In Situ Nitrogen Removal by a Newly Isolated Oligotrophic Aerobic Denitrifier *Zoogloea* sp. N299, in Relations with Temperature and Water Pressure in a Reservoir

S L Zhou [1], Y R Zhang [1], T L Huang [2,*], C H Zhang [2] and K K Fang [2]

[1]School of Environmental Science and Engineering, Hebei University of Science and Technology, Shijiazhuang, 050018, PR China
[2]Key Laboratory of Northwest Water Resource, Environment and Ecology, MOE, Xi'an University of Architecture and Technology, Xi'an, 710055, PR China

Corresponding author and e-mail: T L Huang, huangtinglin@xauat.edu.cn

Abstract. A series of experiments were conducted to explore the nitrogen removal characteristics of oligotrophic aerobic denitrifier *Zoogloea* sp. N299 at different temperature, water pressure, inoculums conditions. In the in situ temperature gradient experiment of hard flask (without water pressure influence), the nitrate removal rate of the surface flask system reaches 99.21 %, the middle reaches 61.1 %, the bottom reaches 57.66 %, and the corresponding TN removal rate reaches 82.42 %, 38.47 % and 27.10 %, respectively, and there is no nitrite accumulation in 96 h. While in the soft flask (with water pressure influence), The TN removal rate of the surface flask reaches 36.40 %, the middle reaches 23.74 %, the bottom reaches 21.41 %. In the different inoculum experiments, the nitrate removal rate of the hard flask and soft flask systems reaches 60.81±0.68 % and 52.4±2.31 %, respectively. From all the results, the water pressure has a disadvantage to the nitrogen removal and the higher temperature, the better performance of denitrification, and there is no difference in the different inoculum experiment. It indicated that Zoogloea sp. N299 is able to achieve effectively denitrification in situ and provide a significant reference to remediate the micro-polluted reservoir water system.

1. Introduction

With more and more nitrogen discarded into the environment, leading to many serious pollution problems, especially in source water reservoir. So the removal of the nitrogen has been a necessary and important topic. However the physical and chemical methods were always used to removal nitrogen of wastewater, and the traditional biological method was also impractical in natural waters. Conventional biological denitrification only occur under anaerobic or anoxic conditions with the reduction of sequence from nitrate to nitrogen gas. The reaction steps are inhibited by oxygen, which are impractical in natural waters, especially in reservoir.

With the discovery of the first aerobic denitrification bacteria *Thiosphaera pantotropha* strain by Robertson and Kuenen (Robertson & Kuenen 1983; Robertson *et al.* 1985)[1,2], it exhibited the possibility of the nitrogen removal from the reservoir ecosystem. The aerobic denitrification has obvious advantages: (1) the nitrification and denitrification can occur in the same treatment system

Zhou, S., Zhang, Y., Huang, T., Zhang, C. and Fang, K.
In Situ Nitrogen Removal by a Newly Isolated Oligotrophic Aerobic Denitrifier Zoogloea Sp. N299, in Relations with Temperature and Water Pressure in a Reservoir.
In *Proceedings of the International Workshop on Environmental Management, Science and Engineering (IWEMSE 2018)*, pages 621-630
ISBN: 978-989-758-344-5

(Schmidt *et al.* 2003)[3]; (2) the denitrification can produce the alkalinity to blance the acid of nitrification. There are recent reports of aerobic denitrification bacteria, such as *Thiosphaera pantotropha* (Su *et al.* 2001)[4], *Alcaligenes faecalis*, *Citrobacter diversus* (Huang & Tseng 2001)[5], *Rhodococcus* sp. (Chen *et al.* 2012)[6], *Klebsiella pneumonia* (Padhi *et al.* 2013)[7], *Microbacterium* sp. (Zhang *et al.* 2013)[8], *Paracoccus versutus* (Shi *et al.* 2013)[9], *Acinetobacter* sp.

Previous studies mainly showed the application of aerobic denitrification in wastewater treatment. For examples, Pai et al. (Pai *et al.* 1999)[10] added the aerobic denitrification bacteria (*Psychrobacter immobilis* T6, *Ochrobactrum anthropi* T23, and *Alcaligenes denitrificans* T25.) to activated sludge to treat a synthetic wastewater; Bouchez et al. (Bouchez *et al.* 2009)[11] showed that the aerobic denitrifier (*Microvirgula aerodenitrificans*) were embedded within an alginate, and alginate fragments adhered to existing flocs and were progressively colonized by the indigenous flora; Gupta et al. (Gupta & Gupta 2001)[12] showed that the alkalinity could be increased through the aerobic denitrifier in nitrification stage (*Thiosphaera pantotropha*); Ma et al. (Wang *et al.* 2007)[13] showed that biological treatment of nitrate wastewater with aerobic denitrifiers in a bioceramic reactor was successful. However, there are rarely reports of aerobic denitrification bacteria isolated from the reservoir for bioremediation of source water ecosystem. Our research team has been researching the aerobic denitrifiers for a while. The goal of the study was to determine the effectiveness of denitrification with a low C/N ratio and a high dissolved oxygen concentration, and the performance of nitrogen removal in different temperature, water pressure and inoculums *in situ*.

