Enhanced Nitrogen Removal of Wastewater at Low Temperature by Iterative Screening of Cold-Tolerant Denitrifying Bacteria

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Abstract

The biological denitrification for wastewater treatment in winter is often seriously compromised due to the effects of low-temperature (<13 °C) on metabolic activity of microorganism. In this study, an excellent cold-tolerant denitrifying bacterium, *Moraxella osloensis* LT-01 was isolated by iterative domestication. The strain LT-01 retained about 60% maximal growth activity at 10 °C. Under initial concentrations of 100 mg/L, average ammonium, nitrate and nitrite removal efficiencies for domestic wastewater (C/N 4:1) at 10 °C were 70.35%, 65.39% and 61.74% in 24 h, respectively. Nitrogen balance analysis showed that about 46% of TN was directed toward in the dissimilation form of gas, and 16% of TN was assimilated for cell growth. Key genes hydroxylamine reductase gene (*HAO)* and nitrite reductase (*NirS*) involved in nitrification and denitrification processes were identified by gene-specific PCR, indicating that strain LT-01 perform nitrogen removal efficiently via unique simultaneous nitrification and denitrification. These results suggest the bacterium LT-01 has great potential as an effective performer for treating domestic wastewater in winter.

Introduction

Excessive reactive nitrogen contents from industrial, agricultural and domestic sewage activities caused an unprecedented increase in the water with negative effects on the aquatic species, human health and ecological environments(Keeley et al., 2020; Ksenofontov, 2020). Therefore, the elimination of active nitrogen in wastewater is essential and urgent due to these adverse effects. Nemours physical, chemical and biological methods have developed to solve this problem of excessive nitrogen in water. Among them, biological nitrogen removal is recognized as the most common, effective and economical strategy. While this processes is dependent on the microorganisms that convert nitrogen compounds into gaseous nitrogen by ammonification, nitrification and denitrification(Rajta et al., 2019). However, environmental factors such as meteorology and hydrology restrict the application of biological methods in actual sewage treatment. Therefore, it is very important to further mining and characterize the microbial resources with excellent nitrogen removal capability and cell viability at low temperature environments.

To date, numerous bacteria with excellent capability of inorganic nitrogen removal have been reported, and research focused on separation, identification, culture, and application of single functional strain has been conducted (Chen et al., 2019; Yang et al., 2015).Such as, Xu(Xu et al., 2017) isolated a strain of *Pseudomonas putida* Y-9 with simultaneous nitrification and denitrification, and the average removal rates of ammonia, nitrate and nitrite were 2.85 mg/($L \cdot h^{-1}$), 1.60 mg/($L \cdot h^{-1}$), and 1.83mg/($L \cdot h^{-1}$), respectively. Ruan(Ruan et al., 2020) isolated an aerobic denitrifying bacteria, the average NO_3^--N, NO_2^--N, and total ammonium nitrogen (TAN) removal rate (>95% removal efficiency) in a batch test was 6.22 mg/($L \cdot h^{-1}$), 6.30 mg/($L \cdot h^{-1}$) and 1.56 mg/($L \cdot h^{-1}$), respectively. And revealed its metabolic pathway by qRT-PCR. Jin(Jin et al., 2015) isolated a highly salt-tolerant *Pseudomonas* ADN-42 from Hymeniacidon perleve. The highest removal rate of heterotrophic ammonium was 6.52 mg/($L \cdot h^{-1}$) in 40g/L NaCl medium. Huang(Huang et al., 2015) screened out a *Pseudomonas stutzeri* strain ZF31. Silva(Carneiro

Fidélis Silva et al., 2019) obtained balearica UFV3 and *Gordonia amicalis* UFV4, evaluated their ammonia removal capability and characterization, and verified the existence of nitrification and denitrification genes via PCR and comparative genomics. However, the above-mentioned functional bacteria with high nitrogen removal efficiencies depend on the optimum temperature at 30-37 °C. In contrast, lower temperature (<13 °C) is very unfavorable for the cell proliferation, resulting in a significant reduction of metabolic capacity. Therefore, although many excellent bacteria with potential in wastewater treatment have been isolated, there are few studies on the treatment of actual urban wastewater with cold -tolerant microorganisms(Laureni et al., 2015; Lotti et al., 2014; Ma et al., 2013) .

Recently, some extremely cold-tolerant microorganisms from low-temperature environment have been isolated and characterized(Laureni et al., 2016). For the unfavorable growth factors caused by low temperature, psychrotrophic bacteria respond by maintaining membrane fluidity, expressing cold shock proteins, regulating enzyme structure, and accumulating compatible solutes(Ayala-del-Río et al., 2010; Ma et al., 2017). Additionally, Extracellular Polymeric Substances (EPS) and intracellular polymer polyhydroxyalkanoates (PHA) produced by microorganisms can also be used to resist the cold(Salama et al., 2016; Williams et al., 2017). Importantly, it has been reported that psychrotrophic bacteria were employed to degrade the Petroleum Hydrocarbons(Cain, 1981; Westlake et al., 1974), chlorophenols(Jarvinen et al., 1994; Margesin et al., 2005), surfactants(Isaksen and Jorgensen, 1996), nitrogen and phosphorus in water environment remediation. Chevalier(Chevalier et al., 2000) successfully isolated four cold-resistant *filamentous cyanobacteria* from the Antarctic and Arctic regions which has a high removal rate of nitrogen and phosphorus at low temperature. Gratia(Gratia et al., 2009) selected the strain *Arthrobacter psychrolactophilus Sp* 31.3 from cold climate areas, which can effectively degrade the protein, starch and lipids in sewage at 10 °C. Margesin(Margesin et al., 2013) isolated the strain *A. sulfureus* BZ73 from South Tyrol, Italy, the strain can completely degrade 12.5 mM phenol at 15 °C within 19 days. Therefore, the application of Psychrophilic bacteria provides a new idea for removing nitrogen from sewage in cold regions.

