Gordon A. McFeters
Editor

Drinking Water Microbiology

Brock/Springer Series in Contemporary Bioscience

12

Biofilms in Potable Water Distribution Systems

E. van der Wende and W. G. Characklis

Institute for Biological and Chemical Process Analysis, Montana State University, Bozeman, Montana

12.1 Introduction

High bacterial populations in potable water distribution systems, sometimes referred to as events or blooms, have troubled utilities because of their possible implications for the hygienic safety and taste and odor of their product. Before considering the contribution of biofilm accumulation to these high bacterial populations in distribution systems, some terminology must be clarified with regard to drinking water bacteriology.

The water utility industry uses the terms "regrowth" and "aftergrowth" synonymously to describe the processes contributing to the increase in number of organisms in distribution systems with distance away from the treatment plant. However, Brazos and O'Connor (1985) proposed the following more specific definitions: **regrowth** is the recovery of disinfectant-injured cells which have entered the distribution system from the water source or treatment plant and **aftergrowth** is growth of microorganisms native to a water distribution system. Both terms implicate the microbial growth process in the phenomenon of increased microbial cell numbers. These terms do not clearly discriminate between the two primary mechanisms by which the microorganisms appear in the distribution system, i.e., breakthrough in the treatment plant and growth within the distribution system.

Breakthrough is the increase in bacterial numbers in the distribution system resulting from viable bacteria passing through the disinfection process. The surviving cells inoculate the biofilms in the distribution system and/or reproduce in the bulk water. Accordingly, injured organisms entering the distribution system recover and contribute to the process of breakthrough. Transport of viable bacterial cells through the disinfection process is very common in drinking water treatment (McFeters et al. 1986). Disinfection only means ridding the water of viable pathogenic organisms.

Growth is the increase in viable bacterial numbers in the distribution

system resulting from bacterial growth downstream of the disinfection process. Growth may occur in biofilms or as planktonic growth in the distribution system water. (*Planktonic cells* are viable bacterial cells suspended in the water phase). An **episode** is an occurrence of "excessive" viable bacteria in the distribution system. Episodes result from breakthrough, growth, and, sometimes, from cross-connections and back-siphoning. Thus, an episode refers to the occurrence while breakthrough and growth refer to its causes.

Excessive viable bacteria are numbers of viable bacteria exceeding drinking water standards. Since present U.S. standards only refer to coliforms, excessive bacteria only refers to these bacteria. Coliforms frequently enter distribution systems via breakthrough without an episode occurring. The standard for the membrane filtration method permits a maximum of one coliform per 100 milliliters based on a monthly average of all samples.

Insufficient disinfectant concentration is a disinfectant residual which permits excessive viable bacteria to exist at some location in the distribution system. Since the disinfectant residual varies spatially and temporally in the distribution system, an insufficient disinfectant concentration may exist in one part of the system (e.g., "dead ends") and not in another (e.g., constantly flowing regions). Disinfectant concentration may also vary radially in the pipe at any given axial position.

12.2 Biofilms

A biofilm is a layer of microorganisms in an aquatic environment held together in a polymeric matrix attached to a substratum (Figure 12.1). The matrix consists of organic polymers that are produced and excreted by the biofilm microorganisms and are referred to as extracellular polymeric substances (EPS). The chemical structure of the EPS varies among different types of organisms and is also dependent on environmental conditions. Biofilms sometimes form continuous, evenly distributed layers but are often quite patchy in appearance. Biofilms in water distribution systems are thin, reaching maximum thicknesses of perhaps a few hundred micrometers. Biofilms from natural environments are generally heterogeneous, frequently containing more than one distinct microenvironment. For example, biofilms with aerobic as well as anaerobic strata are common. This means that different microenvironments inhabited by different microorganisms exist in the direction perpendicular to the substratum. Consequently, the term biofilms does certainly not only refer to a uniform surface community. In addition, the film often contains organic or inorganic debris from external sources. Inorganic particles may result from the adsorption of silt and sediment, precipitation of inorganic salts, or corrosion products.

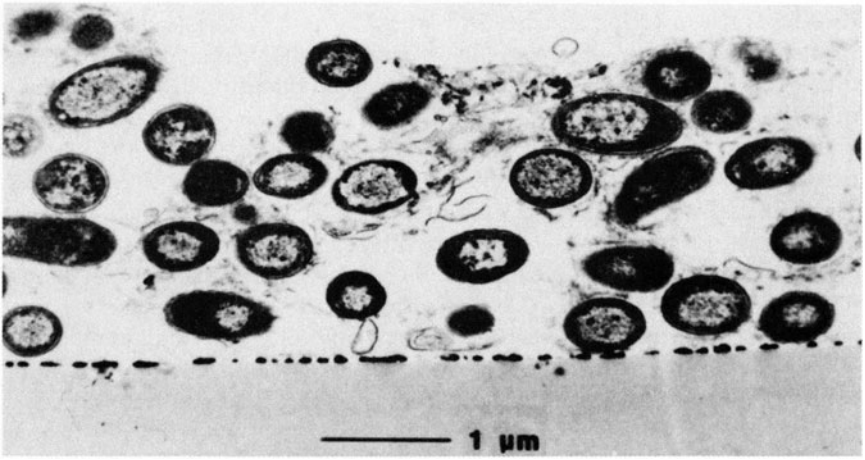

Figure 12.1 Transmission Electron Microscopy (TEM) picture of a cross section of a very thin biofilm.

12.3 Observations of Biofilms in Water Distribution Systems

Limitations in Water Distribution System Research A thorough study of biofilm accumulation and biofilm composition throughout a water distribution system has not been reported, probably because access to these systems is difficult. Most observations consist of analysis of samples obtained during flushing and pigging of water mains, physical main cleaning procedures used to remove biofilm, corrosion products, and sediment from the distribution system. Samples obtained this way consist of biofilm as well as sediments and corrosion products dislodged by the pigging and flushing processes. Results of bacterial enumeration procedures of such samples can not be accurately related to a biofilm surface area because the relation between sample volume and the corresponding pipe wall surface area is unknown. In addition, the extent of biofilm removal from the pipe wall surface is unknown and limits the interpretation of the data.

The biofilm on the often heavily corroded pipe walls is hard to sample and sample handling causes difficulties. For example, instead of water, the pipe surface with the biofilm is often exposed to air for long periods of time preceding viable cell enumerations. This significantly influences these analyses. Direct cell counts on distribution system samples are complicated by the presence of inorganic particles (e.g., corrosion products). Electron micrographs of in situ biofilm of a single pipe surface sample are fragmentary and frequently neglect the spatial heterogeneity of the biofilm.

