

ISOLATION AND IDENTIFICATION OF AN AMMONIA-NITROGEN-DEGRADING BACTERIUM *BACILLUS STERCORIS* SL1 AND DEGRADATION CHARACTERISTICS

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Abstract: This study aimed to screen efficient ammonia-nitrogen-degrading bacteria from natural environments and investigate their taxonomic status and degradation characteristics, thereby providing bacterial resources for the biological treatment of ammonia-nitrogen wastewater. Ammonia-nitrogen-degrading bacteria were enriched and isolated from soil samples collected around South Lake in Wuhan, using ammonium chloride as the primary nitrogen source. The bacterial strain was identified through morphological observation and 16S rDNA sequencing. The effects of initial ammonia-nitrogen concentration (40~120 mg/L), inoculum size (1%~10%, V/V), temperature (25~35 °C), and initial pH (6.0~8.0) on the ammonia-nitrogen removal rate of the strain were investigated using single-factor experiments. A highly efficient ammonia-nitrogen-degrading bacterium, identified as *Bacillus stercoris* SL1 (16S rDNA similarity 99.65%), was isolated. The single-factor experiments revealed that under the conditions of an initial ammonia-nitrogen concentration of 60 mg/L, an inoculum size of 1%, and a pH of 7.0, the strain exhibited a removal rate of 61.50% at 30 °C over a 48-hour period. Notably, when the temperature was increased to 35 °C, the removal rate significantly rose to 70.08% ($P < 0.05$). Additionally, substrate inhibition was observed when the initial ammonia-nitrogen concentration was ≥ 80 mg/L. The highest removal rate (60.41%) was achieved with an inoculum size of 1% over a 48-hour period, while excessive inoculum (10%) reduced the rate to 50.03%. The strain exhibited high degradation activity within the pH range of 6.0~8.0. After culturing under optimal conditions for 48 hours, the nitrite nitrogen accumulation in the culture medium was only 0.016 mg/L, indicating minimal nitrite accumulation during the ammonia-nitrogen degradation process. The strain *Bacillus stercoris* SL1 has significant heat tolerance and efficient ammonia-nitrogen degradation ability, showing great potential in the biological treatment of medium and high temperature ammonia-nitrogen wastewater.

Keywords: Ammonia-nitrogen degradation; *Bacillus stercoris* SL1; Biological denitrification; *Bacillus*

1 INTRODUCTION

Ammonia-nitrogen ($\text{NH}_4^+\text{-N}$) is a major aquatic pollutant that causes eutrophication and oxygen depletion. While physical-chemical treatments for $\text{NH}_4^+\text{-N}$ removal are effective, they suffer from high costs, substantial energy consumption, and secondary pollution. Traditional biological denitrification is limited by the slow growth and environmental sensitivity of autotrophic nitrifying bacteria. In contrast, heterotrophic nitrification-aerobic denitrification (HN-AD) microorganisms have emerged as superior alternatives due to their rapid growth, high stress resistance, and ability to simultaneously remove nitrogen and phosphorus in a single reactor. The genus *Bacillus* is particularly promising for developing HN-AD agents because of its environmental resilience—facilitated by spore formation—and several species within this genus have demonstrated highly efficient $\text{NH}_4^+\text{-N}$ removal [1-3]. *Bacillus stercoris*, recently isolated from compost [4], currently lacks systematic investigation regarding its $\text{NH}_4^+\text{-N}$ degradation capabilities. Therefore, this study aims to explore the ammonia-nitrogen degradation characteristics of *B. stercoris*, thereby enriching the functional understanding of this species and expanding the microbial resources for wastewater treatment.

In this study, a strain of *Bacillus stercoris* SL1 was isolated from the soil around South Lake in Wuhan City, and the effects of initial ammonia-nitrogen concentration, inoculum size, temperature, and initial pH on its ammonia-nitrogen degradation efficiency were systematically investigated, providing bacterial resources and basic data support for the biological enhanced treatment of ammonia-nitrogen wastewater.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Sample source

Soil samples for strain screening were collected from the surface soil (0–10 cm) around South Lake, Wuhan City. A five-point mixed sampling method was adopted. After removing surface debris, the samples were sealed in sterile ziplock bags,

transported to the laboratory in an icebox, and stored at 4 °C for subsequent use. The sampling was conducted in December 2025.

