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18 Biofilms in Drinking Water Treatment and Distribution

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The low nutrient environment present in drinking water treatment plants and distribution systems would not appear to be a hospitable environment for bacterial growth. However, biofilms are found on almost every submerged surface in treatment plants and distribution systems. In treatment, biofilms can be used to reduce the concentration of organic matter that forms disinfection by-products as well as produce biologically stable water that reduces biofilm growth in distribution systems. Biological filters do not appear to support the growth of pathogenic bacteria. Distribution system biofilms are deleterious, in that they can release indicator organisms, and heterotrophic bacteria, and may cause taste and odor problems. Control of these biofilms is difficult. Disinfection alone is usually ineffective. Reduction of organic matter, improved disinfection, and the implementation of corrosion control if unlined iron pipes are present, or a combination of these methods is helpful in controlling distribution system biofilms.

KEY WORDS: biofilms, biological treatment, drinking water, distribution systems

INTRODUCTION

Biofilms occur universally on submerged surfaces in drinking water treatment plants and in distribution systems (Figure 1). Their activity and presence is usually considered to be undesirable, leading to deleterious changes in water quality. Such biofilms are influenced by system operating parameters and environmental variables such as water quality, water demand and the types of surfaces present.

Because biofilms may release organisms of regulatory concern into the water, the industry has been very interested in understanding mechanisms for controlling biofilm processes in distribution systems. The belief has been that all organisms in drinking water are undesirable, and treatment methods have been optimized to reduce the presence of organisms to the lowest levels possible. However, increasing disinfectant concentrations or contact times to control microbial activity has become increasingly difficult due to regulations governing the concentrations of disinfection by-products in water. For this reason, the option of using biofilms in a beneficial manner in drinking water treatment is now gaining acceptance. Since biofilms will inevitably develop in water systems, it is better to design a treatment process where they can

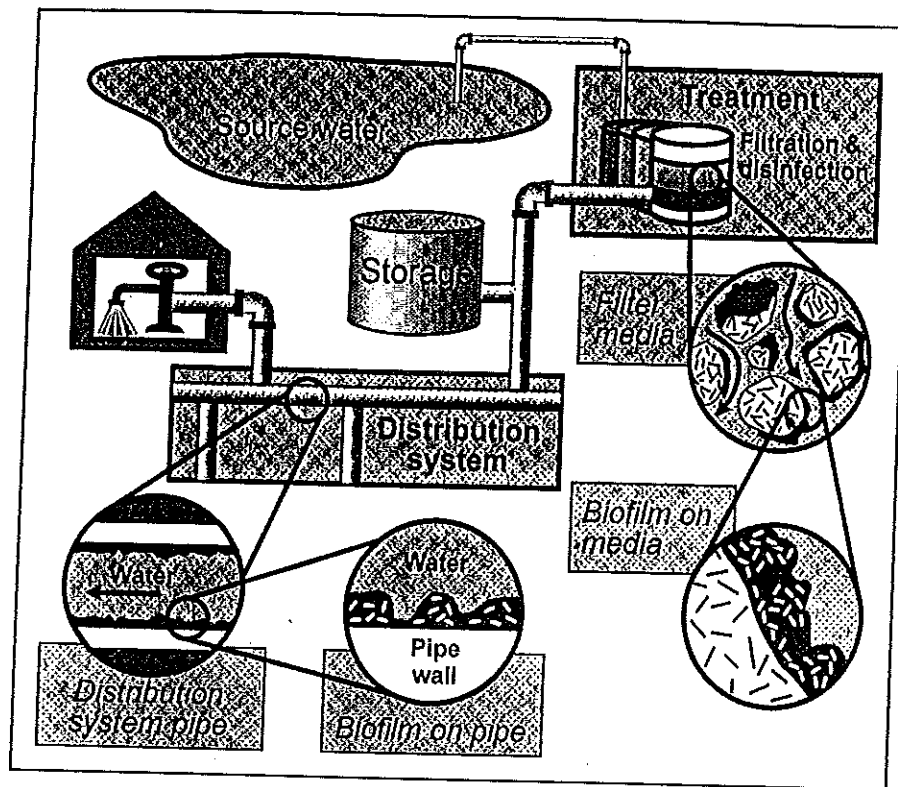


Figure 1 Schematic denoting relevant areas of biofilm activity in drinking water treatment and distribution. Biological treatment involves the encouragement of biofilm growth on filter media. Filtration may be preceded by ozonation to improve removal of organic carbon. The filter media may be anthracite, sand or granular activated carbon. Colonized filter media can be released from the filter and enter the distribution system. A disinfectant is added downstream of the filters before or as the water enters the distribution system to reduce the load of organisms and help mitigate contamination events. Regardless of the level of treatment or the type of disinfectant used, biofilms will grow on pipe surfaces. Note the relatively reduced level of biofilm thickness on the pipe surface compared to that on the filter media. The type of pipe material, the concentration and composition of the disinfectant, corrosion control practices as well as reduction of the organic matter all have the potential to influence the amount of biofilm present on pipe surfaces.

be positively manipulated rather than allow them to cause problems downstream where control is expensive or impossible.

