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Structural differentiation of bacterial communities in indole-degrading bioreactors under denitrifying and sulfate-reducing conditions

Xuan Hong [a,c], Xiaojun Zhang [a,*], Binbin Liu [a], Yuejian Mao [a], Yongdi Liu [b], Liping Zhao [a]

[a] *Key Laboratory of MOE for Microbial metabolism and School of Life Science & Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China*

[b] *College of Resource Science and Environmental Engineering, Eastern China University of Science and Technology, Shanghai 200237, China*

[c] *Xiamen Medical College, Xiamen 361008, China*

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Abstract

The acclimated, anaerobic microbial community is an efficient method for indole-containing wastewater treatment. However, our understanding of the diversity of indole-degrading communities is still limited. We investigated two anaerobic, indole-decomposing microbial communities under both denitrifying and sulfate-reducing conditions. Utilizing a near full-length 16S rRNA gene clone library, the most dominant bacteria in the denitrifying bioreactor identified was β-proteobacteria. Among these, bacteria from genera *Alicycliphilus*, *Acaligenes* and *Thauera* were abundant and thought responsible for indole degradation. However, in the sulfate-reducing bioreactor, Clostridia and Actinobacteria were the dominant bacterial class found and likely the main degrading species. Microbial communities in these bioreactors shared only two operational taxonomic units (OTUs). Differences in the electron acceptors of denitrification or sulfate reduction may be responsible for the higher indole removal capacity in the denitrifying bioreactor (80%) than the capacity in the sulfate-reducing bioreactor (52%). This study is the first detailed analysis of an anaerobic indole-degrading community.

Keywords: Denitrifying bioreactor; Sulfate-reducing bioreactor; Indole degradation; Bacterial community; 16S rRNA gene

1. Introduction

Indole is one of the main N-heterocycle hydrocarbons (NHCs) found in coking wastewater and is degradation resistant. Many researchers have investigated the degradation of indole under aerobic conditions, including its metabolic pathway in many isolates (Bak and Widdel, 1986; Claus and Kutzner, 1983; Dagher et al., 1997). In addition to these aerobic studies on pure cultures, microbial degradation of indole under sulfate-reducing and methanogenic conditions has been studied extensively (Berry et al., 1987; Gu and Berry, 1992; Gu et al., 2002; Madsen and Bollag, 1989; Madsen et al., 1988; Wang et al., 1984). Many studies used anaerobic sewage sludge to acclimate the indole-degrading community. A denitrifying microbial community (Madsen and Bollag, 1989; Madsen et al., 1988) can mineralize indole. Furthermore, under denitrifying conditions, indole degrades more efficiently than under methanogenic conditions (Li et al., 2006). However, previous work focused only on the chemical aspects of degradation, such as the metabolic pathway, and degrading efficiency influenced by other factors. Only one strain of pure culture was studied for anaerobic indole degradation (Johansen et al., 1997). To our knowledge, there are no reports on the diversity of the anaerobic indole-degrading microbial community. Therefore, the knowledge of the microbial community structure and diversity of the functionally important members in anaerobic indole-degrading communities were limited. This paper may be the first detailed

* Corresponding author.
E-mail addresses: amoy.hong@gmail.com (X. Hong), xjzhangsjtu@gmail.com (X. Zhang), binbin.liu@umb.no (B. Liu), nikoxia620@163.com, stoneraining@hotmail.com (Y. Mao), ydliu@ecust.edu.cn (Y. Liu), lpzhao@sjtu.edu.cn (L. Zhao).

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description of a highly efficient, anaerobic indole-degrading microbial community.

The 16S rRNA gene clone library is widely used for analyzing bacterial communities from various environments (Dimitriu et al., 2008; Hwang et al., 2009; Lau et al., 2009). Although new generation sequencing technology is rapidly expanding in microbial ecology (Kirchman et al., 2010; Lau et al., 2009; Miller et al., 2009), clone library technology has been the standard method for identifying this diversity because of its accurate identification of the microorganisms within the community.