2. Material and methods

2.1. Microorganism

The strain was isolated from the sediment samples of Zhoucun reservoir (N34°57′, E117°40′21″) in Shandong Province, China and identified as *Zoogloea* sp. N299 (GenBank No. KP717093). This isolated strain could express periplasmic nitrate reductase which is essential for the aerobic denitrification.

2.2. The relationship between OD value and colony of the N299

In order to reflect the number of colony through the OD value (optical density) of aerobic denitrification bacteria in the medium, it is necessary to study the relationship between OD and colony number. The aerobic denitrifying bacteria N299 was precultured 24 h in 50 mL/150mL Erlenmeyer flask at 30 ℃, 120 rpm, in screening medium (SM) of pH 7.2 (in g/L: CH_3COONa 0.1; $NaNO_3$ 0.02; $K_2HPO_4 \cdot 3H_2O$ 0.02; $CaCl_2$ 0.01; $MgCl_2 \cdot 6H_2O$ 0.01). The N299 was inoculated at 2 % (v/v) into 150 mL/250 mL Erlenmeyer flask 24 h, the cell pellet was prepared by centrifuging a 10 mL sample of broth culture at 5000 rpm for 10 min and then decanted the supernatant after washing twice with distilled water. Then through adding the distilled water, we got a series of OD. The diluents were streaked on a solid screening medium (SM) of pH 7.2 (in g/L: CH_3COONa 0.1; $NaNO_3$ 0.02; $K_2HPO_4 \cdot 3H_2O$ 0.02; $CaCl_2$ 0.01; $MgCl_2 \cdot 6H_2O$ 0.01; agar 20.00) and incubated at 30 ℃ for 5 days. Prominent single colonies were harvested and the number of colonies of every OD suspension was calculated.

2.3. The temperature distribution of the reservoir

Based on the measurement the temperature of 0.5, 2.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 12.5, 15 m in 0, 24, 36, 48, 60, 72 h and 96 h, we got the temperature distribution.

2.4. The temperature gradient experiment of N299 in situ

Based on the temperature distribution of the reservoir, we designed three temperature gradient experiment, 30 ± 2 ℃ in the surface water layer (0.5 m), 18.5 ± 0.5 ℃ in the middle water layer (7.5

m), and 11.5 ± 0.5 ℃ in the bottom water layer (15 m). And we used the hard flask and soft flask to study the influence of the water pressure in the reservoir. The aerobic denitrifying bacteria N299 was precultured 24 h in 50 mL liquid medium (without agar) in 100 mL Erlenmeyer flask at 30 ℃, 120 rpm, in order to be activated. Then the N299 was inoculated at 1 ‰ (v/v) (5 mL/5L) into 5L hard flask and soft flask respectively. The nitrate, nitrite, ammonia, TN, TDN, TOC and cell optical density (OD) were measured to reflect the denitrification performance of N299.

2.5. The *different inoculums* experiment of N299 in situ

In order to study the practical inoculums of N299 to remediate the micro-polluted source water, we designed the different inoculums of N299 experiments which were located in the middle of the reservoir (7.5 m), meanwhile we used the hard flask and soft flask systems to explore the influence of the water pressure to denitrification process in the reservoir. After precultured, the N299 was inoculated in 5 L flask system. The inoculums gradient: 1 mL/5L, 2mL/5L, 5mL/5L, 10mL/5L.

2.6. Analytical methods

The optical density of the culture broth was measured at 600nm (OD_{600}) using a spectrophotometer. Nitrate, nitrite, ammonia, and TN were determined by the procedures detailed in the standard methods (Chinese 2002)[14]. Briefly, Nitrite was determined by N-(1-naphthalene) diaminoethane photometry method; Ammonium was determined by the method of Nessler's reagent spectrophotometry; TN, TDN and nitrate were measured by hydrochloric acid photometry method; TOC analysed by TOC analyser (ET1020A); Aerobic denitrifying bacteria colonies were measured by plate count; T and DO were measured by HQ30d (HACH Company, USA); The samples of nitrate, nitrite, ammonia and TDN were filtrated using a 0.45μm cellulose acetate filter for removing bacteria.

3. Results

3.1. The relationship between OD and colony of the N299

The relationship (figure 1) was showed in the following: y (lg(colony)) = 5.23 + 38.51x (OD_{600}). The correlation coefficient R^2=0.9497. Then, we can get the colonies of the medium through measuring the OD_{600}.

