UTILIZATION OF BIODEGRADABLE ORGANIC MATTER BY BIOFILMS IN DRINKING WATER DISTRIBUTION SYSTEMS

by

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ABSTRACT

Nutrient conditions that contribute to bacterial regrowth in drinking water distribution systems were investigated. The major limiting nutrient for the experimental program was biodegradable organic matter (BOM), which is a surrogate for the amount of available organic carbon. A suite of organic compounds consisting of carboxylic acids, aldehydes, and amino acids was spiked into an existing drinking water source. The spiked tap water was pumped into an annular reactor (AR), which was used to simulate conditions of a section of a distribution system. Bulk (suspended) cell numbers and biofilm cell numbers, as measured by heterotrophic plate counts (HPCs) and total direct cell counts, were used as response variables. A method for removing biofilm cells from a polycarbonate coupon, which is flush-mounted to the side of an AR, was developed as part of the research.

The major experimental factors were: BOM concentration, the mass fraction of amino acids, and hydraulic retention time. It was found that high BOM loading rates (i.e., high BOM concentrations and low retention times) significantly increased the number of biofilm HPCs. The presence of amino acids also increased the number of biofilm HPCs. The net bulk HPCs were found to increase at longer retention times. A high BOM concentration significantly decreased the net bulk HPCs. The amount of amino acids did not affect the net bulk HPCs. In general, total cell counts were less responsive to the three experimental factors than HPC numbers.

Based on the influent organic components dosed to the ARs, a composite BOM was calculated (on a mass carbon basis) for each experimental trial. It was found that external and internal mass transfer to and within the biofilm did not limit BOM removal. Based on eight experimental trials at various BOM loading rates, a first-order rate expression for the removal of BOM was calculated. Pseudo-first-order rate constants for each BOM component were also calculated. The rate constants provide an indication of the relative rate of biodegradability. From this analysis it was shown that formate has the highest rate of biodegradability and glyoxal has the lowest.

The per cent removal of the added amino acids (i.e., aspartic acid, glutamic acid, and serine) was essentially complete. High or near complete removals for formate and formaldehyde were also calculated. Acetate and glyoxal had much lower fractions removed.

Additional experiments were performed whereby three ARs were connected in series and free chlorine was dosed into the first AR. It was found that chlorine was able to mitigate bulk and biofilm numbers in the first AR, however, chlorine had less of an effect on HPC numbers in the subsequent ARs. BOM components were removed by greater than 80 % after the first AR regardless of the influent residual chlorine concentration (i.e. either 0.0 or 0.2 mg/L). Refractory organic matter, as measured by UV absorbance at 285 nm, was found to increase through the three ARs.

An approach for relating bench-scale experiments to full-scale pipes was developed. The critical parameter for this relationship is the ratio between the specific surface area of the bench-scale reactor (e.g. annular reactor) and the specific surface area of the pipe diameter. Based on this approach an AR having a 1 h retention time would approximately correspond to a 7 h retention time in a 100 mm (4 in.) pipe.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOC	Assimilable organic carbon
AODC	Acridine orange direct count
AR	Annular reactor
BAP	Biomass-associated products
BDOC	Biodegradable dissolved organic carbon
BOM	Biodegradable organic matter
CSTR	Continuously stirred tank reactor
DOC	Dissolved organic carbon
GAC	Granular activated carbon
GC/ECD	Gas chromatography with electron capture detection
HPC	Heterotrophic plate count
HPLC	High pressure liquid chromatography
HRT	Hydraulic retention time
NOM	Natural organic matter
NPOC	Nonpurgeable organic carbon
OBP	Ozonation by-product
PFR	Plug flow reactor
ROM	Refractory organic matter
SMPs	Soluble microbial products
THM	Trihalomethane
THMFP	Trihalomethane formation potential
ThOD	Theoretical oxygen demand
TOC	Total organic carbon
TTHMs	Total trihalomethanes
UAP	Utilized-associated products

LIST OF SYMBOLS

A	Area, L^2
a	Specific surface area, L^{-1}
A_d	Detached particle area, L^2
D	Diameter, L
Da	Damköhler number
D_f	Molecular diffusivity in the biofilm, $L^2\ T^{-1}$
$f_d(z)$	Local detachment frequency
J	BOM flux to the biofilm, $M_S\ L^{-2}\ T^{-1}$
k	First-order rate coefficient, T^{-1}
k_1	Utilization-associated SMP formation constant, $M_S\ M_X^{-1}$
k_2	Biomass-associated SMP formation constant, $M_S\ M_X^{-1}\ T^{-1}$
k_d	Detachment coefficient, $T^{-1}\ L^{-1}$
k_{d1}	Growth related detachment coefficient, L^{-1}
k_{d2}	Non-growth related detachment coefficient, $L^{-1}\ T^{-1}$
k_L	Mass transfer coefficient, $L\ T^{-1}$
k_{max}	Maximum specific rate of substrate utilization, $M_S\ M_X^{-1}\ T^{-1}$
K_S	Half-velocity constant, $M_S\ L^{-3}$
L	Equivalent length of pipe, L
L_c	Characteristic length, L
L_f	Biofilm thickness, L
L_l	Liquid boundary layer, L
P	Amount of SMP formed, $M_S\ L^{-3}$
Q	Volumetric flow rate, $L^3\ T^{-1}$
r_d	Biofilm detachment rate, $M_X\ L^{-2}\ T^{-1}$
Re	Reynold's number
$r_{g,b}$	Growth rate of planktonic HPCs, $M_X\ L^{-3}\ T^{-1}$
r_{gf}	Growth rate of biofilm HPCs, $M_X\ L^{-2}\ T^{-1}$
R_L	BOM loading rate, $M_S\ L^{-2}\ T^{-1}$
R_{obs}	Observed reaction rate, $M_S\ L^{-3}\ T^{-1}$
S_b	Bulk liquid BOM concentration, $M_S\ L^{-3}$
S_f	BOM concentration in the biofilm, $M_S\ L^{-3}$

$\overline{S_f}$	Average BOM concentration in the biofilm, $M_S\ L^{-3}$
S_i	Influent concentration of BOM, $M_S\ L^{-3}$
S_{min}	Minimum substrate concentration to achieve a steady-state biofilm, $M_S\ L^{-3}$
S_s	BOM concentration at the surface of the biofilm, $M_S\ L^{-3}$
V	Volume, L^3
v	Linear velocity, $L\ T^{-1}$
v_{max}	Maximum reaction rate for enzyme catalysis with Michaelis-Menton kinetics. $M_S\ L^{-2}\ T^{-1}$
X_b	Bulk liquid concentration of heterotrophic bacteria, $M_X\ L^{-3}$
X_f	Areal biofilm desity, $M_X\ L^{-2}$
$X_{f,V}$	Volumetric bacterial density, $M_X\ L^{-3}$
X_i	Influent concentration of heterotrophic bacteria, $M_X\ L^{-3}$
$Y_{x\,s}$	Yield coefficient, M_X/M_S
Φ	Thiele modulus
Ω	Observable external mass transfer modulus
$\mu(z)$	Biofilm growth rate as function of biofilm depth, T^{-1}
$\rho(z)$	Local biofilm density, $M_x\ L^{-3}$
τ	Hydraulic retention time, T
τ_s	Shear stress, $M\ L^{-1}\ T^{-2}$

CHAPTER 1: INTRODUCTION

The major objective of distribution systems is to act as inert conduits providing safe, clean drinking water to a community of consumers. Unfortunately some distribution systems impart water quality changes which may affect the aesthetic properties of water such as taste, and odour (Suffet *et al.*, 1996). Deterioration of water quality can be associated with the development of bacterial activity in the water mains during distribution (Geldrich, 1996). More serious consequences of water quality deterioration due to microbial activity are episodes of coliform growth (LeChevallier *et al.*, 1996a), an increased likelihood of waterborne disease (Payment *et al.*, 1997; Payment *et al.*, 1991), increased concentration of nitrate (Wilczak *et al.*, 1996), enhanced pipe corrosion (Geldrich, 1996), and a loss of dissolved oxygen (O'Connor *et al.*, 1975).

