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Development of group-specific PCR-DGGE fingerprinting for monitoring structural changes of *Thauera* spp. in an industrial wastewater treatment plant responding to operational perturbations

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ABSTRACT

A *Thauera*-specific nested-PCR denaturing gradient gel electrophoresis (DGGE) method was developed, and its usefulness was demonstrated by monitoring the structural shifts of *Thauera* spp. in an anaerobic-anoxic-oxic fixed-biofilm coking wastewater treatment plant (WWTP) responding to operational perturbations. The specificity of the PCR method was confirmed by the fact that all 16 S rRNA gene sequences, cloned from the amplicons of a biofilm sample, belonged to *Thauera* genus. 16 S rRNA gene V3 region was then amplified from the first round *Thauera*-specific PCR product and applied for DGGE analysis. All *Thauera* clones, with 13 different V3 regions, migrated into 10 positions on DGGE gel, which demonstrated the high resolution of this DGGE method. When the WWTP experienced a gradual deterioration in chemical oxygen demand (COD) removal function due to a mechanical failure of the recirculation pump, biofilm samples were collected from the reactor and analyzed by this method. Principal component analysis (PCA) of the DGGE fingerprinting data showed that the composition of *Thauera* group exhibited a time related trajectory when the plant's COD removal rate decreased from 84.1 ± 2.7% in the first 4 weeks to less than 75% at week 5 and 6, suggesting a concomitant shift of *Thauera* composition and the system's COD removal function. This group-specific PCR DGGE fingerprinting technology has the potential to be a profiling tool for monitoring structural shifts of *Thauera* spp. in industrial WWTPs.

1. Introduction

Structural and functional dynamics of microbial communities in full scale WWTP can be monitored to help engineers in attempts to optimize and stabilize the performance of their plants (Yuan and Blackall, 2002). Previous studies have shown that certain individual populations within a community can have crucial impacts on specific functions of an ecosystem, such as ammonia-oxidizing bacteria (AOB) (Sakano et al., 2002), nitrite-oxidizing bacteria (NOB) (Schramm et al., 1996) and polyphosphate-accumulating bacteria (PAB) (Kortstee et al., 1994). Fluctuation of specific functions of an industrial WWTP may be more directly related to structural changes of functional populations. Therefore, it may be more effective to study the functionally important populations instead of the whole community when investigating the links between community structure and its functions in industrial WWTPs which contain complex microbial communities exerting diverse functions.

In our previous study on a laboratory-scale bioreactor, we found that *Thauera* spp. may be closely associated with quinoline degradation (Liu et al., 2006). The abundance of *Thauera* spp. was dramatically increased (from 4% to 56%) during the acclimation period when the quinoline removal rate was gradually increased from undetectable to 90.2% (Liu et al., 2006). During the past decade, *Thauera* spp. has been found in many other WWTPs as important organic compound degraders (Valle et al., 2004). Studies based on the *Thauera* spp. isolates also showed that they can degrade many kinds of organic compounds, mainly aromatic compounds, such as phenol (Schuhle and Fuchs, 2004), polyphenol (Dibenedetto et al., 2006), halobenzoate (Song et al., 2000) and toluene (Biegert et al., 1996; Shinoda et al., 2004). *Thauera* spp. is thus a critical population for organic load degradation in many industrial WWTPs. Compositional changes in this important group may be closely related with the COD removal functions of these WWTPs. Thus, the structural shifts of *Thauera* spp. may be explored as an important biological indicator for population optimization and performance monitoring.

Many biological methods have been developed for community structural analysis in the past decades, but we still don't have an ideal approach to specifically analyze the structure of *Thauera* spp. 16 S rRNA gene clone library analysis is an effective method for community structural analysis, which has been used in studying the composition of *Thauera* spp. (Liu et al., 2006). But it is difficult to apply it into multiple samples comparison in ecological studies (Eschenhagen

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et al., 2003), more difficult to use it in full-scale WWTP for community structural monitoring. High throughput microarray method has been developed for *Thauera* spp. study (Loy et al., 2005), but it cannot detect unknown species which are very common in industrial WWTPs.

