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Study on the bacterial and archaeal community structure and diversity of activated sludge from three wastewater treatment plants

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ABSTRACT

In this study, the bacterial and archaeal communities along with their functions of activated sludge from three wastewater treatment plants were investigated by Illumina MiSeq Platform. The treatment processes were modified A/A/O, DE oxidation ditch and pre-anaerobic carrousel oxidation ditch, respectively. The taxonomic analyses showed that *Proteobacteria* was the predominant bacterial phylum, and *Nitrosospira* was the dominant nitrification genus. *Candidatus Accumulibacter* was abundant in DE oxidation ditch process, and the main archaea communities were *methanosaeta*-like species which had the capability to anaerobic ammonia oxidation. The results illustrated that anaerobic ammonium oxidation played an important role in the nitrogen metabolism and there might be other unknown phosphate-accumulating organisms (PAOs) performing phosphorus removal in activated sludge. The predicted function analyses indicated that both bacteria and archaea were involved in nitrification, denitrification, ammonification and phosphorus removal processes, and their relative abundance varied metabolic modules differed from each other.

1. Introduction

Activated sludge process had been widely applied as a biological wastewater treatment technology more than one hundred years owing to its advantages in nutrient removal, toxin degradation and high biomass retention (Amanatidou et al., 2015; Tchobanoglous and M. E. Inc., 2003). Activated sludge was a complicated system consisted of bacteria, archaea, protozoan and viruses, in which bacteria and archaea played the leading role (Zhang et al., 2012). The community structure and diversity of microbes along with the interactions in dominant microorganisms determined the performances and functional stability of wastewater treatment plants (WWTPs) (Wagner and Loy, 2002). Therefore, research on the microbial community structure and their functions of activated sludge might give some guidance on sludge population optimization and improving operation of WWTPs.

Biological molecular techniques such as denaturing gradient gel electrophoresis (DGGE), fluorescent in situ hybridization (FISH) (Vanwonterghem et al., 2014) and reverse transcription polymerase chain reaction (RT-PCR) (Chang et al., 2011) were often employed to microbial community analyses. But some conventional methods like DGGE and 16S rRNA clone libraries had been precluded due to the bias of amplification (Aird et al., 2011) and low sequencing depth. Recently, as a powerful and highly efficient tool, high-throughput sequencing (e.g. Illumina, SOLiD and 454 pyrosequencing) (Mardis, 2011) had developed rapidly in assessing genetic diversity of natural samples (Salipante et al., 2014). This method was widely applied to evaluate microbial communities of other environmental samples like marine water (Metcalf and Donk, 2012), drinking water (Jia et al., 2015) and soil (Deng et al., 2014), because it was more reliable and cheaper. In the field of wastewater treatment, Illumina sequencing was used to analyze the microbial diversity in anaerobic digesters (Fu et al., 2016) and study the archaeal community structure in upflow anaerobic sludge blanket (UASB) (Antwi et al., 2017) as well as research the bacterial communities of activated sludge (Zhang et al., 2012; Kong et al., 2017) and anaerobic granular sludge (AGS) (He et al., 2016). Abundant studies had been conducted to explore bacterial community structure in activated sludge, however, they mainly focused on bioreactors or particular bacteria, such as denitrifying bacteria, ammonia-oxidizing bacteria (AOB) (Wu et al., 2016), glycogen accumulating organisms (GAOs) and poly-phosphate accumulating organisms (PAOs), little work had been done to reveal the archaeal communities structure of activated sludge. It was not comprehensive and cannot reflect the real microbial

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communities of full-scale WWTPs. Moreover, bacterial or archaeal communities in activated sludge had been reported separately in many studies; however it was rarely reported simultaneously. Archaea could exist in extreme environments, such as the hot spring and acidic environment (Song et al., 2013); they had even been identified in anoxic/aerobic submerged biofilter system (Fröls, 2013) and reverse osmosis membrane system (Al et al., 2014). Anaerobic ammonia oxidation (ANAMMOX) played an important role in the global nitrogen cycle, through this process; nitrite and ammonium were directly converted into nitrogen, which could remove ammonia nitrogen in wastewater (Sonthiphand et al., 2014). In fact, methanogenic archaea and ammonia oxidizing archaea (AOA) like *Crenarchaeota* also made an important effect on nitrogen and carbon removal processes (Fredriksson et al., 2012).

Therefore, in this study, three activated sludge samples were collected from different full-scale WWTPs, and conducted high-throughput sequencing using the Illumina Miseq platform. The major goals of this study were to research the structure and diversity of bacterial and archaeal communities of three different WWTPs; to analyze the key metabolic functions of different microorganisms comprehensively; to explore the different treatment process's effect on bacterial and archaeal communities.

