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Impact of disinfection on drinking water biofilm bacterial community

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ABSTRACT

Disinfectants are commonly applied to control the growth of microorganisms in drinking water distribution systems. However, the effect of disinfection on drinking water microbial community remains poorly understood. The present study investigated the impacts of different disinfectants (chlorine and chloramine) and dosages on biofilm bacterial community in bench-scale pipe section reactors. Illumina MiSeq sequencing illustrated that disinfection strategy could affect both bacterial diversity and community structure of drinking water biofilm. *Proteobacteria* tended to predominate in chloraminated drinking water biofilms, while *Firmicutes* in chlorinated and unchlorinated biofilms. The major proteobacterial groups were influenced by both disinfectant type and dosage. In addition, chloramination had a more profound impact on bacterial community than chlorination.

Introduction

Microbial growth in drinking water distribution systems (DWDS) can lead to a number of adverse problems, including proliferation of opportunistic pathogenic microorganisms (Berry et al., 2006; Emtiazi et al., 2004; Liu et al., 2013, 2014; Lu et al., 2014). Disinfectants are commonly applied to lower the numbers of microorganisms in DWDS, maintaining a disinfectant residual. In China, the recommended doses of chlorine and chloramine in the water industry were 0.3–4 and 0.5–3 mg/L, respectively (Ministry of Health, 2006). Even at a high dosage, disinfectant application cannot avoid microbial regrowth in DWDS, due to the presence of organic matter and nutrients (Lu et al., 2013; Mathieu et al., 2009; Zhu et al., 2014). Diverse bacterial species can be found both in bulk waters and on pipe surfaces (Berry et al., 2006; Lu et al., 2013; Martiny et al., 2005; Vaz-Moreira et al., 2013; Wu et al., 2014, 2015). Autochthonous microbes may promote the growth of potentially pathogenic bacteria (Berry et al., 2006; Eichler et al., 2006). Therefore, an in-depth knowledge of DWDS microbial community and its influential factors is crucial for the development of effective control strategies (Berry et al., 2006; Lu et al., 2013; Wu et al., 2015). So far, a variety of factors have been found to regulate the structure of DWDS microbial community, such as type of source water, water treatment processes, disinfection, pipe materials, temperature and water age (McCoy and VanBriesen, 2012; Sun et al., 2014;

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Wang et al., 2014; Wu et al., 2015). However, there is still a wide scope for elaborate investigations on the impact of changes in factors governing drinking water microbial communities (Wang et al., 2014). Moreover, the impact of different disinfectants and dosages on DWDS microbial community remains unclear.

Culture-dependent methods and low-profiling molecular biology approaches have greatly contributed to our understanding of drinking water microbes (Lu et al., 2013; Vaz-Moreira et al., 2013). In contrast, high-throughput sequencing, as a next generation sequencing technology, can provide a new opportunity to systematically compare the effects of physiochemical parameters on DWDS microbial communities (Wang et al., 2014; Wu et al., 2015). To date, pyrosequencing analysis has found many applications in characterizing DWDS microbial community (Liu et al., 2012, 2014; Sun et al., 2014; Wang et al., 2014; Wu et al., 2015), however, Illumina MiSeq sequencing, the more recently developed high-throughput sequencing technology, has gained increasing popularity due to its lower costs and greater throughput, compared to pyrosequencing (Caporaso et al., 2012). So far, information on Illumina MiSeq sequencing of drinking water microbial community is still very limited (Wu et al., 2015). Therefore, the main objective of the current study was to systematically investigate the impacts of different disinfectants and dosages on DWDS bacterial community using Illumina MiSeq sequencing.

