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Domesticating a Halotolerant Bacterium of *Vibrio* sp. LY1024 with Heterotrophic Nitrification–Aerobic Denitrification Property for Efficient Nitrogen Removal in Mariculture Wastewater Treatment

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Abstract: Dealing with mariculture wastewater that contains high nitrogenous compounds with efficient biological nitrogen removal technology is challenging but meaningful. The key lies in developing an active microorganism that can spontaneously complete the nitrification–denitrification processes in the marine environment. Herein, a halotolerant heterotrophic nitrification–aerobic denitrification (HN-AD) bacterium of *Vibrio* sp. LY1024 with good nitrogen removal capacity is domesticated to achieve the aforementioned goal. As a result, ammonium (NH_4^+-N) and nitrate (NO_3^--N) removal rates of almost 100% and 98.5% are detected over *Vibrio* sp. LY1024 at the salinity of 3.5%, even further increasing the salinity of wastewater to 5.5%. Its removal capacity towards both NH_4^+-N and NO_3^--N can still maintain at almost 100% and 94.7%, respectively. Further combining these results with those of intermediate product determination, it can be speculated that the ammonium removal is according to the pathway of NH_4^+-N → NH_2OH → NO_3^--N → N_2O → N_2. Moreover, the influence of wastewater temperature on the nitrogen removal efficiency of *Vibrio* sp. LY1024 is also considered. The NH_4^+-N and NO_3^--N removal efficiency over *Vibrio* sp. LY1024 at a relatively low temperature of 15 °C is still up to 97.3% and 76.4%, respectively. Our work provides a promising halotolerant and low-temperature resistance microorganism for the treatment of mariculture wastewater.

Keywords: heterotrophic nitrification; aerobic denitrification; low-temperature resistance; salt tolerance; *Vibrio* sp. LY1024; mariculture wastewater treatment

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1. Introduction

The densely concentrated mariculture model leads to much wastewater that contains highly toxic nitrogenous compounds, such as ammonium (NH_4^+-N) and nitrite (NO_2^--N), etc., being discharged into the marine environment, which will inevitably pose a great threat to the marine ecological system [1–4]. Therefore, it is urgent to develop an effective nitrogen removal technique for tackling mariculture wastewater.

Among various nitrogen removal techniques, the biological method has been regarded as a technique that is feasible and economically reasonable [5–8]. The mechanism of the traditional biological nitrogen removal process relies mainly on the two-stage theory, that is, nitrification and denitrification processes [9]. During this process, NH_4^+-N is first oxidized to NO_2^--N and NO_3^--N under the action of nitrite bacteria and nitrate bacteria under aerobic conditions, and then NO_2^--N or NO_3^--N is in turn transformed into nitrogenous gas by denitrification bacteria in anoxic conditions. Although this has been supposed to be a relatively mature technology, the different requirements between nitrification and denitrification bacteria in the working atmosphere still perplex the nitrogen removal processes.

To conquer this deficiency, a new model of the heterotrophic nitrification–aerobic denitrification (HN-AD) process is proposed. In this process, HN-AD bacteria can simultaneously carry out nitrification and denitrification under aerobic conditions in the same reactor, which greatly simplifies the process flow, and concurrently compensates for the alkalinity consumed during the nitrification process by the denitrification process [10]. Moreover, HN-AD bacteria also preserve the advantages of high growth rate and nitrogen removal capacity. However, within the limits of our knowledge, seldom have reports focused on the application of HN-AD bacteria in the treatment of mariculture wastewater, which might be ascribed to the fact that the growth and reproduction of common HN-AD bacteria would be inhibited in an environment of high salinity and the relatively low temperature [11]. Therefore, the present study aims at domesticating a halotolerant and low-temperature tolerant HN-AD bacteria with high nitrogen removal capacity to treat mariculture wastewater that contains high nitrogenous compounds.

Taking these into consideration, a halotolerant and low-temperature resistant HN-AD bacterium (named *Vibrio* sp. LY1024) is domesticated and identified by 16S rRNA gene sequence analysis. The effects of carbon-to-nitrogen (C/N) ratio, initial pH, temperature, dissolved oxygen, and salinity on the nitrogen removal performance of *Vibrio* sp. LY1024 and its nitrogen removal mechanism under high-salinity and low-temperature conditions are subsequently investigated. In addition, the treatment performance of *Vibrio* sp. LY1024 on stimulated mariculture wastewater is also evaluated. The results obtained from our work will provide a scientific basis for mariculture wastewater treatment.

2. Materials and Methods

2.1. Mediums

All the chemicals were of analytical grade and used as obtained without further purification. The seawater sample (salinity of 3.5%) utilized in this study was sampled from the Jinmeng Bay beach in the city of Qinhuangdao (39°55′ N, 119°38′ E). All the mediums were autoclaved at 121 °C for 20 min before utilization.

