The impact of biofilms on water quality in long pipelines

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Limited water availability and increased water demand necessitates the use of long pipelines to distribute potable and non-potable water for human consumption or other purposes. The effects of microbial growth and activity on the quality of distributed water have been studied for many years, although in recent years much of this focus has shifted to understanding the effects of biofilms, rather than planktonic microorganisms, on water quality.

Recently, it was estimated that 95% of all biomass in water distribution systems is in the form of pipe-wall biofilms, with only 5% of all biomass in the bulk water phase [1]. Under favourable conditions, biofilms can impact water quality by increasing disinfectant demand, creating taste and odour problems, harbouring opportunistic pathogens and contributing to the potential for discoloured water events. More research is required to inform the development of guidelines for the management of biofilms in long pipelines to ensure the delivery of safe drinking water and to minimise impacts on water quality.

The *Australian Drinking Water Guidelines* (ADWG) [2] provide a framework for the management of the catchment, treatment and distribution systems for provision of safe drinking water. The ADWG clearly state that "... the greatest risks to consumers of drinking water are pathogenic microorganisms..."; however, the ADWG does not currently contain any specific information that refers to or provides guidelines for the management of biofilms in water distribution systems. Current monitoring regimes and guideline values are based on the analysis of bulk water samples. Given the relative abundance of sessile microorganisms (biofilms) over planktonic microorganisms (bulk water), this approach at best provides a significant underestimate of the microbiological status of the water distribution system. Recent Australian and international research is shedding new light on the roles and impacts of biofilms, and how they can be managed in order to limit their impact.

Biofilms grow when they are provided with adequate sources of energy, carbon, nutrients, and favourable temperatures. Most water sources contain natural organic matter (NOM). Organic compounds within the NOM provide a suitable source of energy and carbon for growth by heterotrophic microorganisms. Assimilable organic carbon (AOC) has been defined as the fraction of dissolved organic carbon (DOC) which microorganisms use to produce new biomass. Specific tests are used to quantify the AOC content in distributed water in order to help determine the 'biostability' of the water [3, 4], although their use in Australia is limited to research applications.

In some cases, inorganic compounds can act as an important energy source for biofilm growth. In parts of Australia, the less reactive chloramine is used instead of chlorine to maintain disinfection in long pipelines. To achieve chloramination, ammonia is combined with chlorine to produce monochloramine. Ammonia serves as the energy source for growth of ammonia oxidising bacteria (AOB) which oxidise ammonia to nitrite [5]. Loss of ammonia due to nitrification prevents the formation of monochloramine which leads to poor or no disinfection. Nitrifying biofilms have the ability to fix carbon dioxide and therefore act as 'primary producers' by generating organic carbon in the form of new biomass that can then serve as an energy source of heterotrophic microorganisms.

Parts of the Goldfields and agricultural water supply system in the wheat belt of Western Australia are impacted by nitrifying biofilms which prevent the formation of monochloramine [unpublished data]. This system is characterised by long above-ground pipelines with relatively long detention times. In the warmer months, water temperatures regularly exceed 30°C and often exceed 40°C. These elevated temperatures provide ideal conditions for the biofilm growth and correlate well with

