An overview of biofilm formation in distribution systems and its impact on the deterioration of water quality

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Abstract

The impact of biofilms present in water distribution systems on the microbial quality of potable water is reported in this review. The issues covered include the composition of bioflims, factors governing their formation and the effect and significance of biofilms on the microbial quality of drinking water. The review addresses the main factors governing the formation of biofilms such as the types of disinfectants and residual concentrations, resistance of bacteria to disinfectants, the influence of piping material and the effect of temperature. Methods for the enumeration of bacteria in biofilms as well as emerging technologies for *in situ* monitoring of biofilms are discussed. Suggested control measures for managing and controlling the problem of biofilm formation in potable water distribution systems to ensure potable water of an acceptable microbiological quality are dealt with.

Introduction

Deterioration of drinking-water quality during storage or in distribution systems remains one of the major difficulties experienced by potable water suppliers. It is an established fact that the distribution system is often vital in determining the final quality of potable water. Pathogenic and toxigenic microbiological agents in drinking water have long been known to cause disease and death in consumers (Craun, 1986). The health risks associated with these pathogens range from viral and bacterial gastroenteric diseases to infections such as hepatitis A and giardiasis. The International Drinking Water Supply and Sanitation Decade (1981 to 1990) was preoccupied with the construction and expansion of water supplies, and it is only in its latter part that more attention was given to the investigation, protection and control of the installations which supply drinking water (Lloyd and Bartram, 1991).

While water produced in the treatment plant may be of high biological quality, the treated water may be subject to conditions in the distribution network that adversely affect it. The reasons why bacterial numbers increase during distribution are not yet fully understood but two of the main factors have been studied in detail:

- The first factor is usually referred to as mechanical failure. Bacteria can be introduced into the distribution network from external sources by a number of means such as open reservoirs, breakages due to new pipeline construction that may disturb the existing distribution system, mains breaks (which may become an increasing problem as the distribution system ages) and the reduction of the water flow pressure in the system resulting in back siphonage (Rossie, 1975).
- The second factor refers to the situation where the increase of bacteria is due to internal regrowth or aftergrowth of bacteria and the associated formation of bioflims. Several investigators have shown that the multiplication of micro-organisms in biofilms along the distribution systems results in the deterioration of the bacteriological quality of drinking water, the development of odour or colour as well as the acceleration of the phenomenon of corrosion within the pipework (Nagy and Olson, 1985).

Biofilm on surfaces exposed to drinking water in distribution systems may well be the main source of planktonic bacteria since up to 1 000 sessile micro-organisms may be present for each planktonic cell which is detected. The occurrence of biofilms or encrustations that harbour various types of micro-organisms has been described extensively (Van der Kooij and Zoetemann, 1978; LeChevallier et al., 1987). The most alarming results are the presence and multiplication of pathogenic and opportunistic pathogens such as *Pseudomonas, Mycobacter, Campylobacter, Klebsiella, Aeromonas, Legionella* spp., *Helicobacter pylori* and *Salmonella typhimurium* occurring within the biofilms (Engel et al., 1980; Wadowsky et al., 1982; Burke et al., 1984; Armon et al., 1997; Mackey et al., 1998).

This review will focus on biofilms in water distribution networks and will cover issues such as biofilm composition, factors affecting the formation of biofilms, techniques for the investigation of biofilms in distribution systems as well as the deterioration of the water quality and the associated health risks. Suggested measures for controlling the problem of bacterial regrowth or biofilm formation in potable water distribution systems will be presented.

The nature of biofilms in water distribution systems

The process contributing to the increase in microbial numbers, not related to mechanical failure, between the point of entry into the distribution system and the final point of consumption is described by the terms “regrowth”, “aftergrowth” and “breakthrough”. The term regrowth is used when bacteria injured during the treatment process start to multiply after recovering from a form of reversible injury. The term aftergrowth consequently denotes growth of micro-organisms native to a water distribution system and the term

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breakthrough refers to an increase in bacterial numbers in the distribution system after viable bacteria have passed through the disinfection process (Van der Wende and Characklis, 1990).

The term "biofilm" is used to describe a layer of micro-organisms in an aquatic environment held together in a polymetric matrix attached to a substratum such as pipes, tubercules or sediment deposits. Attachment is a first step in the process of microbial colonisation of any surface and may initially limit the rate of the process (Escher and Characklis, 1988). Biofilm development is a result of successful attachment and subsequent growth of micro-organisms on a surface. Under suitable conditions a biofilm develops, initially through the accumulation of organic matter on the metal surface, which is then colonised by bacteria (Wolfaardt and Archibald 1990). Bacteria subsequently develop into a consortium of species within the polysaccharide matrix which imparts the slimy nature to the biofilm.

The matrix consists of organic polymers that are produced and excreted by the biofilm micro-organisms and are referred to as extracellular polymetric substances (EPS). The chemical structure of the EPS varies among different types of organisms and is also dependent on environmental conditions. The formation of glycocalyx has been reported to be critical for cells to attach to exposed surfaces and survive shear forces (Ridgway and Olson, 1981). Moreover, cells which are continually intimately associated with interior walls of water mains may be less susceptible to chemical disinfection processes due to boundary layer effects and the secretion of extracellular coatings (Baylis, 1930). As a result, the extent to which microbes become associated with the inner surfaces of pipes or with suspended particulate matter within the water column could significantly enhance their survival and regrowth potential in distribution lines and reservoirs (Ridgway and Olson, 1981).

Biofilms are sometimes formed as continuous, evenly distributed layers but are often quite patchy in appearance. As the slime layer thickens, micro-environmental changes take place within the biofilm as a result of the activities of the bacteria. Biofilms in water distribution systems are thin, reaching maximum thicknesses of perhaps a few hundred micrometers. In addition, the film often contains organic or inorganic debris from external sources. Inorganic particles may result from the adsorption of silt and sediment and the precipitation of inorganic salts or corrosion products (Van der Wende and Characklis, 1990).

Factors contributing to biofilm formation in potable water distribution systems

The small number of bacteria which can survive the water treatment process or bacteria already present in the distribution system provide a seed which will multiply in the distribution system given the right conditions for growth. The conditions which will enhance growth include factors such as the disinfectants used and the maintenance of a residual concentration in the system, the resistance of micro-organisms to disinfectants, the nature and concentration of biodegradable compounds in the treated drinking water, the kind of piping material used in the system as well as the water temperature.