Trace element solution included the following: 50 g of $C_{10}H_{14}N_2O_8Na_2{\cdot}2H_2O$, 3.92 g of $MgSO_4{\cdot}7H_2O$, 7.28 g of $CaCl_2{\cdot}H_2O$, 5.06 g of $MnCl_2{\cdot}4H_2O$, 5 g of $FeSO_4{\cdot}7H_2O$, 1.57 g of $CuSO_4{\cdot}5H_2O$, 1.61 g of $CoCl_2{\cdot}6H_2O$, and 1 L of distilled water.

The components of activation medium (pH 7.2) employed for activating HN-AD bacteria included the following: 40 g of glucose, 10 g of NaCl, 2 mL of trace element solution, and 1 L of distilled water.

The constituents of domestication medium (pH 7.2) used for halotolerant HN-AD bacteria domestication included the following: 0.382 g of NH_4Cl, 0.5 g of $NaNO_2$, 7.167 g of $C_6H_5NaO_7{\cdot}2H_2O$, 0.2 g of KH_2PO_4, 2 mL trace element solution, (1) 1 L seawater/distilled water solution (v/v, 1/1, labeled as domesticated Medium 1), and (2) 1 L of seawater (labeled as domesticated Medium 2).

The Luria–Bertani (LB) medium (pH 7.2) employed for halotolerant HN-AD bacteria isolation and purification comprised the following ingredients: 5 g of peptone, 1 g of yeast powder, 16 g of agar, 2 mL of trace element solution, and 1 L of seawater.

The heterotrophic nitrification and aerobic denitrification (HN-AD) medium used to determine the nitrogen removal performance of isolated halotolerant HN-AD bacteria included the following: appropriate amount of NH_4Cl, KNO_3, $NaNO_2$, and $C_6H_5NaO_7{\cdot}2H_2O$; 0.2 g of KH_2PO_4; 2 mL of trace element solution; and 1 L of seawater.

2.2. Domestication, Isolation, and Identification of Halotolerant HN-AD Bacteria

The HN-AD bacterial seed was obtained from Jiangnan Biotechnology Co., Ltd., Yichun, China. Before domestication, the bacterial seed was first added to the activation medium and aerobic cultured at 20 °C for 18 h. After two rounds of 48 h of domestication, the resultant bacterial suspension was spread on the solid LB plates. After 36 h of incubation at 30 °C, separate colonies were picked and purified by repeated streaking on fresh LB plates.

2.3. Identification and Phylogenetic Analysis of Isolates

The morphologies of the isolated halotolerant HN-AD strain were observed by scanning electron microscopy (SEM, EM-30AXN, OPTON, Beijing, China). Bacterial 16S rRNA genes were amplified with universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and lower primer 1492R (5′-TACGGYTACCTTGTTACGACT-3′) [12]. The polymerase chain reaction (PCR) protocol of Tamura [13] was followed. The amplified products were sequenced, and the sequence was compared with available 16S rRNA gene sequences in GenBank by BLAST. A phylogenetic tree was constructed using MEGA 5.0 by the neighbor-joining method.

2.4. Investigating the Influence of Different Operation Parameters on Nitrogen Removal over Strain LY1024

To investigate the nitrogen removal performance and related mechanism of the isolated strain, 5 mL of the suspension that contain strain LY1024 (10.7 log CFU/mL) was inoculated into 250 mL of triplicate flasks and contacted with 100 mL of HN-AD medium (170 mg/L of NH_4^+-N or 120 mg/L of NO_3^--N or 130 mg/L of NO_2^--N, COD ≈ 1900 mg/L). The flasks were shaken continuously at 150 rpm to maintain aerobic conditions, and the reaction temperature was kept at 30 °C. A volume of 2.5 mL of samples was taken from the flasks at each 3 h interval.

The concentrations of NH_4^+-N, NO_2^--N, and NO_3^--N chemical oxygen demand (COD) were determined according to the National Standards of the People's Republic of China (HJ 535-2009, HJ/T 346-2007, HJ/T 399-2007, GB/T 7493-87), and the growth of the strain was assayed by measuring the wavelength of 600 nm (OD_{600}) using a spectrophotometer (WFZ-24A, Spectrum Shanghai, Shanghai, China).

The influence of different culture conditions, including C/N ratios of 5, 10, 15, 20, and 25, initial pH values of 5, 6, 7, 8, and 9, different temperatures of 15, 20, 25, and 30 °C, dissolved oxygen concentrations (DO amount changed by setting shaking speed at 100, 130, 150, and 180 rpm), and salinity (0%, 3.5%, 5.5%, 8.5%, and 10%) on the nitrogen (NH_4^+-N ≈ 40 mg/L, NO_3^--N ≈ 25 mg/L) removal performances of strain LY1024 were further investigated.

