7 Biofilm Formation in Potable Water

CONTENTS

7.1 INTRODUCTION

The presence of biofilms in potable water has been evident since 1930 with the reported problem of the existence of the regrowth of *Bacterium coli*.[1,2] Another 7 years passed until research confirmed that microbes were capable of multiplication on the walls of water distribution pipes.[3] However, it took another 42 years for the presence of biofilms associated with potable water to generate concerns when associated with pipe tubercles and encrustations[4] indicating some form of public health problem. Even today, the presence of biofilms within potable water pipes are being observed by a number of researchers using more modern methods, particularly the scanning electron microscope.[5-7] Despite accumulating evidence confirming the presence of biofilms in potable water, it is only recently that any health implications associated with their growth are being documented, particularly as they are known to provide a safe haven for the growth of pathogens, opportunistic pathogens, viruses, and protozoa.[8]

The study of biofilm development in potable water is of great interest to drinking water companies in relation to the effects they have on both the aesthetics and the public's health. In terms of the aesthetics, it is widely acknowledged that potable water can be affected by organisms such as fungi and *Actinomycetes* which are well known to cause taste and odour problems.[9,10] These have been documented as being found as part of biofilms which may well exasperate the taste and odour problems experienced in potable water. It has also been shown that some bacteria found as part of a biofilm can lead to corrosion of pipe material.[11] However, by far the most important concern to a water company is the fact that biofilms can and do have the potential of leading to both heterotrophic and coliform regrowth and providing a safe haven for pathogens and opportunistic pathogens.[12] This may well have very important implications in factorising the increasing numbers of gastroenteritis and noscomial infections being both reported and unreported worldwide.

The adhesion of bacteria to surfaces in potable water have been shown to be a major determinant in protecting microorganisms from chlorine. Therefore, sloughing of the biofilm may well serve as a system for the dissemination of potentially problematic microbes. Despite this possibility, biofilms evident on the pipe walls of potable water systems are not examined routinely as part of an assessment procedure in potable water systems. This is possibly owing to the fact that long runs of water distribution lines cannot be sampled because of the inaccesibility of the water distribution systems. Research that is, therefore, carried out and documented on biofilms has generally used laboratory simulations and small pilot rig systems which cannot truly mimic a water distribution system faultlessly, owing to the presence of so many changing variables which present themselves in these systems. However, it is now accepted that all materials which are exposed to a bathing fluid will support a biofilm and growth of that biofilm. In fact, to date no material has yet been developed which does not allow the adhesion of bacteria.

7.2 ADHESION AND BIOFILMS IN POTABLE WATER

As a material comes into contact with potable water, organic and inorganic particles from this bathing fluid become adsorbed onto pipe surfaces and are then followed by microorganisms. Once microbes are attached to a conditioned surface, aided by the use of EPS, fimbria, and flagella, the microbes grow and multiply, consuming the nutrients evident within the conditioning film. As the water flows past the biofilm, nutrients can diffuse into the biofilm by simple diffusion, allowing for biofilm maturation.

Once bacteria are attached to a surface they may behave in two ways[13]

1. Bacteria are not able to multiply in the potable water system and thus are washed out from the network system (water + biofilm) governed by the flow rate and the dilution rate.
2. Bacteria are able to multiply in the potable water system and remain depending on availability of nutrients. Research has found that eliminating suspended cells entering the network will decrease the biofilm population.[14]

Nutrients are a necessity for the growth of bacteria. These nutrients are classed as biodegradable, measured as either biodegradable organic carbon (BDOC)[15] or assimible organic carbon (AOC). A number of models are available to predict both the impact of BDOC on the biological stability of the waters and the numbers of bacteria in the planktonic and sessile states.[14,16,17] The consequence of the BDOC is correlated with an increase in the number of cells in the biofilm and, subsequently, in the water phase.

