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A heterotrophic nitrification – Aerobic denitrification bacterium

QINGPING HU[1], FENG HE[2] and CAIFANG WEN[3]

College of Life Science, Shanxi Normal University, 1 Gongyuan Street, Linfen City 041 004, China

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ABSTRACT

A bacterial strain named *Pseudomonas stutzeri* ZH-1, which exhibits the ability of efficient heterotrophic nitrification and aerobic denitrification, was isolated from 20 bacterial strains in the sludge of Fenhe River (in Shanxi Province, China) and identified by 16S-rDNA sequencing as a strain of *Pseudomonas stutzeri*. The cells of strain ZH-1 were Gram-negative, non-motile and short rod-shaped. In order to further understand its removal ability, several experiments were conducted to identify the growth and nitrogen removal response at different factors. The results showed that the optimum conditions is sodium citrate as carbon source, C/N 12, shaking speed ≥200 rpm, pH 9, temperature 25-35°C and nitrogen concentration ≥150mg/L. The average removal rate for NH_4^+-N, NO_3^--N, NO_2^--N can be as high as 4.27, 5.53 and 4.30 mg/L/h for synthetic wastewater, respectively. In particular, the strain ZH-1 showed an amazing ability of rapid degradation for NH_4^+-N, NO_3^--N and NO_2^--N under aerobic conditions and it might be a suitable candidate to simultaneously aerobic nitrification/denitrification for future full-scale applications in wastewater treatment.

Key words: Aerobic denitrification, Heterotrophic nitrification, Identification, Nitrogen removal

Nitrogen removal from the wastewater was one of the main purposes for the wastewater treatment. Increases in nitrogen levels in river from key sources such as industrial, agricultural, urban and sanitary wastewater, can cause numerous environmental and health problems. If not treated sufficiently, biological nitrification-denitrification is one of the most effective and economical processes for nitrogen removal from wastewaters (Gupta *et al.* 2001). The most common biological processes include nitrification, which oxidize NH_4^+-N via NO_2^--N to NO_3^--N by autotrophs under aerobic conditions, and denitrification, which converts NO_2^--N and NO_3^--N to N_2 gas by denitrifying bacteria under anaerobic conditions (Ji and Chen 2010; Chang *et al.* 2011). Therefore, this process consists of several distinct metabolic steps, and both aerobic and anaerobic conditions must be prepared. Implementation of such a system was difficult in actual ecosystems due to the requirement for strict anoxic conditions for denitrification.

Conventional nitrogen removal in wastewater treatment systems involves aerobic nitrification by autotrophs and anaerobic denitrification by heterotrophs (Fu *et al.* 2009). Daniel *et al.* (2009) researched that the biofilm was capable of actively oxidizing ammonium and denitrification at high ratios in intermittent intervals within 24 h cycles. The *Pseudomonas stutzeri* YZN-001 strain, which was isolated from swine manure wastewater by Zhang *et al.* (2011), can utilize not only nitrate and nitrite under aerobic conditions but also ammonium. There were some problems from the former researchers in this system, for example, it required separate treatment processes and strict condition control, making these technologies prohibitively expensive. Takebe *et al.* (2012) found a heterotrophic nitrifying bacterium, designated strain DA2, which was isolated from a microbiological agent for enhancing microbial digestion in sewage treatment tanks. Disadvantages to such systems were that it demands large expanses of space to house separate aerobic and anaerobic tanks, the nitrification process tends to be time consuming, so the operating cost was prohibitively high (Khin and Annachhatre 2004). In addition, conventional nitrification can be carried out only after increasing the C/N ratio or diluting the wastewater (Joo *et al.* 2006, Lin *et al.* 2009, Saricheewin *et al.* 2010). According to the report of the Obaja *et al.* (2005), it concluded that heterotrophic nitrogen removal for the treatment of wastewater containing high C/N ratios, because heterotrophic microorganisms often require high concentrations of organic carbon.

In the early 1980s, aerobic denitrifying bacteria that simultaneously utilize oxygen and nitrate as electron acceptors were first reported (Yao *et al.* 2013). It implies that nitrification and denitrification can occur concurrently in the same reaction vessel under identical overall operating conditions. Nowadays, simultaneous nitrification and denitrification (SND) is an attractive method to treat wastewater (Schmidt *et al.* 2003). Kim *et al.* (2005) found that the *Bacillus* strains were able to carry out simultaneously aerobic nitrification/denitrification. Khardenavis *et al.*

[1] e mail: hqp72@163.com; [2] e mail: 1017107855 @qq.com; [3] e mail: 240783014 @qq.com

(2007) had found that *Diaphorobacter* sp. had the ability to perform simultaneous nitrification and denitrification under aerobic conditions which was isolate from activated biomass surviving on wastewater.