Thus, new approach for structural analysis of this functionally important population is needed. Group specific PCR based DGGE has already been developed in environmental ecological studies, such as AOB specific PCR-DGGE (Kowalchuk et al., 1997; Kowalchuk et al., 1999). But to best of our knowledge, there was no study has employed such fingerprinting technique to profile the structure of *Thauera* spp.

In this study, a *Thauera*-specific PCR based DGGE method was developed and its specificity was confirmed with clone library method. Temporal structural shifts of this functionally important population in a full-scale coking wastewater treatment system were analyzed using this method. It was found that the structure of this population was shifted concomitantly with the functional changes of the whole system when the operating condition was changed, demonstrating the potential of this method for monitoring COD removal performance.

2. Materials and methods

2.1. Coking wastewater treatment plant

One coking wastewater treatment plant in Shanghai (China) was used in this study. It is an anaerobic-anoxic-oxic (A1-A2-O) system, in which A2 and O tanks were packed with semisoft fibrous media where biofilms were formed. Effluent of O tank was partially recirculated back to A2 tank at a ratio of 10:1. The average influent flow rate of this WWTP is about 150 m^3/h, the hydraulic retention time (HRT) of A1, A2, O tanks are 6 h, 10 h and 23 h respectively. The temperature of this WWTP was between 25 °C and 38 °C. COD was measured by the standard method which was described by Greenburg et al. (Greenberg, 1992).

2.2. Sampling and DNA extraction

Biofilm samples were collected by scraping the surface of the supporting fibrous media in the center of A2 and O tanks every week during the six weeks sampling period. Samples were pretreated and DNA was extracted according to the method described by Liu et al. (2006). The DNA concentration was determined by DyNA quant™ 200 (Amersham Pharmacia Biotech, USA) and was also checked by electrophoresis on a 0.8% (wt/vol) agarose gel.

2.3. Specificity and sensitivity evaluation of Thauera-specific primers

Thauera-specific probe Thau832 (5′-TGC ATT GCT GCT CCG AAC-3′) has been designed by Loy et al. (2005) for microarray hybridization (Loy et al., 2005). We used this sequence as a reverse primer for *Thauera*-specific PCR. The specificity and sensitivity of this primer was reevaluated against to the most updated database using Probe Match project on Ribosomal Database Project II (RDP) – Release 9 (http://rdp.cme.msu.edu/) by comparing to all the 16 S rRNA gene sequences longer than 1200 bp with the criterion of non-mismatch.

2.4. Thauera-specific PCR amplification and V3 region PCR amplification

A specific primer Thau832 and an universal primer P0 were used in the *Thauera*-specific PCR amplification process (Di Cello et al., 1997; Loy et al., 2005). The PCR mixture (25 μl) contained 1 U of Promega Taq DNA polymerase (Promega Co., USA), 1×PCR buffer (Mg^{2+} free), 2 mM $MgCl_2$, 10 pmol of each primer, 200 μM each deoxynucleoside triphosphate (dNTP), and 10 ng of extracted DNA. The samples were amplified in a thermocycler PCR system (PCR Sprint, Thermo Electron Corp.) using the program: 95 °C for 5 min; 25 cycles of 95 °C for 45 s, 60 °C for 1 min, and 72 °C for 1 min; and finally 72 °C for 6 min. The size of PCR products were assessed by electrophoresis on a 1.2% (wt/vol) agarose gel, and the concentration was determined by DyNA quant™ 200. The *Thauera*-specific PCR products were then diluted and used as templates for V3 region nested amplification with the primers P2 and P3 that were developed by Muyzer et al. (1993). The samples were amplified using the program: 94 °C for 4 min; 25 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min; and 72 °C for 6 min (Liu et al., 2006).

