Analysis of Biofilm-Resistance Factors in Singapore Drinking Water Distribution System

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Abstract. In drinking water distribution system (DWDS), biofilm offers protection of bacterial community within the extracellular polymeric substance (EPS) from outside stressors, including surrounding environment, predatory microorganisms and antibiotics. The growth of biofilm may increase cleaning and maintenance cost for the drinking water decontamination and expose high risk to human immune system. This article critically reviews current Singapore DWDS by analysing the leading and lagging performance of two stages, granulated active carbon (GAC) filtering and secondary disinfection by chloramine, which are highly relevant with the biofilm resistance. Furthermore, other extrinsic factors, such as pipe age and material, hydraulic retention time, seasonal change, primary ultraviolet disinfection, etc. are also reviewed. Finally, top-down approach to address the growth and biofilm resistance are proposed. Practices and technology methodology, e.g. good consumers’ hygiene, nutrient removal, chlorine disinfection, probiotic approach, anti-biofouling coatings are discussed with their associated challenges and opportunities.

Keywords: Singapore drinking water distribution system (DWDS); Biofilm-resistance factors; Granulated active carbon (GAC); Secondary disinfection; Biofilm-resistance control.

1. Introduction of Singapore drinking water distribution system

Biofilm is an aggregation of organic and inorganic, living and dead material collected on a surface. It could commonly appear as a complete film or small patch on pipe surfaces of the water systems. Biofilms in distribution system can be even responsible for a wide range of water quality and operational problems, e.g. loss of disinfectant residuals, increment of bacterial levels, reduction of dissolved oxygen, change of taste and odor, microbial-influenced corrosion, and hydraulic roughness. Setting the pretext for biofilm removal in drinking water distribution pipes, killing of microbials is not equivalent to cleaning. Without removal of the sticky extracellular polymeric substances (EPS), the biofilm remains...
even after killing of resident microbials and dead cell residuals could nourish a new wave of incoming microbials which had survived disinfection process. Thus, even with a marginal amount of nutrients in distributed water, biofilm could persist in pipes and could most likely, never be removed [1]. Therefore, the motivation should not be the complete removal of biofilm, but to attain the desired performance outcome under the prior knowledge that biofilm will always be present within a distribution pipe system. Changes to primary disinfection strategy could have influenced pipe biofilm dynamics, thus, the presence of biofilm should always be considered in every decision process of the treatment plant when deciding the disinfectant type or disinfection mode in Singapore.

The goal of setting Singapore drinking water distribution system (DWDS) is to provide safe, pathogen-free drinking water to Singaporean consumers with reasonable aesthetics appeal as well as low microbial load. Biofilm resistance in drinking water biofilm is defined with respect to higher pathogen survivability as a result of biofilm lifestyle, as opposed to a planktonic existence. The physical manifestation of pathogenic biofilm resistance could be measured on two levels: i) the presence of pathogens in biofilm; or ii) as detached biofilm cells could travel down the distribution network, the presence of (potential) pathogens recovered at the sampled distribution point. The persistence of pathogen occurs even after flocculation, sedimentation, filtration and chemical or UV disinfection, as the waterborne disease can still be transmitted to consumers through the Singapore DWDS. Potable water was found to be the source of *Mycobacterium avium* transmitted to AIDS patients in recent research [2]. In this paper, two stages of Singapore DWDS, which are directly relevant to biofilm resistance: i) filtration with activated carbon granules and ii) secondary disinfection by chloramine, are reviewed. At the meantime, those extrinsic factors, such as pipe age and material, hydraulic retention time from storage reservoirs to consumers, seasonal changes, sudden perturbation in weather and duration of water stagnation at distributed points, which may amplify the biofilm resistance, are also discussed (Fig. 1). Furthermore, position views of the strategies to address this biofilm-resistance issue in Singapore have also been raised.

**Figure 1.** Biofilm contamination of Singapore drinking water distribution system (DWDS).

2. Examining relevant stages of water treatment in existing Singapore DWDS

2.1. *UV irradiation in primary biofilm disinfection of DWDS*

Ultraviolet (UV) disinfection is actively assessed in other countries to replace chlorine as the primary disinfectant. However, it is not considered for Singapore, which uses chlorine and ozone as the primary disinfectants. The key advantage of UV disinfection was the lack of chlorine by-product in treated water,
a key concern for the Stockholm Waterworks [3]. Their study established that changing of disinfection method from chlorine to UV does not jeopardise the microbial quality of treated water as biofilm mass does not present significant changes after the change in disinfection method, although it was speculated that addition of monochloramine as a secondary disinfectant played a greater role to influence this observation.