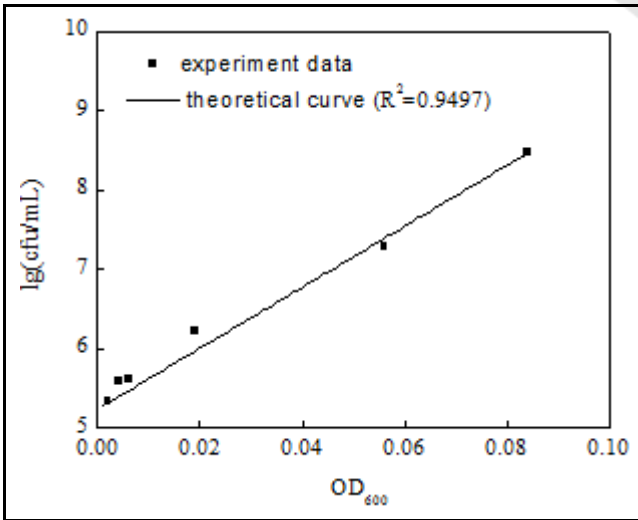

Figure 1. The relationship between OD600 and colonies' number of N299.

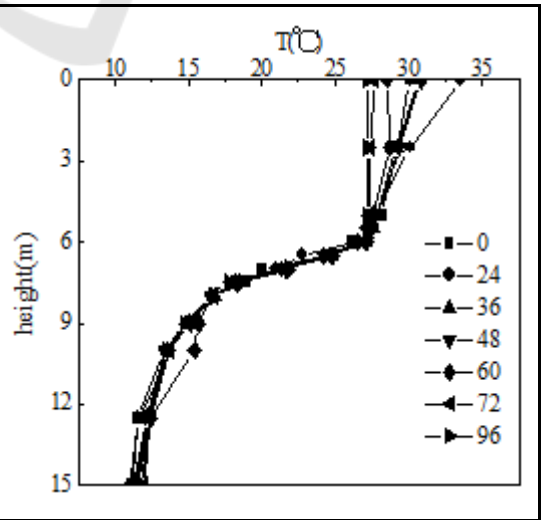

Figure 2. The temperature distribution in the reservoir.

3.2. The temperature distribution of the reservoir

In the figure 2, in 96 h the temperature distribution maintains a stable state. Based on the temperature distribution of the reservoir, we designed three temperature gradient: the surface water layer, the middle water layer, the bottom water layer. The schematic diagram of the flask experiments *in situ* was shown in figure 3. In detail, Figure3-a, the temperature gradient experiment of hard flask in situ; 3b, the temperature gradient experiment of soft flask in situ; 3c, the different inoculums of N299 in hard flask in situ; 3d, the different inoculums of N299 in soft flask in situ

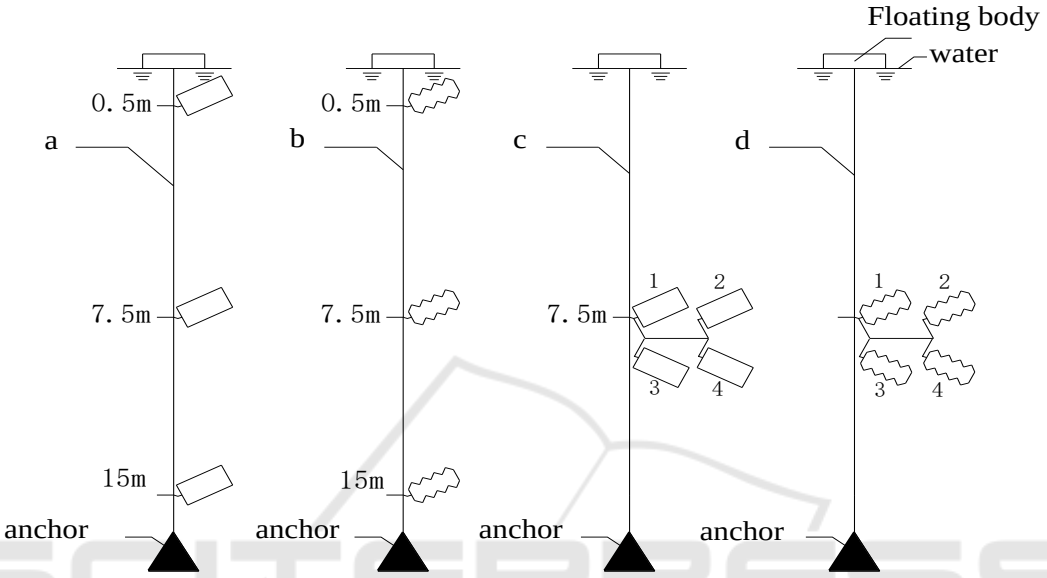

Figure 3. The schematic diagram of the flask experimental systems in situ.