In this research, a novel cold-tolerant bacterium LT-01 with a high nitrogen removal rate at low temperature was isolated. we systematically evaluated the effects of physical-chemical factors (salinity, carbon source, C/N ratio, substrate concentration, dissolved oxygen) on the ammonia removal performance and cell growth of LT-01, and identified the key genes of nitrification and denitrification metabolic pathway. This strain exhibited excellent nitrogen removal capacity at low temperature, making it potentially suitable for domestic sewage treatment under cool conditions.

Materials And Methods

Isolated and identification

The sludge samples (10 g) were mixed with 100 ml sterile saline and inoculate 10mL of mixture into 250 mL Erlenmeyer flask containing 190 mL of enrichment medium and incubated at 10 °C with 200 rpm shaking speed. After 48 hours of incubation, the enrichment medium was carried out by serial dilution,

and streaked on nitrification-denitrification plates at 10 °C for 48 hours. And then, single colonies were picked and inoculated on the sterile plate until the pure strain was obtained. The obtained single colonies were inoculated into 96-well plates containing DM and NM respectively. After inoculated at 10 °C for 24 hours, the chromogenic reagent of nitrite, nitrate, and ammonia were added to check the presence of nitrogen compounds. At last, select favorable strains for further performance verification.

The DNA extraction kit (Hangzhou Baosai Biological Company) was used to extract the genomic DNA of LT-01, the 16S rDNA was amplified by PCR, and the primers were identified as: 27F: AGAGTTTGATCCTGGCTCAG and 1492R: GGTTACCTTGTTACGACTT. The product was purified and sent to sequencing, and the sequencing results were submitted to NCBI. After BLAST comparison, MEGA 5.0 was used to construct a phylogenetic tree(Weber et al., 2009).

Assessment of cell growth at different temperatures

We investigated the growth of strains at different temperatures was examined by incubation at 6 different temperatures (5, 10, 15, 20, 25, 30 and 40 °C). The specific operation is as follows: The seed culture solution (1% v/v) of strains was respectively inoculated into domestic sewage and LB culture solution, incubated with 200 rpm for 48 hours at different temperatures, and OD_{600} value was measured.

Estimation of nitrification and denitrification at low temperature

To verify the aerobic denitrification capacity, the seed culture was inoculated into the DM medium with KNO_3 (100 mg/L) or $NaNO_2$ (100 mg/L) as the sole nitrogen source at 1%(v/v) of the inoculum. For heterotrophic ammonium removal, Perform similar operations in NM medium with NH_4Cl (100 mg/L) as the sole nitrogen source. For progress, cultured at 10 °C and 200 rpm for 72 hours, and detected cell growth (OD_{600} value), NO_3^--N, NO_2^--N, and NH_4^+-N every 6 hours.

Nitrogen transformation pathway and identification of functional genes

Research on nitrogen transformation pathways via nitrogen balance analysis experiments. The obtained bacterium was inoculated into 100 mL LB medium and incubated for 24 hours. Inoculate the above-mentioned LB medium into NM medium with a TN concentration of 98.35 mg/L, inoculation amount is 1%, incubated at 10 °C and 200 rpm for 72 hours. The cell growth, nitrogen biomass, NO_3^--N, NO_2^--N, NH_4^+-N and NH_2OH-H were measured after 72 hours. All experiments were repeated three times.

To further understand the characteristics of heterotrophic nitrification and aerobic denitrification and the potential enzymes of bacterium. Two potential enzyme genes, including *Hao* and *NirS*, were amplified and analyzed by PCR. The bacterial suspension was prepared, and DNA was extracted by the bacterial DNA kit (Hangzhou Baosai Biological Company). Utilize genomic DNA as a template to amplify *HAO* and *NirS* genes. The primers of gene *Hao* are 170F: GTATGAVGCGYTGGTNAAGCGYTA and 939R: TGGAACTGGRAHGTHCVTCTCAAG. The primers of gene *NirS* are 1001F:

CGTGGTGGGAAAYTAYTGGCCKCC and 1242R: CAYGAYGGHGGHTGGGAC. And send the PCR products to the gene sequencing company for sequencing.

Inspect the influence of different factors on nitrification at low temperature

In order to optimize the culture conditions, five characteristics such as salinity, C/N ratio, carbon source, substrate concentration, and dissolved oxygen were verified by single factor experiments. the basic conditions as follows: temperature 10 ℃, pH 7, time 72 h, inoculum size 1% (v / v), rotation 200 rpm and NH_4Cl concentration 100 mg/L. On salinity test, the NaCl concentration set as: 1%, 3%, 5%, 10% and 15%. On the C/N ratio test, set the initial C/N ratio to 2, 5, 8, 15, 20. Test with sucrose, sodium acetate, sodium succinate, glucose and sodium citrate as the sole carbon sources, respectively. In the test, the substrate concentration was set to 20, 50, 100, 200, 500 mg/L. The oxygen mass transfer efficiency between the gas phase and the liquid phase was regulated by controlling the rotation speed during the cultivation process, thus, to control the different dissolved oxygen content in the liquid phase(Mcdaniel and Bailey, 1969; Parker and Jack, 1997; Wittmann et al., 2003), adjust the speed to the dissolved oxygen concentration of 1, 2, 3, 4, 5mg/L. All tests were conducted in triplicate and non-seeded samples were used as blank control.