However, the active role of biofilm in preventing UV damage has been examined in a model system involving *Pseudomonas aeruginosa* whole cell biosensor and simulated biofilm in the form of alginate beads [4]. Genetic engineering was applied to fuse recA promoter of *Pseudomonas aeruginosa* with the lux operon of *Vibrio fischeri* to produce a visible bioluminescence when DNA is damaged. When encapsulated by the alginate bead, a higher survival rate of *Pseudomonas aeruginosa* was observed compared to planktonic cells under the same UVB fluence while UVC irradiation-induced bacterial filamentation, a signature morphology of bacteria under high stress. The EPS matrix provides a physical and particulate shielding effect, as the success of UV disinfectant is highly dependent on penetration depth. The same sentiment was reflected in Pozo et al (2004) where UV-susceptible bacteria were found in a UV-irradiated environment [5].

2.2. Granulated active carbon (GAC) filter

The purpose of the GAC filter was to filter out any potential microbial contamination from primary disinfectant treated water. However, due to the high concentration of bacteria being blocked by these filters, biofilms are known to accumulate on granules and filters, leading to biofouling problems [6]. A cautionary note for using granulated carbon filters and membrane filters, there has been a report which suggests that biofilms on GAC filters may serve as a hotspot for the protozoan congregation, including pathogenic protozoan/free-living amoeba (FLA), which could harbour amoeba-resistant bacteria (ARB). A plausible reason was that biofilm provides a steady source of nutrients for these protozoa as they are known grazers of bacteria living in biofilm. In this situation, the biofilm not only provides FLA with a physical barrier against disinfectant but also a constant source of food, sustaining their growth. There are two problems to consider with the presence of protozoa and FLA in drinking water (Fig. 2):

![Figure 2. Schematic diagrams of microbial contamination and resistance in granulated active carbon (GAC) filter.](image)

1. Protozoa could form oocyst which are highly resistant to chlorine disinfection, which is the main strategy applied to current water treatment systems [7]. Higher exposure time (90 mins) with a higher dose (80 mg/L) were reported to achieve 90% inactivation of Cryptosporidium oocysts [8]. Other than Cryptosporidium, there are other amoeba-of-interests, which include *Acanthamoeba castellanii*,...
Vermamoeba vermiformis (formerly known as Hartmannella vermiformis) and Naegleria fowleri (commonly sensationalized as the "brain-eating amoeba"). Due to the prevalence of these protozoa in Europe DWDS [9]. Meanwhile, same concern would be shown to the treated water in Singapore. In a recent study examining if FLAs exist in both treated and untreated water in Southeast Asian countries (Myanmar, Laos and Singapore), it was revealed that out of 6 treated water samples, none of the samples contain any of the targeted FLA [10]. While in 11 treated water samples of Myanmar from different distribution points, including swimming pools, Vermamoeba vermiformis was found in all. Most alarmingly, water sampled from chlorinated swimming pools were also found positive with Vermamoeba vermiformis which indicates the establishment of a new niche environment, typically disinfected with a high concentration of chlorine. It was postulated that the pipe delivering the chlorinated water contains biofilm which harbors these FLA, protecting them from successful disinfection [11]. Surveying the database, there is only one report studying the presence of pathogenic FLAs in DWDS of Singapore.

2. In addition to being pathogens themselves, there have been reports of amoeba-resistant bacteria (ARB) which can evade phagocytosis within the FLA and reside continually within the host. Among numerous bacteria, some of which are known waterborne pathogens such as Legionella pneumophila (Legionellosis), non-tuberculous Mycobacterium avium and Pseudomonas aeruginosa (Pneumonia) and also emerging strains of pathogens [12]. The association of FLA and ARB is thoroughly reviewed in Thomas (2011) and pathogens like Legionella pneumophila are among the mix. In a theoretical model to explain the high FLA diversity and density in DWDS, biofilms are seen as natural habitats of mixed species biofilm that allow close interspecies relation between FLA and ARB [9]. While in actual biofilm situation, the chance of an ARB encountering and infecting a FLA trophozoite is much higher. In turn, the FLA acts as a carrier that protects ARB until the end of the distribution. ARB strongly benefits from the association to FLA, which allowed continuous intracellular replication, enhanced survival from disinfection and even increase of virulence [2]. Amoeba is eukaryotic organism which has multiple life stages, the most common two stages are: i) actively dividing trophozoite and ii) the sessile non-dividing cyst. Cysts are strongly durable structures which can resist a higher concentration of chlorine disinfectant. By extension, the killing of ARB is highly dependent on the disinfection efficacy for eukaryotic FLA and their cysts. Reports have shown that a higher concentration of chlorine is required to inactivate Legionella when associated with H. vermiformis (> 4 mg/L) and Acanthamoeba cyst (> 50 mg/L) [13].