Ineffective disinfectants

When used at the appropriate concentrations disinfectants are quite effective in the removal of micro-organisms but they may, however, also enhance the formation of easily biodegradable organic substances which can be utilised by micro-organisms as an energy source and promote biofilm formation in distribution systems (Gilbert 1988; Van der Kooij, 1999). Gibbs and his co-workers (1990) observed a quick reduction in bacterial numbers after booster chlorination, but regrowth occurred after the rapid decrease of residual chlorine in the distribution system. LeChevallier et al. (1980) noted that dead-end distribution lines in which no free residual chlorine could be detected contained 23 times the number of standard plate count (SPC) compared with distribution lines with a free chlorine residual. It is well known that chlorine can be consumed in the water phage and at the distribution system pipe walls (Vasconcelos et al., 1996). In the water phage, chlorine is generally consumed by reaction with ammonia (absent normally in drinking water), iron and organic compound (Lu et al., 1999). As demonstrated by Kiéné and Lévi (1996), in the distribution system chlorine disappears due to interactions with deposits, corrosion and biomass at the inner pipe walls and the chlorine decay kinetic depends on the pipe materials and hydraulic conditions (age, diameter, water velocity etc.). Lu et al. (1999) found that fixed biomass chlorine demand in water distribution systems depends on the incubation time and test water quality. According to the authors, the most important parameter to predict the biomass chlorine consumptive is the biodegradable dissolved organic carbon (BDOC).

Certain disinfectants may have properties more conducive to controlling biofilm populations. Less reactive, more persistent compounds, such as chloramine, maintain a higher disinfectant residual throughout the distribution system and may penetrate the biofilm more effectively, and thus control biofilm organisms better than free chlorine (Van der Wende and Characklis, 1990). Momba (1997) reported that biofilm regrowth was limited in laboratory-scale units during the presence of disinfectant residuals. In this study residual concentrations for monochloramine and hydrogen peroxide could be maintained for a longer period of time and these two disinfectants were found to be more effective in controlling the regrowth of biofilms in laboratory-scale units than any of the other disinfectants (chlorine, ozone and UV) used during the study.

Resistance of bacteria to disinfectants

It has been shown that some bacteria can survive and multiply despite the presence of measurable concentrations of disinfectants in the distribution system due to the possible development of resistance towards these compounds (Ridgway and Olson, 1982; Olivieri et al., 1985; LeChevallier et al., 1988a). Biofilm cells were found to be less susceptible to disinfectants than planktonic cells.

For many bacteria, the sensitivity to disinfectants is also lower after nutrient limitation (Pyle and McFeters, 1989; Matin and Harakeh, 1990; Stewart and Olson, 1992a). Limitation or depletion of nutrients affects cell surface properties and membrane functions such as proton motive force (Brown et al., 1990) which may be involved in resistance mechanisms. Changes in the content and composition of lipids, lipopolysaccharides, purines and cations in the outer and cytoplasmic membrane may be related to susceptibility changes of gram-negative bacteria. The type of carbon source may also influence cell physiology and preservative sensibility. Al-Hiti and Gilbert (1980) found that *Pseudomonas aerogina* required three times more phosphate when glycerol was replaced by sodium citrate as the sole carbon source. In this study, the greatest changes in sensitivity towards disinfectants were observed for cultures grown on a different carbon source rather than depletion of carbon, nitrogen or phosphate sources.

Bacterial survival following chlorination has been observed in water in the presence of supposedly adequate residual concentration of disinfectant residual (Mathieu et al., 1992; Momba, 1997). Experience has shown that maintenance of a chlorine residual cannot be relied on to totally prevent bacterial occurrences. It has

also been suggested that chlorine-resistant bacteria may arise as a result of chlorination (Ridgway and Olson, 1982; Leyval et al., 1984), although other investigators found no evidence for this (Haas and Morrison, 1981). Chlorine has been the most widely recognised cause of injury to coliform bacteria in drinking water (Camper and McFeters, 1979) although other biocides and factors such as low concentrations of metals (e.g. copper and zinc), temperature extremes and interaction with other bacteria (LeChevallier et al., 1988a) may also contribute. Both the chlorine and the time of exposure have been shown to influence the degree of injury in coliform organisms (McFeters and Camper, 1983). McFeters et al. (1986) found that enteropathogenic and indicator bacteria become injured in drinking water with exposure to sublethal levels of various biological, chemical and physical substances.

Other factors influencing resistance to halogen disinfectants may include cell aggregation, surface adhesion, spore formation and protective capsules (Pyle and McFeters, 1989). Attachment has been shown to be a major factor in resistance to disinfection (Lechevallier et al., 1988a), potentially protecting attached cells from a chlorine residual (Camper et al., 1999). Attached cells could serve as a reservoir for subsequent spread through the system, following detachment or biofilm sloughing caused by changes in nutrient, disinfectant, or hydrodynamic status (Camper et al., 1999). Experiments have shown that attachment of unencapsulated *Klebsiella pneumonia* to glass microscope slides afforded the micro-organisms as much as a 150-fold increase in disinfection resistance (LeChevallier et al., 1988a). The age of the biofilm, bacterial encapsulation and previous growth conditions (e.g. growth medium and growth temperature) increased chlorine resistance from two- to tenfold (LeChevallier et al., 1988a). The choice of disinfectant residual also influenced the type of resistance mechanisms. LeChevallier et al. (1988a) found that disinfection by free chlorine was affected by surfaces, age of the biofilm, encapsulation and nutrient effects, whereas disinfection by monochloramine was only affected by surfaces. This research showed that these resistance mechanisms were multiplicative (e.g. the resistance provided by one mechanism could be multiplied by the resistance provided by a second).

At present, little quantitative information is available regarding resistance mechanisms of bacterial regrowth to other biocides. Experiments with ozone have shown that ozone may also react with organic material in source water to form nutrients that allow regrowth in the distribution system and increase the growth of biofilm on internal surfaces of distribution pipes (Clark et al., 1994). After studying various combinations of ozone, chlorination and chloramination, Clark et al. (1994) reported that micro-organisms increased as the dissolved organic carbon (DOC) decreased, providing a nutrient source for this biological growth. Lund and Ormerod (1995) reported the microbial regrowth after the action on water of three oxidative disinfection processes (chlorination, UV irradiation and ozonation). This study points out that the greatest biomass production was found from the ozonated water characterised by a high BOD level. It is well known that ozonation of water containing complex organic matter leads to the production of low-molecular organic substances which are easily bio-assimilable, as noted by Lefebvre (1994).

