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Analysis of high-nitrate, high-salinity wastewater in an expanded granular sludge bed reactor and microbial community

Runhua Liao[a,b], Yan Li[b], Jie Du[a], Aimin Li[b,*], Haiou Song[b], Zongyang Shen[a], Yueming Li[a]

[a]*School of Materials Science and Engineering, Jingdezhen Ceramic Institute, Jingdezhen 333403, China, Tel. +86 13 361783526; email: maxlrh@126.com (R. Liao), Tel. +86 15 907984663; email: sallydj@126.com (J. Du), Tel. +86 15 807985955; email: 79226595@qq.com (Z. Shen), Tel. +86 13 767842606; email: 672331953@qq.com (Y. Li)*
[b]*State Key Laboratory of Pollution Control and Resources Reuse, School of the Environment, Nanjing University, Nanjing 210023, China, Tel. +86 18 260033193, email: liyan_0921@126.com (Y. Li), Tel. +86 25 86269876; email: liaimin@nju.edu.cn (A. Li), Tel. +86 13 951785155; email: 79992279@qq.com (H. Song)*

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ABSTRACT

The denitrification of high-strength nitrate wastewater is a major problem for many industries, especially for those involved in the production of petrochemicals, explosives, fertilizers, pectin, nuclear, and many metal-finishing industries, whose wastewater usually contain large amount of salts. What is more, the denitrification of synthetic high-nitrate wastewater containing 14,000 mg N L^{-1} in an expanded granular sludge bed (EGSB) reactor had been achieved in our previous study. The activated sludge culture was acclimatized by a stepwise increase in the nitrate concentration of synthetic waste. In the present work, the denitrification of synthetic high-salinity and high-nitrate wastewater containing 6,000 mg L^{-1} NO_3^--N and 11% (w/v) salinity was achieved in the similar reactor. Meanwhile, The phylogenetic analysis of isolated 16S rRNA gene sequences indicated that *halophilic* species, *Halomonas* sp. and *Marinobacter* sp., were predominant at 11% salinity, suggesting that these bacteria show a high denitrifying activity in the EGSB reactor. The Shannon–Wiener index (*H*), representing the bacterium community diversity of the sludge sample, was calculated as 3.79. The EGSB reactor offers bright prospects for the treatment of high-salinity nitrate wastewater.

Keywords: High-salinity nitrate wastewater; Denitrification; Expanded granular sludge bed (EGSB) reactor; Microbial community

1. Introduction

The removal of nitrogen compounds from wastewater before disposal is closely related to public health and a number of ecological problems, including eutrophication of surface waterbodies and infant methemoglobinemia [1]. The denitrification of high-strength nitrate wastewater is a major problem confronting many industries, especially for those involved in the production of petrochemicals, explosives, fertilizers, pectin, nuclear, and many metal-finishing industries, whose wastewater usually contain large amount of salts such as chlorides (Cl^-),

*Corresponding author.

bicarbonates (HCO_3^-), sulfates (SO_4^{2-}), ammonium (NH_4^+), calcium (Ca^{2+}), sodium (Na^+), and sometimes magnesium (Mg^{2+}) [2–4]. Physical and chemical methods are suitable for low-level nitrate waste and result in a secondary waste, which is concentrated in nitrate [5]. One of the advanced treatment processes used for nitrate removal from the effluent is biological denitrification. Although the effects of salinity have been studied for many decades in the biological field, few studies have been reported on denitrification of high-nitrate wastewater [6–9]. Especially, fewer studies on denitrification of high-nitrate and high-salinity wastewater have been reported [10–13].

In this study, the biological denitrification of high-nitrate and high-salinity wastewater was investigated in a single expanded granule sludge bed (EGSB) reactor. The EGSB reactor enables wastewater to pass through at high up-flow velocity, and can be used to deal with high concentration of wastewater-containing toxics. To the authors' best knowledge, no previous study has addressed high-nitrate and high-salinity wastewater treatment in a single EGSB reactor. The correlation of microbial community and the noted reactor performance is also quite rare. This study aims to cultivate denitrifiers from a single EGSB reactor acclimatized with high nitrate and high salinity. The performance of high-nitrate and high-salinity wastewater treatment using EGSB reactor is determined. The isolated strains, characterized on the basis of 16S rRNA gene sequence, showed the ability to reduce nitrate and nitrite, and growth ability under high saline conditions in EGSB reactor.