1. Materials and methods

1.1. Experiment setup and chemical analysis

In this study, the effect of disinfection on DWDS bacterial community was evaluated using bench-scale pipe section reactors (Fig. 1). Cast iron pipes (25–30 years old; length of 10 cm; diameter of 100 cm) used for the construction of bench-scale pipe section reactors were originally collected in a real DWDS transporting treated surface water. A stirring polyethylene paddle was driven by a motor at the rotating rate of 300 r/min to provide the hydraulic shear. The tap water (ground water previously receiving no disinfection treatment) in the campus of Tsinghua University was used as raw water. The physicochemical parameters of tap water are as follows: pH 7.82 ± 0.02, sulfate 70.5 ± 5.0 mg/L, chloride 19.5 ± 2.5 mg/L, alkalinity 145 ± 10 mg/L as $CaCO_3$, hardness 196 ± 10 mg/L as $CaCO_3$, conductivity 561 ± 20 μS/cm, turbidity 0.22 ± 0.12 NTU, DO 7.64 ± 0.40 mg/L, DOC 0.65 ± 0.15 mg/L, NH^{4+}-N < 0.02 mg/L, NO_2^--N < 0.003 mg/L, and NO_3^--N 0.46 ± 0.12 mg/L. Water pH and conductivity were measured by an electrode probe (HQ11d, HACH, Loveland, Colorado, USA). Turbidity was determined using a Turbidimeter (2011P, HACH, Loveland, Colorado, USA), while dissolved oxygen (DO) using a LDO probe (HQ30d, HACH, Loveland, Colorado, USA). Dissolved organic carbon (DOC) was measured using a TOC analyzer (5000A, Shimadzu, Kyoto, Japan). The concentrations of ammonium, nitrite and nitrate in waters were conducted according to the standard methods described by China Environmental Protection Agency (2002).

The tap water was amended with different levels of NaClO or NH_2Cl for disinfection tests. Pipe section reactors A0–A5 were fed with waters containing NaClO at the levels of 0, 0.04, 0.17, 0.56, 1.02 and 1.76 mg Cl_2/L, respectively, while reactors

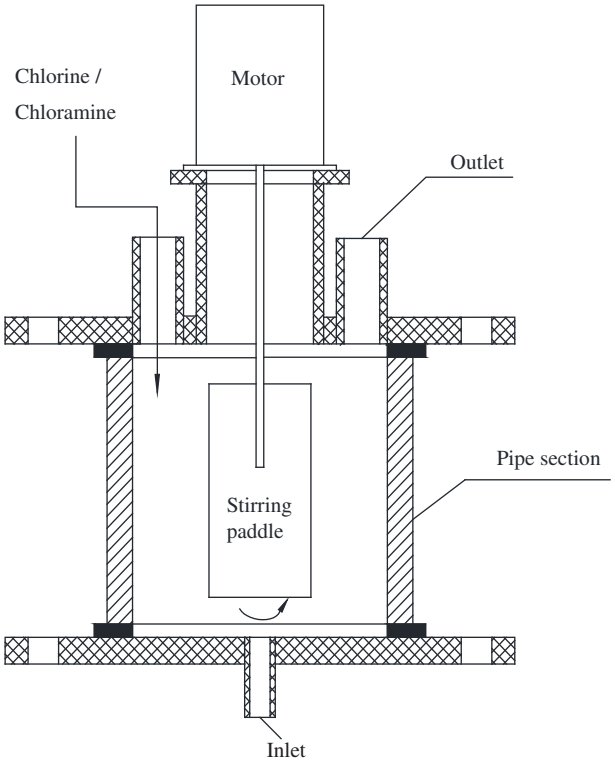

Fig. 1 – Schematic diagram of the bench-scale pipe section reactor.

B0–B6 with waters containing NH_2Cl at the levels of 0, 0.06, 0.20, 0.42, 0.78, 1.16 and 1.41 mg Cl_2/L, respectively. Chlorine and chloramine residual were measured using a HACH Pocket Colorimeter II Chlorine. These reactors were operated in a batch mode for about 2 months at 25°C prior to biofilm sampling. The water in each reactor was totally renewed every two days.

1.2. Molecular analyses

Biofilms were removed from pipes as previously described (Sun et al., 2014). Total genomic DNA was recovered from biofilms using the Powersoil DNA extraction kit (Mobio Laboratories, Carlsbad, CA, USA), and then amplified using the primer sets 515F (5′-GTGC CAGCMGCCGCGG-3′)/R907 (5′-CCGTCAATTCMTTTRAGTTT-3′) targeting V4–V5 hypervariable regions of bacterial 16S rRNA genes (Wang et al., 2015). The amplicons were subjected to Illumina MiSeq sequencing. The reads from the original DNA fragments were merged using FLASH (V1.2.7, http://ccb.jhu.edu/software/FLASH/) and the quality filtering was performed according to the literature (Caporaso et al., 2010). The sequences obtained from Illumina MiSeq sequencing analysis in the present study were deposited in the NCBI short-read archive under accession number SRP049933. UPARSE pipeline was used to cluster bacterial sequences into operational taxonomic units (OTUs) with a maximum distance of 3% and further generated the Shannon diversity index for each biofilm sample (Edgar, 2013). The OTU-based beta diversity analysis was carried out using UniFrac, and Bray–Curtis similarity matrices with QIIME (http://qiime.org/index.html) were used for Unweighted Pair