Because heterotrophic nitrification-aerobic denitrification bacteria does not require an anaerobic environment, so its application promotes nitrogen removal from surface water (Guo *et al.* 2013). However, the isolation of heterotrophic nitrifying-aerobic denitrifying bacteria from natural water environments was extremely limited according to the reported literature describing. In particular, in micro-polluted reservoirs that serve as drinking-water sources, low temperature and low C/N ratio restrain the bioremediation efficiency (Huang *et al.* 2015). During the last three decades, several heterotrophic nitrifying-aerobic denitrifying bacteria had been reported, including *Thiosphaera pantotropha* (Robertson *et al.* 1988), *Microvirgula aer-denitrificans* (Patureau *et al.* 1998), *Bacillus* sp. (Khardenavis *et al.* 2007), *Providencia rettgeri* (Taylor *et al.* 2009), *Bacillus methyltrophicus* (Zhang *et al.* 2012), *Bacillus* sp. strain YX-6 (Song *et al.* 2011), and *Rhodococcus* sp. (Chen and Ni 2012), *Pseudomonas stutzeri* strains YZN-001(Zhang *et al.* 2011,). These bacteria exhibited higher growth rates than autotrophs, tolerated high concentrations of organic loading and performed simultaneous nitrification and denitrification in the same reactor.

Several studies have focused on nutritional and physical factors that adjust the nitrogen removal characteristics of aerobic denitrifying bacteria. Towards this end, in this work, an efficient newfangled aerobic denitrifier, *P. stutzeri* strain ZH-1, a bacterial strain with high efficiency removal of nitrogen, was isolated and identified from the sludge of the Fenhe River. The aim of this study was to separate and screening an efficient heterotrophic nitrifying-aerobic denitrifying bacteria, and further investigate the aerobic nitrification/denitrification ability and factors affecting the optimum performance of *P. stutzeri* ZH-1, and to determine its potential application in biological wastewater treatment systems. It is of great significance in the development of more successful strategies aiming at exploiting more beneficial microbe resources for treatment of wastewater.

MATERIALS AND METHODS

P. stutzeri ZH-1 was originally isolated and identified from Fenhe River (in Shanxi Province, China). After being grown on the solidified beef extract-peptone medium containing 5.0g beef extract, 10.0g peptone, 5.0g NaCl, 18.0g bacto-Agar and 1000ml tap water, the bacterial strain was stored at -80°C in 30%(w/v) glycerol. In the heterotrophic nitrification medium (HNM) and aerobic denitrification medium (ADM-1&2) (Zhu *et al.* 2012) the concentrations of carbon and nitrogen source were adjusted following the subsequent experiments. The initial pH of all the mediums mentioned above were adjusted to 7.2-7.4.

The sludge sample (10 ml) was transferred to 250 ml conical flask containing sterilized water (90 ml) and glass beads and then shaken at 200 rpm to obtain symmetrical bacterial suspension. Than serial dilutions within the range of 10^{-2} to 10^{-7}were inoculated on solidified of beef extract-peptone medium plates. The inoculated media were incubated at 30°C for about 2-3 days under aerobic conditions until obvious colonies had formed. A total of 20 bacterial colonies were selected and streaked on fresh agar plates in order to achieve pure colony. Then, the isolates were streaked onto ADM medium and incubated with steady shaking (120 rpm) at 30°C to test their ability of denitrification. The strain ZH-1 with the highest denitrification ability was singled out for further study.

Micrographs of strain ZH-1 were taken with a scanning electron microscopy. The physiological characteristics of ZH-1 were examined according to the methods of Garrity *et al.* (2004). The 16S-rDNA gene of the isolate was amplified by PCR using bacterial universal primers F27 (5'-AGTTTGATCMTGGCTCAG-3') and R1492 (5'-GGTTACCTTGT-TACGACTT-3'). PCR products were sequenced on both strands. The sequence alignment was performed using the Basic Local Alignment Search Tool (BLASTn). A neighbor-joining tree was constructed in MEGA 6.0 program using neighbor-joining (NJ) method with 1000 bootstrap replicates and the maximum composite likelihood model.