2.1.2 Culture media

Enrichment medium (g/L): NH₄Cl 0.19, tryptone 10.0, yeast extract 5.0, NaCl 5.0, and glucose 5.0. Ammonium chloride and glucose were used as the primary nitrogen and carbon sources, respectively.

Isolation medium: Enrichment medium supplemented with 15 g/L agar.

Heterotrophic nitrification medium (g/L): NH₄Cl 0.38, sodium succinate 4.05, and 50 mL of Trace Elements Solution.

Trace Elements Solution (g/L): K₂HPO₄ 6.55, MgSO₄·7H₂O 2.5, NaCl 2.5, MnSO₄·H₂O 0.038, and FeSO₄·7H₂O 0.05.

LB medium (g/L): Tryptone 10.0, yeast extract 5.0, and NaCl 10.0.

Seed medium: The same as the enrichment medium, without agar.

The pH of all media was adjusted to 7.0–7.2, followed by sterilization at 121 °C for 20 min. Solid media were supplemented with 1.5% agar.

2.2 Methods

2.2.1 Enrichment, isolation, and purification of ammonia nitrogen-degrading bacteria

A 5.0 g soil sample was added to a 100 mL Erlenmeyer flask containing 45 mL of sterile saline (0.85% NaCl). The mixture was oscillated at 30 °C and 150 r/min for 30 min. After settling, the supernatant was collected as the inoculum. A 5% (v/v) inoculum was transferred into 100 mL of enrichment medium and incubated at 30 °C with constant shaking at 150 r/min for 48 h. Subsequently, 5 mL of the enriched culture was transferred into fresh medium for subculturing, and this process was repeated twice.

The final enriched culture was subjected to a 10-fold serial dilution. Aliquots of 100 µL from the 10⁻⁴, 10⁻⁵, and 10⁻⁶ dilutions were spread onto isolation medium plates, with three replicates for each dilution. The plates were incubated upside down at 30 °C for 24–48 h. Single colonies with distinct morphologies were picked and repeatedly streaked on LB plates 3–4 times until pure cultures were obtained. The purified strains were inoculated onto LB slants for short-term storage at 4 °C, and simultaneously preserved in 20% glycerol at -20 °C for long-term storage.

2.2.2 Screening of ammonia nitrogen-degrading bacteria

The purified strains were individually inoculated into LB liquid medium and cultivated at 30 °C and 150 r/min until the logarithmic growth phase to prepare bacterial suspensions with an optical density (OD₆₀₀) of approximately 1.0. A 5% (v/v) inoculum was then transferred into 250 mL Erlenmeyer flasks containing 100 mL of enrichment medium (with an initial ammonium nitrogen concentration of 60 mg/L) and incubated at 30 °C with shaking at 150 r/min for 48 h. At the end of the cultivation, samples were collected and centrifuged at 5000 r/min for 5 min. The supernatant was used to determine the ammonium nitrogen (NH₄⁺-N) concentration and calculate the removal rate. The strain exhibiting the highest NH₄⁺-N removal rate was selected as the target strain for this study.

2.2.3 Identification of the ammonia nitrogen-degrading bacterium

Morphological identification: The purified target strain was streaked onto LB solid medium and incubated at 30 °C for 24 h to observe colony morphology (size, color, edge characteristics, surface texture, etc.). A uniform single colony was picked for Gram staining, and the cellular morphology was observed under an optical microscope.

Molecular biological identification: The purified strain was sent to Hangzhou Yanqu Information Technology Co., Ltd. for 16S rDNA gene sequencing. The testing institution performed PCR amplification and Sanger bidirectional sequencing using bacterial universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The obtained 16S rDNA sequence was submitted to the NCBI GenBank database for BLAST homology comparison. The 16S rDNA sequences of closely related type strains with high homology were downloaded, and a phylogenetic tree was constructed using the Neighbor-Joining method in MEGA 11.0 software with 1000 bootstrap replicates.