The function of a microbial community depends largely on the important populations within that community. The dominant and functionally important members often change when environmental conditions shift (Lee et al., 2008). Therefore, investigation of the community structure is critical for elucidating the microbial community and its functions. For example, by comparing the community structures during different stages of quinoline adaptation in a denitrifying bioreactor, Liu and coauthors speculated that *Thauera* and *Azoarcus*, the predominant bacteria in high efficiency, quinoline removal bioreactors, should be the functionally important members for quinoline degradation (Liu et al., 2006). Data have shown that wastewater treatment technology is the most important factor for constructing a microbial community within the bioreactor. However, the impact of treatment technology on the formation of a diverse anaerobic microbial community in the bioreactor needs further investigation. In this study, the community structures in denitrifying and sulfate-reducing bioreactors including seeding sludge (SS) were compared by using 16S rRNA gene clone libraries to analyze the major anaerobic bacteria communities and the effects of an electron acceptor on determining the structure of the indole-degrading bacterial community.

2. Materials and methods

2.1. Source of seeding sludge and bioreactor operation

The seeding sludge (SS), which was used to acclimate both a denitrifying reactor (DR) and a sulfate-reducing reactor (AR) in this study, was collected from the secondary sedimentation tank of the wastewater treatment plant in the Shanghai Coking & Chemical Factory (Wujing, Shanghai, PR China). The same SS sample was also used for acclimating a quinoline removal reactor in previous report (Liu et al., 2006). All reactors were constructed with plastic rings and synthetic fiber strings packed in 18 L tanks. Indole and glucose were carbon sources for the synthetic wastewater, which was used as an influent to acclimate the reactors. The concentration of indole and glucose was approximately 40 and 180 mg/L, respectively. K_2HPO_4 was the phosphorus source. All conditions were the same except the nitrogen source and electron acceptor was $NaNO_3$ (DR) and $(NH_4)_2SO_4$ (AR). The hydraulic retention time (HRT) was 12 h, and the temperature maintained at 30 °C. The indole removal efficiency of the bioreactors was monitored by HPLC, as described previously (Gu et al., 2002).

2.2. Sampling and DNA extraction

After 6 weeks of acclimation, biofilm samples of DR and AR were collected by scraping the sludge from the surface of the supporting medium. Pre-treated sludge samples (50–100 mg) were suspended in 400 μL of extraction buffer (100 mmol l^{-1} Tris, 100 mmol l^{-1} EDTA, 200 mmol l^{-1} NaCl, 1% PVP, 2% cetyltrimethyl ammonium bromide [CTAB], pH 8.0), including two glass beads. The sample was mixed using a vortex for 5 min. in 400 μL of 2% SDS buffer (100 mmol l^{-1} Tris, 200 mmol l^{-1} NaCl, 2% SDS, pH 8.0); then, 1 μL of Proteinase K (Sigma–Aldrich) (20 mg/ml) and 800 μL of phenol were added. The sample tube was cooled on ice for 5 min. and centrifuged at 13,000 *g* for 15 min. at 4 °C. Afterwards, the supernatant was transferred to a new Eppendorf tube; DNA was extracted successively with phenol–chloroform, chloroform, and ethanol precipitates then suspended in distilled H_2O and stored at −20 °C.

2.3. 16S rRNA gene sequence amplification and library construction

16S rRNA clone libraries were constructed to examine microbial diversity in DR and AR samples. PCR was performed to obtain part of the 16S rRNA gene using the universal bacterial primers 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1391r (5′-GACGGGCGGTGTGTRCA-3′) (Lane et al., 1985). The 50 μL reaction mixture contained 1U Taq DNA polymerase, 5 μL of 10× buffer, 1.5 mmol l^{-1} of $MgCl_2$, 0.2 mmol l^{-1} dNTP, 25 pmol of each primer and 10 ng of genomic DNA template. Amplification was performed using the following program: initial denaturation at 94 °C for 4 min.; 30 cycles at 94 °C for 45 s, 55 °C and 72 °C for 1 min. each; and final extension at 72 °C for 6 min. Purified PCR products were ligated into pGEM-T Easy Vector with T4 DNA ligase, and the ligated product was electroporated into competent *E. coli* DH10B cells. After recombinant identification, 96 clones were chosen for sequencing in each library.

2.4. Sequence analysis of the 16S rRNA gene clone libraries

Partial sequencing was performed with a single primer, 27f, generating sequences of approximately 850 bp. All trimmed sequences were examined for chimeras using CHIMERA_CHECK software and the RDP (Ribosomal Database Project) online service. The clones containing the correct 16S rRNA gene sequence were analyzed. All sequences were submitted to the online alignment service of Greengenes (http://greengenes.lbl.gov); aligned sequences were grouped into provisional operational taxonomic units (OTUs) using Dotur software with 99% similarity as the standard. All sequences were compared to 16S rRNA sequences available in the GenBank databases obtained from the National Center for Biotechnology Information (NCBI) using BLAST search. The aligned representative 16S rRNA gene sequences were used for generating phylogenetic trees using an ARB program package with the ARB neighbor-joining algorithm.