Biofilm Accumulation Studies in Water Mains Allen (1980) examined the occurrence of microorganisms on main encrustations (tubercles) by electron microscopy. Water utilities throughout the United States provided water main tubercles obtained during water main servicing, repair, or replacement. Tubercles from seven different water utilities were examined and the electron micrographs of these tubercles showed a number of common characteristics. The surfaces had a clear porous texture, while beneath the surface veneer a multitude of crystal arrays was found with several tubercles having laminar deposits near the surface. On most tubercles, microorganisms were mainly observed at or near the surface. However, active bacterial colonization was also detected beneath the surface veneer while some cells were found embedded in the granular matrix deep in the tubercle. Allen (1980) stated that bacterial enumeration by conventional methods (scraping of the tubercle surface followed by viable cell counts) greatly underestimates the true bacterial populations present in the tubercles. Certainly, viable counting techniques based on colony growth on solid media only recover a fraction of the living cells, especially when mixed populations are enumerated. Furthermore, electron microscopy cannot distinguish between intact dead cells and living cells.

Ridgway and Olson (1981) used scanning electron microscopy to observe the bacterial colonization of a distribution main consisting of galvanized iron pipe with a 0.5 cm thick hard, black lining, presumably cement or porcelain. The main had been in service 25 years and appeared to be in good condition since iron tubercles were not observed. The main carried unchlorinated ground water for most of the year but it was blended with 10 to 15% chlorinated, fully treated surface water during the hot summer months. A low magnification scanning electron micrograph of a representative area of the surface of the liner showed that it was covered with a thin (10 to 100 μm) amorphous mineral encrustation. The crust contained a network of fissures and cavities, increasing the total available surface area of the pipe, thus providing a variety of potential microhabitats for microorganisms. Aluminum, silicon, phosphorus, sulfur, calcium, and iron were the predominant elements at the pipe surface. This was quite different from the situation in the water phase where both iron and phosphorus were found to be minor soluble constituents. Similarly, manganese and zinc were present in very low concentrations in the water supply but readily detected on the pipe surface. Thus, cells attached to the pipe surface were exposed to concentrations of dissolved compounds that were significantly different from the concentrations in the bulk water. Such altered concentrations of minerals and nutrients could have a profound effect on the growth of attached organisms and their susceptibility towards disinfectants. The surface was examined for microorganisms at high magnification. Many of the observed cells had a coccoid shape and the cells were sometimes linked together in chains like streptococci. Filamentous bacteria that bore a clear morphological similarity to the streptomycetes were the most frequently observed microorganisms. Iron-oxidizing bacteria of the genus *Gallionella* were also frequently observed. *Streptomycetes* and *Gallionella* were also often found as planktonic cells in water

samples. Although the water main had been in service for 25 years, no continuous biofilm was found on its surface. Only sparsely and randomly distributed microcolonies were present on the pipe surface, frequently associated with crevices in the mineral layer. The small amount of biomass accumulation in the system may have been the result of low organic carbon concentrations in the ground water (no data available).

Groundwater often contains smaller amounts of organic carbon than surface water. O'Connor and Banerji (1984) found that the microbial growth on pipe walls was related to total organic carbon (TOC) levels in the water and that TOC levels of 5.0 mg/l or less limited the amount of microbial growth in their laboratory experimental system. Very little growth was observed at an organic-carbon level of 0.5 mg/l. A typical TOC level for treated groundwater is 0.5 to 3.0 mg/l while the TOC level in treated surface water is in the range of 2.0 to 5.0 mg/l.

Characklis (1988) studied the biofilm accumulation on the surface of PVC pipe that was exposed to chlorine-free drinking water prepared from surface water. A biofilm that covered the entire pipe surface and was easily visible had developed after an exposure time of several weeks. Viable cell counts of the biofilm cells after an exposure time of 85 days revealed cell densities of approximately 5×10^{10} cells/m^2. O'Connor and Banerji (1984) found no significant differences in the accumulation of cells on copper, PVC, or iron surfaces. Thus, this relatively high level of biofilm accumulation was probably not a result of different pipe materials. In a somewhat similar situation, Haudidier et al. (1988) studied the accumulation of biomass in a plug-flow system comprised of six serially disposed loops. The influent water was fully-treated river water without any disinfectant. At steady state, the viable biofilm cell density at the beginning of the plug-flow system was almost 10^{11} cells/m^2 while a constant decline was observed with increasing residence time.

Physicochemical and Bacterial Analyses of Water Main Tubercles

Tuovinen (1980) examined tubercles from uncoated or poorly coated iron pipes for their bacterial, physicochemical, and mineral properties. Using selective growth media, Tuovinen isolated a variety of different bacterial groups including sulfate reducers, nitrate reducers, nitrite oxidizers, ammonia oxidizers, sulfur oxidizers, and various unidentified heterotrophic microorganisms. The relative importance of the various groups in the bacterial community could not be estimated since cell numbers were not determined. Oxidation-reduction (redox) potential measurements throughout the tubercles indicated different microenvironments that could explain the presence of microorganisms from various bacterial groups. High redox potentials, reflecting aerobic conditions, were found at the tubercle surface (the oxygen concentration in the bulk water was approximately 8 mg/l). Low redox potentials, reflecting highly reduced conditions, were found at 1 or 2 mm depth in the tubercles. Thus, bacterial niches were available for aerobic, facultative anaerobic, and anaerobic bacteria. The reducing conditions of the tubercle interior may have been a suitable habitat

for the anaerobic, sulfate-reducing bacteria. The obligate aerobic organisms, including those that oxidize inorganic compounds for energy, apparently remain in the exterior layer which is exposed to oxygen where reduced nitrogen and sulfur compounds are accessible from the tubercle interior. The presence of large amounts of reduced iron in the tubercles (an Fe(II)/Fe(III) ratio between 1.2 and 2.0) may contribute to the chlorine demand of the pipe wall and therefore may hinder efforts to control bacteria in the distribution system.

LeChevallier et al. (1987) examined and characterized pipe scrapings, tubercles, biofilm flocs, and sediments from a water distribution system in New Jersey for bacterial and mineral properties. The samples were taken approximately 1.7 km from the water treatment plant from a 15 cm ID, 28 year old, bituminous-coated main. Pipe scrapings were taken just before a flushing and pigging event. At the onset of the flushing, a dark brown floc suspension and a small amount of sediment was collected. More sediment and tubercles were collected during the pigging process. The flox contained 21.4% zinc, probably resulting from accumulation of zinc orthophosphate used for corrosion control. It also contained 2.4×10^6 heterotrophic plate count (HPC) bacteria/ml, mainly *Flavobacterium sp.* and *Pseudomomas vesicularis*. These same species were the predominant planktonic species found in the bulk water. The flushing sediment contained 8.4×10^6 HPC bacteria/gram, mainly *Arthrobacter sp.* The pipe scraping contained 1.0×10^7 HPC bacteria/m^2, again mainly *Flavobacterium sp.* and *P. vesicularis*. The dislodged iron tubercles consisted of 98.7% iron and were the only particulate samples that contained significant numbers of coliforms (>160 colony-forming units (CFU)/gram). The number of HPC bacteria in tubercle material was 1.3×10^7 cells/gram. The number of cells accumulated on the pipe wall surface could not be determined from these data. The chlorine concentration was probably much higher in the bulk water than in the iron tubercles, which may have contributed to the coliform survival in the tubercles. The tubercle environment somehow provided a suitable microenvironment for the coliform bacteria. The most superficial layers (flocs + scrapings) contained essentially the same bacterial species that were found in the water phase, suggesting that the planktonic cells present in the bulk water were mainly cells detached from the biofilms.

