The Journal of Green Energy and Environmental Engineering

Research Paper

Study on the Treatment of Soybean Product Wastewater by Heterotrophic Nitrification-Aerobic Denitrification Acinetobacter

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Abstract. oybean product wastewater (SPW) is organic wastewater generated during the processing of soybean products. Soaking, pulping, boiling, pressing and fermentation load SPW with high organic, N and P content, rendering it easily putrescible and malodorous. If discharged directly without treatment, it will cause serious harm to the receiving environment and human health. Heterotrophic Nitrification-Aerobic Denitrifying Microorganisms (HNADMs) have significant advantages in treating high-nitrogen organic wastewater, but there is currently no research report on using Heterotrophic Nitrification-Aerobic Denitrification (HN-AD) strains to treat SPW. Therefore, this study screened HN-AD strains from epiphytic aggregates of cyanobacteria in Taihu Lake through enrichment culture and gradient dilution-plate streaking, and optimized the process conditions for SPW using methods such as single-factor controlled variable method and response surface design experiments. On this basis, combined with molecular biology techniques such as whole-genome scanning, high-throughput sequencing, and functional gene polymerase chain reaction (PCR) amplification, the nitrogen removal pathways of HNADMs were explored. The main results are as follows: (1) With the existing HN-AD strain Acinetobacter CW-1 treating 3.3-fold-diluted SPW at 25 °C, 240 rpm, pH 5.26 and no external carbon, simultaneous removal of NH_4^+-N, DTN, DTP and COD reached 99.4 %, 85.3 %, 28.0 % and 60.0 %, respectively. (2) Genome and HN-AD gene scans show strain CW-1 funnels NH_4^+-N through three routes: GDH, GS-GOGAT, and the full HN-AD chain ($NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O/N_2$).

Keywords: *Heterotrophic Nitrification-Aerobic Denitrification; Soybean Product Wastewater; Enterobacter; Nitrogen and Phosphorus Removal*

Received on 17 June 2025, Accepted on 23 August 2025, Published on 15 Dec 2025

1 Introduction

Rising global water use has turned eutrophication-driven quality loss and biodiversity crashes into front-page issues. China's 2021 State-of-the-Environment report card lists 23 % of 209 key lakes/reservoirs as lightly eutrophic and a striking 62 % as moderately eutrophic. An EPA survey shows nearly half of U.S. lakes surpass nitrogen limits, 45 % exceed phosphorus benchmarks, and one in four is hypereutrophic. Across the globe, eutrophication touches 53 % of European, 54 % of Asian, 48 % of North-American, 41 % of South-American and 28 % of African lakes . Against this background, algal blooms have become a common problem threatening global water ecosystems and are listed as one of the urgent environmental governance issues to be overcome [2]. Algal blooms—nutrient-driven water pollution sparked by surplus N and P—signal a disrupted lake ecosystem. Human pressures have recently raised both their frequency and intensity, endangering drinking-water security. Human discharge behaviors cause increased nutrient concentrations in water bodies, inducing abnormal algal proliferation, which in turn leads to water quality deterioration and decreased dissolved oxygen (DO) content. This process leads to increased water turbidity, sharp decline in transparency, and causes mass deaths of some aquatic animals (especially fish and shellfish) due to hypoxia, typical cases such as the marine biological disaster

caused by Alexandriumin the St. Lawrence Estuary in 2008 [3]. Nyenje et al. [4] report that sub-Saharan waters are sliding into severe eutrophication: ecological integrity collapses, fish stocks vanish, toxic cyanobacteria bloom, oxygen disappears and pathogens flourish. Furthermore, algae in eutrophic water often secrete harmful substances such as algal toxins, nitrate (NO_3^-), and nitrite (NO_2^-), posing a dual threat to human health and ecosystem stability.

Wastewater treatment processes are one of the most common water resource management strategies, offering many advantages, including reducing eutrophication and carbon footprint, as well as efficient removal of pathogens. Various forms of N present in wastewater, including ammonium (NH_4^+), NO_2^-, and NO_3^-, can cause serious biological stress and may lead to dangerous outcomes such as imbalance in biodiversity. Therefore, research on nitrogen removal processes in wastewater is considered urgent. Traditional nitrogen removal technologies mainly include: biological methods, physico-chemical methods, chemical precipitation, membrane separation, and electrocatalysis.

