# Molecular characterization of bacterial community in aerobic granular sludge stressed by pentachlorophenol

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**Abstract**

To characterize the effects of pentachlorophenol (PCP) on the performance and microbial community of aerobic granular sludge in sequencing batch reactor (SBR), the web-based terminal restriction fragment length polymorphism (T-RFLP) and real-time PCR (RT-PCR) techniques were used to explore the bacterial community structure. When PCP increased from 0 to 50 mg/L, the COD removal rate changed little, while the ammonia removal rate dropped from 100% to 64.9%. The results of molecular characterization showed that the quantity of ammonia oxidizing bacteria (AOB) kept constantly, although the number of bacteria species decreased with the increase of PCP concentration. Significant shift in bacterial community structure at different PCP stresses was observed within aerobic granular sludge. When the PCP was absent, there are 69 strains in aerobic granular sludge detected by T-RFLP method. With the increase of PCP, most of bacteria disappeared and only 19 bacteria existed at all five PCP concentrations. These results contributed to comprehensive understanding of the microbial community structure under the PCP stress and its relationship with the performance for wastewater treatment by aerobic granular sludge.

**Key words**: aerobic granular sludge; bacterial community; real-time PCR; terminal restriction fragment length polymorphism; sequencing batch reactor; pentachlorophenol (PCP)

## Introduction

As a promising process in wastewater treatment, aerobic granular sludge possesses the advantages of both anaerobic granular sludge and aerobic activated sludge because of its granular structure and aerobic growth feature. Compared to conventional aerobic wastewater treatment systems, the granulation system is of several advantages, such as a dense and strong microbial structure, good settle ability, high biomass retention, and ability to tolerate high organic loading rate (Liu *et al.*, 2005). Many researches have focused on the fields including cultivation of aerobic granular sludge (Beun *et al.*, 2002; Liu *et al.*, 2004; Arrojo *et al.*, 2004), removal of nitrogen via simultaneous nitrification and denitrification (SND), and development of aerobic granulation process (Yang *et al.*, 2003; Hu *et al.*, 2005).

Recently, the aerobic granular sludge has been used for the treatment of industrial wastewater. Because the industrial wastewater always contains toxic substances, which will impair the microorganisms in bioreactors through inhibiting the activity of microbial cells or destructing the microbial community (Magbanua *et al.*, 1998). Up to date, many studies have been carried out to explore the effects of toxic compounds on the microbial community within the floc activated sludge. However, with a denser

and stronger granular structure, aerobic granular sludge may have different performance compared to the activated sludge (Tay *et al.*, 2002). Considering the limited knowledge about the effect of toxicants on microbial community in aerobic granular sludge and the different performances of the aerobic granular sludge, it is necessary to study the impact of toxic pollutants on the microbial community in aerobic granular sludge.

With the rapid development of molecular biological techniques, many microbial molecular ecological techniques have been applied to monitor the variance of the bacterial community in activated sludge (Forney *et al.*, 2004). Among these molecular methods, terminal restriction fragment length polymorphism (T-RFLP) seems to be a more practical and useful approach for bio-monitoring the bacterial community (Osborn *et al.*, 2000). On the other hand, because of the importance of the ammonia-oxidizing bacteria (AOB) in the nitrogen removal by aerobic granular sludge, it is interesting to understand the change of AOB quantity under the stress of toxic pollutants. To quantify the AOB in the aerobic sludge, a modified real-time PCR (RT-PCR) method was developed and used to monitor the AOB number in aerobic granular sludge.

As one of most popular toxicants, pentachlorophenol (PCP) was used in this study as a model toxic compound. Therefore, the objectives of this study were as follows:

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(1) to investigate the performance of the aerobic granular sludge in sequencing batch reactor under different concentrations of PCP in wastewater; and (2) to characterize the microbial community in aerobic granular sludge influenced by PCP using web-based T-RFLP and modified RT-PCR methods.