7.3 MICROBIOLOGY OF POTABLE WATER AND BIOFILMS

Potable water generally contains a lot of microorganisms, particularly ones which are injured and dormant, as a result of the disinfection process (chlorination) and water treatment processes. However, this is a poorly defined area, where either the nature of the injury or the degree of dormancy are unknown. The only certain fact is that these injured or dormant bacteria are metabolically active but incapable of replication on culture media, with available culture methods for waterborne bacteria giving results well below the actual total cell count and which vary, however slightly, with the culture method utilised. It is widely acknowledged that the use of agar media for estimating bacteria in water samples produces results which generally underestimate bacteria in water samples.[18] This has led to the development of new rapid techniques for the identification of sessile bacteria.[19]

The majority of bacteria typical of freshwater ecosystems can be found among the flora of water distribution systems, although such organisms are present at very low concentrations. The water flora can be quantified by counting, using an epifluorescence microscope, or by culturing on nutrient agars (total heterotrophic plate counts). Very often, potable water is dominated by gram-negative bacteria,[6,20,21] particularly *Pseudomonas* and yellow/brown pigmented organisms of *Flavobacterium*-like species.[6,22-24] As well as regular harmless flora, pathogenic or potentially pathogenic organisms may be detected (e.g., *Legionella*) which could have serious consequences.

At least 50% of bacteria carried by the water are present as aggregates of dimensions greater than 5 μm or attached to nonbiological particles of over 5 μm in diameter.[5,25] These particles, as soon as they come into contact with water, become colonised by microorganisms from the aquatic environment. This biomass (bacteria, microscopic fungi, protozoa, and yeasts) is attached to either the walls of the pipework in a heterogeneous pattern determined by the shear forces acting on the wall of the precipitates, deposits of sediment, encrustations, or tubercles which form on the pipe wall surface.[4,5]

Although biofilms evident in potable water systems are patchy, a mature biofilm can be up to 200 μm thick which may cause a drop in oxygen levels resulting in anoxic conditions below the surface layer. Such conditions could lead to the growth of sulphate reducing bacteria (SRB) whose activity may corrode and pit the insides of pipelines.[26]

Other effects as a result of biofilms in potable water systems include

- Positive bacteriological tests (coliforms).
- High plate counts.
- Growth of opportunistic pathogens.
- Increased chlorine demands.

- Discolouration of water.
- Taste and odour effects.
- Growth of invertebrates.
- Corrosion and pitting.

Control of the biofilm becomes one of the main objectives of good potable water distribution practice. However, as chlorination does not prevent accumulation of biofilms[27,28] other preventive measures such as mechanical cleaning should be combined with the use of biocides for a greater biofilm removal rate and thus a longer lag phase to regrowth. It should also be noted that chlorination may also induce biofilm development in potable water.[29]

The characteristic of a biofilm, that is, species present, number of cells, specific gravity, and thickness, are controlled by a myriad of factors including number and diversity of the species present in the water, the concentration and nature of the biodegradable organic matter in the water, the hydraulic regime to which the system is subjected, and the characteristics of the support material colonised by the bacteria.[13]

Observations of biofilms in water distribution systems indicate little qualitative or quantitative uniformity. Although some bacterial species are more frequently detected than others, a fairly large variation in population composition can be seen. Cell numbers and distribution in pipe wall linings and corrosion products vary widely. Considering large differences in the water distribution system environments, it is not surprising that differences exist in the quality of the treatment plant effluent and in the structure and chemical composition of the pipe wall.

Within potable water biofilms a large diversity of microorganisms are generally found, most of which may be potentially harmless with possible exceptions (see Chapter 8). The presence of these bacteria suggests that the disinfection process is not inactivating the microorganisms and, therefore, will allow the continual development of biofilms within potable water pipes. This may result in deterioration in water quality hindering the detection of indicator organisms often leading to public health implications. Research suggests that biofilm formation is possibly induced as a result of the low nutrient content of the water, promoting a survival mechanism instigated by the bacteria.

The deterioration of water quality within potable water distribution systems is owing to a number of factors[30] including

- Bacterial colonisation of potable water pipe systems.
- Microbial regrowth.
- Poorly built and operated storage reservoirs.
- Taste and, in particular, odour problems owing to actinomyctes which produce an earthy smell, algae, and fungi.