To further research nitrogen removal efficiency with different initial nitrogen type in the HNM or ADM(1&2), sodium citrate was used as carbon source and NH_4^+-N or NO_3^--N or NO_2^--N was primordially added at a concentrations of 50, 100, 150 and 200 mg/L roughly, with a C/N ratio of 8. In order to assess how carbon sources, aeration, temperature and C/N ratio affected the heterotrophic nitrification-aerobic denitrification efficiency, single factor experiments were conducted in conical flasks. In the carbon sources experiment, sodium citrate, sodium succinate, sodium acetate, glucose, glycerin and sucrose were added separately to the HNM or ADM (1&2) to yield a C/N ratio of 8. In the aeration experiment, the shaking speeds of 0, 50, 100, 150 and 200 rpm were applied using sodium citrate as carbon source at a C/N ratio of 8. The temperature experiment was performed using the same medium in the aeration experiments and the medium was incubated at 20, 25, 30, 35 and 40°C. The effects of C/N ratios were measured using sodium citrate as the sole carbon source, and C/N ratio of 2, 4, 8, 12, 16 and 20, respectively. All of the above experiments were conducted in triplicates with inoculation of 2% (v/v) in 100 ml-conical flask with 50 ml axenic culture medium. During the 24h incubation, cultures were sampled at fixed period to test the optical density (OD_{600}), and concentrations of NH_4^+-N or NO_3^--N or NO_2^--N.

Growth of the bacteria was monitored by measuring the optical density at 600nm (OD_{600}) of the culture broth using a spectrophotometer. Culture samples were centrifuged at 8 000 rpm for 10 min. The concentration of ammonium was analyzed by Nasser's reagent photometry (GB 7497-87). The nitrate concentrations were determined following a UV spectrophotometric method (HJ/T 346-2007) to differentiate

between OD_{220} and $2\times OD_{275}$. The nitrite concentration was determined by N-(1-naphthalene)-diaminoethane photometry method (GB 7493-87). The denitrification ratio was calculated according to the method of Joo *et al.* (2005). The ammonium, nitrate and nitrite removal efficiencies were calculated by the equation:

$$Rv = (T_1 - T_2)/T_1 \times 100\%$$

to assess the nitrification and denitrification ability of strain ZH-1. Note that Rv, T1 and T2 represent ammonium (nitrate or nitrite) removal efficiency, the initial concentration of ammonium (nitrate or nitrite) in medium and the final concentration of ammonium (nitrate or nitrite), respectively. All tests were conducted in-triplicate, with the exception of the growth experiment. Statistical analyses were performed by the SPSS Statistics and differences were considered significant at $p < 0.05$. And drawing software (Origin 9.0) was used.

RESULTS AND DISCUSSION

Isolation, morphological and physiological characteristics

P. stutzeri ZH-1 was found to be Gram-negative and a heterotrophic nitrification-aerobic denitrification bacterium strain. The cells of ZH-1 were observed by scanning electron microscopy and were identified as short non-motile and rod-shaped (1.25 × 0.36μm) (Fig 1). The partial 16S-rDNA sequence (1462 bp) (Fig 2) of strain ZH-1 was determined and deposited in the Gen Bank database (GenBank ID: KM278988). Comparison of its sequence with those in the GenBank databases showed 99% similarity to *Pseudomonas stutzeri* ATCC 17588 (Fig 3). Based on the morphological, biochemical, the *Bergey's Manual of Determinative Bacteriology* and 16S-rDNA gene sequence analysis, strain ZH-1 was recognized as a strain of *Pseudomonas stutzeri* and was thereafter named *Pseudomonas stutzeri* ZH-1.

Assessment of the heterotrophic nitrification-aerobic denitrification of P. stutzeri ZH-1

As shown in Table 1, sodium citrate was the most efficient C-source, presenting removal efficiency of 57.85%, 100% and 99.68% in 24 h, for NH_4^+-N, NO_3^--N and NO_2^--N, respectively. Also, Succinate and Acetate have a very high removal rate for NO_3^--N and NO_2^--N. Especially, sodium acetate has been used as exogenous C-source to remove N in *Agrobacterium* sp. LAD9 (Chen and Ni 2012) and *Acinetobacter sp.* Y16 (Huang *et al.* 2013). The other three types of C-compounds, glucose, sucrose and glycerin were not efficient C-source for the three tested nitrogen of strain ZH-1. The distinction in N removal efficiency of the origin of carbon may be relevant with their oxidoreduction potentials, and the appropriate C-source for N removal might be a series or species characteristic. So sodium citrate was selected as the optimal extraneous C-source for strain ZH-1 in further experiments. In addition to the choice of appropriate C-source, the data in this test also proved that the removal efficiency for the three N-compounds was NO_3^--N > NO_2^--N >NH_4^+-N, which might be correlated with their biological toxicity.