2.5. Clone library construction and analysis

Two clone libraries (a *Thauera*-specific clone library, TL and a universal bacterial 16 S rRNA gene clone library, WL) were constructed for the biofilm sample which was collected from A2 tank at the 3rd week. TL was constructed with the *Thauera*-specific PCR products (840 bp) which were amplified using primers P0 and Thau832. WL was constructed using the near full length 16 S rRNA gene fragments which were amplified using universal bacterial primers P0 and P6 (Di Cello et al., 1997), using the program: 95 °C for 5 min, followed by 20 cycles of 95 °C for 30 s, 56 °C for 30 s, 72 °C for 90 s; and 72 °C for 8 min (Eckburg et al., 2005). PCR products were analyzed by electrophoresis on a 1.2% agarose gel (wt/vol), stained with ethidium bromide (EB). Target bands were excised from gel and purified using DNA Gel Extraction Kit (V-gene Biotechnology Limited, Hangzhou, China) according to the manufacturer's instructions. Purified PCR products were ligated to pGEM-T easy vector (Promega Co., USA) according to the manufacturer's instructions and were electrotransformed to *Escherichia coli* DH10B competent cells.

Clones were sequenced by Chinese National Human Genome Center (Shanghai, China) using ABI PRISM 3730 DNA sequencer (Applied Biosystems). All the sequences were checked in Ribosomal Database Project II (RDP) – Release 8 using Chimera Check (http://rdp8.cme.msu.edu/html/analyses.html), and sequences with chimeric structure were discarded. The remaining sequences were aligned and classified in RDP database using RDP query 2.7 (http://www.simo.marsci.uga.edu/public_db/rdp_query.htm). All the sequences which were classified into *Thauera* genus were selected and used in the following analysis. Sequences were aligned, and distance matrix was computed by ARB (http://www.arb-home.de) (Ludwig et al., 2004). Sequences that had similarity higher than 99% were classified into one operational taxonomic unit (OTU) using DOTUR (http://www.plantpath.wisc.edu/fac/joh/dotur.html) based on the distance matrix (Schloss and Handelsman, 2005). The coverage of clone library was calculated as $[1-(n/N)]\times 100$, where n is the number of singletons and N the total number of sequences (Good, 1953). Phylogenetic tree was constructed based on the representative clone of each OTU by ARB using neighbor-joining method.

2.6. Denaturing gradient gel electrophoresis (DGGE) analysis of PCR amplicons

Thauera-specific V3 amplicons were analyzed on a 8% (wt/vol) polyacrylamide gel with the denaturing gradient from 40% to 58% (100% denaturant corresponds to 7 M urea and 40% deionized formamide) using Dcode System apparatus (Bio-Rad, Hercules, Calif.) (Muyzer et al., 1993). Electrophoresis was performed in 1×Tris-Acetate-EDTA (TAE) buffer at constant voltage (200 V) and temperature (60 °C) for 240 min. Gels were stained by SYBR Green I (Amresco, Solon, Ohio) and were photographed using a UVI gel documentation system (UVItec, Cambridge, UK).

2.7. Principal component analysis (PCA) of DGGE gels

Migration and intensity of DGGE bands were analyzed with Quantity One (version 4.4.0, Bio-Rad Laboratories, Hercules, California) according

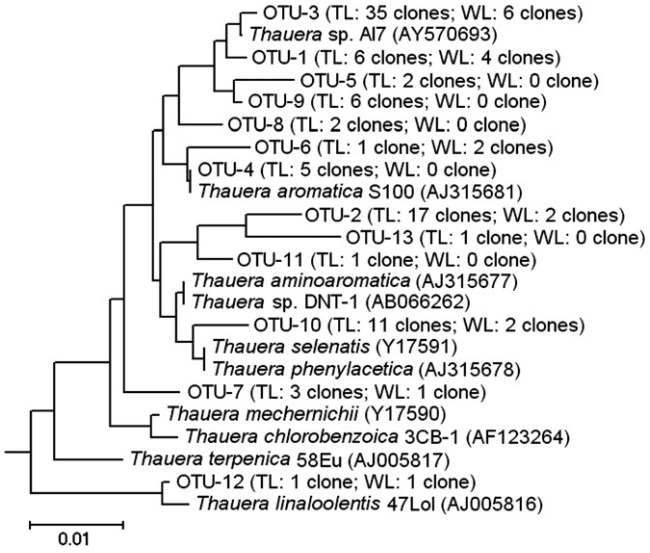


Fig. 1. Phylogenetic tree analysis of the representative clones of all the *Thauera* clones from both *Thauera*-specific clone library (TL) and universal bacterial 16 S rRNA gene clone library (WL). The tree was constructed by ARB using neighbor-joining method. Abundance of each OTU in these two clone libraries was appended in the bracket following each OTU. The bar represents for 1% estimated sequence divergence.