2. Materials and methods

2.1. Experimental equipment and operational conditions

The Plexiglas EGSBR was 60 mm in inner diameter and 91 cm high, giving a total volume of 3.02 L and a working volume of 1.74 L [9]. A peristaltic pump introduced influent at the bottom of the reactor. A gas-washing device collected the generated N_2 gas at the top. A three-phase separator was installed at the top of the reactor to keep the biomass within the reactor. Liquid up-flow velocity (V_{up}) was controlled by inner recirculation. The EGSB reactor was operated under mesophilic condition (35 ± 1 ℃) by water bath.

2.2. Synthetic wastewater

The influent synthetic wastewater was prepared to simulate typical fertilizer and nuclear industry wastewater [7]. The synthetic wastewater (g L^{-1}), composed of 7.0 Na_2HPO_4, 1.5 K_2HPO_4, 0.1 $MgSO_4$, 0.1 NH_4Cl, C:N mole ratio at 2:1, was regulated by adding sodium nitrate and sodium acetate. Trace element solution was added to the synthetic wastewater at a volume ratio of 1:500, which consisted (g L^{-1}) of 5.55 $CaCl_2$, 5.0 $FeSO_4{\cdot}7H_2O$, 5.06 $MnCl_2{\cdot}4H_2O$, 2.2 $ZnSO_4{\cdot}7H_2O$, 1.51 $CuSO_4{\cdot}5H_2O$, 1.61 $CoCl_2{\cdot}H_2O$, 50.00 EDTA, and 1.1 $(NH_4)_6MO_7O_{24}{\cdot}4H_2O$. The pH values of synthetic wastewater were adjusted to 7.15 ± 0.15.

2.3. Experimental procedure

The seed sludge was from the previous successful startup EGSB reactor treatment 2,000 mg L^{-1}·NO_3^--N wastewater [9], whose average biomass concentration reached 25.81 g L^{-1} mixed liquor volatile suspended solids (MLVSS). The reactor was started up with the influent NO_3^--N concentration at 2,000 mg L^{-1} under the optimal process conditions reported by Liao et al. [9], which were V_{up} (3.0 m h^{-1}), hydraulic retention time (HRT) (24 h), the mole ratio of carbon/nitrate nitrogen (C/N) (2.0), and pH (7.0–7.3) of the influent. During five weeks of the operation, the influent NO_3^--N concentration of synthetic wastewater gradually increased from 2,000 to 3,000, 4,000, 5,000, and 6,000 mg L^{-1} with each concentration gradient operation for one week. Then, influent sodium chloride concentration of synthetic wastewater gradually increased from 0 to 1, 3, 4, 5, 7, 9, and 11 (wt.)% for characterizing the tolerability of denitrification in an EGSB reactor. Each sodium chloride concentration would operate for about 15 d.

2.4. Chemical analysis

An ion chromatograph (Dionex ICS-1100) was used to measure the concentrations of nitrate and nitrite in the collected liquor samples following 0.45 μm filtration. Sample separation and elution were performed using an IonPac AG23 AS23 4 mm analysis column with carbonate/bicarbonate eluent (4.5 mmol L^{-1} Na_2CO_3/0.8 mmol L^{-1} $NaHCO_3$ at 1 mL min^{-1}) and a sulfuric regeneration (H_2SO_4, 50 mmol L^{-1} at 1 mL min^{-1}). Mixed liquor suspended solids and MLVSS were measured according to the Standard Method [14]. A pH meter (pHS-25) was used to determine the pH values of liquid samples. Nitrate and nitrite of the effluent were measured every other day during the continuous experiment.

2.5. DNA extraction, PCR amplication, and cloning library construction

Sludge samples were the composite samples, which were taken from the upper, middle, and bottom

part of the reactor on the 165th of the continuous operation (11% NaCl). DNA extraction was the same as previously described [9].

The 16S ribosomal DNA gene fragments used for the clone library construction were amplified using the forward 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse 1492R (5′-GGTTACCTTGTTACGACTT-3′) primers [15]. PCR amplification was performed in 30 μl reaction mixtures containing 1× PCR buffer, 1.5 mmol L^{-1} $MgCl_2$, 200 lmol L^{-1} dNTPs, 0.5 μmol L^{-1} of each primer, 2.5 U of Taq polymerase (TaKaRa, Dalian, China), and about 10 ng of DNA template. The PCR reaction was performed using the following thermal cycles: initial denaturation at 94℃ for 5 min, 35 cycles of 94℃ for 1 min, 55℃ for 1 min, 72℃ for 2 min, and final extension at 72℃ for 10 min. PCR products were analyzed by electrophoresis on 1% (w/v) agarose gel with ethidium bromide in 50 TAE buffer at 120 V for 30 min. Then, PCR products were purified using the DNA Fragment Purification Kit Ver.2.0 (TaKaRa) and were cloned into a pMD19-T vector (TaKaRa) following the manufacturer's instructions. The resulting ligation products were transformed into *Escherichia coli* JM109 competent cells (TaKaRa). After incubation at 37℃ for 1 h, clone libraries were generated on Luria-Bertani plates containing 100 μg mL^{-1} of ampicillin, 80 μg mL^{-1} of X-GAL, and 0.5 mmol L^{-1} of IPTG. White clones were chosen randomly as the possible positive clones [16] and then sequenced using M13 universal primers with a model 3730 (Applied Biosystems, CA USA).