The distance matrix obtained from ARB was analyzed using the Unifrac tool(Lozupone and Knight, 2005).

Two statistical indices, the Shannon–Wiener index (H) and the reciprocal of Simpson's index (1/*D*), were used to estimate the microbial diversity of the two bioreactors by 16S rRNA gene diversity in the two libraries.

Similarities between the two clone libraries and the seeding sludge clone library (Liu et al., 2006) were calculated using the UNIFRAC analysis tool based on a unified phylogenetic tree constructed from the three clone libraries.

2.5. Nucleotide sequence accession numbers

DNA sequences of the two libraries were deposited in GenBank under accession numbers EU136218–EU136304 (DR) and EU136305–EU136386 (AR). The accession numbers for 16S rRNA gene sequences from SS were AY945863–AY945900, reported previously (Liu et al., 2006).

3. Results

3.1. Performance of denitrifying and sulfate-reducing bioreactors

After an initial start-up period of 6 weeks, both bioreactors reached steady state, shown by indole removal efficiency in Fig. 1. The pH was 7.0 and the dissolved oxygen (DO) level was less than 0.1 mg/L, inside the tank. The removal efficiency for indole, for five consecutive days, was 80.4 ± 1.7% in the DR, compared to 52.0 ± 4.5% in the sulfate-reducing bioreactor (AR).

3.2. 16S rRNA gene clone libraries of acclimated bioreactors and seeding sludge

Biofilm samples of AR and DR bioreactors were used for constructing the clone libraries. The number of sequences obtained from the AR and DR clone libraries were 82 and 87, respectively. The SS clone library was reported in a previous study (Liu et al., 2006). The coverage values for these three libraries were, at least, higher than 70%.

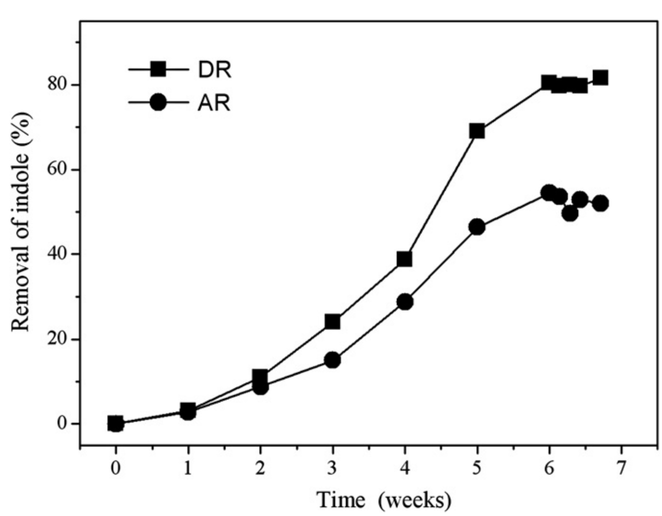

Fig. 1. Percent change in indole removal efficiency during acclimation: (DR) denitrifying reactor (squares) and (AR) sulfate-reducing reactor (circles).

All 16S rRNA gene sequences of SS, DR and AR clone libraries were clustered based on phylogenetic information, and a phylogenetic tree was constructed (Fig. 2). The compositions of each sample at class level are listed in Table 1.

In the DR library, 87 clones were identified and clustered into eight different bacterial groups on the phylum level. Of those clones, 85 were associated with the following classes: Proteobacteria (Alpha, Beta and Delta), Clostridia, Bacteroidetes, Actinobacteria and Chloroflexi. The remaining two clones (OTU66 and OTU45) were not closely related to any recognized microorganisms but were similar to an uncultured bacterium (Fig. 2B). Among these groups, Proteobacteria was the predominant member (82.4%), with β-proteobacteria accounting for greater than 56% (Table 1). The Comamonadaceae and *Thauera* are the most abundant members, representing 32.2% and 9.2% of the total DR clones, respectively. The clone of the largest OTU (OTU41) represents 28.7% of the DR clones and shares 100% sequence similarity with *Alicycliphilus* sp. (DQ342277). The second largest OTU (OTU49), comprising 6.9% of the DR clones, shares 99.9% sequence similarity with the bacterium CYCU-0269 (DQ232423), a member of *Thauera*. The third largest OTU (OTU63), comprising 4.6% of the DR clones, is associated with *Ochrobactrum* sp. M231 (EU604246) in 99% sequence similarity.