Biological treatment utilizes natural bacterial populations to reduce the organic matter in water that would contribute to both disinfection by-product formation and downstream biofilm formation in distribution systems. The concept of biological treatment has been gaining favor, with a considerable amount of research being done to optimize performance and to address the potential for filters to harbor organisms of public health concern.

BIOFILMS IN WATER TREATMENT

Almost all filters in drinking water treatment have some level of biological activity. In the past, this activity has been viewed as undesirable because the organisms are released into the water and may cause increased microbiological counts. Therefore, typical water treatment plant operation has included practices intended to minimize biofilm growth on the filters, including carrying a disinfectant residual onto the filters and using chlorinated backwash water. With increasingly stringent regulations on the presence of disinfection by-products and the growth of biofilms in distribution systems, the potential to enhance biological activity in filters to improve water quality has gained favor.

Drinking water that has been subjected to microbial activity in a controlled manner in a treatment plant is more "biologically stable" or less likely to contribute to microbial proliferation in the distribution system (Cipparone *et al.*, 1997). Biologically treated water typically has lower disinfectant demand and disinfection by-product formation potential than conventionally treated water if the source water is high in organic carbon (Huck and Anderson, 1992; Cipparone *et al.*, 1997). As utilities move to using ozone as a primary disinfectant and for taste/odor/color control, biological filters may be necessary to reduce the concentrations of biodegradable organic carbon entering the distribution system. In some regards, slow sand filtration represents biological treatment, but the term typically refers to the optimization of biofilm activity on rapid rate filters.

Designed and engineered drinking water biological treatment was first implemented in France and other western European countries nearly 20 years ago (Sontheimer *et al.*, 1978; 1979a; 1979b). In the most traditional form, separate granular activated carbon (GAC) filters are located downstream from conventional treatment. In conventional treatment, particle removal is optimized through coagulation, flocculation, sedimentation, and filtration. The water is then ozonated and passed through filters that are optimized for microbial utilization of a portion of the natural organic matter remaining in the water. Biological filters are typically operated with exhausted carbon, that is, the chemisorptive capacity of the GAC has been exceeded. The surfaces of the filter media act as supports for microbial attachment and growth, resulting in a biofilm adapted to using the organic matter found in that particular water. Total organic carbon removals in these filters range from 5 to 75% (Bouwer and Crowe, 1988).

The discussion presented below focuses primarily on secondary biological treatment, *i.e.* biological filters after conventional filtration. However, as will be seen in the last section on this topic, there is a growing interest in using a single filter for both particle removal and biological filtration. Dual operation presents its own unique set of considerations for operation and performance, but many of the principals used to design and operate second stage filters are applicable to single filters also.

Ozonation

A common treatment step before biological treatment is ozonation. Ozone may be applied to reduce taste and odor compounds, remove color, provide primary disin-

fection for protozoan cysts, or to reduce disinfection demand/disinfection by-products by oxidizing some of the organic matter. Water that has been preozonated often has elevated levels of lower molecular weight organic compounds; these compounds have been associated with increased biofilm development downstream (van der Kooij *et al.*, 1989; Price, 1994; LeChevallier *et al.*, 1996b). Goel *et al.* (1995) reported that the fraction of recalcitrant natural organic matter in water made available for microbial growth was increased after ozonation, but the numerical value varied from site to site. This has also been substantiated by van der Kooij *et al.* (1982), Werner and Hamsch (1986), Servais *et al.* (1987) and Speitel *et al.* (1993). Because biofilms can form either in controlled treatment processes (biological filters) or in uncontrolled deleterious locations (distribution systems), the drinking water industry considers biological filtration after ozonation, regardless of the original intent of ozone application.

Importance of Filter Media

GAC vs other media

The selection of filter media (sand, anthracite or GAC) for use in biological filters has been of interest primarily due to capital cost and performance issues. Sand and anthracite are usually less expensive than GAC, but it is important to optimize biological activity and the resultant performance of the filter in terms of net removal of organic carbon from the water. For instance, although rapid sand filters do have the capacity to biologically remove carbon (Eberhardt *et al.*, 1977; Sontheimer *et al.*, 1978; Borbiogot *et al.*, 1982; van der Kooij and Hijnen, 1985) it has been found that GAC typically has superior performance (LeChevallier *et al.*, 1992; *c.f.* DeWaters and DiGiano, 1990 and Hozalski *et al.*, 1995). This is presumed to be the result of the higher amount of biomass that attaches to GAC *vs* anthracite (Niquette *et al.*, 1998). LeChevallier *et al.* (1992) demonstrated that there were more bacteria per unit surface area on GAC than on sand, and that the total organic carbon (TOC) removal rate was 51% *vs* 26%. Another advantage of GAC over other media is that the attached microbial population is less prone to shock from changes in water quality, down time, or accidental application of disinfectant (Bablon *et al.*, 1988; Krasner *et al.*, 1993).