Balancing ecological and economic demands, most treatment plants rely on biological nitrogen removal to strip NH_4^+-N; the classic aerobic-nitrification/anaerobic-denitrification route is now standard practice worldwide [5]. During nitrification microbes step-wise oxidize NH_3 to NO_2^- then NO_3^-; in denitrification the same nitrogen is reduced back to N_2 gas and lost to the air. Conventional nitrification–denitrification is delivered through sequencing-batch reactors (SBR), anaerobic–anoxic–oxic (AAO) or moving-bed biofilm reactors (MBBR). SBR is a single-stage activated sludge system that achieves aerobic nitrification and anoxic denitrification through time-sequence alternation [6]. As a commonly used biotechnology in wastewater treatment, the SBR process has significant advantages such as simple operation, flexible operation, and high automation, but in practical applications, it still has common problems such as insufficient TN removal efficiency and easy acidification during nitrification. The AAO process is the inheritance and development of traditional nitrification-denitrification processes, and is considered to have broad application prospects because it can simultaneously remove nutrients such as N and P from sewage without adding chemicals [7]. However, there are significant differences in water quality, DO, and redox potential distribution within the actual reaction tanks of the AAO process, and this environmental heterogeneity directly affects the distribution characteristics of microbial populations and the nitrogen removal mechanisms of activated sludge in each zone. At the same time, based on actual wastewater treatment needs, it is necessary to optimize the filler addition strategy to specifically enhance the nitrogen removal efficiency of each functional zone of the AAO process [8]. The MBBR process is a new improved process combining biological filters and activated sludge systems. As a typical representative of efficient attached-growth water treatment technology, this process has the dual technical advantages of simple operation and management and excellent treatment performance [9]. However, factors such as DO, biofilm carrier performance, and filler amount always affect the operational performance of MBBR [10]. Therefore, MBBR is often combined with other wastewater treatment processes to achieve COD removal.

Anaerobic ammonium oxidation (Anammox) is a process under strict anaerobic conditions where anaerobic ammonium-oxidizing bacteria use NO_2^--N as an electron acceptor to directly convert NH_4^+-N to N_2. In the late 20th century, Mulder et al. [11] first discovered the Anammox phenomenon in a fluidized bed reactor treating methane-producing wastewater and confirmed the process of oxidizing NH_4^+ to generate N_2 using NO_3^- as the electron acceptor under anaerobic conditions. Graaf et al. [12] devised an autotrophic synthetic feed that enriches anammox bacteria, supplying only NH_4^+-N as electron donor, NO_2^--N as acceptor and carbonate as carbon source. Early work showed anammox activity depends on NO_2^--N presence and the absence of organic electron donors. Strous et al. [13] systematically studied the physiological characteristics of anaerobic ammonium-oxidizing bacterial communities through sequencing batch reactor culture experiments, determining their suitable pH and temperature ranges to be 6.7-8.3 and 20-43°C, respectively, and also found that the concentration of intermediate products such as NO_2^--N significantly affects the Anammox reaction process. Entering the 21st century, Hippen et al. [14] based on industrial and pilot data, deeply discussed the operational stability of the aerobic/anoxic deammonification process and proposed practical experience applicable to the transformation of biofilm systems. Wett [15] developed an innovative deammonification process that significantly improved the energy self-sufficiency capacity of wastewater treatment plants. In mechanism research, Star et al. [16] achieved high-purity, high-yield suspended culture of anaerobic ammonium-oxidizing bacteria, providing new research ideas for the application of membrane bioreactors (MBR) to these bacterial communities. Li et al. [17] systematically analyzed the bacterial diversity in Anammox reactor

communities by analyzing 16S rRNA gene clone libraries and qPCR technology. Kartal et al. [18] pinpointed the core anammox genes and proteins, revealing that NH_4^+-N plus NO_2^--N first form nitric oxide (NO), which is then reduced to hydrazine (N_2H_4)—the pathway's pivotal intermediate. This not only revealed a new biochemical pathway but also highlighted the important role of NO in the evolution of the nitrogen cycle. Kartal and Kelt Jens [19] further clarified that this metabolic process relies on the catalytic and electron transfer functions of c-type heme proteins, which exhibit novel properties in the Anammox process. These findings have triggered a new wave of nitrogen-removal technologies, with PN/anammox and PD/anammox hailed as the most groundbreaking biological upgrades in modern wastewater engineering. Anammox has the characteristic of simultaneously removing NH_4^+-N and NO_2^--N, thus showing unique advantages in treating wastewater containing these two nitrogen pollutants. Because anammox is autotrophic, no external organic carbon is needed, making the process ideal for low-C/N wastewaters. Anammox bacteria also buffer pH, sustain long power-generation cycles and generate minimal sludge, sharply cutting sludge-handling costs.

Relative to strict autotrophs, heterotrophic nitrifiers–aerobic denitrifiers (HNADMs) offer: (i) faster growth, (ii) self-buffering of pH, (iii) tolerance to salinity, metals and antibiotics, and (iv) suitability for low-C/N wastes. Their ability to perform simultaneous nitrification–denitrification under aerobic conditions makes them attractive for modern wastewater treatment. Therefore, the discovery of HNADMs provides new ideas for the development of biological nitrogen removal processes. Single-factor tests evaluated carbon source, C/N, temperature, pH and shaking speed on 3.3-fold-diluted SPW treated by the resident HNADMs, while molecular tools mapped the strain's nitrogen-removal route. Using experimental methods such as enrichment culture-gradient dilution-plate streaking, HNADMs with better SPW treatment performance were screened and isolated, and the purified strains were identified by morphological observation and 16S rDNA sequencing.

