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Section II
BIOFOULING

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Coliform Regrowth and Biofilm Accumulation in Drinking Water Systems: A Review

Anne K. Camper

I. INTRODUCTION

Coliforms in finished potable water are of concern to the U.S. drinking water industry for a variety of reasons. These bacteria are used as indicator organisms; their presence in water is interpreted as evidence of contamination by fecal material. When coliforms are present in the absence of cross connections or disinfection barrier breakthrough, the microbiological quality of the water is unnecessarily questioned. Since the presence of coliforms is regulated, the utility must initiate a response action, including public notification and boil-water orders, which can seriously erode public confidence. The utility must also select a control measure based on unproven technology which may in fact have little chance of controlling the regrowth event. Coliforms which have proliferated in the system may also conceal the presence of those which arise from sources of genuine public health concern.

A recently completed survey of 164 water utilities[1] indicates that a relatively high number of water-distribution systems in the U.S. experience recurring coliform episodes. Many occurrences have been shown to be independent of obvious disinfection barrier breakthrough or cross contamination. It has generally been assumed that these coliforms grow

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in biofilms on pipe surfaces at the expense of nutrients in the bulk water. Biofilm-associated coliform growth in oligotrophic environments such as drinking water distribution systems is plausible, as there are sufficient nutrients present to support the growth of surface-associated bacteria, but not suspended organisms.[2–5]

The European drinking water industry is required to limit the presence of heterotrophic plate count bacteria (HPC) in water. The HPC are a much broader class of organisms than the coliforms, and are therefore more numerous and may arise from diverse sources. However, the same phenomenon of increases in HPC counts in finished water after adequate treatment has been seen, and the implication of growth in biofilms has been made. In addition to regulatory concerns, certain bacteria, such as members of the genus *Actinomycetes*, have been found on distribution surfaces[6] and may contribute to taste and odor problems. It may also be hypothesized that nitrification in monochloraminated systems, which may contribute to taste and odor problems as well as increased HPC growth, is the result of bacterial activity on pipe walls.

A biofilm is an accumulation of cells immobilized at a substratum and frequently embedded in an organic polymer matrix of microbial origin.[7] Biofilms are ubiquitous on surfaces in aqueous environments, and growth of coliforms and other nuisance organisms in biofilms occurs under yet undefined environmental conditions; regrowth events are detected when these organisms leave the biofilm and enter the bulk water. Because of the low-nutrient conditions, drinking water distribution system biofilms may be very thin and occur nonuniformly on the pipe walls.[8,9] Heterogeneities also occur in the distribution of species within the biofilm itself.[10–13] For example, coliforms are not uniformly distributed on pipe surfaces, but have been detected in tubercle materials from water-distribution systems.[14,15]

A wealth of circumstantial information exists, generally anecdotal, which relates operational and environmental variables with the appearance of coliforms in finished drinking water. This information is most often system specific and does not establish a relationship between variables and coliform presence. Factors which may contribute to distribution system microbial growth are (1) seasonal variation in water temperature; (2) availability of growth-promoting nutrients; (3) passage of dormant and/or injured organisms through the treatment process; (4) occurrence of distribution-system corrosion products; (5) distribution-system disinfection practices; and (6) distribution-system hydrodynamics.[1] These factors have been addressed in previous research[2,14,16–25] and two review articles.[26,27]

The broad range of experimental and field conditions reported in the literature, together with the inherent heterogeneities of biofilm systems, make it difficult to derive cause-and-effect relationships between

variables and coliform/HPC occurrences in finished drinking water. An integrated systems approach with standardized, relevant parameters and analyses will be required to generate information which can be used to successfully control regrowth events. Hence, multiple factors which may be important in regrowth as investigated in field studies, pilot scale studies, and laboratory investigations are addressed in this review.

II. TERMINOLOGY

The current literature uses numerous terms to describe the presence of bacteria in finished drinking water. "Occurrences", "episodes", and "events" are used synonymously to describe the phenomenon wherein coliforms in excess of standards are detected in finished water. Chronic or periodic appearance of bacteria in water has been termed "regrowth". Regrowth is presumed to be associated with the proliferation of bacteria within distribution systems, either in the bulk water or at the pipe walls. This contrasts with "breakthrough", which describes bacteria passing through the treatment and disinfection barrier. Regrowth and breakthrough are related, since bacteria must inoculate the system (breakthrough) in order to proliferate or grow at the expense of nutrients in the system (regrowth).