Iron oxide coated sand

Even though the emphasis in drinking water has been on the use of GAC, there is evidence to suggest that iron oxide coated media may be a better choice for removal of natural organic matter (Jacangelo *et al.*, 1995; Owen *et al.*, 1995). Iron oxides have a large potential for the sorption of natural organic matter (McCarthy *et al.*, 1993; Parfitt *et al.*, 1977; Zhou *et al.*, 1994). Under abiotic conditions, humic material is irreversibly held on the surface of iron oxides (Gu *et al.*, 1994; 1996). This property has been used to develop a technique for the removal of natural organic matter (NOM) from water by coating sand particles used in slow sand filter beds with iron oxides (McMeen and Benjamin, 1997). Circumstantial evidence indicates that the bound organic matter is potentially available for biofilm bacteria, since these same investigators stated that the iron oxide-coated olivine used in their filtration studies

continued to remove NOM for a 16 month time period; they suggested that the adsorption sites were being "bioregenerated."

Importance of Operational Conditions

There are at least three operational conditions that have been shown to be important in the performance of biological filters, *viz.* empty bed contact time (EBCT), temperature, and backwash conditions. The EBCT is the residence time of the fluid in the filter calculated as though the entire volume occupied by the filter medium is occupied by water. Both temperature and EBCT have an impact on the amount of organic carbon removal. In the first case, longer contact times allow for longer potential reaction times. Temperature influences the rate at which the reactions occur. If temperatures are higher, the contact time needed to achieve a specific removal can be reduced. Operationally, the contact time can be adjusted, but temperature is extremely difficult if not impossible to control. Backwash strategies will influence the amount of biofilm left on the filter media and therefore impact the organic carbon removal efficiencies after the filter is put back on line.

Empty bed contact time

In most cases, TOC removal in biological filters can be improved by increasing the EBCT. Because of the very large volumes treated by a drinking water plant, a small reduction in EBCT results in substantial savings in filter volume. Experimental EBCTs in biological filters have varied from 2 to 30 min. Sontheimer and Hubel (1987) demonstrated an increase in dissolved organic carbon removal from 27 to 41% when the EBCT increased from 5 to 20 min. LeChevallier *et al.* (1992) reported a 29% removal of TOC with a 5 min EBCT and a 51.5% reduction at 20 min. Prevost *et al.* (1990) suggest that a 20 min EBCT is required for 90% removal of biodegradable organic carbon. However, there are instances where increased EBCT is not beneficial, which is probably a result of the biodegradability of the organic matter present in the water (Hozalski *et al.*, 1995).

Temperature

As stated above, the EBCT required for biological removal of TOC will be temperature dependent. This has been demonstrated at a full-scale biological filtration plant, where 12 min of EBCT was required at 0.5°C for the same percentage removal obtained in 6 min at 10–12°C (Niquette *et al.*, 1998). The importance of temperature has been demonstrated for the removal of biodegradable organic matter as well as for the removal of specific ozonation by-products (Krasner *et al.*, 1993; Coffey *et al.*, 1995).

Backwashing

Optimizing backwash for biological filters may be different than for conventional particle removal filters. The backwash cycle must be designed so that biomass loss

is balanced with the need for decreased headloss and the control of undesirable organisms (*e.g.* nematodes) in the filter.

DiGiano (1992), Miltner *et al.* (1992) and Ahmad *et al.* (1998) evaluated the impact of chlorinated vs non-chlorinated backwash water on the performance of filters. In all cases, filters backwashed with chlorinated water had lower biomass and in general effluent quality suffered.

The mode of backwash may have an effect on biomass removal and subsequent removal of organic carbon. In a model system, Hozalski and Bouwer (1998) demonstrated that water wash without a disinfectant removed only 20–40% of the biomass from filter media, and the retained biomass was capable of maintaining good biological removal of TOC. In pilot plant studies, Niquette *et al.* (1998) showed that filter performance of biologically active carbon filters was not decreased after nonchlorinated backwash, and biomass density increased. This was presumably due to the removal of inorganic material that acted as a diffusion barrier for microbial growth.

In the operation of biological filters, retention of biomass must be balanced with the ability to retain adequate filter run times and reasonable headloss in biological filters. Ahmad *et al.* (1998) determined that water wash alone was not sufficient to clean biological filters. The end result was a buildup of excessive headloss over time. In contrast, air plus subfluidization with water flow followed by a water wash maintained filter run times without seriously impacting biological performance for organic carbon removal. The retention of biological activity was partially explained by the observation that nonbiological particles are more easily removed from filters during backwash than bacteria (Ahmad and Amirtharajah, 1998).

Filters for Particle Removal and Biological Activity

Due to the costs associated with installation of a complete set of dedicated biological filters, treatment plants considering the implementation of biological treatment may wish to promote biofilm development on the existing rapid rate single or dual media filters. This option is being considered by facilities that presently have difficulty meeting disinfection by-product regulations, those that have implemented ozonation to attain primary disinfection requirements, or those that experience regrowth in their distribution systems. It is important to note that particle removal in these filters cannot be compromised, and biological treatment is therefore of secondary importance. This is an area of growing interest, as evidenced by the review article by Urfer *et al.* (1997), which concludes with a section on research needs in the area.

