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Microbial Diversity and their Biofilm Formation Potential in Pipes of Water Distribution System

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Abstract: Microbes are ubiquitous in surface as well as in ground water and some of them can make their way into potable water distribution systems. Contaminated soil with human and animal fecal matter, ill-maintained water and sewage pipelines, poor sanitation and personal hygiene are the main factors responsible for the presence of microbial pathogens in the drinking water. The presence of water-borne microbes in the potable drinking water systems determines its quality. Common microbes present in contaminated water are *Shigella, Escherichia coli, Vibrio cholerae, Pseudomonas* sp, *Salmonella* sp etc. The water-borne pathogens that reside and reproduce in water distribution system causes infection of gastrointestinal tract, urinary tract, skin, and lymph nodes. When these pathogens enter into the water distribution system pipelines they form biofilms. The formation of biofilm is a key component in microbial studies. Biofilm is the sessile aggregation of bacterial cells that adhere to each other on living or non-living surfaces and forms extracellular polymeric substances (EPS). The surface physico-chemical properties of both bacteria and substratum were important for the establishment of bacterial adhesion. Bacteria forming biofilms possesses different growth patterns, responds to specific micro-environmental conditions for the formation of structurally complex mature biofilms. In water distribution systems, adhesion of microbes to the water pipelines initiate biofilm formation which in return reduces the quality of potable water and increases the corrosion of pipes.

Key words: Water-borne microbes, biofilms, extracellular polymeric substances, disinfectants, chlorination.

Introduction

According to the present study, many problems occurring in drinking water distribution networks are of microbial origin. These include biofilm growth, nitrification, microbially mediated corrosion, and the presence of pathogens. Many pathogenic microbes are capable of growing in the water distribution system which may cause infections in different parts of the human body including urinary or gastrointestinal region or the skin, etc (Berry et al., 2006). Biofilms are defined as an association of microorganisms in which the microbial cells are embedded in a self-produced matrix of extracellular polymeric substances (EPS). These biofilms adhere to each other or biotic or abiotic surfaces (Flemming et al., 2016). Biofilm formation is a complex process comprising many species of microbes. It has a high cell density ranging from 10^8 to 10^{11} cells/g wet weight. All higher organisms are colonised by microbes that form biofilms including humans. Biofilms cause infections in humans, plants and animals and also contaminate medical devices and implants including catheters. Furthermore, they also cause biofouling and contamination of water in municipal distribution systems (De Vos, 2015; Flemming, 2011).

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Biofilm formation initiation occurs by adherence of microbial planktonic cells to the surface of water sources followed by the formation of a micro-colony which leads to a complex structure ending into the maturation of the biofilm. Finally, it followed by detachment as shown in Figure 1. It is a multi-step process in which many species of bacteria communicate with each other through a mechanism called quorum sensing. Biofilms are mainly homogenous or heterogeneous bacterial communities composed of proteins including enzymes, DNA, polysaccharides, RNA and water (upto 97%) which is required for the smooth flow of nutrients in the biofilm matrix (Jamal et al., 2015). These biofilms are beyond the access of human defence systems and antibiotics. This is because microbes forming biofilms have higher potential to endure and neutralise the effect of antimicrobial agents (Crossley et al., 2009; Mah and O'toole, 2001). The inner environment of the biofilm matrix not only protects it from antibiotics but also predators, biocides, bacteriophages, amoeba and so on (Gupta et al., 2016).

Previous studies have also reported the significance of material used for water pipe, corrosion, sediments and organic matter, which are thriving grounds for biofilm formations in the water distribution system. Biofilms are considered as the main source of microorganisms in the distribution system fed with partially treated water. They are of major concern in the case of old water distribution systems. The traditional method of chlorine disinfection has proven to be ineffective against these biofilms. The present paper aims to discuss the environmental as well as other factors which are responsible for microbial growth and biofilm formation including microbial diversity in the water distribution system.