2 Experimental Process

2.1 Strain Screening

The HN-AD functional Acinetobactersp. strain CW-1 used in this experiment. The bacterial suspension was preserved in a -80°C ultra-low temperature freezer at a 1:1 ratio with glycerol. SPW was sampled at a Yangzhou soy-processing plant, immediately centrifuged (8 000 r min^{-1}), 0.45-μm filtered and stored at -18°C until use. Post-centrifugation/filtration characteristics of the SPW are listed in Table 1.

Table 1 Water quality indexes of soybean product wastewater after centrifugation and filtration

	COD	TN	TP	NH^{4+}-N	pH
SPW1	4948 mg/L	137.0 mg/L	38.2 mg/L	88.0 mg/L	5.16
SPW2	7840 mg/L	250 mg/L	61.0 mg/L	92.0 mg/L	4.70

2.2 Strain Culture and Detection

LB broth: dissolve 10 g tryptone, 5 g yeast extract and 10 g NaCl in 1 L ultrapure water. Experimental wastewater: The filtered SPW was mixed with deionized water at a ratio of 3:7 to serve as the experimental wastewater.

Filtered DTN was measured by alkaline potassium persulfate oxidation followed by UV spectrophotometry. After potassium persulfate oxidation digestion of the filtered water sample, the ascorbic acid-ammonium molybdate spectrophotometric method was used to determine the dissolved total phosphorus (DTP). NO_3^--N, NO_2^--N and NH_4^+-N were quantified by UV, N-(1-naphthyl)-ethylenediamine and salicylate-hypochlorite spectrophotometry, respectively; COD was measured by $FeSO_4$ titration after $K_2Cr_2O_7$ digestion. Biomass was gauged from OD_{600}; pH was read with a glass electrode.

DOI:

The intracellular nitrogen content, nitrogen removal rate, and nitrogen removal rate in the system were calculated using the formulas TN_o - TN_t, (TN_o - TN_t)/TN_o, and (TN_o - TN_t)/t, respectively. Here, TN_o is the TN content of the inoculated experimental wastewater at the start of the experiment without vacuum pump filtration, TN_t is the TN content of the filtered experimental wastewater at the end of the experiment, and t is the reaction duration.

3 Results and Discussion

3.1 Results of Condition Optimization for Strain CW-1 Treating Soybean Product Wastewater

Figure 1 (mean ± SD, n = 3) summarises how environmental variables affect CW-1-driven nitrogen removal from SPW. As shown in Figure 1a, compared to the treatment groups with glucose or sucrose as carbon source, the treatment groups with sodium acetate or sodium succinate added showed significantly improved NH_4^+-N removal efficiency within 12 h, which is consistent with the experimental results of strain CW-1 applied to simulated nitrogen-containing wastewater [20]. After 36 h, NH_4^+-N removal exceeded 94.6 % in every flask, with no significant difference among carbon-source treatments ($p > 0.05$, ANOVA). Although many microbes favour specific carbon sources [16], SPW's abundant organics are readily used by denitrifiers to accelerate nitrogen removal [18]. Thus carbon-source variation hardly affects NH_4^+-N removal, likely because CW-1 directly metabolises SPW's native organics.