Whilst potable water contains dissolved organic compounds, these can cause a number of problems including bad taste and odours, enhanced chlorine demand, trihalomethane formation, and bacterial colonisation of water distribution lines.[4,17,31-33]

7.4 RELEVANCE OF BIOFILMS IN POTABLE WATER

The depletion of dissolved oxygen, reduction of sulphates to hydrogen sulphide, bad taste, and colour may result from microbial activity within the sessile state. Problems of black water are sometimes caused by *Hyphomicrobium*, an example of which is the biofilm sloughing that occurred in Queensland, Australia.[34] The biofilm had accumulated on the pipe wall and contained high numbers of *Hyphomicrobium* which had formed hypha-like structures which are readily covered with a black precipitate of manganese oxide arising from low concentrations of reduced manganese in water. The cell wall of *Hyphomicrobium* acts like a catalyst in the oxidative precipitation process producing a black precipitate which would be transported in the water.

The occurrence of biofilms can cause many problems[22]

- Bacteria can be the starting point of a trophic food web leading to the proliferation of higher organisms.
- Specific bacterial species may generate turbidity, taste, and odours in the potable water.
- Production of red and black waters which result from the activity of both manganese and iron oxidising bacteria (*Hyphomicrobium*).
- High HPC interfere with detection of coliforms or sanitary indicators.
- Accumulation of attached biomass promotes corrosion, particularly under anaerobic conditions with the production of H_2S.
- Biofilms increase frictional resistance, thus decreasing the capacity of distribution systems to carry water.[35] This leads to a loss of pressure or reduced water flow. The increase in frictional resistance is known also to affect the microbial community in biofilms with an increase in filmanetous bacteria evident when this is high.[36]
- Continued failure of the distribution system to meet all established water quality criteria.

7.5 BIOFILM STRUCTURE IN POTABLE WATER

The structure of a biofilm is very difficult to study, particularly owing to the presence of large amounts of detritus, corrosion products, and inorganic matter, often scale (Figure 7.1), evident at the pipe surface in potable water. Evidence of these materials restricts the techniques which can be used to study the morphology of biofilms in potable water. Therefore, the majority of information which has become available in relation to biofilm structure in oligiotrophic environments, which are indicative of potable water environments, has been obtained from laboratory-based experiments.

Results on biofilm morphology suggests that, as far as potable water is concerned, disinfection processes cause effects on the biofilm structure. In general, it is found that in potable water that is chlorinated, biofilms have a physical appearance that is different from that of unchlorinated biofilms. In chlorinated water, compared to unchlorinated (control), bacterial cells tend to be clumped and the biofilm is found to be more patchy, with the cells approximately 50% smaller than the control cells.[37]

FIGURE 7.1 Evidence of scale in potable water pipes. Reprinted from *Water Research,* 32, Percival, S., Biofilms, mains water and stainless steel, 2187–2201, Copyright 1998, with permission from Elsevier Science.

There is also a shift from longer rod-shaped cells to more rounded organisms, together with a change in the dominant bacteria present in the biofilm.[37]

Biofilms were once considered a homogeneous growth of regular thickness.[26] However, direct examination of biofilms has demonstrated that bacteria grow in a number of microcolonies, the structure of which is dictated by physiological and environmental factors. For a critical review of the structure and function of biofilms, readers are advised to consult the review by Costerton et al.[38] This review draws together the historical and current literature to present biofilms as heterogeneous microniches present on a surface. As such, biofilms are now considered to consist of living, nonviable, or dead microorganisms together with extracellular polymeric substances, organic and inorganic matter in discrete microcolonies through which there are open water channels.[38-40] Biofilms in potable water systems are patchy (Figures 7.2 and 7.3) and heterogeneous with a diverse microbial flora (Figures 7.4).[41,42] A mature biofilm may have microcolony regions up to 200 μm thick. These regions will have differential concentration gradients which would have an effect on the physiology of the microcosm.[43,44] Owing to the thickness of microcolonies, the oxygen concentration would also vary depending on the depth into the microcolony. Hamilton[45] investigated the effect of oxygen on populations of sulphate-reducing bacteria whereas de Beer et al.[46] used microelectrodes to penetrate microcolonies. Anaerobic conditions within biofilms could lead to the growth of sulphate reducing bacteria whose activity may increase corrosion, particularly of the ductile iron once commonly used in water distribution systems.[26,47]