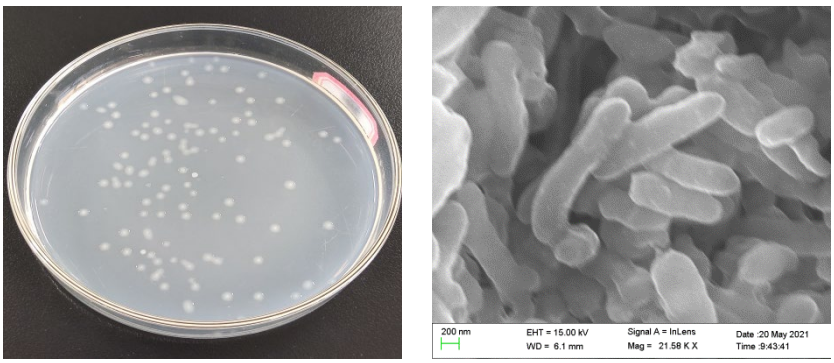
2.5. Treatment of Simulated Mariculture Wastewater by Strain LY1024

The simulated mariculture wastewater was prepared by dissolving 5 g of grouper bait into 4 L of seawater, and then the three flasks that contained 1 L of simulated mariculture wastewater (DO ≈ 4.67 mg/L) were kept at 25 °C. The effect of strain LY1024 on the nitrogen and COD removal of the simulated mariculture wastewater was determined. The physical and chemical properties of the simulated mariculture wastewater were as follows: pH = 7.8, salinity = 35 g/L, NH_4^+-N = 31 mg/L, NO_3^--N = 4.5 mg/L, NO_2^--N = 0.2 mg/L, total nitrogen (TN) = 38.2 mg/L, and chemical oxygen demand (COD) = 526 mg/L.

3. Results and Discussion

3.1. Identification of Isolates

After domestication and isolation, a strain with high nitrogen removal capacity and high salinity tolerance ability was obtained and named LY1024. As shown in Figure 1a, the colonies of strain LY1024 on the LB plate display a round shape with a regular edge and a glossy surface, and the color of these colonies is faint yellow. The micromorphology of strain LY1024 was further characterized by SEM analysis. From Figure 1b, many arc-shaped bacteria with an average size of 0.8–1.6 μm in length and 0.2–0.3 μm in width can be observed, which are typical characteristics of *Vibrio* sp. Bacteria. The subsequent result of sequence comparative analysis reveals that strain LY1024 belongs to the genus *Vibrio*, because it exhibits the closest phylogenetic relative to *Vibrio* sp. A5-15, with a similarity of up to 99.66%, as illustrated in Table 1. In addition, the neighbor-joining phylogenetic tree of strain LY1024 also indicates that strain LY1024 and *Vibrio* sp. belong to the same group (Figure 2). Based on these results, the isolated strain is identified as *Vibrio* sp. LY1024.



(**a**) (**b**)

Figure 1. (**a**) Colony morphology; (**b**) SEM image of strain LY1024.

Table 1. Sequence comparative analysis between strain LY1024 and other bacteria.

Description (16S Ribosomal RNA Gene Partial Sequence)	Per. Ident (%)
Vibrio sp. A5-15	99.66
Vibrio sp. B2-5-1	99.59
Vibrio sp. A5-5	99.52
Vibrio sp. B2-23	99.52
Vibrio sp. C4-5	99.52

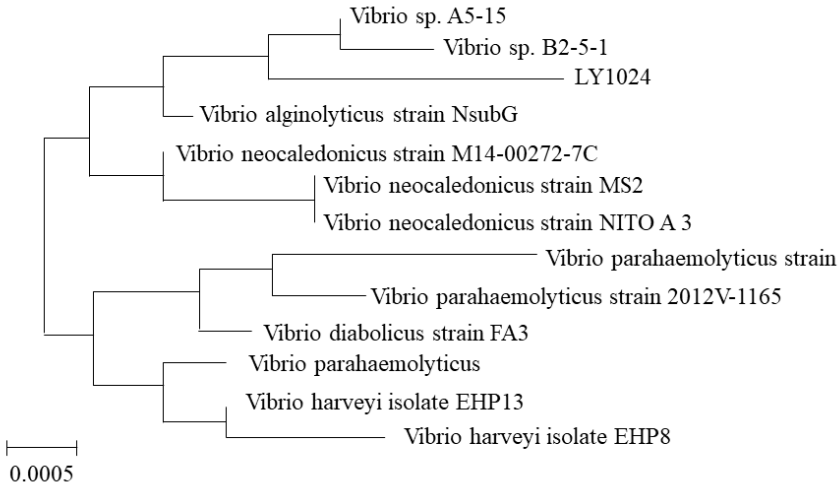


Figure 2. Phylogenetic tree of strain LY1024.