Downstream Effects of Biomass Released from Biological Filters

The end result of biological filtration is conversion of organic carbon in the water into bacterial biomass. Ideally, this biomass is immobilized on the filter media and removed during the backwash cycle. It is possible, however, for colonized filter fines or bacterial aggregates to be shed from the filter media and enter the distribution system. There is evidence to suggest that particle associated bacteria are less susceptible to disinfection (LeChevallier *et al.*, 1984) and that these organisms can then pass the disinfection barrier (Camper *et al.*, 1986; Stewart *et al.*, 1990). There has also been concern expressed by the drinking water industry that biological filters may support

the proliferation and/or concentration of organisms of public health or regulatory importance.

Release of colonized filter media

The type of filter medium and filter operations influences the release of colonized filter medium particles. In a comparison between bacterial counts on released filter fines from biologically activated sand, anthracite, and biologically activated carbon (BAC), the BAC fines contained significantly higher numbers of heterotrophs (LeChevallier *et al.*, 1992). When the release of fines from full-scale anthracite and sand filters was evaluated (Camper *et al.*, 1987), particle counts were similar to those from GAC filters. In the same study, where particles released from GAC filters throughout a filter run were enumerated, higher filtration rates, deeper GAC filter beds, and higher applied water turbidity resulted in higher particle release. The age of the carbon did not influence particle release. These findings were confirmed by Stringfellow *et al.* (1993) who demonstrated that the number of particles released from sand or GAC contactors were similar. They also observed that these particles were frequently colonized by heterotrophs, although no coliforms were detected. Amirtharajah and Westein (1980) showed that an elevated number of particles were released both immediately prior to backwash and shortly after the filter was put back in operation if proper filter-to-waste procedures were not followed. It is probable that colonized filter fines or detached bacteria are also released during these two events, as illustrated by an elevated number of coliforms detected in the filtrate immediately after backwash (Bucklin *et al.*, 1991). Moran *et al.* (1993) showed that there can be breakthrough of turbidity due to detachment of particles from deep laboratory filters at the end of a filter run, and these particle sizes were comparable to the range associated with *Cryptosporidium* oocysts.

In field studies, Camper *et al.* (1987) collected released filter fines from GAC filters by passing water from the underdrain during an entire filter run through a gauze filter in a 47 mm Swinnex. Recognizing that small fines may not have been efficiently retained in the gauze, the average number of particles in a sample was 2,333 ranging in size from 1.0 to $3.5 \times 10^3 \mu\text{m}$ in diameter. Seventeen percent of the filter runs released carbon fines that contained coliform bacteria, and 28% of these coliforms exhibited the fecal biotype. It should be noted, however, that none of these utilities experienced elevated coliform numbers in their distribution systems.

In a similar study, Stewart *et al.* (1990) used a modified Swinnex with a polycarbonate filter to trap carbon particles released from a pilot GAC filter. An average of 36 particles l^{-1} were detected with sizes ranging from 2 to $>40 \mu\text{m}$. It was determined that 200 to 7,000 viable bacteria could be recovered from 1000 particles. The numbers of coliforms were low, with one reported fecal coliform isolated from the released filter fines.

Proliferation of organisms of public health concern

The drinking water industry has expressed concern about the potential for pathogenic organisms to grow on biological filters and then be released into the water. For such organisms to be found in water emanating from a biological filter they must success-

fully colonize and compete with the indigenous organisms (or be retained in large numbers), be released from the filter, and penetrate the disinfection barrier.

To colonize and persist on filter media, the pathogens must be able to compete successfully with the heterotrophic bacterial populations. Competition with the existing microflora may be a key parameter in preventing proliferation of pathogenic organisms. In previous research using laboratory columns containing 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, but the decline of pathogens in the filter and filter effluent was most rapid if pathogens were added to previously colonized GAC. In other studies using groundwater and laboratory columns with virgin GAC, GAC that had been in operation in a filter for a few months, and BAC from a full-scale plant, coliform elimination was most rapid from the BAC filter (LeChevallier *et al.*, 1998). Other laboratory studies (Rollinger and Dott, 1987) with several pathogens on GAC gave 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.

The advent of molecular techniques has led to more detailed investigations of pathogen persistence on filters. The use of PCR can demonstrate pathogen presence even if the organisms cannot be cultured. A recently completed project utilized laboratory columns of filter media inoculated with enteric bacterial pathogens (*Salmonella*, *E. coli* O157:H7). Culturable cells were not detected within 1 d. Bacteria were detected using PCR and fluorescent antibody techniques for 2 weeks, but the pathogen numbers decreased rapidly after inoculation. In companion pilot scale tests, the organisms in filter effluents were reduced three to four logs within 48 h. *Giardia* and *Cryptosporidium* showed similar behavior, suggesting that the bacterial pathogens were not attaching to the filter media in significant numbers. There was no evidence to support the concept that bacteria were growing in biofilms on the filters (Burr *et al.*, 2000).

Impact of released cells/colonized filter fines on the distribution system

At first consideration, it may seem that biological filters would release organisms and filter fines that may have an adverse affect on the distribution system. However, before bacteria from filters reach the distribution system, they must pass the disinfection barrier. They must then be transported in water containing disinfectant, attach to the pipe wall, and proliferate. Evidence from pilot studies has shown that chlorination after biological treatment produced water with lower bacterial numbers than that from conventional treatment (LeChevallier *et al.*, 1998). Presumably this is because the use of chlorine on the conventional filter (including backwash) selected for organisms less susceptible to disinfection. Even if these organisms reach the biofilm, there is ecological evidence showing that high levels of inoculation are required for the colonization and persistence of an allochthonous organism (Warren *et al.*, 1992). Work with filter fines colonized with a coliform has shown that the particles can attach to existing

biofilms, but the organisms on their surfaces have no selective advantage (Morin *et al.*, 1996). In these experiments there was a selective release of the filter fines and most of the coliforms from the biofilm when a disinfectant was applied.