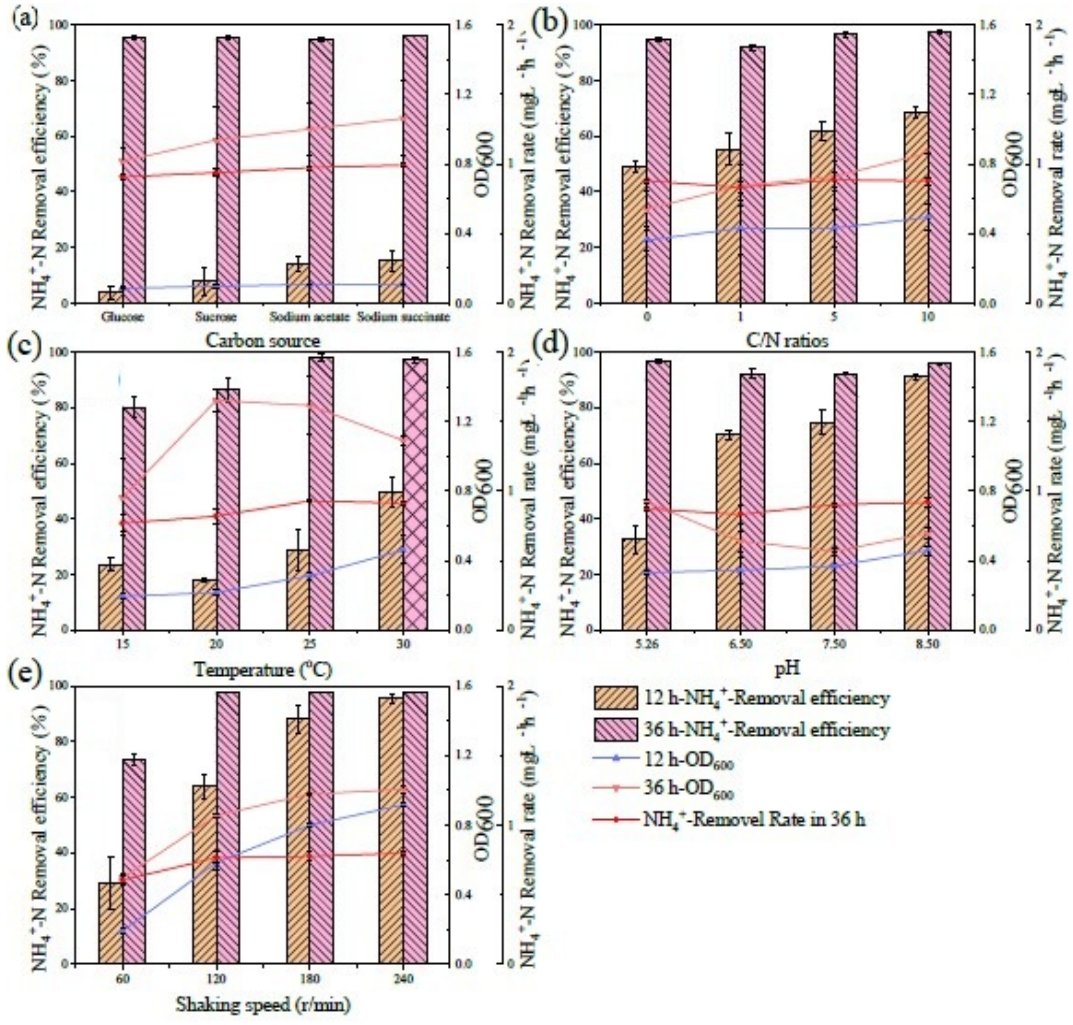

Figure 1 Effects of carbon source (a), carbon nitrogen ratios (b), pH values (c), temperature (d), and shaking speed (e) on the removal of NH4 +-N from SPW by CW-1

The carbon substrate dictates how fast HN-AD cells grow and how much nitrogen they remove [19]. Simple molecules such as acetate feed the TCA, glycolysis and glyoxylate shunt, boosting NADH and delivering electrons to the ammonia and nitrogen-oxide reductases that run the combined nitrification–denitrification process. HNADMs differ in carbon-source usage largely through variable enzyme activities in the associated catabolic pathways; these disparities can be gauged from the transcript abundance of genes encoding the relevant enzymes. Differences in how each HNADM metabolises the carbon supply propagate into distinct electron-transport configurations and, consequently, different nitrogen-removal efficiencies. Although SPW itself contains high concentrations of organic matter, studying carbon source does not negate its own organic load, but rather provides optimization solutions for specific treatment needs or process bottlenecks by regulating microbial metabolic activity, which has important guiding value for engineering practice. By analyzing the utilization effects of strain CW-1 on different carbon sources, its carbon source preference characteristics can be clarified, which provides theoretical support for the optimization of SPW treatment processes based on this strain. Here, sodium succinate was chosen as the model carbon substrate to probe how its concentration steers the NH_4^+-N removal rate. Biomass of strain CW-1 rose markedly across all replicates once C/N was raised from 0 to 10. Relative to the carbon-source screening test, OD_{600} climbed steeply within the first 12 h under every C/N tested, showing that after a brief lag strain CW-1 could exploit the organic carbon present in the synthetic poultry wastewater, lessening its reliance on external carbon. NH_4^+-N removal tracked the OD_{600} trend, improving in parallel with biomass accumulation. After 36 h of cultivation, the treatments with the two lowest carbon source additions achieved NH_4^+-N removal rates of 94.5% and 91.7%, respectively (Figure 1b). Consequently, all later runs were conducted without any extra carbon amendment.

Temperature comprehensively regulates the aerobic denitrification process of bacteria by affecting enzyme activity, microbial community structure, and electron transport chain [20]. Figure 1c shows that strain CW-1 retains robust NH_4^+-N removal at 25 °C and 30 °C, achieving 99.0 % and 97.1 % removal within 36 h, respectively. Notably, at 15 °C growth was sluggish (OD_{600} 0.194) and only 23.5 % NH_4^+-N disappeared in the first 12 h, yet after 36 h the strain still removed 80.2 %—far outperforming reported heterotrophic nitrifiers such as Paracoccus spp. [21]. Literature confirms the genus' cryotolerance: A. calcoaceticus TY1 achieved 97.7 % NH_4^+-N removal at 7.8 °C, while A. sp. HA2 grew normally at 10 °C and reached ~100 % removal within 25 h [22]. The fact that strain CW-1 maintains high NH_4^+-N removal efficiency under low temperature conditions of 15°C significantly expands its application range. Since the environmental temperature in most areas of China (except summer) is generally below 20°C, strain CW-1 may demonstrate significant advantages in practical engineering applications.