2.6. Phylogenetic analysis

The obtained sequences were checked for their phylogenetic affiliation using the Blast search program at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/BLAST/). Sequence similarity was compared with Mothur software, and, if the similarity was greater than 97%, an operational taxonomic unit (OTU) would be identified. Based on OTU, the phylogenetic tree was constructed by Mega 5.05 software according to the neighbor-joining method. The Shannon–Wiener index (H) was used to represent the community diversity and was calculated based on the equation $H = -\sum \frac{n_i}{N} \ln \frac{n_i}{N}$, in which N is the total number of selected positive clones and n_i is the number of clones in each OTU group [17].

2.7. Nucleotide sequence accession numbers

The partial 16S rRNA gene sequences of OTUs were submitted to the NCBI database under accession numbers JX075105-JX075151.

3. Results and discussion

3.1. The performance of the EGSB reactor

In order to investigate the effect of the salinity on the performances of the EGSB reactor, influent sodium chloride concentration of synthetic wastewater gradually increased from 0 to 11(wt.)%. The change of the performances of the EGSB reactor with salinity was shown in Fig. 1. No significant inhibition in the denitrification reaction was observed during the operation of 166 d at HRT of 24 h, even at 11% NaCl. At the same time, pH values of the influent and effluent varied from 9.0 to 9.6 (Fig. 1(c)), and the total alkalinity of the influent varied from 9,000 to 30,000 mg L^{-1}; the scope of corresponding effluent was from 30,000 to 55,000 mg L^{-1} (Fig. 1(d)). However, as the HRT decreased from 24 to 16 h, the nitrate nitrogen and COD removal rate decreased to about 78–45%, respectively, and the nitrite nitrogen concentration of the effluent reached 2,357 mg L^{-1} (shown in Fig. 1(a) and (b)). Furthermore, the pH value of the effluent declined to less than 9.0, and the total alkalinity of the effluent also decreased to below 30,000 mg L^{-1} (Fig. 1(d)). This observation was different from some previous literatures [11,18–22]. For example, Glass and Silverstein [11] increased the salinity stepwise in a denitrifying sequencing batch reactor (SBR), while the influent nitrate was maintained at 5,400 mg L^{-1} NO_3^--N at an ionic strength of 3.0, and nitrate was reduced by approximately 50%, from 3,500 to 1,600 mg L^{-1}. Simultaneously, accumulation of 3,000 mg L^{-1} NO_2^--N was observed. Aminzadeh et al. [18] found that the nitrate nitrogen removal efficiency drastically decreased to 78 and 48% at 4 and 5% NaCl, respectively, in an up-flow packed bed reactor. Vredenbregt et al. [22] reported that denitrification could proceed at a concentration of 4.5% NaCl. Carrera et al. [2] observed a denitrification rate that reached 2.3 g N L^{-1} d^{-1} at a Na_2SO_4 concentration of 1.5–2.0%. However, relative stability in denitrifying activity involving salinity increase under 6.0 g L^{-1} d^{-1} of influent nitrate nitrogen loading rate, as observed in this study, has never been reported. Furthermore, this study demonstrated a denitrification rate of about 6.0 g L^{-1} d^{-1} at 11% salinity in EGSB reactor. This result overturned the generally accepted idea that denitrification rate increased more as salinity decreased. At the same time, this result was also different from the improvement in denitrifying activity involving salinity increase [3,12]. This phenomenon of denitrification potential may be consistent with that in the denitrification performance in EGSB reactor. The results of continuous denitrification experiment indicated that nitrite accumulated during acclimation under high-salinity and