# 1 Materials and methods

## 1.1 Experiment setup and operation

Six Plexiglas columns with an internal diameter of 84 mm and a total height of 260 mm were used as sequencing batch reactors (R0 to R5). The total and working volume of the sequencing batch reactor (SBR) reactors were 1500 and 900 ml, respectively. All reactors were operated in a room with temperature controlled at $28 \pm 2°C$, and pH maintained between 7.0 and 8.0. Air was pumped into the bottom of the reactors, ensuring the dissolved oxygen (DO) concentration maintained in the range of 4.0 and 5.0 mg/L. The reactors were operated at a cycle time of 330 min. One cycle consisted of 20 min for feeding, 300 min for aeration, 2 min for settling, and 8 min for effluent withdrawal. Inoculated granular sludge was cultivated according to Wang *et al*. (2004). In operation stage, the MLSS in the reactors was maintained at about 16 g/L. Influent COD and ammonia nitrogen were 1400–1500 mg/L and 65–75 mg/L, respectively. For the PCP addition, 10, 20, 30, 40, and 50 mg/L PCP were added into reactors R1 to R5, respectively, and bioreactor R0 was served as a control. Before the removal tests, all SBR reactors were operated many cycles until the reactors reached stable.

## 1.2 Feed solution

A synthetic wastewater used in this study was consisted of nutrients and trace metal solution. The nutrient solution consisted of (per liter of distilled water): glucose 0.8 g, yeast extract 0.18 g, $KH_2PO_4$ 0.03 g, $MgSO_4·7H_2O$ 0.05 g, $NH_4Cl$ 0.14 g, $CaCl_2$ 0.04 g, and 2 ml trace metal solution. The composition of the trace metal solution was (per liter of distilled water): $H_3BO_3$ 0.15 g, $CoCl_2·6H_2O$ 0.15 g, $CuSO_4·5H_2O$ 0.03 g, $FeCl_3·6H_2O$ 1.5 g, $MnCl_2·2H_2O$ 0.12 g, $Na_2Mo_7O_{24}·2H_2O$ 0.06 g, $ZnSO_4·7H_2O$ 0.12 g, and KI 0.03 g. PCP was dissolved in 0.02 mol/L NaOH solution and diluted according to the required concentration (ranged from 10 to 50 mg/L).

## 1.3 DNA extraction

Sludge samples were collected from the six SBRs at different time for microbial analysis and preserved at −20°C for DNA extraction. Sludge DNA was extracted using a 3S DNA Isolation Kit (Shanghai Shenerg Biocolor Co., Ltd., China) and the protocol was followed as the manufacturer's instruction.

## 1.4 16S rDNA PCR amplification for T-RFLP analysis

Primers used in this experiment were forward primer 8-27F (5'-AGAGTTTGATCMTGGCTCAG-3'), and reverse primer 1378-1401R (5'-CGGTGTGTGTACAAGGCCCG GGAACG-3') (Heuer *et al*., 1997). The forward primer was labeled with 6-FAM (Shanghai GeneCore BioTechnologies Co., Ltd., China). A thermal cycler (MJ Research, MJ-PTC200, USA) was used for all reactions with the following conditions: each reaction mixture (50 μl) contained 50 ng templates DNA, 1× PCR polymerase buffer, 2.5 mmol/L $MgCl_2$, 0.2 mmol/L each deoxynucleotide triphosphate, 0.4 μmol/L each primer, and 2.5 U of *Taq* DNA polymerase (Fermentas Inc., Lithuania). The following conditions were chosen for amplification of 16S rDNA: 10 min at 94°C, followed by a pause to add *Taq* DNA polymerase, and then 30 cycles of 60 s at 94°C (denaturation), 30 s at 60°C (annealing), and 90 s at 72°C (elongation). PCR cycling was completed with a final elongation step of 10 min at 72°C. The PCR products were quantified in a 1% agarose gel stained by GoldView™ (SBS Genetech Co., Ltd., China) and by comparing the band intensities to the intensity of a λ-*Eco*T14 I digested DNA mass ladder (TaKara Bio Inc., Japan).

## 1.5 Restriction endonuclease digestion

Enzymes were chosen based on digested sequences in the GenBank database by TAP-program (Marsh *et al*., 2000). Enzymes that produced the greatest number of terminal restriction fragments (T-RFs) with different lengths from the bacterial 16S rDNA sequences were tested. All restriction endonuclease enzymes used were purchased from TaKara Bio Inc., Japan. The fluorescence-labeled PCR products were purified by using the DNA purification Kit (TaKara Bio Inc., Japan). Each DNA sample (150 ng) was digested with one of three restriction enzymes: *Alu*I (recognition site AG/CT), *Hha*I (recognition site GCG/C), and *Hae*III (recognition site GG/CC).