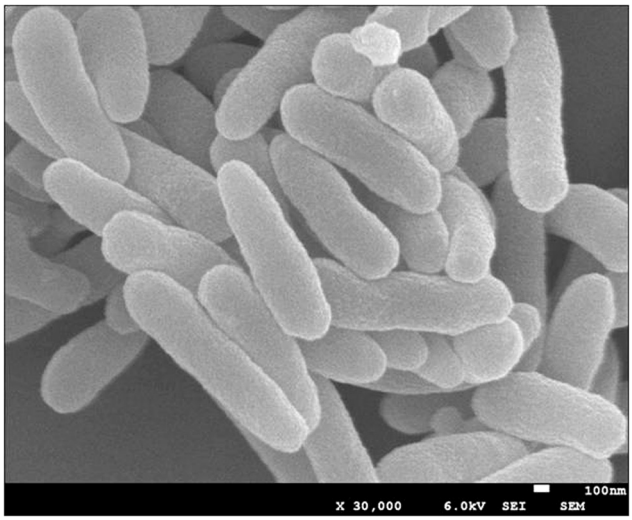

Fig 1 Scanning electron microscopy picture of *P. stutzeri* ZH-1

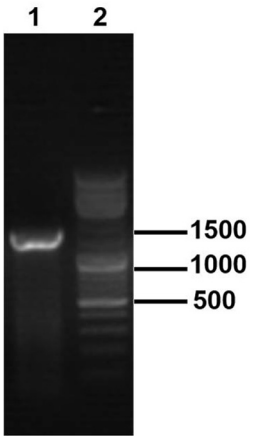

Fig 2 The electrophoretogram shows amplification of strain ZH-1 16S-rDNA. The first lane for the strain of PCR products and the second lane for DNA Marker.

Many times, bacteria depended on the external environment of pH to adapt the environment. As shown in the Table 1, when initial pH was 9, were presenting removal efficiency of 95.62%, 98.12% and 91.32% was presented in 24 h, for NH_4^+-N, NO_3^--N and NO_2^--N, respectively. In addition, as the initial pH increased, nitrogen removal rate for strain ZH-1 on three kinds of nitrogen also increased, but removal rate declined when the initial pH was 10. When the initial pH was 5, the strain ZH-1 on three kinds of nitrogen removal rate was 0. The results showed that the strain ZH-1 was fond of the acid alkali environment, which may be because the different pH which influence the bacterial enzyme activity in the body and affects the bacteria on the absorption of nitrogen source material.

The impact of aeration on N removal was restrained by adjusting the shaking speed. Table 1 showed that the maximum NH_4^+-N removal efficiency (92.09%) was detected in the culture with the highest shaking speed of 100 rpm and greater NH_4^+-N removal when soluble oxygen augmented. The removal of NO_3^--N could accommodate

