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Selective antibiotic resistance genes in multiphase samples during biofilm growth in a simulated drinking water distribution system: Occurrence, correlation and low-pressure ultraviolet removal

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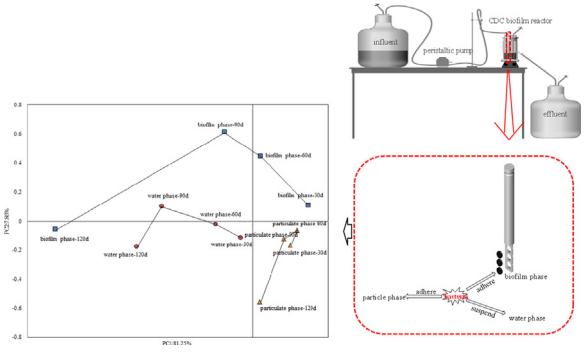
HIGHLIGHTS

- A simulated distribution system was used to explore antibiotic resistance genes (ARGs) occurrence.
- Six ARGs in effluent increased gradually during the running time.
- Six ARGs in samples of three phases have negative correlation with chlorine in pipe.
- Reduction of total organic carbon in water contributes to low-abundance of ARGs.
- Five ARGs in samples of three phases were controlled by low-pressure ultraviolet.

GRAPHICAL ABSTRACT



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ABSTRACT

The aim of this study was to gain comprehensive insights into the characteristics of antibiotic resistance genes (ARGs) in multiphase samples from drinking water distribution pipelines using a simulated biofilm reactor. During 120 d of continuous operation, common parameters and six ARGs (*ermA*, *ermB*, *aphA2*, *ampC*, *sulII*, and *tetO*) in samples of three phases (water, particle, and biofilm) from the reactor were investigated, which demonstrated secondary contamination by ARGs. Abundances of the six ARGs in the reactor effluent increased gradually, and in the 120 d effluent, the relative abundances of *aphA2* and *sulII* were the highest, at 9.9×10^{-4} and 1.3×10^{-3}, respectively, with a 1.5-fold and 2.8-fold increase, compared with those in the influent. The relative abundances of the six ARGs in the biofilm phase increased significantly ($P < 0.05$) at 120 d, which was caused by robust bacteria in biofilm that was newly exposed following the detachment of a large piece of aging biofilm. In the particle phase, four of the ARGs did not change significantly during the 120 d period. The six ARGs in the samples of three phases showed a negative correlation with residual chlorine in the pipe water, which demonstrated that low abundance of ARGs in the samples of three phases was related to the improvement of residual chlorine. The proportion of cultivable bacteria illustrated that the robust and active bacteria were negatively correlated with the six ARGs in the biofilm. Total organic carbon (TOC) in the pipeline showed a positive correlation with the proportion of cultivable bacteria in both the water and biofilm phases, which indicated that a TOC reduction in the pipeline contributed to low abundance of ARGs. With low-pressure ultraviolet (LP-UV) irradiation of

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20 mJ/cm^2, ARGs in the samples of three phases were efficiently controlled, which showed that LP-UV can be used for ARG removal in terminal water for supplemental bactericidal treatment of pipeline effluent.

1. Introduction

The excessive and nonstandard use of antibiotics has led to a significant increase in the amount of antibiotic residues in environmental media, particularly in the aquatic environment (Marti et al., 2013; Bergeron et al., 2015). Residual antibiotics can exert selective pressure, inducing and accelerating the appearance of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Marti et al., 2014). A number of antibiotics and ARGs have been detected in different water sources, effluents of treatment units in water treatment plants (WTPs), and terminal tap water (Jiang et al., 2013; Guo et al., 2014; Xu et al., 2016). Traditional water treatment processes cannot effectively remove ARGs in water (Xi et al., 2009; Bergeron et al., 2017). Some treatment processes, such as chlorine disinfection and activated carbon filtration, enhance the resistance of bacteria, which can contribute to the spread of ARGs in water (Shi et al., 2013; Guo et al., 2014). WTP-treated drinking water is transported through the pipe network before it is finally supplied to the user. The pipe network system acts like a giant reactor, providing a continuous and stable environment for the growth of microorganisms. Although the pipeline system lacks nutrients and contains residual chlorine, bacteria can still grow both in water and as a biofilm (Yang et al., 2011). The bacteria in biofilm can grow together to high density, and then confer better protection and aggregation effects (Butt and Khan, 2015; Proctor and Hammes, 2015). Thus, biofilm on pipe surfaces may result in potential microbiological contamination, resulting in the deterioration of hygienic drinking water quality (Wingender and Flemming, 2004). Owing to the poor nutrition in the water environment, microbial attachment to the biofilm and its subsequent growth are dominant, allowing some favorable genes to adapt better to the environment (Fang et al., 2010). Furthermore, mobile genetic elements, such as transposons and integrons, in distribution systems can promote horizontal transfer and amplification of ARGs (Fang et al., 2010; Dan et al., 2016). It was reported that bacteria in biofilms are 1000 times more resistant than the planktonic bacteria in water (Ceri et al., 1999). A higher level of ARGs was found in water from the pipe network than in WTP effluents and water sources (Xi et al., 2009), which was mainly due to biofilm growth in the pipeline system. As the water distribution systems are the last link for ensuring high quality drinking water for consumers, the threat to human health caused by ARGs in pipeline biofilms cannot be ignored, and an investigation of the distribution and control of ARGs in water distribution systems should be conducted.