Application of bacterium to treat domestic sewage at low temperature

Prepare 10 L of bacterium seed culture and mix with 10 L of domestic sewage. After incubating for 24 hours, put it into a bioreactor (500 L) for processing domestic sewage, and add 10L of mixed nutrient solution of glucose and trace elements at the same time. The NH_4^+-N, NO_3^--N, OD_{600} and cell abundance of the bioreactor were measured every 24 hours for 14 days. The experiment was carried out in winter, and the outdoor temperature was 4-12 °C.

Results And Discussion

Isolation and Identification of *Moraxella osloensis* LT-01

The bacterium LT-01 with the highest nitrogen removal efficiency from six isolates at low temperature was obtained. The 1457 bp DNA fragment of 16S rRNA gene was amplified, and the nucleotide sequence was deposited in the Genbank database under accession number MW031806. The sequence was compared through NCBI Blast, which exhibited a 98% of sequence identity to *Moraxella osloensis* strain. A phylogenetic tree of LT-01 presented as an NJ tree based on the16S rRNA genes of LT-01 and representative denitrify bacteria recently reported, including *Moraxella osloensis* CFP312(MK283753), *Moraxella osloensis* KM7(JF411306), *Enhydrobacer* sp. KB3-12(FN377702) etc. It was clearly found that LT-01 is close to the clades of *Moraxella osloensis* strain (Fig. 1), with 98% homology. Therefore, it is determined that the bacterium is affiliated with the genus *Moraxella* and named it *Moraxella osloensis* LT-01.

The effect of temperatures on Cell growth of *Moraxella osloensis* LT-01

The OD_{600} value was measured by ultraviolet spectrophotometry to verify the cell growth of LT-01. As shown in Fig. 2D that LT-01 can reproduce normally in the temperature range of 5-25 °C, but its proliferation is restricted when the temperature exceeds 30 °C. The optimum growth temperature of LT-01 in LB medium is 20 °C, at which time the OD_{600} reaches the maximum value of 2.07. The OD_{600} value incubated at low temperature (10 °C) is 1.26, which is 60.8% of the OD_{600} value at 20 °C. On the other hand, we observed that the growth trend of LT-01 in domestic sewage is not significantly different from that in LB medium. The optimal incubation temperature of LT-01 in domestic sewage is also 20 °C, the corresponding OD_{600} value is 0.72, and the OD_{600} value of 10 °C is 0.41. Comparing the above results with other studies, such as, Mr. Zheng(Zheng et al., 2011) isolated an aerobic denitrifying bacterium Psychrobacter sp. S1-1, which has an OD_{600} value of 0.2 when incubated at 10 °C for 4 days. The OD_{600} value of *Pseudomonas taiwanensis* obtained by He et al(He et al., 2018) is close to 0.3 after 48 hours incubation at 10 °C. We found that LT-01 has a wide temperature ecological range, higher cell growth performance at low temperatures, good adaptability to the environment, and excellent survival ability. According to Morita's(Morita, 1975) definition of Psychrophilic bacteria, it is judged that LT-01 is a kind of psychrotrophs.

Nitrification and denitrification by *Moraxella osloensis* LT-01 at 10 °C

Experimental results showed that LT-01 could utilize three nitrogen sources including nitrate, nitrite, and ammonia as substrates, at low temperature (10 °C) culture. For the aerobic denitrification process of LT-01, when KNO_3 is used as the sole nitrogen source (Fig. 2A). During the initial 6 hours, bacterial growth is in a lag phase, and nitrate slowly decreases. Within the next 12 hours, the bacterium enters the logarithmic growth phase, at the same time, the concentration of NO_3^--N decreases rapidly, from the initial 98.35 mg/L to 49.71 mg/L. After 18 hours of incubation, the cells in stationary phase, it was observed that degradation rate of nitrate decreased and nitrite accumulated, simultaneously. In the next 12 hours, the cell growth remained stable, with a slight decrease in NO_3^--N concentration, and nitrite gradually disappeared. NH_4^+-N is detected of trace amount at 54 hours, the final removal rate of nitrate was 65.81%, approximately. Make the following analysis for the above phenomenon, the rapid increase in the number of cells in the logarithmic growth phase accelerated the reduction rate of nitrate, which resulted in the accumulation of intermediate product NO_2^--N in the reduction process. The appearance of NH_4^+-N may be due to the ammonification of microorganisms. with $NaNO_2$ as a single nitrogen source (Fig. 2B), the lag period of LT-01 was as long as 30 hours, during which the concentration of A decreased slightly. The cell growth increased exponentially for 30-36 hours, while the concentration of NO_2^--N decreased linearly. After 36 hours, the cell growth enters a stable phase, and the final cell growth is less than when nitrate is used as the sole nitrogen source. it indicates that nitrite has an inhibitory effect on the growth and development of LT-01.

The heterotrophic nitrification capacity of LT-01 is showed in Fig. 2C. When NH_4Cl was used as the sole nitrogen source, the concentration of NH_4^+-N drops from the initial 101.73 mg/L to 30.15 mg/L, the final

removal rate of ammonia is 70.35%, and the bacterial density was also at a high level. The above results indicate that LT-01 belongs to the heterotrophic nitrification-aerobic denitrification type. Besides, compared with nitrate and nitrite, LT-01 has a higher effective utilization rate of ammonium. It is noteworthy that the accumulation of NO_2^--N was detected in the process. These results indicate that LT-01 belongs to heterotrophic nitrifying-aerobic denitrifying bacterium.