2.2.4 Preparation of seed liquid

The preserved target strain was streaked onto an LB solid plate and activated at 30 °C for 24 h. A single colony was picked and inoculated into a 250 mL Erlenmeyer flask containing 100 mL of seed medium, followed by incubation at 30 °C with shaking at 150 r/min for 18 h. The absorbance of the culture was measured at 600 nm and adjusted to an OD₆₀₀ of approximately 1.0 (with a cell concentration of approximately 10⁷ CFU/mL) to serve as the seed liquid.

2.2.5 Single-factor experimental design

Single-factor experiments were conducted in 250 mL Erlenmeyer flasks with a working volume of 100 mL. The initial ammonium nitrogen concentration was provided by NH₄Cl. The baseline cultivation conditions were set as follows: initial ammonium nitrogen concentration of 60 mg/L, inoculum size of 1% (v/v), temperature of 30 °C, initial pH of 7.0, and shaking speed of 150 r/min. When investigating the effect of a specific factor, only the level of that factor was changed, while the other conditions remained at the baseline levels. Each treatment was performed in duplicate, and an uninoculated blank control was set up simultaneously to correct for the natural volatilization loss of ammonia nitrogen.

(1) Initial ammonium nitrogen concentration: Five gradients were set at 40, 60, 80, 100, and 120 mg/L.

(2) Inoculum size: Three gradients were set at 1%, 5%, and 10% (v/v, corresponding to the addition of 1, 5, and 10 mL of seed liquid per 100 mL of medium, respectively).

(3) Temperature: Three gradients were set at 25, 30, and 35 °C.

(4) Initial pH: Three gradients were set at 6.0, 7.0, and 8.0, adjusted using 1 mol/L HCl or NaOH.

Samples were taken at 24 h and 48 h of cultivation, filtered through 0.45 μm membranes, and measured for ammonium nitrogen **concentration** to calculate the removal rate.

2.3 Analytical and Calculation Methods

The ammonium nitrogen ($\text{NH}_4^+\text{-N}$) concentration was determined using Nessler's reagent spectrophotometry according to the standard method HJ 535-2009 [5]. The nitrite nitrogen ($\text{NO}_2^-\text{-N}$) concentration was determined by spectrophotometry according to the standard method GB 7493-87 [6]. OD_{600} refers to the absorbance of the bacterial suspension at a wavelength of 600 nm. The ammonium nitrogen removal rate was calculated using the following formula: Removal rate (%) = $(C_0 - C_t) / C_0 \times 100\%$

Where C_0 is the initial ammonium nitrogen concentration (mg/L), and C_t is the ammonium nitrogen concentration at culture time t (mg/L).

2.4 Data Processing

The experimental data were organized and preliminarily calculated using Microsoft Excel 2019. One-way analysis of variance (One-way ANOVA) and Duncan's multiple range test ($\alpha = 0.05$) were performed using SPSS 26.0. Graphs were plotted using Origin 2024 software.

3 RESULTS AND ANALYSIS

3.1 Isolation and Identification of Ammonia Nitrogen-Degrading Bacteria

3.1.1 Strain isolation and screening

After enrichment cultivation and repeated streaking, two bacterial strains capable of utilizing ammonium chloride as the sole nitrogen source were isolated from the soil samples collected around South Lake, Wuhan. These two strains were individually inoculated into the enrichment medium with an initial ammonium nitrogen concentration of 60 mg/L and incubated at 30 °C for 48 h, after which the ammonium nitrogen removal rates were determined. There were significant differences in the ammonium nitrogen degradation capabilities among the isolates. One strain exhibited the highest removal rate, reaching 61.50%, which was significantly higher than that of the other strain ($P < 0.05$). Consequently, this strain was selected as the target strain for subsequent studies.

