salinity of 8%, and the corresponding removal rate of 8.02 mg/L/h was the highest among all the salinity. Besides, large amounts of NO_2^--N was found, which mostly originated from the conversion of NH_4^+-N and NO_3^--N to NO_2^--N in the process of simultaneous HN-AD by *H. venusta* TJPU05. This phenomenon was consistent with a previous report that the presence of ammonium promoted the reduction of nitrate to nitrite (He et al., 2017). The accumulation of NO_2^--N was the lowest at the salinity of 8%, suggesting that the salinity of 8% was the most suitable for HN-AD by *H. venusta* TJPU05.

Comparing the nitrogen removal performance of *H. venusta* TJPU05 in mixed nitrogen source with that in sole nitrogen source, the maximum removal efficiency of NH_4^+-N (84.06%) in SND-1 medium was slightly lower than that in HNM (86.12%). However, the salinity (10%) and initial nitrogen concentration (313.22 mg/L) of SND-1 medium was much higher than that of HNM (5% and 140.83 mg/L, respectively). Moreover, both NO_3^--N (95.76%) and TN (61.46%) removal efficiencies in SND-1 medium were higher than those in ADM-1 medium (95.68% and 59.79%, respectively). The optimal salinity for nitrogen removal by *H. venusta* TJPU05 in mixed nitrogen source (8%) was higher than that in sole nitrogen source (5%). These results demonstrated that *H. venusta* TJPU05 possessed the capability of simultaneous HN-AD under high salt and high nitrogen environment.

To our knowledge, there were very few reports about evaluating nitrogen removal performance of salt-tolerant bacteria in mixed nitrogen source. Although *B. subtilis* GHSP10 was proved to be salt-tolerant strain and the TN removal efficiency could be up to 96.86% in mixed nitrogen source with the salinity of 1%, the denitrification efficiency was greatly reduced to very low level when salinity was higher than 1% (Zhang et al., 2021). Therefore, *H. venusta* TJPU05 is a promising candidate for the nitrogen removal of wastewater with higher salinity.

NH_4^+-N and NO_2^--N were also used as mixed nitrogen source to determine nitrogen removal performance by *H. venusta* TJPU05. As shown in Fig. 5(B), at the initial salinity of 2.5%, the low removal efficiencies of NH_4^+-N (71.57%) and NO_2^--N (31.49%) were obtained in SND-2 medium. The corresponding removal rates were 2.98 mg/L/h for NH_4^+-N and 1.31 mg/L/h for NO_2^--N, respectively. When the salinity was improved to 5% and 8%, the removal efficiency of NH_4^+-N was increased to 76.34% and 92.9%, and the removal efficiency of NO_2^--N was increased to 49.55% and 50.77%, respectively. The corresponding removal rate also exhibited higher value than that at the salinity of 2.5%. However, the removal efficiencies of NH_4^+-N and NO_2^--N decreased when the salinity was increased to 10%. These results indicated that the highest removal efficiencies of NH_4^+-N and NO_2^--N were all obtained at the salinity of 8%. At the same time, *H. venusta* TJPU05 displayed the highest TN removal efficiency of 77.73% with a corresponding TN removal rate of 7.03 mg/L/h at the salinity of 8%. Too high or too low salinity all reduced the TN removal ability. However, the accumulation of NO_3^--N was the lowest under the salinity of 8%.

According to the above results, *H. venusta* TJPU05 could adapt to high-salt environment and had good performance of nitrification and denitrification in high salinity. However, too high salinity would inhibit cell metabolism, which was not conducive to nitrification and denitrification of *H. venusta* TJPU05. By contrast, the optimal salinity of 8% for *H. venusta* TJPU05 in mixed nitrogen sources was higher than that in sole nitrogen sources (5%), which demonstrated that the high salinity was more beneficial for the HN-AD performance of *H. venusta* TJPU05.

On the other hand, *H. venusta* TJPU05 displayed a lower removal efficiency of NO_2^--N in the presence of NH_4^+-N comparing with that using NO_2^--N as the sole nitrogen source. There were two reasons that might result in the low removal efficiency of NO_2^--N. One was that 220.99 mg/L of initial nitrogen concentration in SND-2 medium was much higher than that in ADM-2 medium (50 mg/L). The other was that simultaneous HN-AD produced more intermediate product nitrite in the whole process. The phenomenon was in agreement with a previous report that the denitrification ability of *P. mendocina* LYX was inhibited by the combination of NH_4^+-N and NO_2^--N with high concentration (Li et al., 2021). Even so, the NH_4^+-N and TN removal efficiencies of *H. venusta* TJPU05 in SDN-2 medium under the salinity of 8% were still higher than those in sole nitrogen source. Moreover, the accumulation of NO_3^--N in SDN-2 medium was considerably lower than that in sole nitrogen source. Therefore, it could be concluded that *H. venusta* TJPU05 possesses simultaneous HN-AD capacity under high salinity conditions.

