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Heterotrophic nitrification–aerobic denitrification by novel isolated bacteria

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Abstract Three novel strains capable of heterotrophic nitrification–aerobic denitrification were isolated from the landfill leachate treatment system. Based on their phenotypic and phylogenetic characteristics, the isolates were identified as *Agrobacterium* sp. LAD9, *Achromobacter* sp. GAD3 and *Comamonas* sp. GAD4, respectively. Batch tests were carried out to evaluate the growth and the ammonia removal patterns. The maximum growth rates as determined from the growth curve were 0.286, 0.228, and 0.433 h^{-1} for LAD9, GAD3 and GAD4, respectively. The maximum aerobic nitrification–denitrification rate was achieved by the strain GAD4 of 0.381 mmol/l h, followed by LAD9 of 0.374 mmol/l h and GAD3 of 0.346 mmol/l h. Moreover, hydroxylamine oxidase and periplasmic nitrate reductase were successfully expressed in all the isolates. The relationship between the enzyme activities and the aerobic nitrification–denitrification rates revealed that hydroxylamine oxidation may be the rate-limiting step in the heterotrophic nitrification–aerobic denitrification process. The study results are of great significance to the wastewater treatment systems where simultaneous removal of carbon and nitrogen is desired.

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Introduction

Conventional ammonium removal consists of two steps: nitrification by autotrophs under aerobic conditions and denitrification by heterotrophs under anaerobic conditions. However, this type of system is difficult to operate due to the low rate of nitrification and the complexity of separating nitrification and denitrification reactors. Recently, bacteria capable of combined heterotrophic nitrification and aerobic denitrification has been investigated as potential microorganisms in biological nitrogen removal systems [2, 4, 9]. These microorganisms, due to their high growth rate and ability to convert ammonium (NH_4^+) to N_2 aerobically, have many advantages as applied for the removal of nitrogen: (1) procedural simplicity, where nitrification and denitrification can take place simultaneously; (2) less acclimation problems; (3) lesser buffer quantity needed because alkalinity generated during denitrification can partly compensate for the alkalinity consumption in nitrification [3]. To date, certain groups of heterotrophic nitrification–aerobic denitrification bacteria, such as *Thiosphaera pantotropha*, *Alcaligenes faecalis*, *Bacillus* sp., and *Providencia rettgeri* have been isolated from soils and wastewater treatment systems [1, 11, 19, 29].

On the basis of the typical strain *Thiosphaera pantotropha*, a speculated model of electron transfer [27] and biochemical mechanisms [3] have been proposed to explain the coupling of heterotrophic nitrification and aerobic denitrification in an organism. In heterotrophic nitrification, the pathway of heterotrophs resembled that of

autotrophs [28]. Ammonia is oxidized to hydroxylamine by ammonia monooxygenase (AMO), and then hydroxylamine is oxidized to nitrite by hydroxylamine oxidase (HAO), which is a key reaction [14, 16]. In aerobic denitrification, co-respiration is an important mechanism, where oxygen and nitrate are simultaneously used as electron acceptors. Constitutive denitrifying enzymes are found in *Thiosphaera pantotropha* [20].

However, in contrast to *Thiosphaera pantotropha*, nitrate cannot be used and nitrate reductase is absent in *Alcaligenes faecalis* [26]. It is clear that there are many differences between bacteria with the ability of combined nitrification and denitrification [13]. Hitherto, it is still difficult to generalize the biochemical mechanisms due to the limitation of the number of tested species. Thus, further investigation on a broader range of species is necessary.

The present study aimed to isolate novel heterotrophic bacteria capable of heterotrophic nitrification–aerobic denitrification from the landfill leachate treatment system. Particular attention was paid to revealing the possible biochemical mechanisms of the isolates. This study is of particular importance to efficient domestic wastewater treatment where simultaneous removal of nitrogen and organics is desired.