Camper (personal communication, 1988) analyzed tubercles and flush sediments from a Connecticut water distribution system for the presence of HPC, iron oxidizing, and sulfate-reducing bacteria. A free-chlorine residual of approximately 1 mg/l was maintained in this system. Tubercle and sediment material contained high levels of HPC bacteria. Iron bacteria and low numbers of sulfate-reducing bacteria were only associated with iron tubercles.

Conclusions on Biofilm Distribution Observations of biofilms in water distribution systems indicate little qualitative or quantitative uniformity. Although some bacterial species were more frequently detected than others, a fairly large variation in population composition was seen. Also, cell numbers and distribution on and in the pipe wall lining or corrosion product layer varied

widely. Considering the large differences in the water distribution system environments, this is not surprising. Differences exist in the quality of the treatment plant effluent (the finished water) and in the structure and chemical composition of the pipe wall. The flow rates may also affect the biomass accumulation at the pipe wall surface. Important water quality parameters appear to include the nutrient level, the presence of disinfectants, and other chemical and physical parameters like pH, hardness, and temperature.

12.4 Relevance of Biofilms in the Water Distribution System

Water Quality Problems The depletion of dissolved oxygen, reduction of sulfate to hydrogen sulfide, occurrence of bad taste and odor, and the occurrence of red water in relation to corrosion of cast-iron mains may result from microbial activity. Lee et al. (1977) conducted a survey to determine the extent and nature of water quality problems in the distribution systems in the United States. The results indicated that 60% of the responding utilities report taste and odor to be their most common water quality problems. Red water caused by ferric iron ranked second, with 48% of the utilities having this problem. Complaints of cloudy and black water (manganese oxides) were the next two most frequently cited water quality problems. In addition, virtually every utility periodically experienced some water quality problem originating in the distribution system. Evaluations of distribution system water quality in five Missouri communities indicated that higher bacterial plate counts were generally observed at locations where consumers reported water quality problems (Banerji, 1978).

Biofilms and Water Quality Problems Waterborne diseases, especially those caused by bacteria, have become rare in the United States and other developed parts of the world, although the number of cases is increasing.

Legionella pneumophila is one of the very few bacterial waterborne diseases that occasionally still causes problems in the United States. *L. pneumophila* sometimes grows in water systems, especially hot-water systems and causes legionellosis, a disease which is often fatal. Colbourne et al. (1988) studied the presence of *L. pneumophila* in public water supplies in England and found culturable *L. pneumophila* to be associated with deposits or biofilms in mains although this organism only appeared in water when these materials were dislodged from the pipe wall.

Serious events of black water are sometimes caused by bacteria of the genus *Hyphomicrobium*. A clear example of this is the biofilm sloughing that occurred repeatedly in a drinking water distribution system in Queensland, Australia (Hamilton, personal communication, 1986). The biofilm that had accumulated on the pipe wall of the distribution system contained high numbers of *Hyphomicrobium*. These bacteria form hypha-like structures that are

readily covered with a black precipitate of manganese oxide arising from low concentrations of reduced manganese present in a dissolved form in the water. The cell wall of *Hyphomicrobium* plays a catalytic role in the otherwise spontaneous oxidative precipitation process. The hypha-like structures yielded a biofilm that was relatively thick and heavy from the manganese oxide precipitate. When a sloughing event occurred in the system, biofilm downstream of the point of initiation would also slough in an avalanche-like event. The result was black water that caused problems such as irreversible staining of laundry.

Controlled Biofilm Experiments in Distribution Systems The role that biofilms play in the chemical and microbiological deterioration of drinking water is clear in cases like the manganese oxide precipitation caused by *Hyphomicrobium* and to a lesser extent in contamination with *Legionella*. However, generally, we do not know the extent to which biofilm cell growth and detachment contribute to the increase in planktonic cell numbers in the distribution water. Experiments designed to examine the contribution of biofilms to elevated planktonic cell numbers in the distribution system were reported by Van der Wende et al. (1989). The purpose of the experiments was to quantify the relative contribution of detached biofilm cells and growth of planktonic cells to the total planktonic cell concentrations in a drinking water distribution system.

The experiments were conducted with a RotoTorque System (RTS; Figure 12.2) which consists of four (4) RotoTorque (RT) reactors (Figure 12.3) in series. Each RT is a continuous flow stirred tank reactor (CFSTR) with a 15 cm (6 in) ID water pipe section forming the outer wall of the reactor. A solid PVC drum spins inside the pipe section. The reactor can be easily dismantled so that pipe sections of different material or varying age can be conveniently substituted in the reactor. The liquid volume of each RT is 1.575 l and the wetted surface area is 0.207 m^2.

The RTS simulates the plug flow nature of a water main in that concentration gradients exist in the direction of flow. Mains of variable length carrying water at variable flow rates can be modelled with this system. The volumetric flow rate determines the hydraulic residence time of the RTS. The rotational speed of the drum creates the shear stress imposed on the inside pipe wall surface of a water main. Therefore, combinations of main length and flow velocity can be modelled by selecting the appropriate combination of rotational speed and hydraulic residence time and shear stress, their effects on biofilm processes can be studied independently. The four RTs serve as "windows" at fixed positions along the modelled main. Water and biofilm samples can be collected from these "windows."

Two RTs were located at a water treatment plant that purified lake water using the "direct filtration" process. Water quality parameters and other physical and chemical properties of the treatment plant effluent are presented in Table 12.1.