Examining the AR library, 80/82 clones cluster into the classes Proteobacteria (Alpha, Beta, Gamma and Delta), Clostridia, Bacilli, Actinobacteria and Chloroflexi. The remaining two clones (OTU122 and OTU129) only show similarities with unclassified bacteria (Fig. 2B). The most abundant group of the AR clones (34.1%) is associated with the class Clostridia, whereas 22.0% of the AR clones belong to Actinobacteria. The dominant bacteria in the AR community are δ- and γ-Proteobacteria (Table 1). Of these, the largest are OTU100 and OTU87, comprising 6.1% of the AR clones, respectively. OTU100 shares 99.5% sequence similarity with uncultured Clostridiaceae (AY261812). OTU87 is distantly affiliated with bacteria from the genus *Propionicimonas*. The second largest OTU, OTU115, comprising 4.9% of the AR clones, is closely related to an uncultured bacterium (AY426450) in *Propionicimonas*, having 99.3% sequence homology.

3.3. Comparison of community structure of seeding sludge with two acclimated bioreactors

A detailed analysis on the effects of treatment technology on the composition of the microbial community required sequence comparison of SS samples with samples collected from both the denitrifying bioreactor (DR) and the sulfate-reducing bioreactor (AR). SS used to acclimate both reactors in this study had been reported previously(Liu et al., 2006). The diversity indices of SS, DR and AR clone libraries were 3.07, 3.06 and 3.10 for Shannon–Wiener index and 10.68, 9.73 and 12.53 for Simpson's index. Both statistical indices suggest that phylotype diversity of samples after acclimation was relatively stable with