Therefore, it is highly recommended to monitor the biofilm on membrane and filters within the water treatment process for eukaryotic genes, to determine if the system is susceptible to colonization by FLA and to allow better respond strategy. The effect of regular membrane cleaning using cross-sectional flow or backwashes could help with improving filtration efficiency and lowering.

2.3. Secondary disinfection by chloramine

Except for GAC filtration, the choice of secondary disinfectant is considered as the other important process in Singapore DWDS, for it would remain in contact with the distributed water right up until consumer’s usage. The biofilm is a heterogeneous structure which allows only diffusion within the densely packed EPS. To elicit any inhibitory effects on pathogens, a secondary disinfectant should diffuse and penetrate through the entire depth of biofilm uniformly. The main purpose of secondary disinfection was to introduce chlorine residuals into treated water meant for distribution to preserve water quality within the pipes. The preservation of water quality occurs in two ways: i) the population of allochthonous microbials which survives the treatment process could be controlled; and ii) autochthonous microbials residing in local biofilms could be constantly exposed to chlorine residuals, thus limiting any unchecked growth.

In Singapore, chloramine is chosen for secondary disinfection as chlorine were known to produce toxic by-product during natural degradation. Outside of Singapore, there has been increasing momentum to switch from free-chlorine disinfectant to chloramine due to regulatory restrictions. To be practically relevant, the paper focuses only on chloramine as a secondary disinfectant, with some comparison
against free chlorine. In terms of diffusion limitation, chloramine is known as a better penetrator of biofilm than free chlorine. Using chlorine and monochloramine microelectrode, direct chlorine/monochloramine concentration within the biofilm was measured to suggest disinfectant penetrability in a nitrifying biofilm. [14] Monochloramine achieved a complete penetration at near substratum depth (250 μm) after 24 hours while only 25% and 0.2% of bulk free-chlorine concentration (2.6 Cl2 mg/L) had penetrated biofilm surface (50 μm) near substratum (250 μm) respectively, after 20 hours. Monochloramine was found to penetrate nitrifying biofilm up to 170 times faster than free chlorine, on the same chlorine equivalency. In biofilms, the presence of carbohydrates, proteins and other macromolecules in EPS constitutes a problem for free chlorine as its highly oxidative nature dictates a faster reaction rate with EPS than diffusion rate through the biofilm. The observed “lack of penetration” could be a result of rapid reaction with biofilm components rather than diffusion limitation within the biofilm. With a higher initial concentration of free chlorine (9.6 mg/L) at 63 hours of experimental time, free chlorine concentration at 61% and 36% of bulk concentration, supporting the theory.

However, the same paper also commented that penetration does not equal to the loss of cell viability. LIVE/DEAD BacLight Bacterial Viability Staining was conducted only on the upper surface layer of biofilm (50 μm) due to limitations of confocal microscopy. The presence of green fluorescence patches in monochloramine-treated biofilm (48 hours) indicates that some cells are still viable after monochloramine treatment. The result contrasted greatly with the completely red fluorescence signals observed after sequential disinfection was applied (chlorine 24 hours and monochloramine 24 hours). The results suggest that chlorine disinfection is strong where it can reach. The same results were replicated under 3D cryoembedding and cross-sectional technique, monitoring cell viability beyond the upper biofilm surface [15].

A similar delay to chlorine penetration was observed in Globes (2002), where Pseudomonas aeruginosa is entrapped in alginate gel bead to simulate an artificial biofilm [16]. It was observed that biofilm kill time is significantly larger than planktonic kill time across all disinfectants (chlorine, glutaraldehyde, 2,2-dibromo-3-nitrilopropionamide (DBNPA) and alkyl dimethyl benzyl ammonium compound (ABAC)). A common trend of lower resistance factor (biofilm kill time divided by planktonic kill time) was observed when an increased concentration of disinfectant was applied. This observation insinuates that biofilm poses a diffusion limitation for all studied disinfectants and disinfection efficacy is concentration dependent.