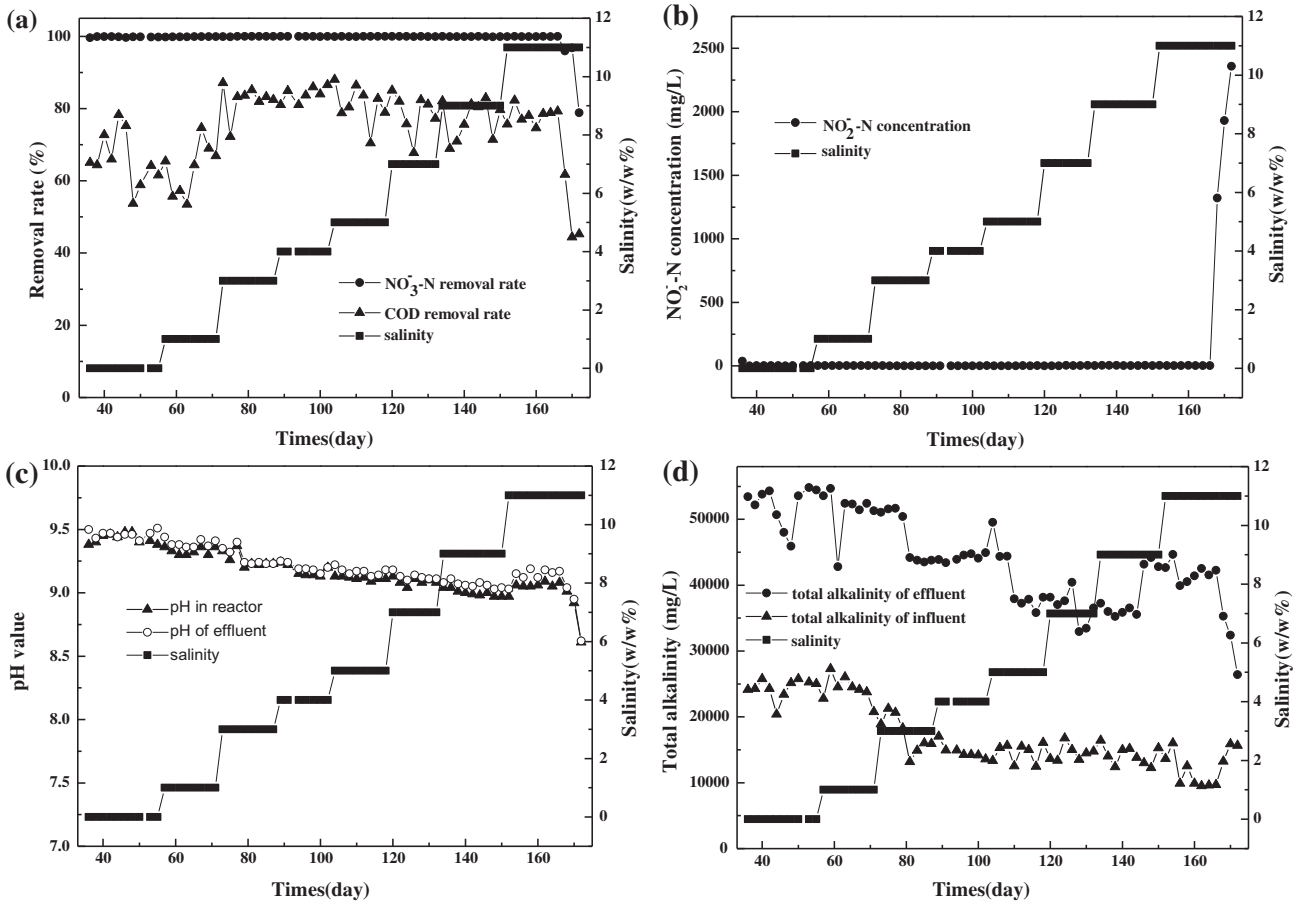

Fig. 1. The change of performance with the salinity in the EGSB reactor: (a) nitrate nitrogen and COD removal rate; (b) nitrite nitrogen accumulated concentration of the effluent; (c) pH in reactor and the effluent and (d) the total alkalinity of the influent and effluent.

high-nitrate concentration. Meanwhile, nitrite accumulation during the acclimation period under high salinity and high nitrate suggested that nitrite reduction was the rate-limiting step for the denitrification of saline wastewater. Such nitrite accumulation has been observed in the study of Yoshie et al. [3,23]. These results might indicate that the activity of nitrite reductase was subject to damage at high salinity and high nitrate.

