2.1 Problems of Biofouling in Drinking Water Systems

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INTRODUCTION

At first glance, it would not seem that conditions in a drinking water distribution system are conducive to microbial growth. Treatment is optimized to remove particles, including bacteria. Temperatures are generally low, in most cases there is little organic carbon present, and disinfectants are deliberately added to suppress microbial growth. Remarkably, microbial growth has been found in all distribution systems examined, including stainless steel pipes delivering ultrapure water.

Biofilms in drinking water became important with the recognition that coliform proliferation on surfaces may result in coliform positive water samples. This is of considerable regulatory concern, since these organisms are used as indicators of the microbiological safety of potable water. In addition to the regrowth issue, biofilms in distribution systems can cause other negative effects on finished water quality. *Actinomycetes* or fungi may result in taste and odour problems (Olson 1982; Burman 1965, 1973), which then lead to consumer complaints. Bacterial biofilms may contribute to the corrosion of pipe surfaces (Lee *et al.* 1980). Iron bacteria can grow on ferrous metal surfaces (Ridgway *et al.* 1981) and particulate iron may be released into the water (Victoreen 1974). In this case, red water may be the outcome. It is also possible to have nitrifying bacteria present in biofilms and these organisms could result in nitrification episodes in systems where chloramines are used (Wolfe *et al.* 1990).

REGROWTH IN DISTRIBUTION SYSTEMS

Coliforms are used as the surrogate, or indicator, of pathogen presence. By definition, an indicator organism should be present only in the event of

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faecal contamination, survive longer than a pathogen, and be present in higher concentrations than the pathogen. The presence of the coliforms is interpreted to represent recent post-treatment faecal pollution (Pipes 1990). One major exception has been accepted in the case of regrowth, where the condition of recent faecal contamination is exempted. It is known that some coliforms can persist in, and sometimes regrow in biofilms of both distribution systems and pipe pilot systems (Camper 1994), with cells detaching and contaminating the bulk water phase. Coliforms released from biofilms may result in elevated coliform positive water samples even though system integrity and disinfectant residual have been maintained (Smith *et al.* 1990; Characklis 1988; Haudidier *et al.* 1988). In these cases, recent faecal contamination is not indicated and a 'false positive' is obtained. If a utility in the U.S. can prove that the presence of coliforms i attributed to a biofilm regrowth event, it can apply for variance from the Total Coliform Rule. Past experience has indicated that this may be reasonable, since no instances of waterborne outbreaks have been associated with regrowth events. However, the opportunistic pathogens, *Klebsiella* spp. and *Aeromonas* spp., are known to be components of biofilms and associated with regrowth events (Geldreich and Rice 1987; Camper 1996; van der Kooij 1988; Havelaar *et al.* 1990).

Regrowth of coliforms and heterotrophs has been attributed to a host of variables including: (i) temperature effects, especially warm water condi tions; (ii) the amount of utilizable carbon for substrate; (iii) inefficiencies in the removal/disinfection of organisms in treatment; (iv) the presence of corrosion products in distribution systems; (v) disinfectant dose/type; and (vi) distribution system hydrodynamics (Smith *et al.* 1990). These topics have been discussed in review articles by Camper (1994), LeChevallier (1990) and Block (1992). A critical variable is the presence of a disinfectant. Intuitively, elevated levels of chlorine should control regrowth, but this is often not the case (LeChevallier *et al.* 1990; Martin *et al.* 1982; Reilly and Kippen 1984; Oliveri *et al.* 1985; Centers for Disease Control 1985; Hudson *et al.* 1983). There is the potential for these same conditions to influence the long-term persistence of pathogenic organisms in biofilms on distribution system pipe surfaces.

PUBLIC HEALTH ISSUES

In the years 1993–1994, the U.S. had 30 disease outbreaks associated with water. Of these, 20 were from groundwater supplies. Ten of the 25 outbreaks of gastroenteritis for which the aetiological agent was identified were caused by protozoa, eight by chemical poisoning, three by *Campylobacter jejuni*, two by *Shigella* spp., and one each by *Salmonella*

typhimurium and a non-O1 *Vibrio cholerae* (Kramer *et al.* 1996). Data from this survey are given to indicate that waterborne disease is still present and that the etiological agents are varied. It is also critical to note that in half of the waterborne outbreaks in the United States, no aetiological agent is identified (Craun *et al.* 1997). It is probable that many of these cases are the result of the inability to detect and culture various viruses responsible for waterborne disease, as well as the difficulties in culturing and identifying bacteriological agents. It is unknown if any of these outbreaks are specifically the result of biofilms in distribution or treatment systems.

