Factors influencing biofilm growth in drinking water distribution systems
by Anne Kosteczko Camper

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Civil Engineering
Montana State University

Abstract:
Conditions contributing to the growth of biofilm bacteria, particularly coliforms, in simulated drinking water distribution systems were investigated. Key experimental variables Were substrate loading, temperature, hydraulic residence time, presence of chlorine, pipe material, and the initial growth rate of the introduced coliforms. Pilot distribution systems comprised (a) annular reactors with carbon steel pipe as the outer cylinder and (b) a five segment pipe loop of 4" carbon steel. The pilot systems and laboratory annular reactors were operated to simulate water quality and operational conditions typical of drinking water distribution systems.

A mathematical model was developed to calculate growth rates of the suspended and attached heterotroph and coliform populations using experimental data. Inclusion of a potential for planktonic growth to that of the biofilm increased the precision of the model.

The initial growth rate of the coliforms was critical to their ability to persist longterm in mixed population biofilms. The longest chemostat residence time tested (20 hr) produced the most competitive organisms.

The influence of substratum material was tested in laboratory systems. Biofilms grown on mild steel contained more heterotrophs and coliforms than those sampled from polycarbonate. However, growth rates on the polycarbonate were higher.

In lab and pilot reactors, higher substrate loading resulted in increased bacterial numbers, but not in increased growth rate. No correlation was found between substrate loading, organism numbers or growth rate.

Temperature increase from 10° to 20° C did not result in elevated coliform numbers or growth rates. Heterotroph growth rate was enhanced.

Chlorination was not effective in controlling coliforms, regardless of the substratum composition. The numbers of culturable heterotrophs on polycarbonate were reduced when chlorine was present; this effect was not seen on mild steel.

Coliforms were more abundant at the shortest (2 hr) hydraulic residence time while heterotrophs were unaffected at increased residence times of 4, 8, and 16 hr.

Comparisons between data from parallel annular reactors and pipe loops showed that the qualitative response of the organisms to operational parameters was similar. The annular reactors are recommended as the preferable tool for experimental and industrial use.

FACTORS INFLUENCING BIOFILM GROWTH IN DRINKING WATER DISTRIBUTION SYSTEMS

by

Anne Kosteczko Camper

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Civil Engineering

MONTANA STATE UNIVERSITY-BOZEMAN
Bozeman, Montana

September 1995

APPROVAL

of a thesis submitted by

Anne Kosteczko Camper

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Dr. Warren L. Jones — (Signature) — Date 9/7/95

Approved for the Department of Civil Engineering

Dr. Robert Oakberg — (Signature) — Date 9/8/95

Approved for the College of Graduate Studies

Dr. Robert Brown — (Signature) — Date 11/17/95

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a doctoral degree at Montana State University-Bozeman, I agree that the Library shall make it available to borrowers under rules of the Library. I further agree that copying of this thesis is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for extensive copying or reproduction of this thesis should be referred to University Microfilms International, 300 North Zeeb Road, Ann Arbor, Michigan 48106, to whom I have granted "the exclusive right to reproduce and distribute my dissertation in and from microform along with the non-exclusive right to reproduce and distribute my abstract in any format in whole or in part."

Signature Anne K. Camper

Date 9/7/95

ACKNOWLEDGMENTS

The completion of a thesis is kin to other "rites of passage;" one is given to reflect on who was instrumental in the journey. This trip began when I was a technician and a theme in conversation was that I should obtain my Ph.D. (thanks Dave and Barry). It became a reality when I submitted a proposal with the encouragement of Bill Characklis. Bill's support and faith in my abilities made it possible to start on this unconventional degree program. Thanks, Bill. Wish you were here for the party. I also thank Debbie Brink and AWWARF for funding this research

Whenever things were too overwhelming, I had the support of two special mentors, Michele Prevost and Warren Jones. Michele, you showed me that someone could switch disciplines, raise a family, work full time, and go to school. You continue to be an inspiration. Warren, thanks for the everyday things including the pep talks, math assistance for the previously inept, and the ability to help me break through my mental blocks. You've shown me how to think like an engineer.

