High-throughput sequencing reveals microbial communities in drinking water treatment sludge from six geographically distributed plants, including potentially toxic cyanobacteria and pathogens

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HIGHLIGHTS

- First study of microbial community of DWTS using Illumina sequencing technology.
- Bacterial community in DWTS is affected by raw water type and geographical distance.
- Relationship between raw water quality and microbial community of DWTS was studied.
- Potentially toxic cyanobacteria and pathogens in DWTS were investigated.

GRAPHICAL ABSTRACT
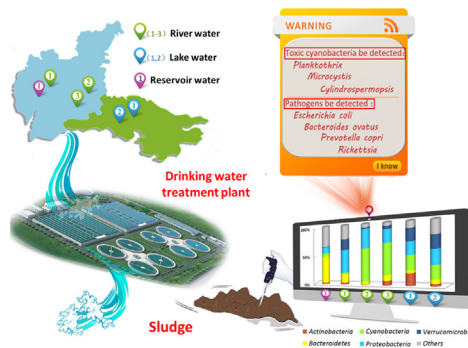
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ABSTRACT

The microbial community structures of drinking water treatment sludge (DWTS) generated for raw water (RW) from different locations and with different source types — including river water, lake water and reservoir water — were investigated using high-throughput sequencing. Because the unit operations in the six DWTPs were similar, community composition in fresh sludge may be determined by microbial community in the corresponding RW. Although *Proteobacteria*, *Cyanobacteria*, *Bacteroidetes*, *Firmicutes*, *Verrucomicrobia*, and *Planctomycetes* were the dominant phyla among the six DWTS samples, no single phylum exhibited similar abundance across all the samples, owing to differences in total phosphorus, chemical oxygen demand, Al, Fe, and chloride in RW. Three genera of potentially toxic cyanobacteria (*Planktothrix*, *Microcystis* and *Cylindrospermopsis*), and four potential pathogens (*Escherichia coli*, *Bacteroides ovatus*, *Prevotella copri* and *Rickettsia*) were found in sludge samples. Because proliferation of potentially toxic cyanobacteria and *Rickettsia* in RW was mainly affected by nutrients, while growth of *Escherichia coli*, *Bacteroides ovatus* and *Prevotella copri* in RW may be influenced by Fe, control of nutrients and Fe in RW is essential to decrease toxic cyanobacteria and pathogens in DWTS.

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1. Introduction

DWTS is a by-product generated by coagulation with inorganic alum-based or ferric-based coagulants, which are used to remove colour, turbidity and humic substances (Razali et al., 2007; Verrelli et al.,

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2009). Recent literature reported that Europe and the United States produce several hundred thousand and over two million tons of solid residuals each year, respectively, and global sludge production might total 10,000 t/day (Gibbons and Gagnon, 2011; Okuda et al., 2014), the equivalent of 4–7% of the total drinking water produced (Razali et al., 2007; Sun et al., 2015). Usually DWTS is not only rich in many contaminants from the natural water source, such as suspended solids, colloidal matter, algae and colour-causing organics, but also includes a large quantity of treatment agents. However, in many countries (especially in developing countries, such as China and India) most of the sludge is discharged into drainage systems directly without any treatment, or disposed of as waste to a landfill after being dewatered (Yang et al., 2006; Ahmad et al., 2016), which will cause serious environmental pollution to the surrounding aquatic or terrestrial ecosystem and waste of resources (Babatunde et al., 2011).

Studies of biodiversity are important for ecology of macroorganisms and microorganisms because of its potential correlation to ecosystem function (Tilman, 1999; Yang et al., 2011). Hence, it is essential to investigate microbial biodiversity of DWTS to understand the influence of DWTS on surrounding ecosystem when sludge is disposed of in sewers or as landfill. Because functional stability of DWTS primarily relies on the dominant microbial activities and interactions within highly diverse communities, a detailed investigation of microbial structure and functions in DWTS provides a more targeted approach that can diminish the harmful effects to surrounding ecosystem.

Due to nutrient over-enrichment of surface waters, toxic cyanobacteria in RW have risen substantially recently, especially in summer and autumn, which then leads to increase of toxic cyanobacteria content in DWTS. Presence of toxic cyanobacteria in DWTS poses a threat to animal and human health because these bacteria can release many types of toxic compound (Pestana et al., 2016; Jin et al., 2017), which can cause serious and even fatal liver, digestive, neurological, and skin diseases in humans (Paerl and Huisman, 2008; Funari et al., 2017). Furthermore, the pathogenic bacteria and viruses in RW can also be enriched in DWTS by coagulation. Wurzer et al. (1995) investigated six different waterworks in different regions of Germany and found that DWTS may contain pathogenic bacteria and viruses, including *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*, *Legionella*, and poliovirus. Hence, the study of structure of bacterial communities in DWTS, including toxic cyanobacteria and pathogenic bacteria and viruses was very important. However, up to now, little attention has been drawn to them.