3.1.2 Morphological characteristics



Figure 1 Colony Morphology of the Strain

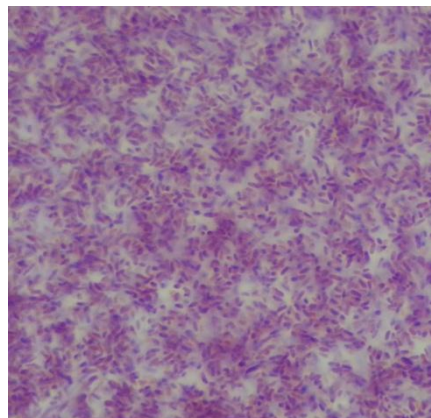


Figure 2 Gram-Stained Microscopic Image of the Strain under an Optical Microscope

After incubation on LB solid medium at 30 °C for 24 h, the target strain formed circular colonies with diameters ranging from 4 to 6 mm. The colonies were opaque, off-white to pale yellow, with a dry and rough surface and irregular serrated edges (Figure 1). Gram staining revealed that the strain was Gram-positive. The cells were rod-shaped, arranged singly or in pairs, and elliptical endospores could be observed (Figure 2). These morphological characteristics are consistent with the typical features of the genus *Bacillus*.

3.1.3 Molecular biological identification

Using the genomic DNA of the strain as a template, a 16S rDNA gene fragment of approximately 1500 bp was amplified via PCR. The assembled sequencing results were submitted to the NCBI GenBank database for BLAST analysis. The results revealed that the 16S rDNA sequence of this strain shared a high homology of 99.65% with *Bacillus stercoris* SL1 (GenBank accession no.: NR_181952.1), 98.72% with *Bacillus australimaris* MCCC 1A05787 (NR_148787.1), and 98.35% with *Bacillus piscis* 16MFT21 (NR_165685.1). The phylogenetic tree constructed using the Neighbor-Joining method (Figure 3) demonstrated that the strain clustered in the same branch as *Bacillus stercoris* SL1 with a 100% bootstrap value, indicating the closest phylogenetic relationship. Based on the combined morphological characteristics and molecular biological identification results, the strain was identified as *Bacillus stercoris*.

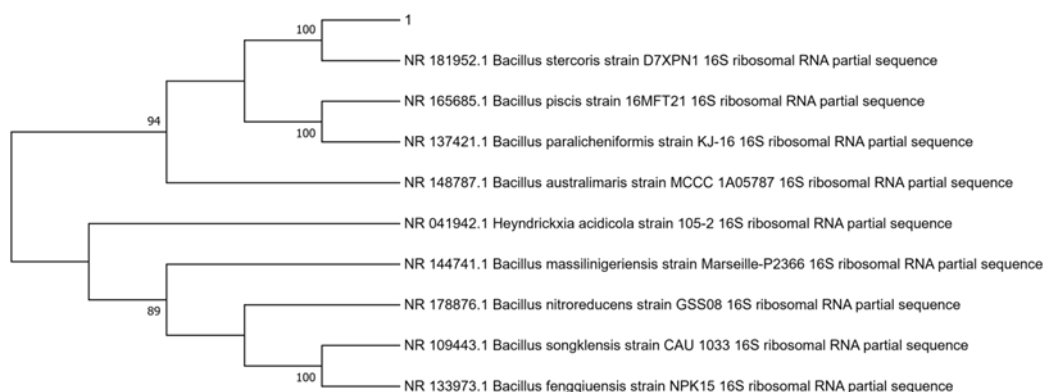


Figure 3 Phylogenetic Tree of the Strain Based on the 16S rDNA Gene Sequence

3.2 Results of Single-Factor Experiments

3.2.1 Effect of initial ammonia nitrogen concentration on degradation efficiency

Under the baseline cultivation conditions (30 °C, inoculum size of 1%, pH 7.0, and 150 r/min), the effects of different initial ammonia nitrogen concentrations on the degradation efficiency of the strain are shown in Figure.