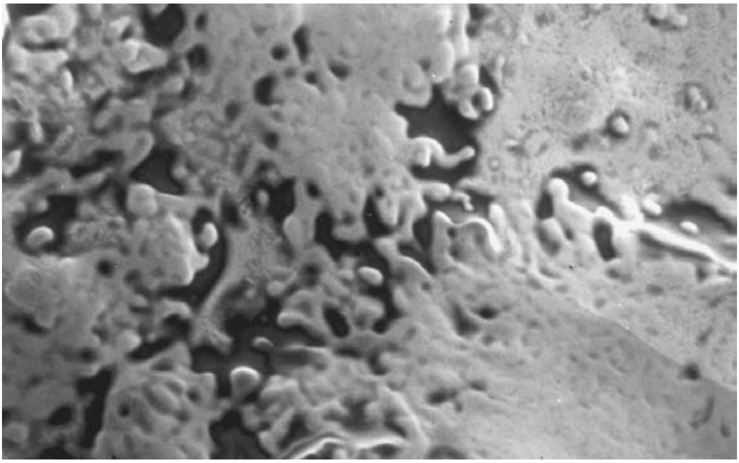

FIGURE 7.2 Evidence of a patchy biofilm formation in potable water. Reprinted from *Water Research,* 32, Percival, S., Biofilms, mains water and stainless steel, 2187–2201, Copyright 1998, with permission from Elsevier Science.

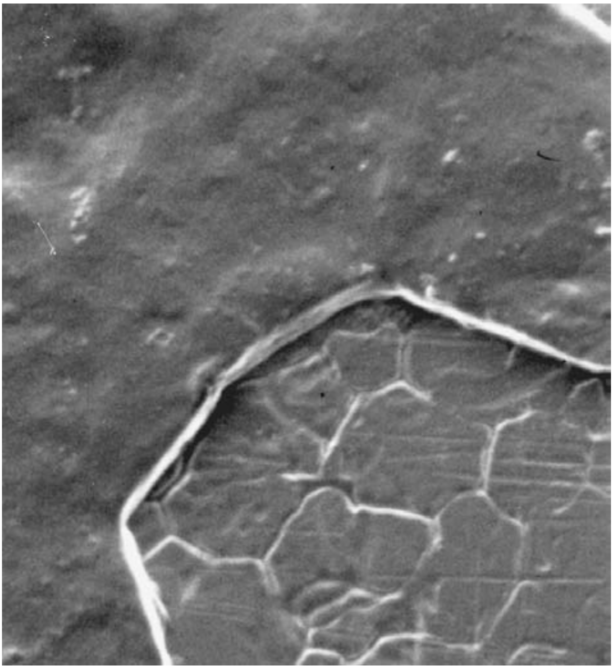

FIGURE 7.3 Evidence of a patchy biofilm formation in potable water. Reprinted from *Water Research,* 32, Percival, S., Biofilms, mains water and stainless steel, 2187–2201, Copyright 1998, with permission from Elsevier Science.