Distribution systems typically do not behave as ideal plug flow reactors (PFRs), consequently many research projects are concerned with modeling the hydraulic processes in pipe networks (e.g., Rossman, 1994). While recognizing the complexities of hydraulic modeling and its importance in understanding distribution systems, this

research will focus on the biochemical reactions which may occur during the distribution of treated waters. Several key microbial and chemical processes which occur in a distribution system are idealized in Figure 1.1.

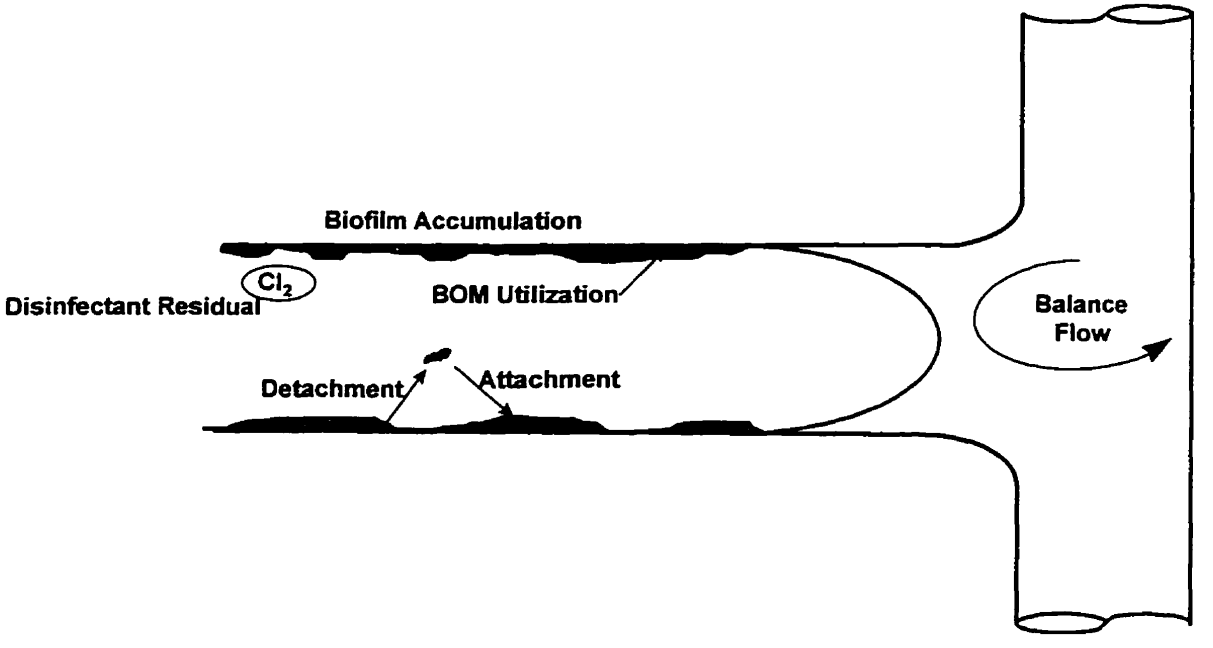

Figure 1.1: Schematic of chemical and microbial processes which may occur in distribution systems.

Microscopic investigations have observed microorganisms attached or fixed to pipe tubercles, or related pipe material during distribution (Allen *et al.*, 1980; Tuovinen *et al.*, 1980). For treated waters, it has been shown that the number of biofilm cells is typically greater than their planktonic (suspended) counterparts (van der Wende *et al.*, 1989). A biofilm consists of cells immobilized at a substratum and frequently embedded in an organic polymer matrix of microbial origin (Characklis and Marshall, 1990). Unlike planktonic cells, biofilm cells form a complex bacterial consortium in which the microbial properties of a given cell may be highly dependent on the metabolic activities of adjacent cells and the diffusive characteristics of the biofilm (Costerton *et al.*, 1994).

Biofilm detachment is assumed to be a critical process balancing the biofilm expansion (Stewart, 1993) and may be responsible for a majority of the number of suspended cells (Camper, 1996).

Because the number of biofilm cells exceeds the number of suspended cells, the flux of BOM to the biofilm is a critical step for most biological processes in drinking water. An idealized biofilm with a homogeneous thickness has been used for modeling substrate utilization by biofilms (e.g., Rittmann and McCarty, 1980). This modeling approach can be generalized for biofilms having a range of thickness (Rittmann and McCarty, 1981), and has been applied to drinking water environments (e.g., Zhang and Huck, 1996; Rittmann, 1990). A critical parameter in the steady-state biofilm model is S_{min}, the minimum substrate concentration required to achieve a steady-state biofilm (Rittmann and McCarty, 1980). S_{min} is particularly relevant to distribution systems as BOM levels in many treated waters may be at or near predicted S_{min} values.

ACHIEVING BIOLOGICAL STABILITY

Drinking water that does not promote bacterial growth during distribution, is considered biologically stable (Rittmann and Snoeyink, 1984). Biological stability can be achieved through the addition of a disinfectant (e.g., chlorine) and/or by the removal of available nutrients which are capable of sustaining microbial growth. Disinfection alone is not an efficient mitigation step. Several studies have demonstrated the ability of bacteria to survive in drinking water after continued exposure to chlorine (e.g., Pedersen, 1994; LeChevallier *et al.*, 1990; Ridgway and Olson, 1982). Also during chlorination, natural organic matter (NOM) reacts with free chlorine forming unwanted organohalides

(e.g., Christman *et al.*, 1983). Many of these chlorinated compounds may be known or suspected carcinogens (Bull and Kopfler, 1991). Consequently, disinfection with chlorine as the sole treatment measure for controlling bacterial regrowth has become less favorable and other measures have been sought.

The goal of biological treatment is to remove the available nutrient sources which may lead to biological instability during distribution. For balanced growth in drinking water a general rule of thumb is that carbon, nitrogen and phosphorus must be present in a ratio of approximately 100:10:1, respectively (Camper, 1994). Meittinen *et al.* (1997) showed that phosphorus is the limiting nutrient for microbial growth in Finnish drinking water. However carbon must be present at significantly higher concentrations than phosphorus to maintain balanced growth. Consequently, most biological processes focus on the removal of biodegradable organic carbon. Aerobic conditions tend to predominate in drinking water as dissolved oxygen concentrations for most treated waters are at or near saturation (Montgomery, 1985). In most biological treatment processes, biodegradable organic matter (BOM) serves as an electron donor and as a carbon source for a select set of aerobic, heterotrophic bacteria known as oligotrophs (Rittmann and Huck, 1989; Bouwer and Crowe, 1988). Therefore an objective of biological treatment is to reduce the level of BOM prior to distribution.

Most methods of biological treatment in drinking water utilize biofilm processes for removal of organic material (Rittmann, 1990). Biological treatment processes include fluidized bed filters, slow sand filters, biologically active granular activated carbon (GAC) filters and soil-aquifer treatment systems (Bouwer and Crowe, 1988). Frequently ozone is applied to water prior to filtration with either a rapid sand filter or a biologically

active GAC filter (Rittmann and Huck, 1989). During ozonation the biodegradability of NOM is enhanced (e.g., Goel *et al.*, 1995), as high molecular weight organic compounds present in NOM are oxidized forming low-molecular weight easily biodegradable organic compounds, commonly referred to as ozonation by-products (OBPs) (Langlais *et al.*, 1991). Several water treatment investigations have shown that many of the OBPs are constituents of BOM and readily removed during filtration (e.g., Gagnon *et al.*, 1997a; Weinberg *et al.*, 1993; Huck *et al.*, 1990).

LeChevallier *et al.* (1996b) surveyed 31 treatment facilities across North America and found that the incidence of coliform regrowth was strongly related to both disinfectant residual and assimilable organic carbon (AOC) concentration (an indicator of BOM). As a result of this survey, a relationship between the number of coliforms, AOC and disinfectant residual was developed (LeChevallier *et al.*, 1996b). This relationship was subsequently modified by Huck and Gagnon (1997) to generalize the relationship associated with BOM, disinfection and bacterial regrowth. Figure 1.2 suggests that for systems with moderate levels of BOM, loss of a disinfectant residual may be expected to cause regrowth problems. Although not shown in the figure, at increased BOM levels the amount of coliform regrowth may be enhanced. Figure 1.2 also implies that utilities need to limit biodegradable organic carbon so that regrowth can be controlled with a reasonable disinfectant residual.