All of the evidence gathered to date from laboratory and pilot experiments as well as full-scale experience points to the ability of biological treatment to produce microbiologically safe drinking water, especially if post-disinfection is practiced.

BIOFILMS IN DISTRIBUTION SYSTEMS

In the last decade the traditionally held view that water quality is solely the result of treatment been refuted. Treatment processes have been optimized to produce water that meets regulatory and end-user requirements, often at great expense. However, this well-treated water may arrive at the consumer's tap modified in taste, odor and microbial content, as well as in other important aspects. Likewise, highly purified and treated industrial water may become compromised in distribution and unsuitable for downstream processes. Many of these changes in quality are the result of the growth of biofilms on the surfaces of distribution systems.

It is reasonably well documented that the increase of bacterial counts through distribution is the result of the detachment of biofilm cells rather than growth of organisms in the water. Published accounts by van der Wende *et al.* (1989) and LeChevallier *et al.* (1990b) demonstrated that elevated bacterial counts in water could not be attributed to replication of suspended cells, but rather was due to biofilm growth. A simple calculation based on mass loading of a distribution system demonstrates the potential for a small amount of organic carbon to produce a large number of organisms. A hypothetical treatment plant produces water with a concentration of total organic carbon of 2 mg l^{-1} , of which 10% can be used for bacterial growth. The treatment plant produces $20,000 \text{ m}^3 \text{ d}^{-1}$, and all this water must pass through the distribution system. If a yield of $0.1 \text{ g dry cell mass g}^{-1} \text{ carbon}$ is assumed, this system could produce approximately 400 grams or 10^{14} new cells each day if the biofilm is at steady state. Another interpretation is that the system would produce enough released biofilm to account for 10^8 total cells ml^{-1} . A distribution system should be viewed as a complex reactor system where many factors, including the growth of biofilms, contribute to water quality deterioration.

Problems Associated with Biofilms in Distribution Systems

Coliform and heterotroph regrowth

The initial interest in biofilm growth in distribution systems arose from the observation that total coliform bacteria were appearing in distributed water that met all the criteria for microbiological quality at the plant. Coliform bacteria are used world wide as indicator organisms, and their presence has been associated historically with fecal contamination of water. When these bacteria were found in systems with well-run treatment plants and properly maintained distribution systems, it was hypothesized that the bacteria were growing in biofilms on the surface and being released

into the water. The fact that bacteria colonize pipe material was reported in the 1970's and early 80's (O'Connor *et al.*, 1975; Allen *et al.*, 1980; Olson, 1982) but it was not until 1984 in the state of Connecticut that the growth of coliforms on distribution system materials was linked to their presence in the water (Centers for Disease Control, 1985). This incident promoted the study of biofilms in distribution systems across the U.S. and Europe.

The diagnosis of a coliform regrowth event is typically done by eliminating all other potential sources of contamination. It is very difficult to prove conclusively that the organisms are growing in the distribution system. Even when coliform counts are high, coliforms may not be found on the surfaces of excavated distribution system materials (LeChevallier *et al.*, 1987; Characklis, 1988). Presumably this is because the organisms are in discrete locations rather than uniformly distributed throughout the biofilm (LeChevallier *et al.*, 1987; Camper *et al.*, 1996).

The regrowth of heterotrophs can be also be of concern, especially for European communities which are required to monitor their presence. Some U.S. utilities will routinely monitor heterotrophs as a general indicator of microbial quality, and may be required to assess their numbers if chlorine residuals are too low. The general heterotrophic population is usually not of public health concern, but with the growing immunocompromised population, many utilities are interested in minimizing the presence of these organisms in their water. Some heterotrophs may be opportunistic pathogens, and for this and other reasons their control is desirable.

Colonization by pathogens and opportunistic pathogens

There is limited information available on the presence of pathogens in distribution system biofilms, primarily because detection is difficult. In spite of this difficulty, there are instances where opportunistic pathogens have been detected, including *Aeromonas* spp., *Mycobacterium* spp. and *Helicobacter pylori*. These organisms are presently on the U.S. EPA's Contaminant Candidate List (USEPA, 1998), and as such, there is much interest in determining the levels at which they exist in distribution systems.

Aeromonas spp. have been found in distribution system biofilms (van der Kooij, 1988; Havelaar *et al.*, 1990). Although these organisms are known to survive under low nutrient conditions, little is known about the factors that govern their growth. There is a positive correlation of growth with residence time (Havelaar *et al.*, 1990), but a mixed correlation with temperature (Burke *et al.*, 1984; Havelaar *et al.*, 1990) in water systems with little or no disinfectant. In controlled laboratory experiments, low level disinfection lead to the elimination of the organism from mixed population biofilms (Camper *et al.*, 1998).