Nitrogen transformation pathway and functional genes of *Moraxella osloensis* LT-01

The results of nitrogen balance analysis are shown in Tab.1. With NH_4Cl as the sole nitrogen source, the initial total nitrogen concentration in the water was 98.35 mg/L. After 72 hours of cultivation, about 63.1% of the nitrogen was removed. In the process of simultaneous nitrification and denitrification, about 46% of nitrogen disappeared in the form of gas such as NOx or N_2, 16% of nitrogen is involved in microbial assimilation for cell proliferation, which is present in cells in the form of organic amines and amino acids.

The traditional heterotrophic nitrification-aerobic denitrification pathways are shown in the Fig. 3A. A large number of studies have shown that *HAO* and *NirS* are the signal labels of nitrification and denitrification(Xia et al., 2020). Based on these studies, we have amplified the *HAO* and *NirS* genes of LT-01. The amplified electropherogram is shown in the Fig. 3B, and the result shows that the bacteria LT-01 has the *NirS* gene, but not the *HAO* gene. This is similar to the gene sequencing results of bacteria *K.pneumoniae* EGD-HPI9-C(Pal et al., 2015) and *Klebsiella* sp.KSND-3(Chen et al., 2019). We speculate that there is a new simultaneous nitrification and denitrification pathway in LT-01, which also indicates the diversity of bacterial nitrification and denitrification metabolic pathways. Padhi suggested that there may be another way of denitrification through hydroxylamine intermediates(Padhi et al., 2013), while PAL(Pal et al., 2015) said that although some unique simultaneous nitrifying and aerobic denitrifying bacteria have incomplete nitrification and denitrification pathways, they can still pass through the ammoniation reaction remove inorganic nitrogen from the system. On this issue, further research is needed to verify the simultaneous nitrification and denitrification process.

Characterization of *Moraxella osloensis* LT-01 on nitrification at 10 °C

Salt stress

Salt stress is one of the important factors affecting the growth of microorganisms. The effect of salinity on the ammonia removal performance and cell viability of LT-01 is shown in the Fig. 4A. As the salinity increases from 1% to 20%, the ammonia removal rate drops from 60.3% to 30.8%, and the OD_{600} value remains above 0.4. During the period, trace amounts of nitrite were detected, and the accumulation of nitrite increased with the increase of salinity. The cause might be that the excessive salinity leads to the increase of osmotic pressure, which induce cell dehydration and rupture(Shapovalova et al., 2008). In addition, the high value of salinity also reduces cell dehydrogenase activity, thereby inhibiting growth and function of bacteria(Duan et al., 2015).

C/N ratios

The C/N ratio required for growth of different microorganisms is different. The experimental results shown in the Fig. 4B, the best C/N ratio for LT-01 to remove ammonia at low temperature (10 °C) is 10. For process, the removal rate of ammonia increases with the increase of the C/N ratio, and then tends to be stable. The reason may be that when the carbon source content is higher than the demand of the bacteria, the carbon source is no longer a limiting factor. For a C/N ratio of 0, the highest cumulative amount of nitrite is 0.11 mg/L, the lowest cumulative amount of nitrite is 0.042 mg/L in a C/N ratio is 20. It indicates that the heterotrophic nitrification-aerobic denitrification reaction is more thorough, and the accumulation of NO_2^--N is less in the process. For sewage with low C/N ratio, for example, when C/N is 0 and 5, the removal rate of ammonia by LT-01 is low, which is 19.32% and 38.75%, respectively, which needs to be optimized in actual sewage treatment.

Carbon source

In the process of heterotrophic nitrification and aerobic denitrification, the carbon source provides electron donors for the denitrification process, as well as energy for the growth and metabolism of microorganism(Anesio et al., 2009), it plays a vital role in the proliferation of bacteria. However, different types of carbon sources have different structures and molecular weights, which affects the proliferation and function of bacteria. As shown in Fig. 4C, using sucrose, sodium acetate, sodium succinate, glucose and sodium citrate as the sole carbon sources, the corresponding ammonia removal rates are 21.4%, 62.2%, 49.7%, 25.1% and 67.3%, the OD_{600} value is 0.53, 1.18, 1.01, 0.63 and 1.25. For sodium acetate and sodium succinate, the ammonia removal efficiency is slightly lower than that of sodium citrate, while for glucose and sucrose, the cell growth level is low, and the ammonia removal effect is worse. The reason is that organic acids with simple structure and small molecular weight are more easily absorbed(Pedersen et al., 1999). Beyond that, sodium citrate, as a precursor of the tricarboxylic acid cycle, is more easily utilized by microorganisms.

Substrate concentration

Ammonium in the water mainly exists in the form of NH_4^+ and NH_3-N. NH_3-N is toxic to microorganisms and inhibits their growth. The ammonia removal of LT-01 at different substrate concentrations are shown in the Fig. 4D. The initial substrate concentration was 20 mg/L, which decreased from 20 mg/L to 6.72 mg/L after 48 hours, and the final OD_{600} value is 0.53. The increase of substrate concentration promoted the degradation of ammonia by LT-01, the cell growth and ammonia removal efficiency were the highest in the medium with the concentration of 200 mg/L. With a high substrate concentration (ammonium concentration is greater than 500 mg/L), LT-01 is not only unable to utilize the excessive ammonia, but also limited its growth and ammonia removal capacity. The reason is that high ammonia concentrations inhibit the activity of microbial enzymes, probably. For the process, the accumulation of nitrite increases along with initial substrate concentration, the affinity of nitrate reductase for electrons is greater than that of nitrite reductase, which causes the degradation rate of nitrate to be greater than that of nitrite.