Materials and methods

Media and isolation

Bacteria were isolated from the bioreactor treating landfill leachate according to the modified Takaya method [24]. Activate sludge (5 ml) was transferred to 200 ml of artificial wastewater medium in 500-ml Erlenmeyer flasks and incubated in a rotary shaker (120 rpm) at 30°C for 3 days. Fresh artificial wastewater medium was sub-inoculated with 10 ml of the culture and incubated at the same conditions. These procedures were repeated three times. Resultant bacterial suspension was streaked on the BTB medium plates at 30°C for 2 or 3 days. Resulting blue colonies were isolated and purified by serially streak plating onto the BTB medium. The composition of Bromothymol Blue (BTB) medium was as follows (per liter of distilled water): 1.0 g L-asparagine; 1.0 g KNO_3; 1.0 g KH_2PO_4; 0.06 g $FeSO_4{\cdot}7H_2O$; 0.2 g $CaCl_2{\cdot}2H_2O$; 1.0 g $MgSO_4{\cdot}7H_2O$; 8.5 g sodium succinate; 1 ml BTB (1% in ethanol); 16–18 g agar; pH 7.0–7.3.

Synthetic mineral medium was used to determine the capabilities of heterotrophic nitrification and aerobic denitrification of the isolates. The composition of medium (A) was as follows (per liter of distilled water): 1.0 g KNO_3, 8.5 g sodium succinate, 1.0 g KH_2PO_4, 0.06 g $FeSO_4{\cdot}7H_2O$, 0.2 g $CaCl_2{\cdot}2H_2O$, 1.0 g $MgSO_4{\cdot}7H_2O$. Medium (B) contained (per liter of distilled water): 0.28 g NH_4Cl, 4.5 g sodium succinate, 1.0 g KH_2PO_4, 0.06 g $FeSO_4{\cdot}7H_2O$, 0.2 g $CaCl_2{\cdot}2H_2O$, 1.0 g $MgSO_4{\cdot}7H_2O$. NH_4Cl or KNO_3 were both sterilized separately and added as needed. Initial pH was set at 7.0 ± 0.2.

Identification of the isolates

Total bacterial DNA was extracted with a genomic DNA extraction kit (TianGen, China) following the manufacturer's instructions. The genes encoding 16S rRNA were amplified from the extracted genomic DNA by PCR using universal primers 27F and 1492R [5]. PCR reaction mixture (50 μl) was composed of 1 μl of DNA template, 2 μl dNTP (10 mmol/l), 5 μl 10 × PCR buffer, 2.5 U *Taq* DNA polymerase, 1 μl of each primer (20 μmol/l) and sterile water to volume. PCR cycling was carried out in a thermal cycler (Bio-Rad, USA) under the following conditions: an initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 40 s and enlongation at 72°C for 1 min; cycling was completed by a final enlongation step of 72°C for 10 min. The PCR products were then sequenced by Sangon Co. Ltd. (Shanghai, China). The sequences and their related references retrieved from GenBank were aligned using Bio-Edit. A phylogenetic tree was constructed by the neighbor-joining method with 500 bootstrap replicates using the Kimura 2-parameters model implemented by the program MEGA 3.1.

Measurement of aerobic nitrification–denitrification rates

To determine the aerobic nitrification–denitrification capabilities of the isolates, the strains were inoculated respectively in 1 l Luria–Bertani medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.04% NH_4Cl) for 24 h. Enriched cultures were harvested by centrifugation at 6,000 rpm for 10 min and washed twice with sterile distilled water. The pellets were resuspended in 50 ml medium (B) for incubation.

Tests were then carried out in 250-ml Erlenmeyer flasks. Each flask was filled with 95 ml of medium (B) and 5 ml of enriched culture with OD value around 1.0. These flasks were shaken in a rotary culture (150 rpm) at 30°C to maintain a homogenous culture and to allow better gas exchange. Flasks without inoculation served as sterility controls. Logistic model (Eq. 1) was used to estimate the maximum growth rate (μ_{max}) from the growth curve, plotted as OD as a function of time. The maximum growth rate was at half the carrying capacity, where $N = K/2$.