Nature and concentration of biodegradable compounds

Regrowth of micro-organisms in drinking water distribution systems is caused by the utilisation of biodegradable compounds which are either present in treated water or originate from materials in contact with drinking water (Van der Kooij, 1999). Micro-organisms, therefore, can be characterised on the basis of the compounds that they use as energy requirement (inorganic or organic hydrogen), carbon requirement (inorganic or organic carbon) and the kind of hydrogen acceptor (oxygen, nitrate, sulphate, carbon dioxide, or organic C). Heterotrophic micro-organisms mainly contribute to the high bacterial counts detected in distribution systems. These organisms use organic carbon compounds as a C-source and energy source and most of them use oxygen as a hydrogen acceptor. Approximately 50% of the absorbed organic carbon is converted into CO_2 (dissimilation) to satisfy the energy requirements of the cells and 50% are used for the new cellular material (assimilation). The compounds of C, N, and P are then required in an approximate ratio of C : N : P = 100 : 10 : 1. In general, compounds that may serve as a source of N are present in concentrations varying from a few tenths of a milligram to a few milligrams of N (mostly in the form of nitrate) per litre, and phosphates are present in concentrations between a few tenths of a microgram and a few hundred micrograms of P /ℓ (Van der Kooij et al., 1982).

Total organic carbon (TOC) has not been found to be a good predictor of bacterial regrowth (Rizet et al., 1982) because the ratio of organic nutrients to TOC is not a constant (Van der Kooij et al., 1982; Hascoët et al., 1986). In most environments, only a small fraction of dissolved organic carbon is actually susceptible to microbial attack, the rest consisting of refractory organic compounds, generally called "humic substances", which are not available for bacterial growth (Ogura, 1977).

Biodegradable organic matter can be broken down into two separate constituents both of which can be determined: BDOC and assimilable organic carbon (AOC). BDOC can be defined as the fraction of the dissolved organic carbon (DOC) which can be metabolised by bacteria with a period of a few days to a few months (Servais et el., 1989). The degree of bacterial regrowth is determined by the overall content of biodegradable organic carbon present in the water and is largely determined by the origin of the drinking water produced. It has been reported that water from lakes and reservoirs contains a much higher concentration of DOC than groundwater (Bernhardt and Classen, 1993). According to Bernhardt and Wilhems (1985), treated water containing a DOC concentration of below 1 mg.ℓ^{-1} is not prone to regrowth. Joret et al.(1991) reported that *E. coli, Klebsiella pneumonia* and *Enterobacter cloacae* can regrow in river water samples with a 3.2 mg·ℓ^{-1} initial DOC and a 1.4 mg·ℓ^{-1} initial BDOC, but are unable to multiply in finished water samples with initial DOCs of 0.4 mg·ℓ^{-1} and 0.8 mg·ℓ^{-1} and BDOCs of <0.1 and 0.1 mg·ℓ^{-1} respectively.

AOC can be determined as the fraction of the biodegradable organic carbon that can be converted into new cellular material (assimilation). This can therefore give an indication of the regrowth potential of a water sample (Van der Kooij et al., 1982), but only measures the carbon that is converted to biomass. The concentration of easily assimilable organic carbon compounds is usually a small part of the DOC concentration, particularly when the water has been exposed to biological processes for a long time, e.g. groundwater (Van der Kooij et al., 1982). Van der Kooij (1992) regards a concentration of AOC in drinking water of 10 µg acetate-C equivalent·ℓ^{-1} or less as an acceptable concentration. At such low concentrations, heterotrophic bacteria will not reproduce in drinking water. Under these conditions, the number of bacteria remains below 100 CFU·mℓ^{-1}. In drinking water such characteristics can be regarded as "biologically stable" (Van der Kooij, 1992).

An experimental study performed by Van der Kooij (1999) on the potential for biofilm development in drinking-water distribution systems revealed a relationship between AOC uptake and biofilm formation. An AOC flux of 15.5 mg C·m^{-2}·d^{-1} corresponded with an increase in biofilm density of 1.9 x 10^7 cfu·cm^{-2}·d^{-1}.

Similarly, an AOC value of 0.375 mg C·m^{-2}·d^{-1} gave a daily increase of 4.5 x 10^5 cfu·cm^{-2}. These calculations indicated that AOC reduction as observed in distribution systems has a significant effect on biofilm formation in drinking water distribution systems. It is also important to note that the AOC concentrations are in the range of μg·ℓ^{-1} and thus low substrate concentrations are sufficient for biofilm accumulation in distribution systems (Van der Kooij, 1999).

Plumbing materials

Pipe materials for water supply and distribution can generally be classified into one of three generic types, i.e. cementitious, metallic or plastic. A wide range of pressure pipe materials is available within these categories, and such materials are used in varying proportions in countries throughout the world. Over the years, many types of material have been used in the construction of water distribution networks, and often material indigenous to the area has been used. Presently material such as concrete, cast iron, steel and plastic is utilised.

It is well known that there is a direct relationship between the material used for the construction of potable water distribution systems and the quality of water (Rogers et al., 1994). Biofilm formation is usually encouraged on the surface of a plumbing material if that material is able to supply the required nutrients for bacterial growth. In countries such as the United Kingdom, the influence of piping material is examined according to British Standard BS6920 before their use is permitted (Anon, 1988). This ensures that the material does not contribute to poor water quality by producing unacceptable tastes or odours, releasing chemicals or encouraging microbial growth. There are a number of examples of how different materials encourage the growth of different micro-organisms in biofilms. The hydrophobic-hydrophilic nature of the surface is known to affect the attachment of aquatic bacterial species to surfaces (Fletcher and Marshall, 1982). Experiments have shown that most pipe surfaces in distribution systems could contain biofilms with bacterial densities as high as 10^9 bacteria·cm^{-2} (Olson, 1982).

The involvement of micro-organisms in the deterioration of concrete has been considered for a number of years, but only recently have attempts been made to understand the basis of microbially mediated decay of concrete. Moreover, the major portion of the work carried out to date on biofilm formation on concrete and biodeterioration of concrete pipes has been conducted on sewage systems, in marine environments (Kulpa and Baker, 1990) as well as with underground concrete structures (Tazawa et al., 1994). The process is believed to be a complex one involving several different groups of micro-organisms such as anaerobic sulphate-reducing bacteria (Kulpa and Baker, 1990; Poulton and Nixon, 1992).

Historically, mild steel protected with thin-film organic coatings has been used as the material for fabrication of pipework in industrial water systems. Research work carried out by Poulton and Nixon (1992) reported that uniform microbial attachment occurred on mild steel, epoxy-coated mild steel, mortar and concrete although no deleterious effect on the concrete and mortar was noted. An investigation performed by Momba (1997) showed that the cement coupons had much lower viable bacteria in the initial treated waters than the stainless steel coupons. The relative difference between the two piping materials diminished with a prolonged exposure time.

