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Quantification and identification of particle-associated bacteria in unchlorinated drinking water from three treatment plants by cultivation-independent methods

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ABSTRACT

Water quality regulations commonly place quantitative limits on the number of organisms (e.g., heterotrophic plate count and coliforms) without considering the presence of multiple cells per particle, which is only counted as one regardless how many cells attached. Therefore, it is important to quantify particle-associated bacteria (PAB), especially cells per particle. In addition, PAB may house (opportunistic) pathogens and have higher resistance to disinfection than planktonic bacteria. It is essential to know bacterial distribution on particles. However, limited information is available on quantification and identification of PAB in drinking water. In the present study, PAB were sampled from the unchlorinated drinking water at three treatment plants in the Netherlands, each with different particle compositions. Adenosine triphosphate (ATP) and total cell counts (TCC) with flow cytometry were used to quantify the PAB, and high-throughput pyrosequencing was used to identify them. The number and activity of PAB ranged from 1.0 to 3.5 $\times$ 10^3 cells ml^{-1} and 0.04–0.154 ng l^{-1} ATP. There were between 25 and 50 cells found to be attached on a single particle. ATP per cell in PAB was higher than in planktonic bacteria. Among the identified sequences, *Proteobacteria* were found to be the most dominant phylum at all locations, followed by *OP3* candidate division and *Nitrospirae*. Sequences related to anoxic bacteria from the *OP3* candidate division and other anaerobic bacteria were detected. Genera of bacteria were found appear to be consistent with the major element composition of the associated particles. The presence of multiple cells per particle challenges the use of quantitative methods such as HPC and *Coliforms* that are used in the current drinking water quality regulations. The detection of anoxic and anaerobic bacteria suggests the ecological importance of PAB in drinking water distribution systems.

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1. Introduction

When distributing drinking water, the regrowth of bacteria and other organisms may occur and lead to water quality deterioration (Ridgway and Olson, 1982; Van Der Kooij, 2000). Depending on the source water and water treatment, more or less planktonic bacteria (PB), as well as particle-associated bacteria (PAB) and biodegradable compounds, are present in the treated water. They enter the drinking water distribution system (DWDS) and may serve as "seeds" for regrowth. The generation of PAB during drinking water treatment is caused by the action of particles as the site for attachment and growth of bacteria (Gregory, 2005; Winkelmann and Harder, 2009). It has been reported that PAB represent a small number compared to the PB population in treated water (Brazos and O'Connor, 1996). Nevertheless, the PAB that may pass through or be generated during treatment have been considered an important source of bacteria entering the drinking water distribution systems both for bacterial regrowth (Camper et al., 1986) and bacteria in accumulated loose deposits (Gauthier et al., 1999; Vreeburg et al., 2008; Liu et al., 2013). PAB have been detected in 41.4% of the samples of granular activated carbon filtered water at water treatment plants (Camper et al., 1986), and in 17% of samples collected from fire hydrants in drinking water distribution systems and water well outlets (Ridgway and Olson, 1981).

Since the attachment and growth of bacteria can lead to biofilm formation on particles (Winkelmann and Harder, 2009), a major concern regarding PAB is their resistance to disinfection (Brazos and O'Connor, 1996; Dietrich et al., 2009; Hess-Erga et al., 2008; Hoadley and Gould, 1977; Lin et al., 2010; Wojcicka et al., 2008). PAB have been proven to be more resistant to disinfection by chlorine (Ridgway and Olson, 1982), ozone (Hess-Erga et al., 2008) and ultraviolet (UV) (Mamane and Linden, 2006; Wu et al., 2005) than PB are. As a result, the regrowth or survival of pathogens in drinking water distribution systems may be enhanced (Herson et al., 1987). Considering the disinfection resistance of PB and PAB, the nutrient limitation approach (Van der Kooij, 1992) to produce biologically stable drinking water is likely to control the regrowth of both PB, PAB and bacteria in biofilms attached to pipe walls.

Another concern regarding PAB is the potential underestimation of bacterial numbers because no matter how many bacteria have been attached to one particle, they will be counted as one by traditional culture methods (Camper et al., 1986). Water quality regulations commonly place quantitative limits on the number of organisms (e.g., heterotrophic plate count and coliforms) and particle densities (e.g., turbidity), resulting in a substantial underestimation of the PAB bacteria present (Dietrich et al., 2007). In addition, PAB may house (opportunistic) pathogens and the dose of microbes may differ significantly if PAB rather than PB are ingested, thereby increasing the potential risk to customers. For instance, Herson et al. (1991) found that a large number of coliforms added to particle-containing drinking water could not be reflected by plate counting because they accumulated as PAB.

All the above-mentioned studies have improved knowledge of the importance of PAB in drinking water. However, limited studies on PAB in drinking water have been conducted, most of which applied cultivation-dependent methods (Camper et al., 1985; Ridgway and Olson, 1982; Wu et al., 2005) or microscopic observations (Brazos and O'Connor, 1996). Considerable bias and underestimation may be introduced by applying these methods. Consequently, PAB in drinking water have been poorly documented. Cultivation-independent techniques for bacterial quantification and identification offer new possibilities to reevaluate PAB in drinking water. The main goals of this study were to determine the presence of PAB in treated water from Dutch drinking water treatment plants by cultivation-independent methods, (i.e., use total cell count (TCC) with flow cytometry to quantify attached bacteria and adenosine triphosphate (ATP) to quantify activity), and use high-throughput pyrosequencing to identify the PAB. This study was undertaken to understand what PAB levels in drinking water are, what the fraction of PAB in the total bacteria levels is; how many bacteria are associated with a single particle; and what the PAB community is, and if the PAB community has a relation to the characteristics of the particles from different water treatment plants.

2. Materials and methods

2.1. Description of water treatment plants

Three drinking water treatment plants with different particle compositions in their treated water were selected: a treatment plant using artificial recharge and recovery (ARR) with river water as source water (TP1), and two groundwater treatment plants (TP2, TP3). TP1 takes source water from the Meuse River. The source water, after pre-treatment, is transported over 30 km to a dune area of natural lakes, where it recharges the groundwater. After an average residence time of 2 months, the water is abstracted from the dunes. Abstracted ARR water is post-treated by softening, powdered activated carbon, aeration, rapid sand filtration, and slow sand filtration before being pumped into the distribution system.

At TP2 anoxic groundwater is treated by aeration and rapid sand filtration, and afterward fed to the distribution system. At TP3, after abstraction, the groundwater is treated by aeration, filtration, softening, carry-over filtration, activated carbon filtration and UV disinfection. The treated groundwater contains somewhat higher levels of iron, manganese and ammonia concentrations than at TP1. The concentrations of these elements are also different between TP2 and TP3 due to the different treatments applied at the two treatment plants. The quality of treated water is summarized in Table S1 in the supplementary data.

2.2. Sampling

The sampling spots are located at the treatment plants just before the water enters the distribution system. PAB were collected with a specially designed multiple-particle filtration system (MuPFiS, Fig. 1). Each line of MuPFiS consists of 47 mm Swinnex filter holder followed by a flow meter. Multiple samples can be collected at the same time, and with the recorded water volume, the concentration of quantified PAB