3.3. The temperature gradient experiment of hard flask in situ

As shown in figure 4a, the nitrate of surface system decreased from 3.77 mg/L to 0.03 mg/L, the middle system from 3.77 mg/L to 1.47 mg/L, the bottom system from 3.77 mg/L to 1.60 mg/L, in 96 h. It is obvious that the performance of the nitrogen removal of the surface system is the best. In figure 4b and figure 4c, the nitrate removal rates of surface system reached 99.21 %, the middle reached 61.1 %, the bottom reached 57.66 %, and no nitrite accumulation in 96 h. The removal rate of nitrate correlated strongly with the growth rate of isolate N299. Because carbon is essential for cell growth and nitrate reduction processes, the optimal quantity of carbon is a key parameter in the denitrification process. With the death of N299, the ammonia started to release into the water. In figure 4d, the ammonia in surface and middle system had a light increase, but 48 h, the ammonia began to decrease which correlated strongly with the growth rate of isolate N299. As shown in figure 4e and figure 4f, the 82.42 % of TN in surface system, 38.47 % in middle system, and 27.10 % in bottom system changed into gas and achieved removal.

In the figure 4e, the C/N from 4.67 reached 6.78 in surface system, 0.93 in middle system, 0.68 in bottom system, because of the lack of the carbon source, the denitrification can't continue further. In 0 ~ 24 h, the strain was in logarithmic phase and had the highest performance of denitrification. Moreover, the temperature maintained stable and the DO was in 4 ~ 8 mg/L.

3.4. The temperature gradient experiment of soft flask in situ

As shown in figure 4g, figure 4h and figure 4i, the nitrate removal rate of surface system reached 48.98 %, the middle reached 53.45 %, the bottom reached 56.08 %, and no nitrite accumulation in 96 h. The removal rate of nitrate correlated strongly with the growth rate of isolate N299. With the death

of N299, the ammonia started to release to the water, which correlated strongly with the growth rate of isolate N299. As shown in figure 4k and figure 4l, the 36.40 % of TN in surface system, 23.74 % in middle system, and 21.41 % in bottom system changed into gas and achieved removal. Compared with the hard flask experiment, the water depth (hydrostatic pressure) was disadvantage to denitrification process. In the figure 4k, the C/N from 4.67 reached 1.15 in surface system, 0.75 in middle system, 0.82 in bottom system, because of the low C/N, the denitrification can't continue further. Moreover, the temperature maintained stable and the DO was in 4 ~ 8 mg/L.