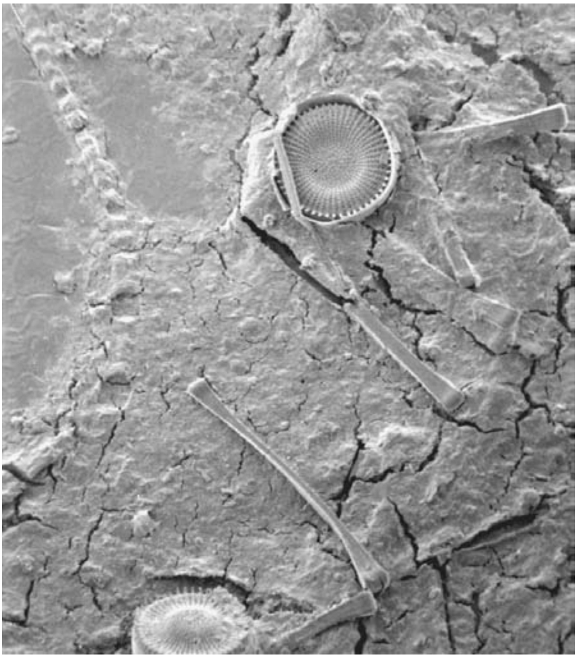

FIGURE 7.4 Evidence of a patchy heterogeneous biofilm. Reprinted from *Water Research,* 32, Percival, S., Biofilms, mains water and stainless steel, 2187–2201, Copyright 1998, with permission from Elsevier Science.

7.6 FACTORS WHICH AFFECT THE ACCUMULATION OF BIOFILMS IN POTABLE WATER

Within potable water, the main area of concern is that of proliferation of coliforms and heterotrophic bacteria in biofilms. Factors which are known to affect the growth of these include[37]

1. Utilisable carbon.
2. Temperature.
3. Ineffeciences in the removal/disinfection of organisms in treatment.
4. Disinfection dose/type.
5. The hydrodynamics of the distribution system.[48]

Whilst it is suggested that several parameters govern biofilm accumulation in water systems,[49] the most important variables to consider are particularly the water hydraulics, flux of cells, nutrients (AOC or BDOC), characteristics of the substratum, temperature, and disinfectants. The main parameters which are known to affect biofilm development within potable water will be discussed next.

7.6.1 WATER HYDRAULICS

Water hydraulics have been instrumental in affecting microbial growth on pipe surfaces present in potable water. It is generally portrayed that high water velocities cause a greater flux of nutrients to a pipe surface, greater transport of disinfectants, and greater shearing of biofilms. Any changes in water velocity in potable water systems is owing mainly to seasonal fluctuations. Particular known causes are owing to consumer demand in the warm summers. Other factors known to affect water velocity include fire hydrants, pipe network design, breaks in pipes, or breaks in systems owing to pipe flushing.[50] Areas of pipe work which have stagnation zones are known to have adverse effects on biofilm in potable water. Particular effects include a loss in disinfectant residual and accumulation of sediment, debris, and, ultimately, increases in microbial growth.[51-53] The increase in microbial growth evident in these dead legs could then lead to high bacterial counts evident at the customer's taps.[54,55]

7.6.2 NUTRIENT AVAILABILITY

The availability of nutrients is a necessity if bacteria are to grow in potable water. The general requirements for heterotrophic bacteria include carbon, nitrogen, and phosphorus in a ratio of about 100:10:1. This ratio is necessary for the maintenance of effective growth. Trace elements and cofactors, necessary chemicals for enzymes to function, are also required as well as these principal nutrients. The availability of these nutrients is one of the main factors known to affect the regrowth of bacteria within potable water which can be controlled during the water treatment processes.

Nutrients available to bacteria are generally in the form of AOC. This is often measured by the techniques initially utilised by van der Kooij, Visser, and Hijiner.[56] and have been used by a number of researchers in their studies[57] to demonstrate the influence of nutrients on growth. Production of water with a low AOC has been used within water distribution systems to reduce the total viable count and the presence of biofilms.[56] Other ways of measuring carbon in water include total organic carbon (TOC). This includes the total amount of soluble and insoluble organic carbon compounds, dissolved organic carbon (DOC), which includes the soluble fraction of TOC and AOC. This is the fraction of DOC that can be utilised for growth by microbes present in the water.