When the hydraulic residence time in the RTS is significantly shorter than

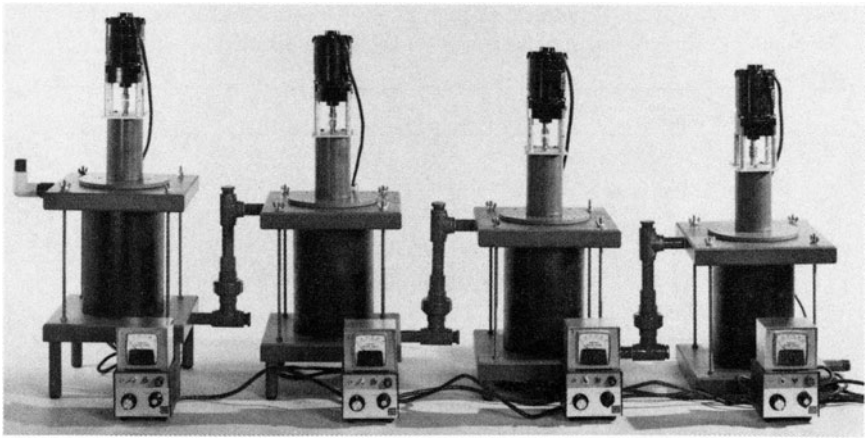

Figure 12.2 A RotoTorque system as used at the water treatment plant.

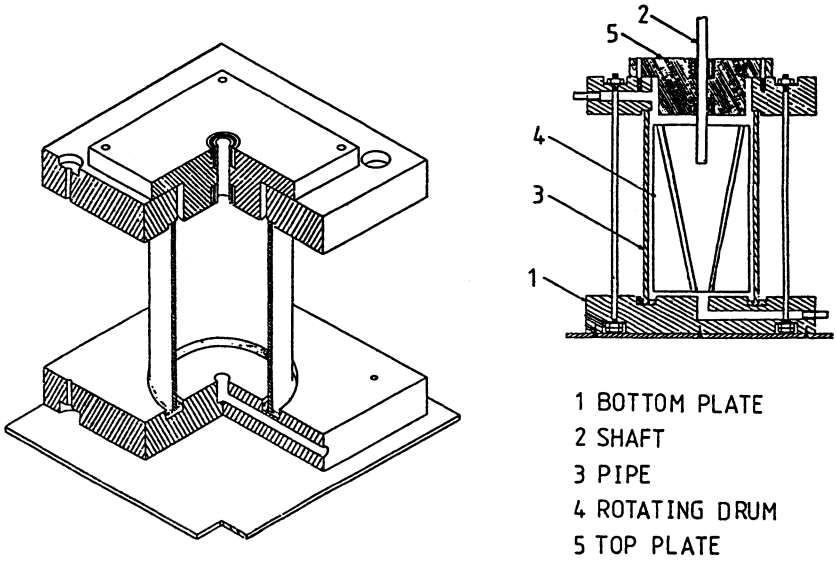

Figure 12.3 Schematic illustration of a RotoTorque reactor.

the generation time of the planktonic cells, replication of planktonic cells in the RTS is negligible. As a consequence, the measurement of biofilm (X_b) and planktonic cell numbers (X) in the RTS for two suitable hydraulic residence times (V/Q), one short and one long, permits the calculation of the specific growth rate in the water (μ) and in the biofilm (μ_b) by means of material

Table 12.1 Physical and chemical properties of the water treatment plant effluent (RTS influent) during the period of biofilm accumulation (average values)

Parameter	Value
pH	7.2
Alkalinity	15 mg/l (as $CaCO_3$)
TOC	2 mg/l (as carbon)
Color	1 Color Unit
Turbidity	0.3 nepholometric turbidity unit (NTU)
Ammonia	0.1 mg/l (as nitrogen)
Nitrate	0.2 mg/l (as nitrogen)
Temperature	21.1 °C

balances. The following mathematical expressions describe the balance for cells in RT1:

Planktonic cell balance in RT1:

$$V\frac{dX_1}{dt} = Q[X_0 - X_1] + \mu X_1 V + r_d X_{bl} A \qquad 1$$

rate of accumulation	net rate of transport out of reactor	net rate of growth in the bulk liquid	net rate of detachment from the biofilm

Biofilm cell balance in RT1:

$$A\frac{dX_{bl}}{dt} = \mu_b X_{bl} A - r_d X_{bl} A \qquad 2$$

rate of accumulation	net rate of growth in the biofilm	net rate of detachment from the biofilm

At steady state, equation 1 and 2 simplify to:

$$\frac{Q}{V}[X_1 - X_0] = \mu X_1 + r_d X_{bl}\frac{A}{V} \qquad 3$$

$$\mu_b = r_d \qquad 4$$

The equations contain three unknowns: μ, μ_b and r_d. However, at high flow rates (short hydraulic residence times), dilution rates are much larger than the

specific planktonic cell growth rate (μ) and the planktonic cell growth rate (μX_1) is negligible.

$$\frac{Q}{V}[X_1 - X_0] = r_d X_{bl} \frac{A}{V} \qquad 5$$

Thus, the set of equations is determinant and r_d can be calculated from equation 5 while μ_b can be determined from equation 4. The values for μ_b and r_d determined for the short hydraulic residence time can be used to determine μ in the long hydraulic residence experiments (equation 3).

The RTS hydraulic residence times used for the experiments were 7.3 h ($Q = 0.87\ l\ h^{-1}$) and 0.22 h ($Q = 28.2\ l\ h^{-1}$). Dilution rate (D) is the reciprocal of hydraulic residence time (V/Q). Thus, the corresponding dilution rates are 0.14 h^{-1} and 4.5 h^{-1}, respectively. The dilution rate of 0.14 h^{-1} is low enough to expect cellular reproduction in suspension in addition to growth in the biofilm. A dilution rate of 4.5 h^{-1} is too high for significant microbial cell replication in suspension (van der Kooij and Hijnen, 1982; van der Kooij et al., 1982). In order to improve the accuracy of the experiment, RTS influent was filtered during the conditions of the high dilution rate so that the planktonic cell concentration in the influent (X_0) was zero, thus eliminating background noise in the measurement of cell numbers. The rotational speed in all of the experiments provided a shear stress at the reactor surface simulating 0.92 m s^{-1} (3 ft s^{-1}) flow velocity in a pipe of 0.15 m (6 in) ID. The pipe sections in the RT were made of new cement-lined iron pipe with a bitumastic coating.

Most microbial activity occurred near the inlet to the system as indicated by the relatively high numbers of biofilm cells and the large increase in planktonic cell numbers (Figure 12.4). Thus, cell growth-rate calculations were made for RT1 using equations 3, 4, and 5. The calculated specific growth rate for biofilm cells was 0.0025 h^{-1}. This means that the cell turnover time or cell generation time in the biofilm (the reciprocal of the specific growth rate) was approximately 17 days. The specific growth rate of the planktonic cells was $-0.12\ h^{-1}$. The negative value may be explained by analytical error (a small error in the count of detached biofilm cells at the high flow rate results in a large error in the calculation of the specific planktonic cell growth rate) or by decreasing cell viability or cell dieoff in RT1 water. The latter is quite possible as suggested by the declining viable bacterial numbers in the consecutive RT operating at the low flow rate (Figure 12.4). The decrease in cell numbers with distance (time) through the reactor may reflect a nutrient limitation.