to the manual. The bands which shared identical migration position were considered as the same species. The matrix which contained the band's migration and intensity data was exported from Quantity One and analyzed in Matlab 7.04 (The Mathworks, Natick, MA). Before analysis, the band intensity data of DGGE were normalized so that the total intensity of bands in each lane was brought to 100. PCA was then performed with program princomp.m according to the manual.

2.8. Nucleotide sequence accession numbers

The sequences used in this study were deposited in GenBank with accession numbers EU369920–EU370028.

3. Results

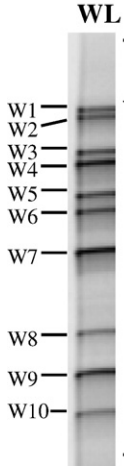
3.1. Establishment of Thauera-specific PCR method

In this study, universal forward primer and *Thauera*-specific reverse primer were used to develop *Thauera*-specific PCR method. Thau832, a *Thauera*-specific probe designed by Loy et al. (2005) was used as the reverse primer in our method. The specificity and sensitivity of this probe was first reevaluated against the most updated RDP database. Comparing with the existing 16 S rRNA gene sequences which were longer than 1200 bp length, we found that 96.2% of the perfectly matched sequences were affiliated to *Thauera* genus and the rest 3.8% were affiliated to unclassified betaproteobacteria. 90.5% of existing *Thauera* spp. sequences in the database perfectly matched with this primer. Among 45 *Thauera* isolates with 16 S rRNA gene sequences longer than 1200 bp, only one strain (*Thauera chlorobenzoica* 3BB1) did not match with this probe. The 16 S rRNA gene sequence of this strain had two insertions and one unidentified base in the primer binding region. These results demonstrated the high specificity and high sensitivity of this probe/primer for detection of known *Thauera* species.

Clone library analysis was then used to test the specificity and sensitivity of the *Thauera*-specific PCR with Thau832 as the reverse primer and the universal primer P0 as the forward primer. A *Thauera*-specific clone library (TL) was first constructed with the PCR products amplified from the 3rd A2 biofilm sample using this primer set. Totally, 91 sequences were obtained after 2 potential chimeric sequences were removed. RDP query analysis showed that all these clones in TL belonged to *Thauera* genus, indicating the high specificity of the *Thauera*-specific PCR process with this primer set. At 99% similarity cutoff, these 91 clones were classified into 13 OTUs with a library coverage of 93.4%.

To further evaluate the sensitivity of this primer set, a universal bacterial 16 S rRNA gene clone library (WL) was also constructed for the same sample. After removal of 5 potential chimeric sequences, 157 sequences were used for further analysis. RDP query analysis showed that 18 clones in WL belonged to *Thauera* genus which accounted for 11.46% of the clone library, indicating a quite high abundance of this population in the A2 tank of this plant. With the same 99% similarity cutoff, these 18 *Thauera* clones were classified into 7 OTUs.

All the 109 *Thauera* clones from these two libraries were pooled together for constructing a phylogenetic tree with the same 99% similarity cutoff for OTU differentiation (Fig. 1). The same set of 13 OTUs as those in TL were classified in this tree, which suggests that all the 7 *Thauera* phylotypes existed in WL was also detected by the *Thauera*-specific PCR method. These 7 phylotypes shared between these two libraries were generally predominant. In addition, compared to WL, 6 more OTUs of *Thauera* spp. were found in TL, showing high sensitivity of this method. This *Thauera*-specific PCR method thus provided deeper and more complete description of the species composition of *Thauera*.



