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Combining flow cytometry and 16S rRNA gene pyrosequencing: A promising approach for drinking water monitoring and characterization

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ABSTRACT

The combination of flow cytometry (FCM) and 16S rRNA gene pyrosequencing data was investigated for the purpose of monitoring and characterizing microbial changes in drinking water distribution systems. High frequency sampling (5 min intervals for 1 h) was performed at the outlet of a treatment plant and at one location in the full-scale distribution network. In total, 52 bulk water samples were analysed with FCM, pyrosequencing and conventional methods (adenosine-triphosphate, ATP; heterotrophic plate count, HPC). FCM and pyrosequencing results individually showed that changes in the microbial community occurred in the water distribution system, which was not detected with conventional monitoring. FCM data showed an increase in the total bacterial cell concentrations (from $345 \pm 15 \times 10^3$ to $425 \pm 35 \times 10^3$ cells mL^{-1}) and in the percentage of intact bacterial cells (from $39 \pm 3.5\%$ to $53 \pm 4.4\%$) during water distribution. This shift was also observed in the FCM fluorescence fingerprints, which are characteristic of each water sample. A similar shift was detected in the microbial community composition as characterized with pyrosequencing, showing that FCM and genetic fingerprints are congruent. FCM and pyrosequencing data were subsequently combined for the calculation of cell concentration changes for each bacterial phylum. The results revealed an increase in cell concentrations of specific bacterial phyla (e.g., *Proteobacteria*), along with a decrease in other phyla (e.g., *Actinobacteria*), which could not be concluded from the two methods individually. The

Abbreviations: ANOSIM, analysis of similarity; ATP, adenosine tri-phosphate; a.u., arbitrary unit; FCM, flow cytometer / flow cytometry; HD-PE, high-density polyethylene; HNA, high nucleic acid; HPC, heterotrophic plate count; LNA, low nucleic acid; MDS, multidimensional scaling; OTU, operational taxonomic unit; PCR, polymerase chain reaction; PET, polyethylene terephthalate; TCC, total bacterial cell count; TOC, total organic carbon; %HNA, percentage of high nucleic acid content bacterial cells.

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combination of FCM and pyrosequencing methods is a promising approach for future drinking water quality monitoring and for advanced studies on drinking water distribution pipeline ecology.

1. Introduction

Drinking water should be biologically stable in order to limit unwanted bacterial growth within distribution systems. Bacterial growth can cause operational problems such as pipeline bio-corrosion or fouling, resulting in maintenance issues and customer complaints, and in the worst-case hygienically-related problems. It is therefore important to rapidly identify distribution systems areas with bacterial growth issues in order to undertake early maintenance actions. However, the occurrence of such situations may also require long-term improvement of the distribution conditions and maintenance strategies, which can only be achieved with an in-depth understanding of microbial dynamics in distribution pipelines. There is therefore a need for rapid, sensitive and accurate tools for microbial monitoring but also a need for quantitative and qualitative tools for detailed characterization of microbial communities in water samples.

The value of flow cytometry (FCM) for assessment and monitoring of total and intact bacterial cell concentrations during drinking water treatment and distribution has been highlighted before (Hoefel, 2003; Hammes et al., 2008, 2010; Ho et al., 2012; Lautenschlager et al., 2013; Liu et al., 2013a). The method is easy and rapid, with results obtained in 15 min from sampling. Moreover, FCM is quantitative, highly reproducible (less than 5% error) and sensitive (detection of change down to 3% from initial value) (Prest et al., 2013). In addition, correctly performed FCM measurements also generate so-called fluorescence fingerprints (De Roy et al., 2012; Koch et al., 2013a), which are unique to each sample and apparently dependent on the bacterial community composition and DNA content (De Roy et al., 2012; Vila-Costa et al., 2012; Koch et al., 2013a, 2013b; Müller et al., 2010). FCM fingerprints thus provide information on the bacterial community characteristics that is not obtained with FCM cell counting alone. The combination of FCM cell counting and fluorescence fingerprinting can have value for both monitoring purposes and for advanced studies in distribution pipelines, by providing rapid and quantitative information on the bacterial community characteristics, also revealing changes that are not reflected in the total cell concentration (e.g. a bacterial community turnover due to continuous attachment and detachment from pipe wall biofilms; Liu et al., 2013b). A recent laboratory-scale study has shown that the fingerprints can be quantified and used in combination with the total cell concentration for accurate detection of events affecting the bacterial community in water (Prest et al., 2013). However this approach has not yet been tested on real, full-scale drinking water distribution systems, where changes may well be less pronounced than those created under controlled laboratory conditions.

Sequencing methods have also gained considerable interest for microbial community characterization during drinking water treatment and distribution (Henne et al., 2008; Pinto et al., 2012; Liu et al., 2013c). Pyrosequencing is a next-generation sequencing technology that provides insight on the microbial community composition (identity) and structure (proportion). It does not require labelled primers/nucleotides or gel electrophoresis and allows a large number of samples to be pooled (Ronaghi, 2001; Fakruddin and Chowdhury, 2012). This technique has recently been applied for the identification of species present in water during treatment (Wakelin et al., 2011; Pinto et al., 2012) and distribution (Henne et al., 2008; Hong et al., 2010; Hwang et al., 2012; Lin et al., 2013; Liu et al., 2013a; Lautenschlager et al., 2013). The studies using pyrosequencing have proved the value of identifying bacterial groups, for the evaluation of e.g. disinfection (Hwang et al., 2012) or residence time (Lautenschlager et al., 2013) effects on bacterial community composition. Pyrosequencing can therefore provide meaningful qualitative information on drinking water distribution pipeline ecology.

Combining highly quantitative FCM data with detailed qualitative pyrosequencing data could provide adequate tools for both monitoring and detailed investigations of full-scale drinking water treatment and distribution systems. To date, only few recent studies have applied both FCM and pyrosequencing. The studies were either applied to different fields than drinking water (e.g. seawater bacterial community identification, Vila-Costa et al., 2012) or were lab-scale batch experiments under controlled conditions (Bombach et al., 2010). Two recent studies applied both methods on full-scale drinking water systems, one focussing on the characterization of particle associated bacteria (Liu et al., 2013c), the other exploring the variations in bacterial community characteristics in a distribution network (Lautenschlager et al., 2013). The latter study showed that relatively small changes in bacterial cell concentration and community composition can occur during water distribution and can be detected using FCM and pyrosequencing individually.

The objective of this study was to evaluate the combination of FCM bacterial cell counting, newly-developed FCM fingerprinting and 454-pyrosequencing data for the detection and characterization of microbial changes occurring in full-scale drinking water distribution systems. For this purpose, we moved a step forward from previous studies by (i) evaluating the complementary nature of data derived from these methods, particularly comparing FCM fingerprints with pyrosequencing-derived genetic fingerprints and (ii) combining data sets obtained independently by the two methods, for the generation of new quantitative information