Also thanks are due to my research group, particularly Jason Hayes and Lu Goodrum. Nick, keep on "rotating." To Peg , Susan, Alma and my extended family at the Center, you'll never know how much you've helped.

To Randy, who put up with more than anyone (how many years have I been a student?) and Hali, who's become a strong young woman and helped me in so many ways, hugs and kisses. Mom, you always believed. Dad, wish you could be here......

TABLE OF CONTENTS

LIST OF TABLES

Table

Table

LIST OF FIGURES

Figure

Figure

Figure

ABSTRACT

Conditions contributing to the growth of biofilm bacteria, particularly coliforms, in simulated drinking water distribution systems were investigated. Key experimental variables were substrate loading, temperature, hydraulic residence time, presence of chlorine, pipe material, and the initial growth rate of the introduced coliforms. Pilot distribution systems comprised (a) annular reactors with carbon steel pipe as the outer cylinder and (b) a five segment pipe loop of 4" carbon steel. The pilot systems and laboratory annular reactors were operated to simulate water quality and operational conditions typical of drinking water distribution systems.

A mathematical model was developed to calculate growth rates of the suspended and attached heterotroph and coliform populations using experimental data. Inclusion of a potential for planktonic growth to that of the biofilm increased the precision of the model.

The initial growth rate of the coliforms was critical to their ability to persist long-term in mixed population biofilms. The longest chemostat residence time tested (20 hr) produced the most competitive organisms.

The influence of substratum material was tested in laboratory systems. Biofilms grown on mild steel contained more heterotrophs and coliforms than those sampled from polycarbonate. However, growth rates on the polycarbonate were higher.

In lab and pilot reactors, higher substrate loading resulted in increased bacterial numbers, but not in increased growth rate. No correlation was found between substrate loading, organism numbers or growth rate.

Temperature increase from 10° to 20° C did not result in elevated coliform numbers or growth rates. Heterotroph growth rate was enhanced.

Chlorination was not effective in controlling coliforms, regardless of the substratum composition. The numbers of culturable heterotrophs on polycarbonate were reduced when chlorine was present; this effect was not seen on mild steel.

Coliforms were more abundant at the shortest (2 hr) hydraulic residence time while heterotrophs were unaffected at increased residence times of 4, 8, and 16 hr.

Comparisons between data from parallel annular reactors and pipe loops showed that the qualitative response of the organisms to operational parameters was similar. The annular reactors are recommended as the preferable tool for experimental and industrial use.

CHAPTER 1

INTRODUCTION

When coliform bacteria are present in treated drinking water in the absence of cross-connections, disinfection barrier breakthrough, or other sources of immediate public health concern, the water utility is said to be experiencing a regrowth event. Regrowth is presumed to be associated with the proliferation of bacteria in the distribution system, either in the bulk fluid or on pipe surfaces. Evidence suggests that the coliform bacteria must be growing on pipe surfaces in biofilms and subsequently detaching into the bulk water. When these detached bacteria are detected in routine monitoring, the microbiological quality of the water is considered unnecessarily compromised. Presence of these indicator bacteria in water regardless of their source is regulated by federal law through the Coliform Rule of the Safe Drinking Water Act; thus the utility must initiate a response action, including public notification, and “boil water” orders. These actions may seriously erode public confidence in the water supply. Utilities are forced to select a "best guess" control measure which in fact may have little chance of controlling the regrowth event. Coliforms arising from a regrowth event may also conceal the presence of coliforms that arise from sources of genuine health concern.

In response to the urgent needs of water utilities, the American Water Works Association Research Foundation issued a request for proposals for research on "Factors

Limiting Microbial Growth in the Distribution System." This thesis reports results of a research project that was subsequently awarded by AWWARF. A comprehensive research plan was developed to investigate those variables that govern the growth of bacteria, particularly coliforms, in distribution system biofilms. The approach included (a) laboratory studies to determine the importance of bacterial physiology and substratum composition (pipe materials) on the surface colonization potential of coliforms; (b) controlled experiments in pilot scale distribution systems to determine the influence of temperature, substrate loading and measured assimilable organic carbon (AOC) concentration, disinfectant concentration, and hydraulic residence time on bacterial colonization; and (c) a field study of 31 drinking water distribution systems to identify field conditions that correlate with regrowth events. Laboratory and pilot data were collected by the project's principal investigator and are presented in this thesis. The field data were gathered and processed by Dr. Mark LeChevallier of the American Water Works Service Company and Darrell Smith of the South Central Connecticut Regional Water Authority.