There is strong evidence available that suggests a correlation between AOC and regrowth of heterotrophic bacteria.[58] The potential threshold for assimilable organic carbon has been set at 10 mg of carbon per litre for heterotrophs[58] and 50 mg of carbon per litre for coliforms (LeChevallier, Schulz, and Lee, 1991).[59] It has also been found that AOC levels decrease with travel time through a potable water distribution system. With this in mind, it has been suggested that this decrease is owing to carbon utilisation by the sessile bacteria.[55] Despite evidence of a correlation between AOC levels and bacterial regrowth, particularly coliforms, other research has found no such evidence.[53]

Nitrogen, as well as carbon, is also considered a necessity for microbial life to develop amino acids and genetic material, and it is present in a number of different forms in water which can be utilised by bacteria. These include organic nitrogen, ammonia, nitrate, and nitrite. Despite the evidence of these in potable water, there is, at present, a lack of research and documented evidence on the effects of nitrogen levels on bacterial regrowth and biofilm formation. One piece of research by Donlan and Pipes[60] has indicated that no correlation exists between organic nitrogen, ammonia, nitrate, and nitrite, and bacterial growth in potable water. As with carbon, nitrogen is not presumed to be limiting in potable water, owing to the low concentrations which are necessary for bacterial growth. Phosphorus is also not presumed to be limiting in potable water. In the environment, phosphorus occurs as orthophosphate (PO_4^{3-}). Phosphate may be added to potable water to control corrosion. These have shown to have no real effect on the growth of bacteria.[61] However, some studies have found that low phosphorus concentrations may restrict microbial growth in potable water.[62]

7.6.3 DISINFECTION

Whilst chlorination is used to destroy potential pathogens and feacal coliforms in potable water, it is well known that increases of chlorine into potable water will not control biofilms or coliforms. Concentrations as high as 12 mg per litre have been shown to be inadequate to control biofilms in potable water.[63,64] It has also been found that the disinfection process can have profound effects on biofilm structure and morphology.[37] This suggests the development of a survival strategy by the biofilms caused by exposure to adverse conditions. The effects of disinfection on biofilm development will be discussed in Chapter 11.

7.6.4 TEMPERATURE

Temperature is well known to increase the microbial population within potable water systems with evidence of greater bacterial regrowth in potable water during summer months owing to higher water temperatures. Colbourne et al.[65] have demonstrated that coliform occurrence in potable water is associated with temperatures exceeding 20°C in comparison to a decrease when temperatures fell to less than 14°C. Donlan and Pipes[66] have also found a close relationship between water temperature and the development of biofilms. Increases in temperature above 15°C have led to general increases in the microbial content of potable water.[67-69] Fransolet, Villers, and Masschelein[68] have also found that water temperature not only has an effect on the growth rate of bacteria, but also the lag phase and cell yield. The length of lag time is important to the survival rate of bacteria in potable water. Temperature affects microbial growth rates, disinfection efficiency, dissipation of disinfectant residuals, corrosion rates, distribution system hydraulics, and water velocity through customer demand.[70] However, water companies have little control over changing water temperature. Therefore, any efforts should be focused on other parameters which temperature is known to affect. This can be reflected in the use of disinfectant residual adjustments.

7.6.5 SUBSTRATUM COMPOSITION

Pipe material is well known to affect biofilm development in potable water. Different materials are used to transport potable water ranging from cast iron to unplasticised polyvinyl chlorides. A number of construction materials have been shown to stimulate bacterial growth. These include rubber, silicon, polyvinyl chloride (PVC), polyethylene, and bituminous coatings.[71-73] After several days of being immersed in potable water, it is possible to rank different materials used to transport potable water according to the number of attached cells: cast iron has more attached cells than cement lined cast iron which has more attached cells than stainless steel.[74] Plastic materials such as PVC and medium density polyethylene (MDPE) are in the process of replacing many of the older traditional materials such as cast iron for transporting potable water. However, like most materials which are exposed to potable water, their biofilm-forming potential has not been investigated to any large extent. Work completed recently has established that there are significant differences in terms of biofilm accumulation between cast iron, MDPE, and unplasticised PVC (uPVC) in drinking water.[75-77] Cast iron is noted in contributing to the deterioration of water quality and has been documented as being prone to microbial colonisation and, ultimately, microbial regrowth in potable water.[78-80]

It is possible to measure the biofilm formation potential (BFP) of materials which come into contact with potable water.[81] The test is based on measuring the biofilm density on materials which are incubated at 25°C in biostable drinking water as a function of time.[82] For glass, the BFP has been calculated at 10 pg ATP cm^{-2} compared to PVC (uPVC) of more than 100 pg ATP cm^{-2}.[82]

The relationship between the different surface characteristics and number of attached bacteria depends in particular on wettable area and the surface roughness of materials exposed to potable water.[7,83] Therefore, this suggests that any pipes used for the transport of potable water should be regarded as a potential health risk as the degree of surface roughness of piping systems ultimately will affect the adhesion of microorganisms.