Prevalence of Microbes in Water Distribution System and Health Risks

Waterborne pathogens may enter the water distribution system through sewage-disposal tank, raw water reservoirs or leakage in the systems. Despite chlorine disinfection, few microbes still prevail at low concentrations in water, which include pathogenic strains of *Mycobacteria, Legionella, Acinetobacter* spp, *Pseudomonas aeruginosa* and *Aeromonas* spp. Biofilms formed in the water distribution systems are not as continuous slime or films but rather they are patchy and heterogeneous in nature. Bacteria form biofilms both in flowing and stagnant water conditions. Thermophilic bacteria mainly thrive in water storage tanks as they are the main sites for the microbial biofilm

Process of biofilm formation

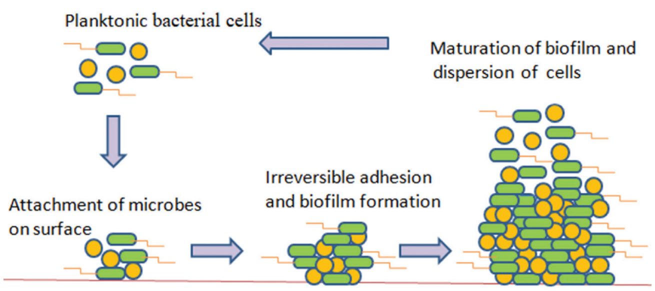

Figure 1: Steps of biofilm formation.

development at favourable temperatures. Although algae, fungi and protozoans are also present, the predominant microorganisms that form biofilms in water distribution system pipes are Gram-negative bacteria. When the microbes form biofilms at or near the point of water distribution channels, they act as a repository for microbes which disperse into the water stream. After dispersion from the water source, these microbes may colonise the instruments, aerators, showers, taps, medical devices and environmental surfaces; thus, spreading infections and diseases (Exner et al., 2005; Ortolano et al., 2005). Biofilms produced by multispecies of bacteria are more resistant to disinfectants such as chlorine dioxide and chlorite as compared to single species of bacterial strain. Inadequate disinfection strategies and lapses in the chlorination process leads to increased resistance of the bacteria that produce biofilms.

By studying the microbial diversity of the municipal water distribution system, many researchers have isolated different bacterial strains (Table 2). According to a study by Tokajian et al. (2005), among the heterotrophic bacteria, Gram positive, alpha, beta and proteobacteria are mostly found in the chlorinated water. Types and dosage of disinfectants as well as strategies of disinfection have a great impact on biofilm formation (Berry et al., 2006). Bacterial strains isolated from the water distribution system were mostly heterotrophic in nature i.e., they derive their nutrition by using organic matter such as carbon and various energy sources. Colonisation of bacteria on the inner surface of pipelines in the water distribution network depends upon several factors as mentioned earlier and was first observed by Ridgway and Olson in 1981. These isolated microbes are pathogenic in nature and among them, many are opportunistic pathogens. They may cause gastrointestinal tract, skin and lymph node infections (Boe-Hansen, 2001; Szewzyk et al., 2000).

Factors Contributing to Microbial Growth and Biofilm Formation in Water Distribution System

Various factors influence microbial growth and enhance the biofilm formation in the pipes, which participate in water distribution system. These factors include temperature, rainfall, pipe material, hydraulics of the system, nutrient availability, corrosion of pipes and disinfectant residuals (Table 1). Microbes are always present in the drinking water even if strict precautions are taken in the distribution system. The adherence of the microbes to the water pipes surface is a well-known phenomenon (LeChevallier et al., 1988). The choice of surface, as well as pipe material, is of great importance for inhibiting biofilm formation. If the inner side of the pipes is smooth and in good condition without any cracks, joints and bends, it will pile fewer nutrients and sediments comparatively. Therefore, there are fewer chances of microbial growth. The interface between the water and the pipe wall in the water distribution network is the main site where microbial cells,

Table 1: Factors affecting microbial growth in the water distribution system pipes