The goal for the project was to establish criteria to limit microbial growth in drinking water distribution systems. Project objectives were the following:

(1) Determine the extent of coliform growth in mixed population biofilms.

(2) Experimentally determine conditions that permit coliforms to establish and persist in mixed population biofilms in model distribution systems. Important variables included a) growth rate of the introduced coliforms, b) nutrient loading, particularly assimilable organic carbon, c) temperature,

d) hydraulic regime, particularly residence time, e) the presence of disinfectant, and f) pipe surface composition.

(3) Establish correlations between operational and environmental variables and the establishment and persistence of coliforms on pipe surfaces by experimentation. Verify laboratory and pilot observations with field results.

(4) Design, construct, and evaluate model distribution system(s) for investigating biofilm accumulation on pipe walls.

(5) Establish the validity of the pilot distribution system(s) for simulating actual distribution systems.

Research Approach

Developing relevant information on distribution system microbial growth that can be effectively applied by the drinking water industry requires an integrated research effort. Microbial and chemical processes occur in the entire treatment plant/distribution system, in individual pipes, and on the surface of the pipes in biofilms. Research for the AWWARF project and this thesis was defined in terms of scales of observation (Fig. 1.1). As illustrated, microscale research focuses on measurements and interactions within the biofilm. At the mesoscale, fluid dynamics and system geometries become important, since biofilm processes are dependent on mass transfer phenomena. The macroscale, or treatment plant/distribution system network, is influenced by system operating parameters and environmental variables including influent water quality, water demand, treatment methods, and regulations regarding water quality. Experiments at the micro- and mesoscale must be performed under relevant conditions so that the results can be extrapolated to the macroscale.

Because it is difficult to identify cause-and-effect relationships in full scale distribution systems, the research effort was coordinated to include laboratory and pilot scale experiments to test perceived relationships between factors and regrowth events. A parallel effort, which is not reported here, was conducted in the form of a field survey of thirty one utilities. A systematic examination of data from the utilities was performed to determine if coliform regrowth events could be correlated with measured parameters. An overview of the research plan for the entire project is illustrated in Figure 1.2. This figure also provides a platform for describing the manner in which the individual papers written for this thesis are integrated. First, the literature review "Coliform Regrowth and Biofilm Accumulation in Drinking Water Systems: A Review," which has been published in the book *Biofouling and Biocorrosion in Industrial Water Systems*, provided the input for determining the important variables for investigation. The microscale research results are described in the paper "Effect of Growth Conditions and Substratum Composition on the Persistence of Coliforms in Mixed Population Biofilms" submitted to Applied and Environmental Microbiology. Results from the microscale experiments were used to design colonization strategies for the pilot plant and to interpret responses seen in mesoscale experiments. These results satisfy objectives 1 and part of objective 2 of the research plan. Mesoscale work is described in two papers "Physical Distribution System Models for Assessing Regrowth: Pipe Loops and Annular Reactors" and "Influence of

Water Quality Parameters on Biofilm Growth in Model Drinking Water Distribution Systems," both submitted to the Journal of the American Water Works Association. The first of these provides a physical description of the pilot plants and gives a statistical comparison of the reactor types on the basis of the response of biofilm organism to operational parameters. This paper specifically addresses objective 4. The second paper describes the response of the biofilm organisms to the tested parameters in fulfillment of objectives 2 and 3. The final paper, "Estimated Biofilm Organism Growth Rates as Influenced by Distribution System Parameters" has been submitted to Water Research and describes modeling efforts conducted to explain the behavior of the organisms in the simulated distribution systems. Integration of all results from the entire project has been provided as a final chapter of this thesis.