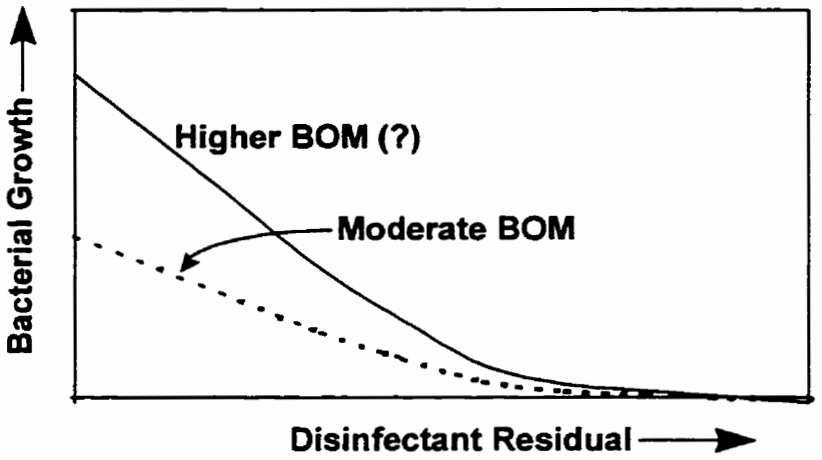

Figure 1.2: Inferred relationship between bacterial regrowth, disinfectant residual and the amount of BOM (After: Huck and Gagnon, 1997).

The determination of what constitutes "moderate" and "high" BOM levels is difficult, and the impact of a given BOM level in a specific system will depend on several factors, such as temperature, water chemistry, and BOM composition. Results presented in the next section will discuss the range of BOM levels encountered in treated waters.

QUANTIFYING BIOLOGICAL STABILITY

The concentration of substrate (BOM) is typically used to determine the extent to which a treated water is considered biologically stable. Several surrogate measurements have been developed for quantifying BOM in drinking water and were reviewed in detail by Huck (1990). Table 1.1 summarizes some of the available surrogate measurements and other relevant methods currently used for characterizing BOM in drinking water.

Table 1.1: Parameters Available for Characterizing BOM in Drinking Water

BOM Parameter	Analytical Approach	Comment
AOC	Growth of two microbial strains	• able to compare stability of treated waters • limited by microbial activity of two strains
BDOC	Removal of DOC in a specified bio-reactor	• measure the DOC removal by indigenous biofilm population • site and method specific
TOC	several Standard Methods (e.g., Infra-red detection)	• does not differentiate between biodegradable and recalcitrant organic carbon
BOM Components: Aldehydes Amino Acids Carboxylic Acids	 Gas Chromatography High Pressure Liquid Chromatography Ion Chromatography	 • able to quantify BOM on molar basis • limited to specific BOM component

AOC

Assimilable organic carbon (AOC) quantifies the available amount of organic carbon which is able to support the growth of two specific strains of bacteria common to drinking water (van der Kooij *et al.*, 1982; van der Kooij and Hijnen, 1984). An advantage of the AOC assay is that it can be readily standardized, allowing several treated waters to be compared quantitatively for their potential to support microbial growth. However, a major drawback of the AOC method is that it relies on the microbial activity of only two bacterial strains. In drinking water treatment and distribution a much broader and diverse population of microorganisms has been reported in the literature (El-Masry *et*

al., 1995; LeChevallier *et al.*, 1987). Noble *et al.* (1996) showed that AOC only partially explains the capabilities of heterotrophic growth in drinking water.

Kaplan *et al.* (1994) surveyed 109 treated waters across North America for BOM related parameters, such as AOC, biodegradable dissolved organic carbon (BDOC), and coliform growth response. It was found that AOC concentrations ranged from 18 to 322 μg/L with a median concentration of 110 μg/L. In another full-scale survey, which was similar in scope, LeChevallier *et al.* (1996b) found that AOC levels ranged from 1 to 1300 μg/L in finished waters. A histogram of the AOC values reported by LeChevallier *et al.* (1996b) is shown in Figure 1.3.

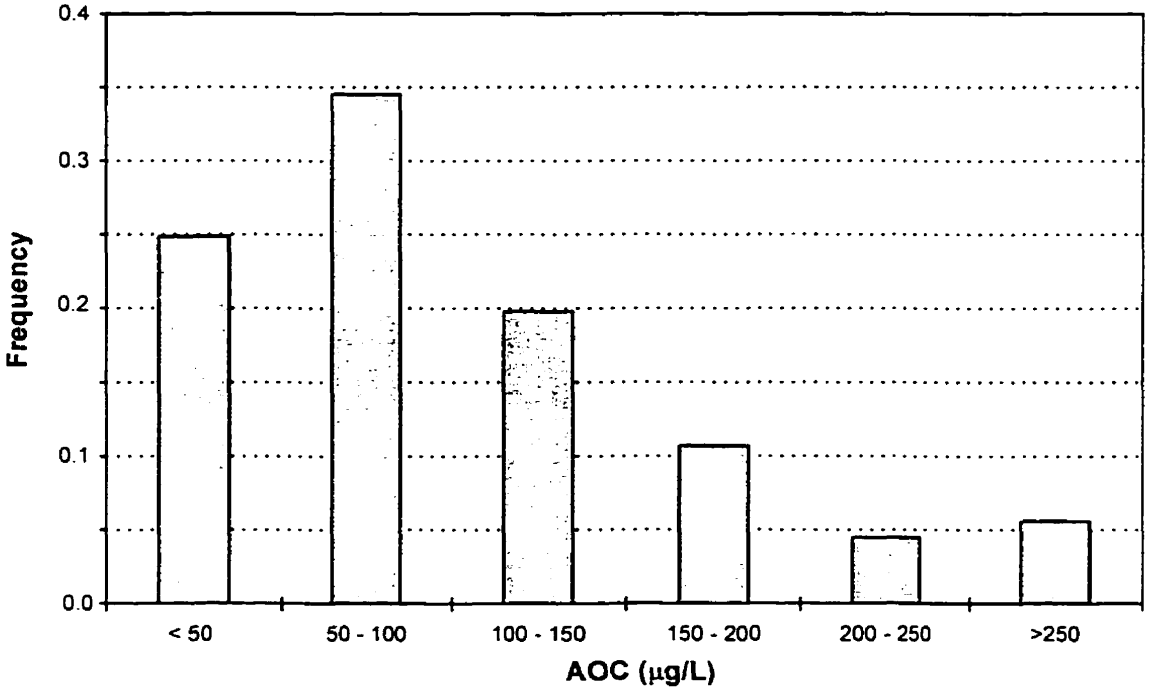

Figure 1.3: Distribution of AOC in treated waters from a North American survey (Data source: LeChevallier *et al.*, 1996b).

BDOC

Another parameter used to quantify BOM is the concentration of biodegradable dissolved organic carbon (BDOC). Joret and Levi (1986) and Servais *et al.* (1987)

developed the original method for determining BDOC in drinking water. In the method developed by Servais *et al.* (1987), BDOC is defined as the fraction of the dissolved organic carbon (DOC) which can be metabolized by heterotrophic microorganisms in a batch water sample which is incubated at 20°C for a period of 10-30 days. The method has since been modified and revised by several researchers (Allgeier *et al.* 1996; Carlson and Amy, 1995; Kaplan and Newbold, 1995; Frias *et al.*, 1992), however the underlying concept of the assay (i.e. removal of DOC after a specified incubation period in a given bio-reactor), has remained constant.

The BDOC procedure has the advantage of relying on the catabolic activities of the indigenous bacteria from a specific water source, rather than two species. However, bacteria from different watersheds may have dissimilar nutritional requirements, making quantitative comparisons of BDOC values difficult for various treated waters.

