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Identification of Bacterial Populations in Integrated PAC/UF System for Ammonia Nitrogen Removal in Drinking Water Treatment

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In this study, we investigated the use of ultrafiltration (UF) combined with adsorption on powdered activated carbon (PAC) for the removal of ammonia nitrogen. This system was able to remove more than 80% ammonia nitrogen. The aim of this study was to identify bacterial populations in integrated PAC/UF system by analyzing 16S rRNA-based clone libraries. The results from this study further improve our understanding of the molecular diversity and bacterial population dynamics of drinking water microbial communities.

Key words: PAC/UF; drinking water treatment; bacterial populations; 16S rDNA.

Powdered activated carbon coupled to ultrafiltration (PAC-UF) is a promising water treatment option for the removal of organic micropollutants from drinking water[1]. PAC/UF joins the adsorption capacity of PAC with the UF membrane ability to retain microorganisms and particles (including PAC particles), therefore allowing the removal of low molar mass compounds which could not be removed by the UF membrane itself (large pore size). PAC/UF is a low-pressure (<1 bar) process, and as so has a relatively low operating cost[2]. Much of the previous PAC/UF research addressed the optimization of the operating parameters through the model experiments[3-5]. Presently, adequate measures to prevent or reduce biofouling are lacking. The microbiological and physical processes associated with biofilm formation and biofouling in the PAC/UF system are poorly understood.

Bacteria populations are typically based on morphological and physiological characterization of bacteria after cultivation on artificial media[6]. The conventional plating and colony isolation methods showed the presence of a wide variety of species on the feed and permeative surfaces of biofouled cellulose acetate, polyetherurea thin-film composite, or polyamide thin-film-composite membranes[7-9]. However, by cultivation-dependent methods, information about only 0.01 to 3% of the population in natural environments is obtained[10]. In recent years, the microbial community structure was examined using 16S rRNA gene clone libraries and fluorescence in situ hybridization methods and using PCR-denaturing gradient gel electrophoresis (DGGE) and sequence analysis of constructed clone libraries containing larger PCR fragments of the 16S rRNA gene[11, 12]. While, the new culture-dependent approaches have been successful at gathering bacterial presence data, they have been criticized for their inability to accurately characterize the microbial diversity in natural environments.

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In drinking water treatment nitrification can be considered as a promising process in the removal of ammonia nitrogen. As known nitrification is a two-step process. The first step consists of the oxidation of ammonia to nitrite by ammonia-oxidizing bacteria (AOB) such as Nitrosomonas and Nitrosospira. Then, nitrite is converted to nitrate by nitrite-oxidizing bacteria (NOB) such as Nitrobacter and Nitrospira[13]. Although the processes occurring in PAC/UF system are well-understood, the nitrifier community has not been investigated in detail.

The aim of this study is to investigate bacterial populations in integrated PAC/UF system for drinking water treatment. The microbial communities in the sludge mixed PAC were investigated by using 16S rRNA gene clone library analysis in order to fully characterize nitrifier communities and activities.

MATERIALS AND METHODS

Total DNA extraction

Samples were taken from sludge mixed PAC in integrated system.The nucleic acid extraction was performed using FastDNA SPIN kit (MP Biomedicals) according to the manufacturer's instructions with a few modifications. Samples were mechanically bead beaten for 10 s at maximum speed (Mini BeadBeater-8, Biospec Products) for DNA isolation instead of Fast Prep Instrument. The amount and purity of DNA was estimated spectrophotometrically by measuring the optical density at 260 and 280 nm. DNA was diluted to obtain suitable PCR amplicons, as templates for subsequent PCRs.

PCR amplification

Full-length bacterial 16S rDNA fragments were amplified for slot-blot hybridization with general bacterial primers, 27 forward (AGA GTT TGA TCC TGG CTC AG) and 1492R (GGT TAC CTT GTT ACG ACT T). Then the PCR products of the excised target bands were purified, inserted into pUCM-T Simple Vector (Shengong, China) and transformed into Escherichia coli DH5α by the heat-shock method. Positive clones were selected using ampicillin selection and blue/white screening.

Phylogenetic analysis

The 16S rDNA bands were compared with GenBank reference sequences (http://www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST) program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to classify the taxonomic position of the microbial strains. The multiple sequence alignments were evaluated by the CLUSTAL_X program. Then the phylogenetic tree was constructed using the neighborhood-joining and maximum-parsimony protocol and bootstrap percentages based on 1000 replications and MEGA 5.05 was also used to construct the phylogenetic tree.

RESULTS AND DISCUSSION

The research on removal effect of ammonia, nitrite, nitrate and organic pollutants is conducted by using integrative membrane bioreactor. The removal rate of ammonia reaches 73.9% as expected after run of two weeks. The amount of nitrite in effluent water increases firstly and then decreases. The removal rate of UV410 is 100%. The decontaminate efficiency of UV_{254} and COD_{Mn} declines over time. The removal rate of UV_{254} is higher than COD_{Mn}. PAC benefits to improve removal effect of UV_{254} in oligotrophic water environment.