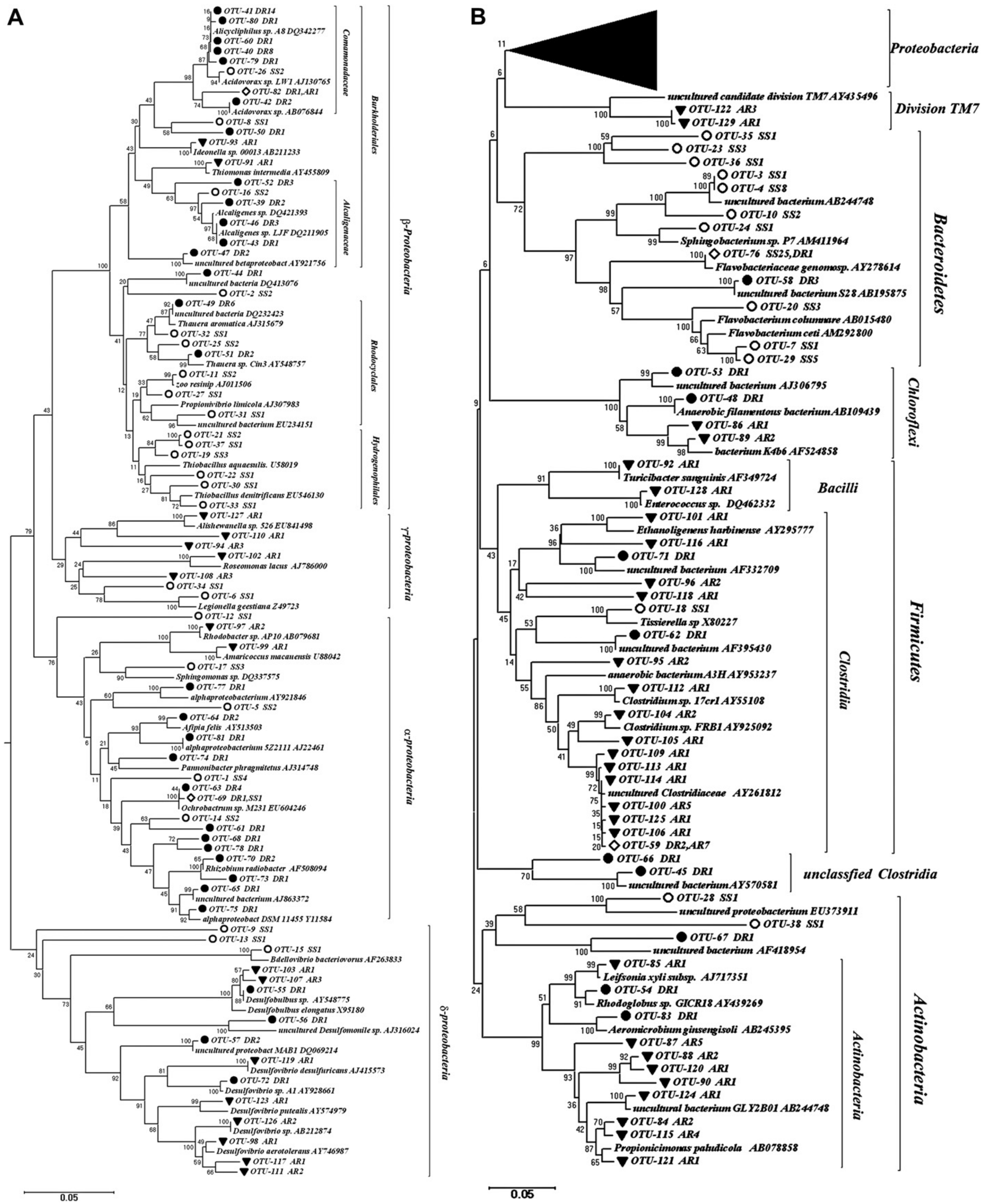

Fig. 2. Dendrogram of 16S rRNA gene sequences showing phylogenetic relationships of operational taxonomic units (OTUs) from the three bioreactors. The phylogenetic tree separates into one bacterial tree Proteobacteria (A) and remaining bacteria (B) in the other. Construction of neighbor-joining tree is from 16S rRNA gene sequences of representative clones from each OTU with sequences retrieved from the GenBank database. Sources and clone numbers use OTU nomenclature. Source of OTUs: open circle (SS), solid circle (DR), solid, inverted triangle (AR), and open diamond (OTUs found in more than one bioreactor). The bar in Fig. 2A and B represents 5% estimated sequence divergence.

Table 1
Relative clone frequencies of different phylogenetic groups in three clone libraries.

Phylogenetic groups		Percent (%) in SS (95 clones)	Percent (%) in DR (87 clones)	Percent (%) in AR (82 clones)
Phylum	Class			
Proteobacteria	α-Proteobacteria	13.7	18.4	4.9
	β-Proteobacteria	24.2	56.3	3.7
	γ-Proteobacteria	2.1	0	8.5
	δ-Proteobacteria	1.1	5.7	14.6
Acidobacteria	Acidobacteria	1.1	0	0
Bacteroidetes	Bacteroidetes	0	3.4	0
	Flavobacteria	35.8	1.1	0
	Sphingobacteria	12.6	0	0
Firmicutes	Clostridia	1.1	5.7	32.9
	Bacilli	0	0	2.4
Actinobacteria	Actinobacteria	0	3.5	22.0
Chloroflexi	Chloroflexi	0	2.3	0
Other group or unclassified bacteria		7.4	3.4	11.0

Significant increases in comparison to SS are underlined; Significant reductions are highlighted (dark background).

only a slight increase in the AR cloning library. All three clone libraries include highly diverse microbial communities with limited sharing of very few bacterial species. Only two OTUs were common between DR and SS (OTU69 and OTU76), whereas two other OTUs were common between DR and AR: OTU82 (Acidovorax) and OTU59 (Clostridiaceae), shown in Fig. 2A and B, respectively. The UNIFRAC distance values of DR and AR, DR and SS, AR and SS are 0.8404, 0.8795 and 0.9509, respectively, and show significant differences between the three clone libraries.

Bacteroidetes and Proteobacteria populations dominated the early communities of SS samples but changed to two different communities during the acclimation of DR and AR bioreactors (Table 1). Some bacterial groups, for example, Actinobacteria, Choroid, Verrucomicrobia, and Bacteroidetes, were washed out of the community. However, there were specific effects due to acclimating conditions on microbiota in the bioreactors. For example, δ-proteobacteria, Clostridia and Actinobacteria greatly increased in the AR reactor, whereas β-proteobacteria significantly increased in the DR reactor, becoming a dominant but smaller population in AR (Table 1).