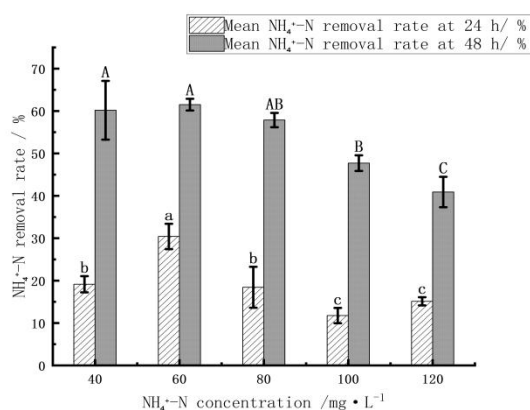


Figure 4 Effect of Initial Ammonia Nitrogen Concentration on Ammonia Nitrogen Removal Rate

As shown in Figure 4, at 24 h, the 60 mg/L treatment group exhibited the highest ammonia nitrogen removal rate (30.41%), which was significantly higher than that of the other concentration groups ($P < 0.05$). When the initial ammonia nitrogen concentration increased to 80 mg/L or above, the removal rate at 24 h decreased significantly, indicating that high concentrations of ammonia nitrogen exerted a pronounced inhibitory effect on the initial activity of the strain. At 48 h, the removal rates for the 40 mg/L and 60 mg/L treatment groups reached 60.18% and 61.50%, respectively, both of which were significantly higher than those of the 80 mg/L and above groups. The 48 h removal rates for the 100 mg/L and 120 mg/L treatment groups were significantly lower, demonstrating that the strain's tolerance to excessively high concentrations of ammonia nitrogen was limited and subject to evident substrate inhibition.

The inhibitory effect of high-concentration ammonia nitrogen on microorganisms is primarily mediated by free ammonia (FA). Numerous studies have demonstrated that FA can exert a significant inhibitory effect on the biological nitrogen

removal of wastewater [7]. FA can freely diffuse across the cell membrane into the cell; upon dissociation, it causes the dissipation of the proton gradient, interferes with oxidative phosphorylation, and inhibits ATP synthesis. Simultaneously, FA can directly bind to the active sites of key denitrifying enzymes, thereby inhibiting their enzymatic activities. This inhibition primarily compromises wastewater nitrogen removal performance by reducing the activity of denitrifying bacterial communities and restructuring the microbial community structure [8]. The *Bacillus amyloliquefaciens* strain screened by Zhang et al. achieved a 48 h removal rate of 92.32% for 100 mg/L ammonia nitrogen [1]. In contrast, the 48 h removal rate of the strain in this study for 100 mg/L ammonia nitrogen was only 47.71%, indicating its relatively limited tolerance to high-concentration ammonia nitrogen. Furthermore, the *Lysinibacillus sphaericus* N-2 strain isolated by Gu et al. exhibited a 91.0% removal rate for 100 mg/L ammonia nitrogen within just 10 h [9], further confirming that significant differences exist in substrate tolerance among different *Bacillus* strains. Based on the above analysis, the suitable initial ammonia nitrogen concentration for *Bacillus stercoris* SL1 is 40–60 mg/L.

3.2.2 Effect of inoculum size on degradation efficiency

The effects of different inoculum sizes on the ammonia nitrogen removal rate of the strain are shown in Figure 5.

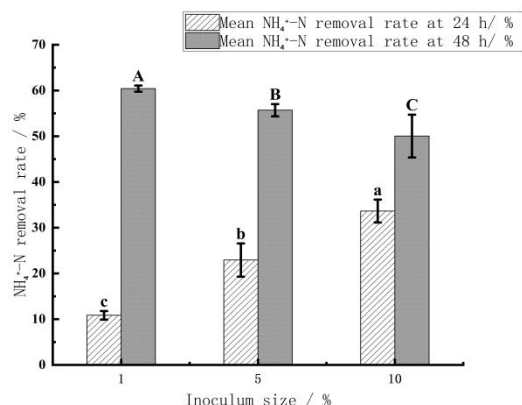


Figure 5 Effect of Initial Ammonia Nitrogen Concentration on Ammonia Nitrogen Removal Rate

As shown in Figure 5, at 24 h, the ammonia nitrogen removal rate increased significantly with increasing inoculum size ($P < 0.05$). The removal rate in the 10% inoculum group reached 33.64%, which was 1.5-fold and 3.1-fold that of the 5% group (22.93%) and the 1% group (10.85%), respectively. This is because a higher initial cell density can rapidly utilize substrates for metabolism, thereby shortening the lag phase and increasing the initial degradation rate. This result is consistent with the findings of Yang et al. regarding *Bacillus* sp. JD-014 [2]. Research on *Bacillus cereus* YB3 has also confirmed that an increased inoculum size can significantly accelerate the initial ammonia nitrogen removal rate [10].