A total of 95 different colonies were collected from one of the sludge samples in September and their phyogentic affiliation was determined using 16S rDNA gene analysis. In order to measure the potential bacterial richness and abundance within each sampled library, the 16S rDNA sequences with ≥90% sequence similarity were clustered into operational taxonomic units (OTUs) and the OTU abundance pattern was determined by ranking and plotting the relative abundance data for each sample. The results yielded 58 distinct OTUs in the sample. A variety of the OTUs (16 OTUs) were found to belong to the *Beta-proteobacteria*. The *Gamma-proteobacteria* and *Alpha-proteobacteria* affiliated OTUs were found to be clustered into 10 and 8 OTUs.2 OTUs each belong to the *Delta-proteobacteria, Actinobacteria, Planctomycetacia, Acidobacteria* and *Cyanobacteria.* Respectively, only 1 OTU was found to belong to the *Spirochaetes*, *Bacteroidetes*, *Chlorobia* and *Verrucomicrobiae.* The rest 10 OTUs belong to novel lineages for

which there are no currently available culturable representatives.

Representatives of each OTU were subjected to a phylogenetic analyses (Fig. 1 and 2). The experiment of PAC/UF process showed a good removal efficiency, especially in the removal of organic matter and nitrogen material. It can be found that pollutant removal is closely related with microbial species composition. The nitrogen removal related microorganism mainly is the *Beta proteobacteria*. The dominant *Burkholderiales* belongs to the *Beta Proteobacteria* and its nitrogen removal effect is obvious. The *Comamonadaceae*, *Methylophilaceae* and *Rhodocyclaceae* are common species of nitrogen removal process. The *Nitrosomonas* is the nitrosation communities. *Alpha Proteobacteria* have a good denitrification effect, and in later period of the experiment, denitrification reaction also indeed found in the transition zone and the membrane tank. The