It is known that organisms of the genus *Mycobacterium* can grow in biofilms, and that there is the potential for selection for these organisms in water due to their resistance to chlorine (Collins *et al.*, 1984; Schulze-Robbecke and Fischeder, 1989; Briganti and Wacker, 1995). These organisms were found in biofilms on the surfaces of pilot systems that received conventionally and biologically treated water, with a lower frequency of detection when the systems received biologically treated water. These studies showed that no mycobacteria were found in pipes receiving biologically treated water followed by chlorination (LeChevallier *et al.*, 1998). Of particular

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concern are the MAC, or *Mycobacterium avium* complex, implicated in infections in the immunocompromised population, particularly those with acquired immunodeficiency syndrome (Horsburgh, 1991; Nightingale *et al.*, 1992).

There is presently a great deal of interest in determining the ability of *Helicobacter pylori* to survive in biofilms. Laboratory studies have shown that it can persist in water for extended time periods (West *et al.*, 1992; Shahamat *et al.*, 1993) and it has been isolated from drinking water (Klein *et al.*, 1991). Mackay *et al.* (1999) found that in a mixed population biofilm, this organism can be detected by PCR for up to 192 h. This suggests that it can at least persist if the inoculum is sufficiently high.

There is even less information about the survival of frank bacterial pathogens (*Salmonella* spp., *Escherichia coli* O157:H7, *Vibrio cholerae*) in distribution system biofilms than for opportunistic pathogens. Pathogens in distribution system biofilms would be subjected to the same ecological pressures as were described above for biological filters. It is therefore unlikely that pathogens would persist. The rationale presented above for the pathogen survival in biological filters would likely apply to distribution systems also.

There are no known instances where frank bacterial pathogens have been recovered in distribution system biofilms, even after that system has suffered a water-borne disease outbreak. There are laboratory studies where pathogens have been shown to persist, but these systems may not be representative of drinking water conditions. For example, *Salmonella enteritidis* has been shown to grow in pure culture biofilms when fed glucose at a concentration of 100 mg l⁻¹ (Jones and Bradshaw, 1996). *Campylobacter* spp., as detected by fluorescent antibody staining, could survive for over 42 d in mixed population biofilms fed filter sterilized tap water when inoculated at a concentration of ca 10⁶ cells ml⁻¹ (Buswell *et al.*, 1998). Studies done with *E. coli* O157:H7 showed that even after a high population inoculum, the organisms could not be detected by fluorescent antibody methods in mixed population biofilms after a few days. In contrast, *Salmonella typhimurium* persisted for over 50 d, but there was no evidence of proliferation (Camper *et al.*, 1998). All these studies suggest that the organisms may be present, but presence alone must be separated from viability and/or infectivity.

Control of Biofilms in Distribution Systems

Almost every water distribution system is prone to biofilm formation, regardless of the purity of the water, type of pipe material, or biocide treatment used. It is known that there can be substantial changes in metal concentrations, bacterial populations, disinfectant residuals and disinfectant by-products, and esthetic qualities (taste, odor, and color) in the water through distribution. There is an interaction between surface-mediated reactions (corrosion, biocide/disinfectant demand, immobilization of substrates for bacterial growth), mass transfer and mass transport processes, and bulk fluid properties (*e.g.* concentration and type of biocides, general water chemistry, and organic concentration) on the microbial ecology of the biofilm and subsequent water quality changes. These interactions can be exceedingly complex, which means that control measures are not obvious and are often system specific.

There are a variety of operational and environmental parameters that appear to encourage the growth of biofilms in distribution systems. Surveys of the industry have

shown that temperature, organic carbon levels, the concentration and type of disinfectant, and the presence or absence of a corrosion control regime when corrodible materials are used in the distribution system are of importance (LeChevallier, 1990; Smith *et al.*, 1990). Some of these factors have been studied, and evidence to support their importance is given below.

Reduction of organic carbon concentrations

Regulations on disinfection by-product formation and the desire to produce biologically stable water has lead to the identification of options to reduce the concentration of organic matter in water. The three most likely options are enhanced coagulation, activated carbon adsorption, and biological filtration. For any of these options, identifying design criteria for the amount of organic carbon in water that is capable of supporting biofilm growth has been of key interest.

There have been several methods developed to measure the amount of organic carbon in water that is available for bacterial growth. The assimilable organic carbon (AOC) test was developed by van der Kooij *et al.* (1982) and measures the increase in numbers of a known bacterial culture when grown in unamended drinking water. Threshold levels of AOC to limit heterotrophic biofilm growth have been set at $10 \mu\text{g C l}^{-1}$ for heterotrophs (van der Kooij, 1992) and a recommended $50 \mu\text{g C l}^{-1}$ for coliform control (LeChevallier *et al.*, 1991). A second measurement is the biodegradable organic carbon (BDOC) test, where the water is inoculated with an undefined mixed bacterial population in suspension or on sand particles and the change in dissolved organic carbon is measured over time. A guideline BDOC value of 0.15 mg l^{-1} (Servais *et al.*, 1993) for biological stability has been suggested, with Joret (1994) recommending that the levels be adjusted for temperature.