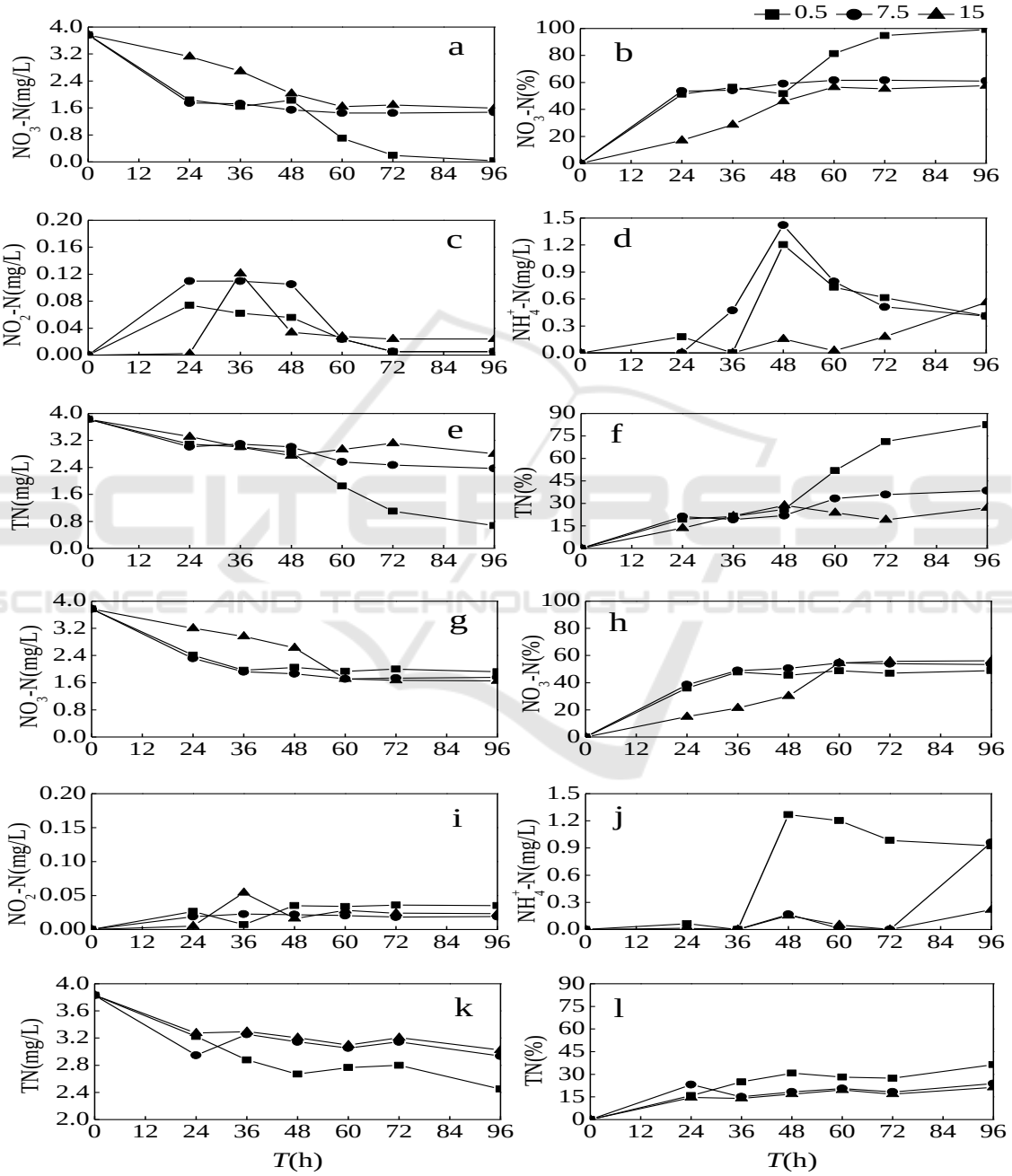

Figure 4. The temperature gradient experiment in hard flask and soft flask systems, respectively.

3.5. The different inoculums of N299 in hard flask

The different inoculums experiment chose the temperature of in the middle of the reservoir (7.5 m) to simulate the temperature of the whole reservoir. We designed four inoculums gradient: 1mL/5L system, 2mL/5L system, 5mL/5L system, 10mL/5L system.

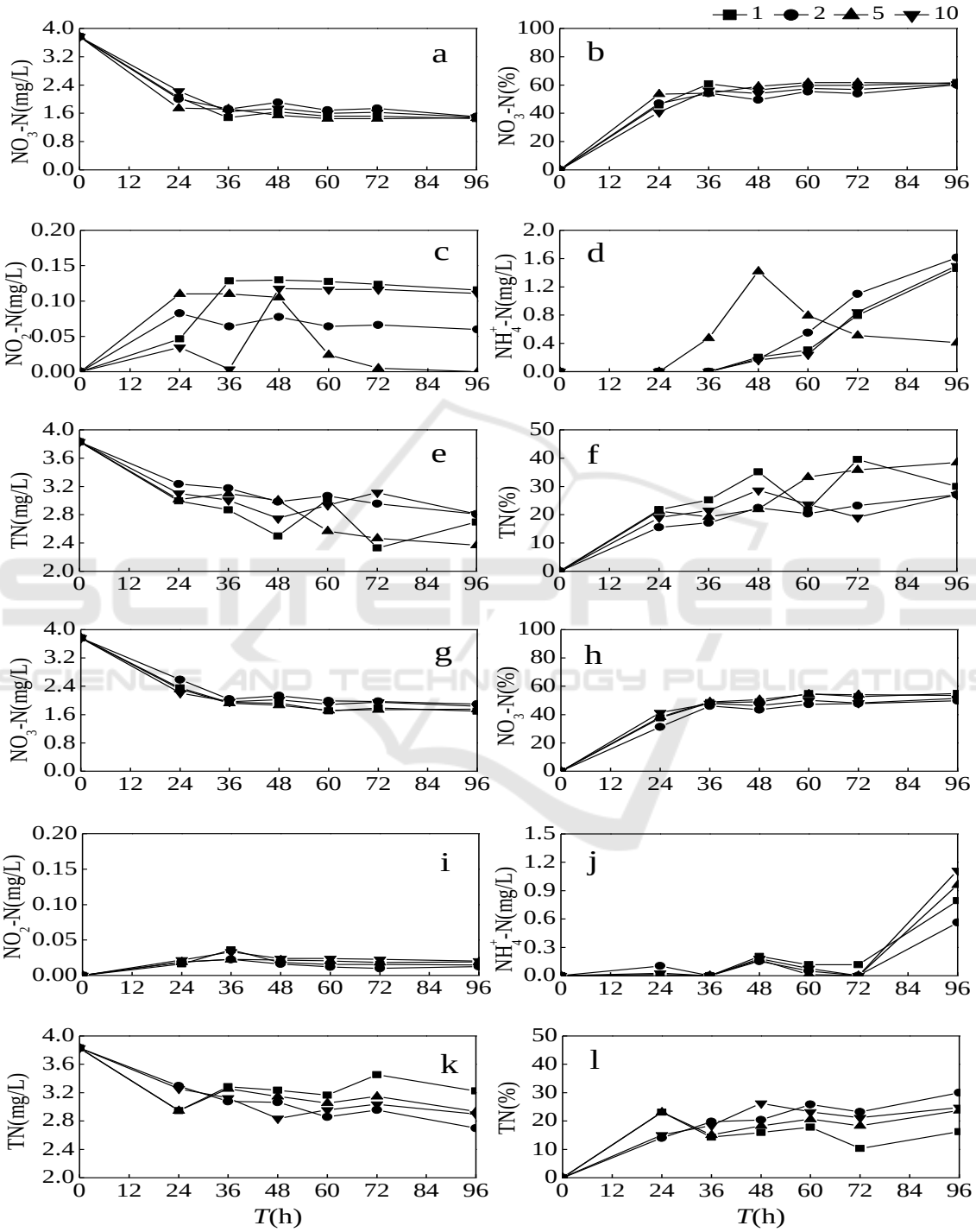

Figure 5. The different inoculums experiment in the hard flask and Soft flask system, respectively.

