Application of the biofilm coupon to bacterial regrowth potential
by Xiaoming Xu

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
Montana State University

Abstract:
The growth of biofilm in drinking water distribution systems often contributes to most of the increase in bacterial numbers from the treatment plant effluent to the customer tap. When coliforms are shed from distribution system biofilms, their appearance in water samples is taken as indicative of a public health risk. To seek strategies for controlling regrowth of coliforms in distribution systems, methods should be developed to determine the bacterial growth potential. The biofilm coupon technique, a patented device of the Center for Biofilm Engineering, has been improved and used to study the factors affecting bacterial regrowth in the laboratory and in a pilot scale pipe loop distribution system in the Bozeman Drinking Water Treatment Plant. This research is a part of the project "Factors Limiting Microbial Growth in the Distribution System", which has been funded in part by the American Water Works Association Research Foundation. The experimental results showed that the coupon performance was affected by organic carbon loading, temperature and chlorine residual. The growth kinetic parameters (maximum growth rate; Atmax, yield Yx/s, half-saturation constant Ks and temperature constant 6) were estimated from the experimental data for two species of bacteria (Klebsiella pneumoniae and Pseudomonas fluorescens P17) . The threshold chlorine concentration for K. pneumoniae growth in ¦ the biofilm coupon also was determined experimentally. Based on the laboratory results, a mathematical model was used to predict the effect of organic carbon concentration, temperature and biocide residual on bacterial growth in the biofilm coupons. The experimental results in the pilot system showed the bacterial growth rate in the biofilm coupon correlated significantly with the biofilm growth on the pipe walls, and qualitatively validated the laboratory and model results. It demonstrated that the biofilm coupon can be used to simulate biofilm growth in distribution system and as an indicator of microbial growth potential. >

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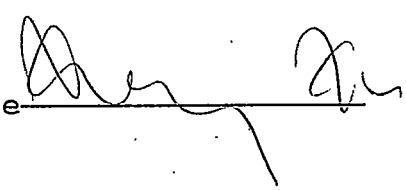
Signature 

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ABSTRACT

The growth of biofilm in drinking water distribution systems often contributes to most of the increase in bacterial numbers from the treatment plant effluent to the customer tap. When coliforms are shed from distribution system biofilms, their appearance in water samples is taken as indicative of a public health risk. To seek strategies for controlling regrowth of coliforms in distribution systems, methods should be developed to determine the bacterial growth potential. The biofilm coupon technique, a patented device of the Center for Biofilm Engineering, has been improved and used to study the factors affecting bacterial regrowth in the laboratory and in a pilot scale pipe loop distribution system in the Bozeman Drinking Water Treatment Plant. This research is a part of the project "Factors Limiting Microbial Growth in the Distribution System", which has been funded in part by the American Water Works Association Research Foundation. The experimental results showed that the coupon performance was affected by organic carbon loading, temperature and chlorine residual. The growth kinetic parameters (maximum growth rate μ_{max}, yield $Y_{x/s}$, half-saturation constant K_s and temperature constant θ) were estimated from the experimental data for two species of bacteria (*Klebsiella pneumoniae* and *Pseudomonas fluorescens* P17). The threshold chlorine concentration for *K. pneumoniae* growth in the biofilm coupon also was determined experimentally. Based on the laboratory results, a mathematical model was used to predict the effect of organic carbon concentration, temperature and biocide residual on bacterial growth in the biofilm coupons. The experimental results in the pilot system showed the bacterial growth rate in the biofilm coupon correlated significantly with the biofilm growth on the pipe walls, and qualitatively validated the laboratory and model results. It demonstrated that the biofilm coupon can be used to simulate biofilm growth in distribution system and as an indicator of microbial growth potential.

INTRODUCTION

Occurrences of excessive bacterial populations in drinking water distribution systems, especially coliforms, have troubled utilities because of their possible implications for the hygienic safety of their product. The growth of biofilm in drinking water distribution systems contributes to most of the increase in bacterial numbers from the treatment plant effluent to the customer tap.