The evaluation of water associated disease outbreaks such as hospital acquired (nosocomial) respiratory infections by organisms in water (*Legionella, Pseudomonas, Mycobacterium*) or other mild gastroenteritis (*Aeromonas*) are not included in typical drinking water surveys. This is because the ability to associate disease caused by some of these organisms with water is difficult. In fact, an overview of risk assessment on health effects from microbes shows that there are many organisms responsible for disease and that there are significant problems associated with establishing risk (Sobsey *et al.* 1993). With this overview in mind, the following sections on the organisms perceived and known to be responsible for waterborne and water associated disease and their potential association with biofilms have been prepared. It should be noted that there appears to be the potential for a direct link with biofilms in several instances.

BACTERIA

General Heterotrophs

There are three 'categories' of bacteria of potential public health concern that can be isolated from drinking water. The first are the general heterotrophic bacteria. In the past, these bacteria have not been considered to be of significance, but their presence in finished water suggests that the water is capable of supporting microbial growth. With the growing number of immunocompromised people in the population, it is probable that interest in the number of heterotrophs present in drinking water will increase. The studies of Payment *et al.* (1991; 1993) indicated that populations drinking conventionally distributed water may be at higher risk for gastroenteritis than those drinking the same water after reverse osmosis treatment. There was no correlation of illness with coliform levels, but there was an association with the increased number of heterotrophs enumerated at 35° C, as seen with longer hydraulic residence times. The correlation was attributed to regrowth of the heterotrophs rather than contamination of the distribution system.

Opportunistic Pathogens

The second set of organisms are the opportunistic pathogens. These organisms do not cause disease in healthy individuals, but have been implicated with health effects in the portion of the population with lower immune responses (the very young, very old, and those with immunodeficiencies due to chemotherapy, therapy for implants or organ replacement and HIV infection). A review of opportunistic pathogens in drinking water has been prepared by Geldreich (1991).

The *Aeromonas* spp. are an example of a group of opportunistic pathogens that have received scrutiny in the past few years. A review of the information available on this genus reveals a general lack of understanding of how these bacteria behave in the aquatic environment and also demonstrates the controversy surrounding their public health significance.

Aeromonas spp. have been isolated from drinking water (Versteegh *et al.* 1989; Burke *et al.* 1984b; LeChevallier *et al.* 1982; Havelaar *et al.* 1990; van der Kooij 1988). They are considered water-based rather than waterborne organisms, since they are indigenous to aquatic environments and physiologically adapted for growth within the drinking water system (Rippey and Cabelli 1979). Aeromonads appear to be a small and variable component of the overall heterotrophic population (Havelaar *et al.* 1990), with conflicting evidence supporting the correlation of aeromonads with the overall heterotrophic plate count population (Havelaar *et al.* 1990; LeChevallier *et al.* 1982). They can use a wide range of biopolymers; this may be important for aeromonads to maintain viability or grow in a distribution system, particularly if the aeromonads are growing in the biofilm and using products from more predominant bacteria (van der Kooij 1988). It has been shown that the presence of other microflora, such as pseudomonads, in bottled water enhanced the survival of *Aeromonas* (Warburton *et al.* 1994).

The factors governing aeromonas regrowth appear highly complex and are not well understood. In some studies, positive correlation has been made with temperature and residence time (Havelaar *et al.* 1990), although temperature has been shown to have no correlation in other investigations (Burke *et al.* 1984b). However, these studies collectively agree that aeromonas numbers have no correlation to coliform levels. Colbourne *et al.* (1991) demonstrated that *Aeromonas hydrophila* remained as a component of the biofilm even when the monochloramine concentration was 0.6 mg L^{-1}.

The potential for waterborne aeromonas to act as aetiological agents in a disease outbreak is also poorly understood. It has been shown (Burke *et al.* 1984a, b) that aeromonas numbers in the distribution system correlated with the numbers of clinical gastroenteritis cases associated with

aeromonas. In a survey of studies investigating the presence and absence of aeromonas in faeces, particularly in patients suffering from diarrhoea (van der Kooij 1988), it was found that isolation rates varied widely (<1 to 20%), with highest isolation frequencies in tropical regions and lowest in Europe and the USA. The most common isolate was *A. caviae*. He also noted that the isolation of aeromonas in the absence of other pathogens is not adequate evidence of it being the aetiologic agent.

Concerns associated with aeromonas have led to some attempts at regulation of these organisms. For example, health authorities in the Netherlands have defined 20 colony-forming units (CFU) 100 mL^{-1} in drinking water at the production plant and 200 CFU 100 mL^{-1} during distribution, as maximum allowable values (van der Kooij 1988). In Canada, a limit of 0 CFU 100 mL^{-1} in bottled water has been proposed (Warburton *et al.* 1994).

Overall, it can be concluded that while *Aeromonas* demonstrably has a niche in distribution system microbiota and possesses the potential for causing waterborne disease, direct cause and effect between the presence of the bacteria in water and a disease outbreak is questionable.

Other opportunistic pathogens found to be capable of proliferating in drinking water distribution systems include *Mycobacterium* spp. (Engel *et al.* 1980; Kaustova *et al.* 1981; Collins *et al.* 1984), *Legionella* spp. (Dennis *et al.* 1982; Tobin *et al.* 1981b; Wadowsky *et al.* 1982; Rogers and Keevil 1992; Colbourne *et al.* 1988) and *Pseudomonas* (Geldreich 1990; Gambassini *et al.* 1990).

