#### Bioresource Technology 117 (2012) 72-79

Contents lists available at SciVerse ScienceDirect



**Bioresource Technology** 



journal homepage: www.elsevier.com/locate/biortech

# Microbial community structures in different wastewater treatment plants as revealed by 454-pyrosequencing analysis

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### HIGHLIGHTS

- ▶ Sixteen wastewater treatment systems were investigated by 454-pyrosequencing.
- ▶ The structures of microbial communities in different samples were not the same.

► The Simpson's diversity index and evenness index of MBR samples were the lowest.

► Treatment processes might have effects on the structure of microbial communities.

#### ARTICLE INFO

Article history: Received 21 February 2012 Received in revised form 17 April 2012 Accepted 18 April 2012 Available online 26 April 2012

Keywords: Activated sludge Microbial community Pyrosequencing Wastewater treatment plant

#### ABSTRACT

In this study, 454-pyrosequencing technology was employed to investigate the microbial communities in 12 municipal wastewater treatment plants (WWPTs) with different treatment processes. In total, 202,968 effective sequences of the 16S rRNA gene were generated from 16 samples that widely represented the diversity of the microbial communities. While *Proteobacteria* was found to be the dominant phylum in some samples, in other samples it was *Bacteroidetes*. The Simpson's diversity index and evenness index were lowest in samples from membrane bioreactors (MBRs), possibly due to the long sludge retention time (SRT) and low food/microorganism ratio (F/M). For one WWTP which had two disparate treatment processes operating in parallel, the structures of microbial communities in the two systems were compared. The differences found between the two indicated that the treatment process likely had effects on the structure of microbial communities.

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## 1. Introduction

Biological treatment has been broadly applied in WWTPs because of its high treatment efficiency and low cost. Different from physical treatment and chemical treatment, biological treatment depends on the metabolism of microbial communities to remove organic and inorganic pollutants, or transform them into nontoxic compounds. A better understanding of microbial communities in WWTPs can not only provide important guidance in design and stable operation of wastewater treatment systems, but also enrich the theory of microbial ecology (Oerther et al., 2001; DeAngelis et al., 2011). Researchers have studied microbes in wastewater treatment systems using a diverse set of methods, including fluorescence in situ hybridization (FISH) (Egli et al., 2003), denaturing gradient gel electrophoresis (DGGE) (Stamper et al., 2003), terminal restriction fragment length polymorphism (T-RFLP) (Wang et al., 2011), clone library (Figuerola and Erijman, 2007). As these conventional molecular biological techniques do not require isolation or cultivation of microorganism, they enable detection of many microbial species. However, due to its inherent limitations, the entire information about the microbial community structures can hardly be revealed by such conventional molecular biological methods. For example, the number of sequences that can be generated by the clone library method, which is time-consuming and expensive, is very limited (DeSantis et al., 2007). A limitation of PCR-DGGE is that one band may contain more than one species (Muyzer and Smalla, 1998). Thus, to more fully investigate the microbial community, more sensitive detection is needed.

Towards this goal, new technologies have been developed, such as the second-generation high-throughput sequencing, which can elucidate the characters of microbial community more completely and accurately (Roesch et al., 2007). Pyrosequencing, developed by Roche 454 Life Science, was the first second-generation DNA sequencing platform to be commercially available (Margulies et al., 2005). Compared to other second-generation platform, the critical advantage of 454-pyrosequencing is its read-length, which is typically ~400 bp (Shendure and Ji, 2008). This technology was first applied by other groups to study the composition of microbial communities found in the ocean (Sogin et al., 2006), and soil

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