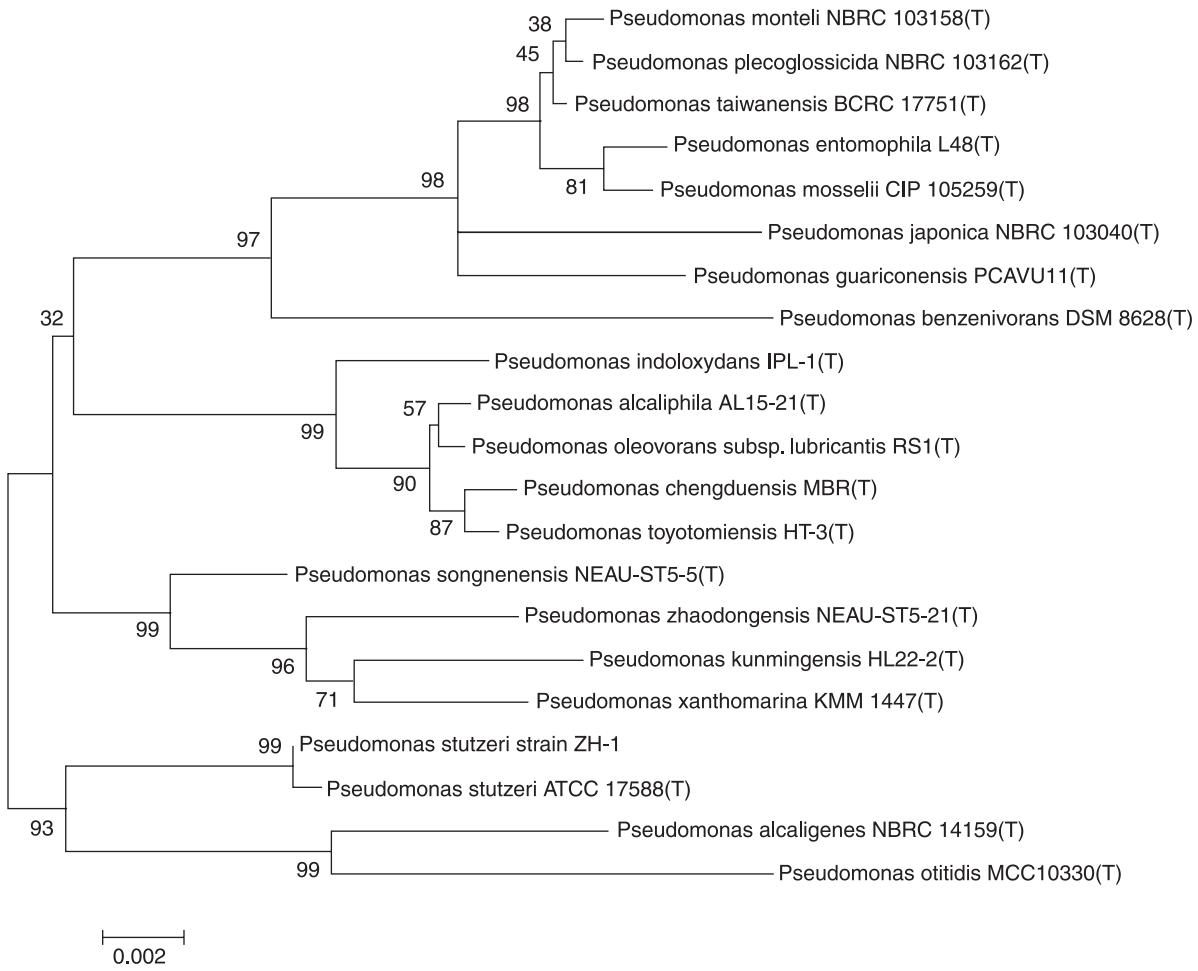

Fig 3 Phylogenetic tree based on the 16S rDNA gene sequence of strain ZH-1 and other reference sequences

with extensive soluble oxygen, more than 99% of NO_3^--N could be removed at the shaking speeds of 200 rpm. The maximum NO_2^--N removal efficiency was 99.7% at a shaking speed of 100 rpm. This result was consistent with *Pseudomonas stutzeri* YG-24 and reasonable because the NO_3^--N reduction required lower soluble oxygen concentration compared with NH_4^+-N removal. These results also proved that, when using strain ZH-1 to remove different kinds of nitrogenous compounds effectively, the aeration condition must be restrained according to different sources of wastewater in practical application.

Growth of strain ZH-1 and nitrogen removal can tolerate a wide range of temperature. Under the temperature of 20 and 40°C, the strain ZH-1 for ammonia nitrogen removal rates were 66.8% and 79.27%, for nitrite removal rate were 29.55% and 53.77%, respectively. But for nitrate removal rate seems to have greater adapting ability to the temperature, the highest removal rate can reach about 99.94%. The similar and high NH_4^+-N, NO_3^--N and NO_2^--N removal efficiencies were achieved between 25 and 35°C and removal rates were decreased at 20°C and 40°C. This phenomenon might be due to the strain ZH-1 belong to a thermophilic bacterium. It was worth noting that the NO_3^--N removal efficiency was still high for 97.84% at 20°C, which was similar to the highest removal efficiencies for NH_4^+-N and NO_2^--N. Previous reports said that *P. stutzeri* ZF-31 (Huang *et al.* 2015) could remove 97% of nitrate nitrogen after 16h, without nitrite accumulation. The results showed that the strain ZH-1 had stronger adaptability to tolerate the external temperature changing ceaselessly, in the meanwhile, the removal efficiency for NO_3^--N the removal rate of NO_3^--N was above 97.8%.

The data in Table 1 showed that C/N ratio conspicuously affected the N-removal efficiency ($p < 0.05$). The removal efficiency was increased with the C/N ratio going up from 2 to 8, but a little decreased when at a C/N ratio of 20. The highest removal efficiency of 98.56%, 100% and 99.82% for NH_4^+-N, NO_3^--N and NO_2^--N, respectively, was observed at a C/N ratio of 12. The leftover of nitrogen at low C/N ratios (e.g. 2 and 4) was mainly because the C-source had been exhausted for heterotrophs. This optimum C/N ratio was similar to that of *Bacillus* strain (Kim *et al.* 2005).