In general, BDOC values are greater than AOC values for most finished waters. In the survey conducted by LeChevallier *et al.* (1996b) it was found that BDOC values ranged from 240 to 540 μg/L. It was also found that the system with the highest AOC level corresponded to the system which had the highest BDOC value. However, the variability between the two assays was substantial and the results were not significantly correlated (LeChevallier *et al.*, 1996b).

In recent years, target effluent concentrations for both AOC (van der Kooij, 1992; LeChevallier *et al.*, 1991) and BDOC (Servais *et al.*, 1995a) have been recommended to the water industry. Using a pilot-scale distribution system, Sibille *et al.* (1997) compared the biological stability of water which was biologically filtered (effluent BDOC of 350 μg C/L) with water treated by nanofiltration (effluent BDOC less than 100 μg C/L). Sibille *et*

al. (1997) suggested that a measurable BDOC concentration that prohibits bacterial growth during distribution may not exist.

TOC

Total organic carbon (TOC) is a well documented measurement (Eaton *et al.*, 1995) and probably the most frequently used parameter for characterizing NOM. TOC does not differentiate between the biodegradable and more recalcitrant portions of NOM. However, TOC is a reliable quality control measure which can be used in combination with other water quality parameters for characterizing the performance of many water treatment processes. Raw water TOC in combination with the amount of UV absorbance at 254 nm in the raw water can indicate the portion of TOC which will not adsorb to the added coagulant during coagulation/flocculation (Edwards, 1997). It has been shown that in comparison with biological filtration, optimizing the coagulant dosage offers a more effective means of accomplishing substantial TOC removal (Hozalski *et al.*, 1995). The presence of recalcitrant portions of TOC in finished waters may be an important factor associated with bacterial regrowth in the extremities of some distribution systems.

BOM Components

BOM components which have been identified in treated waters at full-scale are aldehydes (Weinberg *et al.*, 1993), amino acids (Dossier-Berne *et al.*, 1994), and carboxylic acids (Gagnon *et al.*, 1997a). By analyzing BOM components researchers have the ability to quantify major organic species on a molar basis and calculate their respective biodegradation rates from controlled bench- and pilot-scale studies. Ultimately this type of information could identify organic constituents which play a role in causing

biological instability. However, by focusing solely on easily biodegradable and currently measurable components, other biodegradable organic compounds (i.e. humic and fulvic acids, carbohydrates, proteins) are not accounted for. The extent to which these other components affect biological stability is unclear and would be specific to a particular treated water.

To date most investigations have studied BOM components during treatment, and the information regarding BOM components during distribution is relatively sparse. Aldehydes, such as formaldehyde and acetaldehyde, are easily removed by biological filtration, whereas the fraction of glyoxal removal is typically lower (Krasner *et al.*, 1993). The removal rates of aldehydes have been found to be slower at lower temperatures (Coffey *et al.*, 1995). Studies have also indicated that carboxylic acids, such as acetate and formate, can be removed during biological filtration (Urfer and Huck, 1997). However, a full-scale investigation has shown that complete removal of some carboxylic acids does not occur during biological filtration, which may have an impact on the extent of bacterial regrowth in the distribution system (Gagnon *et al.*, 1997a).

The most significant nitrogen-containing compounds with respect to BOM are amino acids and proteins. One study observed that greater than 96 % of the total amino acids were associated with aquatic humus (Lyttle and Perdue, 1981). In a pilot plant investigation, which examined the efficacy of nanofiltration, it was shown that amino acids represented approximately 63 % biodegradable dissolved organic carbon (BDOC) in the permeate (Agbekodo *et al.*, 1996). The remaining BDOC fractions consisted of sugars (22 %), aldehydes (5 %), and fatty/aromatic acids (10 %).

EXPERIMENTAL INVESTIGATIONS IN DISTRIBUTION SYSTEMS

Full-Scale

Full-scale studies provide valuable information regarding the bacterial and chemical changes which occur in "real" systems. However, most full-scale investigations focus less on the experimental design approach (e.g., controlling input parameters) and more on the collection of data. This is understandable given that the primary concern with full-scale systems is public health, which precludes compromising water quality during an investigation. Furthermore, in larger municipalities the residence time in a distribution system is not easily established.

Two factors that may affect bacterial regrowth in distribution systems are residence time and bacterial nutrients. By comparing residence times of 1 h and 11 h, Prévost *et al.* (1997) found that the number of suspended cells (as measured by HPC counts and epifluorescence microscopy) increased at the longer retention time (e.g., 11 h). However, the free chlorine residual at the longer residence time was also lower, which may have contributed to the higher HPC numbers observed by Prévost *et al.* (1997). Water reservoirs, which essentially increase the residence time of treated waters during distribution, have also been shown to substantially elevate bacterial numbers (Kerneïs *et al.*, 1995).

Donlan *et al.* (1994) showed that a bacterial population was established on the pipe surface of a cast iron pipe within 30 days after continued exposure to a low monochloramine residual (0.1 - 0.9 mg Cl_2/L). In that study temperature had a profound effect on biofilm formation (increased number of biofilm HPCs at higher temperatures). However, other water quality parameters that were measured by Donlan *et al.* (1994) (pH,

alkalinity, organic nitrogen, nitrite, nitrate, total phosphorus, orthophosphate, and TOC) were poorly correlated with the extent of biofilm accumulation. More sensitive indices of BOM (i.e. AOC, BDOC) were not measured by Donlan *et al.* (1994).

The effect of BOM, as measured by AOC and BDOC, has been shown to influence the degree of bacterial regrowth in distribution systems. LeChevallier *et al.* (1991) found that of the various nutritional parameters measured (nitrate, nitrite, ammonia, orthophosphate, total phosphorus, TOC, and AOC) only AOC was reduced in concentration from the plant effluent and through a short distribution system. A significant correlation has been shown to exist between the AOC concentration in plant effluents and the number of suspended cells in distributed waters (van der Kooij *et al.*, 1992). Similarly, Servais *et al.* (1995a) reported a significant correlation (in the absence of a chlorine residual) between finished water BDOC and fixed biomass in the distribution system. The extent to which specific components of BOM may be utilized during distribution has not been demonstrated at full-scale.

Pilot-Scale and Bench-Scale

Pilot-scale distribution systems generally are pipe networks which are smaller in distance and complexity than full-scale systems, but operate at similar flow rates through representative piping material using pipes of similar size to the smallest ones in full-scale systems. Two common designs for pilot-scale systems are the once-through system, similar to a plug flow reactor (PFR), (Holden *et al.*, 1995; LeChevallier *et al.*, 1990) and the recirculating system, similar to a continuously stirred tank reactor (CSTR) (Camper, 1996; Piriou and Levi, 1994; Haudidier *et al.*, 1988). The once-through system offers a

closer approximation to full-scale, however, from an experimental point of view, data analysis and kinetic evaluation are simplified considerably with the recirculating design. Once-through systems also require substantially more water than the recirculating systems.

Because of their size and cost (both operational and capital) annular reactors (ARs) and other bench-scale models of distribution systems are currently being used with greater frequency by the industry. ARs are generally assumed to represent a discrete section of a distribution system (Characklis, 1988). Recent investigations of direct relevance to the water industry include: examining the effect of corrosion inhibitors (Rompré *et al.*, 1996), evaluating biocide (e.g., chlorine) efficacy (Camper, 1996), and assessing biofilm accumulation/bacterial regrowth for various NOM sources (Butterfield *et al.*, 1996).

A comprehensive study by Camper (1996) looked at several important factors and their impact on the number of coliform and HPCs from a pilot-scale and bench-scale level. The factors examined in this study were temperature, AOC and chlorine dose. Each of the three factors was controlled at two levels. The water temperature was controlled at either 10°C or 20°C using a recirculating water bath. The model distribution systems were fed filtered tap water with either no additional AOC or an AOC cocktail of five organic compounds at an organic carbon concentration of 500 μg/L. Finally, chlorine was either dosed at level of 1.0 mg/L (as free chlorine) or not dosed into the model system.