Corrosion of water plumbing systems (particularly copper) of a few institutional buildings in certain soft water areas has been identified as being caused by the presence of biofilms.[57,84] This has led to the suggestion that alternative metals should be used where copper has shown to corrode prematurely. Corrosion provides a protective surface for microorganisms. Pits and nodules formed during the corrosion process are known to aid in the concentration of nutrients and to protect bacteria from water shear.[85] Coliforms have also been shown to be found in iron tubercles.[55] Although plastics would seem to be ideal materials of choice, some have been shown to impart nutrients, encouraging development of biofilms and pathogens, in particular, *Legionella pneumophila*.[75,86] One particular alternative to plastics, iron, and steel pipes for transporting potable water that is now being extensively researched is stainless steel.[6,7] Overall, the use of materials that will reduce biofilm development, owing to their own characteristics, will obviously be advantageous in potable water and thus prevent/reduce the public health potential which can exist in biofilms.

7.7 COLIFORM REGROWTH IN POTABLE WATER

Coliforms are used as indicator organisms in potable water to confirm the presence of faecal contamination. If they are present in potable water, they are generally evident owing to a number of reasons but, in particular, disinfection failures. In the U.S., it has been documented that of 164 water utilities surveyed, a high number of these experience recurring coliform episodes.[87] These have been owing mainly to disinfection barrier breakthroughs.

Despite evidence of coliform regrowth in potable water, there is still little evidence that conclusively supports coliform proliferation on pipe walls. This has been owing to a number of reasons relating predominantly to the difficulty of sampling potable water pipe systems and the heterogeneity of biofilms known to be evident in potable water. Contrary to this, a large number of researchers have found evidence of coliform regrowth and consider this a public health concern.

7.8 PROBLEMS OF STUDYING POTABLE WATER BIOFILMS IN EXPERIMENTAL MODELS

The study of biofilms within potable water distribution systems and mimicking the process in laboratory-based systems are difficult. This is because these systems are chaotic and fluctuate continually, owing to the large number of variable changes known to be present at any moment in time. These range from sudden increases in temperature which, in turn, lead to increases in turbidity owing to increasing nutrient levels. Even more difficult to model are the hydraulic regimes and constantly fluctuating pressure and flow conditions in water distribution systems together with changes and/or hot–low spots of disinfectants.

As Camper[88] portrayed, there are two basic types of pilot systems developed to study biofilms. These are once through systems[7] and recirculating systems.[7] Possibly, the most extensively researched study of biofilms in potable water have taken place using laboratory-based simulations and large scale pilot rig systems.[14,18,89,90] Possibly, the largest was undertaken at Thames Water, U.K.[91]

7.9 CONCLUSION

Potable water biofilms are not well characterised. It should be presumed that all surfaces within potable water systems have a biofilm presence. By reviewing the literature, it is possible to draw a number of conclusions with respect to biofilm formation in potable water. It is evident that organic carbon is by far the most important nutrient source able to support the growth of sessile communities which can range from opportunistic and pathogenic bacteria to normal noninfectious heterotrophic plate count bacteria. This has been shown to be the case in laboratory-based studies using pilot and full scale rig systems. It is also now acknowledged that temperature, linked closely to nutrient and, therefore, turbidity levels, has important effects on biofilm formation in potable water. However, with this in mind, very little work has been done in this area. It is, nevertheless, feasible to suggest that increasing temperature leads to increasing biofilm growth up to an optimum and has pronounced effects on disinfection efficiency.

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