## 1.6 T-RFLP analysis

Digested PCR products were analyzed by Shanghai GeneCore BioTechnologies Co., Ltd. Samples were precipitated by ethanol and then dissolved in 9 μl of Hi-Di formamide (Applied Biosystems, USA), with 0.5 μl each of Genescan Rox 500 (Applied Biosystems, USA) size standards. The DNA was denatured at 95°C for 5 min and snap-cooled in ice for 10 min. Samples were run on an ABI Prism™ 310 Genetic Analyzer at 15 kV and 60°C. T-RFs size was performed on electropherogram output from Genescan software.

In this study, we used a web based analysis tool for T-RFLP analysis. This tool allowed the researcher to automate the task of phylogenetic assignment from T-RFs profiles produced by multiple digests (Kent *et al*., 2003). In brief, the user selects a database file, the associated digest files, and the bin sizes to use in the computation and then assigns a specific restriction enzyme present in the selected database to each of the digest files, and then the program runs the phylogenetic assignment tool (PAT) filtering algorithm to determine phylogenetic matches. The results contain three files. The first result file contains the phylogenetic matches. The record for each match contains the lane identification, matched organisms and their access numbers, peak area, and length of the fragment from

each digest. One second file contains an analysis of the completeness of the matching algorithm and the abundance of unmatched fragments from each restriction digest. The final file contains a list of unmatched fragments. It includes lane IDs and peak areas for T-RFs that were not matched to known organisms by the program.

### 1.7 Phylogenetic analysis

The phylogenetic trees were inferred with the neighbor-joining algorithm using the NEIGHBOR program in MEGA3 according to the following accession numbers, which were obtained through web search: X97070; X97071; AJ009448; AB015265; AJ009481; AJ223780; U40078; AJ009467; AF078758; AB025790; AB000699; AF170354; L35509; U48244; AF013527; AF013522; U33316; AJ241011; U85473; AJ240984; AJ009453; U75647; U14905; AF001636; AF121885; X93482; U89300; L33974; L33975; AF060671; AF050493; D14515; D14509; L04168; X55602; M24580; X73441; X55587; X62171; AF121886; M83548; AF114033; M59061; M34132; M34139; M24290; AF084947; X87275; AF099059; L06166; X78419; AF005749; M23932; L08642; X573037; X70321; M94277; M94278; M34114; M34125; AJ240990; AF099064; AF099065.

### 1.8 AOB quantification by improved real-time PCR

The forward primers CTO189fA/B (5'–3') GGAGRAAAGCAGGGGATCG and CTO189fC (5'–3') GGAGGAAAGTAGGGGATCG were used with a 2:1 ratio. The reverse primer is RT1r (5'–3') CGTCCTCTCAGACCARCTACTG. The primer set will produce 116 bp fragment. The Taqman probe TMP1 (5'–3') CAACTAGCTAATCAGRCATCRG CCGCTC was labeled with a reporter dye (6-carboxyfluorescein, FAM) attached at the 5' end and a quencher dye (6-carboxy-tetramethylrhodamine, TAMRA) attached at the 3' end. The primers and probe were designed specifically for the V2 region of the 16S ribosomal of AOB (Hermansson and Lindgren, 2001). All the primers and probes were synthesized by Jikang Biological Engineering Company, Shanghai.

The modified RT-PCR method for AOB quantification was described in detail by Li *et al.* (2007). The whole process includes three steps, that are construction of a standard sample DNA, identification of recombinant plasmid, and development of standard curves for AOB quantification.

Generally, the V2 region of the 16S rRNA of AOB was amplified using the above PCR primers and was cloned into T-easy vector. After the recombinant plasmid was constructed, a standard curve relating the $C_T$ value and DNA numbers was generated with a 10-fold dilution series of the recombinant plasmid containing 16S rDNA. There was a strong linear ($R^2 = 0.999$) inverse relationship between $C_T$ and the log cell numbers ranged from $10^2$ to $10^8$ copies of DNA, and the standard deviation of the $C_T$ value was all below 0.15, suggesting that the standard curve were of high accuracy and reproducibility (data not shown).

