Investigation of total bacterial and ammonia-oxidizing bacterial community composition in a full-scale aerated submerged biofilm reactor for drinking water pretreatment in China

Ying-Ying Qin, Dao-Tang Li & Hong Yang
School of Life Science and Biotechnology, Shanghai Jiaotong University, Shanghai, China

Correspondence: Hong Yang, School of Life Science and Biotechnology, Shanghai Jiaotong University, No. 800 Dongchuan Rd., Shanghai, 200240, China. Tel.: +86 21 34205343; fax: +86 21 34205709; e-mail: hongyang@sjtu.edu.cn

Received 9 July 2006; revised 16 November 2006; accepted 17 November 2006. First published online 18 December 2006.

DOI:10.1111/j.1574-6968.2006.00571.x

Editor: Elizabeth Baggs

Keywords
biological water pretreatment reactor; bacterial community; ammonia-oxidizing bacteria; clone library.

Abstract
The community composition of total bacteria and ammonia-oxidizing bacteria in a full-scale aerated submerged biofilm reactor for drinking water pretreatment was characterized by analysis of 16S rRNA gene and the functional gene amoA, respectively. Sampling was performed in February and in July. 16S rRNA gene clone libraries revealed 13 bacterial divisions. At both sampling dates, the majority of clone sequences were related to the Alpha- and Betaproteobacteria. A minor proportion belonged to the following groups: Gammaproteobacteria, Deltaproteobacteria, Nitrosospira, Firmicutes, Acidobacteria, Verrucomicrobia, Actinobacteria, Planctomycetes, Chloroflexi, Gemmatimonadetes and the Cytophaga–Flavobacterium–Bacteroides group. Some sequences related to bacteria owning high potential metabolic capacities were detected in both samples, such as Rhodobacter-like rRNA gene sequences. Surveys of cloned amoA genes from the two biofilm samples revealed ammonia-oxidizing bacterial sequences affiliated with the Nitrosomonas oligotropha lineage, Nitrosomonas communis lineage. An unknown Nitrosomonas group of amoA gene sequences was also detected.

Introduction
Water quality deterioration of surface water supplies is one of the major concerns in drinking water treatment, particularly in developing countries. In China, for example, more than half of the major surface water resources have been polluted to a level that has a COD_{M} of 6 mg L\(^{-1}\) or higher and an ammonia level of more than 3 mg L\(^{-1}\) (Xia et al., 2000), which presents great difficulties and challenges to the performance of conventional water treatment processes. Using biological pretreatment as the first process in the water treatment train could improve the conventional treatment processes for better dissolved organics and ammonia removal (Takasaki et al., 1990). Bioreactors such as aerated submerged biofilm reactors enhance microbial activity for biological nutrient removal by providing a surface for indigenous water microorganisms to attach and grow. Meanwhile, it could help to reduce microbial regrowth potential and formation of carcinogenic disinfection by-products in drinking water distribution systems (Takasaki et al., 1990; Lim & Shin, 1997). Owing to the relatively low maintenance cost and effective dissolved organic matter and ammonia removal, biological pretreatment has become an attractive additional unit process in drinking water plants in China (Wang & Liu, 1999).

Although hundreds of biological reactors have been constructed for drinking water pretreatment in China, some of the processes had fluctuating nutrient removal efficiency (Wang & Liu, 1999). While several studies have focused on the impacts of various operating characteristics and design parameters on process performance (Lim & Shin, 1997; Wu, 1999), the microbial communities in the reactors, which directly govern the substrate utilization performance of the process, are poorly understood. Recently, molecular-based techniques have been frequently and successfully used to study the bacterial community in a variety of habitats, including freshwater lakes (Sekiguchi et al., 2002) and estuaries (Bernhard et al., 2005), biological waste water treatment plants (Cole et al., 2002; Park & Noguera, 2004; Gómez-Villalba et al., 2006) and biofilms in drinking water distribution systems (Lipponen et al., 2004). However, relatively little work has been carried out to learn microbial communities in the biological process used to pretreat drinking water.