Targeted samples in water distribution systems in previous research were commonly divided into the water phase and pipe biofilm phase (Boe-Hansen et al., 2002; Henne et al., 2012). However, particles in the pipeline are considered an important source of bacteria entering drinking water distribution systems both for bacterial regrowth and for bacteria found in accumulated loose deposits (Liu et al., 2013a). It was found that between 25 and 50 cells were attached to a single particle and that their resistance to disinfection and their metabolic index (ATP per cell on particulate matter) were higher than those for planktonic bacteria in water (Liu et al., 2013a). Therefore, rather than only two phases, the existence of bacteria in distribution systems should be divided into three phases: the water phase, biofilm phase on the pipe wall, and particle phase. However, the correlations between the ARGs and other parameters in samples of three phases from the pipeline are currently unclear. Furthermore, to date, no treatment processes have been considered to use in terminal water purifiers to control the ARGs contamination in multiphase samples in the pipeline.

In the present study, a Centers for Disease Control and Prevention (CDC) biofilm reactor was used to simulate the distribution pipe system (Ling and Liu, 2013). During 120 d of continuous operation, the presence of six ARGs, namely *ermA* and *ermB*, *aphA2*, *ampC*, *sulII*, and *tetO*, with resistance to erythromycin, aminoglycoside, β-lactam, sulfonamides, and tetracycline, respectively, were investigated to determine the occurrence of ARGs in the multiphase system. Furthermore, the correlations between the six ARGs and other parameters were analyzed and removal efficiencies of the six ARGs via low-pressure ultraviolet (LP-UV) irradiation were examined.

2. Materials and methods

2.1. Simulation of the water distribution system

The CDC biofilm reactor used to simulate the drinking water distribution system is shown in Fig. 1. The reactor consisted of a 1 L cylindrical glass vessel. There were eight separate, removable polypropylene rods on the top; a cover with an inlet; a sampling port; and a gas exchange port. Three detachable polycarbonate coupons, to provide a surface for biofilm growth, were fixed to each rod. The inside of the vessel was fitted with a diaphragm and a rotor, which drove the partition to rotate and provided a certain shearing force to the coupons, to instill water conditions similar to those in an actual water supply network. In the experiment, the rotational speed was set to 200–300 rpm, ensuring uniform shear force and stable hydraulic conditions for the coupons in the reactor.

2.2. Quality parameters of the CDC reactor's influent

During the continuous operation, the CDC reactor was fed tap water from the city of Beijing, China, and the main quality parameters for the influent were as follows: turbidity: 0.10–0.28 NTU, pH value: 7.5–7.8, total hardness: 117–126 mg/L, total dissolved solids: 135–158 mg/L, sulfate: 36–42 mg/L, chloride: 14–17 mg/L, fluoride: 0.22–0.24 mg/L, and nitrate nitrogen: 1.0–1.3 mg/L. Tap water flowed into an influent vessel for regulation and adjustment of water quality before flowing to the reactor. Chlorine was supplemented to the influent vessel to maintain an initial residual chlorine of around 0.8 mg/L.

2.3. Collection of the samples of three phases

Samples of influent, effluent, biofilm, and particles were collected separately. For water-phase samples, 10 L influent and 10 L effluent were collected and filtered through a 0.22-μm polyether sulfone membrane filter (GPWP, Millipore). For particle-phase samples, 50 L effluent was filtered through a 1.2-μm glass Swinnex filter (Millipore SX0004700). The filters of the water-phase and particle-phase samples were placed on a clean bench, and the microbial floras on the filters were scraped off with a spatula. To rinse the bacteria on the scraper and filter surface, 10 mL of sterile phosphate buffered saline (PBS) solution was used, and the suspension was collected in a 50 mL centrifuge tube. For biofilm-phase samples, three coupons were placed in one 50 mL centrifuge tube with 15 mL sterile PBS solution, and then vortexed for 2 min. To detach bacteria from the coupons, ultrasonication (500 W, 40 kHz, 20 min) was applied in combination with vortexing or handshaking for 30 s every 10 min, and then the coupons were removed (Luo et al., 2014; Li et al., 2017). The water-phase, biofilm-phase, and particle-phase suspensions were centrifuged at 10,000 ×*g* for 10 min