To seek strategies for controlling growth of coliforms in drinking water distribution systems, the research project "Factors Limiting Microbial Growth in the Distribution System" is ongoing in the Center for Biofilm Engineering (CBE) at Montana State University. The project has been funded by the American Water Works Association Research Foundation (AWWARF) and the National Science Foundation (NSF).

One of the objectives of the AWWARF project is to monitor biofilm development and determine growth potential under different conditions in laboratory scale (microscale) and pilot scale (mesoscale) experimental systems. Several techniques have been used for this purpose. However, sampling and examination using these existing techniques are exhausting and time-consuming. Easier and more rapid methods need to be developed.

The biofilm coupon, a microbial growth sensing device, was invented and patented by CBE researchers in 1989. It is a

rapid method to monitor the water quality *in situ*. The bacterial growth rate in the biofilm coupon is affected by the nutrition and physical-chemical factors in the water environment; thus the biofilm coupon may be used to determine the bacterial growth potential.

Since the biofilm coupon technique is an innovation, a variety of technological problems relating to coupon design, preparation and application must be solved. The goal for the research is to verify the feasibility of the biofilm coupon as applied to the drinking water distribution system. The objectives are:

a. Improve the procedure of biofilm coupon preparation to obtain uniform cell distribution, low baseline noise and high mechanical strength.

b. Determine the relationships between coupon performance and environmental factors, such as temperature, organic loading and disinfectant.

c. Experimentally determine the kinetic parameters of *K. pneumoniae* (New Haven isolate) in acetate and glucose media, and the diffusion resistance in the gel matrix of the biofilm coupon.

d. Apply the biofilm coupon technique in the pilot distribution system. Compare the biofilm coupon performance with other techniques to monitor *in situ* growth potential.

e. Use the Biomass Accumulation Model (BAM) to simulate the biofilm coupon performance.

LITERATURE REVIEW

Biofilm in Drinking Water Distribution Systems

Biofilm refers to microbial cells immobilized at the pipe surface or on a particle. In almost all drinking water distribution systems, even in the absence of chlorine, the extent of suspended growth is negligible. Thus, biofilms contribute significantly to bacteria and especially coliform regrowth (Characklis, 1988; Herson, 1991).

Attachment of bacteria to surfaces in flowing oligotrophic environments, such as drinking water, has important ecological considerations (Fletcher, 1982): (1) macromolecules tend to accumulate at solid-liquid interfaces, creating a favorable environment in a nutrient deficient situation; (2) low nutrient concentration in the water plus high flow rates can transport tremendous quantities of nutrients to fixed microorganisms, (3) Extracellular polymers (EPS) anchors attached bacteria and may also be a factor in nutrient capture; and (4) bacteria embedded in EPS matrixes are protected from disinfectants by a combination of physical and transport phenomena.

The formation of biofilm is governed by at least four factors which are already well known (Bryers and Characklis, 1982; Trulear and Characklis, 1982; Bryers, 1987). These are:

- deposition and adsorption of both living and dead

microorganisms from the aqueous phase onto the solid phase;

- growth of attached microorganisms;
- the death of attached microorganisms;
- continual detachment or erosion of the biomass by the flow of the water.

Growth and detachment generally are the major processes which govern biofilm formation.

Coliforms in Drinking Water

Almost all the aquatic microorganisms habitually encountered in river water possibly are isolated from drinking water distribution networks if satisfactory sampling and testing methods are used (Maul and Vagost 1991). Some of them are potential pathogens (Table 1). Among them, *Klebsiella pneumoniae* is an opportunistic pathogen (Orskov, 1981). Often it is one of the dominant populations in industrial wastewaters (Seidler, 1981). It also has been monitored widely in drinking water distribution systems (Lechevallier et al.,1987).

According to the Bergey's manual, *K. pneumoniae* has been characterized as straight rod, 0.3-1.0 μm in diameter and 0.6-6.0 μm in length, capsulated, Gram-negative, nonmotile and facultatively anaerobic, having both a respiratory and a fermentative type of metabolism (Orskov, 1984).