3.2. Heterotrophic Nitrification and Aerobic Denitrification Ability of Strain LY1024 at High Salinity

Ammonium (NH_4^+-N) was first selected as the sole nitrogen source to explore the heterotrophic nitrification and aerobic denitrification ability of strain LY1024 under high-salinity conditions, and the corresponding results are shown in Figure 3. It can be seen from the growth curve of strain LY1024 (Figure 3a) that, during the first 9 h of cultivation, the OD_{600} value of strain LY1024 displays a relatively low value of 0.28, indicating its growth rate was rather slow; correspondingly, relatively low removal rates of 11.5% and 21.5% toward NH_4^+-N and COD are observed on strain LY1024. Further prolonging the cultivation time to 27 h, the OD_{600} value rapidly increases from 0.28 to 1.6, demonstrating that the growth of strain LY1024 enters the logarithmic growth phase. As a result, both the concentrations of NH_4^+-N and COD are also degraded rapidly. Finally, approximately 98.8% of NH_4^+-N and 90.2% of COD are removed after 36 h of cultivation. The average ammonium removal rate was 4.74 mg/(L·h), which is higher than that of *Alcaligenes faecalis* C 16 (1.06 mg/(L·h)) [14] and *Rhodococcus* sp. CPZ 24 (3.4 mg/(L·h)) [15] and similar to that

of *Alcaligenes faecalis* strain NR (4.35 mg/(L·h)) [16]. Moreover, by detecting the variation of nitrate (NO_3^--N) concentration during the NH_4^+-N removal process (Figure 3b), it can be found that the concentration of NO_3^--N is improved continuously, with a maximum accumulation concentration of 16.3 mg/L when the reaction time reaches 21 h, indicating that strain LY1024 has the ability of heterotrophic nitrification. Through this process, NH_4^+-N is transformed into NO_3^--N. Further prolonging the reaction time to 36 h, the concentration of NO_3^--N displays a gradual degradation trend, implying that strain LY1024 also presents the ability of aerobic denitrification. Almost no accumulation of NO_2^--N was observed during the whole culture process.

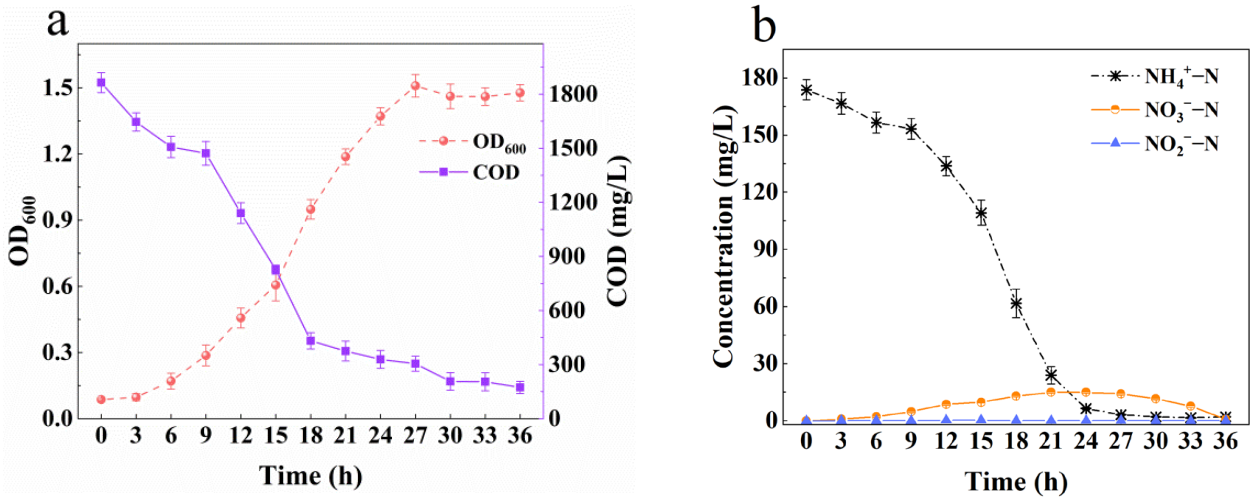


Figure 3. (**a**) The growth curve and COD removal capacity of strain LY1024; (**b**) heterotrophic nitrification performance of strain LY1024.

To verify the aforementioned inference, similar contrast experiments were carried out with NO_3^--N or NO_2^--N as the sole nitrogen source. As shown in Figure 4a, the growth trend of strain LY1024 with NO_3^--N as the nitrogen source is similar to the heterotrophic nitrification process, and 0–9 h is the adaptive stage of strain growth, during which the growth rate of LY1024 is slow. After 9 h, the strain enters the logarithmic growth stage, and the bacteria begin to grow and reproduce rapidly. In addition, the concentration of COD decreases rapidly with the growth of strain LY1024, and the removal rate reaches 90.1% after 36 h. In Figure 4b, NO_3^--N rapidly decreases from 121.58 mg/L to 0, and its removal rate is 100% after 24 h. There is almost no accumulation of NO_2^--N in the whole aerobic denitrification process. Therefore, it is preliminarily inferred that the intermediate binding of heterotrophic nitrification and aerobic denitrification of strain LY1024 is NO_3^--N.