Results from both the pipe loop and "industrial" annular reactor (AR) experiments (e.g., larger ARs operated at the treatment plant) are presented in Tables 1.2 and 1.3, respectively. The trends from both reactors were similar, the major discrepancy being that

the ARs consistently had larger numbers of biofilm and bulk liquid organisms. The three input factors (temperature, AOC concentration, and chlorine residual) that influenced regrowth at the 5 % level of significance are indicated in both Table 1.2 and 1.3. Increases in AOC concentration significantly increased the number of coliforms and HPCs in the biofilm for both reactor designs. Also bulk HPCs increased in both reactors with increasing AOC. Practically, these results suggest that if a chlorine residual has dissipated, increased AOC may result in enhanced regrowth.

Temperature had no significant effect on bulk or biofilm coliforms for either reactor design. In the pipe loop system an increase in temperature decreased both the number of bulk and biofilm HPC bacteria. Similarly in industrial ARs, higher temperatures increased the number of bulk biofilm HPCs.

Finally, an influent concentration of 1.0 mg/L as free chlorine did not significantly impact either bulk or biofilm HPC bacteria in either reactor. In fact, the presence of chlorine only caused a significant decrease in bulk coliforms in the pipe loop system. This suggests that HPCs and coliforms in the biofilm are essentially resistant to chlorine under the described experimental conditions. The actual chlorine residuals in the systems were not reported by Camper (1996).

Table 1.2: Significant Effects from Pipe Loop Experiments[†]

Parameter	Coliform		HPC	
	Bulk	Biofilm	Bulk	Biofilm
Temperature	-	-	Increase	Increase
AOC	-	Increase	Increase	Increase
Chlorine	Decrease	-	-	-

[†] *The table shows the response of the bulk water or biofilm to an increase in the stated parameter.*

Table 1.3: Significant Effects from Industrial Annular Reactor Experiments[†]

Parameter	Coliform		HPC	
	Bulk	Biofilm	Bulk	Biofilm
Temperature	-	-	Increase	Increase
AOC	-	Increase	Increase	Increase
Chlorine	-	-	-	-

[†] *The table shows the response of the bulk water or biofilm to an increase in the stated parameter.*

MODELING BIOFILM ACCUMULATION IN DISTRIBUTION SYSTEMS

A thorough review of the current approaches for modeling biofilm growth and the utilization of BOM in distributions systems was conducted by Gagnon *et al.* (1997b). The capabilities of the available models were analyzed on the basis of several factors: chlorine kinetics, BOM kinetics, mass transfer considerations, biofilm detachment kinetics, pipe material and aqueous chemistry. Future research needs from both a modeling and experimental perspective were also discussed.

All of the biofilm models (i.e., Dukan *et al.*, 1996; Lu *et al.*, 1995; Servais *et al.*, 1995b; Camper *et al.*, 1994) reviewed by Gagnon *et al.* (1997b) are useful research tools capable of examining processes associated with BOM utilization and biofilm accumulation. In their current form, however, all of the available biofilm models are too

complex for most practical situations. Some of the major conclusions drawn from the review by Gagnon *et al.* (1997b) are:

- Sensitivity analyses of all of the models are necessary for identifying highly uncertain and/or practically redundant processes/parameters. The SANCHO model (Servais *et al.*, 1995b), for example, assumes that any limitations due to substrate diffusion into and in the biofilm are negligible. This is in direct contrast to the steady-state biofilm model presented by Lu *et al.* (1995), which relies on diffusion with bio-reaction implicitly.
- Experimental data are required to quantify several important processes/parameters in drinking water distribution systems, such as: substrate (BOM) removal kinetics, biofilm detachment kinetics, mass transfer limitations and the role of pipe material as a nutrient source.

SUMMARY

This brief review presented some of the major factors which may lead to biological instability during the distribution of drinking water. Nutrient sources are important factors associated with bacterial regrowth and can be controlled during treatment. Other factors such as temperature are essentially controlled by the raw water source. The main energy source (e.g., electron donor) in drinking water is biodegradable organic matter (BOM), which is a surrogate for the amount of available organic carbon. Relationships have been established between bacterial regrowth and BOM (as measured by surrogate measurements) at full-scale and pilot-scale levels. Analyzing BOM components in drinking water has primarily been limited to studies investigating the

formation and removal of specific organic compounds during biological treatment. In general, further experimental data are required to quantify the rate of BOM utilization, from a component basis and its effect on bacterial regrowth in distribution systems.

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CHAPTER 2: RESEARCH OBJECTIVES

The primary objective of this research is to evaluate the effect specific biodegradable organic compounds have on bacterial growth in drinking water distribution systems. The specific compounds studied are carboxylic acids (formate, acetate), aldehydes (formaldehyde, glyoxal), and amino acids (aspartate, glutamate, serine). The concentration range of these compounds will be representative of most treated waters (e.g. 50 - 500 μg/L). Autochthonous microorganisms from the local groundwater will be used as an inoculum for all experiments. The experiments were performed using bench-scale models of distribution systems.

A number of secondary objectives which evolved from the primary objective include:

- development of a standardized method for removing bacteria from polycarbonate surfaces;
- an evaluation of the kinetics associated with the utilization of specific biodegradable organic compounds from drinking water;

- an examination of the factors influencing the formation of refractory organic matter in distribution systems; and
- an approach for relating results from bench-scale reactors to full-scale systems.

ORGANIZATION OF THESIS

All experimental work in this research used annular reactors (ARs) to model the physical and biochemical processes of a distribution system. Prior to discussing any results, Chapter 3 presents a detailed description of the AR and generalities of the bench-scale system. Also discussed in Chapter 3 are the assays and statistical procedures common to a majority of the research.

The AR is constructed of polycarbonate and houses removable slides which enable researchers to quantify biofilm activity. Only polycarbonate slides were used in this research and a standard procedure was developed for removing biofilm cells from these coupons for this research. The removal method is presented in Chapter 4.

The effect of BOM concentration, retention time, and the BOM composition (specifically the presence of free amino acids) on biofilm accumulation is examined in Chapter 5. Factorial experiments involving these three input factors are used to quantify their effect on biofilm accumulation and the production of suspended cells.

Chapter 6 presents a kinetic evaluation of the experiments performed in Chapter 5. A first-order relationship between the observed BOM removal rate and the effluent BOM concentration was found. The results suggest that Monod kinetics could be simplified to a first-order relationship for the removal of BOM in drinking water

distribution systems. An empirical investigation regarding the rate of mass transfer relative to BOM removal rate is also presented in Chapter 6.

In Chapter 5, it was found that bulk cells were more positively influenced by the hydraulic retention time. The influent substrate (BOM) concentration significantly increased the number of biofilm heterotrophic bacteria. By comparing the relative biofilm to bulk numbers it was apparent that the biofilm cells utilized most of the BOM. An examination of the possible mechanisms associated with biofilm detachment is presented in Chapter 6.

For the experiments presented in Chapter 7, three ARs were connected in series to create a model distribution system. The influent nutrient conditions were essentially similar to those presented in Chapter 5, however, half of the experiments discussed in Chapter 7 were also operated in the presence of a chlorine residual. The purpose of Chapter 7 is to examine the factors affecting biofilm accumulation in a model distribution system and to examine the processes associated with formation of soluble microbial products in treated waters.

Finally, Chapter 8 proposes the surface area to volume ratio (specific surface area) of a bench-scale reactor as a possible parameter for relating the extent of biofilm accumulation in ARs to that in full-scale systems. Calculations presented in Chapter 8 are primarily conceptual; further experimentation and modeling would be necessary to confirm the importance of specific surface area in biofilm studies.

CHAPTER 3: MATERIALS AND METHODS

INTRODUCTION

The purpose of this chapter is to explain in detail the physical characteristics and standard operating procedures of the bench-scale system. Also a description is provided for many chemical or microbiological assays, which were used for most experimental designs described in Chapters 4, 5, and 7. Finally, a general description of the statistical analyses performed on the data is provided.