4. Discussion

Due to the higher degrading efficiencies, denitrification treatment technology is widely applied to treat wastewater-containing NHCs. However, the microbial ecology of the denitrifying degrading community is far from being understood, despite studies on a few isolates. Using quinoline and glucose as the carbon source, in a previous study we characterized the microbial community structure in an acclimated denitrifying bioreactor. Community diversity decreased significantly after 6 weeks of acclimation, and bacteria from a smaller group dominated the acclimated community (Liu et al., 2006). In the present study, we investigated another denitrifying community acclimated from the same SS, using indole and glucose as the main carbon source. Although the population of β-proteobacteria was also enriched during acclimation, an indole-acclimated denitrifying community maintained high microbial diversity in contrast to a quinoline-acclimated community. This may be due to the lower toxicity of indole than quinoline to microorganisms. When compared with the bacterial community of quinoline in a denitrifying, degradative bioreactor, we found only one *Thauera* bacteria (OUT-51) being commonly shared between these two bioreactors (Hong et al., 2008). OTU-51 was the most abundant species found in a quinoline-degrading community but only 2.3% of the population in the indole-degrading community.

Denitrifying β-proteobacteria are the most efficient bacteria in breaking down aromatic compounds in various environments, which include bioreactors used in industrial wastewater treatment (Lemmer et al., 1994; Miura et al., 2007; Nielsen and Nielsen, 2002; Snaidr et al., 1997; Sofia et al., 2004). We found that 56.3% of the total clones in DR bioreactors belong to the β-proteobacteria. *Alicycliphilus* (DQ342277), a member of Comamondadceae, which was the most common and dominant bacterium in the DR reactor with respect to the 16S rRNA gene. DQ342277, obtained from a chlorate-reducing enrichment culture (Weelink et al., 2007) is benzene-degrading and most similar to the predominant bacterium in the DR, with respect to the 16S rRNA gene. In another study, we found that bacteria similar to *Alicycliphilus denitrificans* (K601) became dominant in an anoxic denitrifying bioreactor of a coking wastewater treatment plant where SS was collected from (unpublished data). The denitrifying degradation of aromatic compounds by Comamonadaceae bacteria has been reported (Boon et al., 2001; Khan et al., 2002). In our DR samples, analyzed sequences from *Alcaligenes* were dominant. Among isolates from coking wastewater treatment plants, 96 strains of phenol-degrading *Alcaligenes* sp. were dominant denitrifiers (Zhang et al., 2004). Indole degradation by bacteria of *Alcaligenes* had also been reported previously (Claus and Kutzner, 1983; Madsen and Bollag, 1989). Another important group in DR bioreactors is *Thauera*-related bacteria. The *Rhodocyclaceae*, including *Thauera*, *Azoarcus* and other closely related species, were found frequently in wastewater denitrifying communities (Ginige et al., 2005; Juretschko et al., 2002; Liu et al., 2006; Mechichi et al., 2002; Song et al., 2001; Zhou et al., 1995). *Rhodocyclaceae* are versatile bacteria for aromatic compounds degradation (Thomsen et al., 2007). *Thauera* bacteria were among the dominant bacterial species in coking wastewater treatment bioreactors (Mao et al., 2008) and in quinoline-acclimated bioreactors (Liu et al., 2006). *Ochrobactrum* sp. M231 (EU604246), abundant in DR, was isolated previously from a quinoline-degradating bioreactor acclimated using the same seeding sludge (Hong et al., 2008).

To understand further the effects of electron acceptors on the composition of the microbial community, we compared the microbial community structures of two anaerobic bioreactors (DR and AR). They were acclimated from the same seeding sludge under similar conditions (for example, dissolved oxygen concentration, hydraulic retention time) except that a different electron acceptor supplemented the synthetic wastewater. Different microbial communities formed in the two bioreactors. β-proteobacteria denitrifiers were almost non-existent, whereas Clostridia and Actinobacteria bacteria became the dominant species in the AR bioreactor. Sequences of a dominant OTU in an AR reactor were closely associated with Clostridia, cloned from UASB granular sludge microbial community used in treating phenol wastewater. Another dominant OTU in an AR reactor was closely related to Propionicimonas bacteria, which contain strains reported to be trichlorobenzene-degradative (von Wintzingerode et al., 1999).

We found higher indole degradating efficiency when the bioreactor operated under denitrifying conditions than when it operated under sulfate-reducing conditions. The analysis of bacterial community structures showed that the electron acceptors used in two different conditions had a strong effect on the community composition. The difference between predominant bacteria was due to differences in the two electron acceptors, causing discrepancy in the indole-degrading capacity of the two bioreactors.

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References

Bak, F., Widdel, F., 1986. Anaerobic degradation of indolic compounds by sulfate-reducing enrichment cultures, and description of *Desulfobacterium indolicum* gen. nov., sp. nov. Arch. Microbiol. 146, 470–476.