It is important to note that these measurement techniques were developed by European investigators responsible for the monitoring and operation of European systems where many of the confounding variables influencing regrowth have been minimized. For example, European systems are operated without a substantial disinfectant residual. It is not uncommon for the upper limit for disinfectant leaving European sources to be set at $0.2\text{--}0.4 \text{ mg l}^{-1}$, while US facilities typically use much higher concentrations ($1\text{--}4 \text{ mg l}^{-1}$). For this and other reasons, the reasonable success of these methods in European systems has not been as straightforward in the US. There are instances where associations between AOC/BDOC and biofilm are not clear cut. In a field survey, regrowth was seen in systems with average AOC levels both $>$ and $< 100 \mu\text{g l}^{-1}$ (LeChevallier *et al.*, 1996a). In pilot experiments, there was a weak correlation between biofilm and influent AOC concentrations, but no correlation with the concentration of AOC in the reactors (Camper, 1996). Studies where known organic carbon sources were used also demonstrated similar results; increased organic carbon loading did not always result in higher biofilm cell numbers (Ellis *et al.*, 1999). There appear to be interactions in the distribution system that complicate the ability to use AOC/BDOC as an independent assessment of regrowth potential.

Regardless of how appropriate or inappropriate a specific test or the values from these tests are for predicting regrowth, the concept of reducing the amount of organic carbon entering a distribution system is still valid. It is only logical to assume that limiting the amount of substrate for heterotrophs will lead to less biofilm growth,

fewer detached cells, and improved water quality. This has been demonstrated in pilot studies where, in the absence of a disinfectant, biological treatment has reduced the number of organisms on pipe surfaces (LeChevallier *et al.*, 1998; Volk *et al.*, 1998). Likewise, a detailed study of two full-scale systems demonstrated that biological treatment produced water that had the same microbiological quality as conventional treatment, with less residual disinfectant required (LeChevallier *et al.*, 1998).

Controlling biofilms with disinfectants

Biofilms are notoriously less susceptible to disinfectants than suspended cells, and the literature contains many references to this effect. It is generally believed that increasing the concentration of a disinfectant should control regrowth, but many instances exist where the opposite effect is seen (Martin *et al.*, 1982; Hudson *et al.*, 1983; Reilly and Kippen, 1984; CDC, 1985; Oliveri *et al.*, 1985; LeChevallier *et al.*, 1987). Hudson *et al.* (1983) found no correlation between free chlorine residuals and the number of heterotrophic plate count (HPC) organisms per unit surface area. An interesting observation has been that the presence of chlorine can enhance the numbers of coliforms in biofilms (Camper *et al.*, 1996; 1999).

There are several reasons why this lack of disinfection efficacy may occur. First, there may be mass transfer limitations that prevent the penetration of the disinfectant into the biofilm (van der Wende and Characklis, 1990; LeChevallier *et al.*, 1990a; 1993; deBeer *et al.*, 1994; Srinivasan *et al.*, 1995; Koudjonou *et al.*, 1997). Disinfectants may react with the total organic carbon in the water, making it more available for microbial growth (Bryant *et al.*, 1992). Low level chlorination (*ca* 0.2 mg l⁻¹ residual) has also been observed to increase the growth rate of heterotrophs in biofilms (Ellis *et al.*, 1999). It has also been shown that biofilm bacteria are physiologically different from suspended cells, and this may account for their reduced susceptibility to disinfection (Gilbert and Brown, 1985). It is also possible that the disinfectants increase corrosion, which, as shown below, is an important factor in biofilm development on pipe surfaces.

With the increased emphasis on controlling disinfection by-products as well as controlling regrowth, utilities have become more interested in using monochloramine as a secondary disinfectant. Monochloramine is typically not used as a primary disinfectant because of its high concentration x time (CT) requirements, but it has been found to be a superior disinfectant over free chlorine for biofilms (Griebe *et al.*, 1994; Srinivasan *et al.*, 1995; Olson, 1996; Ollos *et al.*, 1997). When full scale distribution systems were studied, chloramines were more effective at reducing the number of biofilm total coliforms and HPC than chlorine (Neden *et al.*, 1992). Donlan and Pipes (1988) demonstrated that elevated monochloramine concentrations were associated with reduced attached bacterial populations. A field study of 31 utilities showed that 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. The same study showed that the average density of coliforms in chlorinated systems was 35 times higher than that in the chloraminated systems (LeChevallier *et al.*, 1996b).

Monochloramine may also have an advantage over chlorine if corrodible surfaces are present. A study in a model pipe loop system composed of several materials showed that biofilms on galvanized, copper, or 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.*, 1990a). Abernathy (1998) showed that monochloramine was more effective at reducing bacterial counts on ductile iron surfaces than free chlorine, and the effect was more pronounced if a corrosion inhibitor was also used.