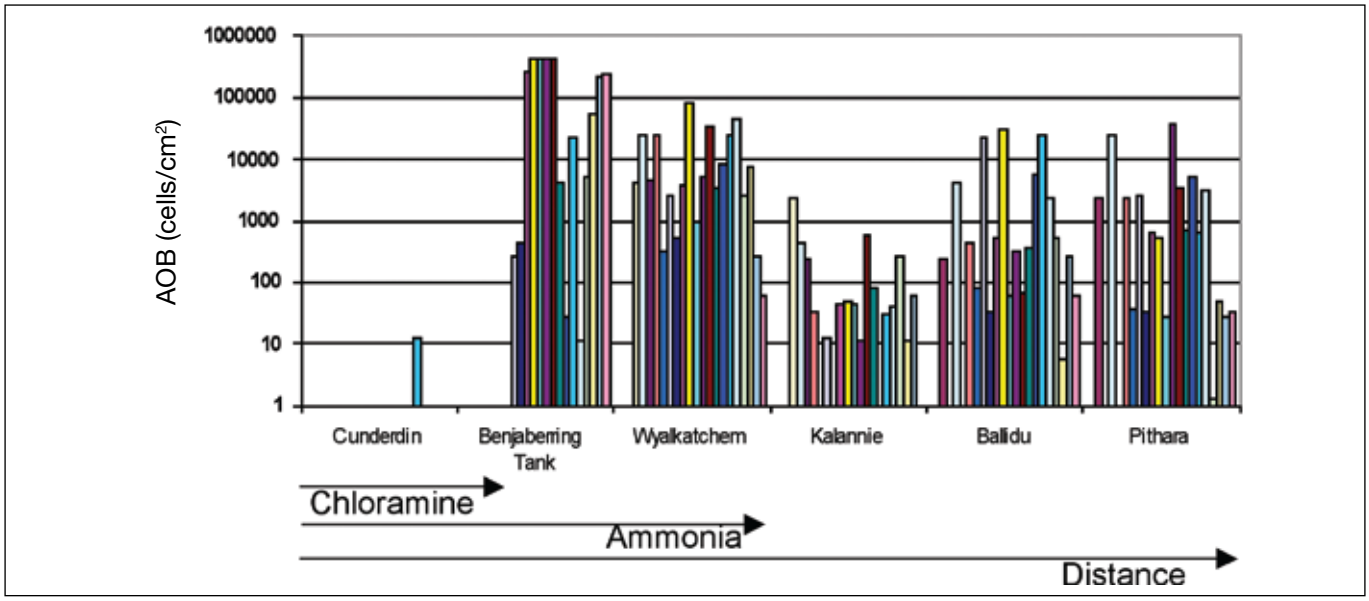

Figure 1. Numbers of ammonia oxidising bacteria (AOB) per cm² at different locations within parts of a chloraminated distribution system in Western Australia. Individual columns represent monthly or bimonthly samples collected from each site between early 2006 and mid 2008. Arrows indicate penetration of chloramine and ammonia and increasing distance through the system.

increases in ammonia oxidising bacteria in pipewall biofilms and concomitant loss of free ammonia in solution (Figure 1). Recent studies show that novel strains of nitrifying bacteria comprise a significant part of pipewall biofilms in the most active nitrifying zones [Ginige *et al.* in preparation].

Conditions of elevated water temperatures and little or no disinfectant residual biofilm growth provide an abundant food source for protozoa and other bacterivorous microorganisms. *Naegleria fowleri,* the causative agent of primary amoebic meningoencephalitis (PAM), is a thermotolerant free-living amoeba (Figure 2) that is occasionally found (to date in at least three Australian states) in pipewall biofilms growing at temperatures >20°C. Although infections with *N. fowleri* are rare, they are usually fatal. No cases of PAM have been reported in Australia in recent decades, although several fatal cases have occurred in the USA this decade, some due to poorly disinfected drinking water.

Detection of *N. fowleri* is therefore an important part of water quality monitoring in potentially affected distribution systems. The current testing procedure employs a culture-based method to enrich and purify pure strains of thermotolerant *Naegleria* which are then typed using a PCR-based molecular test [6]. This testing procedure takes at least 2-3 days before a positive detection is confirmed due to the need to culture the *Naegleria* spp. A new PCR-based method has recently been developed that is capable of specifically detecting *N. fowleri* in bulk water or biofilm [Puzon *et al.* in preparation]. This method is at least as sensitive, and more rapid, than the current testing procedure as it does not rely on the need to culture *Naegleria* spp. This test has the ability to provide a quantitative estimate of *N. fowleri* cell numbers in less than 24 hours. The application of this new method will hopefully lead to improved monitoring and management of distribution systems to prevent colonisation by *N. fowleri.*

While they do not represent a health risk, water aesthetics issues such as taste and odour problems, or discoloured water events, are significant issues due to their ability to rapidly generate negative public perceptions. These issues often generate the greatest number of customer complaints made to water utilities. The formation of mature pipewall biofilms can lead to the generation of water aesthetics problems. A 'swampy odour' due to the formation of dimethyl trisulphide in distributed water was attributed to the actions of biofilm microorganisms [7]. Biofilm reactor experiments showed that dimethyl trisulphide production from potential organosulphur precursors like cysteine and methionine was insignificant in the absence of biofilms. Mature biofilms can metabolise organic compounds and are also able to oxidise and accumulate inorganic materials such as metals.