Copper has also been used as piping material for distribution systems. Scanning electron microscopy (SEM) has revealed two distinct layers: a layer of extracellular polymeric substance in direct contact with the copper and a second layer consisting of bacteria which were not embedded in the extracellular polymeric substance, in the direction of the luminary. Some of the micro-organisms of this layer showed holes. Bacteria in direct contact with a disinfection solution showed a rough thick end-surface indicating the existence of capsule substances (Exner et al., 1983).

From the 1950s, synthetic materials were used in the construction of potable water distribution systems. Polyethylene (PE) and polybutylene (PB) have been adapted for use as pipe and tubing material for fluid conveyance (Crowson and Chambers, 1985). Because of their many advantageous properties, such as resistance to chemicals and corrosion, electrical non-conductivity, competitive cost, flexibility and ease of handling, storage and installation, these piping materials have gained widespread acceptance and use within the water industry (Crowson and Chambers, 1985). Despite their many advantages, they also contribute to biofilm formation in drinking water. A recent study performed by Lu et al. (1999) demonstrated a relationship between the piping materials and the chlorine demand of biofilms in water distribution systems. The authors stipulate that, in a distribution network, the classification of parameters which consume chlorine needs to consider separately synthetic pipes and metallic pipes. In the first case, most of the chlorine is consumed by deposits, water and biomass. For example for a 250 mm diameter synthetic pipe, total chlorine consumption in 2 h is equal to 0.22 mg·ℓ^{-1} whereas for a cast-iron pipe, chlorine consumption after 2 h is equal to 0.50 mg·ℓ^{-1}. In this case, biomass chlorine demand becomes negligible, and chlorine is principally consumed by the material, deposits and then water. To control material corrosion and then chlorine demand, the authors suggest a replacement of gray cast-iron pipes by cement-lined ductile cast iron. It is also important to minimise the organic matter level in water in order to reduce chlorination by-product formation and, at the same time, to decrease chlorine demand due to biomass and the water itself (Lu et al., 1999).

New plastic pipe materials, for example unplasticised polyvinyl chloride (uPVC) and medium density polyethylene (MDPE), are currently replacing the much older cast-iron pipes in water distribution systems. Although their biofilm forming potential has not fully been investigated, it well known that any new material should not support greater amount of biofilm than existing ones. Comparing uPVC and MDPE to cast-iron pipes, Keer et al. (1999) found that the number of bacteria on each material increased exponentially between 0 and 11 d when the biofilm viable count remained constant. The mean doubling times of the heterotrophic population on the materials during the exponential phase was 13.2 h for cast iron and 15.6 h for MDPE and uPVC. This investigation concluded that MDPE and uPVC supported the lowest numbers of bacteria in a steady biofilm in the short term (21 d) and over a long term (7 months). The diversity of heterotrophic bacteria was greatest on cast iron.

Temperature

The ability of bacteria to grow and survive over a wide range of temperatures has been demonstrated. LeChevallier et al. (1980) noted that several trends were apparent in the seasonal distribution and species diversity of the SPC population present in distribution water. For example, there was greater species diversity in the warmer period than during the cold winter months. After incorporating *L. pneumonia* into both the planktonic and biofilm phases of the model system at 20, 40 and 50°C, Rogers et al. (1994) noted the overall trend of growth was related to temperature as well as piping material.

Propagation, enumeration and monitoring of biofilm bacteria in water distribution systems

Propagation devices

Studies have been directed at monitoring adhesion processes, biofilm formation and the subsequent advantages of this mode of growth. The following devices have been designed for the *in situ* development of sessile microbial populations in water distribution systems. A number of devices have been described in literature for the *in situ* development of biofilms in water distribution systems. One of the first devices used was the Robbins device (Costerton and Robbins, 1980). It consists of a cylinder which allows it to be inserted directly into a pipe or a bypass system. The surface of the removable metal studs forms an integral part of the metal surface available for colonisation. A variation is the Chemostat-coupled modified Robbins device which provides a large number of sample surfaces for monitoring both the formation and control of biofilms over extended periods of time (Jass et al., 1995). Another device is the Pedersen device which consists of microscope slides fitted into acrylic plastic holders. It was developed in order to study microbial biofilms in flowing-water systems with special reference to the flow conditions in electrochemical concentration cells (Pedersen, 1982). Biofilms propagated in this manner are mostly used to quantify the bacteria present.

Enumeration techniques

Apart from the classical enumeration methods which rely on the culturing of organisms, other techniques have also been used to enumerate bacteria present in bioflims. Epifluorescence direct counting is one of the important methods for the evaluation of bacterial counts in natural waters as well as in biofilms. Early studies were done with acridine orange [3,6-bis (dimethylamino) acridium chloride], but this fluorescent dye was replaced by 4', 6-diamidino-2-phenylindole (DAPI), which has more stable fluorescence (Porter and Feig, 1980). The DAPI is believed to be very specific for DNA and it is thus used to count total (including nonviable and viable but nonculturable) bacteria in water (Porter and Feig, 1980).The use of other fluorochromes with epifluorescent microscopy for the enumeration, or determination of viability or activity of bacteria in biofilms has also been described by a number of investigators (Newby, 1991; Patterson et al., 1991; Rodriguez et al.,1992; Yu and McFeters,1994). One of the disadvantages of direct microscopic techniques is the lack of sensitivity. They require at least 10^3 organisms per mℓ in small samples for reliable detection (Mittelman, 1995). Another factor is that the type (s) of bacteria involved in the contamination are not readily determined with the microscopy examination. To overcome this problem fluorescent *in situ* hybridisation (FISH) could be used. In this technique fluorescent-labelled probes are used to identify specific microbes within biofilms.

Physiological stains are also often used in conjunction with total cell stains to determine the active portion of a population . Yu and McFeters (1994) found that 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) and rhodamine 123 (Rh 123) were effective indicators of metabolically active cells in biofilms, although other investigators reported a lack of sensitivity with CTC in chlorinated biofilms (Morin and Camper, 1997).

Confocal microscopy has been used extensively for observation of planktonic bacteria, disrupted biofilms and intact biofilms. This technique has some advantages over epifluorescence microscopy although it is also limited to excitation at specific wavelength, e.g. a Kr-Ar-laser excites at 4888, 568 and 647 nm (Camper et al., 1999). Confocal microscopes are linked to computer image analysis capabilities. Images obtained from the separate excitation wavelengths can be compiled and enhanced. Moreover, objects on the slide can be optically sectioned in the z-plane, which means that thick objects (like biofilms) that cannot be brought into focus in their entirety by epifluorescence can be visualised after reconstruction of the individual thin-section confocal images (Camper et al., 1999).