Dissolved oxygen

Dissolved oxygen has significant effects on nitrification and denitrification performances of microorganisms(Jin et al., 2019). In the process of biological oxidation, oxygen molecules act as electron acceptors. As expected, it was found that LT-01 showed high ammonia removal efficiency under different rotating speeds (DO levels), resulting in the removal rate over 70% (Fig. 4E), indicating that LT-01 can efficiently transfer electrons no matter what level of dissolved oxygen it is at, so as to achieve ammonia removal. With the increase of rotating speed, the content of dissolved oxygen increases, while the accumulation of nitrite gradually decreases, the reason may be that the growth and development of microorganisms are affected by dissolved oxygen, leading to the limitation of their biological reaction rate.

Application for domestic sewage treatment at low temperature

To solve the problem of nitrogen removal from sewage in cold areas, we applied LT-01 to bioreactors at low temperatures and treated domestic sewage for 14 days. The data shows (Fig. 5) that during the experiment, the number of cells per unit volume in the bioreactor increased from 0.14 $\times 10^3$ CFU/mL to 1.67$\times 10^3$ CFU/mL. and the OD_{600} value also reached 0.45. This shows that even in a complex environment, LT-01 can still adapt to the environment well and maintain a high biomass. In addition, we monitored that TN dropped from 53.25 mg/L to 13.33 mg/L, removing 74.96% of nitrogen, and ammonia continued to show a downward trend, from 43.58 mg/L to 9.23 mg/L, the ammonia removal rate is remained at 78.82% stably. During this period, a small amount of NO_3^--N accumulates, which fluctuates in the range of 0-8 mg/L, and eventually approaches zero. The above results show that LT-01 can adapt to the environment well at low temperature, and play a stable and continuous function, and has a significant effect on the removal of nitrogen. It is suitable for the purification of domestic sewage in cold areas and provides a low consumption. Efficient biochemical treatment options can also be used to optimize processes.

Conclusion

The novel psychrotrophs denitrifying bacterium, named strain LT-01, was identified as *Moraxella osloensis* based on 16S rRNA gene sequence. The strain LT-01possessed excellent cold resistance and was able to remove 65.39% of nitrate, 61.74% of nitrite and 70.35% of ammonium at 10 °C. It converted ammonium to gaseous denitrification products, such as N2. Under the condition of 10 °C, the evaluated different C/N ratio, salt stress, carbon source and other factors will affect the nitrification process of strain LT-01. It was worth noting that the PCR result proves that the bacteria LT-01 has the nitrate reductase (*NirS*) but no hydroxylamine oxidase (*HAO*), which means that LT-01 may have a novel simultaneous nitrification-denitrification pathway. The application experiment results show that the strain LT-01 can maintain high abundance and nitrogen removal rate in a complex and cold environment, is a promising candidate for nitrogen removal from wastewater at low temperatures.

Declarations

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and material

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Code availability

Not applicable

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ruojin Zhao, Yinyin Chen and Peng Jin. The first draft of the manuscript was written by Jin Qu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

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Not applicable

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Tables

Tab. 1. Nitrogen balance during the heterotrophic nitrification-aerobic denitrification by LT-01at 10 °C.

Initial TN	Nitrogen concentration (mg/L)						N Loss
(mg/L)	$NO_3^- - N$	$NO_2^- - N$	$NH_4^+ - N$	NH_2OH-N	Intracellular N	Nitrogen Gas	(%)
98.35	0	0	36.29	0	16.44	45.62	63.10