The main goal of this study was to investigate the diversity of general bacterial community in a full-scale...
aerated submerged biofilm reactor for drinking water pretreatment by phylogenetic analysis of their 16S rRNA genes. Additionally, the functional genes for ammonia monoxygenase (amoA) were analyzed to examine the community composition of ammonia-oxidizing bacteria (AOB) due to the particular ammonia removal required in the processes.

**Materials and methods**

**Sampling of biofilms**

Biofilm samples were collected from the aerated submerged biofilm reactor run by Huinan Waterworks of Shanghai in China, which receives Dazhi River as the source of raw water. The reactor has been operated since 1998, and it treats c. 1.2 × 10^5 m^3 of raw water per day with a hydraulic retention time of 1.45 h. Biofilm was allowed to build naturally on the media of fibrous material in the initial operation stage. Samples collected during two seasons were designated as Bio-Feb (winter sample, sampled in February) and Bio-Jul (summer sample, sampled in July). Details of the operational parameters, influent characteristics and removal efficiencies on sampling days are listed in Table 1. For each sampling time, biofilm samples were collected at three different depths (the top, middle and bottom) in the middle part of the reactor and mixed well, and then transported to the lab on ice and stored at −20 °C before analysis.

**DNA Extraction**

DNA was extracted from biofilms using a modification of the method described by Rowan et al. (2003). The modified step involved addition of 50 μL of proteinase K (TaKaRa Biomedicals, Tokyo, Japan) during the lysing step, and the mixture was incubated for 30 min at 37 °C. After that, 600 μL of phenol/chloroform/isoamyl alcohol (25:24:1) was added. The extracted DNA was checked by 1% agarose electrophoresis using ethidium bromide as the staining dye, and quantified spectrophotometrically at 260 nm and diluted to 50 μg mL⁻¹ for PCR amplification.

**PCR amplification of target genes from total bacterial and AOB populations**

PCR amplification of bacterial 16S rRNA gene fragments was performed using the universal bacterial primers 27F and 1492R (Brosius et al., 1978). The reactions were carried out in a 50 μL volume containing 1 μL DNA template solution, 5 μL 10 × PCR Buffer [100 mM Tris-HCl (pH 8.3), 500 mM KCl, 15 mM MgCl2] (TaKaRa), 2.5 U of Taq DNA Polymerase (TaKaRa), 20 nmol of each deoxyribonucleoside triphosphate and each primer at a concentration of 10 pmol. PCR was conducted using the GeneAmp2400 thermal cycler (Applied Biosystems, Foster City, CA). The thermocycling program was 94 °C for 3 min, 30 cycles of 94 °C for 30 s, 58 °C for 45 s, 72 °C for 1 min, followed by a 7-min final extension at 72 °C.

The betaproteobacterial AOB were analyzed using PCR amplification with primer sets amoA-1F and amoA-2R (Rotthauwe et al., 1997), which amplify a 491 bp fragment (453 bp without primers) from the amoA gene of the ammonia oxidizers. Reactions were performed in a solution described above. The thermocycling program was: 94 °C for 3 min, 30 cycles of 94 °C for 30 s, 58 °C for 45 s, 72 °C for 105 s, followed by a 7-min final extension at 72 °C.

**Cloning and sequence analysis**

PCR products of all the reactions were purified with Agarose Gel DNA Purification Kit Ver.2.0 (TaKaRa), ligated into the vector pMD19-T (TaKaRa) and cloned into competent cells of *Escherichia coli* according to the manufacturer’s instruction. Clones were randomly selected, and cloned inserts were identified using primers M13-47 and RV-M (TaKaRa) that targeted the vector sequences.

For 16S rRNA gene clone libraries, all screened clones were sequenced on an ABI 377 Sequencer (Applied Biosystem) using fluorescently labeled primers M13-47 and RV-M. Operational taxonomic units (OTUs) were grouped for the Bio-Feb and Bio-Jul 16S rRNA gene clone libraries according to the 97% similarity threshold for OTU assignment (Stackebrandt & Goebel, 1994). For each amoA gene library, representative clones identified by restriction enzyme analysis separately using MspI, HhaI and Rsal (TaKaRa) were selected for sequencing. The closest matching sequences in the GenBank database were identified using BLAST (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/BLAST/) (Altschul et al., 1997). Sequences that did not closely match a GenBank sequence...
were examined for chimeras with the CHECK-CHIMERA software of the Ribosomal Database Project (Maidak et al., 1999). To assess the completeness of the clone libraries, coverage was calculated according to the formula: coverage = \[ 1 - \frac{n1 \times N^{-1}}{N} \] \times 100\%, where n1 is the number of OTUs consisting of only one sequence and N is the number of all clones in the library (Good, 1953).