Low pH boosts N_2O build-up in heterotrophic denitrification: acid conditions favour electrons to NO_3^- and NO_2^- reductases over N_2O reductase, whereas the latter remains competitive at pH 7–9, preventing net N_2O accumulation . This shows that pH deeply affects the survival and function of bacteria by directly acting on cell structure and indirectly regulating the microenvironment. Figure 2-1d reveals that at pH 5.26 only 32.5 % NH_4^+-N disappeared after 12 h, far below values recorded at higher pH, indicating pronounced inhibition of CW-1 under mildly acidic conditions. At the end of cultivation, the differences in NH_4^+-N removal efficiency and removal rate among different pH treatments were not significant ($p > 0.05$, ANOVA). Mildly alkaline pH is widely regarded as the sweet spot for HN-AD microbial growth [24]. Nevertheless, the strain ultimately attained 96.4 % removal at pH 5.26 (Figure 1d), mirroring Acinetobacter JR1 which cleared ~96 % NH_4^+-N across pH 4.5–10 [24]. Because raw SPW sits at pH 5.26 and CW-1 still removes >96 % NH_4^+-N, the strain can be dosed directly without costly alkali addition. With anaerobic effluent typically at pH 8.0–8.5 [12] and CW-1 performing equally well in this range (Figure 1d), the bacterium can be coupled with upstream anaerobic treatment to polish NH_4^+-N without pH correction.

Shaking speed is a key parameter for cultivating aerobic denitrifying bacteria to treat wastewater, as this parameter affects nitrogen transformation and removal pathways by changing dissolved oxygen [25]. Figure 1e shows that agitation markedly influences CW-1: both OD_{600} and NH_4^+-N removal rose with shaker speed during the first 12 h. At rotational speeds other than 60 rpm, all treatments achieved NH_4^+-N removal efficiencies exceeding 97.6%. Comparable outcomes are commonly reported in other high‑nitrogen anaerobic processes. For instance, research by Yang et al. [26] demonstrated that elevated agitation rates facilitated the complete elimination of NH_4^+-N, whereas reduced shaking intensities markedly impaired the NH_4^+-N removal capacity of the bacterial strain Pseudomonas putida NP5. Because strain CW-1 cleared NH_4^+-N most effectively within the

first 12 h at 240 rpm, this agitation rate was kept for all later work. Ultimately, NH_4^+-N removal from SPW by strain CW-1 was tested at 25 °C, 240 rpm, with no pH adjustment and no external carbon. Following the introduction of strain CW-1 into SPW2, the NH_4^+-N concentration exhibited a sharp decline, dropping from 32.9 mg L^{-1} to 14.4 mg L^{-1} within the first 24 hours, and ultimately reaching 0.2 mg L^{-1} by the conclusion of the cultivation period (Figure 2a). Over the course of cultivation, the treatment of SPW that did not accumulate NO_2^--N resulted in the removal of approximately 32.7 mg L^{-1} NH_4^+-N, 10.1 mg L^{-1} NO_3^--N, and 73.7 mg L^{-1} DTN (Figure 2b). These findings suggest that strain CW-1 possesses considerable potential for application in SPW treatment across various operational stages. All data presented in Figure 2 are reported as the mean value plus or minus the standard deviation from three independent experimental replicates.

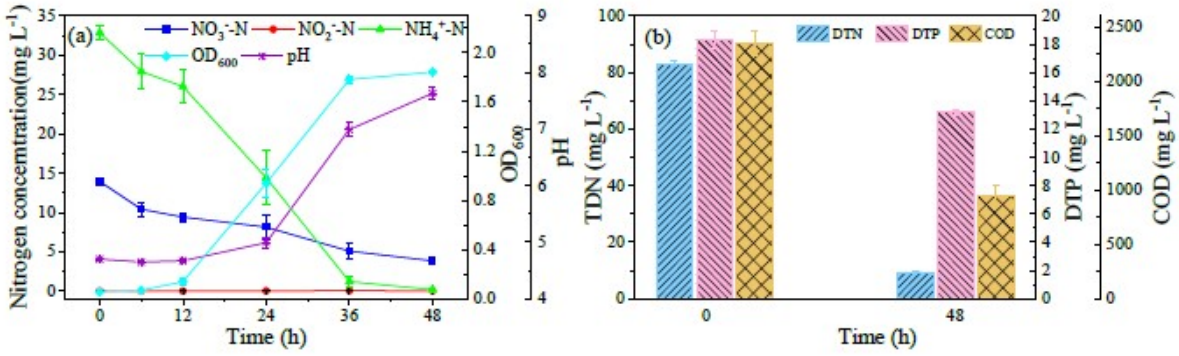

Figure 2 Application of strain CW-1 for SPW treatment under optimized conditions:(a) Changes in different forms of nitrogen concentration and OD600 during the cultivation process, (b) Initial and Final Concentrations of DTN, DTP, and COD

To further assess the high-nitrogen anaerobic digestion (HN-AD) capability of strain CW-1, the data obtained from the nitrogen mass balance experiment were computed and examined (Table 2-6). Mass-balance of initial vs. final TN and intracellular N showed 17.3 % lost through the HN-AD route, whereas 71.4 % was locked up in biomass. In contrast to certain other Acinetobacter species, the nitrogen removal efficiency of strain CW-1 is lower than that reported for Acinetobacter baumannii [27], which achieved a removal rate of 45.9%. As indicated in Table 2, SPW is characterized by a high concentration of organic nitrogen. Within 48 hours of cultivation, about 85.3% of the dissolved organic nitrogen was assimilated into bacterial biomass. Some HNADMs readily turn organic-N into biomass-N yet struggle to complete the final step to N_2; SPW's high organic-N load therefore limits the aerobic-denitrification contribution to overall TN removal. It is worth noting that when considering inorganic nitrogen species alone, the proportion of nitrogen eliminated through the HN-AD pathway reached 30.8%, exceeding the removal efficiency of 22.4% reported for Acinetobacter indicusZJB20129 under equivalent conditions [28].