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right) \quad (1)$$

where N was the biomass; r was the instantaneous growth rate; K was the carrying capacity.

The rates of the aerobic nitrification–denitrification (R_{AND}) were calculated by the following Eq. (2):

$$R_{AND} = \frac{NH^{+}_{4\,oxidized} - NO^{-}_{x\,produced}}{t} \quad (2)$$

where $NO^{-}_{x\,produced}$ was the sum of nitrite and nitrate remaining at time t, in mmol/l; $NH^{+}_{4\,oxidized}$was calculated as the total ammonia consumption at time t, in mmol/l; t was the total reaction time.

Analytical methods

Cell growth was monitored by measuring the absorbance at 600 nm using a SmartSpec™ Plus spectrophotometer (Bio-Rad, USA). The liquid samples were centrifuged at 10,000 rpm for 5 min, and then the supernatants were diluted as required for different analyses. Nitrite was determined by *N*-(1-naphthyl)-ethylene diamine spectrophotometry and nitrate was determined by ultraviolet spectrophothometry. The amount of total nitrogen (TN) was calculated by adding concentrations of ammonia, nitrate, and nitrite.

Hydroxylamine oxidation and nitrate reduction were both measured in cell-free periplasms extracted by the osmotic shock method [22]. Protein concentration in the periplasms was measured by the Folin-phenol reagent [12]. Nitrate reduction was assayed using the following reaction mixture: 50 mM potassium phosphate (pH 7.5), 10 μM KNO_3, 200-μl cell periplasms and distilled water to make a final volume of 1 ml. Formation of nitrite from nitrate in the reaction mixture was taken as a measure of nitrate reductase (NAP) activity [10]. Hydroxylamine oxidase (HAO) activity was determined by the appearance of nitrite from NH_2OH in the reaction mixture, which contained (ml^{-1}): 10 mM Tris/HCl pH 8.0, 1 mM NH_2OH and 200-μl cell periplasms.

The collection numbers were CGMCC No. 2962 for LAD9, CGMCC No. 2964 for GAD3 and CGMCC No. 2963 for GAD4.

Results

Isolation and identification

In a typical experiment, 15 strains positive in BTB plate assays were screened. These strains were screened further by culturing them in flasks containing medium (A) under aerobic conditions. Denitrification was periodically monitored by measuring nitrite and nitrate concentrations in the medium. The strains that demonstrated more than 85% TN removal efficiency were selected and named as LAD9, GAD3, and GAD4, respectively. To assess the capabilities of ammonia oxidation by the three isolates, they were then cultured in the medium (B), and ammonium removal patterns were determined. After 24 h of the incubation under standard conditions, 100% of ammonium was removed by the three isolates. With the decrease of ammonia, nitrite or nitrate accumulation was very low. It was suggested that the three isolates were capable of heterotrophic nitrification–aerobic denitrification.

To identify the isolates, phenotypic observation and genotypic analysis were conducted. Phenotype showed that LAD9, GAD3, and GAD4 were all Gram-negative, rod-shaped species with sizes of (0.3–1.5) μm × (0.6–1.0) μm, (0.5–1.2) μm × (0.5–2.6) μm and (0.5–1.2) μm × (0.5–2.6) μm, respectively. The nucleotide sequences of their 16S rRNA genes alignment (Table 1) and a phylogenetic analysis (Fig. 1) revealed that LAD9 was a member of the genus *Agrobacterium* in the α subclass of the class *Proteobacteria*, while GAD3 and GAD4 were members of the genus *Achromobacter* and *Comamonas*, both in the β subclass of the class *Proteobacteria*.

Aerobic nitrification–denitrification by the isolates

Aerobic nitrification–denitrification capabilities of the isolates were assessed in the medium (B) with NH_4Cl as the sole nitrogen source and sodium succinate as the carbon source. Growth curves of the isolates were established by measurement of the OD value at 600 nm.