The growth and COD removal capacity of strain LY1024 with NO_2^--N as the only nitrogen source are shown in Figure 4c. It follows that, after 6 h, strain LY1024 enters the logarithmic growth phase, and after 18 h, it enters the flat growth phase, and the OD_{600} value of the strain is stable at about 1.51, and the COD removal rate is 91.1% after 36 h. During the logarithmic growth phase, NO_2^--N is rapidly removed (Figure 4d). After 15 h, NO_2^--N is rapidly reduced from 132.5 mg/L to 0.06 mg/L, and the removal rate is nearly 100%. In the process of reaction, part of NO_3^--N is accumulated, the maximum amount of which is 8.58 mg/L, and then it is rapidly degraded.

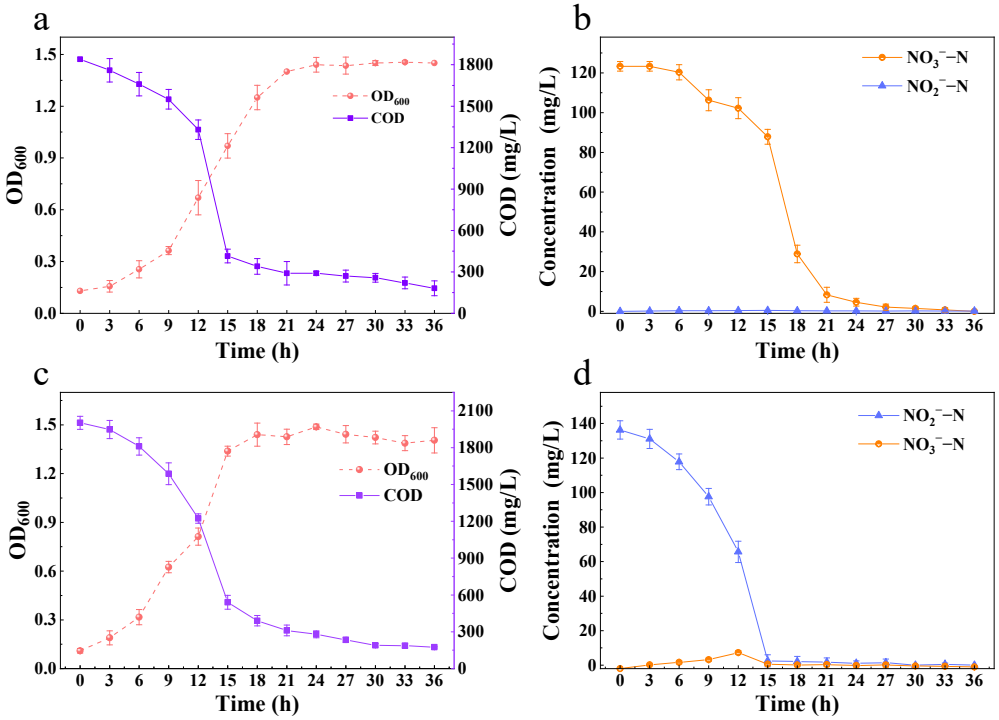


Figure 4. Aerobic denitrification characteristics of strain LY1024 with NO_3^--N (**a**,**b**) and NO_2^--N (**c**,**d**) as the solo nitrogen source.

3.3. Influence of C/N Ratio, Initial pH Value, Temperature, DO, and Salinity on Nitrogen Removal over Strain LY1024

Owing to the fact that carbon and nitrogen sources are the necessary energy sources for microbial growth and reproduction, to investigate the influence of the C/N ratio on the nitrogen removal capacity of HN-AD bacteria, a series of contrast experiments were conducted. As shown in Figure 5, it can be seen that when the C/N ratio is higher than 15:1 (including 15:1, 20:1, and 25:1), almost 100% of NH_4^+-N and more than 95% of NO_3^--N could be removed after 24 h of cultivation. However, when the C/N ratio reduces from 15:1 to 10:1 to 5:1, 92.7% and 51.5% of NH_4^+-N and 58.3% and 49.2% of NO_3^--N can be removed. It follows that the reduction of the C/N ratio has a greater effect on the removal rate of nitrate nitrogen than ammonia nitrogen. This can be ascribed to the lack of a carbon source for microbial growth and an electron donor for denitrification [9,17–19]. Moreover, it should be noted that with the C/N ratio increasing from 15 to 25, there is no significant difference in the NH_4^+-N removal rate. Therefore, to ensure sufficient NH_4^+-N removal, the C/N of 15 is selected in the following experiments.

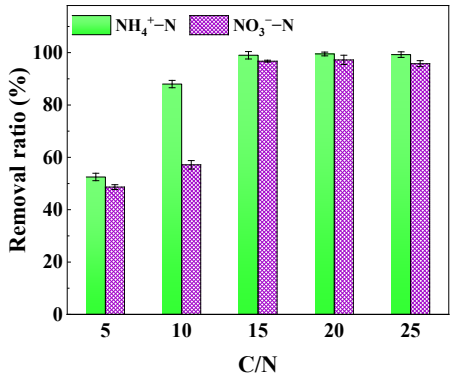


Figure 5. Influence of C/N on nitrogen compounds removal on *Vibrio* sp. LY1024.