As shown in figure 5, the nitrate removal rate of the 1 my/5L system reached 61.60%, the 2 mL/5L reached 60.02 %, the 5 mL/5L reached 61.08 %, and the 10 mL/5L reached 60.55 %, in 96 h. And there was no nitrite accumulation in whole experiment (figure 5c).

The removal rate of nitrate correlated strongly with the growth rate of isolate N299. With the death of N299, the ammonia started to release to the water, the ammonia began to increase which correlated strongly with the growth rate of isolate N299. As shown in figure 5, the 29.94 % of TN in 1 mL/5L system, 27.10 % in 2 mL/5L, 38.47% in 5 mL/5L, and 27.10 % in 10 mL/5L were changed into gas and achieved nitrogen removal. In the figure 5e, the C/N from 4.67 reached 0.54 in 5 mL/5L system, in 36 h. Because of the lack of the carbon source, the denitrification can't continue further. In 0 ~ 24 h, the strain was in logarithmic phase and had the highest performances of denitrification. Moreover, the temperature maintained stable and the DO was in 4 ~ 8 mg/L.

3.6 The different inoculums of N299 in soft flask

As shown in figure 5g and figure 5h, the nitrate removal rate of the 1 mL/5L system reached 55.03%, the 2 mL/5L reached 49.77 %, the 5 mL/5L reached 53.45 %, and the 10 mL/5L reached 51.35 %, in 96 h. And there was no nitrite accumulation in whole experiment（figure 5i）. The removal rate of nitrate correlated strongly with the growth rate of isolate N299. With the dead of N299, the ammonia started to release to the water, in figure 6j, the ammonia began to increase which correlated strongly with the growth rate of isolate N299. As shown in figure 5k and figure 5l, the 16.24 % of TN in 1 mL/5L system, 29.94 % in 2 mL/5L, 23.74 % in 5 mL/5L, and 24.77 % in 10 mL/5L were changed into gas and achieved removal. The 2 mL/5L system owned the best denitrification ability. The water pressure had a bad effect on denitrification.

The TDN removal rate reached 42.31 % in 1 mL/5L, 42.05 % in 2 mL/5L, 35.55 % in 5 mL/5L, and 32.43 % in 10 mL/5L, in 96 h. In the figure 5k, the C/N from 4.67 reached 0.93 in 1 mL/5L system, 0.74 in 2 mL/5L system, 0.99 in 5 mL/5L, 0.92 in 10 mL/5L, in 36 h. Because of the lack of the carbon source, the denitrification can't continue further.

4. Discussion

Based on the temperature distribution of the reservoir, we designed three temperature gradient experiments. Denitrification process is sensitive to temperature, and denitrification rate doubles with every 4 ℃ increase (Zaitsev *et al.* 2008)[15]. As shown in Figure 4, temperature had a pronounced effect on nitrogen removal by isolate N299. The nitrate removal percentage of hard flask experiment increased from 57.66 % at 11.5 ± 0.5 ℃ to 99.21 % at 30±2 ℃ in 96 h. The TN removal percentage of hard flask experiment increased from 27.10 % at 11.5 ± 0.5 ℃ to 38.47 % at 18.5 ± 0.5 ℃ to 82.42 % at 30 ± 2 ℃ in 96 h. Meanwhile, the nitrogen removal of hard flask was consistent with the soft flask experiment. The TN removal percentage of soft flask experiment increased from 21.41 % at 11.5 ± 0.5 ℃ to 23.74 % at 18.5 ± 0.5 ℃ to 36.40 % at 30 ± 2 ℃ in 96 h. A remarkable decrease in nitrate and TN removal were found when the temperature increased from 11.5 ± 0.5 ℃ to 30 ± 2 ℃. The optimal temperature for nitrogen removal was 30 ± 2 ℃ for N299, and the nitrogen removal rate was higher than that of other bacteria capable of aerobic denitrification (Wei *et al.* 2010; Wei *et al.* 2012)[16,17]. Moreover, the excellent adaptability to low temperature presented by strain N299 is beneficial for nitrogen removal from water in cold regions.