The growth of the isolates in batch cultures all resulted in sigmoidal curves (Fig. 2a–c), where three distinguished phases were found. The maximum growth rates (μ_{max}) as determined from the growth curve were 0.32, 0.31, and 0.38 h^{-1} for LAD9, GAD3 and GAD4, respectively.

Furthermore, as depicted in Fig. 2, an excellent ammonia nitrogen removal of about 95% was achieved by all the isolates in 12 h. The corresponding nitrite release during the ammonia degradation was extremely low. However, the evolution of nitrate demonstrated different patterns. In LAD9, nitrate accumulation was not detected and the final

Table 1 16S rRNA sequence similarities between three strains and related bacteria

Strain	GenBank No.	Affiliation	Similarity (%)
LAD9	FJ639330	*Agrobacterium* sp.	99
GAD3	FJ639331	*Achromobacter* sp.	97
GAD4	FJ639332	*Comamonas* sp.	99

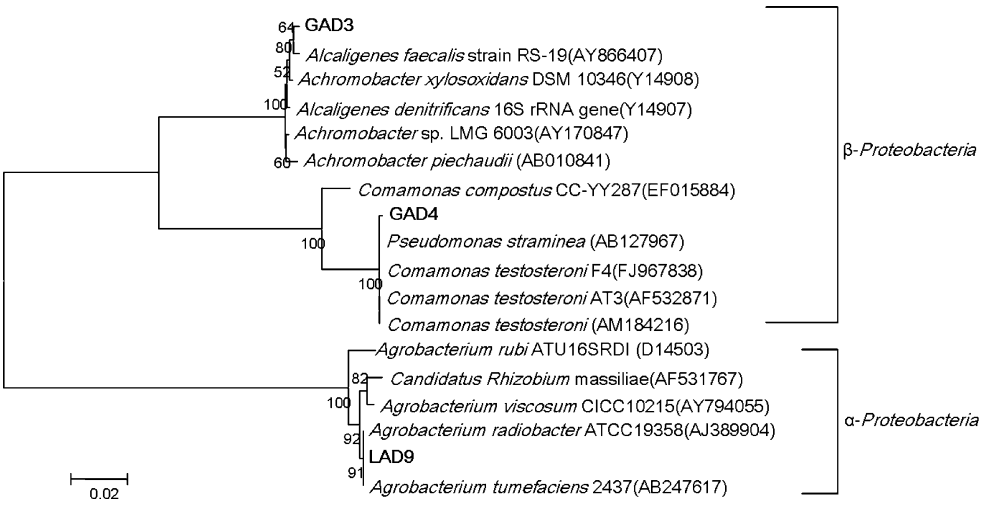

Fig. 1 Phylogenetic tree based on 16S rDNA sequences of the isolates and related standard bacteria (*the numbers* at the *forks* indicate the bootstrap values in percentage. *Bar* indicates the nucleotide difference per sequence position. The accession numbers of the sequences are given in *parentheses*)