The mycobacteria are found in drinking water distribution systems throughout the United States (duMoulin *et al.* 1988; Fischeder *et al.* 1991; von Reyn *et al.* 1993; Glover *et al.* 1994). Specific isolates have been shown to survive for up to 41 months in a distribution system (von Reyn *et al.* 1994). The organisms infect the lungs and may cause a tuberculosis-type disease. Of particular interest are the organisms belonging to the *M. avium* complex (*M. avium* and *M. intracellulare*; MAC). These are acid-fast rod shaped bacteria that are found in natural waters, including drinking water, throughout the United States (Haas *et al.* 1983; duMoulin *et al.* 1986, 1988; Carson *et al.* 1988; Fischeder *et al.* 1991; von Reyn *et al.* 1993, 1994; Glover *et al.* 1994). It appears that these organisms are actually growing in these environments, since they have been shown to reproduce in water with no added nutrients (von Reyn *et al.* 1994). Several investigators have recovered these organisms from hospital water systems (Carson *et al.* 1988; duMoulin *et al.* 1988; von Reyn *et al.* 1993) in numbers ranging from 1–10 000 CFU per 100 ml water. The source of these bacteria is believed to be shed biofilm fragments. This is substantiated by the observation by Schultz-Robbecke *et al.* (1989, 1992) that 45 of 50 biofilms removed from municipal or domestic water sources contained mycobacteria. Another adaptive mechanism for

survival in drinking water is their ability to tolerate disinfection by chlorine (Haas *et al.* 1983; Carson *et al.* 1978; Collins *et al.* 1984). A recently completed study found that *Mycobacterium* spp. were present in five of eight biofilm samples grown in conventionally treated water. These numbers could be reduced if the water was depleted in organic carbon or if a free chlorine residual was maintained (LeChevallier *et al.* 1998).

The MAC is of sufficient public health concern that they are one of the top three bacterial 'emerging pathogens' in the drinking water industry. Consequently, they have been added to the U.S. Environmental Protection Agency's (EPA) Contaminant Candidate list, which identifies organisms of concern worthy of further research for risk assessment. These organisms are also candidates for possible regulation by the EPA.

In distribution systems, *Legionella* is believed to be most prevalent in biofilms, since it depends on other organisms for growth-supporting substances (Wadowsy and Yee 1983, 1985; Stout *et al.* 1985; Rogers *et al.* 1994). These organisms colonize and grow in water heaters, shower heads and cooling towers, where their release can lead to respiratory disease in sensitive individuals. A review paper by Lin *et al.* (1998) suggests that hospitals take routine samples for the organism in their distribution system and the efficacy of any disinfection processes be monitored before and after analysis for the presence of *Legionella*.

The pseudomonads are ubiquitous in nature. In most cases they are benign, but have been associated with infections in burn patients, eye infections, and in the special case of cystic fibrosis.

Frank Pathogens

The third subset of bacteria are the frank pathogens. These are organisms known to be associated with waterborne disease outbreaks and include the genera *Salmonella, Shigella, Escherichia, Campylobacter* and *Vibrio*. It is generally believed that these organisms do not last long outside of their hosts and are easily disinfected. To colonize and persist in distribution systems, the pathogens must be able to successfully compete with the heterotrophic bacterial populations. Competition with the existing microflora may be a key parameter in preventing proliferation of pathogenic organisms. In research using laboratory columns containing granular activated carbon (GAC), Camper *et al.* (1985) found that a suite of pathogenic bacteria could survive on GAC when fed a sterile source of surface water. However, if the pathogens were challenged with organisms present in unsterilized surface water, the numbers of pathogens declined. If pathogens were added to the carbon simultaneously with the autochthonous heterotrophs, they declined more rapidly than in the first instance. Finally, if pathogens were added to previously colonized GAC, the decline

of pathogens in the filter and filter effluent was the most rapid. In recently completed experiments using groundwater and laboratory columns with virgin GAC, GAC that had been in operation in a filter for a few months and biologically activated carbon (BAC) from a full-scale plant, coliform elimination was the most rapid from the BAC filter (LeChevallier *et al.* 1998). Other laboratory studies (Rollinger and Dott 1987) with several pathogens on GAC reported similar results. The pathogens persisted when introduced to sterile GAC and fed sterile water, but were eliminated from the medium when they were subsequently challenged by autochthonous bacteria from tap water. It should be noted that GAC filter effluents have been found to contain GAC particles colonized with a variety of potential pathogens including *Escherichia coli*, *Klebsiella pneumoniae* and *Aeromonas hydrophila* (Brewer and Carmichael 1979; Tobin *et al.* 1981a; Camper *et al.* 1986).

