Effects of Drainage and Stagnation on Bacterial Communities in Biofilm and Bulk Drinking Water

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Introduction

Diverse microbial communities inhabit the microenvironments of drinking water systems. While treatment processes such as filtration and disinfection remove or inactivate a great portion of microorganisms found in raw water, the treated water is ultimately not sterile. In DWDSs the majority of this microbial ecology can be found in biofilms attached to pipe walls[1], as well as in the bulk water phase. Microbial communities are ubiquitous in DWDSs even in the presence of disinfectants. The presence of microbial communities is especially relevant because they may compromise consumer microbial water quality through biofilm sloughing and general drinking water flow.

Biofilm formation can pose significant challenges for the drinking water industry because they can be a source of contamination, including pathogens. Research has shown that biofilms can serve as a reservoir for pathogens by providing a long-term habitat for pathogens and fecal indicator bacteria where they may persist in a viable but non-culturable state (VBNC) and develop a resistance to disinfectants[2]. One important issue that is still debated is how operating conditions of DWDSs affect the growth and microbiology of biofilms.

Intermittent Water Supply (IWS), in which pipes experience significant pressure fluctuations, stagnation, drainage, and other conditions is likely to affect bacterial communities in both biofilm and bulk water. IWS can be defined as piped water supply that is available for less than 24 hours per day, and is a condition that is prevalent worldwide. IWS can be caused by water scarcity, inadequate infrastructure and other factors and can be problematic due to risks of contamination and microbial regrowth[3-6]. The specific aim of this study is to understand how bacterial communities in bulk water and biofilms are affected by stagnation and drainage, which are key features of IWS.

Methods

Stagnation and drainage conditions were simulated using three annular reactors (ARs). Annular reactors use pressure and rotational speed to mimic pipe flow, and are advantageous because it allows for the manipulation of these variables. An intermittency period of three days was selected for the stagnant and drained reactor (i.e. four days of the week, these two reactors operated continuously, and for three days, the water supply was cut off, with one reactor drained and the other left stagnant). A third AR was set up to simulate constant continuous flow and to serve as a control. Each reactor was subjected to routine measurements prior to and after each intermittency event. The reactors were tested for temperature, pH, total chlorine and free chlorine. Samples were prepared by quenching chlorine residuals with 0.04% thiosulfate. SYBR Green and propidium iodide (PI) stains were used to determine total and intact cell count in each of the reactors using flow cytometry. Bulk water samples were filtered through a 0.2 μm filter and DNA was extracted from bulk water samples using a QIAGEN PowerSoil Pro kit with protocol modifications.

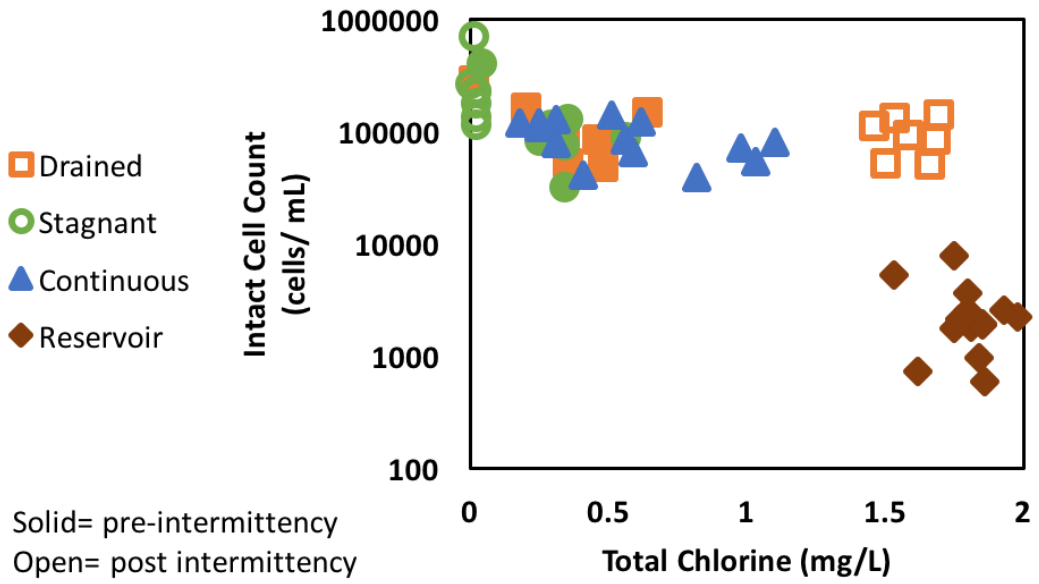

Figure 1. Intact cell counts versus chlorine concentrations before and after intermittency period for each reactor, where the Reservoir indicates tap water that serves as the source water for all reactors.

Results and Discussion

Results from the cell count experiment (Figure 1) suggest the formation of a biofilm within the reactors, which is apparent by observing that all reactor cell counts are greater than those of the reservoir by two orders of magnitude. Additionally, various trends emerge. When all reactors operate continuously their chlorine concentrations converge at approximately 0.5 mg/L and have similar cell counts at approximately 10^5 cells/mL. In contrast, after a period of intermittency, the stagnant reactor contains higher cell counts nearing 10^6 cells/mL and consumes the initial chlorine concentrations by nearly 100%. Lastly, the drained reactor exhibits unique results by containing both higher cell counts and chlorine concentrations. After experiencing an intermittency in water supply, the drained reactor was refilled with reservoir water and operated for 3-5 minutes before sampling, thus, the immediate leap from 10^3 to 10^5 cells/mL is indicative of biofilm sloughing. This sloughing emulates a first-flush period in a DWDS (when supply first returns after intermittency).

The annular reactor study demonstrated that different operating conditions, specifically stagnation and drainage alters a water supply's chlorine residuals and affect the magnitude of its microbial presence. Future research includes using extracted DNA to perform a metagenomic analysis of the microbial communities of each reactor. The results from this analysis will elucidate the way stagnation and drainage may affect the profile and composition of microbial communities in simulated DWDSs, which may be useful to improve the reliability of drinking water infrastructure.

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