### 1.9 Analytical methods

COD, ammonia, nitrite, nitrate, pH, DO, and MLSS were determined according to the standard methods (APHA, 1998).

## 2 Results

### 2.1 COD and nitrogen removal at different PCP concentration

The influence of PCP on the aerobic granular sludge was carried out with the PCP concentration ranging from 10 to 50 mg/L. As shown in Fig.1a, the COD removal was slightly influenced when PCP was below 40 mg/L, and only slightly lagged when PCP concentration increased to 50 mg/L. Meanwhile, when PCP increased from 0 to 50 mg/L, ammonia removal rate dropped from 100% to 64.9%, indicating the ammonia removal ability of the aerobic granular sludge was impaired by high strength of PCP.

To understand the inhibitory effect on the nitrification and denitrification process, the ammonia, nitrite, nitrate, and total nitrogen concentration were monitored at different PCP concentrations (Fig.1b). When the PCP was below 10 mg/L, the effluent concentrations of $NO_2^-$-N and $NO_3^-$-N were below 0.04 and 0.02 mg/L, respectively, suggesting the aerobic granular sludge had excellent SND ability for nitrogen removal. With the increase of PCP, the nitrogen in effluent increased gradually because of the decrease of the SND ability. However, based on the accumulation profiles of ammonia, nitrite, and nitrate nitrogen, it can be deduced to different inhibitory effects of PCP. When PCP concentration increased to 30 mg/L,
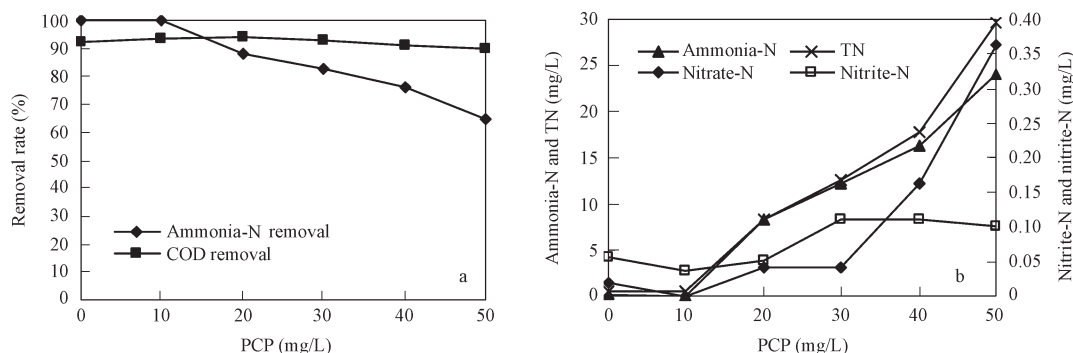


**Fig. 1** COD and ammonia-N removal (a) and profiles of different kinds of nitrogen (b) under different PCP concentrations.

the nitrite and nitrate nitrogen reached to 0.11 and 0.04 mg/L, respectively, owing to the inhibition of nitrification and denitrification processes. However, when the PCP was 50 mg/L, the nitrite nitrogen concentration was stable and the nitrate nitrogen increased rapidly. This phenomenon indicated that the denitrification process was influenced seriously. Because the nitrification and denitrification processes were carried out by AOB and various denitrifiers, respectively, the profiles of these intermediates supplied different inhibitory model of bacterial community by PCP. Moreover, since the ammonia and total nitrogen was accumulated, the nitrification process was also inhibited.

## 2.2 Effect of PCP on AOB number in aerobic granular sludge

The quantity of ammonia oxidizing bacteria (AOB) was monitored in samples of aerobic granular sludge at different PCP concentrations using RT-PCR. As shown in Fig.2, without PCP, the quantity of AOB in aerobic granular sludge was $(4.28 \pm 0.544) \times 10^7$ cells/g dried sludge. When PCP was below 50 mg/L, AOB quantity always maintained at about $10^7$ cells/g dried sludge. Because the ammonia removal decreased with the increase of PCP, it seems that PCP probably inhibits the AOB activity instead of killing the AOB cells. Although the long term effect of PCP on the AOB population should be carried out in detail, it is somewhat unexpected with our previous hypothesis that the AOB number would be always positively related with the ammonia removal ability.