In the temperature gradient experiment, in 7.5 m, the TN removal rate of the hard flask reached 38.47 %, however the soft flask system reached 23.74 %; in 15 m, the TN removal rate of the hard flask reached 27.10 %, however the soft flask system reached 21.41 %. Table 1 showed a clear relationship between environment variables and uncovered nitrogen in hard and soft flask experiment systems, obviously. The bivariate analysis has indicated that uncovered nitrogen (gaseous N removal) significantly correlated with the height (R=-0.9468) and (R=-0.9291), and temperature (R=0.9813) and (R=0.9702) in hard and soft flask systems, respectively. Obviously, the hard flask system had a

better nitrogen removal performance than the soft flask system, which indicated that the water pressure had a disadvantage to denitrification process. As we all known, the Carbon source, DO concentration, C/N, and Temperature were the critical factors of denitrification, while the strains, reactors and other unknown conditions also had some effect on the nitrogen removal (Tanner *et al.* 1999)[18]. The previous studies (Bartlett 2002; Picard & Daniel 2013)[19,20] showed that the high static water pressure could influence on microbial growth and metabolic processes, which were consistent with our results. There were some combined factors (DO, pH, and static water pressure) in this *in situ* flask experiment, therefore, we only conducted a qualitative analysis between static water pressure and denitrification. In order to explore the mechanism obviously, we would study further in the future. In the different inoculums experiment, the nitrogen removal rate of the hard flask system was better than the soft flask system. All in all, the nitrogen removal rate of the hard flask was higher than the soft flask in the same water layer. The water pressure had a bad influence in the nitrogen removal.

Table 1. Correlation analysis of environment variables and uncovered nitrogen in hard and soft flask experiment systems.

System	Parameters	Depth	Temperature	DO	OD	TN R.E.[a]
Hard flask experiment system	Depth	1				
	Temperature	-0.9910	1			
	DO	0.6820	-0.7737	1		
	OD	-0.4096	0.5280	-0.9465	1	
	TN R.E.	-0.9468	0.9813	-0.8811	0.6815	1
Soft flask experiment system	Depth					
	Temperature	-0.9910	1			
	DO	0.7595	-0.8397	1		
	OD	0.7107	-0.6103	0.0822	1	
	TN R.E.	-0.9291	0.9702	-0.9463	-0.4001	1

[a] TN R.E., mean TN removal rate; uncovered nitrogen means gaseous nitrogen.

It is known that insufficient carbon supply impairs both microbial growth and electron donor for denitrification (Lin *et al.* 2010; Zheng *et al.* 2012)[21,22]. Xu et al. (Zhu *et al.* 2012)[23] showed that an aerobic denitrifier (*Pseudomonas mendocina* 3-7) could exhibit the aerobic denitrification characteristic under the low substrate level (TOC, 48 mg/L and nitrate 4 mg/L), the removal efficiencies of nitrate and TN were 31.7 % and 45.0 %, at 30 ℃ with a shaking speed of 150 rpm, respectively. Zhao et al. (Zhao *et al.* 2010)[24] pointed out that an aerobic denitrifier (*Acinetobacter calcoaceticus* HNR) ,isolated from a Membrane Bioreactor (MBR), could removal 40.2% of ammonia at 30 ℃ with a shaking speed of 120 rpm. Yang et al. (Yang *et al.* 2011)[25] found out an aerobic denitrifier (*Bacillus subtilis* A1) from ammonium-rich wastewater *in situ*, which could removal TN stabilized at approximately 81.3% at 28 ℃ with a C/N of 6. However, in this study, the removal efficiencies of nitrate and TN were 99.21 % and 82.42 %, at 30 ± 2 ℃, and inoculums had a slight effect on nitrogen removal, which is consistent with our previous studies. Hence, fewer requirements for C/N ratio and inoculum by strain N299 would be favourable for the treatment of oligotrophic source water.

5. Conclusions

The OD of the strain N299 and the colony of the strain N299 had a good linear relationship. In the temperature gradient experiment, the nitrogen removal rate increased significantly, and the strain N299 showed a nice denitrification from 11.5 ± 0.5 ℃ to 30 ± 2 ℃. Based on the hard flask experiment and soft flask experiment, this study concluded the water pressure was not advantage to the nitrogen removal. However, their removal rates did not significantly increase if the inoculum ranges from 1 mL/5L to 10 mL/5L. Considering the practical requirements of biological inoculation, the low inoculum period had practical significance

Acknowledgment

This study was funded by the National Science and Technology Pillar Program (No. 2012BAC04B02). Specially thanks to the anonymous reviewers for their valuable remarks on the manuscript.

References

[1] Robertson L A and Kuenen J G 1983 *Thiosphaera pantotropha* gen.nov.sp.nov.a Facultatively Anaerobic Facultatively Autotrophic Sulphur Bacterium *Journal of General Microbiology* 129 2847-55

[2] Robertson L A, Kuenen J G and Kleijntjens R 1985 Aerobic denitrification and heterotrophic nitrification by *Thiosphaera pantotropha Antonie van Leeuwenhoek* 51, 445

[3] Schmidt I, Sliekers O, Schmid M, Bock E, Fuerst J, Kuenen J. G, Jetten M S M and Strous M 2003 New concepts of microbial treatment processes for the nitrogen removal in wastewater. *FEMS Microbiology Reviews* 27(4) 481-92