3.2. Microbial community in the EGSB reactor

The phylogenetic tree, based on 16S rRNA gene sequences, consisted of sequences of clones detected in the sludge sampled from EGSB reactor. Fifty-two true-positive clones were randomly selected, and 45 different bacterial OTUs (named CSE1–CSE45) were identified by the amplified ribosomal DNA restriction analysis and correspondent sequencing results of the analyses are shown in Fig. 2. Phylogenetic reconstruction (Fig. 2) revealed that as a result of the phylogenetic analysis of the clones detected from EGSB reactor under high-salinity condition (11%), *Halomonas* sp. were found to be the most predominant (89.36% of the total number of clones) in the bacterium community under such a condition. Blast search results (Fig. 3(a) and (b)) revealed that the majority of the bacterium OTUs, accounting for 89.36% of the total library, represented *Halomonas*, especially the order *ventosaes* (29.79%). Most OTUs belonged to *Gamma-Proteobacteria*, and most were similar to the genera *Halomonas* (42 clones) and *Marinobacter* (3 clones), and the fraction of clones belonging to the *Gamma-Proteobacteria* reached more than 95% of the total clones (*Halomonas* 89.36%, and *Marinobacter* 6.38%). The Shannon–Wiener index (*H*), representing the bacterium community diversity of the sludge sample, was calculated as 3.79. The above analysis indicated the genera *Halomonas* and *Marinobacter* played an important role in denitrification for high-nitrate, high-salinity

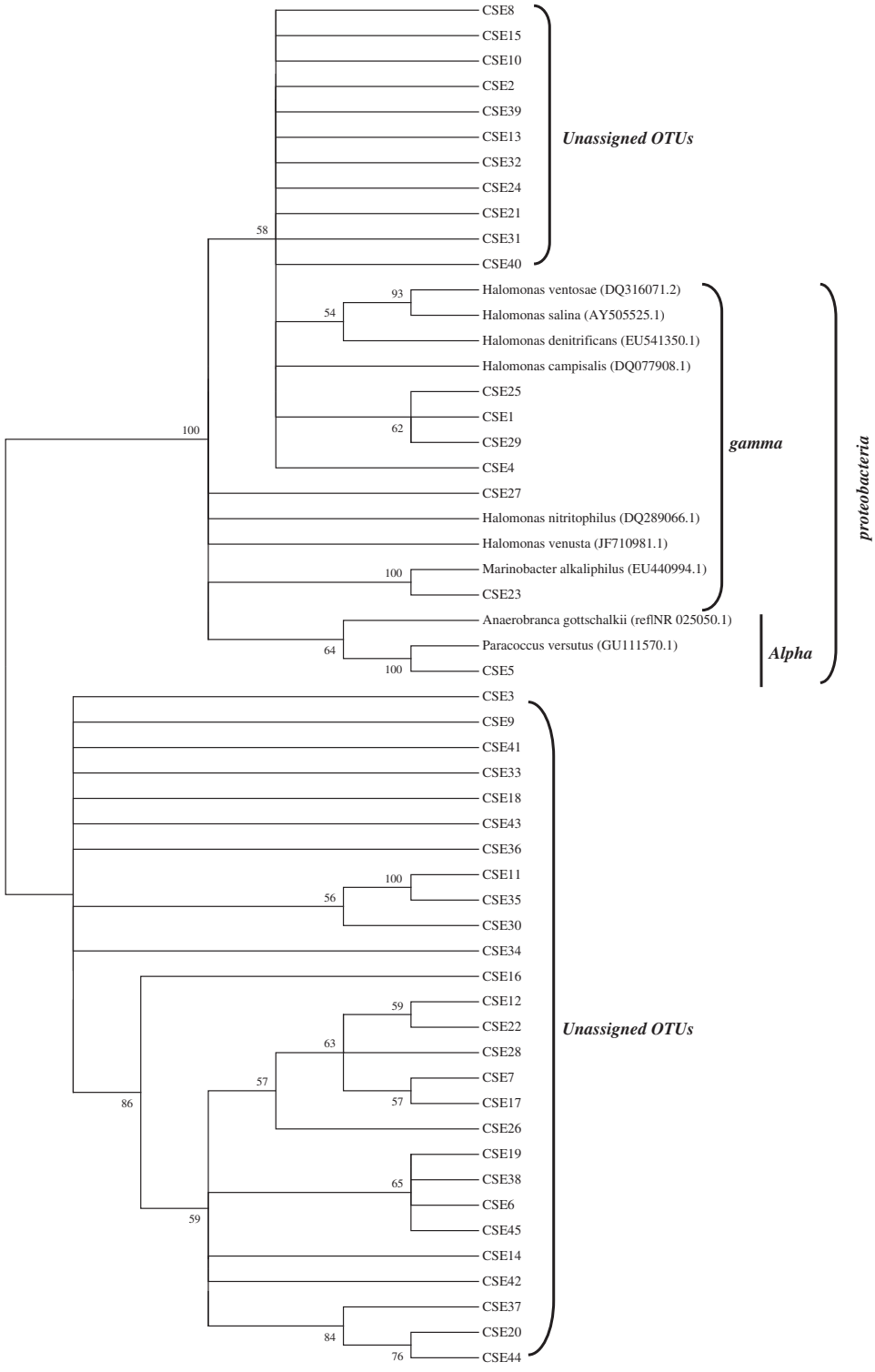

Fig. 2. Neighbor-joining phylogenetic tree based on partial 16S rRNA gene sequences of isolates from EGSB reactor. A single sequence was chosen as representative of an OTU with more than 97% similarity; Bootstrap values greater than 50% are shown.