To further explore the influence of the initial pH value on the NH_4^+-N and NO_3^--N degradation performances of strain LY1024, a group of contrast experiments were set at an initial pH value that varied from 5 to 6, 7, 8, and 9. From Figure 6, it can be observed that strain LY1024 presents excellent NH_4^+-N and NO_3^--N removal abilities at a wide pH value range from 6 to 9, where after 24 h of cultivation, 93.1%–99.2% of NH_4^+-N and 95.9%–99.1% of NO_3^--N can be degraded. In contrast, only 68.5% of NH_4^+-N and 46.4% of NO_3^--N are removed when the initial pH value is set at 5, suggesting that strain LY1024 is expected to display optimal nitrogen removal performance under a neutral or weak alkalinity solution. The pH values of all reaction systems increase after 24 h of cultivation (Table 2), further proving that the denitrification process is an alkali production process.

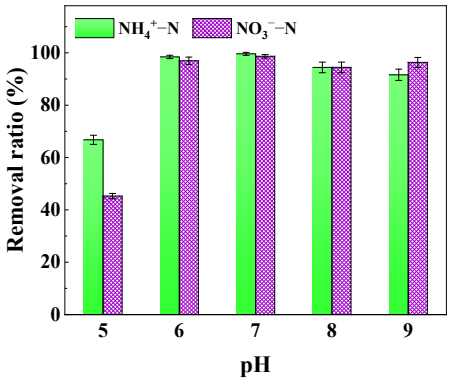


Figure 6. Influence of initial pH value on nitrogen compounds removal on *Vibrio* sp. LY1024.

Table 2. Variation of solution pH value before and after culture.

Initial pH Value	5	6	7	8	9
pH value after 24 h	5.12	6.80	7.75	8.67	9.05

The nitrogen compound removal properties of LY1024 at different temperatures are also investigated, and the related results are shown in Figure 7. After 24 h, the removal efficiencies of strain LY1024 towards NH_4^+-N at 15 °C, 20 °C, 25 °C, and 30 °C are 97.3%, 97.8%, 97.9%, and 98.5%, while its performance for NO_3^--N removal can also reach 76.4%, 95.3%, 96.3%, and 97.4%, respectively. These results demonstrate the strain LY1024 possesses excellent nitrogen removal ability in a wide temperature range, which is superior to most heterotrophic nitrification and aerobic denitrification bacteria already reported, as they were mainly carried out under mesophilic conditions (30–37 °C) [20–22]. The low-temperature-tolerance property endows strain LY1024 great potential to be employed to deal with practical mariculture wastewater, because the water temperature of mariculture wastewater treatment reactors is usually below 20 °C.

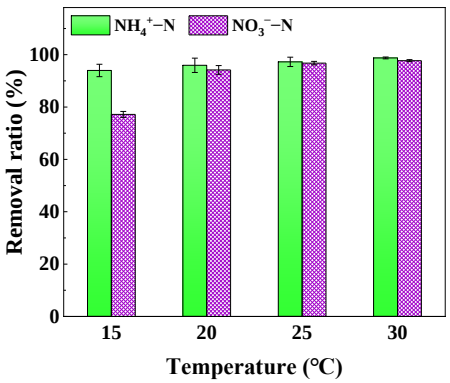


Figure 7. Influence of temperature on nitrogen compounds removal on *Vibrio* sp. LY1024.

In addition, the influence of contacted dissolved oxygen (DO) on the nitrogen removal activities of strain LY1024 is considered, and the degree of contacted DO is controlled by adjusting the shaking speed. From Figure 8, almost 100% of NH_4^+-N could be removed after 24 h of cultivation, regardless of whether the shaking speed is set at 100 rpm (DO ≈ 4.05 mg/L), 130 rpm (DO ≈ 4.67 mg/L), 150 rpm (DO ≈ 5.12 mg/L), or 180 rpm (DO ≈ 5.71 mg/L). Moreover, after 24 h of cultivation, the efficiencies of strain LY1024 towards NO_3^--N removal are 91.3%, 98.0%, 98.1%, and 98.0%, respectively. Thus, it can be concluded that an appropriate shaking speed is helpful to improve the nitrogen degradation rate owing to the increase in the mass transfer coefficient of oxygen. However, when the amount of DO reaches a certain level, an excessive increase in DO has no significant effect on the nitrogen removal rate.