On-line monitoring techniques

A number of monitoring techniques have been developed which allow for the non-destructive *in situ* qualitative monitoring of biofilms. Once immersed into a water system, biofilms will develop not only on the walls but also on the tip of an optical fibre. The crucial part of the sensor is the probe head which incorporates the optical fibre tip. Light is transmitted to a well-defined measuring area on the surface of the probe to which the biofilm has attached and reflected light is used as a measure of biofilm formation (Cloete, 1997). Commercial turbidity measurement devices using the same principle to determine the rate of biofilm formation on the detector window could be used. Fourier transformation infrared spectrometry allows the detection of bacterial biofilms as they form on a crystal of zinc selenite or germanium (Nichols et al., 1985). The amide stretching of the proteins and ether stretch of the carbohydrates are clearly detectable when bacteria attach to surfaces. This non-destructive technique holds promise as an on-line monitoring tool for bacterial colonisation in purified water systems.

Other techniques which have also been used include the use of fluorometry for on-line monitoring of biofilm development (Khoury et al., 1992) and electrochemical monitoring such as impedance spectroscopy (Dowling et al., 1988) and vibrating quartz crystal microbalance (QCM) technology (Nivens et al., 1993b).

Significance and public health concerns associated with biofilms

The microbial composition of potable water reflects the microflora characteristics of the raw water source. These may be broadly classified into four groups: bacteria, viruses, protozoa and fungi. Biofilm serves as a focal point where bacteria and other microorganisms interact. A large variety of different heterotrophic bacteria have been isolated from the biofilm both in chlorinated and non-disinfected water distribution systems (Colbourne et al., 1988). The presence of *E. coli, Pseudomonas Aeromonas, Artrobacter, Caulobacter, Klebsiella Bacillus, Enterobacter, Citrobacter, Acinetobacter, Prosthescomicrobium, Alcaligenes, Serratior* and *Actinolegionella* has been reported (Van der Kooij and Zoetemann, 1978; Ridgway and Olson, 1981; Olson, 1982; Olivieri et al., 1985; Herson et al., 1987; Schindler and Metz, 1991). Although no single medium, temperature, or choice of incubation time will ensure recovery of all organisms present in water, selected members of the bacterial population can be measured accurately and their numbers can be related to water treatment efficiency and quality deterioration in the distribution system. According to Brazos and O'Connor (1989), bacteria originating from the source water or their successor cells (regrowth bacteria) present in the biofilm, made a much greater contribution to the planktonic population of the distributed water than bacteria originating from aftergrowth.

Potable water of good bacteriological quality is generally regarded as that containing less than one total coliform per 100 mℓ

of water sample. Although the detection of coliform bacteria is the primary concern, attention should also be directed towards controlling the general bacteria population as many of the heterotrophic bacteria isolated from distribution systems have been related to secondary opportunistic pathogens in humans. Attachment of pathogenic bacteria such as *Pseudomonas, Mycobacter, Klebsiella, Aeromonas, Legionella* spp., *Yersinia enterocolitica, Salmonella typhimurium* and enterotoxigenic *E. coli* to surface in water distribution systems has been noted (Engel et al., 1980; Wadowsky et al., 1982; Burke et al., 1984; Camper et al., 1985). Recently there have also been reports of the survival of *Campylobacter* spp., *Helicobacter pylori* and *Cryptosporidium parvum* in biofilms (Armon et al., 1997; Buswell et al., 1998; MacKay et al., 1998). Not only have bacterial cells and protozoan cysts been identified in water distribution systems in both the water phage and the biofilms, but also yeast, fungi and algae (Sibile, 1998). Although the majority of these organisms are not pathogens, nevertheless potentially pathogenic bacteria (*Legionella, ...*), faecal bacteria (coliforms, *E. coli*), and pathogen protozoan cysts (*Giardia intestinalis, Cryptosporidium parvum...*) can transitorily find favourable conditions for their proliferation in the networks (Sibile, 1998) .

Control strategies

Amongst the various treatment barriers between the consumer and water-borne diseases, disinfection occupies a key position, as it is indispensable for the prevention of water-borne infectious diseases. Disinfection of drinking water can be regarded as the single most significant public heath measure of this century. The destruction of pathogens in drinking water has dramatically reduced the incidence of water-borne diseases in all industrialised countries. The final disinfection step in the treatment of drinking water is introduced to remove bacteria or other micro-organisms which escape prior treatment processes. Oxidative disinfectants e.g. chlorine, chloramine, ozone or hydrogen peroxide are most commonly used.

Increased awareness of the magnitude of water-borne diseases suggests that new and improved strategies are needed to reduce the health risk from microbes in drinking water. The approach by the water treatment industry to provide safe drinking water and prevent outbreaks of water-borne disease should be based on the concept of multiple barriers. This includes the protection of surface and groundwater quality at its source, multiple treatment technologies applied at the utility (including coagulation, flocculation, sedimentation, filtration and disinfection) as well as the management of the distribution systems. Factors contributing to biofilm formation should be controlled. Efforts should be made to utilise materials in the network which will suppress the attachment of bacteria as well as controlling the AOC levels.

Increasing or modifying present conventional treatment practices, especially disinfection, is likely to reduce such health risks. As mentioned previously, the maintenance of a chlorine residual cannot totally be relied on to prevent bacterial occurrences. Bacterial survival following chlorination has been reported even in the presence of supposedly adequate residual concentration of chlorine (Mathieu et al., 1992, Momba et al., 1998). Researchers have shown that less reactive, more persistent, monochloramine maintained a longer disinfectant residual throughout the distribution systems and could penetrate the biofilm more effectively, resulting in better control of biofilm formation than free chlorine (LeChevallier et al., 1990; Van der Wende and Characklis, 1990). A study performed by Momba (1997) showed that the maintenance of a disinfectant residual in potable water could not be relied on to prevent bacterial adhesion on stainless steel coupons, cement coupons and glass surfaces. Even in the presence of significant residual disinfectant concentrations of 12.5 to 19.0 $mg \cdot \ell^{-1}$ hydrogen peroxide, 0.8 to1 $mg \cdot \ell^{-1}$ monochloramine and 0.2 to 0.5 $mg \cdot \ell^{-1}$ free chlorine, bacteria were detected on the coupons. In the absence of a disinfectant residual concentration, a high increase in biofilm micro-organisms was detected, whereas a low increase was recorded in the presence of the residual concentrations. Similar to the other studies mentioned, monochloramine and hydrogen peroxide were found to be much more effective in controlling the growth of biofilm in laboratory-scale units.