S. No.	*Factors influencing microbial growth*	*Description*	*References*
	Temperature	It influences microbial growth in the water distribution system pipelines by affecting the efficiency of treatment plant, water velocity and microbial growth rate, dissipation of disinfectant residuals, disinfection efficiency, corrosion rates, and hydraulics of water distribution system. Most of the studies reported that microbial activity significantly increased in water at 15°C or higher temperature. Microbial cells washed out of the distribution system at low temperatures before any significant growth could be achieved.	Zhang et al., 2006; Donlan et al.,1994; Else et al., 2003
	Rainfall	Rainfall acts as a catalyst for the growth of microbes in the water distribution system hence deteriorating the drinking water quality.	Lowther and Moser, 1984; LeChevallier, 1990
	Nutrient availability	Generally, the water distribution systems are considered as oligotrophic environments i.e. having low nutrient contents of carbon, nitrogen and phosphorous. To grow, microbes must derive all the substances from the environment for the synthesis of cell materials and generation of energy.	Bowden and Li, 1997
	Pipe material	The water distribution pipes having rough surfaces are good substrate for the bacterial cells to adhere and form biofilms. The other fitting materials like faucets, elastic sealants, pump lubricants and pipe coating and pipe gaskets promotes bacterial growth. Hydrophobic, non-polar surfaces are good adhering materials such as teflon and other plastics rather than glass or metals which are hydrophilic.	Chowdhury, 2012; Donlan, 2002; Yu et al. 2010
	Disinfectant residual	The concentration of the disinfectant residuals also influences the biofilm formation in the water pipes. The commonly used disinfectants are chlorine, chlorine dioxide, chloramines and UV radiations. The presence of residual chlorine in the water distribution system promotes microbial biofilm development.	Berry et al., 2006
	Hydraulic effects	The construction materials and the water consumption rate regulate the hydraulic conditions. The corrosion and the accumulation of the sediments in the water pipes are mainly due to the hydraulics of the water distribution system.	Boe-Hansen, 2001
	Corrosion	Corrosion in the water distribution system pipelines forms cracks and crevices and releases iron which promotes the growth of several opportunistic pathogens including coliforms, nitrifying and denitrifying and sulphate reducing bacteria.	Nawrocki, 2010

sediments and organic matters gather. It is also a site for microbial multiplication. Corrosion of pipes forms corrosion tubercles which are considered as niches for the microbes to grow (Batte et al., 2003; Flemming and Geesey, 1990)

Table 2: Different biofilm producing microbes in the water distribution system

Microbes isolated from water distribution system	*References*
Escherichia coli *Legionella* sp *Helicobacter* sp *Mycobacterium intracellulare* *Aeromonas* sp *Nitrospira* *Klebsiella pneumoniae* *Mycobacterium xenopi* *Pseudomonas vesicularis* *Flavobacterium* *Gallionella* *Bacillus* sp *Burkholderia* *Citrobacter* *Enterobacter* *Staphylococcus* *Acinetobacter* *Alcaligenes*	LeChevallier, 1990; Schwartz et al., 2003; Berry et al. 2006; Liu et al., 2013; Simões et al., 2010; Mattila-Sandholm et al., 1992; Flemming et al., 2016; Loveday et al., 2014; O'Toole et al., 2000; Mahapatra et al., 2015; Ashbolt, 2015

Strategies to Control Biofilm Formation

It is a crucial step to control biofilm formation in the water systems to inhibit the spread of microbes to different sources through the water stream. The biofilms, once established, are difficult to remove. Therefore, it is necessary to eliminate the planktonic bacteria via regular water disinfection. For this purpose, the biocides used to eliminate biofilm-forming organisms must penetrate the extra polymeric substances matrix formed to get access to the microbial cells (Meyer, 2003).

Materials used for the water pipes should be carefully selected that do not promote biofilm formation. Factors like leaching and accumulation of organic material in the water pipes may also promote biofilm formation. In hot water systems, metal corrosion is high, it promotes biofilm growth by providing iron to the microbes. Dead legs in the piping and capped pipes allow ideal conditions for biofilm growth as the water remains stagnant there. These conditions in the water systems should be avoided wherever possible (Guardian, 2018). The smooth internal surfaces provide a good internal environment as microbes find it difficult to attach to the smooth surfaces.

The flow rate in the water systems determines the adherence ability of the bacteria to the pipe surfaces. So, by maintaining a continuous flow in the water distribution system, we can prevent the adherence of the planktonic cells to the surfaces. Valves in the water systems should be carefully selected. Good valves do not allow water accumulation at particular areas and also avoids leakage. This is because leakage in the valves allows external microbes to enter the water system (Sandle, 2017).

Conclusion

The bacterial study in the water distribution system is still not fully discovered qualitatively as well as quantitatively. It is very much clear from the study that the chlorination strategies are inadequate for controlling the microbial growth and biofilm formation in the water distribution network. It can only be improved by exploring the microbial ecology. This study investigated many factors that play an important role in biofilm formation. Microbial growth can be controlled by a better understanding of environment and other related factors that leads to corrosion of pipes.

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