## 2.3 Variance of bacterial diversity influenced by PCP

To investigate the influence of PCP on the bacterial diversity within the aerobic sludge, the bacterial species number was calculated according to the T-RFs numbers, which were obtained from the T-RFLP analysis (data not shown). All the peaks with relative area percentage exceeded 1% were taken into account and the area of all these T-RFs were more than 90% (data not shown), indicating that the T-RFs were sufficient to represent the microorganisms in the aerobic granular sludge. Fig.3 shows the relationship between the T-RFs numbers and the PCP concentration. According to the results, the T-RFs numbers decreased with the increase of the PCP concentration. This result suggested that the PCP destructed the bacterial community within the aerobic granular sludge. Taking into consideration of the performances of COD and ammonia
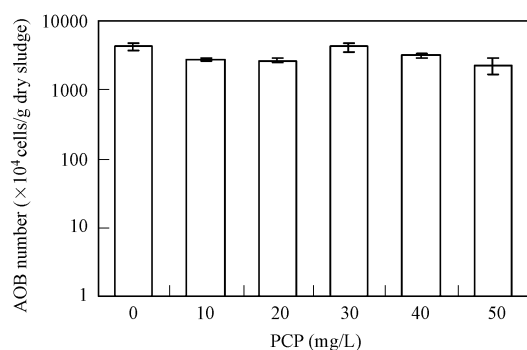
removal, it is obvious that the bacterial diversity is closely relative to the ammonia removal, instead of COD removal. Dai *et al.* (2000) also reported that the PCP selectively inhibited the methanogens in anaerobic granular sludge other than hydrolytic and fermentative bacteria below 10 mg/L and the COD removal was slightly influenced.

## 2.4 Impact of PCP on bacterial community composition

Based on the variance of bacterial species numbers in aerobic granular sludge, it is interesting to further investigate the detailed change of the bacterial composition under the stress of PCP. Significant shift in microbial community structure at different PCP stress was observed using T-RFLP method (Fig.4). It was obtained according to *Hha* I profiles and only T-RFs with area percentage $A$p $\geq$ 1% in samples were considered (Lukow *et al.*, 2000). As shown in Fig.4, relative areas of fragment 194, 202, and 207 bp ranged from 7% to 35.6%, suggesting that the bacterial strains represented by these fragments were more than others in the sludge. In contrast, the relative peak areas of the 90 and 95 bp fragments averaged <6% of the total peak areas. When PCP was increased to 10 mg/L, organisms represented by fragment 64 bp disappeared, but the other bacteria strains are still reserved. However, when PCP reached 20 mg/L, the bacteria strains represented by fragments 89, 160, and 212 bp could not be detected any more, indicating these bacteria could not tolerate a high PCP concentration. Organisms represented by fragment
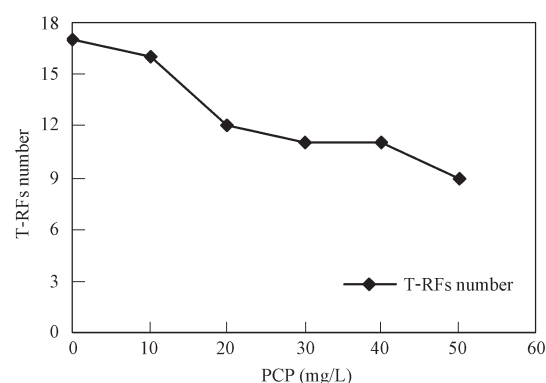


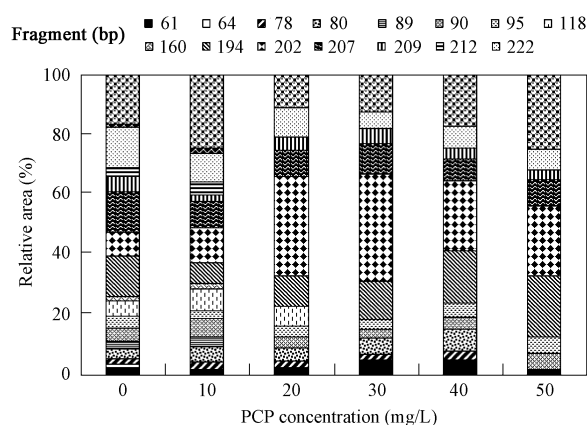**Fig. 3**   Effect of PCP on T-RF number in aerobic granular sludge.