Figures

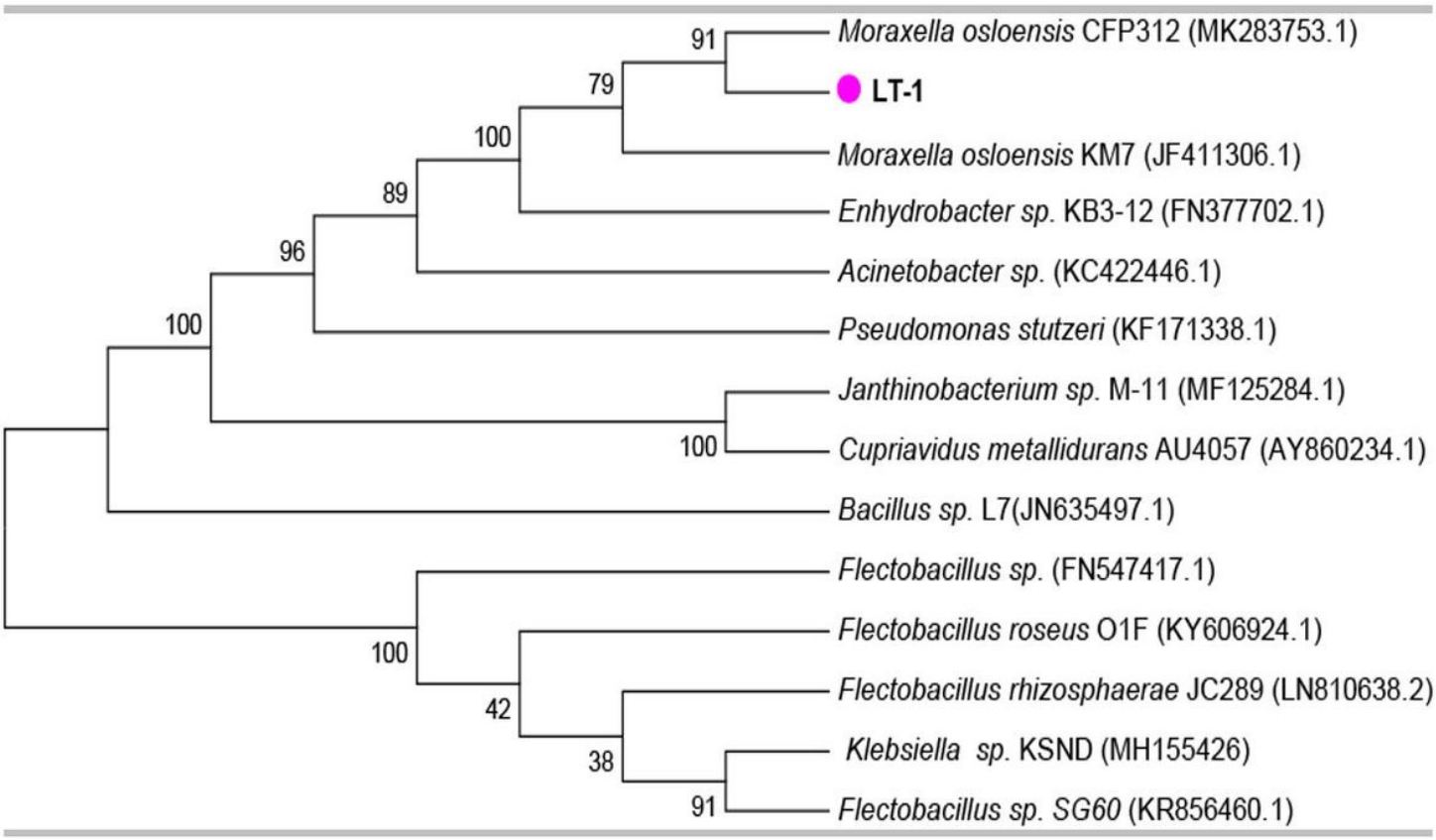

Figure 1

Phylogenetic analysis of the 16S rRNA sequence. The phylogenetic tree of LT-01 was constructed using the neighbour-joining (NJ) method. One thousand bootstrap replications were performed using the software MEGA5.

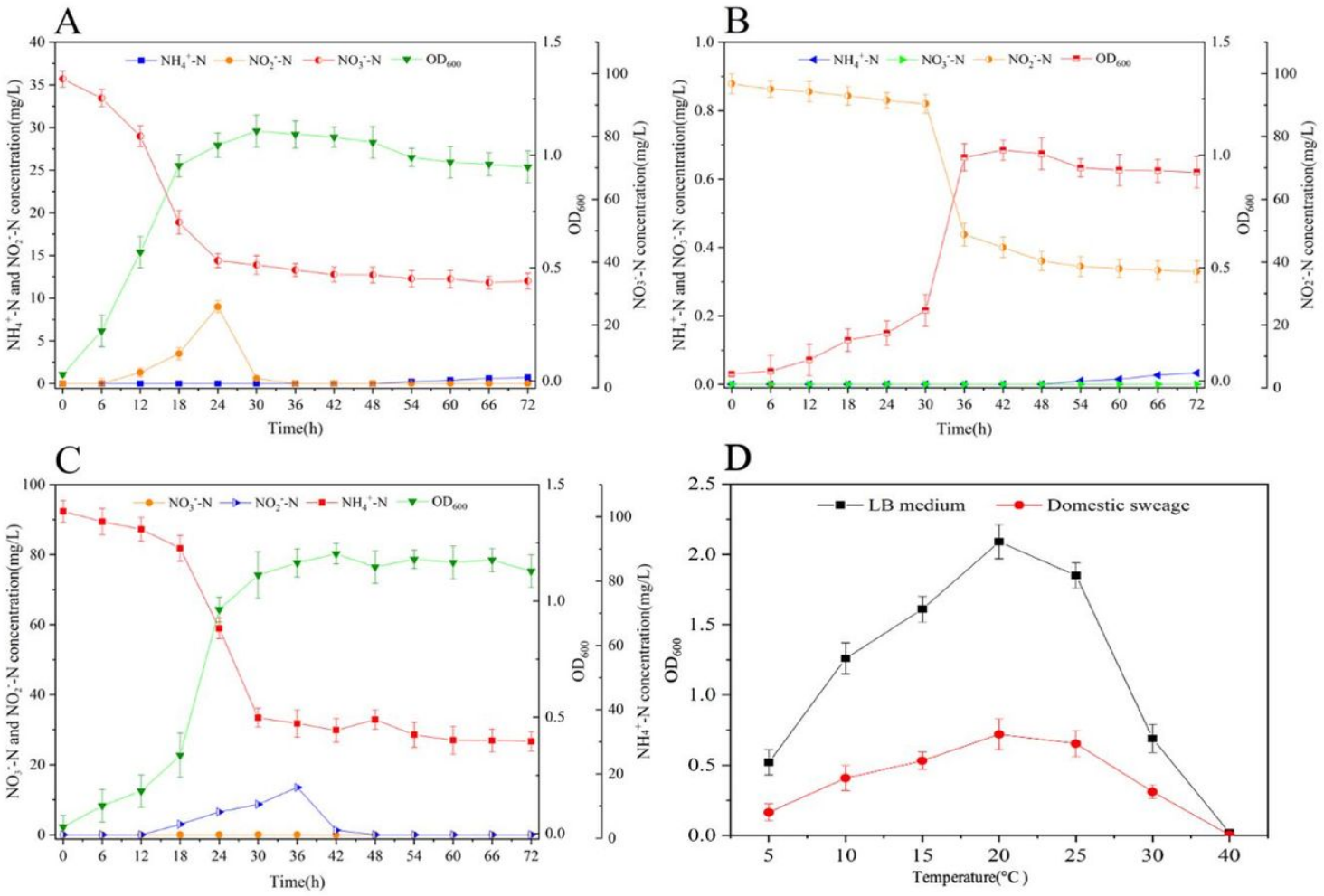

Figure 2

Changes in nitrogen compound at 10 °C and growth of Moraxella osloensis LT-01. (A) Nitrate; (B) Nitrite; (C) Ammonia. (D)The growth curve of LT-01 at different temperatures.