The above results were further verified by studying the enzyme activity of *H. venusta* TJPU05 under aerobic conditions. As shown in Fig. 3, the successful expression of AMO, HAO, NAR and NIR proved that *H. venusta* TJPU05 had the ability of HN-AD. The activity of NAR was higher than that of NIR, indicating that the accumulation of NO_3^--N in HNM was invariably higher than that of NO_2^--N, regardless of the culture conditions. Therefore, the mechanism that *H. venusta* TJPU05 adopted to remove nitrogen through complete nitrification and denitrification pathways was consistent with that of *P. aeruginosa* P-1 (Wei et al., 2021) and our previous work (He et al., 2019).

3.5 Nitrogen removal performance of *H. venusta* TJPU05 in landfill leachate

Although *H. venusta* TJPU05 possessed excellent simultaneous HN-AD ability, its performance in treating AW with high concentration of salt and nitrogen remained to be determined. To verify the practical application value of *H.*

venusta TJPU05, the nitrogen removal capacity of this strain in AW (i.e. landfill leachate) was further investigated. As shown in Fig. 6, in control experiment (i.e. without inoculation of *H. venusta* TJPU05), 7.11% of NH_4^+-N, 37.7% of NO_3^--N and 7.9% of TN were removed after 24 h incubation, respectively. There was no obvious nitrite accumulation during the whole process. These results indicated that the indigenous microbial population had a slight influence on the nitrogen removal of AW (Zhang et al., 2019b). By contrast, the removal efficiencies of NH_4^+-N, NO_3^--N and TN were up to 50.96%, 47.28% and 50.92% after 24 h of incubation respectively when *H. venusta* TJPU05 was inoculated.

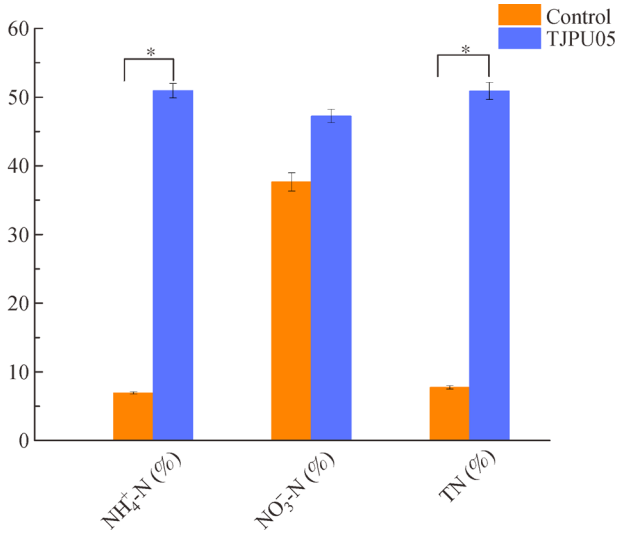

Fig. 6 The removal performance of NH_4^+-N, NO_3^--N and TN by *H. venusta* TJPU05 in AW. Statistical significance of data between control group and experimental group was evaluated: * $P < 0.05$.

In addition, there was almost no accumulation of NO_2^--N in the process of AW treatment by *H. venusta* TJPU05. Compared with other reports, most bacteria capable of HN-AD such as *Vibrio diabolicus* (Duan et al., 2015) and *Diaphorobacter* sp. (Ge et al., 2015) can not be used in treating actual primary wastewater with high salt concentrations. Therefore, these results indicated that *H. venusta* TJPU05 exhibited great potential for AW treatment with high concentration of salt and nitrogen.

4 Conclusions

A novel salt-tolerant bacterium, isolated from Salt Lake and identified as *H. venusta* TJPU05, showed excellent HN-AD ability in SW and AW with high concentration of salt and nitrogen. The salinity had an important effect on the nitrogen removal performance of *H. venusta* TJPU05 by influencing the activities of related enzymes. The highest activities of AMO, HAO, NAR and NIR were obtained at the salinity of 5% and 8%, respectively. Correspondingly, the optimal salinity for the treatment of SW by *H. venusta* TJPU05 was 5% and 8% in sole and mixed nitrogen sources, respectively. Furthermore, the co-expression of various enzymes proved that *H. venusta* TJPU05 possessed the ability of HN-AD. The concentration of nitrogen treated by *H. venusta* TJPU05 reached as high as 313.22 mg/L. The highest removal efficiencies of NH_4^+-N and NO_3^--N were 92.9% and 95.76% respectively in mixed nitrogen source, which were higher than those in sole nitrogen source. The TN removal efficiency of *H. venusta* TJPU05 in mixed nitrogen sources was higher than that in sole nitrogen sources at the salinity of 8%. More significantly, *H. venusta* TJPU05 exhibited good HN-AD ability to remove nitrogen from AW with high concentration of salt and nitrogen. The simultaneous HN-AD ability under high salt and high nitrogen condition makes *H. venusta* TJPU05 a broad application prospect in the field of wastewater treatment.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant No. 22075207) and the Training Program of Innovation and Entrepreneurship for Undergraduates (No. 201910058052).

Conflict of 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.

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