Other conceptual terms are "operational" and "environmental". Operational variables are those which can be controlled by the operator or system designer, e.g., disinfection and assimilable organic carbon (AOC). Environmental variables or parameters are outside the realm of control by the operator, and include seasonal effects (temperature, consumer demand, raw water quality), hydraulic regime, and pipe composition.

Two terms have been developed to describe the organic carbon fraction in drinking water which is utilized by bacteria as a carbon source. These are operationally based and depend on the type of analysis performed to obtain the information. AOC represents the small, readily degradable fraction of dissolved organic carbon and is traditionally defined as a calculated quantity based on the number of organisms which grow in the test water. Biodegradable organic carbon, biologically degradable organic carbon, and biodegradable dissolved organic carbon (BDOC) refer to the change in total organic carbon in the water after it has supported the growth of microorganisms. The BDOC content of a given water may be greater than the AOC value due to the more diverse microbial population used in the assay. The various procedures used to obtain AOC and BDOC values are summarized in a review article by Huck.[28]

III. FIELD STUDIES

A. Biofilms on Distribution-System Materials

Large numbers of bacteria were shown to be associated with distribution-system materials as early as 1979 when Allen et al.[8] reported that organisms were found in and on water-distribution main tubercles and encrustations. These findings were supported by Tuovinen et al.,[29,30] and more recently by other investigators.[2,14,15] More direct evidence has come through scanning electron microscopic observations of drinking water distribution-system surfaces.[6,9,14,31]

There is little conclusive evidence for the proliferation of coliforms on pipe surfaces, even though significant numbers of these bacteria may be found in the water. This is most likely due to the difficulty in sampling distribution systems and the patchy or heterogeneous manner in which the coliforms may proliferate on these surfaces. However, Olson[6] has recovered *Escherichia coli* from pipe surfaces. LeChevallier et al.[14] have enumerated coliforms from iron tubercles in one distribution system, and have concluded that the indicator organisms found in the bulk fluid originated from surface-associated growth.

Organisms that colonize the pipe materials in distribution systems originate from a variety of sources, but the majority are those which can be found in the source water of the system. This may also include coliform bacteria, especially total coliforms. These bacteria may pass the treatment and disinfectant barriers (breakthrough), enter the finished water in an injured state, and later recover either in the planktonic state or in biofilms. The injured bacteria may be undetected if bacteriological surveillance media such as m-Endo are used to evaluate the quality of drinking water. For example, Bucklin et al.[32] reported that filter effluent produced immediately after filter backwash could be characterized by high levels of turbidity and injured coliforms. Likewise, McFeters et al.[33] demonstrated that a significant number of injured coliforms (5.7 to 67.5 colony-forming units per 100 ml) were present in various drinking water samples while <1 per 100 ml were detected using m-Endo LES medium. Additional information on the significance of injured coliforms may be found in a book chapter by McFeters.[34]

B. Cause-and-Effect Investigations of Regrowth in Distribution Systems

When evaluating field data, it is important to recognize that separation of variables is often impossible. Distribution systems must provide drinking water to their consumers and, as such, cannot be operated as experimental systems. It is difficult to determine direct cause-and-effect

relationships in these systems when several variables change at once. For example, elevated summer temperatures are often accompanied by increased nutrient levels and turbidities; all have been implicated in influencing regrowth events. Comparisons between systems are also complicated by differences in hydraulic regime, distribution-system piping materials, raw and finished water quality, and disinfectant type and concentration. Reported general categories of variables which may control regrowth are chemical constituents and physical parameters.

1. Chemical Constituents

a. Nutrients

The growth of heterotrophic and coliform bacteria requires the presence of various nutrients, including carbon, nitrogen, and phosphorous. A general rule of thumb is that these substances must be present in a ratio of approximately 100:10:1, respectively, for balanced growth. Carbon-containing compounds are the source of energy (electron donor) and carbon (for anabolic processes) for these bacteria, while nitrogen and phosphorous are required for biosynthesis. Other growth factors such as trace metals and organic cofactors may also be necessary for bacterial growth, although these are generally presumed to be nonlimiting in a mixed microbial population. Heterotrophs also require a terminal electron acceptor, which in these systems is typically oxygen. It is possible, however, for the fermentative organisms such as coliforms to use an internal organic compound as their terminal electron acceptor in the absence of oxygen.