Band	Richness in WL (%)[a]	OTU	Nearest species	Accession No.	Similarity (%)
W1	5.56	OTU-10	*Thauera* sp. B4P	AJ315678	99
W2	5.56	OTU-12	*Thauera linaloolentis* 47Lol	AJ005816	99
W3	5.56	OTU-10	*Thauera* sp. G3DM-88	EU037291	98
W4	22.22	OTU-2/6[b]	*Thauera* sp. R-24450	AM231040	98
W5	5.56	OTU-3	*Thauera* sp. Al7	AY570693	99
W6	5.56	OTU-3	*Thauera* sp. Al7	AY570693	99
W7	16.67	OTU-3	*Thauera aromatica* LG356	AJ315680	99
W8	5.56	OTU-3	*Thauera sp.* Al7	AY570693	99
W9	22.22	OTU-1/7[b]	*Thauera* sp. S2	AJ315677	99
W10	5.56	OTU-7	*Thauera aromatica* 3CB3	AF229882	99

Fig. 2. DGGE pattern of the 13 *Thauera* clones with unique V3 region and their relationship with OTUs in the clone library WL. [a]Richness in WL: corresponding clone richness of each band; [b]two OTUs in one band.

3.2. Development and evaluation of Thauera-specific PCR-DGGE method

After establishment of the *Thauera*-specific PCR, we tried to adapt it to a PCR-DGGE fingerprinting method. Due to the fact that the fragments (840 bp) achieved by *Thauera*-specific PCR with Thau832 and P0 was too long to be resolved on DGGE, the V3 regions which were embedded in these large fragments were reamplified and used for DGGE analysis. 13 *Thauera* clones with different V3 regions from WL were used in this *Thauera*-specific PCR-DGGE method development. Target fragments were amplified from these clones, followed by nested PCR with primers P2 and P3 (Muyzer et al., 1993), V3 regions of these clones were achieved for optimizing the denaturing gradient of DGGE. Using the denaturing gradient from 40% to 58%, the 13 different V3 fragments migrated into 10 different positions (Fig. 2), showing relatively high resolution of this *Thauera*-specific PCR-DGGE. More diverse V3 fragments migrating behaviors were found in DGGE profile compared to the number of phylotypes (7 OTUs in WL) which were divided based on the large *Thauera*-specific fragments. 3 OTUs in WL (OTU-3, OTU-7 and OTU-10) each had more than one type of V3 regions, and exhibited multiple bands on DGGE (Fig. 2). On the other hand, 2 types of V3 regions with the same migration behavior (band W4 and W9) each contained two different OTUs.

The 3rd A2 biofilm sample was analyzed by this *Thauera*-specific PCR-DGGE method. Comparing to V3-DGGE analysis of WL (10 bands) made from the same sample, 4 predominant bands (T3, T5, T7 and T8) were detected (lane 3rd A2 sample, Fig. 3). Comigration analysis showed that bands T3, T5, T7 and T8 were identical to W1, W4, W7 and W9 respectively. Although only 4 out of the 13 V3 fragments in WL were detected, these 4 bands already covered 66.7% *Thauera* clones and 6 OTUs (85.7%) in WL (Fig. 2).

The representative of corresponding clones of each predominant band was analyzed in NCBI (http://www.ncbi.nlm.nih.gov/blast) (Fig. 2). The corresponding clones of band T3, T5, T7 and T8 shared very high similarity with *Thauera* sp. B4P (99%), *Thauera* sp. R-24450 (98%), *Thauera aromatic*a LG356 (99%) and *Thauera* sp. S2 (99%) respectively (Mechichi et al., 2002; Heylen et al., 2006). Strain B4P and LG356 were isolated from sewage WWTP activated sludge, S2 from anoxic ditch sludge, all of these strains showed aromatic compound degradation capability (Mechichi et al., 2002). Strain R-24450 was isolated from activated sludge of a municipal WWTP, and was identified as a denitrifier which contained nitrite reductase gene *nirS* (Heylen et al., 2006). These results imply that these predominant *Thauera* species may represent the important COD removers in this WWTP.