**Fig. 4**   Abundance and diversity of microbial community of aerobic granular sludge at different PCP concentrations.



**Fig. 2**   Profile of AOB quantity at different PCP concentrations.

118 bp could survive when PCP concentration below 30 mg/L PCP. When PCP was at 50 mg/L, the bacterial strains represented by fragments 194, 202, and 207 bp dominated in the reactor.

Up to now, little was known about the detailed bacteria composition under PCP stress in aerobic granular sludge during industrial wastewater treatment process. In this work, the web-based PAT method was used to identify the specific bacteria with tolerance of PCP in the SBRs (Kent *et al.*, 2003). Table 1 listes 19 specific bacteria emerged in all six PCP reactors.

### 2.5 Phylogenetic analysis of bacteria in aerobic granular sludge

To investigate the relationship between PCP and bacteria, the phylogenetic tree of all bacterial 16S rDNA sequences occurred in aerobic granular sludge without PCP was constructed by PAT method (Fig.5). Based on the phylogenetic tree, all the disappeared and existed bacterial strains at different PCP concentrations were marked in the tree to show their phylogenetic position with other bacteria. According to the tree, it can be divided into 11 groups named from groups I to XI. Groups II, VIII and IX, and subgroups S III-1, S IV-1, S VI-3 and S XI-1, were sensitive to PCP at PCP concentration of 20 mg/L, where they disappeared. The populations detected at PCP 40 and 50 mg/L, which was clustered into several relative groups and belonged to groups V, VI and X, could endure a higher PCP concentration. Generally, most of these T-RFLP peaks fell together within the same fragment, were phylogenetically closely related, and the bacteria in the same group showed similar behavior under the influence of PCP.

## 3 Discussions

Generally, the performance of aerobic granular sludge showed that nitrogen removal was closely dependent on the PCP concentration, while COD removal was not inhibited when PCP was below 50 mg/L (Figs.1 and 2). It was apparent that PCP influenced the nitrogen removal, even though the AOB quantity was not decreased proportionally with the rise of PCP concentration (Fig.3). Therefore, it is suggested that the reduced nitrogen removal probably was attributed to the activity inhibition of AOB by PCP instead of decreased AOB cells. It is somewhat unexpected with our conventional view that the AOB quantity is always positively relative to the nitrogen removal by activated sludge for wastewater treatment. Although this point should be further verified in a long term operation by aerobic granular sludge, it seams that the AOB quantity may be unsuitable to be used as an index to characterize the nitrogen removal ability. However, in accordance with other results elsewhere, the nitrogen removal ability seems to be more easily impaired by PCP than that of COD removal. On the other hand, it was not found that the decreased bacterial diversity always lead to a reduced COD removal in the previous study (Li *et al.*, 2006, 2007), although we reported that the COD removal by aerobic granular sludge is positively related with the bacterial species numbers. This result proved the complexity of relationship between the bacteria population and the COD removal by the aerobic granular sludge.

The microbial abundance and diversity within aerobic granular sludge at different PCP concentrations were quite different (Fig.4), indicating that microbial community patterns shifted obviously with PCP concentration change. Because the signal intensity of an individual T-RF linearly was correlated to the amount of fluorescently-labeled DNA, it means that the signal intensity of an individual peak directly corresponded to the abundance of the respective T-RF (Lukow *et al.*, 2000).

When the PCP was absent, there were 69 strains in aerobic granular sludge detected by T-RFLP method (Fig.5). The number was larger than that in a previous report using conventional culture method to investigate the bacterial composition in aerobic granular sludge (Ruan, 2004), indicating that the T-RFLP method is more sensitive than