Table 2 The results of the nitrogen balance analysis

	TN/mg	NO^{3-}-N/mg	NH^{4+}-N/mg	NO^{2-}-N/mg	Nitrogen*/mg	Nitrogen loss /mg
Starting	16.62 ± 0.22	2.79 ± 0.10	6.57 ± 0.18	0	0	-
End	13.75 ± 0.23	0.77 ± 0.06	0.04 ± 0.01	0.01± 0.00	11.87 ± 0.26	2.88 ± 0.17

HNADMs are heterotrophic bacteria that can effectively remove NH_4^+-N and organic matter. Furthermore, some HNADMs exhibit significant phosphorus accumulation ability. To determine whether carbon and phosphorus were co-removed alongside NH_4^+-N, key parameters were analyzed at the start and conclusion of the experiment. The findings indicated removal efficiencies of 28.0% for dissolved total phosphorus (DTP) and 60.0% for chemical

oxygen demand (COD) (Figure 2b). Therefore, strain CW-1 can not only serve as an independent treatment solution for SPW but also as a valuable auxiliary device for the anaerobic treatment process of SPW. In SPW remediation, microalgae and photosynthetic bacteria draw interest for their dual role in pollutant removal and generation of biomass or value-added products. Compared to these microorganisms, the COD and TP removal efficiencies of strain CW-1 treating SPW are slightly lower.

3.2 Strain Identification Results

An HN-AD strain was isolated from the epiphytic bacterial community of cyanobacteria in Taihu Lake. Its morphology, scanning electron microscopy, and Gram staining results are shown in Figure 3. The strain is a Gram-negative bacterium, about 0.6 μm (1.0-1.3) μm in size, belonging to short rod-shaped bacteria. Colonies appear milky-white and opaque, displaying smooth surfaces and flat margins.

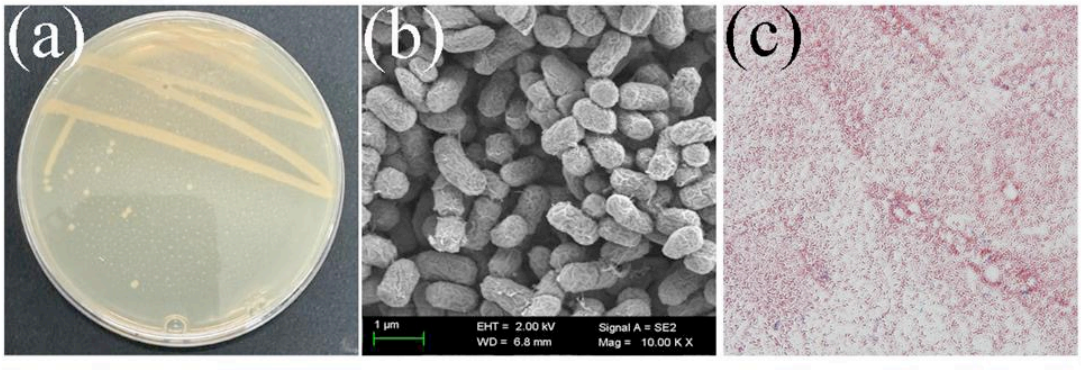

Figure 3 Strain identification results: (a) strain morphology, (b) scanning electron microscope, (c) gram staining