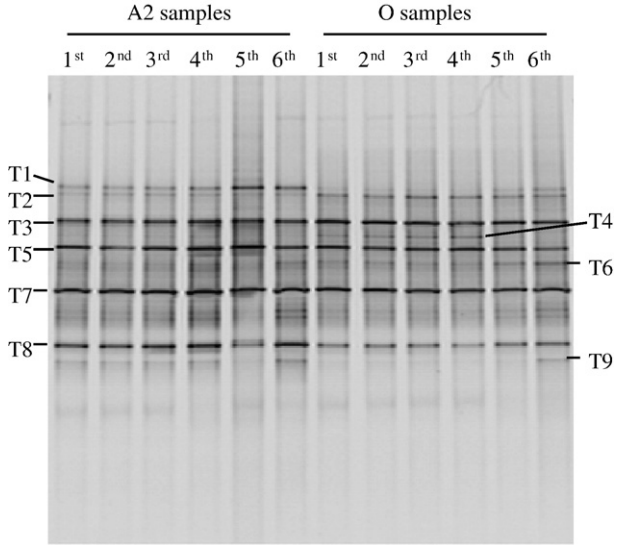


Fig. 3. *Thauera*-specific PCR-DGGE analysis of biofilm samples from A2 and O tanks of a coking wastewater treatment plant (WWTP) during a 6 weeks sampling period. The denaturing gradient was from 40% to 58%. 1st–6th: six sampling time points from week 1 to week 6. A2 samples: biofilm samples collected from the center of anoxic (A2) tank; O samples: biofilm samples collected from the center of oxic (O) tank.

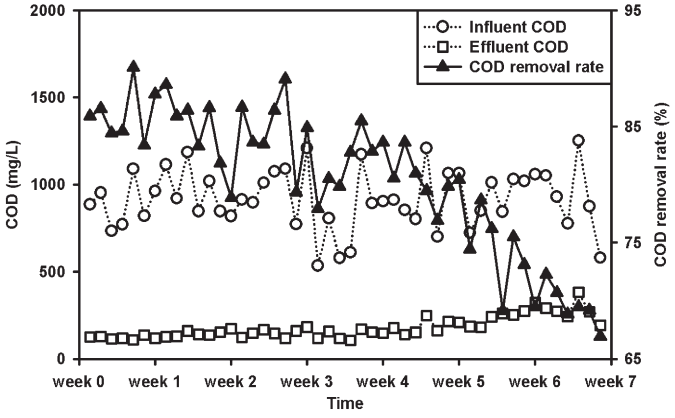


Fig. 4. COD removal efficiency of the coking wastewater treatment plant (WWTP). The day collected the first biofilm sample was defined as week 1, the recirculation flow was stopped one day after week 4 sampling time point.

3.3. Structural monitoring of Thauera spp. in the coking WWTP responding to operational perturbations

Removal of COD is one of the main functions of the A1-A2-O coking WWTP. During the six weeks sampling period, in the first four weeks (1st, 2nd, 3rd and 4th), the WWTP was under normal operating conditions, and COD removal efficiency ranged from 78.9% to 90.1%. One day after the 4th sampling time point, the recirculation pump, which used to pump partial effluent of O tank back into A2 tank, was stopped due to mechanical failure. It means denitrification in A2 tank would be stopped due to lack of electron acceptor (nitrate and nitrite which were produced by nitrification in O tank). The COD removal rate was then gradually decreased to 74.4% at week 5 and 69.5% at week 6 (Fig. 4).

The compositions of *Thauera* spp. in the 12 biofilm samples obtained from both A2 and O tank of the coking WWTP during the six weeks period were analyzed by this *Thauera*-specific PCR-DGGE method (Fig. 3, the result was repeated twice). Totally nine discernable bands were detected. The four predominant bands (band T3, T5, T7 and T8) remained relatively unchanged during the whole period. Structural shifts due to changes of the other relatively minor bands were observed in both tanks.