Notably, a reverse trend was observed at 48 h: the 1% inoculum group exhibited the highest removal rate (60.41%), whereas the 10% group showed the lowest (50.03%). This phenomenon of a "high final removal rate at a low inoculum size" can be explained from the perspective of nutrient limitation in a closed cultivation system. Under high inoculum conditions, the cells underwent rapid proliferation in the initial stage and consumed a substantial amount of carbon sources. As the cultivation progressed into the later stage, carbon source depletion forced a portion of the cells into the endogenous respiration phase or even caused cell autolysis. The intracellular nitrogen-containing organic compounds released by autolysis were subsequently re-ammonified, leading to the release of ammonia nitrogen and consequently decreasing the apparent removal rate. Mason et al. systematically elucidated this phenomenon from a microbial ecology perspective [8], pointing out that in a closed system, an excessively high initial cell density intensifies nutrient competition, leading to cell death and autolysis in the later stage, which paradoxically reduces the net removal efficiency of the target pollutant. These results indicate that for this strain, an inoculum size of 1% (v/v) achieves the highest ammonia nitrogen removal rate within 48 h, representing the optimal inoculation condition.

3.2.3 Effect of temperature on degradation efficiency

The ammonia nitrogen degradation efficiency of the strain at different temperatures is shown in Figure 6.

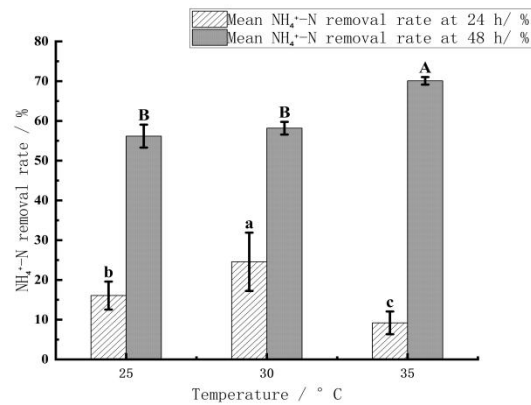


Figure 6 Effect of Temperature on Ammonia Nitrogen Removal Rate

As shown in Figure 6, at 24 h, the 30 °C treatment group exhibited the highest removal rate (24.56%), which was significantly higher than those of the 25 °C group (16.07%) and the 35 °C group (9.20%) ($P < 0.05$). The relatively low removal rate at 24 h under the 35 °C condition may be attributed to the sudden temperature increase; the cells were required to synthesize heat shock proteins to adapt to the environmental stress, which temporarily reduced the energy allocation for degradation. Alternatively, the activity of certain enzyme systems associated with substrate transport might have been temporarily inhibited during the initial phase of the high-temperature exposure.

At 48 h, the 35 °C treatment group demonstrated a robust degradation capacity, reaching a removal rate of up to 70.08%, which was significantly higher than those of the 30 °C group (58.17%) and the 25 °C group (56.17%) ($P < 0.05$), whereas the difference between the 25 °C and 30 °C groups was not significant ($P > 0.05$). This result clearly demonstrates that the optimal degradation temperature for *Bacillus stercoris* SL1 is 35 °C, at which its key enzymes involved in ammonia nitrogen degradation possess the highest catalytic activity. The denitrification-related enzyme systems in the genus *Bacillus* are predominantly mesophilic enzymes, with optimal catalytic temperatures typically ranging from 30 to 40 °C. The excellent performance of the strain at 35 °C is consistent with the characteristics of thermotolerant denitrifying bacteria reported by Yan et al. [11]. Furthermore, the optimal degradation temperature for *Pseudomonas aeruginosa* CH1, isolated from seawater by Chen et al. [10], was also 35 °C. Additionally, *Bacillus huajintanensis* SLWX2, as reported by Wang et al. [12], exhibited excellent ammonia nitrogen removal performance within the range of 28–40 °C. The 48 h removal rate of the present strain reaching 70.08% at 35 °C indicates its favorable tolerance to moderate and high temperatures, conferring unique application advantages for the treatment of moderately high-temperature wastewater in summer or in tropical regions.