Salmonella is well recognized as a pathogenic organism of faecal origin. It therefore should not be present in a well-run potable water distribution system. If found in water, it is regarded as more a case of survival rather than proliferation of the organism.

With the exception of the very large outbreak in Riverside, California, there seems to be a level of about 1–100 cases per year of waterborne salmonellosis in the U.S. (Craun 1986). There is considerable potential for non-detection, given phenomena such as viable non culturable (VBNC) cells with *Salmonella*. In an investigation with *Salmonella enteritidis* in river water microcosms (Roszak *et al.* 1983), salmonellae rapidly became non culturable. This was initially reversible following nutrient addition, but after 3 weeks resuscitation failed to give culturable cells, although the cells remained viable. Therefore, even if water during a disease outbreak tests negative for salmonellae, there remains the possibility of transmission by VBNC cells.

The importance of attachment to environmental surfaces in the survival of this organism has been previously investigated. Camper *et al.* (1985) found *Salmonella* readily colonized and persisted on GAC in water, although attachment was at a lower rate and the organism decreased in numbers more rapidly in the presence of other heterotrophic bacteria. Suspended pathogenic cells died away faster than attached cells. We have completed experiments where *S. typhimurium* was able to colonize simulated drinking water distribution system biofilms and remain detectable by fluorescent antibodies for up to two months. Some of these organisms could be cultured after recovery in a non-selective medium (Warnecke 1996). However, there is no information available on the virulence of these bacteria.

Some strains of *E. coli* such as the enterohemorrhagic O157:H7 strain (ECO157) have a demonstrated pathogenicity to humans. This strain has several physiological differences from typical isolates, including a lack of

the enzyme β-glucuronidase (often used for *E. coli* detection) and poor or no growth at 45° C (Rice *et al.* 1992). Therefore, this strain is non-detectable by standard methods used for the routine detection of *E. coli*. It has been shown to persist at a similar rate as typical *E. coli* strains under drinking water conditions, confirming the premise that typical *E. coli* strains would be effective in indicating ECO157 presence (Rice *et al.* 1992). However, this study did not account for differential survival on particulates or in biofilms. Survival on surfaces has been shown to be the major long-term persistence mechanism of *E. coli* in lake waters (Brettar and Höfle 1992). Pathogenic and non-pathogenic *E. coli* strains have been shown to have similar growth rates to an environmental isolate of *E. coli* under growth conditions relevant to drinking water distribution systems (Camper *et al.* 1991). Colonization by an environmental *E. coli* isolate of a pre-existing biofilm has been demonstrated under water distribution system conditions (Robinson *et al.* 1995). In addition, this organism was shown to persist in a model distribution system biofilm for over 12 days (Block 1992) and for at least 21 days in another system even in the presence of 0.3 mg L^{-1} monochloramine (Colbourne *et al.* 1991). A faecal origin, non-benzoate degrading *E. coli* has been shown to be able to colonize a reactor containing a biofilm of benzoate degrading bacteria and subsequently re-enter the water phase. In this study, 5 mM benzoate was the sole carbon source, demonstrating consortial feeding by this organism (Szewzyk *et al.* 1994). The same investigator had also demonstrated that a pathogenic strain of *E. coli* could colonize a single species biofilm (Szewzyk and Manz 1992). Studies in our laboratories have shown that *E. coli* O157:H7 did not persist for over one week in a mixed-population biofilm grown under drinking water conditions (Warnecke 1996).

VIRUSES AND PROTOZOANS (*GIARDIA* AND *CRYPTOSPORIDIUM*)

These two types of pathogens have been grouped by the characteristic that they do not have the ability to grow in the distribution system. In the case of bacteria, there is always the chance that a pathogen can colonize and grow in a biofilm, while this is not possible for the pathogenic viruses and the specific protozoans listed above. Virus and cyst/oocyst interactions with biofilms would be more similar to that of particles.

There are several groups of viruses believed to be of importance in waterborne outbreaks, although no information exists in the open literature as to their potential presence or survival in biofilms. The enteroviruses, including those that cause polio, can be very resistant to disinfection and there have been unsubstantiated reports that a polio outbreak was

attributed to a drinking water source (Mosley 1966). More information on these viruses in potable water is being collected under the Information Collection Rule in the United States. Hepatitis A virus (HAV) can survive for more than four months in water (Sobsey *et al.* 1988) and has been identified as the causative agent in more than 20% of waterborne disease outbreaks in the U.S. where the aetiological agent has been determined (Lippy and Waltrip 1984). However, it should be noted that many of these outbreaks were associated with groundwater rather than surface water sources. Although the virus is sensitive to disinfectants, it has been found in samples with a free chlorine residual of 0.2 mg/L (Bosch *et al.* 1991). Again, no direct evidence for a biofilm/virus interaction presently exists.