BENCH-SCALE DISTRIBUTION SYSTEM

Annular Reactor

The annular reactors (ARs) used in this research were manufactured by BioSurfaces Technologies (1994 model; Bozeman, MT). The ARs are made of polycarbonate and stainless steel, and can be autoclaved in their entirety. Each AR consists of a stationary outer cylinder housing twelve flush-mounted removable slides (Figure 3.1). An inner drum rotates *via* a variable speed motor. The rotational speed of

the inner drum determines the shear stress at the wall. The inner drum also contains draft tubes to ensure good vertical as well as horizontal mixing. Residence time is controlled by the dilution rate. The volume of water contained by the AR was measured as 670 mL, this corresponded to a wetted surface area of 0.18 m^2. The reactors are capable of receiving multiple influent lines, which allows for substantial flexibility in operation.

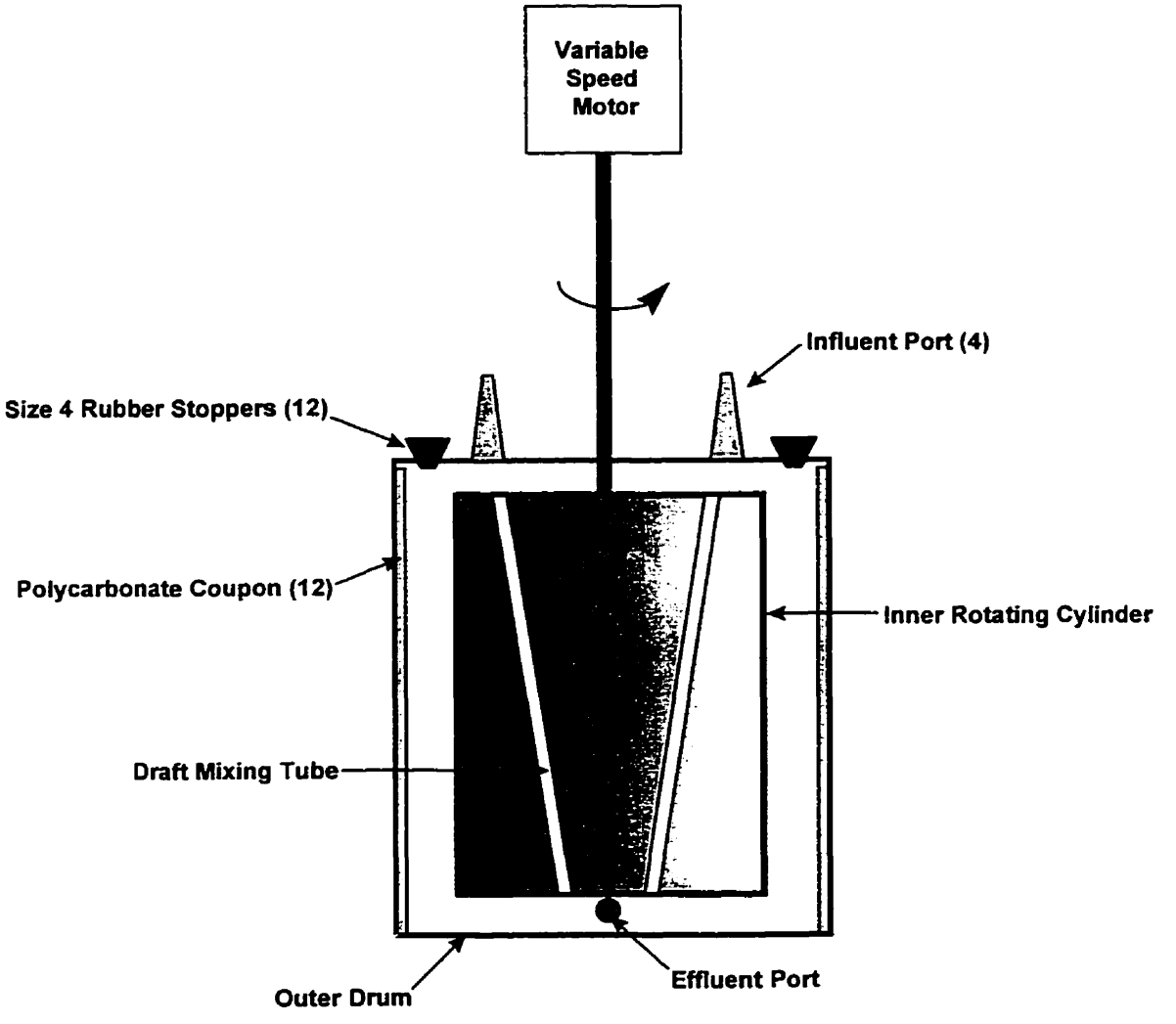

Figure 3.1: General schematic of an annular reactor.

Several advantages ARs provide for simulating distribution systems include: the AR is a completely mixed reactor; fluid shear and residence time are independently

controlled parameters, and; microbiological and/or chemical analysis is easily performed without disturbances to the reactor (Characklis, 1988). The annular reactor was critically evaluated by Gjaltema *et al.* (1994) for its limitations as a biofilm reactor. Gjaltema *et al.* (1994) found that non-homogeneous flow and shear patterns existed in the AR, which created longitudinal and lateral variations in biofilm structure on the polycarbonate coupon. It was concluded that the ARs are less suited to quantitative physiological studies, but can provide useful information for "black box" experiments. Although a quantitative analysis of the data will be performed, the experiments in this study are largely concerned with differences in gross parameters that can be generalized for the entire reactor rather than the details concerning biofilm processes and structure. Consequently, ARs should provide relevant information concerning biofilm accumulation in treated waters.

In Chapter 7 a different set of ARs (Model 920LJ; BioSurfaces Technologies, Bozeman, MT) are used. The major design differences between the two models are presented in Table 3.1. Experimentation by the author found that negligible differences in terms of biofilm accumulation rate and the number of steady-state biofilm bacteria were observed.

Table 3.1: Comparison of the Design Details in Two AR Models

Design Parameter	1994 Model	Model 920LJ
Motor Location	Top	Bottom
Coupon Location	Outer shell	Inner cylinder
Working Volume, mL	670	950
Number of Coupons	12	20
Number of Sampling Ports	12	1
Outer Shell Material	Polycarbonate	Glass

Physical Configuration

The experiments employed two ARs operating in parallel. As an initial treatment step, feed water (tap water which was essentially high quality groundwater) was passed through two GAC filters, with a combined empty bed contact time of 30 min, to remove chlorine and background organic matter (Figure 3.2). Sterile influent cocktails consisting of BOM components were also pumped into each AR. To ensure the system was carbon limiting, ARs were also dosed with a separate cocktail of nitrogen ($NaNO_3$) and phosphorous (K_2HPO_4 and KH_2PO_4) containing compounds. The cocktails were contained in 4 L amber glass bottles and pumped into the ARs with a C:N:P ratio of 100:20:5 (molar basis). In general, the GAC filter effluent contained negligible concentrations of the measured BOM components added in the cocktails.

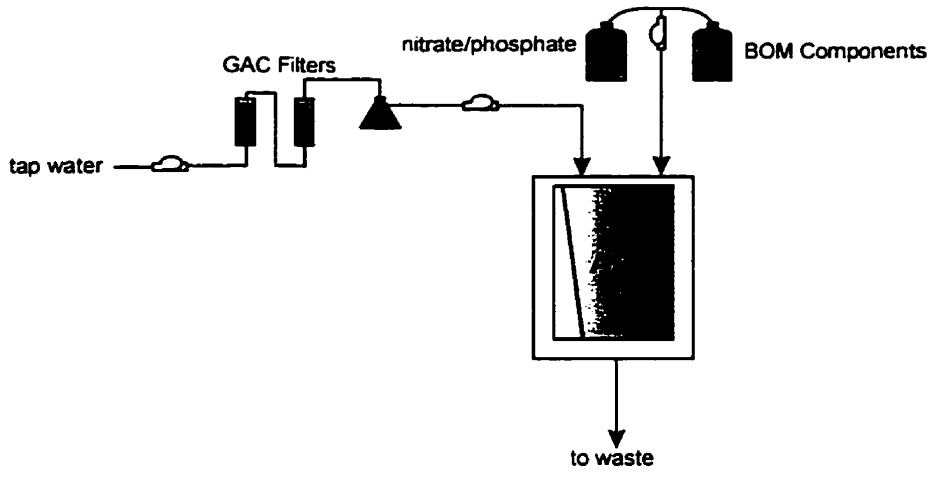

Figure 3.2: General schematic of bench-scale system.