[4] Su J J, Liu B Y and Liu C Y 2001 Comparison of aerobic denitrification under high oxygen atmosphere by *Thiosphaera pantotropha* ATCC 35512 and Pseudomonas stutzeri SU2 newly isolated from the activated sludge of a piggery wastewater treatment system *Journal of Applied Microbiology* 90(3) 457-62

[5] Huang H K and Tseng S K 2001 Nitrate reduction by *Citrobacter diversus* under aerobic environment *Applied microbiology and biotechnology* 55(1),90-4

[6] Chen P Z, Li J, Li Q X, Wang Y C, Li S P, Ren T Z and Wang L G 2012 Simultaneous heterotrophic nitrification and aerobic denitrification by bacterium Rhodococcus sp. CPZ24. *Bioresour Technol* 116,266-70

[7] Padhi S K, Tripathy S, Sen R, Mahapatra A S, Mohanty S and Maiti N K 2013 Characterisation of heterotrophic nitrifying and aerobic denitrifying *Klebsiella* pneumoniae CF-S9 strain for bioremediation of wastewater. *International Biodeterioration & Biodegradation* 78, 67-73

[8] Zhang D, Li W, Huang X, Qin W and Liu M 2013 Removal of ammonium in surface water at low temperature by a newly isolated *Microbacterium* sp. strain SFA13 *Bioresource technology* 137 147-52

[9] Shi Z, Zhang Y, Zhou J T, Chen M X and Wang X J 2013 Biological removal of nitrate and ammonium under aerobic atmosphere by *Paracoccus versutus* LYM. *Bioresource technology* 148 144-8

[10] Pai S L, Chong N M and Chen C H 1999 Potential applications of aerobic denitrifying bacteria as bioagents in wastewater treatment. *Bioresource technology* 68(2) 179-85

[11] Bouchez T, Patureau D, Delgenès J P and Moletta R 2009 Successful bacterial

incorporation into activated sludge flocs using alginate *Bioresource technology* 100(2) 1031-2

[12] Gupta A B and Gupta S K 2001 Simultaneous carbon and nitrogen removal from high strength domestic wastewater in an aerobic RBC biofilm *Water Research* 35(7) 1714-22

[13] Wang H Y, Ma F, Su J F, Zuo W, Zhang J and Zhang X X 2007 Aerobic denitrification of nitrate wastewater and changes of microbial community structure in a bio-ceramic reactor *Journal of Environmental Sciences* 28(12), 2856-60

[14] Chinese N 2002 Water and wastewater monitoring methods *In, Chinese Environmental Science Publishing House* p. 806

[15] Zaitsev G, Mett änen T and Langwaldt J 2008 Removal of ammonium and nitrate from cold inorganic mine water by fixed-bed biofilm reactors. Minerals Engineering 21(1) 10-5

[16] Wei W, Huang T L and Li N 2012 Denitrification characteristics of in-situ biological inoculation under conditions of low temperature and poor nutrient *Water Technology (in Chinese)* 6, 8-12

[17] Wei W, Huang T L, Su J F, Wang C Y, Huang Z and Li N 2010 Isolation and identification of an oligotrophic and aerobic denitrification and its denitrification characteristics. *Ecol. Environ. Sci.* 19 2166-71

[18] Tanner C C, D'Eugenio J, McBride G B, Sukias J P and Thompson K 1999 Effect of water level fluctuation on nitrogen removal from constructed wetland mesocosms *Ecological Engineering* 12(1) 67-92

[19] Bartlett D H 2002 Pressure effects on in vivo microbial processes. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology* 1595(1) 367-81

[20] Picard A. and Daniel I. (2013). Pressure as an environmental parameter for microbial life—A review *Biophysical chemistry* 183, 30-41

[21] Lin Y, Kong H, Wu D, Li C, Wang R. and Tanaka S 2010 Physiological and molecular biological characteristics of heterotrophic ammonia oxidation by *Bacillus* sp. LY. *World Journal of Microbiology and Biotechnology* 26(9) 1605-12

[22] Zheng H Y, Liu Y, Gao X Y, Ai G M, Miao L L and Liu Z P 2012 Characterization of a marine origin aerobic nitrifying–denitrifying bacterium *Journal of Bioscience and Bioengineering* 114(1) 33-7

[23] Zhu L, Ding W, Feng L J, Dai X and Xu X Y 2012 Characteristics of an aerobic denitrifier that utilizes ammonium and nitrate simultaneously under the oligotrophic niche. *Environmental Science and Pollution Research* 19(8), 3185-91

[24] Zhao B, He Y. L, Hughes J and Zhang X F 2010 Heterotrophic nitrogen removal by a newly isolated *Acinetobacter calcoaceticus* HNR *Bioresource technology* 101(14) 5194-200

[25] Yang X P, Wang S M, Zhang D W and Zhou L X 2011 Isolation and nitrogen removal characteristics of an aerobic heterotrophic nitrifying–denitrifying bacterium, *Bacillus subtilis* A1 *Bioresource technology* 102(2) 854-62