An example of this ability is shown in Figure 3, where an iron-rich upper layer was found to cover a biomass-rich lower layer. Iron and manganese oxidising bacteria in water distribution systems have been well studied [8, 9], although there is limited information on the rates of iron and manganese accumulation within mature biofilms and the factors that influence this, e.g. bulk water Fe and Mn concentrations, disinfectant residuals, biofilm age or thickness. Recent laboratory findings show that even with acceptably low concentrations of Fe and Mn (0.05 and 0.02mg/L respectively) in the bulk water, over time, stable biofilms have the ability to accumulate sufficient Fe and Mn to provide the potential for a discoloured water event [Ginige *et al.* submitted]. This finding suggests that prevention of biofilm formation is at

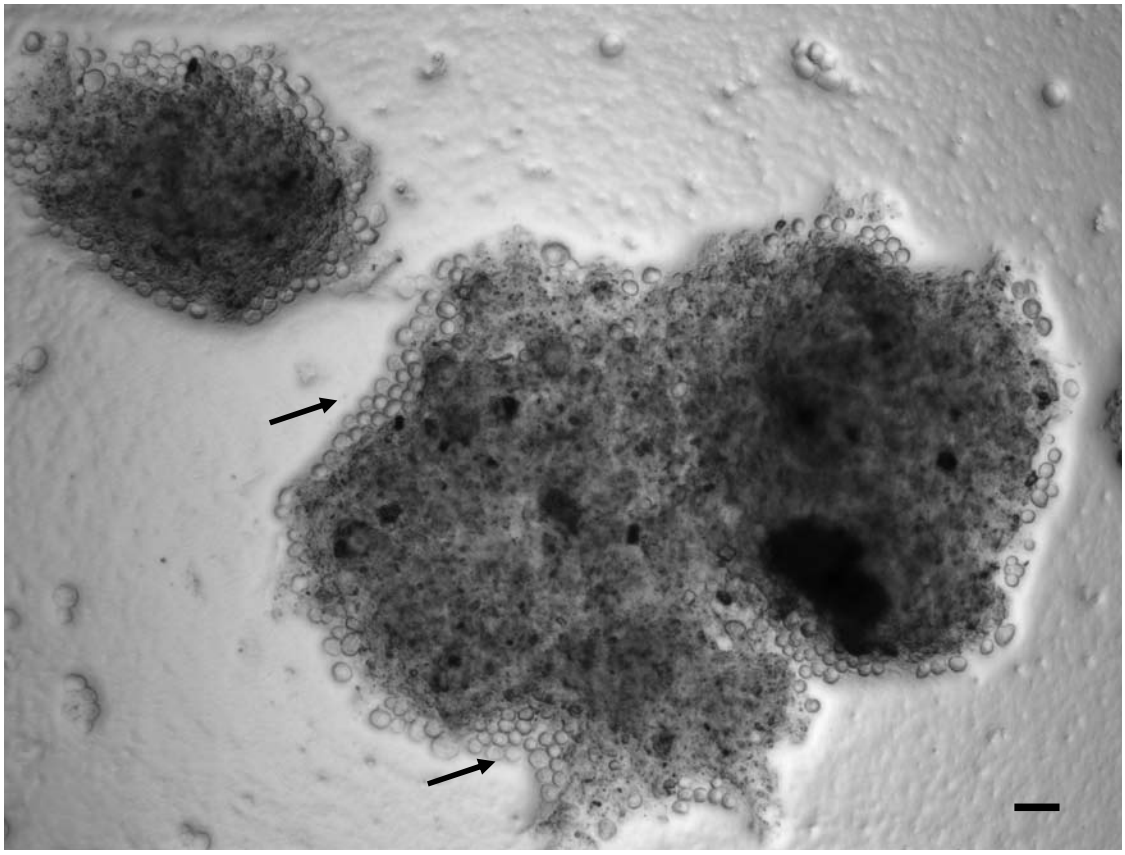

Figure 2. Photomicrograph of *N. fowleri* cysts enriched on a lawn of *E. coli* cells. Arrows indicate cysts. Scale bar=20μm.

Figure 3. Photograph of a mature biofilm with metal-rich upper layer above a biomass-rich lower layer on the pipewall surface within a distribution system (photo by Luke Zappia).

least as important as the effective treatment of water to achieve low concentrations of Fe and Mn.

Pipelines distributing lower quality non-disinfected water (e.g. stormwater, re-use water, untreated third pipe systems) likely provide more favourable conditions for biofilm formation. The level of management or control of biofilm formation within these systems will be dependent on the end-use of these waters and other considerations. Enhanced biofilm growth due to lower water quality will pose many of the same challenges as those outlined above; however, it has the potential to pose significant additional problems for treatment and distribution infrastructure through increased fouling of membrane-based filtration systems and also increased microbially induced corrosion.

Improved treatment of source water to remove AOC or other energy sources for microbial growth, combined with advanced disinfection strategies, represents the best way to prevent biofilm formation in drinking water distribution systems. For non-potable water distribution systems, more research is needed to better understand the likely impacts of biofilm growth on treatment and supply infrastructure, and to assess any potential hazards to human and environmental health.

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