The Ecology of Enteric Viruses within Distribution Pipe Biofilms

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ABSTRACT

Whilst there is sufficient evidence to suggest that enteric virions may accumulate and persist within distribution pipe biofilms, a surprising paucity of research has been undertaken in this important area of public health microbiology. The presence of enteric virions within distribution pipe biofilms may pose a potential underlying health risk to consumers, yet despite this fact, a quantification of the nature of this risk and an understanding of the mechanisms that permit this phenomenon to occur remains to be investigated.

As a first step in addressing this deficiency of research, a range of methods and models for the analysis of distribution pipe biofilms was evaluated. The synergistic combination of sonication and stomaching was shown to give an optimal yield of bacteria and indicator bacteriophages from biofilms formed on coupon surfaces. A multi-parametric analysis of biofilm biomass provided a holistic assessment of microbial biofilms that could be used to evaluate the potential health risks associated with distribution pipe biofilm growth. The simultaneous quantification of total heterotrophic bacteria (HPC on R2A), total direct counts of bacteria *(Bac*Light™ LIVE/DEAD®), total biofilm protein (NanoOrange® Protein Quantitation) and carbohydrate (phenol-sulphuric) content, and a range of indicator organisms (total coliforms, *Escherichia coli,* enterococci, and φXI74, MS2 and B40-8 bacteriophages) could be adopted by water utilities for the routine monitoring of biofilms within a distribution system.

Relatively inexpensive biofilm coupon devices, Biofilm Exosamplers™ and Biofilm Reactors™ were shown to provide comparable information on biofilm growth within a distribution system when compared to *in situ* modified Robbins Devices. A field sampling site, termed a Biofilm Sampling Site (BSS) designed and constructed within a municipal distribution system assisted in the establishment of cause and effect relationships between distribution pipe biofilm growth and prevailing physical and chemical water parameters (free and total chlorine, pH, hydraulic demand and water temperature).

Within the Rouse Hill Development Area (RHDA), New South Wales there was more biofilm growth in the recycled water distribution system, which had been serviced by potable water since commissioning of the system and throughout the duration of this thesis, than the potable water

distribution system. The physicochemical effects of substrata and hydraulic demand were shown to be the most important parameters influencing biofilm accumulation on surfaces, contradicting traditional beliefs that pH, disinfection, and to a lesser extent, water temperature are the major predetermining factors affecting biofilm growth within distribution systems.

Model enteric virions (φXI74, MS2 and B40-8 bacteriophages) were shown to be incorporated into potable water biofilms at concentrations representing approximately one percent of virions present in the adjacent bulk water phase. A breakthrough of 10^4 virions.L^{-1} from a recycled water treatment plant could therefore be taken up by biofilms within a distribution system. Consequently, over distances exceeding 2.5 km within a 150 mm (ID) distribution system, virions may not be detected in the bulk water, subsequently masking a potential underlying health risk to consumers. More importantly, a very persistent sub-population (approximately one percent) of these sessile virions remained infectious within biofilms throughout an experimental period of 30 days, inferring their potential to accumulate within distribution pipe biofilms over time. Furthermore, B40-8 bacteriophages and alternate model enteric virions (0.02 μm fluorescent micro spheres) were incorporated into bacterial microcolonies, environments that are known to sequester bacterial pathogens from the effects of disinfection.

Enteric virions can therefore accumulate within distribution pipe biofilms and be subsequently released in concentrated numbers to a distribution system through biofilm sloughing, where they may infect consumers through the direct or indirect consumption of water, or through the inhalation of aerosols. In water that meets NSW Recycled Water Guidelines (one infectious enteric virion in 100 L water, for example) sufficient numbers of enteric virions may accumulate within biofilms within a one metre length of 150 mm (ID) distribution pipe, and during a 90% biofilm sloughing event, present an annual risk of infection of 1.7×10^{-4} to consumers for the accidental daily consumption of one millilitre of recycled water. This value exceeds the USEPA risk benchmark of 10^{-4} *(i.e.* one infection in 10 000 incidences of exposure *per annum).*

Herein there lies a substantial need to better understand the functioning of distribution systems and the ecology of microorganisms contained within them in order to better assess the microbiological quality of water after it leaves a treatment plant. The results of this study may provide a platform for more detailed modelling of the health risks associated with pathogen accumulation within distribution pipe biofilms. Furthermore, an appreciation of enteric virion ecology within distribution pipe biofilms may also elucidate a currently unknown potential health risk associated with reticulated water quality that could be taken into consideration in the development of recycled water guidelines.