Conclusions

The formation of biofilms associated with events of regrowth or aftergrowth in water distribution systems is one of the main reasons for the deterioration of the bacteriological quality. It has been established that for each planktonic bacterium detected there might be close to a 1 000 organisms present in the biofilm. Biofilms consist mainly of a consortium of species entrapped within a polysaccharide matrix.

Typical factors which may enhance biofilm formation are efficiency of the type of disinfectants used and the ability to maintain adequate residual concentrations, the nature and concentration of biodegradable compounds in the drinking water, the nature of the materials used for the construction of the drinking-water distribution systems as well as prevailing water temperature. The management of all these factors is important for the control of biofilms to ensure compliance with bacteriological quality standards of the drinking water

Although the detection of coliform and faecal coliforms bacteria is the primary concern, attention should also be directed towards controlling the general bacterial population as many of the heterotrophic bacteria present in biofilms have been relegated to secondary opportunistic pathogens in humans. Apart from the presence of the heterotrophic organisms detected in biofilms, a number of pathogenic and toxigenic microbiological agents have been detected in biofilms. The health risks associated with these pathogens range from viral and bacterial gastro-enteric diseases to infections such as hepatitis A and giardiasis. Recently there have also been reports of the survival of *Campylobacter* spp., *Helicobacter pylori* and *Cryptosporidium parvum* in biofilms.

Measures suggested for the control of biofilm formation include the use of less reactive, more persistent, monochloramine or hydrogen peroxide which maintained a longer disinfectant residual throughout the distribution system. The disinfectants can also penetrate the biofilm more effectively, resulting in better control of biofilm formation than the more reactive disinfectants such as free chlorine. To ensure adequate protection chlorine might be used for the final disinfection stage during water treatment and this will be followed by the use of monochloramine to ensure a persistent concentration disinfectant residual throughout the distribution system.

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References

AL-HITI MMA and GILBERT P (1980) Changes in preservative sensitivity for the USP antimicrobial agents effectiveness test micro-organisms. *J. Appl. Bacteriol.* **49** 119-126.

ANONYMOUS (1988) British Standard 6980. Suitability of Non-metallic Products for Use in Contact with Water Intended for Human Consumption with Regard to Their Effect on the Water Quality. Growth of Aquatic Microorganisms. British Standards Institution London. Section 2-4.

ARMON R, STAROSVETZKY J, ARBEL T and GREEN M (1997) Survival of *Legionella pneumophila* and *Salmonella typhimurium* in biofilm systems. *Water Sci. Technol.* **35** (11/12) 293-300.

BAYLIS JR (1930) Bacterial aftergrowth in water distribution systems. *Water WKS Sewer* **77** (10) 335.

BERNHARDT H and WILHELMS A (1985) Erfahrungen mit der Desinfektion von Trinkwasser durch Chlordioxid. *38 Int. CFBEDEAU Konf. - CEBEDOC.* Liège. 193-231.

BERNHARDT H and CLASSEN J (1993) Eutrophication control as an essential condition for an optimum disinfection. *Water Supply* **11** (3) 89-108.

BRAZOS BJ and O'CONNOR JT (1989) Relative contribution of regrowth and aftergrowth of the number of bacteria in a drinking water distribution system. In: *Proc. AWWA Water Qual. Tech. Conf.*, Philadelphia, Pennysylvannia, November.

BROWN MRW, COLLIER PJ and GILBERT P (1990) Influence of growth rate on susceptibility to antimicrobial agents: Modification of the cell envelope and batch and continuous cultural studies. *J. Antimicrob. Chemother.* **34** 1623-1628.

BURKE V, ROBINSON J, GRACEY M, PETERSEN D and PARTRIDGE K (1984) Isolation of *Aeromonas hydrophila* from a metropolitan water supply: Seasonal correlation with clinical isolates. *Appl. Environ. Microbiol.* **48** 361-366.

BUSWELL CM, HERLIHY YM, LAWRENCE LM, McGGUIGGAN JTM, MARSH PD, KEEVIL CM and LEACH SA (1998) Extended survival and persistence of *Campylobacter* spp. water and aquatic biofilms and their detection by immunofluorescent-antibody and -rRNA staining. *Appl . Environ. Microbiol.* **64** 733-741.

CAMPER AK and McFETERS GA (1979) Chlorine injury and the enumeration of waterborne coliform bacteria. *Appl. Environ. Microbiol.* **37** (3) 633.

CAMPER AK, LECHEVALLIER MW, BROADWAY SC and McFETERS GA (1985) Growth and persistence of pathogens in granular activated carbon filters. *Appl. Environ. Microbiol.* **50** 1378-1382.

CAMPER MB, ELLIS B, BUTERFIELD P and ABERNATHY C (1999) Development and structure of drinking water biofilms and techniques of their study. *J. Appl. Microbiol. Symp. Supp.* **85** 1S-12S.

CLARK RM, LYKINF BW, BLOCK JC, WYMER LJ and REASONER DJ (1994) Water quality changes in a simulated distribution system. *J. Water SRT-Aqua* **43** (6) 263-277.

CLOETE TE (1997) Technology Scan Report - Eskom on International Workshop on Industrial Biofouling and Biocorrosion. Mülheim an der Ruhr - Germany. 4 -5 September.

COLBOURNE JS, DENNIS PJ, TREW RM, BERRY C and KESEY G (1988) Legionellis and public water supplies. In: *Proc. Int. Conf. on Water and Wastewater Microbiol*. Newport Beach, California.

COSTERTON JW and ROBBINS JR (1980) The Robbins device for the *in situ* development of sessile bacterial populations. Patent, Canada and USA.

COSTERTON JW, CHENG KJ, GEESEY GG, LADD TI, NICKEL JC, DASGUPTA M and MARRIE TJ (1987) Bacterial biofilms in nature and disease. *Ann. Rev. Microbiol.* **41** 435-464.

CRAUN GF (1986) *Waterborne Diseases in the United States*. CRC Press, Inc. Boca Raton. Florida. 295 pp.

CROWSON DL and CHAMBERS R (1985) Polyethylene and polybutylene pipe and tubing: A status report. *J. AWWA* **77** (11) 45-46.

DOWLING NJE, STANSBURY EE, WHITE DC, BORENSTEIN SW and DANKO JC (1988) On-line electrochemical monitoring of microbially induced corrosion. In: *Microbial Corrosion*, Palo Alto, Calif. 5-17.