## Phylogenetic analysis

The general bacterial 16S rRNA gene sequences from the biofilm samples were aligned with published sequences from GenBank database in program CLUSTAL W version 1.4 (Thompson et al., 1994). Matrices of evolutionary distance were computed from the sequence alignment with the program DNADIST implemented in the software package PHYLIP version 3.65 (http://evolution.genetics.washington.edu/phylip.html). The neighbor-joining method was applied (Thompson et al., 1994). Matrices of evolutionary distance were computed from the sequence alignment with the program DNADIST implemented in the software package PHYLIP version 3.65 (http://evolution.genetics.washington.edu/phylip.html). The neighbor-joining method was applied to calculate the phylogenetic trees from the distance matrices and reconstructed with 1000 replicate bootstraps.

The amoA sequences were translated into amino acid sequences and aligned in CLUSTAL W. Protein distances were inferred using PROTDIST program in PHYLIP with the Dayhoff PAM 001 matrix as the amino acid replacement model. Trees were constructed from the distances using the FITCH method. Protein parsimony was applied with 1000 times bootstrap resamplings.

## Nucleotide sequence accession numbers

Sequences determined in this study have been deposited in the GenBank database under accession numbers EF110587 to EF110612, DQ363589 to DQ363632 and DQ363639 to DQ363663.

## Results

### General bacterial communities of biofilms revealed by 16S rRNA gene clone libraries

In total, 26 and 44 OTUs were obtained from the Bio-Feb and Bio-Jul clone libraries, respectively. The coverage of the two libraries was calculated as described by Good (1953); it was shown that the retrieved 16S rRNA gene clones covered 95.1% (Bio-Feb library) and 71.4% (Bio-Jul library) of the expected OTU diversity.

Phylogenetic analysis indicated that sequences recovered in February and in July samples distributed in roughly the same bacterial divisions (Table 2). Among the 13 bacterial divisions detected from the two libraries, sequences related to either Alphaproteobacteria or Betaproteobacteria were mostly found (Table 2). A large fraction of the Alphaproteobacteria were Rhodobacter-like sequences (13.11% and 8.16% of all clones) (Fig. 1). Sequences affiliated with cultured species of Phaeospirillum, Devosia, Zoogloea, Beijerinckia and Methylocystis were also found in this subclass. The remaining alphaproteobacterial clones were closely related to uncultured environmental clones retrieved from Changjiang River in China (Sekiguchi et al., 2002), subsurface water of the Kalahari Shield in South Africa (DQ354731 and DQ336968), freshwater reservoir in USA (Stein et al., 2002) and activated sludge from an industrial sewage treatment plant in Germany (Juretschko et al., 2002). In the beta subclass of the Proteobacteria, clones were closely related to Ideonella, Xylophilus, Acidovorax, Burkholderia, Dechloromonas, Massilia, Lautropia, Methylophaga and Nitrosomonas spp. and four environmental clones obtained from Meiliang Bay of Lake Taihu in China (DQ166474), farm soil adjacent to a silage storage bunker (Tringe et al., 2005), biofilms of polluted Elbe River in Germany (Brummer et al., 2003) and subsurface water of the Kalahari Shield in South Africa (DQ223206). Clones from Gammaproteobacteria were presented with minor proportions, accounting for 14.75% and 13.27% of the Bio-Feb and Bio-Jul libraries, respectively. 4.92% and 3.06% of the clones in Bio-Feb and Bio-Jul library came from Deltaproteobacteria (Fig. 1).