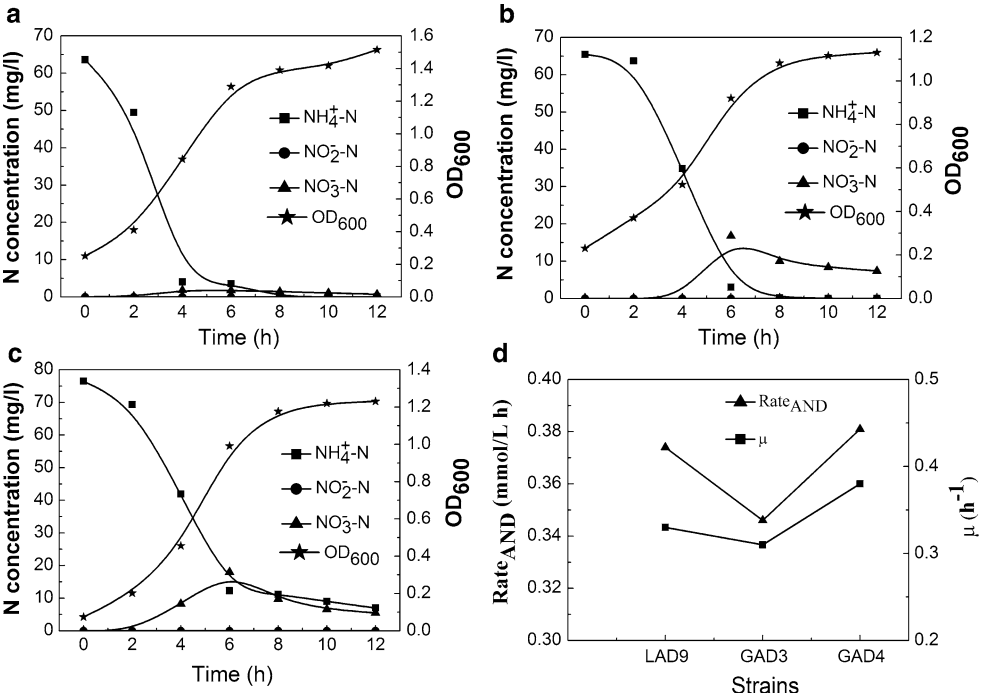

Fig. 2 Temporal variation of nitrogen compounds concentration of LAD9 (**a**), GAD3 (**b**), GAD4 (**c**), R_{AND} and specific growth rate of the three strains (**d**)

concentration was as low as 0.71 mg/l; while in GAD3 and GAD4, the appearance of nitrate was obvious, where nitrate concentration gradually reached the peak at 6 h, and decreased to a certain level at the end. In addition, a distinct lag phase was found in the strain GAD3 in the initial 2 h for ammonia degradation, but its cell growth increased two-fold when analyzing the growth profile (Fig. 2b), which was absent in the strains LAD9 and GAD4. This may be attributed to the proliferation of GAD3 prior to the nitrification process. The occurrence of the lag phase is also found in aerobic denitrifiers [6]. The variance in metabolism by different subclass of *proteobacteria* may be the possible reason for it and the proliferation by assimilation may take place before the catabolism process.

R_{AND} (calculated by Eq. 1) was used to determine the capabilities of aerobic nitrification–denitrification by the isolates. The results showed that strain GAD4 exhibited the highest R_{AND} (0.381 mmol l^{-1} h^{-1}), while GAD3 performed the lowest, with the R_{AND} of 0.346 mmol l^{-1} h^{-1}. In the case of LAD9, R_{AND} was 0.374 mmol l^{-1} h^{-1}. The order of R_{AND} was the same as it of μ_{max}, indicating better removal rate correlated well with the growth rate (Fig. 2d).

Enzyme activities

To identify the possible pathway of the combined nitrification and denitrification, HAO and NAP activities were tested in cell periplasms as they have been suggested to be

Table 2 Specific HAO and NAP activities of the three strains

Strain	Specific HAO activity (U/g)	Specific NAP activity (U/g)
LAD9	0.094 ± 0.008	0.094 ± 0.012
GAD3	0.031 ± 0.006	0.220 ± 0.088
GAD4	0.178 ± 0.003	0.754 ± 0.259

crucial enzymes involving in this process. The results showed that they were both successfully expressed in all the isolates (Table 2). Maximum activity of HAO was found in GAD4 with a specific activity of 0.178 U/g protein, followed by 0.094 and 0.031 U activities in the case of LAD9 and GAD3. GAD4 also exhibited the highest NAP activity. The corresponding activity was 0.754 U/g protein. The NAP activities of GAD3 and LAD9 were 0.094 and 0.220 U, respectively. It was clear that the order of HAO activity was different from it of NAP activity.