ENGEL HWB, BERWALD LG and HAVELAAR AH (1980) The occurrence of *Mycobacterium kansasii* in tap water. *Tubercle* **61** 21-26.

ESCHER AR and CHARACKLIS WG (1988) Microbial colonization of a smooth substratum: A kinetic analysis using Image Analysis. *Water Wastewater Microbiol. Water. Sci. Tech.* **20** (11/12) 277-283.

EXNER M, TUSCHEWITZKI GJ and THOFERN E (1983) Observation of bacterial growth on a copper pipeline of a central disinfection dosage apparatus. *Zbl. Bkt. Hyg. I. Abt. Orig. B.* **177** 170-181.

FLETCHER M and MARSHALL KC (1982) Bubble contact angle method for evaluation of substratum interfacial characteristics and its relevance to bacterial attachment. *Appl. Environ. Microbiol.* **44** 797-732.

GIBBS RA, SUTT JE and CROLL BT (1990) Microbiological and trihalomethane responses to booster chlorination. *J. IWEM* **4** (1) 131-139.

GILBERT E (1988) Biodegradability of ozonation products as a function of COD and DOC elimination by the example of humic acid. *Water Res.* **92** 123-126.

HAAS CN and MORRISON EC (1981) Repeated exposure of *Escherichia coli* to free chlorine: Production of strains possessing altered sensitivity. *Water Air Soil Pollut.* **16** 233-242.

HASCOËT MC, SERVAIS P and BILLEN G (1986) Use of biological analytical methods to optimize ozonation and GAC filtration in surface water treatment. Paper presented at Annual Meeting, AWWA, Denver, Col.

HERSON DS, McGONIGHE B, PAYER MA and BAKER KH (1987) Attachment as a factor in the protection of *Enterobacter cloacea* from chlorination. *Appl. Environ. Microbiol.* **53** 1178-1180.

JASS J, COSTERTON JW and LAPPIN-SCOTT HM (1995) Assessment of a chemostat-coupled modified Robbins device to study biofilms. *J. Ind. Microbiol.* **15** 283-289.

JORET JC, LEVI Y and VOLK C (1991) Biodegradable dissolved organic carbon (BDOC) content of drinking water and potential regrowth of bacteria. *Water Sci. Technol.* **24** (2) 95-101.

KEER CJ, OSBORN KS, ROBSON GD and HANDLEY PS (1999) The relationship between pipe material and biofilm formation in a laboratory model system. *J. Appl. Microbiol. Symp. Suppl.* **85** 29S-38S.

KHOURY AE, NICHOLOV R, SOLTES S, BRUCE AW, REID G and DICOSMO F (1992) A preliminary assessment of *Pseudomonas aeroginosa* biofilm development using fluorescence spectroscopy. *Int. Bioterior. Biodegrad.* **30** 187-199.

KIENE L, LU W and LEVI Y (1996) Relative importance of phenomena responsible of the chlorine consumption in drinking water distribution systems *Proc. of WQTC AWWA*, Boston, MA. 21 Nov. 117 pp.

KULPA CF and BAKER C (1990) Involvement of sulfur-oxidizing bacteria in the concrete deterioration In: Whiting D (ed.) *Paul Klieger Symp. on Performance of Concrete*, USA. SP. 122-127, 313-322.

LECHEVALLIER MW, SEIDLER RJ and EVANS TM (1980) Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. *Appl. Environ. Microbiol.* **40** (5) 922-930.

LECHEVALLIER MW, BABCOCK TM and LEE RG (1987) Examination and characterization of distribution system biofilms. *Appl. Environ. Microbiol.* **53** (12) 2714-2724.

LECHEVALLIER MW, CAWTHON CD and LEE RG (1988a) Mechanisms of bacterial survival in chlorinated drinking water. *Water Sci. Technol.* **20** (11/12) 145-151.

LECHEVALLIER MW, LOWRY CD and LEE RG (1990) Disinfecting biofilms in a model distribution system. *J. AWWA* **82** 87-99.

LEFEBVRE E (1994) Modification de la matière organique lors des treatments conventionnels de potabilisation. *Proc. Congrès GRUTTEE. Poitiers* **21** 1-13.

LEYVAL C, ARZ, C, BLOCK JC and RIZET M (1984) *Escherichia coli* resistance to chlorine after successive chlorination. *Environ. Tech. Lett.* **5** 359-364.

LOYD BJ and BARTRAM JK (1991) Surveillance solutions to microbiological problems in water quality control in developing countries. *Water Sci. Technol.* **24** (2) 61-75.

LU W, KIENE L and LEVI Y (1999) Chlorine demand of biofilms in water distribution systems. *Water Res.* **33** (3) 827-835

LUND V and ORMEROD K (1995) Influence of disinfection processes on biofilm formation in water distribution systems. *Water. Res.* **29** (4) 1013-1021.

MACKAY WG, GRIBBON LT, BARER MR and REID DC (1998) Biofilms in drinking water systems - A possible reservoir for *Helicobacter pylory. Water Sci. Technol.* **38** (12) 181-185.

MATHIEU L, PAQUIN JL, HARTEMANN P and COLIN F (1992) Paramètres controlant l'accumulation de bactéries dans les réseaux de

distribution: approche expérimantale. Séminaire International. Matière organique biodégradable dans les réseaux de distribution, Nancy.

MATIN A and HARAKEH S (1990) Effect of starvation on bacterial resistence to disinfectants. In: McFeters GA (ed.) *Drinking Water Microbiology: Progress and Recent Developments*. Springer-Verlag. New York. 88-103.

McFETERS GA AND CAMPER AK (1983) Enumeration of indicator bacteria exposed to chlorine. *Adv. Appl. Microbiol*. **20** 177-193.

McFETERS GA, KIPPIN JJ and LECHEVALLIER MW (1986) Injured coliforms in drinking water. *Appl. Environ. Microbiol.* **51** 1-5.

MITTELMAN MW (1995) Biofilm development in purified water systems. In: Lappin-Scott HM and Costerton JW (eds.) *Microbial Biofilms.* Cambridge Univ. Press, Cambridge, UK. 133-147.

MOMBA MNB (1997) The Impact of Disinfection Processes on Biofilm Formation in Potable Water Distribution Systems. Ph.D Thesis, Univ. of Pretoria, South Africa.

MOMBA MNB, CLOETE TE, VENTER SN and KFIR R (1998) Evaluation of the impact of disinfection processes on the formation of biofilms in potable surface water distribution systems. *Water Sci. Technol.* **38** (8/9) 283-289.

MORIN P and CAMPER AK (1997) Attachment and fate of carbon fines in simulated drinking water distribution systems biofilms. *Water Res.* **31** 399-410.