Each AR was maintained at a fixed rotational speed of 50 rpm resulting in a calculated shear stress of 0.25 N/m^2 at the outer wall. This shear stress approximately corresponds to a flow of 0.30 m/s (1 fps) in a 100-mm (4 in.) diameter smooth pipe. All exposed surfaces (i.e., AR, tubing, feed tank) were covered by opaque plastic to reduce the potential of phototrophic growth in the bench-scale system. The experiments were conducted at ambient temperature resulting in a liquid phase temperature range of 19 ± 2°C.

Start-Up Protocol and Operating Procedures

Prior to use the AR was washed with hot distilled water in a dishwasher. The AR was then fitted with PharMed® tubing (Norton Co.) and autoclaved at 121°C and 101 kPa (15 psi) for an exposure time of 15 min. The autoclaved equipment was allowed to cool overnight, with all ports closed to maintain sterile integrity.

The influent feed solutions were prepared by diluting a stock solution of a specific compound into 3500 mL of sterile deionized water. The compounds were transferred into the feed bottles in a pre-sterilized containment hood. The feed bottles were changed

approximately every 4 to 6 days during an experimental trial. For quality control purposes the tubing carrying the influent cocktails was occasionally monitored for bacterial growth. It was found that the amount of growth in the lines was generally less than in the influent feed water. For the few occasions when excessive growth had occurred the feed solution and respective tubing were replaced immediately and the experimental trial was allowed to run for additional amount of time to allow the system to recover.

Prior to connecting the influent solutions to the AR, the pumps were calibrated to the appropriate settings. The pumps were turned on after the appropriate connections were made, and the ARs were allowed to fill. After approximately one changeover period (e.g., specific retention time for the experimental trial) had occurred, biomass and substrate samples were taken. This initial sampling was taken as the beginning of the acclimation period.

ANALYTICAL PROCEDURES

Carboxylic Acids

An ion chromatographic method (Peldszus *et al.*, 1996; Peldszus *et al.*, 1997) was used to analyze the water samples for carboxylic acids. Without any preparation, samples were injected directly into the ion chromatograph using a large sample loop (740 μL). Utilizing a sodium hydroxide gradient (8 mM/10 min - 13 min - 125 mM/10 min) the organic acids were separated on an anion exchange column (AS 10, Dionex, Sunnyvale, CA) with conductivity detection. Low background conductivity was achieved by an anion self generating suppresser (ASRS I, 4 mm, Dionex, Sunnyvale, CA), which was operated

in the external water mode because of the relatively high sodium hydroxide concentration at the end of the gradient (125 mM).

The samples for this investigation were preserved in 0.1 % $CHCl_3$ and stored at 4°C immediately after sampling. Samples were analyzed within 2 weeks of their collection. It was found that samples could easily be contaminated from skin and/or glassware. Consequently, many precautions (e.g., wearing latex gloves, rinsing glassware with deionized water) were taken when handling water samples, especially those samples which were measured for organic acids.

Fresh standards (tap water and deionized water) were prepared on the day of analysis by diluting a 100 mg/L stock to 1 mg/L and 100 µg/L. Included in the stock standards were (in order of elution time): hydroxybutyrate, acetate, glycolate, butyrate, formate, pyruvate, ketobutyrate, and oxalate. Method blanks (tap and deionized water) were included in all sample runs. For any given analysis, approximately one standard was prepared for every 6 samples. Replicate analysis occurred on a randomized basis and/or if one or more data points appeared questionable (e.g., abnormally high value due to contamination).

Unless noted otherwise all chemical products were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). Deionized water was prepared from a Milli-Q system (Millipore Corp.; Bedford, MA).

Aldehydes

Immediately after the ARs were sampled for aldehydes, 20 mL of sample was transferred into glass vials for aldehyde analysis. In addition to the AR samples deionized

water was used as a sample blank. The samples derivatized overnight at room temperature with 1 mL σ-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine hydrochloride (PFBHA) and preserved with 32.5 g of mercuric chloride ($HgCl_2$) and 32.5 g of ammonium chloride (NH_4Cl_2). The aldehydes were extracted from the sample with hexane as described by Sclimenti *et al.* (1990). Briefly, 4 mL of hexane containing an internal standard (dibromopropane) and 4 drops of concentrated sulphuric acid (H_2SO_4) were added to the sample. The sample was homogenized for approximately 5 min. After the organic and aqueous phases had separated 10 mL of 0.1 M H_2SO_4 was added to the sample to clear the organic layer. The sample was homogenized for an additional 5 min and allowed to stand for phase separation. The organic layer was transferred to vials containing sodium sulphate (Na_2SO_4) and stored at 4°C for up to 30 days. The aldehyde samples were analyzed by gas chromatography equipped with electron capture detection (GC/ECD) (HP 5890 series II, Hewlett-Packard; Sunnyvale, CA).

One day prior to analysis fresh standards and method blanks (deionized water) were prepared. For any given analysis, approximately one standard was prepared for every 10 samples. Because the system was controlled and there were on average 10 sampling periods for a given experimental trial, aldehyde samples were not analyzed in replicates. However, replicate analysis occurred on a randomized basis and/or if a data point(s) appeared questionable (e.g., abnormally high value due to contamination).

Amino Acids

Precolumn derivatization with orthophthaldialdehyde (OPA) followed by analysis with high pressure liquid chromatography (HPLC), as described by Dossier-Berne *et al.*

(1994), was used for measuring amino acids. The mobile phase (mobile phase A was acetate/phosphate buffer, pH 7.4; mobile phase C was 75 % methanol) was introduced to the column using a 600 E Multi Solvent Delivery System (Waters Corp.; Milford, MA). The sample was introduced to the system with a Waters 717 Autosampler (Waters Corp.; Milford, MA). Amino acids were quantified with a Waters 470 Scanning Fluorescence Detector (Waters Corp.; Milford, MA). These samples were analyzed by a chemist (either L. Liao or V. Goodfellow) within the NSERC Chair for Industrial Research in Water Treatment.

Non-purgeable Organic Carbon (NPOC)

NPOC was measured using a Xertex Dohrmann DC-180 Total Carbon Analyzer (Rosemount Analytical; Santa Clara, CA) using *Standard Methods* (Eaton *et al.*, 1995). Each reported value was typically the average of 3 determinations. These samples were analyzed by either L. Liao or V. Goodfellow.

Heterotrophic Plate Count (HPC) Bacteria

Microbiological samples were taken from the influent and effluent of each AR. Biofilm coupons were aseptically sampled and placed into 100 mL of sterile deionized water. Biofilm was removed from the coupon and homogenized for two minutes in the diluent using a Stomacher 400 Lab Blender (Seward Ltd.; London, UK).

Heterotrophic plate counts (HPCs) were determined by serially diluting the sample into sterile deionized water and spreading 100 μL of diluent onto R2A agar (Eaton *et al.*, 1995). Each dilution series was plated in duplicate. Plates were incubated for seven days at room temperature (19 ± 2°C). Plates containing between 30 and 300

colonies were selected for enumeration. Biofilm and bulk (suspended) HPCs were reported on a CFU/cm^2 and CFU/mL basis, respectively.

Autoclaved deionized water was used for blank samples. Contaminated dilution water was rarely found as the cycle time for preparing culture tubes was typically less than a week. In general, the agar plates were more easily contaminated than the dilution water. Any agar plates that were contaminated prior to plating were discarded. The coefficient of variation for HPC numbers ranged from 1.3 % to 3.7 %, on a log-scale.

All microbiological media were purchased from BBL Products (Becton Dickinson Microbiology Systems; Cockeysville, MD).

Total Direct Cell Counts

Bacterial samples were stained with acridine orange and enumerated using a Nikon Labophot 2A fluorescence microscope (Nikon Canada Inc.; Mississauga, ON) equipped with barrier filters. The staining and enumeration procedure for measuring total direct cells followed the method described by Fry (1990). Homogenized biofilm and effluent samples were preserved in 2% (final concentration) formaldehyde solution at 4°C until analysis, maximum storage of 30 days. The samples were incubated for 10 min., with 0.01% acridine orange and filtered through a Nuclepore (Millipore, Corp.; Bedford, MA) polycarbonate membrane filter (pore size 0.22μm; diameter 25 mm). Cell numbers were reported as the mean count from 10 fields of view at 1000x magnification.