3.2.4 Effect of initial pH on degradation efficiency

The ammonia nitrogen degradation efficiency of the strain under different initial pH conditions is shown in Figure 7.

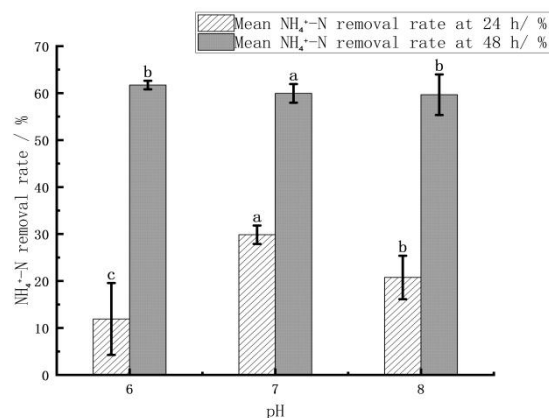


Figure 7 Effect of Initial pH on Ammonia Nitrogen Removal Rate

pH regulates microbial metabolism by affecting cell membrane surface charge, substrate ionization state, and the conformation of enzyme active sites.

As shown in Figure 7, at 24 h, the strain exhibited the highest ammonia nitrogen removal rate at pH 7.0 (29.85%), which was significantly higher than those of the pH 8.0 group (20.75%) and the pH 6.0 group (11.91%) ($P < 0.05$). At 48 h, the removal rate of the pH 7.0 group was 59.94%, showing no significant differences compared to the pH 6.0 group (61.72%) and the pH 8.0 group (59.66%) ($P > 0.05$), with all three groups achieving removal rates of over 59%. This indicates that

the strain can effectively degrade ammonia nitrogen within the pH range of 6.0–8.0, demonstrating a broad adaptability, although the initial degradation rate was the fastest at pH 7.0.

The heterotrophic nitrification-aerobic denitrification strain H1, reported by Xie et al. [13], exhibited the highest nitrogen removal efficiency within the pH range of 7.0–8.0. Wang et al. found that *Bacillus huajintanensis* SLWX2 demonstrated excellent ammonia nitrogen removal performance under weakly acidic, neutral, and weakly alkaline conditions. The present strain achieved its highest 24 h removal rate at pH 7.0, with an adaptable pH range of 6.0–8.0, which is consistent with the optimal pH range of most *Bacillus* species.

3.3 Determination Results of Nitrite Nitrogen

After 48 h of cultivation under the optimal degradation conditions (35 °C, initial ammonia nitrogen concentration of 60 mg/L, inoculum size of 1%, pH 7.0), the NO₂⁻-N concentration in the culture medium was only 0.016 mg/L, indicating almost no nitrite accumulation. Similarly, *Lysinibacillus sphaericus* N-2 reported by Gu et al. achieved rapid ammonia nitrogen removal within 10 h via bacterial assimilation [9], without nitrate or nitrite accumulation. The low nitrite accumulation characteristic of this strain suggests that it may possess the capability to rapidly convert nitrite.

3.4 Discussion

3.4.1 Strain identification and degradation potential

The strain isolated in this study was identified as *Bacillus stercoris* through 16S rDNA sequence analysis, sharing a 99.65% homology with the type strain. Adelskov et al. first isolated and named this species from compost [4], and Patel et al. completed its whole-genome sequence analysis [14]. Currently, there are few reports on the ammonia nitrogen degradation function of this bacterium. Notably, *B. subtilis* subsp. *stercoris* C5 achieved a degradation rate of 94.7% in 72 h in aquaculture wastewater with an ammonia nitrogen concentration of 320 mg/L; however, systematic reports on the ammonia nitrogen degradation characteristics of the wild-type *Bacillus stercoris* are still lacking. This study systematically investigated the ammonia nitrogen degradation characteristics of this strain for the first time, enriching the functional understanding of this species and providing a new strain resource for the application of *Bacillus* in the field of environmental remediation.