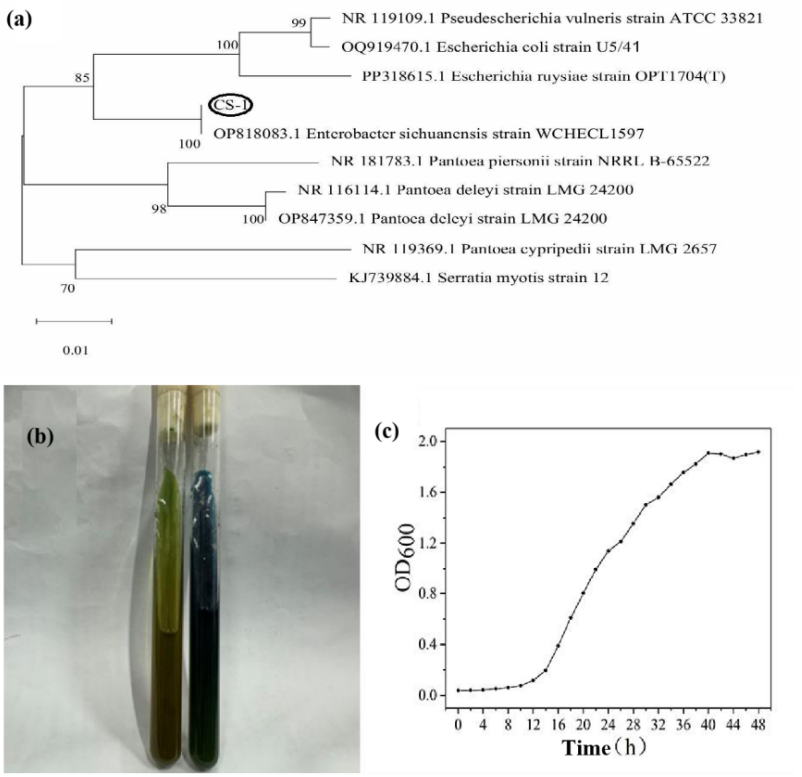

Figure 4 phylogenetic tree of strains (a); strain culture results: (b) BTB inclined plane culture, (c) strain growth curve

The isolated strain underwent DNA extraction, PCR amplification targeting the 16S rRNA gene, and subsequent sequencing. The sequence was submitted to GenBank under accession PQ663255. A neighbour-joining tree for the isolate was generated with MEGA and is shown in Figure 4a. Homology comparison results showed that the screened strain has 100% similarity to EnterobacterStrain WCHEC1 1597 in the NCBI database. Combined with the morphological characteristics of the strain, it was determined that the strain belongs to the genus Enterobacter, hence the strain was named EnterobacterCS-1.

In the experiment of slant culture of the strain, compared to the control group without bacterial culture, the color of the BTB medium in the experimental group changed from yellow-green to dark blue after the bacteria grew for a period of time (Figure 4b). This indicates that the strain produces alkaline substances during growth, causing the pH to increase, suggesting that the strain is an aerobic denitrifying bacterium. Microbial growth follows lag, log, stationary and decline phases; Figure 4c shows the lag phase persisted for 0–10 h. During this time, growth was relatively slow because the microorganisms had just entered a new culture environment and needed to adapt. Within 12-30 h, the strain was in the logarithmic growth phase. During this stage, the bacteria fully utilized the nutrients in the medium for rapid growth, so the number of bacteria increased sharply, showing an exponential growth trend. Within 32-40 h, the bacteria had gradually adapted to the culture environment, but the low nutrient content and the toxicity of metabolites to the bacteria limited the growth rate. Within 40-48 h, the bacteria entered the decline phase. Therefore, there is a maximum value (OD_{600} = 1.917) during the stationary growth phase of the bacteria.

3.3 Nitrogen Removal Performance of the Strain

During the denitrification assessment where NO_3^--N served as the exclusive nitrogen source, the concentration of NO_3^--N showed a progressive decline throughout incubation. This was accompanied by an accumulation of NO_2^--N, which peaked at 14.2 mg L^{-1} (Figure 5a). This pattern is likely attributable to a slower reduction rate for NO_2^--N relative to NO_3^--N during the strain's aerobic denitrification process. After 72 h, NO_3^--N removal peaked at 95.3 % with a top rate of 4.4 mg L^{-1} h^{-1}. Throughout this period, the NH_4^+-N concentration remained consistently low, at levels below 1 mg L^{-1}.