The specific growth rate of planktonic cells in the finished water was very low. Thus, the significant increase of cell numbers in the bulk water was primarily due to detachment of biofilm cells. Net cell production rate is the product of specific growth rate and population cell numbers. In the biofilm, the low specific cell growth rate was countered by a high density of cells resulting in a net biofilm cell production rate that was relatively high compared to the planktonic cell production rate and the input rate of cells with the influent. Another determination also suggests that planktonic growth can be neglected

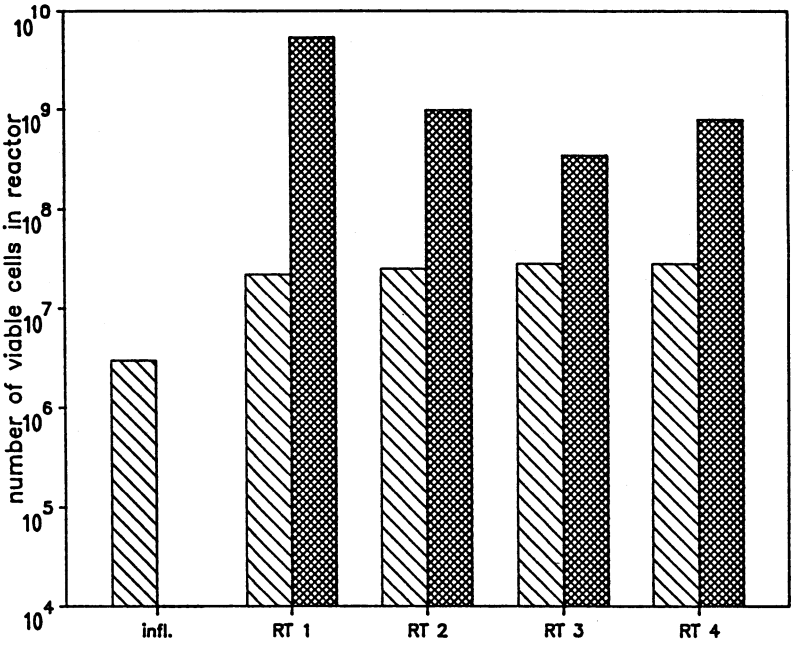

Figure 12.4 Number of viable cells (Heterotrophic Plate Count) in the bulk water (= planktonic cells) and biofilm in a RotoTorque system after an exposure time of 85 days to chlorine-free water. The overall hydraulic residence time was 7.3 hours. The data represent cell numbers per RotoTorque or per 1.575 l (= RT volume) influent.

▨ = planktonic cells. ▩ = biofilm cells.

relative to the number of cells detaching from the biofilm. If all microbial growth was occurring in suspension, μ would have to be approximately 0.5 h^{-1} to account for the growth observed in RT1 for the low flow rate. Such a high growth rate may be possible for bacteria living under rather ideal conditions but not for organisms in unsupplemented tap water at low temperature (van der Kooij and Hijnen, 1982; van der Kooij et al., 1982).

LeChevallier (1987) observed a large increase in coliforms between a water treatment plant effluent and water samples taken 1.1 km down stream of that plant. The hydraulic residence time in this 1.1 km stretch moved from 91 to 102 min. The large increase in coliform numbers could be explained by the planktonic growth of these bacteria only if they had a specific growth rate of more than 2 h^{-1}. This is much too high for the low nutrient conditions, low temperature, and high chlorine residuals found in the water main. High pipe line pressures and other operating parameters ruled out cross connections and backsiphonage as causes of the coliform episode. The only remaining possible explanation was that these coliform bacteria came from biofilms in the water main.

12.5 Disinfectants and Biofilm in the Water Distribution System

Drinking Water Disinfection Water utilities usually apply one or more disinfectants during or at the end of the water treatment process. The most often applied disinfectant in the United States is chlorine, although the use of chloramines is on the increase. In some cases, chlorine dioxide is applied. After a disinfectant has been applied to the water, the disinfectant residual concentration begins to decline due to the reaction with various dissolved and suspended compounds in the water phase and with biofilm and pipe wall materials. The disinfectants are reactive compounds which oxidize substances like Fe^{2+}, Mn^{2+}, sulfide, and organic compounds (Characklis et al., 1980). Disinfectant booster stations throughout the distribution system are sometimes used to maintain appropriate disinfectant residuals in the extremities of the system.

Effect of Chlorine on Bacteria in the Water Distribution System Van der Wende et al. (1988) studied the effect of different chlorine levels on biofilm accumulation and bacteriological water quality in the RTS system described earlier. RTS influent was prepared by mixing chlorine-free filter effluent with chlorinated treatment plant effluent in two feed tanks equipped with overflows. One RTS received influent with a chlorine concentration of 0.8 mg/l free chlorine while another RTS received influent with a free chlorine concentrations of 0.2 mg/l. Chlorine reacted with various components of the system resulting in declining chlorine concentrations through the RTS. The flow rate through both systems was 1.8 l/h and the rotational speed of the drums was 150 rpm, simulating a flow velocity of 0.92 m/s (3 ft/s).

Biomass accumulation in the system with the low chlorine concentration (0.2 mg/l) was determined for exposure times of 17 and 45 days. Biofilm cell numbers increased through the RTS (Figure 12.5a and b). The accumulation of planktonic cells was correlated with biofilm accumulation, i.e., as biofilm accumulation increased, planktonic cell numbers increased. The maximum number of planktonic cells observed at low chlorine concentrations was approximately the same as for the chlorine-free system. However, the free-chlorine residual in the bulk water never decreased below 0.05 mg/l at any point in the system.

Biomass accumulation in the system with the high chlorine concentrations (0.8 mg/l) was determined for exposure times of 17 and 38 days (Figure 12.6a and b). Not surprisingly, viable cell numbers were lower than in other experiments due to higher chlorine levels. For the first 17 days, the RTS remained essentially free of cells. However, after approximately 38 days, a "patchy" biofilm had accumulated throughout most of the RTS. At the same time, low numbers of planktonic cells were found throughout the RTS. Free-chlorine residuals never decreased below 0.5 mg/l in the bulk water at any point of the system.