Many studies have analyzed microbial diversity of activated sludge communities in wastewater treatment plants and found that there were obvious spatial and temporal changes in microbial diversity in different wastewater treatment plants, which may be due to the bioreactor operating parameters (Boon et al., 2002; Xia et al., 2010; Jo et al., 2016). Similarly, quality of RW in different geographic locations and of different types (e.g. river water, lake water, and reservoir water) may vary, which then may lead to differences in microbial communities in DWTS after coagulation. The relationship between RW quality and bacterial diversity in DWTS should be investigated to better understand the differences. To the best of our knowledge, there have been no relevant studies.

Recently, high-throughput sequencing technology has shown considerable advantages for analysis of microbial communities and can provide a deeper insight into the participating bacterial populations (Caporaso et al., 2012; Ibarbalz et al., 2013). It has been widely applied to investigations of bacterial community structure in drinking water (Chao et al., 2013), sea water (DeLong et al., 2006), activated sludge in sewage treatment plants (Yang et al., 2011; Zhang et al., 2012), and soil (Fierera et al., 2012). As regards the microbial communities related to drinking water systems, researchers have mainly focused on the bacterial communities of RW and treated water (Pinto et al., 2012; Chao et al., 2013). Although Chao et al. (2015) studied a pertinent investigation applying high throughput

sequencing to the biofilms formed on drinking water distribution pipelines. There is basically no metagenomic work investigating the microbial structure of DWTS in drinking water treatment plants. Hence, in the present work, high-throughput sequencing technology was used to investigate the microbial communities, especially toxic cyanobacteria and pathogenic bacteria, of 6 DWTS samples from different regions of China. The relationships between RW quality and microbial composition in DWTS were also investigated. In addition, water quality of the extracted water after DWTS dewatering was assessed and compared.

2. Materials and methods

2.1. Drinking water treatment plants and sample collection

Location and detailed information for each plant are shown in Fig. 1(a) and Table 1. As can be seen, four DWTPs were in Shandong Province and two DWTPs were in Jiangsu Province, China. Treatment processes in the six DWTPs were similar, including coagulation, sedimentation, filtration and disinfection, which are used in most of the Chinese water treatment plants. The RW sources for DWTP1 to DWTP6 were (in order): Yellow River, Shu River, Yi River, Hongze Lake, Luoma Lake, and Huangqian Reservoir. In the Yellow River basin, most areas are agriculture-dominated (Yu et al., 2010). There is also mainly agricultural area around Shu River and Yi River. Luoma Lake mainly has three inflowing rivers (Yi River, Zhongyun River, and Fangting River), which are mainly the agricultural area (Ren et al., 2016). Huai River is the mainly inflowing river of Hongze Lake, contributing 87% of the total amount of freshwater (Tian et al., 2015). In recent years, Huai River has a certain degree of pollution and the main pollutant sources were industrial and municipal point sources and agricultural nonpoint sources (Xia et al., 2011). The stream water flowing from Mount Tai is the major water source of Huangqian Reservoir and the reservoir is mainly surrounded by agriculture and tourism (Wei et al., 2014). It is worth noting that DWTP1 was close to DWTP6, DWTP2 was next to DWTP3, and DWTP4 was close to DWTP5. The coagulant used in DWTP1 was polymeric aluminium ferric chloride, while poly-aluminium chloride was applied in the other DWTPs (Table 1).

DWTS samples were collected from the sedimentation tank of the six Chinese DWTPs when they were formed in the drinking water treatment plant immediately in October 2016, along with samples of the corresponding RW from the respective water source. T (temperature), pH and DO (dissolved oxygen) of the samples were measured immediately after they were collected. Sludge and RW were then taken in sterile polyethylene bottles and transported to the laboratory on ice, where sludge samples were immediately centrifuged at 6000 rpm for 10 min and the extracted water (i.e. the centrate) was collected.

Portions of this dewatering water and RW were used to measure COD (chemical oxygen demand). The other portions of the extracted water and RW were filtered through a 0.45 μm cellulose acetate membrane before characterizing MCs (microcystins), CYN (cylindrospermopsin), TOC (total oxygen carbon), Cl^-, SO_4^{2-}, Al, Fe, TN (total nitrogen), TP (total phosphorus), NH_3-N (ammonia nitrogen) and NO_3-N (nitrate). The dewatered sludge was stored at −26 °C and then was used for DNA extraction, 16S rRNA gene PCR amplification, and Illumina MiSeq sequencing. To evaluate the experimental repeatability, a replicate sample of the dewatered sludge from DWTS1 was also analyzed (DWTS1–2).

Table 1 also describes the main characteristics of the six DWTS samples. The temperature and pH were similar, and furthermore there were no obvious differences in MLSS (mixed liquor suspended solids) or DO among the six DWTS samples, which implies that the drinking water treatment operations in the six DWTPs were similar.