Discussion

Three heterotrophic nitrification–aerobic denitrification strains were isolated from the landfill leachate treatment system. The μ_{max} of the isolates are between 0.3 and 0.4 h^{-1}, which is five to ten times than that of the autotrophic bacteria (the μ_{max} of *Nitrosomonas europaea* is about 0.03–0.05 h^{-1}) [20]. For heterotrophs, Kim reported that the specific growth rates of *Bacillus* spp. strains were in the range of 0.43–0.55 h^{-1} [11]; the maximum growth rate of *T. pantotropha* was about 0.25–0.45 under different growth conditions [20]; In *A. faecalis* No. 4, a value of 0.2 h^{-1} was addressed [7]. It is shown that the μ_{max} in our study is comparable with the bacteria reported in the literature.

For the C/N ratio greatly affects the simultaneous occurrence of nitrification and denitrification, the aerobic nitrification–denitrification rates in our isolates were compared to previously published data at similar C/N ratios (Table 3). At the same C/N ratio of 10, the aerobic rate of GAD4 is somewhat higher than that of *A. faecalis* No. 4, while the rates of three isolates are about five times higher than that of *A. faecalis* OKK17 and two times higher than that of *Providencia rettgeri*. It is obvious that the isolated strains in our study demonstrate comparative capabilities of heterotrophic nitrification–aerobic denitrification. In view of higher growth rates and prominent abilities of conversion of ammonia to nitrogen gas, the strains would be of great potential in practical wastewater treatment.

Moreover, the aerobic nitrification–denitrification by the isolates leads to little accumulation of nitrate and even less nitrite during the ammonia degradation, which is coincident with the results of Robertson [21] and Patureau [17]. However, accumulation of nitrite instead of nitrate was found in *Diaphorobacter* sp. [10]. It is possible that the denitrification enzymes ceased to function before those of heterotrophic nitrification, and the processes would no longer be in balance, resulting in an accumulation of nitrite [26].

Further investigations have shown that ammonia, nitrite, and nitrate could all be used by the isolates under aerobic conditions (data not shown) and both HAO and NAP were successfully expressed in the cell periplasms in our study. Similar results were obtained with *Thiosphaera pantotropha* [19], *Bacillus* sp. [6], and *Pseudomonas* sp.[23]. It is speculated that these bacteria all exhibit a fully nitrification and denitrification pathway (NH_4^+–NH_2OH–NO_2^-–N_2O–N_2). However, in contrast, neither nitrite nor nitrate was utilized as electron accepters by *Alcaligenes faecalis* and *Acinetobacter calcoaceticus* [30]; meanwhile, the activity of nitrite or nitrate reductase was not detected in them [8]. As a result, denitrification in these bacteria is via hydroxylamine rather than nitrite or nitrate (NH_4^+–NH_2OH–N_2O–N_2). These results suggest that there may be two main pathways involved in the combined nitrification and denitrification process. The difference between them is concentrated on the aerobic denitrification route [18]. One is through nitrite or nitrate, while the other is through hydroxylamine.

Table 3 Aerobic nitrification–denitrification rates of the isolates compared with published results

Organism	C/N	Activity	Reference
Agrobacterium LAD9	10	1.66 mmol h^{-1} g^{-1} protein (0.374 mmol l^{-1} h^{-1})	This study
Achromobacter GAD3	10	1.38 mmol h^{-1} g^{-1} protein (0.346 mmol l^{-1} h^{-1})	This study
Comamonas GAD4	10	1.91 mmol h^{-1} g^{-1} protein (0.381 mmol l^{-1} h^{-1})	This study
T. pantotropha	6–7	0.59 mmol h^{-1} g^{-1} dry wt	[20]
A. faecalis OKK17	10	0.27 mmol h^{-1} g^{-1} protein	[15]
A.faecalis No. 4	10	1.8 mmol h^{-1} g^{-1} protein	[7]
Pseudomonas sp.	12	0.034 mmol l^{-1} h^{-1}	[23]
Diaphorobacter sp.	8	0.21 mmol l^{-1} h^{-1}	[10]
Providencia rettgeri YL	10	0.179 mmol l^{-1} h^{-1}	[25]