Berry, D., Madsen, E., Bollag, J., 1987. Conversion of indole to oxindole under methanogenic conditions. Appl. Environ. Microbiol. 53, 180–182.

Boon, N., Goris, J., De Vos, P., Verstraete, W., Top, E.M., 2001. Genetic diversity among 3-chloroaniline- and aniline-degrading strains of the Comamonadaceae. Appl. Environ. Microbiol. 67, 1107–1115.

Claus, G., Kutzner, H.J., 1983. Degradation of indole by *Alcaligenes* sp. Syst. Appl. Microbiol. 4, 169–180.

Dagher, F., Déziel, E., Lirette, P., 1997. Comparative study of five polycyclic aromatic hydrocarbon degrading bacterial strains isolated from contaminated soils. Can. J. Microbiol. 43, 368–377.

Dimitriu, P.A., Pinkart, H.C., Peyton, B.M., Mormile, M.R., 2008. Spatial and temporal patterns in the microbial diversity of a meromictic soda lake in Washington State. Appl. Environ. Microbiol. 74, 4877–4888.

Ginige, M., Keller, J., Blackall, L., 2005. Investigation of an acetate-fed denitrifying microbial community by stable isotope probing, full-cycle rRNA analysis, and fluorescent in situ hybridization–microautoradiography. Appl. Environ. Microbiol. 71, 8683–8691.

Gu, J., Berry, D., 1992. Metabolism of 3-methylindole by a methanogenic consortium. Appl. Environ. Microbiol. 58, 2667–2669.

Gu, J., Fan, Y., Shi, H., 2002. Relationship between structures of substituted indolic compounds and their degradation by marine anaerobic microorganisms. Mar. Pollut. Bull. 45, 379–384.

Hong, X., Liu, B., Zhang, X., Zhao, L., 2008. Comparison of microbial community structure of quinoline and indole acclimated denitrifying bioreactor. Wei Sheng Wu Xue Bao 48, 503–507.

Hwang, C., Wu, W., Gentry, T.J., Carley, J., Corbin, G.A., Carroll, S.L., Watson, D.B., Jardine, P.M., Zhou, J., Criddle, C.S., Fields, M.W., 2009. Bacterial community succession during in situ uranium bioremediation: spatial similarities along controlled flow paths. ISME J. 3, 47–64.

Johansen, S.S., Licht, D., Arvin, E., Mosbæk, H., Hansen, A.B., 1997. Metabolic pathways of quinoline, indole and their methylated analogs by *Desulfobacterium indolicum* (DSM 3383). Appl. Microbiol. Biotechnol. 47, 292–300.

Juretschko, S., Loy, A., Lehner, A., Wagner, M., 2002. The microbial community composition of a nitrifying-denitrifying activated sludge from an industrial sewage treatment plant analyzed by the full-cycle rRNA approach. Syst. Appl. Microbiol. 25, 84–99.

Khan, S., Horiba, Y., Yamamoto, M., Hiraishi, A., 2002. Members of the family Comamonadaceae as primary poly(3-hydroxybutyrate-co-3-hydroxyvalerate)-degrading denitrifiers in activated sludge as revealed by a polyphasic approach. Appl. Environ. Microbiol. 68, 3206–3214.

Kirchman, D.L., Cottrell, M.T., Lovejoy, C., 2010. The structure of bacterial communities in the western Arctic Ocean as revealed by pyrosequencing of 16S rRNA genes. Environ Microbiol..

Lane, D.J., Pace, B., Olsen, G.J., Stahl, D.A., Sogin, M.L., Pace, N.R., 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc. Natl. Acad. Sci. U. S. A. 82, 6955–6959.

Lau, M.C., Aitchison, J.C., Pointing, S.B., 2009. Bacterial community composition in thermophilic microbial mats from five hot springs in central Tibet. Extremophiles 13, 139–149.

Lee, C., Kim, J., Shin, S.G., Hwang, S., 2008. Monitoring bacterial and archaeal community shifts in a mesophilic anaerobic batch reactor treating a high-strength organic wastewater. FEMS Microbiol. Ecol. 65, 544–554.

Lemmer, H., Roth, D., Schade, M., 1994. Population densities and enzymatic activities of heterotrophic bacteria in sewer biofilms and activated sludge. Water Res. 28, 1341–1346.

Li, Y., Li, W., Gu, G., 2006. Cometabolic biodegradation of nitrogenous heterocyclic compounds indole and pyridine under anoxic conditions. Huan Jing Ke Xue 27, 300–304.