<table>
<thead>
<tr>
<th>Phylogenetic group</th>
<th>Bio-Feb sample</th>
<th>Bio-Jul sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of OTUs</td>
<td>No. of clones (%)</td>
</tr>
<tr>
<td>Alphaproteobacteria</td>
<td>6</td>
<td>41 (33.61)</td>
</tr>
<tr>
<td>Betaproteobacteria</td>
<td>7</td>
<td>35 (28.69)</td>
</tr>
<tr>
<td>Gammaproteobacteria</td>
<td>2</td>
<td>18 (14.75)</td>
</tr>
<tr>
<td>Deltaproteobacteria</td>
<td>3</td>
<td>6 (4.92)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>1</td>
<td>1 (0.82)</td>
</tr>
<tr>
<td>Nitrospira</td>
<td>1</td>
<td>1 (0.82)</td>
</tr>
<tr>
<td>CFB group</td>
<td>1</td>
<td>1 (0.82)</td>
</tr>
<tr>
<td>Acidobacteria</td>
<td>1</td>
<td>7 (5.74)</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>1</td>
<td>5 (4.10)</td>
</tr>
<tr>
<td>Chloroflexi</td>
<td>1</td>
<td>3 (2.46)</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>1</td>
<td>1 (0.82)</td>
</tr>
<tr>
<td>Planctomycetes</td>
<td>1</td>
<td>1 (0.82)</td>
</tr>
<tr>
<td>Gemmatimonadetes</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Proportion of clones in the library.

ND, not detected.

Clones affiliated with the Nitrospira phylum comprised 13.27% of the Bio-Jul library but only 2.46% of the Bio-Feb library (Table 2). Clones from the Firmicutes, the Cytophaga–Flavobacterium–Bacteroides group, Acidobacteria, Verrucomicrobia, Actinobacteria and Planctomycetes were present in both libraries, but at much lower fractions (Fig. 2). Sequences related to Chloroflexi were only detected in the Bio-February library while sequences from Gemmatimonadetes were only found in Bio-Jul library.

---

**Table 2. Summary of bacterial sequence identification according to the closest matched sequences in the GenBank database showed by BLAST**

<table>
<thead>
<tr>
<th>Phylogenetic group</th>
<th>No. of OTUs</th>
<th>No. of clones (%)</th>
<th>No. of OTUs</th>
<th>No. of clones (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alphaproteobacteria</td>
<td>6</td>
<td>41 (33.61)</td>
<td>9</td>
<td>21 (21.43)</td>
</tr>
<tr>
<td>Betaproteobacteria</td>
<td>7</td>
<td>35 (28.69)</td>
<td>11</td>
<td>28 (28.57)</td>
</tr>
<tr>
<td>Gammaproteobacteria</td>
<td>2</td>
<td>18 (14.75)</td>
<td>5</td>
<td>13 (13.27)</td>
</tr>
<tr>
<td>Deltaproteobacteria</td>
<td>3</td>
<td>6 (4.92)</td>
<td>2</td>
<td>3 (3.06)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>1</td>
<td>1 (0.82)</td>
<td>4</td>
<td>6 (6.12)</td>
</tr>
<tr>
<td>Nitrospira</td>
<td>1</td>
<td>1 (0.82)</td>
<td>1</td>
<td>13 (13.27)</td>
</tr>
<tr>
<td>CFB group</td>
<td>1</td>
<td>1 (0.82)</td>
<td>6</td>
<td>6 (6.12)</td>
</tr>
<tr>
<td>Acidobacteria</td>
<td>1</td>
<td>7 (5.74)</td>
<td>1</td>
<td>2 (2.04)</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>1</td>
<td>5 (4.10)</td>
<td>1</td>
<td>1 (1.02)</td>
</tr>
<tr>
<td>Chloroflexi</td>
<td>1</td>
<td>3 (2.46)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>1</td>
<td>1 (0.82)</td>
<td>1</td>
<td>2 (2.04)</td>
</tr>
<tr>
<td>Planctomycetes</td>
<td>1</td>
<td>1 (0.82)</td>
<td>1</td>
<td>1 (1.02)</td>
</tr>
<tr>
<td>Gemmatimonadetes</td>
<td>ND</td>
<td>ND</td>
<td>2</td>
<td>2 (2.04)</td>
</tr>
</tbody>
</table>

*Proportion of clones in the library.