Besides, comparing the HAO activity to the R_{AND}, we find that the higher the HAO activity, the faster the reaction rate. However, the NAP activity and the R_{AND} are uncorrelated, indicating that nitrate reduction is not limiting in this process. In conventional ammonia removal, nitrification occurs less efficiently and the nitrification rate is 10–100 times lower than the denitrification rate, indicating that nitrification is the rate-limiting step in the autotrophic ammonium removal. Similarly, hydroxylamine oxidation may be the rate-limiting step in the combined heterotrophic nitrification–aerobic denitrification process. Analysis of produced gases and nitrogen balance in this process are undergoing to describe the mechanisms in details.

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References

1. Anderson IC, Poth M, Homstead J, Burdige D (1993) A comparison of NO and N_2O production by the autotrophic nitrifier *Nitrosomonas europaea* and the heterotrophic nitrifier *Alcaligenes faecalis*. Appl Environ Microbiol 59:3525–3533
2. Bai YH, Sun QH, Zhao C, Wen DH, Tang XY (2009) Aerobic degradation of pyridine by a new bacterial strain, *Shinella zoogloeoides* BC026. J Ind Microbiol Biotechnol 36:1391–1400
3. Gupta AB (1997) *Thiosphaera pantotropha*: a sulphur bacterium capable of simultaneous heterotrophic nitrification and aerobic denitrification. Enzyme Microb Technol 21:589–595
4. Gupta AB, Gupta SK (2001) Simultaneous carbon and nitrogen removal from high strength domestic wastewater in an aerobic RBC biofilm. Water Res 35:1714–1722
5. Heuer H, Krsek M, Baker P, Smalla K, Wellington EMH (1997) Analysis of *Actinomycete* communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. Appl Environ Microbiol 63:3233–3241
6. Huang HK, Tseng SK (2001) Nitrate reduction by *Citrobacter diversu*s under aerobic environment. Appl Microbiol Biotechnol 55:90–94
7. Joo HS, Hirai M, Shoda M (2005) Characteristics of ammonium removal by heterotrophic nitrification-aerobic denitrification by *Alcaligenes faecalis* No. 4. J Biosci Bioeng 100:184–191
8. Joo HS, Hirai M, Shoda M (2005) Nitrification and denitrification in high-strength ammonium by *Alcaligenes faecalis*. Biotechnol Lett 27:773–778
9. Joo HS, Hirai M, Shoda M (2007) Improvement in ammonium removal efficiency in wastewater treatment by mixed culture of *Alcaligenes faecalis* No. 4 and L1. J Biosci Bioeng 103:66–73
10. Khardenavis AA, Kapley A, Purohit HJ (2007) Simultaneous nitrification and denitrification by diverse *Diaphorobacter* sp. Appl Microbiol Biotechnol 77:403–409
11. Kim JK, Park KJ, Cho KS, Nam SW, Park TJ, Bajpai R (2005) Aerobic nitrification-denitrification by heterotrophic *Bacillus* strains. Bioresour Technol 96:1897–1906
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275
13. Matsuzaka E, Nomura N, Nakajima-Kambe T, Okada N, Nakaharai T (2003) A simple screening procedure for heterotrophic nitrifying bacteria with oxygen-tolerant denitrification activity. J Biosci Bioeng 95:409–411
14. Moir JWB, Crossman LC, Spiro S, Richardson DJ (1996) The purification of ammonia monooxygenase from *Paracoccus denitrificans*. Febs Lett 387:71–74