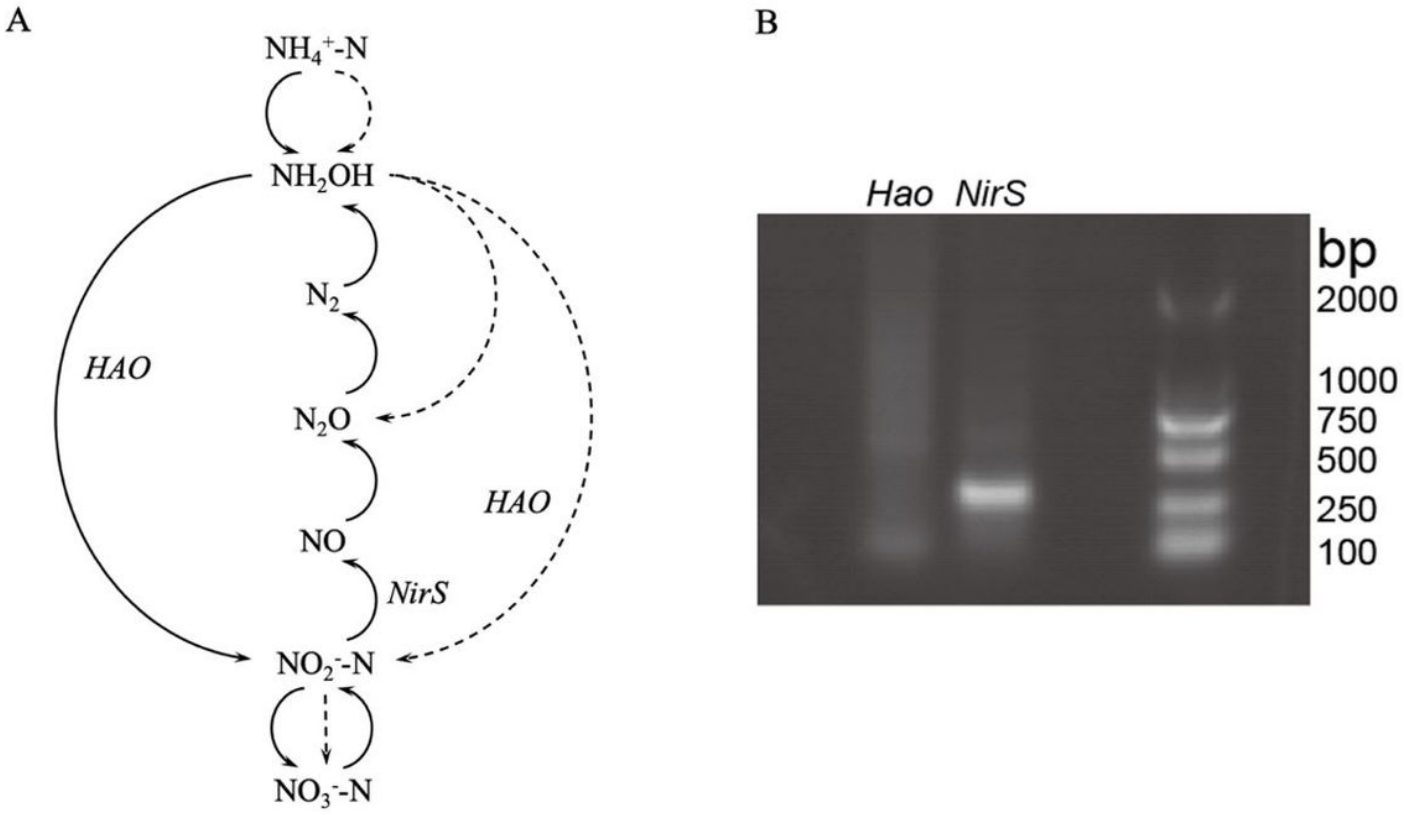

Figure 3

Nitrogen metabolism pathways and genes. (A) The traditional synchronous nitrification-denitrification process. (B)Amplification profiles of NirS and HAO genes from Moraxella osloensis LT-01.