ND, not detected.
Characterization of betaproteobacterial AOB by amoA gene clone libraries

AOB clone libraries of amoA gene were constructed from the Bio-Feb (76 clones) and Bio-Jul (79 clones) samples. Clones were grouped into different patterns by restriction enzyme analysis, six different patterns were obtained in the February sample while 19 patterns were gained from the July sample. This grouping means, the retrieved amoA gene clones covered 97.4% (Bio-Feb library) and 87.3% (Bio-Jul library).

![Phylogenetic analysis of the proteobacterial 16S rRNA gene clones from Bio-Feb and Bio-Jul libraries in this study with the closest known relatives obtained from GenBank.](https://academic.oup.com/femsle/article-abstract/268/1/126/594500)

**Fig. 1.** Phylogenetic analysis of the proteobacterial 16S rRNA gene clones from Bio-Feb and Bio-Jul libraries in this study with the closest known relatives obtained from GenBank. The tree was constructed using the neighbor-joining algorithm with the Kimura parameter in the PHYLIP software package. Bootstrap values of >50% are expressed as percentage of 1000 trees. The scale bar indicates the expected number of changes per sequence position.
of the expected OTU diversity. Phylogenetic analysis of these 25 sequences showed that all the amoA gene sequences retrieved from the two samples could be clustered into three groups within the genus Nitrosomonas (Fig. 3). Group 1 contained closely related clones that exhibited 87.0–98.9% nucleotide acid and 98.0–100% amino acid identity with each other, and fell into the Nitrosomonas oligotropha lineage. These sequences were closely related to published AmoA sequences of N. oligotropha, Nitrosomonas ureae and several undescribed species Nitrosomonas sp. AL212, Nitrosomonas sp. Nm59 and Nitrosomonas sp. Nm86 (Fig. 3). Clone Jul-amoA2 showed 98.8% amino acid identity with an environmental clone P22-3, which was recovered from sediment samples from a low-salinity site in Plum Island.
Sound estuary in Massachusetts (Bernhard et al., 2005). Group 2 contained 24.2% of the Bio-Feb clones and 69.6% of the Bio-Jul clones. These clones had 96.2–99.6% nucleotide acid and 97.0–99.4% amino acid identity with each other, and formed a separate branch within the *Nitrosomonas communis* lineage (Fig. 3). This cluster also included an environmental clone UCT-5, which was retrieved from activated sludge from a wastewater treatment plant with enhanced biological phosphorous removal in Wisconsin (Park & Noguera, 2004). Clones in this group showed 93.3–96.4% sequence identity to the closest described AOB species *Nitrosomonas nitrosa* at the amino acid level.

The third group was comprised of 27.3% clones from the February sample and 10.1% clones from the July sample that shared 91.6–99.6% nucleotide acid and 95.1–100% amino acid identity with each other. Phylogenetic analysis did not support the association of this group with any previously reported AOB lineage (Fig. 3). They represented an independent cluster within the genus *Nitrosomonas* with several undefined environmental clones from wastewater treatment plants (e.g. clone RI-14, GLII-20 and B3-3) and a sequence CT1-28 from Chesapeake Bay sediment (Francis et al., 2003). The closest amino acid sequence identity to a recognized AOB was 92.5% between clone Feb-amO11 and *N. nitrosa*.

**Discussion**

16S rRNA gene sequences from 13 bacterial divisions were retrieved in the general bacterial clone libraries of two seasonal biofilm samples from the biological drinking water pretreatment reactor, indicating the complex bacterial community structure in the reactor. Sequences related to the *Alpha-* and *Betaproteobacteria* were most frequently found in both libraries, with a slightly lower frequency of occurrence of the *Gammaproteobacteria* (Table 2). Schwartz et al. (1998) performed in situ hybridization experiments to investigate the bacterial composition in biofilms from embankment filtered drinking water and found that *Betaproteobacteria* were the most frequently found, but also *Alpha-* and *Gammaproteobacteria* could be detected in significant minor percentages. The alpha and beta subclass of the *Proteobacteria* were also found to be the dominant or typical bacterial groups in biofilms of drinking water networks (Kalmbach et al., 1997; Schmeisser et al., 2003). It is likely that the prevalence of these bacteria divisions might be a common feature of most drinking water systems.