Liu, B., Zhang, F., Feng, X., Liu, Y., Yan, X., Zhang, X., Wang, L., Zhao, L., 2006. *Thauera* and *Azoarcus* as functionally important genera in a denitrifying quinoline-removal bioreactor as revealed by microbial community structure comparison. FEMS Microbiol. Ecol. 55, 274–286.

Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71, 8228–8235.

Madsen, E., Bollag, J.-M., 1989. Pathway of indole metabolism by a denitrifying microbial community. Arch. Microbiol. 151, 71–76.

Madsen, E., Francis, A., Bollag, J., 1988. Environmental factors affecting indole metabolism under anaerobic conditions. Appl. Environ. Microbiol. 54, 74–78.

Mao, Y., Zhang, X., Yan, X., Liu, B., Zhao, L., 2008. Development of group-specific PCR-DGGE fingerprinting for monitoring structural changes of *Thauera* spp. in an industrial wastewater treatment plant responding to operational perturbations. J. Microbiol. Methods 75, 231–236.

Mechichi, T., Stackebrandt, E., Gad'on, N., Fuchs, G., 2002. Phylogenetic and metabolic diversity of bacteria degrading aromatic compounds under denitrifying conditions, and description of *Thauera phenylacetica* sp. nov., *Thauera aminoaromaticasp*. nov., and *Azoarcus buckelii* sp. nov. Arch. Microbiol. 178, 26–35.

Miller, S.R., Strong, A.L., Jones, K.L., Ungerer, M.C., 2009. Bar-coded pyrosequencing reveals shared bacterial community properties along the temperature gradients of two alkaline hot springs in Yellowstone National Park. Appl. Environ. Microbiol. 75, 4565–4572.

Miura, Y., Watanbe, Y., Okabe, S., 2007. Membrane biofouling in pilotscale membrane bioreactors (MBRs) treating municipal wastewater: impact of biofilm formation. Environ. Sci. Technol. 41, 632–638.

Nielsen, J., Nielsen, P., 2002. Enumeration of acetate-consuming bacteria by microautoradiography under oxygen and nitrate respiring conditions in activated sludge. Water Res. 36, 421–428.

Snaidr, J., Amann, R., Huber, I., Ludwig, W., Schleifer, K., 1997. Phylogenetic analysis and in situ identification of bacteria in activated sludge. Appl. Environ. Microbiol. 63, 2884–2896.

Sofia, A., Liu, W.T., Ong, S.L., Ng, W.J., 2004. In situ characterization of microbial community in an A/O submerged membrane bioreactor with nitrogen removal. Water Sci. Technol. 50, 41–48.

Song, B., Palleroni, N.J., Kerkhof, L.J., Haggblom, M.M., 2001. Characterization of halobenzoate-degrading, denitrifying *Azoarcus* and *Thauera* isolates and description of *Thauera chlorobenzoica* sp. nov. Int. J. Syst. Evol. Microbiol. 51, 589–602.

Thomsen, T.R., Kong, Y., Nielsen, P.H., 2007. Ecophysiology of abundant denitrifying bacteria in activated sludge. FEMS Microbiol. Ecol. 60, 370–382.

von Wintzingerode, F., Selent, B., Hegemann, W., Gobel, U.B., 1999. Phylogenetic analysis of an anaerobic, trichlorobenzene-transforming microbial consortium. Appl. Environ. Microbiol. 65, 283–286.

Wang, Y.T., Suidan, M.T., Pfeffer, J.T., 1984. Anaerobic biodegradation of indole to methane. Appl. Environ. Microbiol. 48, 1058–1060.

Weelink, S.A., Tan, N.C., Ten Broeke, H., van Doesburg, W., Langenhoff, A.A., Gerritse, J., Stams, A.J., 2007. Physiological and phylogenetic characterization of a stable benzene-degrading, chlorate-reducing microbial community. FEMS Microbiol. Ecol. 60, 312–321.

Zhang, X., Gao, P., Chao, Q., Wang, L., Senior, E., Zhao, L., 2004. Microdiversity of phenol hydroxylase genes among phenol-degrading isolates of *Alcaligenes* sp. from an activated sludge system. FEMS Microbiol. Lett. 237, 369–375.

Zhou, J., Fries, M., Chee-Sanford, J., 1995. Phylogenetic analyses of a new group of denitrifiers capable of anaerobic growth of toluene and description of *Azoarcus tolulyticus* sp. nov. Int. J. Syst. Bacteriol. 45, 500–506.