The importance of materials

Distribution system pipe materials apparently have a marked effect on biofilm formation. In particular, unlined iron (mild steel, cast and ductile iron) pipes have a profound positive effect on the number of attached bacteria (Camper, 1996; Camper *et al.*, 1996; LeChevallier *et al.*, 1993; 1996a; 1996b). A utility survey has shown a positive relationship between the number of miles of unlined metal pipes and coliform occurrences (LeChevallier *et al.*, 1996b). The influence of iron pipes can be substantial, with one experiment demonstrating a >100 fold increase in biofilm cell numbers on iron compared to polyvinylchloride (PVC) surfaces (LeChevallier *et al.*, 1998) and another showing a two-fold increase on the same surfaces (van der Kooj and Oorhuizen, 1997). Other pilot system experiments have supported an 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, while 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. Delanoue *et al.* (1997) also noted that unlined iron pipes had higher biofilm densities than non-ferrous materials, and that mild steel was more heavily colonized than cast and ductile iron. In another study where cast iron, medium density polyethylene and unplasticized polyvinyl chloride surfaces were tested, the cast iron surface had the highest doubling time for biofilm bacteria, an average of 97% more organisms than the other materials, and the highest species diversity (Kerr *et al.*, 1999).

The same influence of materials has been demonstrated for coliforms. Laboratory experiments have shown that mild steel surfaces supported ten-fold more heterotrophs and two to ten fold more coliforms than polycarbonate surfaces. The effect was also seen in effluent cell counts. The presence of a small amount of mild steel (10% on the basis of surface area) in an otherwise plastic system resulted in the same elevated biofilm counts on all surfaces (Camper *et al.*, 1996).

There are several reasons why iron surfaces may be more conducive to bacterial growth than inert materials. It is probable that the metal surfaces exert a disinfectant demand (Knocke, 1988; Knocke *et al.*, 1994; Vasconcelos *et al.*, 1996), which would limit disinfectant efficacy against biofilms. There is circumstantial evidence for this effect, because biofilms on ferrous metal surfaces have been found to be less susceptible to free chlorine than those on inert materials (LeChevallier *et al.*, 1987; 1988; 1990a; 1998; Chen *et al.*, 1993; Kerneis *et al.*, 1994). In addition, increased corrosion rates decrease the efficacy of free chlorine against biofilm organisms (LeChevallier *et al.*, 1993).

It is also possible that corrosion products change the availability of natural organic matter to biofilm bacteria. Aquatic humic substances readily adsorb to iron oxide surfaces (Tipping, 1981; Gu *et al.*, 1994; Parfitt *et al.*, 1997; Varadachari *et al.*, 1997),

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and this may change the chemical and biological properties of the molecules. When attached to surfaces, humic substances uncoil due to collapse and binding with the surface, which results in the exposure of sugars and peptides previously concealed in the hydrophobic molecule (Gu *et al.*, 1994). Experiments in the author's laboratory have demonstrated that humic substances can be used as a sole carbon and energy source for biofilms (Camper *et al.*, 1999). In addition, the presence of an iron oxide surface with bound humic substances enhances the attachment of bacteria and the subsequent growth rate when humics are provided in the bulk water (Qui, 1999).

Corrosion control

If corroding surfaces contribute to increased biofilm accumulation, it should follow that corrosion control may reduce biofilm cell numbers. There is evidence to demonstrate that corrosion control has mitigated coliform regrowth in full scale systems (Martin *et al.*, 1982; Hudson *et al.*, 1983; Lowther and Moser, 1984; Schreppel *et al.*, 1997). The mechanism by which corrosion control influences regrowth could be based on less reactivity of the disinfectant with the surface, fewer bacterial attachment sites, or other factors. For example, LeChevallier *et al.* (1993) showed that corrosion control reduced biofilm numbers but attributed the response to increased chlorine efficacy. It may be that control is dictated by water quality and the type of disinfectant used. For example, an increase in pH (a common corrosion control scheme) may decrease chlorine efficacy because of the shift in the equilibrium towards the less effective hypochlorite ion. This effect would not be seen if phosphate was used to control corrosion. It may also be that corrosion control supercedes disinfection; Martin *et al.* (1982) showed that increasing the pH to 9 in a chlorinated system reduced bacterial counts. Other studies have cited that corrosion control was more important than disinfection in reducing biofilm densities (LeChevallier *et al.*, 1998). As supporting evidence, the author and colleagues have noted that at near neutral pH, in the absence of corrosion control, the presence of low levels of disinfectant 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). Abernathy (1998) has shown that biofilm density is directly proportional to the mass of corrosion products present. The reduction of corrosion product concentration and a concomitant decrease in biofilm was accomplished by adding phosphates or increasing the pH or by changing from chlorine to monochloramine. The best results were obtained when phosphates were used in conjunction with monochloramine. In another pilot experiment, the results indicated that corrosion control was more important in reducing bacterial numbers in biofilms than decreasing the amount of useable organic matter in the water or the maintenance of a free chlorine residual (LeChevallier *et al.*, 1998).

CONCLUSIONS

As with all other aqueous environments, drinking water treatment and distribution systems offer habitats suitable for biofilm growth. Biofilm accumulation in treatment, which has historically been viewed as extremely undesirable, is now being optimized

as a component of water treatment. Biological treatment can then reduce the potential for biofilm formation in distribution systems where control is very difficult. Biofilm growth in distribution systems is governed by a host of variables that may be system specific. Some of the factors that appear to be important in biofilm control are the type and concentration of disinfectant, the organic content of the water, the type of pipe material, and the use of a corrosion control scheme. It is important to note that each system is unique and a biofilm control strategy that is successful for one utility may not be appropriate for another.

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