As shown in Table 1, the strain ZH-1 for ammonia nitrogen removal efficiency stay at a high level with 98.31%, 98.28% and 97.05% , and nitrite removal rates were 71.35%, 85.77% and 99.42%, respectively, by using initial N concentrations around 50, 100 and 150 mg/L. The

Table 1 NH_4^+-N, NO_3^--N and NO_2^--N removal efficiency under different carbon sources, Initial pH, shaking speeds, temperatures, C/N ratios and initial nitrogen contents by *P. stutzeri* ZH-1 in 24 h. Values represent the mean ± standard deviation of three replicates

Factors	Level	Removal efficiency (%)		
		NH^+_4–N	NO^-_3–N	NO^-_2–N
Carbon source	Citrate	57.85 ± 0.3 a	100 a	99.68 ± 0.11 a
	Succinate	35.41 ± 2.02 b	89.92 ± 1.15 b	99.03 ± 0.66 a
	Acetate	43.66 ± 0.26 c	100 a	100 a
	Glucose	32.27 ± 1.78 d	59.35 ± 2.60 c	7.21 ± 2.41 c
	Sucrose	7.31 ± 1.00 e	4.60 ± 1.36 d	0.07 ± 0.03 d
	glycerin	31.27 ± 0.56 d	57.78 ± 3.5 c	15.48 ± 3.37 b
Initial pH	5	-	-	-
	6	57.79 ± 0.24 e	87.98 ± 1.19 b	60.65 ± 2.94 c
	7	69.22 ± 1.69 d	94.60± 0.73 c	72.70 ± 3.94 c
	8	78.01 ± 0.27 c	96.06 ± 0.36 c	84.27 ± 3.86 d
	9	95.62 ± 0.49 a	98.12 ± 0.15 d	91.32 ± 5.54 b
	10	85.58 ± 0.98 b	99.52 ± 0.12 d	78.39 ± 5.65 a
Shaking speed (rpm)	0	52.22 ± 2.06 e	99.18 ± 0.13 a	99.63 ± 0.04 a
	50	72.01 ± 1.13 d	99.35 ± 0.18 a	99.70 ± 0.09 a
	100	92.09 ± 0.71 a	99.63 ± 0.13 b	99.70 ± 0.16 a
	150	88.00 ± 0.85 b	99.59 ± 0.99 c	99.39 ± 0.32 a
	200	79.23 ± 0.60 c	99.42 ± 0.11 c	96.19 ± 2.01 a
	250	77.64 ± 0.48 c	98.67 ± 0.52 c	52.16 ± 2.56 b
Temperature (°C)	20	66.81 ± 1.93 c	97.84 ± 1.90 a	29.55 ± 0.88 d
	25	80.82 ± 0.84 b	99.86 ± 0.03 a	40.69 ± 2.09 c
	30	86.75 ± 0.27 a	99.89 ± 0.02 a	99.05 ± 0.12 a
	35	84.58 ± 0.38 a	99.93 ± 0.02 a	52.56 ± 1.03 b
	40	79.27 ± 0.23 b	99.94 ± 0.02 a	53.77 ± 1.83 b
C/N ratio	2	18.92 ± 1.67 d	6.82 ± 2.52 c	16.74 ± 2.37 d
	4	29.79 ± 3.53 c	27.89 ± 2.72 b	45.59 ± 2.51 c
	8	88.09 ± 1.32 b	89.32 ± 1.61 b	99.34 ± 0.18 a
	12	98.56 ± 0.53 a	100 a	99.82 ± 0.19 a
	16	94.19 ± 1.20 a	100 a	99.24 ± 2.08 a
	20	97.16 ± 0.58 a	83.27 ± 1.38 a	79.45 ± 1.78 a
Initial nitrogen concentration (mg/L)	50	98.31 ± 0.84 a	100 a	71.35 ±4.01 b
	100	98.28 ± 1.72 b	100 a	85.77 ± 6.66 b
	150	97.05 ± 1.35 c	100 a	99.42 ± 0.13 a
	200	89.75 ± 1.47 c	92.86 ± 1.16 d	92.89 ± 1.54 a

Values followed by different letters in the same column are significantly different at $p < 0.05$. "-"is on behalf of no value.

nitrate was completely eliminated in 24 h by strain ZH-1 using initial N concentrations around 50, 100 and 150 mg/L. At the N content around 200 mg/L, the efficiencies by strain ZH-1 were significantly decreased, but still maintained at acceptable levels: 89.75% for ammonia nitrogen, 92.86% for nitrate, and 92.89% for nitrite. In conclusion, the strain ZH-1 could effectively remove NO_3^--N and NH_4^+-N at concentrations ≤150 mg/L. So the strain ZH-1 was expected to be applied into the biological denitrification process.