Giardia and *Cryptosporidium* cysts and oocysts are notoriously resistant to disinfection and can survive for extended periods of time in cold waters. As in the case for the viruses, there is no documented evidence that these organisms have accumulated in distribution systems and subsequently caused disease. There is some evidence suggesting that protozoan cysts and oocysts can associate and remain in laboratory grown biofilms for several months, but there is no evidence that these organisms are still virulent (Rogers and Keevil 1995). Practical experience indicates that once the water carrying the cysts/oocysts starts to leave the system, there are decreasing instances of disease. There are no reported instances where there has been a reoccurrence of a protozoan disease outbreak in the same distribution system once the original source of the organism was removed or corrected. This may provide indirect evidence that the cysts (i) do not accumulate in numbers sufficient to cause disease if biofilm is sloughed from the surface or (ii) lose infectivity and virulence with time even if they do accumulate.

MANAGEMENT OF BIOFILMS IN DISTRIBUTION SYSTEMS

Practical experience has shown that there are a variety of water quality parameters that tend to support biofilm growth in drinking water systems. These include temperature, the concentration and type of organic carbon and disinfectant and the presence or absence of a corrosion control regime when corrodible materials are used in the distribution system. Many of these interactions have been supported in laboratory studies or at the pilot scale using experimental conditions similar to those in full-scale distribution systems. However, there are still conflicting and confusing aspects to this work. Some researchers have shown that the concentration of organic carbon measured as assimilable organic carbon (AOC) or biodegradable organic carbon (BDOC) influences biofilm cell numbers, while others have less conclusive results. In fact, biofilms are known to occur in ultrapure

water stainless steel distribution systems where organic levels should be extremely low. There are also conflicting results on the ability of chlorine or monochloramine to limit biofilm proliferation. In many cases, the findings appear to be system specific and indicate the complexity of the response of the biofilm to an interrelated set of environmental conditions. One general observation can be made; biofilms will not be eliminated from distribution systems by any of the current methods available now or in the future. Our primary challenge is to control rather than eradicate biofilms from distribution systems.

Reducing Organic Levels

Reducing organic carbon concentrations in treatment is gaining favour as an acceptable option for reducing biofilm growth, controlling the formation of disinfection by-products and reducing disinfectant demand. As regulations become increasingly more stringent, the relative economic gains from organic removal are increasing.

There are a variety of means for reducing organic matter in drinking water. Although it is beyond the scope of this chapter to provide detailed descriptions of the processes, a list of technologies is provided. These include changing the source water, enhanced coagulation, activated carbon adsorption, membrane filtration and biological filtration. The choices available to a specific utility will be limited by water quality and physical and economic resources. Regardless of the type of treatment, a reduction in the amount of organic carbon can potentially reduce the amount of biofilm development in the distribution system.

Attempts have been made to define potential threshold concentrations of organic carbon that limit biofilm growth. van der Kooij (1992) has suggested an AOC level of 10 μg C l^{-1} for heterotrophs, while LeChevallier *et al.* (1991) recommended a level of 50 μg C l^{-1} for coliform control. In terms of BDOC, Servais *et al.* (1991) have associated biological stability of water with a level of 0.2 mg l^{-1}, although, Joret (1994) has stated these levels are temperature dependent (0.15 mg l^{-1} at 20° C and 0.30 mg l^{-1} at 15° C). These numbers have been used with some success as design criteria in European systems where the confounding influence of high levels of secondary disinfectant is not present. There are instances where there has not been a clear association between AOC/BDOC and biofilm development. For example, Kerneis *et al.* (1994) reported that there was no correlation between distribution system BDOC measurements, suspended bacteria and fixed biomass. Conflicting information was also obtained in field studies; regrowth was seen in systems with average AOC levels both greater than and less than 100 μg L^{-1} (LeChevallier *et al.* 1996a). In experiments where we have used mild steel pipe loops and annular reactors

there was a weak correlation between biofilm and influent AOC concentrations, but no correlation with the concentration of AOC in the reactors (Camper 1996). These results suggest that utilizable organic matter as a sole parameter in determining the potential for a water to support biofilm is not always appropriate.

Optimizing Disinfection

An explanation for the lack of consistent correlation between utilizable organic matter and biofilm formation is the presence of a disinfectant. There appear to be interactions between the organic matter, the pipe surface and the biofilm that influence the number of organisms present. It is generally believed that increasing the concentration of a disinfectant should control regrowth, but many instances exist where the opposite effect is seen (LeChevallier *et al.* 1987; Martin *et al.* 1982; Reilly and Kippen 1984; Oliveri *et al.* 1985; CDC 1985; Hudson *et al.* 1983). When distribution system biofilms were examined, no correlation was found between free chlorine residuals and the number of heterotrophic plate count (HPC) organisms per unit surface area (Hudson *et al.* 1983). Many reports exist that demonstrate the relative lack of sensitivity of biofilm cells to disinfectants. This effect is even more pronounced if the biofilms are grown on reactive iron surfaces (Kerneis *et al.* 1994; LeChevallier *et al.* 1990; Chen *et al.* 1993). It has been noted that increased corrosion rates decrease the efficacy of free chlorine against biofilm organisms (LeChevallier *et al.* 1993).