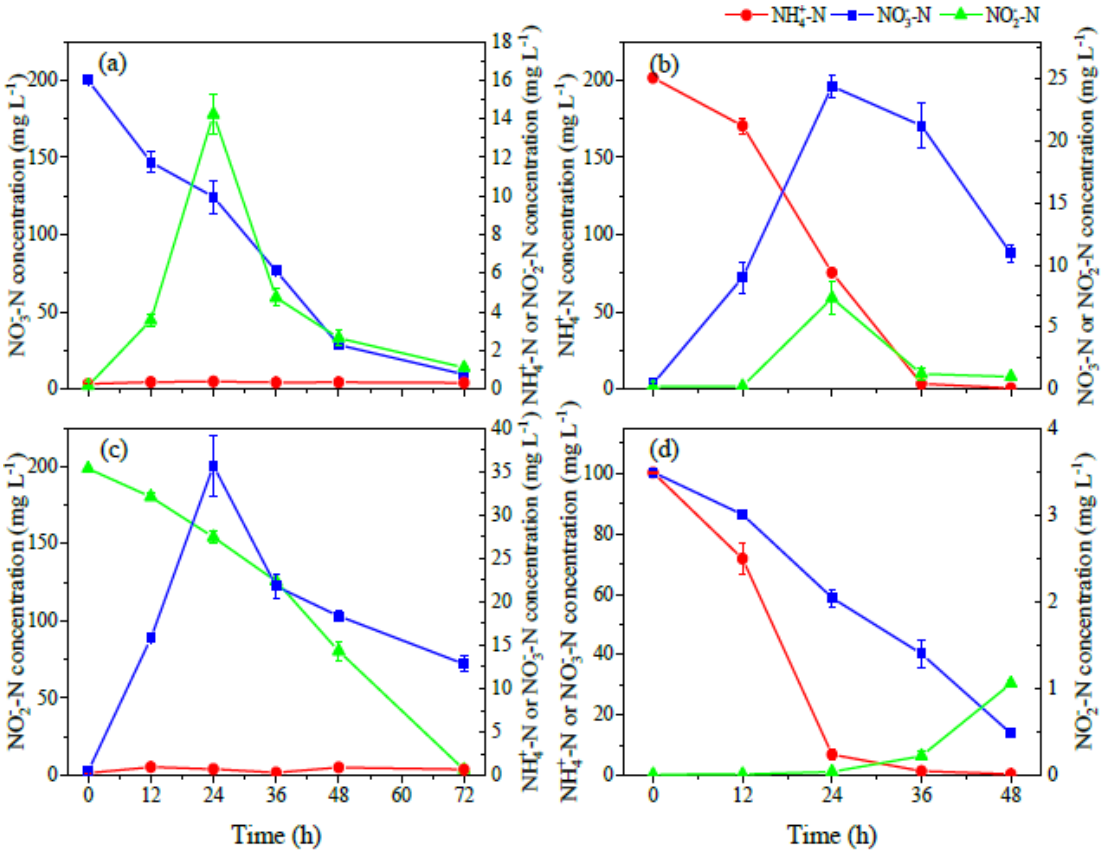

Figure 5 Nitrogen removal capability of strain CS-1 under different nitrogen sources: NO3–-N (a), NH4+-N (b), NO2–-N (c), and NH4+-N+NO3–-N (d)

The strain's capacity to treat wastewater with elevated NH_4^+-N levels was assessed using NH_4^+-N as the sole nitrogen source. As depicted in Figure 5b, following a 24-hour cultivation period, the NH_4^+-N concentration decreased rapidly from an initial 200 mg L^{-1} to 75.5 mg L^{-1}, approaching nearly zero after 48 hours. The overall NH_4^+-N removal efficiency and the peak removal rate achieved were 99.7% and 7.9 mg L^{-1} h^{-1}, respectively, demonstrating the strain's robust capability in eliminating NH_4^+-N. Notable accumulation of both NO_3^--N and NO_2^--N was observed at the 24-hour mark. However, by 48 hours, the NO_2^--N concentration had diminished to near zero, while NO_3^--N levels stabilized at 9.5 mg L^{-1}. The transient accumulation of these nitrogen oxides during NH_4^+-N removal provides evidence of heterotrophic nitrification activity in strain CS-1. Figure 5c displays denitrification with NO_2^--N as sole N-source: concentration fell from 198.6 mg L^{-1} to 125.7 mg L^{-1} within 36 h, then declined rapidly to give 98.1 % removal. Removal peaked at 3.8 mg L^{-1} h^{-1} between 36 h and 48 h. Concurrently, NO_3^--N accumulated to 35.7 mg L^{-1} at 24 h and subsequently decreased, yet remained 12.8 mg L^{-1} at harvest. Throughout the incubation period, NH_4^+-N levels were consistently maintained below 1 mg L^{-1}. To evaluate the strain's capability for concurrent NH_4^+-N and NO_3^--N removal, experiments were conducted using a dual nitrogen source (NH_4^+-N + NO_3^--N). The results revealed that concentrations of both nitrogen forms decreased rapidly within the initial 24 hours of cultivation. However, the rate of NH_4^+-N reduction was statistically significantly greater than that of NO_3^--N ($p < 0.01$, ANOVA). By 48 hours, the strain achieved removal efficiencies of 99.6% for NH_4^+-N and 86.2% for NO_3^--N (Figure 5d). Removal efficiency with the mixed-N feed was markedly inferior to that achieved when NO_3^--N was supplied alone. Throughout the process, NO_2^--N only accumulated in small amounts at the end of cultivation, with a maximum value of about 1.1 mg L^{-1}.

4 Conclusion

AcinetobacterCW-1 was isolated and identified due to its excellent nitrogen removal ability and was applied to SPW treatment. Whole-genome sequencing and successful amplification of HN-AD functional genes indicated that this strain mainly achieves nitrogen removal through the direct GDH pathway or the indirect GS-GOGAT assimilation pathway, as well as the complete HN-AD pathway (NH_4^+-N → NH_2OH → (NO) → NO_2^--N (→ NO_3^--N → NO_2^--N) → NO → N_2O/N_2). In the treatment of SPW, strain CW-1 demonstrated notable resilience to low temperatures, acidic conditions, and a versatile capacity to utilize various carbon substrates. Under operational parameters of 25°C, 240 rpm, an initial pH of 5.26, and in the absence of an external carbon source, strain CW-1 achieved removal efficiencies of 99.4% for NH_4^+-N, 85.3% for DTN, 28.0% for DTP, and 60.0% for COD. These results underscore the significant potential of this strain for practical wastewater treatment applications. However, regardless of the acclimation method used, AcinetobacterCW-1 cannot grow normally in undiluted SPW, which greatly reduces the comprehensive nitrogen removal efficiency and rate of this strain. Therefore, subsequent experiments will the isolation and screening of HNADMs to obtain a HNADM that can be directly applied to the nitrogen removal treatment of undiluted SPW. On this basis, its nitrogen removal potential and pathways for treating SPW will be explored.

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