PCA was used in this study to analyze the DGGE patterns of these12 samples (Fig. 5). First two principal components (PC1 and PC2)

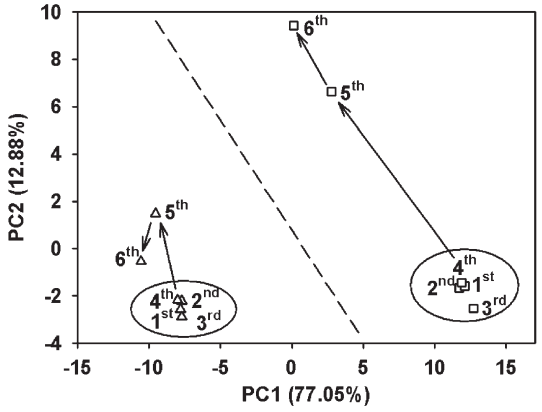


Fig. 5. Score plot of PCA analysis of *Thauera*-specific PCR-DGGE profile. (△): biofilm samples collected from anoxic (A2) tank; (□): biofilm samples collected from oxic (O) tank. 1st–6th: six sampling time points from week 1 to week 6.

explained considerable variance (PC1 = 77.05% and PC2 = 12.88%). In PCA score plot (PC1 vs PC2), A2 samples were grouped negatively, while O samples grouped positively along the PC1 axis, indicating that A2 and O samples were clearly distinct from each other. Time related trajectories were found both in A2 and O tanks before and after the perturbation. The first four sampling points (1st, 2nd, 3rd and 4th) of both A2 and O samples were each grouped together with low PC2 scores. One day after 4th sampling time point, the recirculation pump was stopped. In the following two weeks period, the 5th and 6th sampling points moved from space with lower PC2 scores to higher scores. At the same time, COD removal rate of the whole system was decreased from 84.1 ± 2.7% to less than 75%. The loadings of band T1, T4 and T6 were plotted far from the zero point along the PC1 loading axis, suggesting that these three bands made significant contributions to separation between A2 and O samples. Loadings of band T4 and T6 were plotted far from the zero point along the PC2 loading axis, suggesting that these two bands were significantly changed in response to operational perturbations (data not shown). These results revealed that the fluctuation of COD removal efficiency was closely associated with the structural shifts of *Thauera* spp. in the WWTP.

4. Discussion

Thauera spp. with high versatile organic substrate degrading capacity (Biegert et al., 1996; Schuhle and Fuchs, 2004; Dibenedetto et al., 2006) was shown to be quite abundant and important in many WWTPs (Valle et al., 2004; Liu et al., 2006; Thomsen et al., 2007). Therefore, a *Thauera*-specific PCR based DGGE method was developed in this study to specifically profile the structure of this population. Its structural shifts in a full-scale coking WWTP were then monitored for investigating the relationship between the composition of *Thauera* spp. and the COD removal function of the system.

In recent years, group specific PCR has increasingly been proven as a powerful tool, not only in structural analysis of complex microbial communities, but also in detection, isolation, characterization and identification of targeted bacteria (Yamada et al., 1999; De Clerck et al., 2004). In most of these specific PCR methods, both the forward and reverse primers are group-specific. Alternatively, in our study a semi-specific primer set was used for *Thauera*-specific PCR amplification. Although similar strategy has already been used in investigations of *Clostridium leptum* subgroup (Shen et al., 2006), *Paenibacillus* spp. (da Silva et al., 2003), *Bacillus* spp. (Garbeva et al., 2003) and ammonia oxidizing bacteria (Calvo et al., 2004), our understanding on the reliability of this kind of selective PCR method was still limited. Therefore, the performance of this group-specific PCR method was carefully evaluated by a *Thauera*-specific clone library. Just as expected, this primer set has high specificity and all the sequenced clones belonged to *Thauera* genus. Although unspecific amplification has been found in previous studies (da Silva et al., 2003; Garbeva et al., 2003; Calvo et al., 2004; Shen et al., 2006), this was possibly due to the limitation of the specific primer they used which can capture some species out of the target group (da Silva et al., 2003; Garbeva et al., 2003), not due to the combination of a universal primer. The quality of the specific primer can thus assure the specificity of this PCR method.