Contrastingly, the advantages of using chloramine could be counteracted by several disadvantages. The process of producing chloramine is one of in-situ production by reacting free-chlorine and ammonia in 4:1 ratio. The presence of free ammonia poses a higher selective advantage for ammonia- oxidizing bacteria e.g. Nitrosomonas which feeds on ammonia to produce nitrite, leading to nitrification problems and disinfectant depletion when left unchecked. Nitrifiers are known colonizers of pipe biofilms and by providing free ammonia that encourages their growth, this will also increase the potential for new biofilm formation. Disadvantageously, the use of chloramine selects for non-tuberculous mycobacterium (NTM) which are known opportunistic pathogens residing in DWDS biofilm [17]. The selective accumulation of NTM in water-main biofilm was demonstrated in a recent study involving two full-scale DWDS which used different disinfection strategies: i) initial chloramine residual of 3.8 mg L-1 in the United States; and ii) minimum free chlorine residual of 0.08 mg L-1 in Norway [18]. As planktonic NTM were already known for their chlorine and chloramine resistance, the biofilm could have allowed an alternate phenotypic expression in NTM, allowing their higher abundance within the biofilm, although the exact mechanism is not known. Although a higher abundance of Mycobacterium was observed in water main biofilm of chloramine-treated system, it was observed that Mycobacterium diversity has remained low, perhaps due to specific species selection with the high chloramine concentration used, favouring Mycobacterium gordonea, a relative less virulent pathogenic than Mycobacterium avium.
3. Extrinsic factors affecting the biocide disinfection of Singapore DWDS

Biofilm resistance is suspected as the main causative factor in the presence of pathogens in Singapore DWDS. However, a discretionary note will be set up on biofilm resistance, to impress upon the great complexities in solving pathogen persistence in DWDS. Between untreated raw water to distributed water from consumer tap, several factors unrelated to biofilm resistance also affects the survivability of pathogen, until their detection at sampled points. These extrinsic factors include the age of biofilm in distributed pipes (Fig. 3), hydraulic retention time (HRT), the distance between sampled point and water treatment plant, climate and seasonal changes in which Singapore DWDS is located. These extrinsic factors could potentially interact with some biofilm resistance mechanisms, e.g. phenotypic adaptation to applied selective pressure, contributing to enhanced pathogenic survivability.

The Singapore drinking water distribution system is a meandering network of pipes which radiates outwards from the treatment plant or storage reservoir into distribution points. These pipe networks range from young (within 5 years) to very old (20 years plus) with different hydraulic retention time and distance from the treatment plant. In distribution network of Singapore, it was shown that biofilm in distal pipe regions (HRT: >60 h) contains distinct bacteria ecology at a higher cell abundance as compared to proximal pipe region (HRT <1 h) relative to storage reservoirs, which contains relatively uniform communities with lower cell abundance. The observation was attributed to the diminishing concentration of residual chlorine from proximal (chlorine concentration: 0.25 mgL\(^{-1}\)) to distal pipe region (chlorine concentration: ~0.04 mgL\(^{-1}\)). This reflects the importance of water distribution network designs which might unwittingly lead to uneven distribution of disinfectant residual along the pipe. The sub-lethal exposure of disinfectant at the distal pipe could have caused phenotypical adaptation in biofilm species leading to higher abundance and distinct bacterial biofilm ecology. However, solving this problem by reconfiguring and shortening pipes will not be possible due to the sheer scale, cost and complexity of operation. Thus, the solution to solve diminished concentrations of disinfectant residuals should be approached from another angle.

![Figure 3](image-url)

**Figure 3.** Schematic diagrams of microbial contamination and resistance in DWDS pipe networks.

The temperature of water and seasonal changes alter the concentration of free-living amoeba within untreated water sources such as lakes and rivers. In the event after heavy rain, the sudden surge of nutrients being introduced to raw water sources also encourages the boom in microorganisms which feast on these nutrients. Sudden perturbation induces a higher burden on the treatment plant to remove the excess concentration of microbials, which might lead to a higher proportion of FLA escaping disinfection and ending up in the distributed water [9]. In a study of four DWDS in Paris, sudden flooding of the River Seine was suspected as the cause of higher relative abundance in Legionella and Escherichia detected through high-throughput sequencing method during the month from June to July [19]. The rain swept soil organic nutrients and soil particles into the river, contributing to a higher organic load which might affect the efficiency of water treatment.
4. Control Strategies to overcome biofilm resistance of pathogens in Singapore DWDS