**Table 1**   List of bacteria existing in all the six SBRs

| Species match | Length of fragment (bp) | | |
| --- | --- | --- | --- |
| | *Hha*I | *Hae*III | *Alu*I |
| *Marinococcus halophilus* NCIMB 2178 (T). (X62171) | 60.93 | 240.55 | 254.46 |
| *Bdellovibrio stolpii* strain UKi2 ATCC 27052 (T). (M34125) | 90.48 | 232.08 | 248.24 |
| Strain LSv23. (AF099059) | 95.07 | 259.57 | 207.52 |
| *Brevibacillus laterosporus* ATCC 64 (T). (X57307) | 193.69 | 292.54 | 198.87 |
| *Acidimicrobium ferrooxidans* strain ICP DSM 10331 (T). (U75647) | 201.65 | 213.45 | 229.13 |
| Clone SJA-102. (AJ009481) | 201.65 | 213.45 | 225.34 |
| *Leptothrix discophora* strain SS-1 ATCC 43182 (T). (L33975) | 202.07 | 214.57 | 248.16 |
| *Leptothrix mobilis* strain Feox-1 DSM 10617 (T). (X97071) | 202.07 | 214.57 | 230.83 |
| Clone SJA-15. (AJ009453) | 202.07 | 214.57 | 230.83 |
| *Burkholderia* NF100. (AB025790) | 207.1 | 220.96 | 234.64 |
| symbiont of *Crithidia* sp. (L29303) | 207.23 | 218.47 | 232.93 |
| Clone T23. (Z93988) | 207.23 | 253.61 | 234.64 |
| *Ectothiorhodospira marismortui* strain BN 9410; Oren EG-1 DSM 4180 (X93482) | 207.23 | 253.61 | 234.64 |
| *Kurthia zopfii* strain F64/100; K5 NCIMB 9878 (T). (X70321) | 209.19 | 232.08 | 248.24 |
| *Burkholderia cepacia*. (X87275) | 209.21 | 202.74 | 234.64 |
| *Rhodoferax antarcticus* strain ANT. BR. (AF084947) | 209.21 | 202.74 | 150.39 |
| *Kingella denitrificans* strain UB-294 CCUG 28284. (L06166) | 209.21 | 254.89 | 234.64 |
| Clone T58. (Z93982) | 209.21 | 253.61 | 234.64 |
| *Nitrosomonas* sp. strain AL212. (AB000699) | 369.84 | 220.96 | 234.64 |
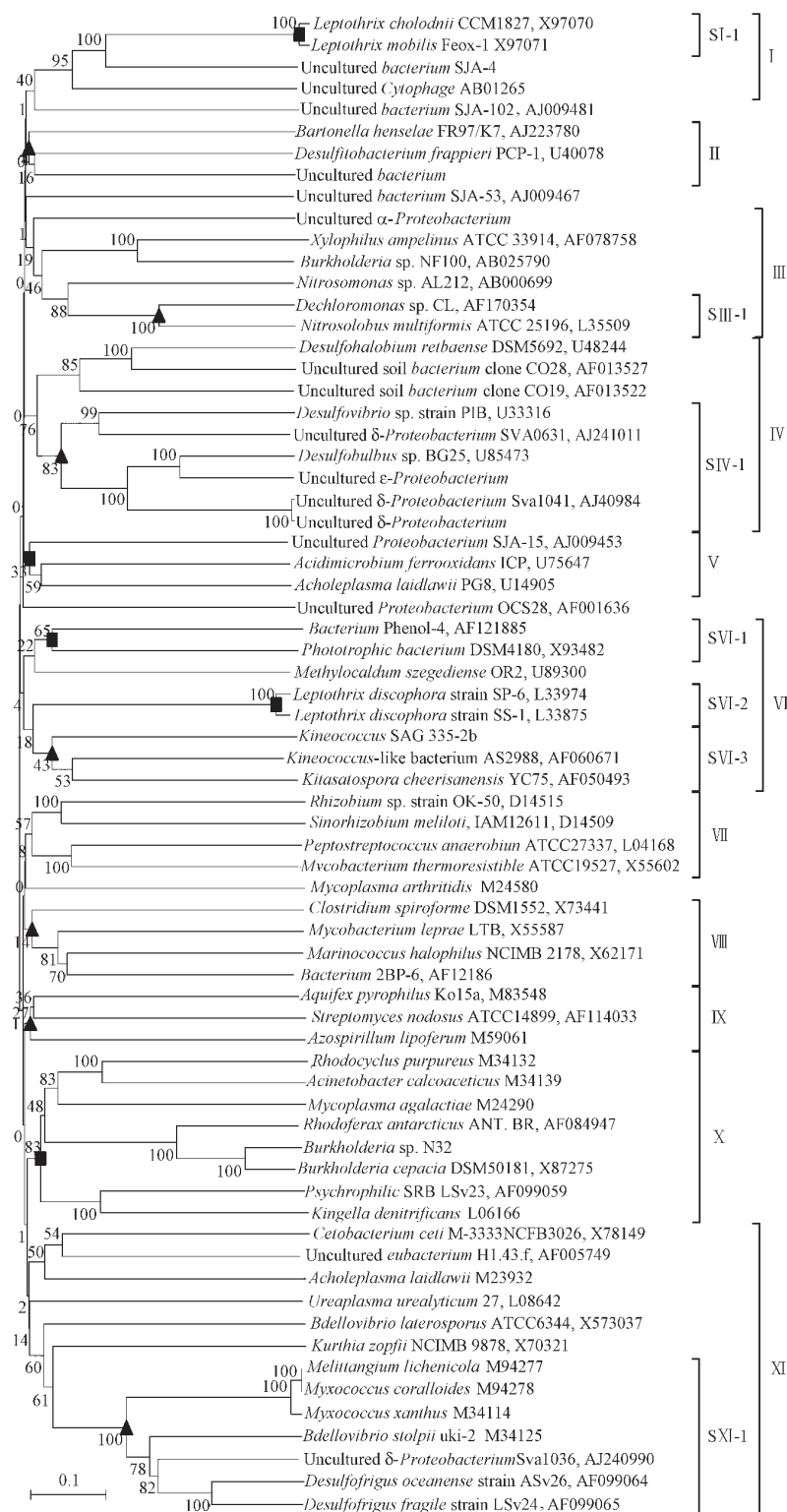
**Fig. 5** Neighbor-joining tree derived from an alignment of bacterial 16S rDNA sequences in R0. The used sequences were searched in GenBank according to the access numbers obtained by phylogenetic assignment tool.