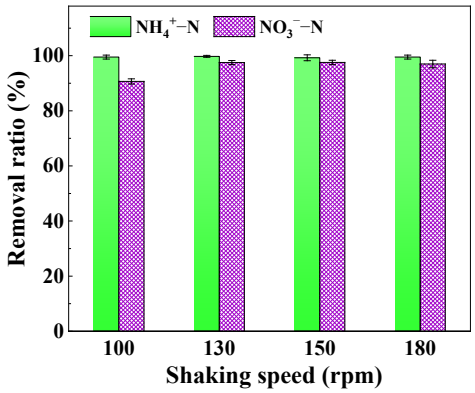


Figure 8. Influence of shaking speed on nitrogen compounds removal on *Vibrio* sp. LY1024.

Last but not the least, it is very necessary to investigate the salinity tolerance of LY1024 for achieving the goal of being employed to deal with mariculture wastewater, due to the latter usually containing high salinity. As shown in Figure 9, strain LY1024 exhibits relatively stable nitrogen removal efficiency with almost 100% of NH_4^+-N, and 81.1%–98.5% of NO_3^--N can be efficiently degraded in the salinity range of 0%–8.5% after 24 h of cultivation. However, further increasing the salinity to 10%, a sharp decrease in the NO_3^--N removal efficiency to 69.3% and a slight reduction of the NH_4^+-N removal rate to 86.4% are detected, respectively. These can be ascribed to the dehydration of cells caused by high salinity, which affects the biological activity of the microorganism [23]. The excellent nitrogen removal ability of strain LY1024 at the wide salinity range of 0%–8.5% greatly expands its application scope, which is also superior to most reported halotolerant strains, such as JR1, CL1502, etc. [24,25].

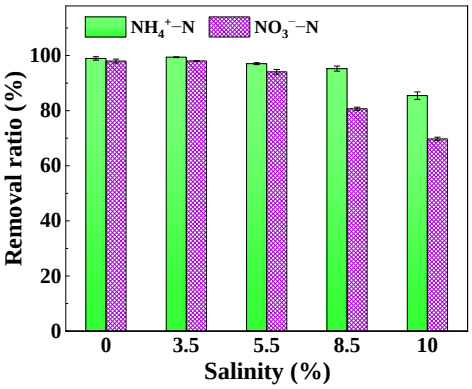


Figure 9. Influence of salinity on nitrogen compounds removal on *Vibrio* sp. LY1024.

3.4. Aerobic Nitrogen Removal Mechanism and Pathway by Strain LY1024

As mentioned above, when NH_4^+-N is used as the sole nitrogen source, the obvious accumulation of NO_3^--N and the significant decrease in COD appear during the reaction process, which indicates that obvious heterotrophic nitrification occurs in the process of nitrogen removal with organics as carbon sources. NO_3^--N accumulates to a certain extent and then gradually decreases under aerobic conditions, indicating that aerobic denitrification occurs in the process of nitrogen removal. The experimental results with NO_3^--N and NO_2^--N as the sole nitrogen source also well prove that *Vibrio* sp. LY1024 has good aerobic denitrification capacity.

At present, it is universally accepted that the aerobic denitrification process can be realized over bacteria through two main pathways [26], as depicted in Figure 10. One is NH_4^+-N is first converted into NH_2OH by the catalysis of ammonia monooxygenase (AMO), oxidized into NO_3^--N and NO_2^--N by hydroxylamine oxidase (HAO), reduced to NO and N_2O, and finally converted to N_2 released into the atmosphere [27–29]. The other is that NH_4^+-N is first converted into NH_2OH and then directly transformed into N_2O and N_2 without the conversion to intermediate, such as NO_3^--N and NO_2^--N [30–33].

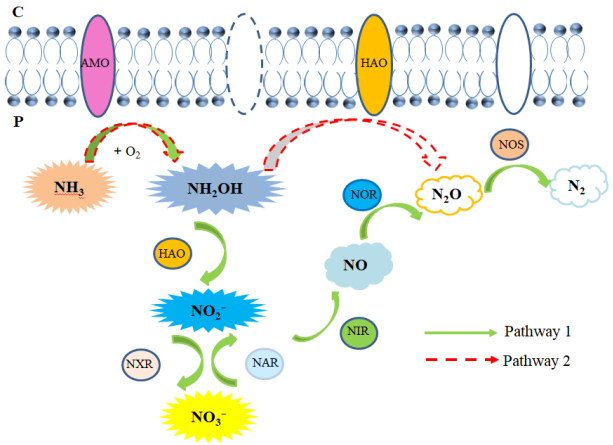


Figure 10. Possible pathways of aerobic nitrogen removal. Note: C: cytoplasm, P: periplasm, AMO: ammonia monooxygenase, HAO: hydroxylamine oxidase, NXR: nitrite oxidoreductase, NAR: nitrate reductase, NIR: nitrite reductase, NOR: nitric oxide reductase, NOS: nitrous oxide reductase.