Achievement of efficient nitrogen removal when treating synthetic wastewater

As shown in Fig 4, strain ZH-1 could simultaneously remove NH_4^+-N, NO_3^--N and NO_2^--N together. In this analysis, the growth curves of strain ZH-1 presented by OD_{600} were similar in the three mediums, HNM and ADM-1&2: a lag phase occurred in the first 3h, followed by the exponential phase from the 3rd to 15th h similarity, then the stationary and death phases, respectively. For NH_4^+-N, NO_3^--N and NO_2^--N removal from 85, 100.42 and 65.8 mg

L decreased to 0.15, 0.76, 1.27 /mg L after incubated for 15, 18, 15h, respectively, coupled with the change of growth curves. The average removal rate (mg/ L h) was 4.27 ± 0.22 for NH_4^+-N, 5.53 ± 0.37 for NO_3^--N and 4.30 ± 0.13 for NO_2^--N. Strain ZH-1 also presented some differences in the removal of NH_4^+-N, NO_3^--N and NO_2^--N. During heterotrophic nitrification (Fig 4 a), 5.23 mg L^{-1} of NO_3^--N was formed in the beginning because the NH_4^+-N oxidation and the NO_3^--N increased to 15.75 mg/ L after15h of shake-flask cultured but decreased to 10.59 mg/ L after 24h of incubation cultured. In this process, there was no NO_2^--N had been detected, but little amount of NH_4^+-N were detected at the end of cultured, which might be released by the dead bacterial cells. In the aerobic denitrification of the NO_3^--N as the sole source of nitrogen medium (Fig 4 b), 8.31 mg L of NO_2^--N was detected in the beginning may of be because the NO_3^--N deoxidation, and then it decreased to zero in 18 h. In the aerobic denitrification of NO_2^-N as the sole source of nitrogen medium (Fig 4 c), NO_3^--N had been detected, which was up to the maximum of 32.72 mg L at the 6th h, and decreased and remained at 19.02 mg L until the end (24th h) of incubation. Nitrate production further evidence that strain ZH-1 had the heterotrophic nitrification ability at the same time. So far, there were not many reports about nitrite of aerobic denitrification bacteria. Strain ZH-1 showed amazing ability of rapid degradation under aerobic conditions and had broad application prospects in the respect of nitrite degradation.

Although higher NH_4^+-N removal rate (28.9 mg /L·h) was reported of *A. faecalis* No. 4 (Joo *et al.* 2006), it could not remove NO_2^--N and NO_3^--N. In addition, the NH_4^+-N removal rate of strain ZH-1 (4.27 mg/ L·h) was much higher than that of *B. methylotrophicus* (2.14 mg/ L h) (Zhang *et al.* 2012). The average NO_2^--N removal rate (4.30 mg/ L h) of strain ZH-1 was much higher than other reported bacteria, such as *Pseudomonas tolaasii* Y-11 (1.72 mg/ L h) (He *et al.* 2016) and *Pseudomonas* sp. yy7 even at the moderate temperature of 25°C (Wan *et al.* 2011). Furthermore, the average NO_3^--N removal rate (5.53mg /L h) of strain ZH-1 was much higher than that of *Klebsiella pneumoniae* CF-S9 (2.2 mg/ L h) (Padhi *et al.* 2013). Above all, strain ZH-1 could be a better candidate than those reported bacteria for the simultaneous removal of ammonia, nitrate and nitrite. Therefore, strain ZH-1 had an extensive application prospect in terms of wastewater treatment.

About 20 bacteria strains which have the function of denitrification were isolated and selected from Fenhe River, found one of the strains with high nitrogen removal efficiency, and had been carried on the morphological identification and 16S-rDNA gene identification and named as *Pseudomonas stutzeri* ZH-1 strain ultimately. The optimal conditions for NH_4^+-N, NO_3^--N and NO_2^--N removal were: Citrate as carbon source, C/N 12, shaking speed ≤200 rpm, temperature 25-35°C and nitrogen concentration ≤150 mg/L. For synthetic wastewater of initial concentration of 85 mg/ L for NH_4^+-N, 100.42 mg/L for NO_3^--N, and 65.8 mg/L for