the conventional isolation method. With the rise of PCP, most of bacteria disappeared and only 19 bacteria existed at all five PCP concentrations.

By applying the web-based T-RFLP method, all the PCP tolerant bacterial strains were also identified (Table 1). According to the phylogenetic analysis, the disappeared and existing bacteria were clustered in different groups in the

phylogenetic tree. Among the 19 bacteria strains, 8 strains (42%) were belonged to β-*Proteobacteria*. In addition, 21% of 19 existing strains were belonged to Gram-positive organisms, 58% of that were classified as Gram-negative bacteria, and the rest was unidentified. The Gram-negative bacteria dominated in the existing bacteria strains, and it was coincided with previous work which reported that

Gram-negative bacteria accounted for a large amount of bacteria in wastewater treatment system (You, 2001). It has been reported that Gram-negative strains of *Norcadia*, *Pseudomonas*, *Bacillus* and *Mycobacterium coeliacum* can be used to remove chlorophenols in aerobic conditions (Uotila *et al.*, 1992; Fulthorpe and Allen, 1995). Because that in Gram-negative bacteria, a number of responses can be used to counteract the destructive effect by PCP which is likely to limit the fluidity of cell membrane (Segura *et al.*, 1999).

In conclusion, the results of present work highlighted the importance of using microbial community structure analysis in combination with main chemical parameters for the assessment of new biotechnological processes specifically designed for toxic wastewaters treatment. The clarification of the microbial ecological mechanisms may provide significant insights into the further improvement of the performance of the aerobic granular sludge for wastewater treatment (Yuan *et al.*, 2002).

## 4 Conclusions

(1) When PCP concentration was ranged from 0 to 50 mg/L, COD removal of aerobic granular sludge was slightly influenced, whereas ammonia removal decreased gradually with the rise of PCP concentration. (2) The AOB quantity in aerobic granular sludge does not change significantly with the reduced ammonia removal ability, suggesting that PCP probably inhibit the AOB activity instead of killing the AOB cells. (3) With the increase of PCP concentration, significant shift in bacterial community structure at different PCP stress was observed within aerobic granular sludge. (4) According to phylogenetic analysis, the survivors in aerobic granular sludge were distributed closely in the phylogenetic tree, in which 42% were belonged to β-*Proteobacteria*. Among the 19 existed bacteria strains, 21% were belonged to Gram-positive organisms, 58% of which were classified as Gram-negative bacteria, and the rest was unidentified.

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