As a result of changing drinking water regulations and an increased emphasis on the presence of disinfection by-products, utilities may use monochloramine as a secondary disinfectant. In the past, monochloramine was not viewed favourably because of its high CT requirement as compared to chlorine, but an interesting beneficial effect on biofilms was noted. In a distribution system comparison, chloramines were more effective at reducing the number of biofilm total coliforms and HPC than chlorine (Neden *et al.* 1992). In another study where statistical evaluation of the influence of chloramine concentration on attached microbial populations in a distribution system was made, an inverse relationship was established (Donlan and Pipes 1988). A study in a model pipe loop system composed of several materials showed that biofilms on galvanized steel, copper, or polyvinylchloride (PVC) surfaces were readily disinfected by free chlorine or monochloramine (1 mg l^{-1}), while iron pipe surface-associated bacteria were more susceptible to monochloramine than free chlorine (4 mg l^{-1}) (LeChevallier *et al.* 1990). A field study of 31 utilities showed systems that used chloramines had 0.51% coliform positives in 35 159 water samples as compared to 0.97% of 33 196 samples in chlorinated systems. These same data showed that the average density of coliforms in the water of the

chlorinated systems was 35 times higher than in the chloraminated systems (LeChevallier *et al.* 1996b).

Corrosion Control

An interesting recent development in biofilm management occurred when utilities implemented corrosion control to comply with the Lead and Copper Rule. Anecdotal reports both of decreased bacterial numbers and increased disinfectant residual have been given, especially if the distribution system has a substantial amount of unlined iron-containing pipe. Iron pipes have been implicated as a key component in microbial regrowth in distribution systems (Camper 1996; Camper *et al.* 1996; LeChevallier *et al.* 1996b; LeChevallier *et al.* 1993). This has been supported by observations that utilities with a large proportion of unlined ferrous metal pipes are more prone to coliform regrowth. A utility survey has also shown a positive relationship between the number of miles of unlined metal pipes and coliform occurrences (LeChevallier, *et al.* 1996b). Pilot system experiments have supported the interaction between corroding iron pipes and biofilms. Neden *et al.* (1992) found that bacterial populations on unlined cast iron were the highest, while PVC was colonized with the lowest number and Block (1992) determined that there was a progressive decrease in bacterial densities on surfaces from cast iron, tinned iron, cement lined cast, to stainless steel. The influence of iron pipes can be quite dramatic, with one experiment demonstrating a >100 fold increase in biofilm cell numbers on iron compared to PVC surfaces (LeChevallier *et al.* 1998). It has also been demonstrated that biofilms on ferrous metal surfaces were less susceptible to free chlorine than biofilms on inert materials, (LeChevallier *et al.* 1987, 1988, 1990), even in the presence of measurable chlorine residuals (LeChevallier *et al.* 1998). This may be because the metal exerts a chlorine demand. The ability for reduced iron to react with disinfectants has been documented (Knocke 1988; Knocke *et al.* 1994; Vasconcelos *et al.* 1996). Research in our laboratories has confirmed that pipe material has a dramatic impact on biofilm cell numbers. Mild steel surfaces were consistently colonized by nearly ten-fold more heterotrophs and two to ten-fold more coliforms than polycarbonate. The effect was also seen in effluent cell counts. Interestingly, the presence of a small amount of mild steel (10% on the basis of surface area) in an otherwise polycarbonate reactor resulted in elevated biofilm counts on all surfaces; the plastic surfaces supported the same numbers of bacteria as seen on the steel itself (Camper *et al.* 1996).

As stated above, there is evidence to demonstrate that corrosion control has mitigated coliform regrowth in full scale systems (Hudson *et al.* 1983; Lowther and Moser 1984; Martin *et al.* 1982; Schreppel *et al.*

1997). It is unknown if the reason is improved disinfection, reduced attachment sites for bacteria or other factors. LeChevallier *et al.* (1993) showed that corrosion control reduced biofilm numbers but attributed the response to increased chlorine efficacy due to decreased corrosion rates. This may not be the case if the corrosion control scheme is an increase in pH, since chlorine speciation and disinfection is adversely influenced by elevated pH. However, Martin *et al.* (1982) showed that increasing the pH to 9 in a chlorinated system actually reduced bacterial counts. In this case it may be inferred that corrosion control superseded the effects of reduced disinfection efficacy. We have noted that at near neutral pH in the absence of corrosion control, the presence of low levels of disinfectant actually increases biofilm density on ductile or steel surfaces. This is presumably because corrosion was enhanced and the disinfectant consumed at the surface (Camper 1996; Abernathy 1998). Recently completed laboratory and pilot work has shown that the number of biofilm bacteria is directly related to the mass of corrosion products present; reduction in biofilm and corrosion product accumulation can be achieved by corrosion control schemes or by changing from chlorine to monochloramine to produce less corrosion (Abernathy 1998). In another pilot experiment, the results indicated that corrosion control was more important in reducing bacterial numbers in biofilms than decreasing the amount of BDOC in the water or the maintenance of a free chlorine residual (LeChevallier *et al.* 1998).