Table 1. Bacteria found in drinking water distribution systems and their possible significance (Dott et al., 1986)

Bacterial genus	Potential effects
Acinetobacter	Potential rival to other bacterial indicators
Acromonas	Potential pathogen, opportunistic
Alcaligenes	--
Arthrobacter	Colored water, possible rival
Bacillus	Nitrate reduction, corrosion, rival to other bacterial indicators
Beggiatoa	Rust-colored water, oxidation of sulphur
Clostridium	--
Corynebacterium	--
Crenothrix	Rust-colored water (iron bacteria)
Desulfovibrio	Black water, production of H_2S, corrosion
Edwarsiella	Potential pathogen, opportunistic
Enterobacter	--
Escherichia	Indicator of fecal pollution
Flavobacterium	Opportunistic pathogen, potential rival to other bacterial indicators
Gallionella	Rust-colored water, corrosion (iron bacteria)
Klebsiella	Potential pathogen
Legionella	Potential pathogen
Leptothrix	Rust-colored water (iron bacteria)
Methanomonas	Oxidation of methane
Micrococcus	Nitrate reduction, corrosion, rival to other bacterial indicators
Moraxella	Opportunistic pathogen
Mycobacterium	Potential pathogen
Nitrobacter	Nitrate production
Nitrosomas	Nitrate production
Nocardia	Potential pathogen
Proteus	Possible rival to other indicators
Providencia	Opportunistic pathogen
Pseudomonas	Opportunistic pathogen, rival to bacterial indicators
Salmonella	Potential pathogen
Serratia	Opportunistic pathogen
Sphaerotilus	Rust-colored water
Staphylococcus	Potential pathogen
Streptococcus	Indicator of fecal pollution
Streptomyces	Taste and odor
Yersinia	Potential pathogen

The strain of *Klebsiella pneumoniae* isolated from the New Haven, Connecticut system during coliform growth episodes was shown to be capable of significant growth under low nutrient concentrations, even in double glass distilled water (0.2 -0.8 mg/L TOC) (Camper et al., 1991). The suspended growth rate of *K. pneumoniae* (New Haven isolate and clinical isolate) in various levels of yeast extract in batch culture at 25 ^{0}C is listed in Table 2.

Table 2. Kinetic parameters for two strains of *K. pneumoniae* grown on yeast extract (Camper et al. 1991).

Isolate	Substrate co-efficient,Ks (mg liter^{-1})	Maximum growth rate, μ_{max} (h^{-1})	Cell yield on carbon basis, Y_{carbon} (mg mg^{-1})	Cell yield on cell basis, Y_{number} (cells mg of C^{-1})
New Haven isolate	0.06	0.32 ± 0.064	$0.07\pm8.9\times10^{-4}$	$1.7\times10^{9}\pm2.2\times10^{7}$
Clinical isolate	0.12	0.16 ± 0.038	$0.01\pm3.8\times10^{-4}$	$2.4\times10^{8}\pm9.6\times10^{6}$

The strain of *K. pneumoniae* (New Haven isolate) was widely used in previous projects on drinking water research in the Center for Biofilm Engineering (Characklis, 1988; Camper, 1992). It also is being used in the ongoing AWWARF project. Thus this *K. pneumoniae* (New Haven isolate) was used to prepare biofilm coupons in order to compare the biofilm coupon results with the results from other investigators.

Factors Influencing Biofilm Growth in Distribution Systems

Recent investigations have shown the influence of the

following factors on bacterial regrowth are important (LeChevallier, 1990a): (1) the availability of nutrients, especially organic carbon sources, (2) environmental factors such as temperature and pH, (3) the ineffectiveness of disinfectant residuals, (4) hydraulic effects, and (5) corrosion and sediment accumulation.

Nutrients

For coliform and HPC bacteria, the principal nutrient sources required are phosphorous, nitrogen and organic carbon. Because bacteria consume these nutrients in a ratio of approximately 1:10:100 (P:N:C), organic carbon is often a growth-limiting nutrient (LeChevallier, 1990a).

A US EPA survey showed that the nonpurgeable total organic carbon (NPTOC) concentration in drinking water in 80 locations ranged from 0.05 to 12.2 mg/L, with an average concentration of 1.5 mg/L (Symons et al., 1975). Recently, AOC (Assimilable Organic Carbon) has been widely used as an indicator for water quality (van der Kooij, 1991). With this method, drinking-water supplies in North America have been found to contain AOC between 1 and 2000 μg acetate carbon equivalents (ac-C eq)/L (Characklis, 1988; LeChevallier et al., 1987).