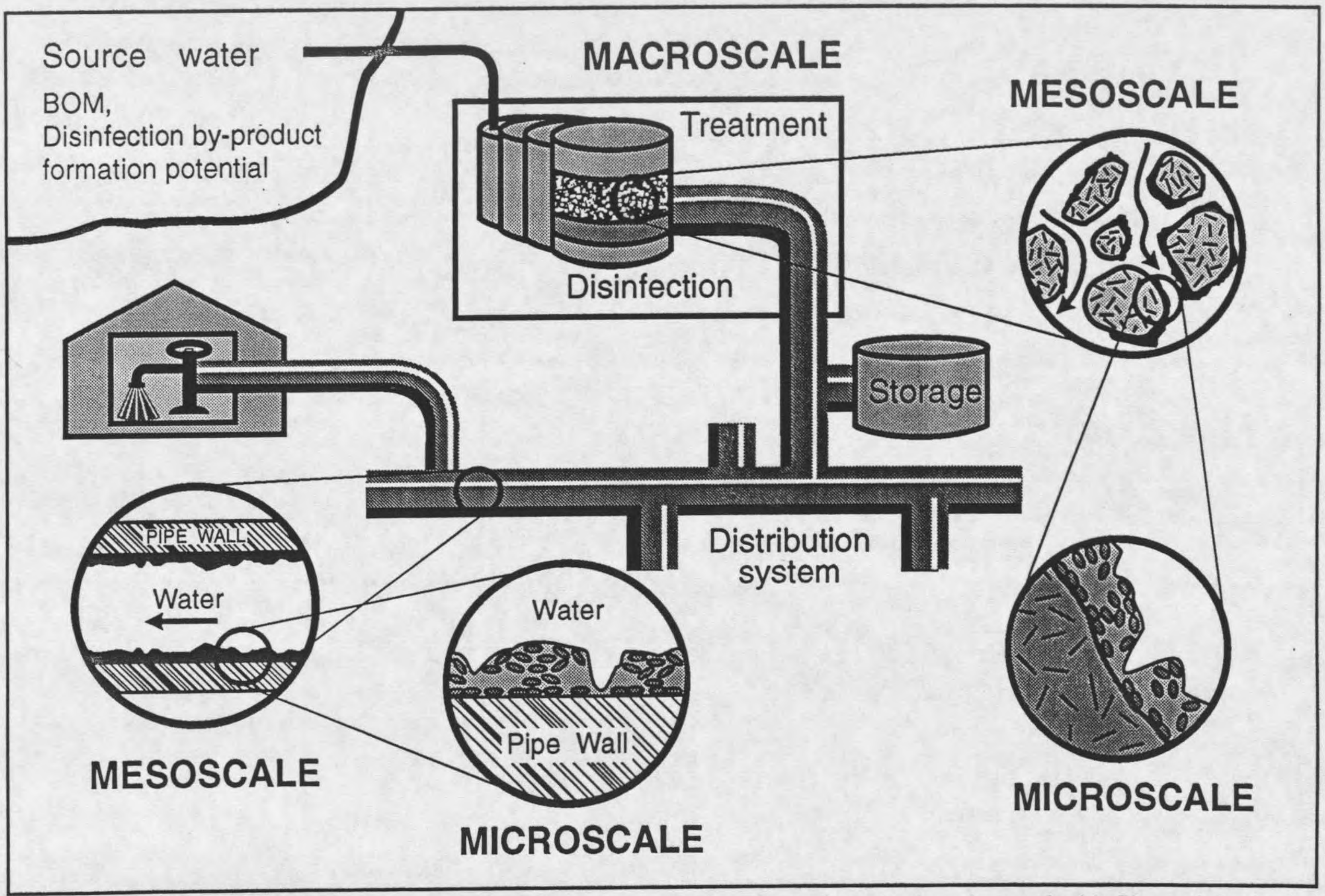

Figure 1.1. Scale-up based on observations at the micro-, meso- and macroscales. At the microscale, biofilm activity is similar regardless of system configuration. System geometry and related hydrodynamics become important at the mesoscale. Investigations at the macroscale provide insight on the importance of system operations and the environment on regrowth.