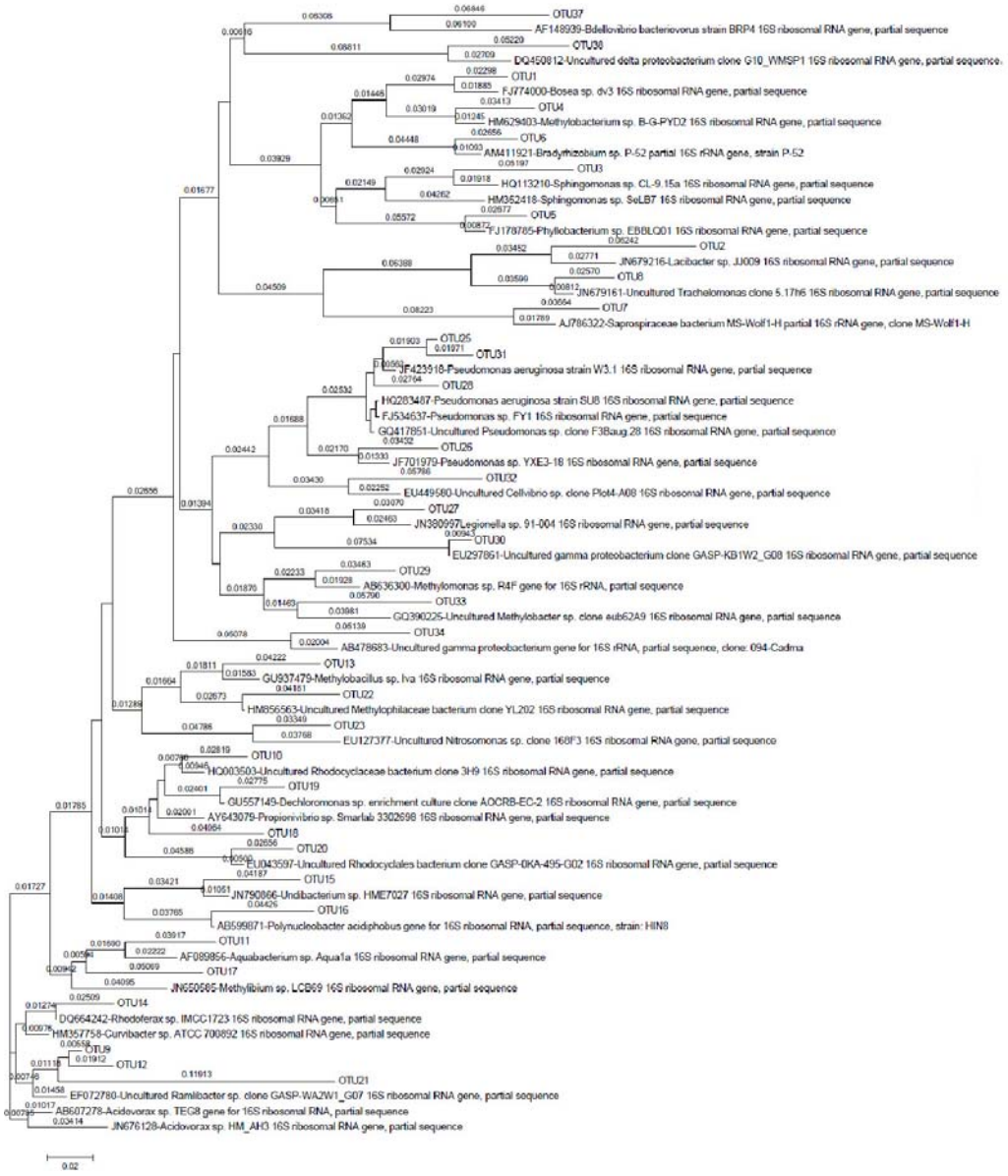

Fig. 1. Phylogenetic tree of OTUs belong to the class *Proteobacteria*

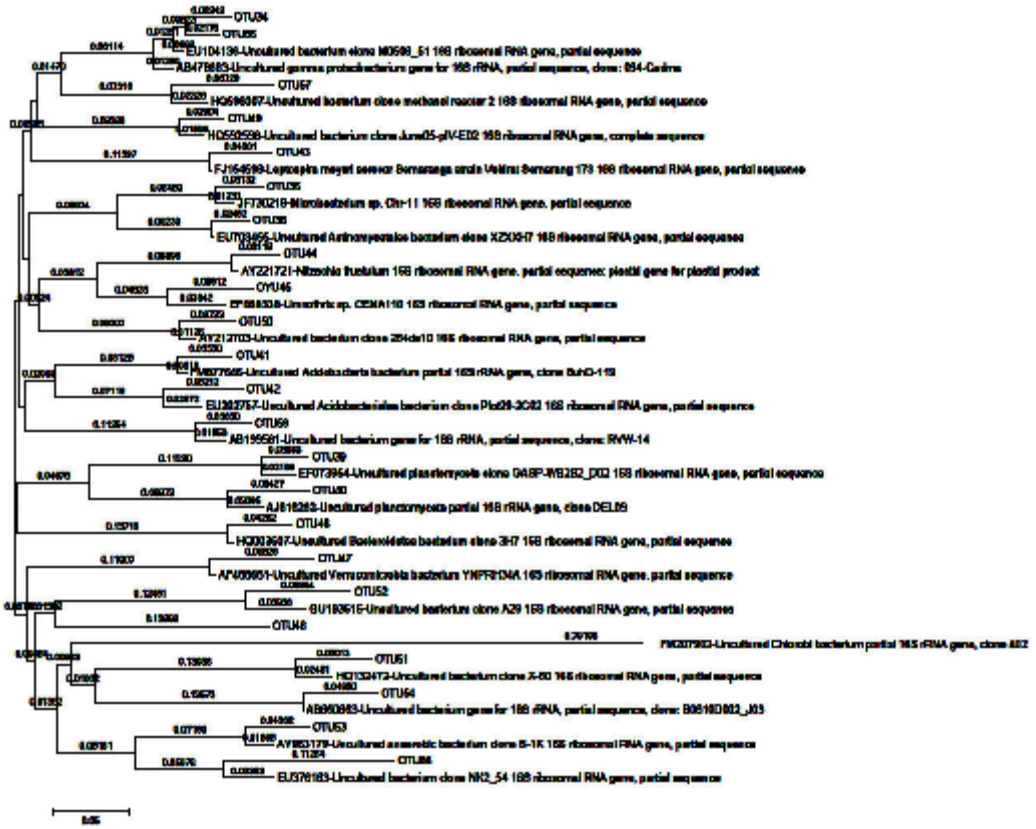

Fig. 2. Phylogenetic tree of OTUs belong to other class

Planctomycetacia, although the amount is not much, can remove nitrogen in the aerobic and anaerobic conditions. The dominant bacteria for nitrogen removal are mostly take organic matter as carbon source, therefore they play an important role in reduce effluent organic matter. Especially the γ *Proteobacteria* can be used for the removal of various pollutants , which also includes the ammonia nitrogen and organic material. They are also important biological treatment quality indicator parameters. The *Methylomonas* can remove organic matter in aerobic environment, and its known mainly carbon source is methane, methanol and formaldehyde.

Some microorganisms measured in clone database also have outstanding performance in specific pollutant removal, such as the degradation of PCBs, PCP, herbicides and other pollutants. The *Actinomycetes* bacteria can degrade polycyclic aromatic hydrocarbons, fenamiphos and other pollutants. Although such indicators did not monitor in this study, special pollutant removal can also serve as the research content, and for further understanding of PAC-UF technology characteristics.

CONCLUSION

The microorganism in the reactor is in a large quantity, and concentrated in biological flocculation tank. The number of microorganism is in the range of 4000 to 8000 CFU/ml in the phase of stable operation. The bacterial diversity of the sludge in flocculation tank is studied by the construction of a 16S rDNA clone library. The bacterial community in the sludge is highly diverse, including plenty of organisms which can be easily found in wastewater and drinking water treatment progress. The top three fractions are Beta-proteobacteria, Gamma-proteobacteria and Alpha-

proteobacteria. There are many bacteria beneficial to the removal of organics, ammonia nitrogen and other special pollutants. All of them compose a stable ecological system in the PAC-UF reactor, and play an important role in degrading pollutants and guaranteeing quality of effluent.

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