4.1. Proper distribution system maintenance
As it would not be practical to completely eliminate existing biofilm from DWDS (no elimination) and the detached biofilm would always make its way to consumer’s tap, so the available methods to prevent and decrease the chances of new biofilm formation is to conduct proper maintenance for the distribution system. For this, holistic perspectives should be applied to encompassing all stages of DWDS from raw water sourcing to consumer’s tap. Ideally, the microbial load of treated water at the origin of the distributed point should be as low as possible to prevent the introduction of new bacterial gene pool into existing biofilms and to prevent establishment of new biofilms [1]. For biofilms at distribution point, the only means of intervention come from educating consumers regarding presence of biofilm in showerhead and preventing prolonged water stagnation [19]. Except for regular flushing, pigging, pipe replacement, and maintain adequate residuals, other novel technology such as wellhead protection program and cutting-edge piping materials with surface characteristics can also be applied [20, 21].

4.2. Simultaneous removal of nutrients, FLA and bacteria
Introduce methods that could simultaneously remove nutrients, FLA and bacteria. By preventing FLA access to the DWDS, one mechanism of pathogen persistence could be cut off. Lower concentration of nutrients meant that biofilm has lower nutrient availability, precluding growth of new biofilm. For this purpose, ultrafiltration is considered as the most effective method to achieve an integrated result of higher nutrient, particulates, bacteria and FLA removal as compared to other options [22, 23]. Of course, the standard prerequisite steps including sedimentation, flocculation and rapid sand filter must still be in-place and smoothly operated to reduce the burden on filtration membrane. Moreover, biologically activated filters would become new options to address the biofilm resistance in Singapore.

4.3. Increase free chlorine residual
Despite the regulatory requirement to maintain a constant disinfection concentration, the DWDS is non-uniform in disinfectant distribution at different segments of the pipe [24]. The importance of maintaining concentration is even more pertinent, learning that diffusion limitations within biofilm can be reversed with the application of higher disinfectant dose. Alternatively, it also ensures that consumer at different length of the pipe is exposed to low amount of disinfectant by-product. With a single high dose at the beginning, consumer at the distal end of the distribution could see higher concentration of disinfectant by-product and lower disinfectant residuals.

4.4. Probiotic approach
By recognising that bacteria can never be excluded from the DWDS, it could be beneficial to take the benefit of bacteria in turn [25]. The concerns with bacteria in DWDS are limited to pathogen, not all the bacteria. By encouraging competitive growth of harmless bacteria against pathogens, nutrients and resources are depleted for pathogens strain, which could be outcompeted and crowded out. Species-specification antagonistic interactions between certain bacteria strain and pathogen could also be encouraged for selective elimination. Applying ecological concept, keystone species playing an important role to sustaining pathogen could be selectively knocked out. For a probiotic approach specifically targeting FLA, it is plausible to employ amoeba phage that act only on amoeba.

5. Risks and opportunities
In the drinking water distribution system of Singapore, there are two typical stages, which are directly relevant to biofilm resistance: i) filtration with activated carbon granules and ii) secondary disinfection with chloramine. In the former, biofilm provides nutrients for eukaryotic grazers which are hosts of pathogenic bacteria. Biofilm, as a hotspot for bacteria and amoeba congregation provides a platform to facilitate their close multi-species association, increasing chances of encounter and infection. In the latter, the effectiveness of secondary disinfection is closely associated with the choice of disinfectant as
the diffusion limitation imposed by the biofilm is different for different disinfectant. Besides, in a comparison between chlorine and monochloramine disinfectant, it is discovered that the monochloramine acts as a better penetrator of biofilm, while the chlorine enables higher disinfection efficacy. In the context of DWDS, monochloramine is preferred due to the lower concentration of toxic by-product it generates and the selective disinfection it conferred. Besides, the apparent pathogenic persistence is also subjected to effects from other extrinsic factors such as UV primary disinfection process, pipe age and material, hydraulic retention time, distance from storage reservoirs, seasonal changes, sudden perturbation in weather and duration of water stagnation at distributed points. When extrinsic factors could not be properly managed, effects of biofilm resistance could be amplified and translated into the undesirable outcome of transmitted waterborne diseases from potable water.

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