MONITORING AND TESTING FOR BIOFILMS IN DISTRIBUTION SYSTEMS

In general, access to full-scale distribution systems for biofilm samples is extremely limited. As a consequence, it is unlikely that a utility can obtain much information on biofouling directly from pipe sections. Because of this limitation, the tendency is to infer biofilm responses based on easily obtained water samples. There are problems with this approach. If a disinfectant is present, the detached biofilm cells in the water may be reduced, giving the false indication that the biofilms are also controlled. There are no acceptable methods for directly correlating numbers of bacteria in the water with the number in biofilms. Because of these limitations, water distribution personnel may wish to use a side-stream or pilot device to obtain biofilm samples. It should be noted that these devices are appropriate for providing data on trends in biofouling, but it is not appropriate to infer actual numbers of cells in distribution systems from those obtained in side-stream or pilot devices.

Monitoring Devices; Pipe Loops and Reactors

Pilot-scale distribution facilities are typically constructed to provide a platform for determining the influence of water quality parameters on full-scale distribution system performance. This is required when access to the full scale system is limited, when the parameters to be tested may significantly alter the quality of water delivered to the consumer, or if more tightly controlled conditions are required (flow, water quality, pipe composition, etc.). Since pilot system results are extrapolated to the distribution system, it is critical that the reactors be designed to simulate relevant operational conditions, including shear stress, pipe materials, temperature, disinfectant types and concentrations and other water quality parameters such as organic carbon concentrations. Coupons for sampling surfaces should be flush mounted to reduce perturbations in the local hydrodynamics. Ideally, the reactors should be relatively compact, easily controlled, have minimal water demand and be inexpensive to construct, maintain and operate. An additional consideration is the manner in which the pilot system can be mathematically modelled, i.e. either plug flow or completely mixed reactor behaviour.

Historically, pilot systems have been designed to provide information on corrosion (Levin and Schock 1991; Gardels and Sorg 1989; Heumann 1989; Birden *et al.* 1985; AWWA 1985) and more recently to investigate biological processes in distribution systems (LeChevallier *et al.* 1990; Haudidier *et al.* 1988; Camper 1996). For example, the pipe loop at Nancy in France was designed so that experiments to examine biofilm accumulation and detachment of bacteria, disinfectant decay and efficacy against biofilm and suspended organisms and disinfection by-product formation are possible (Haudidier *et al.* 1988).

As an alternative to pipe loops, Characklis (1988) and van der Wende and Characklis (1990) utilized annular reactors in series to simulate the hydraulic conditions found in a distribution system. Annular reactors consist of a stationary outer cylinder with flush mounted coupons for surface analyses and a rotating inner cylinder with an annular space occupied by the water. Rotation of the inner drum controls shear stress, which can be scaled to that found in a circular pipe. Annular reactors are also well-mixed, and when staged, the entire series can simulate long residence times typical of municipal systems.

These reactors have been used by a number of utilities as side-stream devices in treatment plants and distribution systems and to provide advance information about proposed changes in treatment or disinfection practices. For example, a utility may choose to pilot corrosion control in two reactors. One would receive water as it is currently treated and the other would receive water amended with the proposed corrosion control scheme.

Direct comparisons on biofouling between the two systems could then be obtained.

Researchers at the University of Nancy in France have developed a reactor called the Propella® with design criteria somewhat similar to that of the annular reactor. The Propella® has a stationary outer cylinder made of pipe material with removable coupons. There is an inner cylinder with a propeller at the top that forces water down through the inner cylinder, out the bottom and along the inside of the outer cylinder to simulate flow conditions in a distribution system.

Analysis of Biofilm Samples

Culturing

Traditionally, environmental bacteria have been studied by culture-dependent methods. For biofilm samples, the cells are scraped from the surface, dispersed by methods such as homogenization and the bacteria enumerated on a variety of media. Optimal results for heterotrophic bacteria can be obtained by using the spread plate method on R2A agar incubated for one week at room temperature. Other culturing methods can be used if specific subsets of bacteria are being targeted.

It is now well established that cultural methods underestimate the numbers and diversity of environmental bacteria. For example, Wagner *et al.* (1993) found that viable plate counts of bacteria from activated sludge were about 1% of direct microscopic counts. Regardless of the shortcomings of culturing methods, they still have their value. They have regulatory significance since most contaminant levels are based on culturable counts. There are some types of samples, including those with very high concentrations of corrosion products and detritus, that are not amenable to any other type of enumeration method currently available. In this case, culturing using optimal recovery methods provides the best estimate of bacterial numbers.