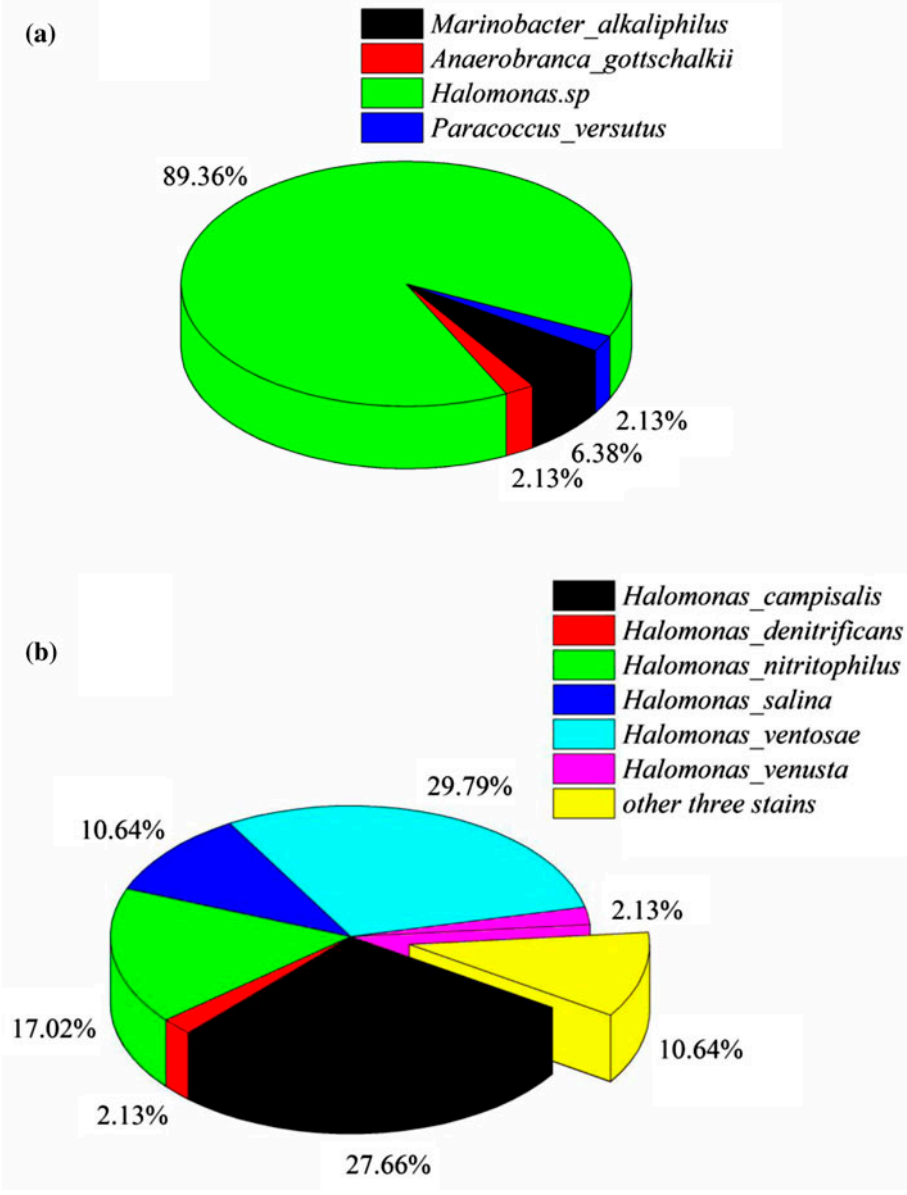

Fig. 3. Proportions of every genus in bacterium clone libraries of sample: (a) represent the proportions of every phylum in the clone libraries of sample and (b) represent the proportions of every order in the phylum *Halomaonas* to the total in the clone libraries of sample.

wastewater. This was similar with other related researches reported [4,13,21,24]; a study by Osaka et al. [21] suggested that the genera *Halomonas* and *Marinobacter* play an important role in acetate-fed reactor for high-nitrate removal at 0–10% NaCl. Furthermore, Yoshie et al. [4,24] and Liao et al. [13] found that *Gammaproteobacteria* also played a significant part in high-rate denitrification of saline wastewater. While, this was different from the result reported by Liao et al. [9], who concluded that Sporolactobacillaceae incertae sedis play some part in denitrification.