The presence of chlorine caused reduced accumulation of biofilm and in-

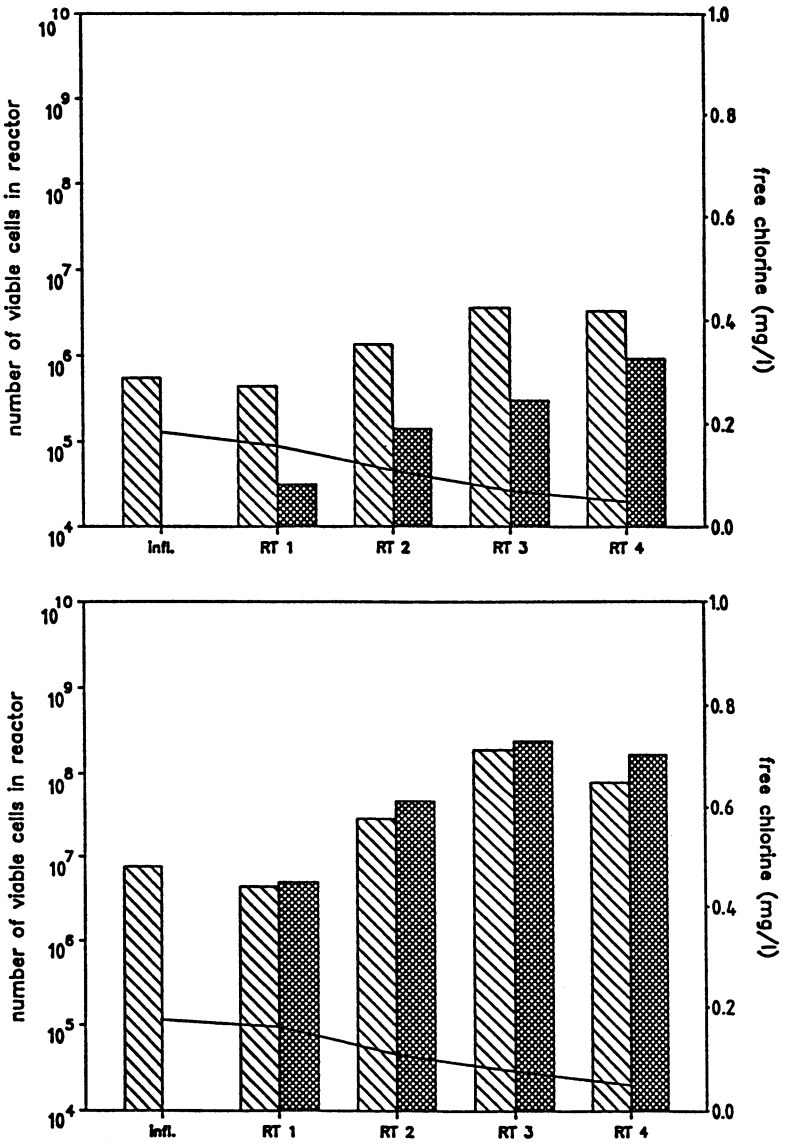

Figure 12.5 a. Numbers of viable cells (Heterotrophic Plate Count) in the bulk water (= planktonic cells) and biofilm of a RotoTorque System after an exposure time of 17 days to water with a low chlorine concentration. The overall hydraulic residence time was 3.5 hours. The data represent cell numbers per RotoTorque or per 1.575 1(= RT volume) influent. b. Numbers of viable cells (Heterotrophic Plate Count) in the bulk water (= planktonic cells) and biofilm of a RotoTorque System after an exposure time of 45 days to water with a low chlorine concentration. The overall hydraulic residence time was 3.5 hours. The data represent cell numbers per RotoTorque or per 1.575 1 (= RT volume) influent.

= planktonic cells. = biofilm cells. — = chlorine.

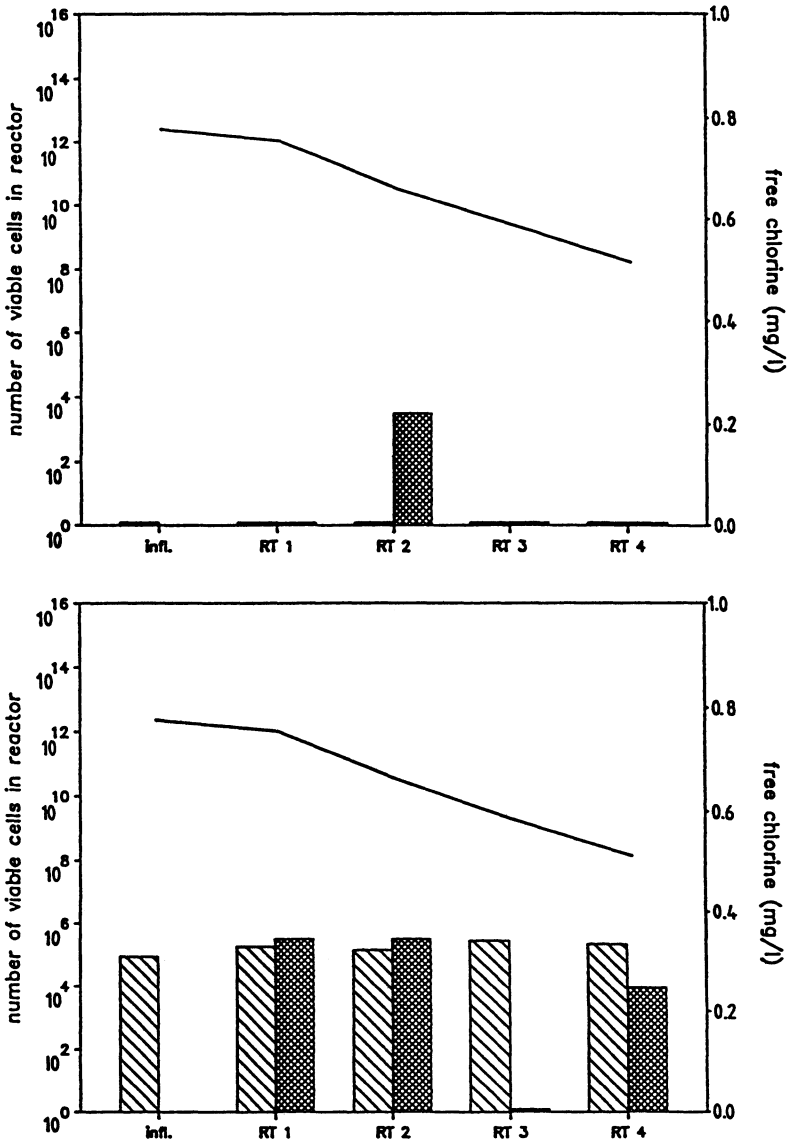

Figure 12.6 a. Numbers of viable cells (Heterotrophic Plate Count) in the bulk water (= planktonic cells) and biofilm of a RotoTorque System after an exposure time of 17 days to water with a high chlorine concentration. The overall hydraulic residence time was 3.5 hours. The data represent cell numbers per RotoTorque or per 1.575 1 (= RT volume) influent. b. Numbers of viable cells (Heterotrophic Plate Count) in the bulk water (= planktonic cells) and biofilm of a RotoTorque System after an exposure time of 38 days to water with a high chlorine concentration. The overall hydraulic residence time was 3.5 hours. The data represent cell numbers per RotoTorque or per 1.575 1 (= RT volume) influent.