The relationships between AOC level and bacterial growth in drinking water were studied by different researchers. Some research verified that coliform bacterial growth was usually observed in waters containing AOC level > 50 μg/L

(LeChevallier et al., 1990b). Investigation showed that heterotrophic plate count (HPC) bacterial growth in distribution water will not occur at an AOC level of 10 -15 μg/L. AOC levels of 15 - 50 μg/L produced variable growth results (van der Kooij, 1982).

A variety of factors may play a role in making low levels of nutrients more available to biofilm bacteria. Extracellular polymers may play a role in accumulating nutrients from bulk water (Geesey 1987). Some research showed that substantial coliform growth was stimulated by the particles of the corrosion deposits found in distribution system tubercles (Herson, 1991), while others found no evidence of such an effect (Camper et al., 1992).

Hydraulic Effects

Flow velocity may regulate microbial growth on pipe surfaces in several ways. Increasing velocities allow for greater flux of nutrients to the pipe surface, greater transport of disinfectants, and greater shearing of biofilm from the pipe surface. The net effect on biofilm formation varies from system to system. Some investigators showed that increased fluid shear stress resulted in partial detachment of biofilm (Characklis, 1988).

Temperature

Water temperature is one of the most important rate-

controlling parameters in the biofilm growth process. Water temperature influences not only the growth rate, but the lag phase and cell yield as well (Fransolet et al., 1985). The temperature of drinking water varies with seasons. Microbial activity was observed in water at 15 ^{0}C or higher (Fransolet et al., 1985). However, significant bacterial growth was also found in a distribution system where year-round water temperatures remain near 0 ^{0}C (Emde et al., 1992).

Chlorine residuals

Disinfection of biofilm bacteria is more difficult than disinfection of unattached bacteria (LeChevallier et al. 1984). The major factors influencing disinfection efficiency for biofilms include the transport of the disinfectant into biofilm (LeChevallier, 1988), the composition of pipe material (LeChevallier, 1990c), and the accumulation of corrosion deposits (Emde et al., 1992). The concentration of chlorine required to inhibit bacterial regrowth varies from system to system. Table 3 lists some results reported by different investigators.

Chlorine and monochloramine are the most common disinfectants used in the drinking water industry in the U.S.A. (JMCEI, 1985). Chlorine is much more effective in inactivating the free cells than monochloramine (van der Wende, 1992). However, monochloramine is more effective for disinfection of biofilm (Griebe et al, 1993; LeChevallier,

1990c). The additional advantages of monochloramine include reaction specifically with microorganisms and less toxic by-products (Mattila-Sandholm 1992).

Bacterial activity can be restored following recovery from injury under suitable conditions (Singh et al., 1990). The injured coliforms from the water treatment plant will potentially recover and thus seed the biofilms in distribution networks. The knowledge of coliform recovery from biocide injury will be helpful to understand the regrowth phenomenon.

Table 3. Biofilm disinfection under various conditions

System	Substratum	Organisms	Disinfectant	Concentration and exposure (mg/L)	Biofilm growth	Reference
Model distribution system	PVC	Mixed culture	Free chlorine or monochloramine	continuously exposed to 1 or 4 mg/L	1 mg/l HOCL or NH_2CL inactivated bacteria on galvanized, copper, or PVC pipes; Bacteria on iron pipes survived in 4 mg/L HOCL but exhibited a more than 3-log die-off in 4 mg/L NH_2CL	LeChevallier 1990
	Galvanized					
	Copper					
	Iron					
Batch reactor	Glass slide	K. pneumoniae	Free chlorine	150 mg • min/L	99.6 % reduction	LeChevallier 1988
			Monochloramine	3 mg • min/L	99.9 % reduction	
Batch reactor	Granular activated carbon	E. coli	Free chlorine	2 mg/L for 1 hour	No significant decrease	LeChevallier 1984
Annular reactor	PVC	Mixed culture	Total chlorine	0.8 mg/L, continued	Reduced significantly but continued growth	Characklis 1988