Because carbon must be present in significantly greater quantities than the other nutrients, a major focal point in recent investigations on regrowth has been the concentration of the utilizable organic carbon fraction in drinking water, which ranges from 3 to >1000 $\mu g\ l^{-1}$.[25,35,36] This is an operational parameter which could be controlled at the treatment plant, particularly through the use of biological treatment. Van der Kooij[37] has found a significant correlation between AOC concentrations and the regrowth of HPC in Dutch distribution systems. Growth at levels <10 $\mu g\ C\ l^{-1}$ was limited. In an investigation at a New Jersey facility, LeChevallier et al.[25] determined that the occurrence of coliform bacteria in the distribution system could be related to a number of factors, including AOC concentrations in excess of 50 $\mu g\ C\ l^{-1}$. In an earlier study[14] it was shown that AOC concentrations decreased with travel time of the water from the treatment plant. It was hypothesized that this decrease was due to utilization of the carbon by biofilm organisms. The loss of utilizable organic material with travel time from the plant has also been demonstrated with the BDOC procedure.[38] In contrast, Opheim and

Smith[39] demonstrated no correlation between AOC concentrations and the presence of coliforms in their distribution system.

Nitrogen is present in water in the form of organic nitrogen, ammonia, nitrate, and nitrite. All forms are utilizable by different microorganisms. Studies of nitrogen concentrations in distribution systems as related to microbial regrowth events are limited. Donlan and Pipes[21] indicated that there was no correlation between organic nitrogen, ammonia, nitrate, or nitrite with attached microbial population density. In another investigation, concentrations of nitrogen in water, which were adequate for balanced microbial growth, did not change with travel through a distribution system, whereas the AOC concentration decreased.[14,25] It is generally assumed that nitrogen is not limiting in distribution systems due to turnover of existing cellular nitrogen and the low concentrations which are required for growth and maintenance.

Similar to nitrogen, phosphorous is required in very low concentrations for microbial growth. It is also not presumed to be limiting in drinking water systems,[21] although one study has shown that low phosphorous concentrations may have restricted microbial growth in a section of distribution main.[40]

b. Disinfectants

It is generally recognized that simply raising the chlorine concentration in drinking water often does not control biofilms or the presence of indicator organisms.[14,17,18,23,41–43] In extreme cases chlorine doses up to 12 mg l^{-1}, which are far in excess of usual concentrations, were inadequate in attempts to control coliform regrowth.[44,45] When distribution-system biofilms were examined, no correlation was found between free chlorine residuals and the number of HPC organisms per unit surface area.[46]

Chloramines have become increasingly popular as a drinking water disinfectant. In a recent distribution-system comparison, chloramines were more effective at reducing the number of biofilm total coliforms and HPC than chlorine.[47] In another study where statistical evaluation of the influence of chloramine concentration on attached microbial populations in a distribution system was made, an inverse relationship was established.[21]

The reasons for the differences in disinfectant performance cannot be assessed in drinking water distribution systems due to the multitude of variables which influence disinfectant efficacy. However, these variables can be controlled in pilot and/or laboratory experiments, and their importance will be addressed in the following sections on pilot plant studies and laboratory investigations.

2. *Physical Attributes*

a. Temperature

As an interdependent variable, temperature has been implicated as contributing to increased microbial populations in distribution systems. Most, but certainly not all, regrowth events occur in summer months when water temperatures are highest. LeChevallier et al.[25] demonstrated an association between coliform regrowth and water temperatures above 15°C. In London, it was reported that coliform occurrences increased when the water temperature exceeded 20°C and declined when the temperature was less than 14°C.[48] Likewise, Donlan and Pipes[21] reported a direct relationship between water temperature and the attached microbial population density.

b. Precipitation

Increases in nutrients associated with runoff from rainfall have been proposed by some investigators as a contributing factor to regrowth events.[25,45] The nutrients are collected in surface water sources, pass through the treatment plant, and are then available for microbial growth in the distribution system.