= planktonic cells. = biofilm cells. — = chlorine.

fluenced its distribution in the RTS. For the chlorine-free system, most biofilm accumulated at the entrance to the reactor system, probably through the rapid consumption of nutrients. Less biofilm accumulation was observed in the remaining RTS (Figure 12.4). A low chlorine concentration (0.2 mg/l) in the RTS influent reduced the biofilm in the first reactor, thus leaving a larger potential for biofilm accumulation in the subsequent reactors where the free-chlorine residual was lower (Figure 12.5). Planktonic cell numbers increased to 1 to 2 orders of magnitude in conjunction with increased biofilm accumulation between the exposure times of 17 and 45 days.

At the higher chlorine concentration (0.8 mg/l), planktonic cells were observed only in the presence of biofilm (Figure 12.6). Planktonic cells appeared only after an initial biofilm had been established. These results are consistent with the results from the growth rate experiment which showed the importance of biofilm cell growth in the increase of planktonic cell numbers.

Biofilm cell growth and detachment may be even more dominant in water distribution systems with low chlorine concentrations than in chlorine-free systems. The biofilm environment is believed to protect cells against the activity of chlorine by diffusional resistance and neutralization of chlorine as a result of the reaction with biofilm and pipe wall materials. Thus, microbial growth in the biofilm will be less inhibited than the growth of planktonic cells which experience a higher chlorine concentration.

Bacterial species less susceptible to chlorine may accumulate selectively in the distribution system. Biofilm and planktonic cells isolated from the RTS at high chlorine concentration after 38 days represented only a few species. An estimated 75 to 90% belonged to one species of the genus *Pseudomonas*. Wolfe et al. (1985) found that these bacteria can be highly chlorine tolerant. The colonies of this species, on R_2A-agar, were clearly of the "smooth type," indicating the production of significant amounts of extracellular polymer substances (EPS). Rate and extent of EPS formation in situ is influenced by many environmental conditions but it is clear that this species has the potential of producing significant amounts of EPS. The large difference in species diversity between chlorinated and unchlorinated water was also observed by other experimenters (Brazos and O'Connor, 1985; Maki et al., 1986). Ridgway and Olson (1982) described large differences in chlorine and chloramine susceptibility between bacteria obtained from chlorinated drinking water and those from a chlorine-free system. Wolfe et al. (1985) found that a red pigmented *Flavobacterium sp.* was highly chlorine-tolerant. Flavobacteria have been frequently isolated from disinfected drinking water. Thus, chlorine may lead to the selection of a limited number of microbial species in the early stages of biofilm accumulation. For example, in chlorine-free water, different species may randomly adsorb but subsequent cell growth will be dominated by the "fast growers." In chlorinated water, organisms less susceptible to the damaging effects of chlorine may adsorb to the pipe surface and can selectively accumulate in the system. Further enhancement of chlorine tolerance may be achieved by different processes such as continued selection, mutation, and/or recombi-

nation of genetic material (Kelly et al., 1983). In addition, a film of organisms containing high amounts of EPS or accumulating various reduced species characterized by a high chlorine demand may further insulate other organisms from the effects of chlorine. Although this protective mechanism is often suggested by researchers, little, if any, evidence is available that EPS protects planktonic cells against disinfection. LeChevallier et al. (1987) studied the effect of cell encapsulation with EPS on the cell susceptibility towards chlorine and monochloramine. They used two strains of *Klebsiella pneumoniae*, an encapsulated strain and a mutant which was incapable of EPS production. Dilute suspensions of the cells in low nutrient water were exposed to the disinfectants. Despite large differences in the amount of EPS, no change in the susceptibility to free chlorine or monochloramine was observed between the two strains. Obviously, cell encapsulation was not a sufficient penetration barrier to cause a measurable difference in the level of disinfection at the disinfection concentration examined. However, EPS exists in many chemical forms and some may be more protective than others depending, on the reaction kinetics with chlorine. Furthermore, a protective function of EPS may be more important in biofilms where the large quantity of EPS protects the cells deeper in the biofilm.

Disinfectant Chemistry and Biofilm Control LeChevallier et al. (1987) conducted experiments examining the disinfection of biofilms with chlorine, monochloramine, and chlorine dioxide. They first determined the disinfectant doses (CT = concentration $\times$ time) that caused 99% kill in dispersed biofilm cell populations. These doses (different for the various disinfectants) were then applied to the initial biofilm to determine the percentage kill in these sessile cell populations. The results indicated the relatively high biofilm disinfection efficacy of monochloramine as compared to free chlorine (HOCl and OCl^-). Apparently, monochloramine is more successful in penetrating the biofilm than free chlorine. The extent of penetration is dependent on two competing rate processes: diffusion and reaction. Siegrist and Gujer (1985) studied the diffusion rate of several different molecules and ions in biofilm accumulated on a permeable membrane. The biofilm had a total solids content of 1.8 to 2.6% and reduced the molecular diffusion rate of the test compounds to 50 to 80% of the value in water. The biofilm diffusivities of the species (glucose, sodium, and bromide) were linearly related to their diffusivities in water. The diffusivity of monochloramine will probably be similar to that of free chlorine. However, there is a large difference in the reactivity of chlorine and monochloramine with biological matter.

In the last decade, researchers have determined that chlorine reacts much faster and with a larger variety of biological organic compounds than the chloramines. Chlorine causes oxidation, hydrolysis, or deamination of virtually every component of the bacterial cell. In contrast, monochloramine reacts rather specifically with nucleic acids, tryptophan, and sulfur-containing amino acids (Jacangelo and Olivieri, 1985) but does not react with EPS or sugars such as ribose. The reaction of chloramines with sensitive amino acids has been re-

ported by several researchers but not the reaction with nucleic acids. Olivieri et al. (1980) reported that chlorine reacted much faster with RNA than mono- and dichloramine did. These chemical properties of chloramines do not make them particularly good primary disinfectants. Wolfe et al. (1985) studied the inactivation of heterotrophic bacterial populations in finished drinking water. They found that prereacted chloramines were less effective disinfectants than chlorine. Members of the genera *Pseudomonas, Enterobacter, Acinetobacter, Moraxella,* and *Alcaligenes* which were inactivated after 1 min exposure to 1.0 mg/l free chlorine were recovered in low levels after 30 and 60 min of exposure to 1.0 mg/l prereacted chloramines. Water consumers that are situated close to a water treatment plant often receive water with a very short disinfectant contact time. Approximately 25% of the U.S. water utilities report contact times of less than 10 min between disinfectant application and first consumer. However, the same chemical properties that negatively influence the efficacy of this compound as a primary disinfectant increase the biofilm penetration capabilities of this disinfectant. Thus, cells deeper in the biofilm are not as well protected against the activity of this disinfectant. The chemical properties of chloramines also make them more effective than chlorine in inhibiting biofilm accumulation in the extremities of a water distribution network or in parts of the system with low flow rates. A highly reactive compound like chlorine will not even reach such areas that are far from the point of chlorine addition.