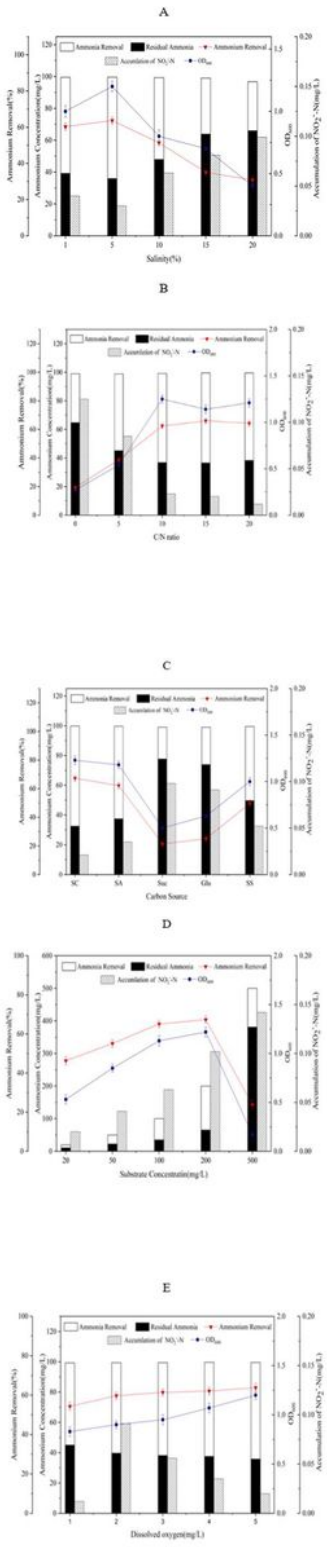

Figure 4

Effects of physical-chemical factors on growth and ammonium removal of Moraxella osloensis LT-01after 72 hours of incubation in NM medium at 10 °C. (A) Salinity -1, 5, 10, 15, 20%; (B) C/N ratio-0, 5, 10, 15, 20; (C) Carbon source - the carbon sources used were sodium citrate (SC), sodium acetate (SA), sucrose (Suc), glucose (Glu) and sodium succinate (SS); (D) Substate concentration-20, 50, 100, 200, 500 mg/L; (E) Dissolved oxygen-1, 2, 3, 4, 5 mg/L.

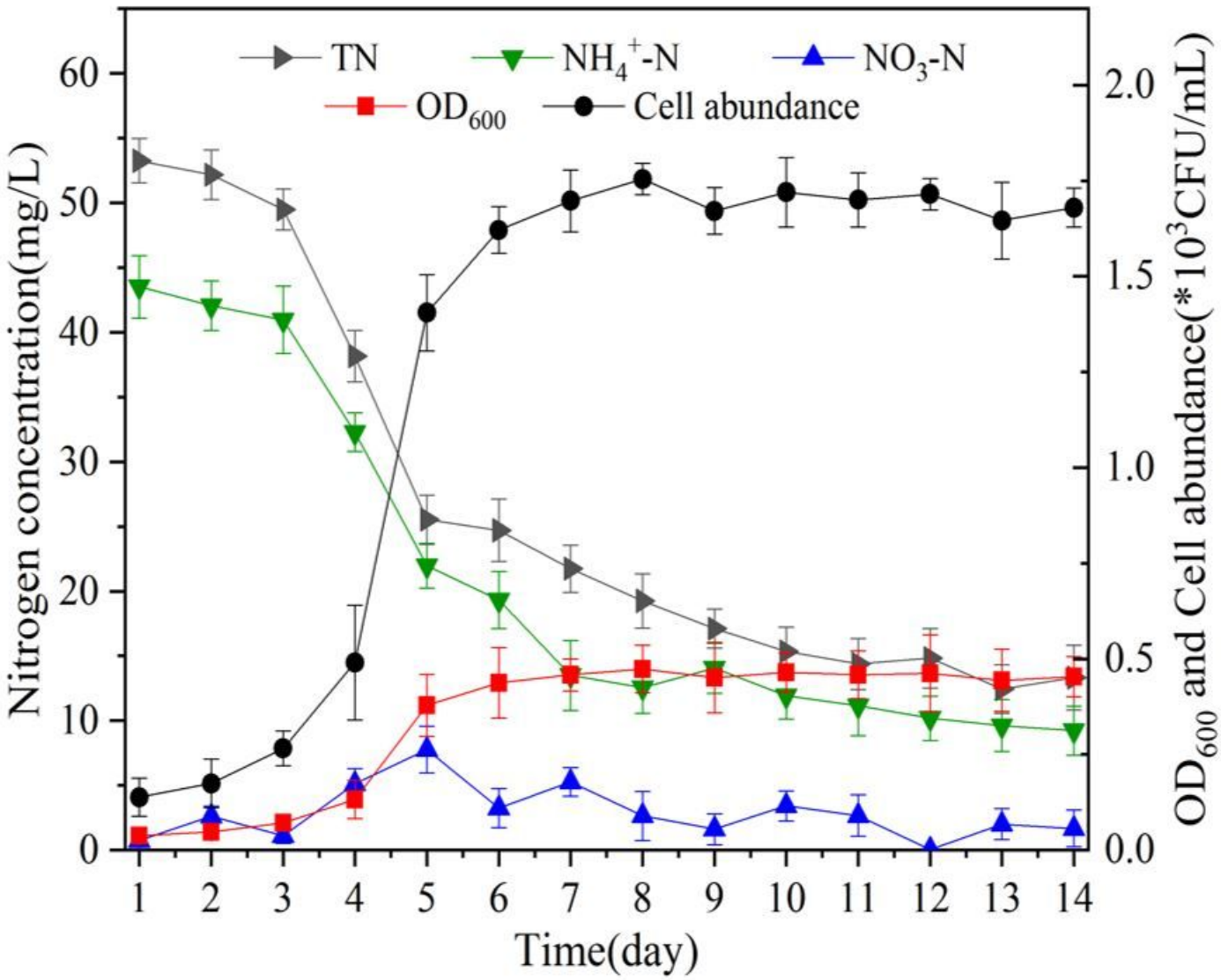

Figure 5

Utilize the LT-01 to treat the nitrogen concentration change and cell growth of domestic sewage in the bioreactor.