c. Pipe Materials

Various pipe materials are used in distribution systems, including cement lined, asphaltic coated, mild steel, ductile iron, and PVC. When examining steel and iron surfaces, the interaction between the ferrous metal and disinfectants must be considered, as well as the production of corrosion products and tubercles. As stated before, coliforms have been found in association with tubercles in some distribution systems,[14,15] while not in others. When pipe materials have been compared, the number of bacteria on an unlined cast-iron pipe were the highest, while PVC-pipe biofilm densities were the lowest.[47] The relative importance of corrodible pipe materials in regrowth events has been shown indirectly by the reduction or control of coliform bacteria by application of corrosion inhibitors,[17,45] although this is not universally true.[14]

d. Hydraulic Regime

Hydraulic regime in distribution systems is controlled by original design and layout, but is also heavily influenced by customer water demand. Large daily fluctuations can occur between peak use periods (mornings, evenings) and night. Low flow conditions can lead to decreased disinfectant concentrations and nutrient transport. Higher flow rates result in increased nutrient transport to attached organisms, but also increases disinfectant flux and shear stresses at the surface. Associations between

flow rates in distribution systems and regrowth events are therefore difficult to establish, although one study has shown an inverse relationship between flow rates and biofilm density.[21]

Residence time of the water in a system may also be an important factor in determining where organisms are most likely to occur on pipe surfaces, since AOC and BDOC concentrations have been shown to decrease as water moves through a distribution system.[14,25,37,38]

IV. PILOT PLANT STUDIES

A pilot system provides flexibility in conducting controlled experiments and accessing data under conditions which are far more controlled than those found in an operating system of water mains. Pilot systems must be carefully designed to simulate critical parameters in operating drinking water distribution systems and maximize separation of variables. These systems provide an important "proving ground" for relevancy of laboratory findings or the clarification of presumed phenomena in the field. Unfortunately, the presence of well-constructed and operated pilot facilities for research on regrowth are limited due to expense of construction and operation.

Two basic types of pilot pipe systems are found in the literature: (1) once-through and (2) recirculating. Examples in a review on pipe-loop systems for investigating corrosion by Levin and Schock[49] and others[50–55] indicate a majority of pilot systems have once-through flow. Characklis[2] has developed a staged pilot water-distribution system using RotoTorques with controllable residence time to represent plug-flow conditions and residence times up to several days, as found in the field. Once-through pilot systems lack this important capability. Haudidier et al.[5] describe the use of six pilot pipeline recirculation loops, configured in series, in a pilot system developed in Nancy, France, to conduct studies of attached microbial films in water-distribution systems. The French system is designed so that each loop can be operated as a perfectly mixed reactor and, when operated in series, can simulate the plug flow of a pipeline with a high axial-dispersion coefficient. An additional benefit of the latter system is the ability to separate residence-time effects from those of shear stress.

A. Cause-and-Effect Investigations

Few published papers are available on the use of pilot systems to investigate cause-and-effect relationships between drinking water quality parameters and biofilm accumulation. Using RotoTorque systems, it was

demonstrated that the amount of biofilm which accumulated in sequential reactors depended upon chlorine concentration.[2,16] When no chlorine was present, most biofilm accumulation occurred at the entrance to the system. It was hypothesized that this occurred due to rapid consumption of available nutrients. At low chlorine concentrations, biofilm accumulation was reduced at the entrance to the system where chlorine concentrations were higher; as chlorine was consumed at greater residence times in the reactor, biofilm accumulation increased at the expense of the nutrients. In similar experiments with the same system, it was shown that a chlorinated backwash of the upstream pilot mixed-media (anthracite/sand) filter decreased the amount of biomass on the filter media with a subsequent increase in the amount of total organic carbon in the filter effluent. There was a concomitant increase in the amount of biofilm within the RotoTorques, indicating that the amount of available carbon influenced biofilm accumulation.[2] Using a pipe loop system, Levi and Joret[38] report that virtually all BDOC was consumed within 24 h. Both groups of investigators, although using different systems, concluded that the numbers of planktonic organisms in the water can originate only from detached biofilm organisms and not growth in the bulk phase. In a study using the French recirculating pipe-loop system, biofilm accumulation and AOC depletion were greatest in the first loop (40-h residence time).[5] LeChevallier et al.[50] utilized a model pipe system to determine the influence of chlorine and monochloramine disinfectant efficacy on biofilms which had accumulated on several pipe materials. Biofilms on galvanized, copper, or PVC surfaces were readily disinfected by free chlorine or monochloramine (1 mg l^{-1}). Iron-pipe surface-associated bacteria were more susceptible to monochloramine than free chlorine, but higher monochloramine concentrations (4 mg l^{-1}) were required than on the other surfaces.

V. LABORATORY INVESTIGATIONS

Although an abundance of basic laboratory research has been performed on biofilms,[7] the focus of this section will be restricted to those directly related to drinking water research. In well-designed laboratory investigations, proper control of variables is possible. However, the scope of these studies is often so narrow that applicability of the results to actual distribution systems is difficult. Proper design of experiments which take into account features relevant to pipe systems is critical.