Combining these theories with our determination results, *Vibrio* sp. LY1024 could oxidize NH_4^+-N under aerobic conditions, with obvious NO_3^--N and trace NO_2^--N accumulation appearing in the process of NH_4^+-N removal, meaning that the roles of AMO, HAO, and nitrite oxidoreductase (NXR) in the strain are expressed. In addition, *Vibrio* sp. LY1024 also displays the ability to degrade NO_3^--N and NO_2^--N under aerobic conditions, meaning that the roles of nitrate reductase (NAR) and nitrite reductase (NIR) in the strain are expressed. Therefore, the nitrogen removal mechanism over strain LY1024 is speculated via the pathway of NH_4^+-N $\rightarrow$ NH_2OH $\rightarrow$ NO_3^--N $\rightarrow$ N_2O $\rightarrow$ N_2.

3.5. Treatment of Stimulated Mariculture Wastewater by Vibrio sp. LY1024

Vibrio sp. LY1024 was finally employed to treat stimulated mariculture wastewater, and the corresponding result is shown in Figure 11. It can be seen from Figure 11 that strain LY1024 displays excellent activities on NH_4^+-N, NO_3^--N, and COD degradation. After 24 h of reaction, the removal rates of strain LY1024 towards NH_4^+-N, NO_3^--N, and COD reach 99.0%, 97.1%, and 88.5%, respectively. Moreover, almost no NO_2^--N accumulated in the process, further demonstrating that the nitrogen removal is mainly through the heterotrophic nitrification–aerobic denitrification (HN-AD) pathway. These experiment results imply that strain LY1024 has great potential in tackling the nitrogenous contaminants in practical mariculture wastewater.

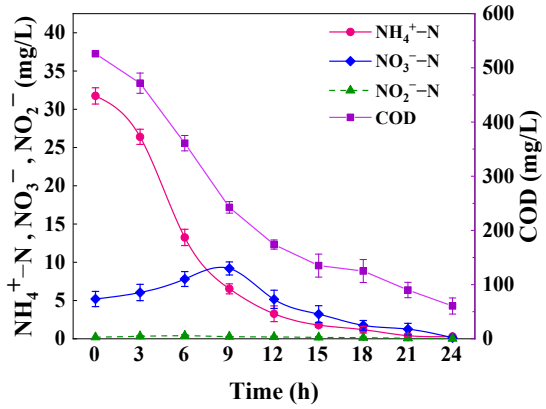


Figure 11. Treatment performance of simulated mariculture wastewater by *Vibrio* sp. LY1024.

The Monod model is usually used to describe the kinetics of microbial growth and substrate utilization. When it is used to describe the kinetics of substrate utilization, its formula can be expressed as:

$$v = (v_{max} \times S)/(K_s + S) \quad (1)$$

where v is the specific substrate removal rate, v_{max} is the maximal specific substrate removal rate, S is the substrate concentration, and K_s is the Monod constant.

In this study, the Monod model is used to describe the nitrogen removal reaction kinetics of the simulated mariculture wastewater and the fitting results are shown in Figure 12. The fitting results show the nitrogen removal reaction kinetics can be well described by the Monod model with the correlation coefficient $R^2 = 0.973$. The maximal nitrogen (total inorganic nitrogen) removal rate v_{max} is calculated to be 2.17 mg/(L·h), which is comparable to most reported results [34,35], indicating the good denitrogenation performance of *Vibrio* sp. LY1024, and the Monod constant K_s is calculated to be 4.28 mg/L.

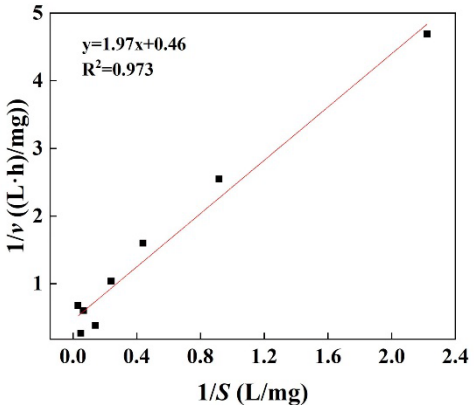


Figure 12. Fitting results of denitrification experimental data with Monod model.

4. Conclusions

Overall, a halotolerant HN-AD bacterium of *Vibrio* sp. LY1024 with high nitrogen removal capacity is domesticated and isolated. *Vibrio* sp. LY1024 exhibits excellent nitrogen removal performance under the conditions of a C/N ratio greater than 15, an initial pH value of 6–9, a temperature of 15–30 °C, a DO of 4.05–5.71 mg/L, and a salinity of 0%–10%. During the treatment of the simulated mariculture wastewater, 99.0%, 97.1%, and 88.5% of NH_4^+-N, NO_3^--N, and COD can be removed by *Vibrio* sp. LY1024 in 24 h of reaction time, and the nitrogen removal pathway is speculated as NH_4^+-N → NH_2OH → NO_3^--N → N_2O → N_2. Such a wide range of application conditions endows *Vibrio* sp. LY1024 with a great application potential in mariculture wastewater treatment.

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