2. Materials and methods

2.1. Sample collection

Three activated sludge samples were collected from secondary clarifiers of different wastewater treatment plants in Wuhan, Hubei province, China, which were B1 from Erlangmiao WWTPs (30°18′5.8″N, 114°10′17.4″E), B2 from Tangxun Lake WWTPs (30°26′9.3″N, 114°14′9.5″E) and B3 from Luobuzui WWTPs (30°47′11.3″N, 114°18′7.3″E). These WWTPs treat mainly municipal wastewater and the treatment processes were modified A/A/O (anaerobic/anoxic/aerobic), DE oxidation ditch and pre-anaerobic carrousel oxidation ditch processes, respectively. The mixed liquid suspended solids (MLSS) of B1, B2, B3 were 3540 mg/L, 3342 mg/L and 3587 mg/L, respectively. The mixed liquor volatile suspended solids (MLVSS) of B1, B2, B3 were 2339 mg/L, 2441 mg/L and 2690 mg/L, respectively. And the hydraulic retention times (HRT) of B1, B2, B3 were 14.0 h, 7.8 h and 13.2 h, respectively. The average qualities of influent and effluent qualities of three WWTPs were showed in Table 1. Sludge samples were taken on June, 22, 2017. For each sample, we collected 500 mL activated sludge from three sites of the secondary clarifier and mixed them uniformly. Centrifuge for 5 min at 8000*g*, remove supernatant, and then keep at −20 °C for further treatment.

2.2. DNA extraction, PCR amplification and pyrosequencing

Bacterial and archaeal genomic DNA were extracted by using E.Z.N.A. soil DNA isolation kit (OMEGA Biotek Inc., Norcross, GA, USA) following the manufacturer's instruction. Fragments of the 16S rRNA gene were amplified by PCR using universal primer 338F (5′-ACTCCT ACGGGAGGCAGCAG-3′) and reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Kumar et al., 2011) for bacteria, using barcoded primers set 340F (5′-CCCTAYGGGGYGCASCAG-3′) and 1000R (5′-GGCCATGCACYWCYTCTC-3′) (Gantner et al., 2011) for archaea targeting the V4–V5 hypervariable regions. Q5 High Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA) was used for DNA amplification. The PCR mixture of 25 μL contained the following: 0.25 μL polymerase, 5 μL 5× Reaction buffer, 5 μL 5× High GC buffer, 0.5 μL dNTPs, 1 μL forward primer, 1 μL reverse primer, 1 μL template DNA, and 11.25 μL ultrapure water. PCR was performed under following thermocycle: initial denaturation at 98 °C for 30 s followed by 25 cycles of denaturation at 98 °C for 15 s, annealing at 50 °C for 30 s, and an extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min.

Table 1
Average qualities of influent and effluent of three WWTPs.

Sample	B1		B2		B3	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
BOD_5 (mg/L)	52.13	10.41	61.52	7.87	75.6	7.52
COD_{Cr} (mg/L)	101.32	17.14	150.13	26.65	120.32	17.54
NH_3^+-N (mg/L)	11.21	1.67	25.12	1.62	28.13	7.47
TN (mg/L)	16.41	8.66	28.54	16.23	32.11	15.74
TP (mg/L)	2.98	0.86	3.22	0.76	1.44	0.41

The PCR products were checked by agarose (2.0%) gel electrophoresis, which were recycled by a DNA purification kit (Axygen Biosciences, Inc., CA, USA) and quantified with Nanodrop 2000 spectrophotometer. The MiSeq platform (Illumina, San Diego, CA) was applied for sequencing of the complete genome of three samples at Personal Biotechnology Co., Ltd. of China. DNA library quality was verified on an Agilent Bioanalyzer with a High Sensitivity DNA chip. The original gene sequences were available at NCBI Sequence Read Archive (SRA, http://www.ncbi.nlm.nih.gov/sra/, accession numbers of the bacterial sequences were SRR5895948, SRR5895947, SRR5895949 and the accession numbers of archaeal sequences were SRR5957102, SRR5957103 and SRR5957101 for B1, B2 and B3 respectively).

2.3. Sequence analysis

Using a sliding window approach to filter the low quality sequences, FASTA files were generated from the resultant sequences according to the barcodes of individual samples. Then software QIIME (Bokulich et al., 2013) (ver1.8.0) was used to identify the interrogative sequence, invoking USEARCH (ver.5.2.236) of software QIIME to check and eliminate the chimera and interrogative sequence.

Software QIIME and UPARSE were used as a sequence alignment tool to assign operational taxonomic units (OTUs) at the levels of 97% similarities. The representative sequences from each OTU were subjected to the RDP-II Classifier of the Greengenes databases. Alpha diversity and OTU networks were generated using software QIIME ver. 1.8.0. Rarefaction curves and the diversity indices (Ace and Chao) were determined based on the calculated OTUs using the same software. Differences in OTUs abundance at phylum and genus levels were determined using Metastats via a web interface (http://metastats.cbcb.umd.edu/detection.html). Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (Langille et al., 2013) was used to predict the functional content of the bacterial operational taxonomic units. Bacterial and archaeal functional profiles were compared at Kyoto Encyclopedia of Genes and Genomes (KEGG) modules level 2.

3. Results and discussion

3.1. Diversity of microbial communities

A total of 431,051 effective sequence reads (124,268 for bacteria and 306,783 for archaea) were yielded out by the MiSeq pyrosequencing after excluded the low-quality reads, then used for microbial community analyses (Table 2). The amount of archaeal sequences was 2.5 times as much as the bacterial sequences, and the average sequence quantity we obtained was more abundant than other activated sludge samples taken from WWTPs (Zhang et al., 2012; Sánchez et al., 2013). As shown in Table 2, the effective sequence reads varied inconsistent with operational taxonomic units (OTUs) and differed between samples. The OTUs of archaea were less than bacterial OTUs, the effective archaeal sequence reads were B3 > B2 > B1 and the OTUs were