Determining the response of bacteria to potential nutrients has been of key importance, and has been described in the section on AOC, BDOC, and regrowth-potential methods. This has not been the only area of interest. The ability of coliforms isolated from drinking water to

grow on extremely low carbon concentrations and temperatures has been established.[56] The source of growth-stimulating compounds has also been investigated. Fedorak and Huck[57] demonstrated that cyanobacterial products, which may be a source of nutrients in drinking water, were readily mineralized by HPC bacteria. The importance of crushed tubercle material in enhancing coliform growth has been substantiated by several researchers[8,17,58,59] and refuted by one.[56]

Much of the fundamental research on efficacy of disinfectants against biofilms has been conducted in laboratory studies. Initially, it was shown that attachment of organisms to surfaces resulted in decreased disinfection by chlorine.[60–63] In more elaborate studies using biofilms on relevant surfaces, Berman et al.[63] reported that monochloramine was no more effective than chlorine. In another study, LeChevallier et al.[64] showed that there was decreased sensitivity to chlorine conveyed to organisms simply by being attached to a surface and that this effect was enhanced in older biofilms. When these biofilms were treated with monochloramine, more disinfection was observed than with free chlorine. In a related study, the interaction of oxidizing disinfectants, biofilms, and various pipe surfaces was investigated.[65] Monochloramine was less effective than free chlorine against suspended organisms, but the reverse effect was seen with biofilm bacteria. The same effect was seen in analyses where the CT values (concentration-time of biocide exposure for 99% inactivation) of biofilms and suspended (dispersed biofilm) cells of *Pseudomonas aeruginosa* exposed to chlorine and monochloramine were calculated.[66] A laboratory system for assaying the efficacy of monochloramine against biofilms containing coliforms has also been developed.[48] This device supports a stable heterotrophic bacteriological population representative of that found in their distribution system. When challenged with a coliform, it became incorporated into the biofilm and remained as a component. Monochloramine concentrations of 0.6 mg l^{-1} caused a loss of the coliform; at lower disinfectant concentrations it persisted.

VI. INTEGRATING SCALES OF OBSERVATION

Developing relevant information on distribution-system microbial growth which can be effectively applied by the water industry requires an integrated research effort. Microbial and chemical processes occur in the water-distribution system as a whole, within individual pipes, and in biofilms at the surface of the pipe. To investigate the problem, research

must be defined in terms of scales of observation. Microscale research, e.g., millimeters in size ($<10^{-3}$ m), focuses on measurements and interactions at the cellular level within the biofilm and is represented in the above material by laboratory studies. At the mesoscale (1 mm to 10 m), fluid dynamics and system geometries become important, since the activity of the biofilm is dependent on the transport and interfacial-transfer phenomena created by these conditions (within a pipe). Well-designed pilot scale research falls within this category. The macroscale (>10 m), or field system, is influenced by system-operating parameters and environmental variables, such as influent water quality and water demand, as well as regulations regarding water quality. Experiments at the micro- and mesoscale must be carefully designed so the results can be "scaled up" to the macroscale. For example, microscale observations in a biofilm must be done under conditions relevant to the fluid dynamics in a pipe system (mesoscale) in order to model an operating distribution system (macroscale).

While the overview presented in this paper touches on the relevance of phenomena, parameters, and processes at each scale of observation, it is clear that integration of scales has yet to be accomplished. Nevertheless, some complimentary conclusions appear by comparing results at the various scales:

- Organic carbon is probably the most important nutrient in a distribution system, as it has been shown to support coliform growth in the laboratory as well as being correlated with regrowth in pilot and full-scale systems.
- While little work on the effect of temperature has been done at the micro- or mesoscale, and macroscale phenomena are often linked with other variable variations (e.g., nutrients, turbidity), it is reasonable to expect higher coliform proliferation with increased temperature due to increased enzyme kinetics. These organisms often have optimum growth temperatures well above those found in distribution systems and are quite sensitive to changes in the lower temperature range.
- Disinfection efficacy appears to vary widely with system parameters and variables as well as disinfectant type. It is tempting to conclude that slower-acting biocides (e.g., monochloramine) are superior to highly reactive compounds (e.g., free chlorine) in treating biofilms. However, there is insufficient supporting evidence at this time. Nevertheless, it is also clear that disinfectant alone may not be capable of controlling regrowth events.

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