Nucleic Acid Stains and Physiological Indicators

If clean samples are available, direct microscopy with a variety of nucleic acid and physiological probes can be used to assess the number and activity of biofilm cells. These stains can be used on intact or dispersed (scraped and homogenized) biofilm samples.

Overviews of the use of fluorochrome staining and direct microscopic observation of bacterial cells have been prepared by McFeters *et al.* (1995) and Kepner and Pratt (1994). They and others have noted the general use of the nucleic acid stains acridine orange (AO) and 4',6-diamidino-2-phenylindole

(DAPI) for obtaining total cells counts. Physiological stains vary from those that measure membrane integrity to those that detect specific metabolic functions. Each fluorochrome has its advantages, although it is recommended that a suite of fluorochromes be used to assess overall activity. For example, Yu *et al.* (1994) reported that 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) and rhodamine 123 (Rh 123) were effective indicators of metabolically active cells in biofilms, but Morin and Camper (1997) reported a lack of sensitivity with CTC in chlorinated biofilms.

Nucleic acid stains and physiological fluorochromes do not identify species but may be used in conjunction with specific antibodies or nucleic acid probes, provided the emission spectra of the different fluorochromes are dissimilar enough to allow separate detection of each component. Hicks *et al.* (1992) combined DAPI for total cell counts with a specific oligonucleotide probe labelled with tetramethyl rhodamine isothiocyanate (TRITC). Pyle *et al.* (1995) used CTC and a fluorescent antibody for *E. coli* O157:H7 in the same assay to detect actively respiring cells.

Fluorescent Antibodies

In specific instances, fluorescent antibodies have been shown to be effective at targeting bacterial cells in intact or dispersed biofilms. Rogers and Keevil (1992) have used fluorescent antibodies to target *Legionella pneumophila* in thin, laboratory grown biofilms. A similar approach has been taken by Szwerinski *et al.* (1985) to identify nitrifying bacteria in thick biofilms. We have used fluorescent antibodies to target *Klebsiella pneumoniae* in mixed species biofilms. Experience has shown that this method is best applied in relatively clean samples and is probably inappropriate for most distribution system samples due to nonspecific binding of the antibody, interference of inorganic matter and difficulty in ascertaining penetration of the biofilm by the antibody.

Molecular Probes in Biofilm Research

Increasingly, environmental microorganisms, including those in biofilms, are detected by so-called 'molecular' methods. A broad interpretation of the term includes methods that do not require bacterial growth on selective or non-selective media. A narrow interpretation is limited to methods that target nucleic acids or proteins. Either whole cells or purified cell extracts of DNA, RNA, or proteins can be analysed. Whole cells can be extracted and/or concentrated from samples or analysed *in situ*. *In situ* detection of undisturbed whole cells, especially in biofilms, is essential in order to determine the spatial distribution of species. A list of molecular tools includes nucleic acid and protein stains, physiological indicators, labelled

antibodies, nucleic acid amplification, nucleic acid probes and gel electrophoresis of nucleic acids and proteins. An integrated molecular approach to studying planktonic or biofilm bacteria would include methods for determining their identity, abundance, and physiological status in single or parallel assays.

Molecular probes are gaining popularity in drinking water biofilm studies. Because of current limitations in their practical application there are still few reports of successful use in these samples, and routine use by the industry is still not feasible. Szewzyk *et al.* (1994) suggested that oligonucleotide probes can penetrate biofilms found in oligotrophic environments, while labelled antibodies may only detect target cells on the biofilm surface. Manz *et al.* (1993) inserted glass slides into a Robbins device installed in a drinking water distribution system and noted microcolony formation with phase contrast microscopy within 3–8 weeks. The biofilms were hybridized with oligonucleotide probes and subsequently stained with DAPI. The universal probe (EUB338) was detected in about 70% of the attached cells but only about 40% of the planktonic cells, based on DAPI total counts. This disparity was considered evidence of higher rRNA content of attached cells.

FUTURE DIRECTIONS

The drinking water industry is driven by two major groups, the regulators and the customers. Complying with certain regulations can have a negative effect on the ability of water utilities to comply with other regulations and can impact the public's perception of the water. For example, increasing chlorine concentrations to decrease microbial activity in distribution systems can increase disinfection by-products (a regulatory concern) and result in complaints from customers. To ensure safe, palatable water at an economical cost, it will be necessary to obtain fundamental information on the interaction of a variety of water quality and distribution system parameters.

In the area of microbiology, adaptation and implementation of the suite of new tools being developed in the area of molecular microbial ecology will provide valuable insight on the factors that control biofilm formation in distribution systems. Of key importance will be direct targeting of potentially pathogenic bacteria so that relevant measures of the safety of water can be obtained.

Connected with improved microbiological monitoring methods is the need for sound risk assessment and epidemiological studies on the health effects of a variety of waterborne and water associated organisms. When this information is available, sound decisions on the appropriate means for

ensuring microbiologically and chemically safe water at a reasonable cost can be made.

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