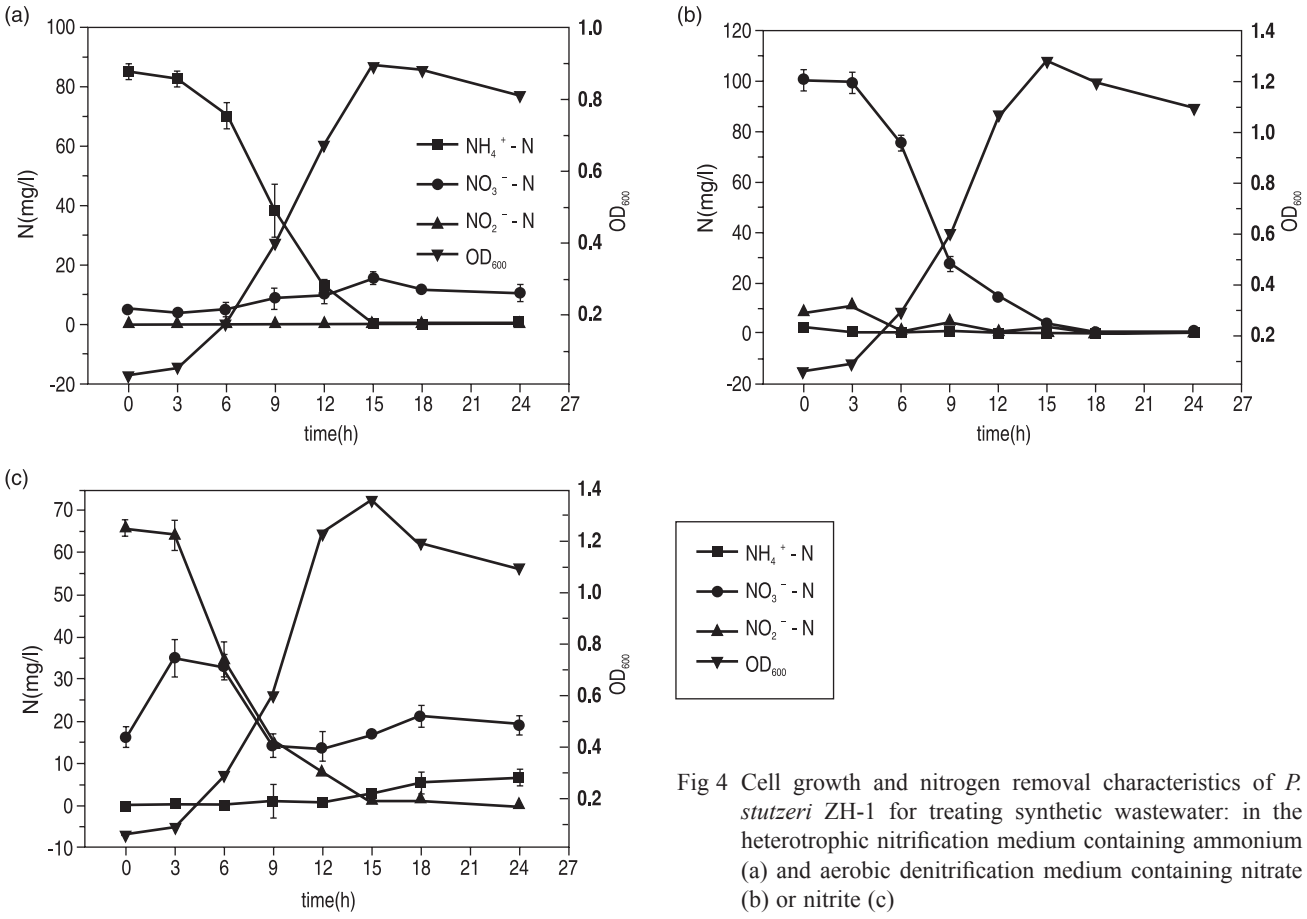

Fig 4 Cell growth and nitrogen removal characteristics of *P. stutzeri* ZH-1 for treating synthetic wastewater: in the heterotrophic nitrification medium containing ammonium (a) and aerobic denitrification medium containing nitrate (b) or nitrite (c)

NO_2^--N, respectively, the remove efficiency can be as high as 99.82%, 99.24%, 98.06%. These values are higher than most bacterial species with similar functions such as *Pseudomonas stutzeri* YG-24, *Pseudomonas stutzeri* strain ZF31, *P. putida* BD2 (Janek *et al.* 2013). These results suggested that strain ZH-1 had potential applications and can be a suitable candidate to remove nitrogen in wastewater treatment. It would be worthy to probe into the particular mechanisms of heterotrophic nitrification and aerobic denitrification bacteria with *Pseudomonas stutzeri strain* ZH-1.

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