NAGY LA and OLSON BH (1985) Occurrence and significance of bacteria, fungi and yeasts associated with distribution pipe surfaces. *Water Supply* **11** (3-4) 365-376.

NEWBY PJ (1991) Analysis of high-quality pharmaceutical grade water by a direct epifluorescence filter technique microcoly method. *Letters in Appl. Microbiol.* **13** 291-293.

NICHOLS PD, HENSON JM, GUCKERT JB, NIVENS DE and WHITE DC (1985) Fourier transform-infrared spectroscopic methods for microbial ecology: Analysis of bacteria, bacteria-polymer mixtures, and biofilms. *J. Microbiol. Meth.* **17** 199-213.

NIVENS DE, CHAMBERS JQ, ANDERSONT R and WHITE DC (1993b) Long-term, on-line monitoring of microbial biofilms using a quartz crystal microbalance. *Anal. Chem.* **65** 65-69.

OGURA N (1977) High molecular weight organic matter in seawater. *Mar. Chem.* **5** 534- 549

OLIVIERI VP, BAKALIAN AE, BOSSUNG KW and LOWTHER ED (1985) Recurrent coliforms in water distribution systems in the presence of free residual chlorine. In: Jolley RL, Bull RJ, Davis WP, Katz S, Roberts MH (Jr) and Jacobs VAO (eds.) *Water Chlorination: Chemistry, Environmental Impact and Health Effects.* Lewis Publishers, Inc., Chelsea, MI. 651-666.

OLSON BH (1982) Assessment and implication of bacterial regrowth in water distribution systems. *EPA.* 600/52-82-072 U.S. Environmental Protection Agency .

PATTERSON MK, HUSTED GR, RUTKOWSKI A and MAYETT DC (1991) Bacteria: Isolation, identification, and microscopic properties of biofilms in high-purity water distribution systems. *Ultrapure Water* **8** (4)18-24.

PEDERSEN K (1982) Method for studying microbial biofilms in flowing water systems. *Appl. Environ. Microbiol.* **3** 6-13.

PORTER KG and FEIG YS (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* **25** 943-948.

POULTON WIJ and MIXON M (1992) Investigation into the Degradation of Mortar Linings and Concrete by Microorganisms in Industrial Water Systems. Water Research Commission Report No 398/1/93. SA.

PYLE BH and MCFETERS GA (1989) Iodine sensitivity of bacteria isolated from iodine water systems. *Can. J. Microbiol.* **35** 520-523.

RIDGWAY HF and OLSON BH (1981) Scanning electron microscopy evidence for bacterial colonization of a drinking water distribution system. *Appl. Environ. Microbiol.* **41**(1) 974-987.

RIDGWAY HF and OLSON BH (1982) Chlorine resistance pattern of bacteria from drinking water systems. *Appl. Environ. Microbiol.* **44** 972-987.

RIZET M, FIESSINGER F and HOUEL N (1982) Bacterial regrowth in a distribution system and its relationship with the quality of the feed water: Case studies. In: *Proc. AWWA. Water Qual. Tech. Conf*., Miami Beach. 1199-1214.

RODRIGUEZ GG, PHILIPPS D, ISHIGURO K and RIDGWAY HF (1992) Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. *Appl. Environ. Microbiol.* **58** 1801-1808.

ROGERS J, DOWSETT AB, DENNIS PJ, LEE JV and KEEVIL CW (1994) Influence of plumbing materials on biofilm formation and growth of *Legionella pneumophila* in potable water systems. *Appl. Environ. Microbiol.* 1832-1851.

ROSSIE WLJR (1975) Control of water quality in transmission and distribution systems. *J. AWWA* **67** (8) 425.

SCHINDLER PRG and METZ H (1991) Coliform bacteria in drinking water from South Bavaria: Identification by the API 20G system and resistance patterns. *Water Sci. Tech.* **24** (2) 81-84.

SERVAIS P, ANZIL A and VENTRESQUE C (1989) Simple method for determination of biodegradable dissolved organic carbon in water. *Appl. Environ. Microbiol*. **55** (10) 2732-2734.

SIBILE I (1998) Biological stability in drinking water distribution systems: A review. *L'année biol.* **37** (3) 117-161.

STEWART MH and OLSON BH (1992a) Impact of growth condition on resistance of *Klebsiella pneumoniae* to chloramine. *Appl. Environ. Microbiol.* **58** 2649-2653.

TAZAWA EI, MORINAGA T and KAWA K (1994) Deterioration of concrete derived from metabolites of micro-organisms. In: Malhotra VM (ed.) *3rd Int. Conf. On Durability of Concrete* , Nice, France. 1085-1093.

VAN DER KOOIJ D and ZOETEMAN BCJ (1978) Water quality in distribution systems. *Proc. IWSA 12th Congress, Kyoto.*

VAN DER KOOIJ D, ORANGE JP and HIJNEN WAM (1982) Growth of *Pseudomonas aeruginosa* in tap water in relation to utilization of substrates at concentrations of few micrograms per liter. *Appl. Environ. Microbiol.* **44** 1086-1095.

VAN DER KOOIJ D (1992) Assimilable organic carbon as an indicator of bacterial regrowth. *J AWWA* **84** 57-65.

VAN DER KOOIJ D (1999) Potential for biofilm development in drinking water distribution systems. *J. Appl. Microbiol. Symp. Supplement* **85** 39S-44S.

VAN DER WENDE E and CHARACKLIS WG (1990) Biofilms in potable water distribution systems. In: McFeters GA (ed.) *Drinking Water Microbiology: Progress and Recent Developments.* Springer-Verlag. New York. 249-268.

VASCONCELOS JJ, BOULOS PF, GRAYMAN WM, KIENE L, WABLE O, BISWAS P, BHARI A, ROSSMAN LA, CLARK RM and GOODRICH JA (1996) Characterization and modelling of chlorine decay in distribution systems. *AWWA* Research Foundation.

WADOWSKY RM, YEE RB, MEZMAR L, WING EJ and DOWLING NJ (1982) Hot water systems as sources of *Legionella pneumophila* in hospital and non-hospital plumbing fixtures. *Appl. Environ. Microbiol.* **43** 1104-1110.

WOLFAARDT GM and ARCHIBALD R (1990) Microbially induced corrosion or biocorrosion in industrial water systems. *Tech. SA*. 1-7.

YU PF and MCFTERS GA (1994) Rapid *in situ* physiological assessment of bacteria in biofilm using fluorescent probes. *Am. Soc. for Microbiol. J. Microbial. Meth.* **20** 1-10.