15. Nishio T, Yoshikura T, Mishima H, Inouye Z, Itoh H (1998) Conditions for nitrification and denitrification by an immobilized heterotrophic nitrifying bacterium *Alcaligenes faecalis* OKK17. J Ferment Bioeng 86:351–356
16. Otte S, Schalk J, Kuenen JG, Jetten MSM (1999) Hydroxylamine oxidation and subsequent nitrous oxide production by the heterotrophic ammonia oxidizer *Alcaligenes faecalis*. Appl Microbiol Biotechnol 51:255–261
17. Patureau D, Zumstein E, Delgenes JP, Moletta R (2000) Aerobic denitrifiers isolated from diverse natural and managed ecosystems. Microb Ecol 39:145–152
18. Richardson DJ, Wehrfritz JM, Keech A, Crossman LC, Roldan MD, Sears HJ, Butler CS, Reilly A, Moir JWB, Berks BC, Ferguson SJ, Thomson AJ, Spiro S (1998) The diversity of redox proteins involved in bacterial heterotrophic nitrification and aerobic denitrification. In: 665th meeting of the Royal-Irish-Academy-lecture on peptide metabolism in cytoplasm of brain cells. Hants, England
19. Robertson LA, Kuenen JG, Kleijntjens R (1985) Aerobic denitrification and heterotrophic nitrification by *Thiosphaera pantotropha*. J Microbiol 51:445
20. Robertson LA, van Niel EWJ, Torremans RAM, Kuenen JG (1988) Simultaneous nitrification and denitrification in aerobic chemostat cultures of *Thiosphaera pantotropha*. Appl Environ Microbiol 54:2812–2818
21. Robertson LA, Kuenen JG (1990) Combined heterotrophic nitrification and aerobic denitrification in *Thiosphaera pantotropha* and other bacteria. Antonie van Leeuwenhoek 57:139–152
22. Shaw JG, Kelly DJ (1991) Binding-protein dependent transport of C4-dicarboxylates in *Rhodobacter capsulatu*s. Arch Microbiol 155:466–472
23. Su JJ, Yeh KS, Tseng PW (2006) A strain of *Pseudomonas* sp. isolated from piggery wastewater treatment systems with heterotrophic nitrification capability in Taiwan. Curr Microbiol 53:77–81
24. Takaya N, Catalan-Sakairi MAB, Sakaguchi Y, Kato I, Zhou ZM, Shoun H (2003) Aerobic denitrifying bacteria that produce low levels of nitrous oxide. Appl Environ Microbiol 69:3152–3157
25. Taylor SM, He YL, Zhao B, Huang J (2009) Heterotrophic ammonium removal characteristics of an aerobic heterotrophic nitrifying-denitrifying bacterium, *Providencia rettgeri* YL. J Environ Sci 21:1336–1341
26. van Niel EWJ, Braber KJ, Robertson LA, Kuenen JG (1992) Heterotrophic nitrification and aerobic denitrification in *Alcaligenes faecalis* strain TUD. Antonie van Leeuwenhoek 62:231–237
27. Wehrfritz JM, Reilly A, Spiro S, Richardson DJ (1993) Purification of hydroxylamine oxidase from *Thiosphaera pantotropha*-identification of electron accepters that couple heterotrophic nitrification to aerobic denitrification. Febs Lett 335:246–250
28. Wehrfritz JM, Carter JP, Spiro S, Richardson DJ (1996) Hydroxylamine oxidation in heterotrophic nitrate-reducing soil bacteria and purification of a hydroxylamine-cytochrome c oxidoreductase from a *Pseudomonas* species. Arch Microbiol 166:421–424
29. Zhao B, He YL, Huang J, Taylor S, Hughes J (2010) Heterotrophic nitrogen removal by *Providencia rettgeri* strain YL. J Ind Microbiol Biotechnol 37:609–616
30. Zhao B, He YL, Hughes J, Zhang XF (2010) Heterotrophic nitrogen removal by a newly isolated *Acinetobacter calcoaceticus* HNR. Bioresour Technol 101:5194–5200