The phylogenetic analysis of isolated 16S rRNA gene sequences during acclimation under high-salinity condition revealed that bacteria of the genera *Halomonas* and *Marinobacter* have been predominant at 11% salinity condition. Clone analysis of bacterial member indicated that these two bacterial groups accounted for about 95% of the total number of clones. Other clones were similar to the genera *Anaerobranca gottschalkii* and the genus *Paracoccus versutus*, which are frequently reported to be detected from saline environment and have denitrification ability [25–27].

However, 16S rRNA-encoding gene-targeted analysis used in this study cannot clarify whether or not detected *halophilic* bacteria are truly involved in denitrification activity. Previous studies, which include the results of denitrification activity test using isolated bacteria [4] and community analysis on the basis of denitrification functional genes *nirS* and *nirK* [28], support the possibility that dominant bacterial groups (i.e., *Halomonas* spp. and *Marinobacter* spp.) are responsible for denitrification activity in the denitrificaiton system. Furthermore, introduction of new method such as SIP [29], MAR-FISH [30], and 454-pyrosequencing [9,13] would lead to the answer of this question. For example, Liao et al. [13] concluded that Sporolactobacillaceae incertae sedis play some part in denitrification by 454-pyrosequencing.

4. Conclusion

This study demonstrated relative stability in denitrifying activity involving salinity increase under 6.0 g $L^{-1} d^{-1}$ of influent nitrate nitrogen loading rate, and a denitrification rate of about 6.0 g N $L^{-1} d^{-1}$ at 11% salinity in EGSB reactor. Isolated from the EGSB denitrifying system with 11% salinity, the strains *halophilic* bacteria, *Halomonas* sp. and *Marinobacter* sp., suggested that these bacteria show a high denitrifying activity at 11% salinity.

The long-term acclimated sludge used in this study resulted in high denitrification performance for a high-salinity and high-nitrate used EGSB reactor, which indicated that the design of a high-performance denitrification system for high-salinity and high-nitrate wastewater will be possible.

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References

[1] D. Forman, Nitrate exposure and human cancer, Nitrate Contamination, RD NATO ASI Series 30 (1991) 281–288.
[2] J. Carrera, J.A. Baeza, T. Vicent, J. Lafuente, Biological nitrogen removal of high-strength ammonium industrial wastewater with two-sludge system, Water Res. 37 (2003) 4211–4221.
[3] S. Yoshie, T. Ogawa, H. Makino, H. Hirosawa, S. Tsuneda, A. Hirata, Characteristics of bacteria showing high denitrification activity in saline wastewater, Lett. Appl. Microbiol. 42 (2006) 277–283.
[4] S. Yoshie, H. Makino, H. Hirosawa, K. Shirotani, S. Tsuneda, A. Hirata, Molecular analysis of *halophilic* bacterial community for high-rate denitrification of saline industrial wastewater, Appl. Microbiol. Biotechnol. 72 (2006) 182–189.
[5] A. Kapoor, T. Viraraghavan, Nitrate removal from drinking water-review, J. Environ. Eng. 123(4) (1997) 371–380.
[6] L. Foglar, F. Briski, L. Sipos, M. Vukovic, High nitrate removal from synthetic wastewater with the mixed bacterial culture, Bioresour. Technol. 96 (2005) 879–888.
[7] P.B. Dhamole, R.R. Nair, S.F. D'Souza, S.S. Lele, Denitrification of high nitrate waste, Bioresour. Technol. 98 (2007) 247–252.
[8] R.R. Nair, P.B. Dhamole, S.S. Lele, S.F. D'Souza, Biological denitrification of high strength nitrate waste using preadapted denitrifying sludge, Chemosphere 67 (2007) 1612–1617.
[9] R. Liao, K. Shen, A. Li, P. Shi, Y. Li, Q. Shi, Z. Wang, High-nitrate wastewater treatment in an expanded granular sludge bed reactor and microbial diversity using 454 pyrosequencing analysis, Bioresour. Technol. 134 (2013) 190–197.
[10] B. Duncan, Effect of Ionic Strength on Denitrification of a High Salt, High Nitrate Brine Simulating Rocky Flats Evaporation Pond Water using Activated Sludge, M.S. Thesis, University of Colorado, Boulder, CO, 1995.
[11] C. Glass, J. Silverstein, Denitrification of highnitrate high-salinity wastewater, Water Res. 33(1) (1999) 223–229.
[12] P. Chanjae, The effect of high salinity on denitrification of concentrated nitrate wastewaters, M.S. Thesis, University of Nevada, Reno, 2000.
[13] R. Liao, Y. Li, Z. Wang, Y. Miao, K. Shen, P. Shi, Y. Ma, W. Li, A. Li, 454 pyrosequencing analysis on microbial diversity of an expanded granular sludge bed reactor treating high NaCl and nitrate concentration wastewater, Biotechnol. Bioproc. Eng. 19 (2014) 183–190.
[14] American Public Health Association (APHA), Standard Methods for the Examination of Water and Wastewater, seventeenth ed., American Public Health Association, New York, NY, 1998.
[15] H. Song, Z. Li, B. Du, G. Wang, Y. Ding, Bacterial communities in sediments of the shallow Lake Dongping in China, J. Appl. Microbiol. 112 (2011) 79–89.
[16] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning—A Laboratory Manual, second ed., Cold Spring Harbor Laboratory Press, New York, NY, 1989.
[17] C.E. Shannon, W. Weaver, The Mathematical Theory of Communication, University of Illinois Press, Urbana, 1964.
[18] B. Aminzadeh, A. Torabian, A.A. Azimi, R. Nabi, R.N.B Gh, N Mehrdadi, Salt inhibition effects on simultaneous heterotrophic/autotrophic denitrification of high nitrate wastewater, Int. J. Environ. Res. 4 (2010) 255–262.