3.4.2 Effect of temperature on degradation efficiency

Temperature was the most significant factor affecting degradation efficiency, with the optimal temperature of 35 °C yielding a 48 h removal rate of 70.08%, significantly higher than those at 30 °C (58.17%) and 25 °C (56.17%). This peak performance at 35 °C aligns with the typical optimal range (30–37 °C) for denitrification-related enzymes in the genus *Bacillus*, as similarly reported for strains like *Bacillus thuringiensis* and *Bacillus megaterium* [15]. The notable decline in efficiency at normal temperatures (25–30 °C) reveals a distinct temperature preference and high activity of its denitrification enzyme systems specifically at moderately high temperatures. Consequently, this strain exhibits strong thermotolerance and is particularly recommended for the targeted treatment of moderately high-temperature ammonia nitrogen wastewater, such as summer livestock effluent and anaerobic digestion liquid, rather than conventional ambient-temperature wastewater.

3.4.3 Substrate Concentration Inhibition And Inoculum Size Effect

Obvious substrate inhibition occurred when the initial ammonia nitrogen concentration was ≥ 80 mg/L. The inhibition of microorganisms by high-concentration ammonia nitrogen is primarily mediated by free ammonia. In the inoculum size experiment, the removal efficiency of the low inoculum size group overtook that of the high inoculum size group in the later stage, indicating that excessive bacterial cells accelerated carbon source consumption, leading to cell autolysis and subsequent nitrogen release in the later stage. This result suggests that in practical engineering applications, the sludge retention time (SRT) should be optimized according to the influent carbon-to-nitrogen (C/N) ratio to avoid a decline in treatment efficiency caused by excessively high sludge concentrations.

3.4.4 pH adaptation range

The strain exhibited the highest degradation efficiency at pH 7.0, with an adaptation range of 6.0–8.0. This adaptation range is consistent with the optimal pH range (6.5–8.5) of most *Bacillus* species [3]. A slightly alkaline environment facilitates the conversion of NH₄⁺ to free NH₃, which may have promoted the strain's utilization efficiency of ammonia nitrogen. The broad pH adaptation range of this strain provides it with good application flexibility in actual wastewaters with varying pH levels.

3.4.5 Comparison with similar strains

The present strain was compared with recently reported ammonia nitrogen-degrading *Bacillus* strains (Table 1). Under normal temperature conditions (30 °C), the 48 h removal rate of this strain (61.50%) was at a moderate level compared to similar strains; however, under moderately high temperature conditions (35 °C), the removal rate could reach 70.08%, demonstrating good thermotolerant characteristics. Furthermore, the ability of this strain to form spores facilitates long-term preservation and microbial agent preparation, indicating good application potential in the treatment of seasonal moderately high-temperature wastewater.

Table 1 Comparison of ammonia nitrogen degradation performance among different *Bacillus* strains

Strain name	Initial ammonia nitrogen (mg/L)	pH	Temperature (°C)	Time (h)	Removal rate (%)	Reference
<i>Bacillus amyloliquefaciens</i>	100	7.0	30	48	92.32	[1]

<i>Lysinibacillus sphaericus</i> N-2	100	8.0	31	10	91.0	[9]
<i>Bacillus thuringiensis</i> (Strain 8)	Approx. 453 (NO ₃ ⁻ -N)	6.8	35	48	87.0 (NO ₃ ⁻ -N)	[15]
<i>B. stercoris</i> SL1	60	7.0	30	48	61.50	This study
<i>B. stercoris</i> SL1	60	7.0	35	48	70.08	This study

4 CONCLUSIONS AND PROSPECTS

In this study, an ammonia nitrogen-degrading bacterium, *Bacillus stercoris* SL1, was isolated from Wuhan soil and achieved a 70.08% removal rate within 48 h under optimal conditions (35 °C, 60 mg/L, 1% inoculum, pH 7.0) with negligible nitrite accumulation (0.016 mg/L). Although its performance was inhibited by high substrate concentrations (≥ 80 mg/L) and excessive inoculation (10%), the strain demonstrated a broad pH adaptability (6.0–8.0). Given its distinct preference for moderately high temperatures, rapid nitrite conversion capability, and spore-forming advantage, this strain holds promising application potential for the biological treatment of seasonal moderately high-temperature ammonia nitrogen wastewater.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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