In addition to specificity, sensitivity was another important aspect of group-specific PCR method. Compared to clone library constructed with universal primer set (WL), approximately two times more *Thauera* phylotypes (OTUs) were detected in the *Thauera*-specific clone library (TL). In addition, all the phylotypes existed in WL were covered by TL. This indicates a much higher sensitivity of this group specific PCR method compared to universal PCR method.

Group-specific PCR-DGGE analysis based on nested amplification of the hypervariable V3 regions from larger group specific fragments has been successfully used by Freitag et al. in ammonia oxidizing bacteria analysis (Freitag and Prosser, 2003). Our work also showed the high resolution of this method, in which 13 different V3 fragments were resolved into 10 DGGE bands. The method also has relatively high coverage, where 66.7% clones or 85.7% of OTUs were detected. Combining with relative high throughput, these advantages make it suitable for monitoring the structural shifts of *Thauera* spp. in full-scale WWTPs in relation to functional changes caused by operational perturbations.

It has been well recognized that the performance of a WWTP is mainly determined by the structure and activity of its microbial community (Wagner et al., 2002; Cook et al., 2006). However, our understanding on the relationship between microbial community structure and function is still limited and even contradictory. It has been shown that the substrate removal function of bioreactors changed concomitantly with microbial community structural shifts in response to perturbation (LaPara et al., 2000) or during acclimation (Forney et al., 2001; LaPara and Ghosh, 2006; Liu et al., 2006), and stable function was associated with stable community structure (Ayala-Del-Rio et al., 2004). On the other hand, it was found that in a functionally stable bioreactor the microbial community structure was fluctuating (Fernandez et al., 1999; Fernandez et al., 2000), which may be due to the phenomenon that the same physiological function can be shared by different microorganisms i.e. the functional redundancy of the microbial community (Franklin and Mills, 2006). Furthermore, a more stable community showed less functional stability in response to glucose perturbation (Fernandez et al., 2000; Hashsham et al., 2000). This may be due to the changes of functionally important but quantitatively minor populations, which may escape the detection by community structural analysis targeting general populations.

Thauera spp. can live, and may play important roles both in anoxic and oxic conditions (Biegert et al., 1996; Shinoda et al., 2004). Therefore, in this study, biofilm samples were collected from both anoxic (A2) and oxic tank (O) of the coking WWTP for *Thauera*-specific PCR-DGGE analysis. DGGE fingerprinting showed that the predominant *Thauera* species in these two tanks were quite similar. This is mainly due to the high flexibility of *Thauera* species in switching between denitrification and aerobic respiration (Shinoda et al., 2004; Mao et al., unpublished), and also due to the well communication of microorganisms between these two tanks. The predominant *Thauera* spp. may represent the important COD remover in this system (Liu et al., 2006), because phylogenetic analysis of their corresponding clones showed that they were close to the aromatic compound degraders. After perturbation, time related trajectory analysis with PCA score plot of *Thauera*-specific DGGE profile showed that the structure of *Thauera* spp. was shifted concomitantly with the fluctuation of COD removal function of the whole system, which was in agreement with LaPara's studies (LaPara et al., 2000; LaPara et al., 2002). And more significant community structural shifts were observed in the O tank, where more COD was removed than in the A2 tank. Band T4 and T6, which were more significantly changed in response to perturbation, may represent species sensitive to environmental fluctuations, and may be used as indicators for monitoring performance of WWTPs (Hartmann and Widmer, 2006).

In conclusion, a *Thauera*-specific PCR-DGGE method was developed in this study, and its specificity and sensitivity were confirmed by clone library method. Combined with principal component analysis (PCA), this approach has been successfully applied to monitor a full-scale WWTP in response to perturbations. The result showed that the structural shifts of this population were closely associated with the functional fluctuation of the whole system. This method has the potential to be used as a tool for engineers in diagnosis and optimization of microbial community structures in WWTPs for best performance.

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