12.6 Summary

Biofilms accumulate in drinking water distribution systems primarily by growth at the expense of nutrients in the water. The biofilms are continuously seeded with planktonic organisms entering the system via breakthrough. Biofilm cells are generally less susceptible to disinfectants than planktonic cells. As a consequence, excessive viable cell numbers may accumulate in the bulk water from detachment of cells from the biofilm in areas where insufficient disinfectant residual is maintained.

Certain disinfectants may have properties more conducive to controlling biofilm populations. Less reactive, more persistent compounds, such as chloramines, maintain a higher disinfectant residual throughout the distribution system and may penetrate the biofilm more effectively and, thus, control biofilm organisms better than free chlorine. However at present, little quantitative information is available regarding disinfection kinetics in biofilms.

The authors gratefully acknowledge the financial support and in-kind services from the South Central Connecticut Regional Water Authority and the American Water Works Service Company. The National Science Foundation, the Office of Naval Research, and the IPA Industrial Associates are also acknowledged for their partial support.

References

Allen, M.J., Taylor, H., and Geldreich, E.E. 1980. The occurrence of microorganisms in water main encrustations. *American Water Works Association Journal* 72:614–625.

Banerji, S.K. 1978. Quality deterioration in mains. *Journal of the Missouri Water & Sewage Conference* 1:46–56.

Brazos, B.J. and O'Connor, J.T. 1985. A transmission electron micrograph survey of the planktonic bacteria population in chlorinated and nonchlorinated drinking water. *Proceedings, Water Quality Technology Conference: Advances in Water Analysis and Treatment*. Houston, TX, 275–305.

Characklis, W.G. 1988. *Bacterial Regrowth in Distribution Systems*. Research Report. American Water Works Association Research Foundation. Denver, CO, 107–114.

Characklis, W.G., Trulear, M.G., Stathopoulos, N., and Chang, L.C. 1980. Oxidation and destruction of microbial films. pp. 349–368 *in* Jolley, R.L., W.A. Brungs, R.B. Cumming, and V.A. Jacobs (eds.), *Water Chlorination: Environmental Impact and Health Effects*, Ann Arbor Science Publishers, Inc., Ann Arbor, MI.

Colbourne, J.S., Dennis, P.J., Trew, R.M., Berry, C., and Vesey, G. 1988. *Legionella* and public water supplies. *Proceedings, International Conference on Water and Wastewater Microbiology*. Newport Beach, CA.

Haudidier, K., Paquin, J.L., Francais, T., Hartemann, P., Grapin, G., Colin, F., Jourdain, M.J., Block, J.C., Cheron, J., Pascal, O., Levi, Y., and Miazga, J. 1988. Biofilm growth in drinking water network: a preliminary industrial pilot plant experiment. *Proceedings, International Conference on Water and Wastewater Microbiology*. Newport Beach, CA.

Jacangelo, J.G. and Olivieri, V.P. 1985. Aspects of the mode of action of monochloramine. pp. 575–586 *in* R.L. Jolley, R.J. Bull, W.P. Davis, S. Katz, M.H. Roberts, Jr., and V.A. Jacobs (eds.), *Water Chlorination, Chemistry, Environmental Impact and Health Effects*, Lewis Publishers Inc., Chelsea, MI.

Kelly, A.J., Justice, C.A., and Nagy, L.A. 1983. Predominance of chlorine tolerant bacteria in drinking water systems. *Abstracts Annual Meeting American Society for Microbiology* 0, Q122.

LeChevallier, M.W., Babcock, T.M., and Lee, R.G. 1987. Examination and characterization of distribution system biofilms. *Applied and Environmental Microbiology* 53:2714–2724.

Lee, S.H., O'Connor, J.T., and Banerji, S.K. 1977. Biologically mediated deterioration of water quality in distribution systems. *Proceedings, American Water Works Association 5th Annual Water Quality Technology Conference*. Kansas City, MO.

Maki, J.S., LaCroix, S.J., Hopkins, B.S., and Staley, J.T. 1986. Recovery and diversity of heterotrophic bacteria from chlorinated drinking water. *Applied and Environmental Microbiology* 51:1047–1055.

McFeters, G.A., Kippin, J.S., and LeChevallier, M.W. 1986. Injured coliforms in drinking water. *Applied and Environmental Microbiology* 51:1–5.

O'Connor, J.T. and Banerji, S.K. 1984. *Biologically Mediated Corrosion and Water Quality Deterioration in Distribution Systems*. EPA-600/52-84-056. U.S. Environmental Protecting Agency, Cincinnati, OH.

Olivieri, V.P., Dennis, W.H., Snead, M.C., Richfield, D.T., and Kruse, C.W. 1980. Reaction of chlorine and chloramines with nucleic acids under disinfection conditions. pp. 651–653 *in* Jolley, R.L., W.A. Brungs, R.B. Cumming, and V.A. Jacobs (eds.), *Water Chlorination: Environmental Impact and Health Effects*, Ann Arbor Science Publishers, Inc., Ann Arbor, MI.

Ridgway, H.F. and Olson, B.H. 1982. Chlorine resistance pattern of bacteria from 2 drinking water systems. *Applied and Environmental Microbiology* 44:972–987.

Ridgway, H.F. and Olson, B.H. 1981. Scanning electron microscope evidence for bacterial

colonization of a drinking-water distribution system. *Applied and Environmental Microbiology* 41:274–287.

Siegrist, H. and Guyer, W. 1985. Mass transfer mechanisms in a heterotrophic biofilm. *Water Research* 19:1369–1378.

Tuovinen, O.H., Button, K.S., Vuorinen, A., Carlson, L., Mair, D.M., and Yut, L.A. 1980. Bacterial, chemical and mineralogical characterization of tubercles in distribution pipelines. *Journal of the American Water Works Association.* 72:626–635.

van der Kooij, D. and Hijnen, W.A.M. 1982. Nutritional versatility of a starch-utilizing flavobacterium at low substrate concentrations. *Applied and Environmental Microbiology* 45:804–810.

van der Kooij, D., Visser, A., and Oranje, J.P. 1982. Multiplication of fluorescent pseudomonas at low substrate concentrations in tap water. *Antonie van Leeuwenhoek* 48:229–243.

van der Wende, E., Characklis, W.G., and Smith, D.B. 1989. Biofilms and bacterial drinking water quality. *Water Research*, in press.

Wolfe, R.L., Ward, N.R., and Olson, B.H. 1985. Inactivation of heterotrophic bacterial populations in finished drinking water by chlorine and chloramines. *Water Research* 19:1393–1404.