Clones related to *Rhodobacter* spp. of the *Alphaproteobacteria* had significant proportions in both 16S rRNA gene libraries in this study. Members of *Rhodobacter* possess an extensive range of metabolic capabilities. The great capacity
of this genus to practice photoheterotrophy (to use light as the energy source and an organic compound as the carbon source) makes them adapt well in all types of aquatic environments (Madigan et al., 2003).

The phylogenetic analysis revealed that 16S rRNA gene sequences affiliated with *Nitrosospira* spp. existed in both February and July samples (Fig. 2). *Nitrosospira*-like microorganisms were found to be the dominating nitrite oxidizers in various drinking water systems (Regan et al., 2002; Williams et al., 2004) and other ecosystems like wastewater treatment plants (Daims et al., 2001; Dionisi et al., 2002) or soils (Bartosch et al., 2002).

AOB sequences closely related to *N. oligotropha* were identified in the two bacterial 16S rRNA gene clone libraries (Fig. 1). To investigate the diversity of AOB population in the reactor in detail, *amoA* gene sequences specific for betaproteobacterial AOB were, respectively, retrieved from the February and July samples. The *amoA* sequences analysis confirmed the presence of *N. oligotropha* lineage members in both samples (Fig. 3). AOB members belonging to the *N. oligotropha* lineage were often recovered from oligotrophic environments, including drinking water distribution systems (Lipponen et al., 2004), freshwater (Cébron et al., 2003) and wastewater treatment systems with low-ammonia influents (Limpiyakorn et al., 2005). Members of this lineage have been found to adapt well in environments with low ammonia concentrations because of their low *Ks* values ranging from 1.9 to 4.2 μM NH₃ (Koops & Pommerening-Röser, 2001), resulting in a lower ammonia threshold concentration for growth. It seemed that the limiting amounts of ammonia in the biological drinking water pretreatment reactors made members of the *N. oligotropha* lineage strong competitors among AOB populations.

Sequences closely related to *N. nitrosa*, which belongs to the *N. communis* lineage, were also detected in both *amoA* gene libraries. Strains of *N. nitrosa* were commonly isolated from eutrophic freshwaters (Koops & Pommerening-Röser, 2001). Owing to their relatively high-affinity constants for ammonia, it is likely that factors other than the ammonia concentration influence the existence of *N. nitrosa*-like AOB in our reactor. AOB sequences related to *N. nitrosa* have been retrieved from diverse environments like industrial wastewater treatment plants (Dionisi et al., 2002), biological phosphorus removal activated sludge (Park & Noguera, 2004), submerged filter biofilms for urban wastewater treatment (Gómez-Villalba et al., 2006), freshwater estuary (Cébron et al., 2003), soils from rice field (Nicolaisen & Ramsing, 2002) and now in this study, indicating the ecological versatility in this species.

A number of previous studies have suggested the distribution of *Nitrosospiroa* lineage in various natural and man-made aquatic systems (Hiorns et al., 1995; Whitby et al., 2001; Hollibaugh et al., 2002). For example, Hiorns et al. (1995) demonstrated that *Nitrosospiroa* spp. were widespread in the environment and low-ammonia environments commonly harbored *Nitrosospiroa* spp. However, in this study, both 16S rRNA and *amoA* gene approaches indicated the dominance of the *Nitrosomonas* genus over *Nitrosospiroa* in AOB populations in our drinking water pretreatment reactor. This result is in accordance with findings of several studies on AOB communities in biofilms from drinking water production systems. Regan et al. (2002) found a predominance of *Nitrosomonas*-like over *Nitrosospiroa*-like AOB in a pilot-scale chloraminated drinking water distribution system using molecular techniques. In the developing biofilms in two full-scale drinking water distribution networks in Finland, only *Nitrosomonas*-like sequences were recovered as AOB members (Lipponen et al., 2004).

This study provides a first glance at the community composition of general bacteria and AOB in a full-scale aerated submerged biofilm reactor for drinking water pretreatment, which is the first step toward optimizing the operation and enhancing the performance of the reactor. To understand the correlation between microbial community structure and the functionality of the biological process in the reactor, our continuing objective is to investigate the active bacterial communities by technologies based on molecule RNA under different nutrient removal efficiency.

Acknowledgements

This project was supported by National Natural Science Foundation of China (No. 50278053 and No. 20377030).

References