[19] B.U. Bae, Y.H. Jung, W.W. Han, H.S. Shin, Improved brine recycling during nitrate removal using ion exchange, Water Res. 36 (2002) 3330–3340.
[20] J.D. Gu, W. Qiu, A. Koenig, Y. Fan, Removal of high NO_3-N concentrations in saline water through autotrophic denitrification by the bacterium Thiobacillus denitrificans strain MP, Water Sci. Technol. 49 (2004) 105–112.
[21] T. Osaka, K. Shirotani, S. Yoshie, S. Tsuneda, Effects of carbon source on denitrification efficiency and microbial community structure in a saline wastewater treatment process, Water Res. 42 (2008) 3709–3718.
[22] L.H.J. Vredenbregt, K. Nielsen, A.A. Potma, G.H. Kristensen, C. Sund, Fluid bed biological nitrification and denitrification in high salinity wastewater, Water Sci. Technol. 36 (1997) 93–100.
[23] S. Yoshie, N. Noda, T. Miyano, S. Tsuneda, A. Hirata, Y. Inamori, Characterization of microbial community in nitrogen removal process of metallurgic wastewater by PCR-DGGE, Water Sci. Technol. 46 (2002) 333–336.
[24] S. Yoshie, N. Noda, T. Miyano, S. Tsuneda, A. Hirata, Y. Inamori, Microbial community analysis in the denitrification process of saline-wastewater by denaturing gradient gel electrophoresis of PCR-amplified 16S rDNA and the cultivation method, J. Biosci. Bioeng. 92 (2001) 346–353.
[25] J.A. Mata, J.M. Cánovas, E. Quesada, V. Béjar, A detailed phenotypic characterization of the type strains of Halomonas species, Syst. Appl. Microbiol. 25 (2002) 360–375.
[26] N.M. Gortshkova, E.P. Ivanova, A.F. Sergeev, N.V. Zhukova, Y. Alexeeva, J.P. Wright, D.V. Nicolau, V.V. Mikhailov, R. Christen, Marinobacter excellens sp. nov., isolated from sediments of the sea of Japan, Int. J. Syst. Evol. Microbiol. 53 (2003) 2073–2078.
[27] M. Nakano, T. Inagaki, S. Okunishi, R. Tanaka, H. Maeda, Effect of salinity on denitrification under limited single carbon source by *Marinobacter* sp. isolated from marine sediment, J. Basic Microbiol. 50(3) (2010) 285–289.
[28] S. Yoshie, N. Noda, S. Tsuneda, A. Hirata, Y. Inamori, Salinity decreases nitrite reductase gene diversity in denitrifying bacteria of wastewater treatment systems, Appl. Environ. Microbiol. 70 (2004) 3152–3157.
[29] S. Radajewski, P. Ineson, N.R. Parekh, J.C. Murrell, Stable isotope probing as a tool in microbial ecology, Nature 403 (2000) 646–649.
[30] N. Lee, P.H. Nielsen, K.H. Andreasen, S. Juretschko, J.L. Nielsen, K.H. Schleifer, M. Wagner, Combination of fluorescent *in situ* hybridization and microautoradiography—A new tool for structure-function analysis in microbial ecology, Appl. Environ. Microbiol. 65 (1999) 1289–1297.