Blank samples were prepared with filter sterilized deionized water which contained 2 % formaldehyde. The blank samples were stained and enumerated as previously described. Blank samples were measured prior to staining any other samples.

Blanks were typically measured after every tenth sample. In the event of contamination (cell count exceeding 1.0×10^4 cells/mL, which roughly corresponds to a total of 20 cells counted per 10 fields of view) fresh acridine orange was prepared. The coefficient of variation for total cell counts ranged from 0.7 % to 1.4 %, on a log-scale.

Diagnostic Measurements

Perturbations in temperature, dissolved oxygen, and pH were measured in the influent and effluent of each AR during an experimental trial. Ion selective electrodes used for quantifying dissolved oxygen and pH were calibrated prior to sampling.

STATISTICAL ANALYSIS

Most of the experiments performed in this thesis have a two-level factorial design. The basic idea of a two-level factorial experiment is to determine which "factors" (usually two or more) influence the "response" of a process. For each factor an experiment is run at a "high" and "low" level. Therefore, if three factors were used for a particular investigation, a total of eight experimental trials (e.g., 2^3) would be necessary to complete the design. Factorial experiments are efficient, requiring relatively few runs per factor. However, the factorial approach (at two levels) is limited by its inability to fully explore specific regions in the factor space. Therefore an experiment which has a factorial design is useful for indicating major trends and identifying new regions for further experimentation (Box *et al.*, 1978).

Results from the factorial experiments were analyzed using analysis of variance (ANOVA) tests and student t-tests. Unless otherwise noted, the level of significance (α) for these tests was 5 %.

Linear regressions were calculated for many variables in this thesis. Standard tests such as normalized plots, residual plots, ANOVA tables, and r^2 values as outlined in Draper and Smith (1981) were calculated for all regressions. Also care was taken to ensure that the models made physical sense and were not the result of happenstance or unplanned data (Box, 1966).

Plate counts and total counts exhibited non-constant variance over the range of experimental observations. One of the major reasons for an increased variability with the mean number of HPCs was the effect of serial dilution. To minimize this variability the cell count data was transformed onto a log-scale as recommended by Berthouex and Brown (1994). In most cases the mean of the transformed data will be presented, along with the symmetric 95 % confidence limits (transformed scale). The arithmetic mean refers to the mean on the original scale, and the geometric mean refers to the antilog of the transformed mean.

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CHAPTER 4: A METHOD FOR THE REMOVAL OF BIOFILM CELLS FROM A POLYCARBONATE COUPON

INTRODUCTION

In drinking water distribution systems the number of biofilm cells generally exceeds the number of their planktonic counterparts (Camper, 1996). Accordingly, the focus of many investigations in distribution systems concerns the accumulation of biofilm cells under different operating and environmental conditions (LeChevallier *et al.*, 1990; Donlan and Pipes, 1988) and the characterization of biofilm cells present (LeChevallier *et al.*, 1987). In granular activated carbon (GAC) filters operating biologically, bacteria are typically liberated from the GAC particles using a homogenizer (Pernitsky *et al.*, 1997; Stewart *et al.*, 1990; Camper, 1985). However, biofilm coupons that are mounted on the sides of an annular reactor (AR) have a higher surface area to volume ratio than GAC particles, and are relatively rigid. Removing cells from the coupon with a homogenizer is not feasible for routine analysis.

Bacteria attached to biofilm coupons in an AR have typically been removed or scraped from the coupon with a utility knife into a known aliquot of sterile water (e.g., Camper *et al.*, 1996; Baribeau *et al.*, 1996). The advantage of this method is its simplicity, however scraping could compromise sterility and be highly subject to individual variation and human error. To the author's knowledge this method has not been compared to other available desorption techniques, particularly under drinking water conditions. However, scraping has been commonly adopted as the standard method for removing cells attached to AR coupons by *de facto*.

The purpose of this chapter is to describe the development of a method that can remove and resuspend biofilm cells efficiently, consistently and with a good recovery rate. Several methods available for the removal of attached cells are examined. Techniques used for homogenizing flocculated cells are also critically evaluated. Finally, different buffer solutions are compared to determine the impact buffers may have on maintaining the integrity and viability of the recovered cells.

MATERIAL AND METHODS

Experimental Configuration

In general, the ARs were operated similar to the physical set-up described in Chapter 3. The ARs were fed groundwater filtrate, sodium acetate (as the sole carbon source), nitrogen ($NaNO_3$) and phosphorus (KH_2PO_4/K_2HPO_4). The cocktails were contained in 4 L amber glass bottles and pumped into the ARs with a C:N:P ratio of 100:20:5 (molar basis). Acetate was pumped into the ARs at an influent concentration of 500 μg/L. The ARs were operated at a hydraulic retention time (HRT) of 2 h. The reactors

were run for at least two weeks to ensure that the number of biofilm heterotrophic bacteria, as measured by heterotrophic plate counts (HPCs), was at an approximate steady-state (Camper, 1996).

Detachment of Biofilm Cells

Three methods used to detach cells from the polycarbonate coupons were: scraping with a utility knife, swabbing, and stomaching. Coupons were scraped either 5 or 10 times to determine if the scraping frequency was a significant parameter.

The coupon was aseptically removed from the reactor and was partially submerged in a 250 mL beaker containing 100 mL of sterile deionized water prior to scraping. Biofilm was then scraped from the coupon into the water either 5 or 10 times with a utility knife which had been flame sterilized with 95 % ethanol. The detached cells were manually shaken for 30 sec, this also served as the control for the homogenization step.

Sterile cotton swabs were first moistened in the sterile water and then pressed against the coupon, which was partially submerged in a 250-mL beaker containing 100 mL of sterile deionized water. The swabs were then rolled down the coupon starting from the top (i.e. the portion furthest from the water). The coupons were swabbed for several square centimeters and then were rinsed in the sterile water. This process was repeated until the entire surface of the coupon had been swabbed. To remain consistent with the scraping technique the entire coupon was swabbed from top to bottom 5 times. Similar to the scraping method, the detached cells were manually shaken for 30 sec.

For the stomacher method, coupons were transferred into a sterile stomacher bag (177 mm by 304 mm) and rinsed in the bag with 100 mL of sterile deionized water. The bag was then placed in the stomacher (Stomacher 400 Lab Blender; Seward Ltd., London, UK) for processing. The stomacher was operated on normal speed (230 rpm ± 5 %) for 2 min. No additional mixing occurred after this step.

Homogenization of Detached Cells

After the cells from the coupon were removed into 100 mL of sterile water, aliquots of this solution were divided for comparing mixing efficacy. Four options selected for homogenizing cells were: a tissue blender, vortexing the sample, the stomacher, and a sonicator. In addition, an aliquot which received minimal mixing was used as a control.

An aliquot of 10 mL was homogenized in the pre-sterilized tissue blender, Pother-Slvehem homogenizer. The blender consisted of an outer glass tube that housed the sample, and a Teflon plunger, which was used for homogenizing the sample. The sample was blended for approximately 2 min with a slow, consistent plunging action.

Another method required 10 mL of the buffering solution for mixing with a vortex mixer (Fisher Vortex, Genie 2™; Fisher Scientific Corp.). The sample was transferred into a pre-sterilized culture tube and vortexed for 2 min.

Regardless of the previous detachment technique 50 mL of the biofilm solution was stomached in a stomacher for 2 min at normal speed. To evaluate mixing efficacy the stomacher bag did not contain the biofilm coupon.

The remaining 20 mL of solution was divided into two equal aliquots. One aliquot was used as a control, and the remainder was placed into a glass culture tubes for subsequent sonication. Three glass tubes were then placed in a 500 mL beaker, which was submerged in the sonicator (Bransonic® Ultrasonic Cleaner, Model 5210; Branson Ultrasonic Corp., Danbury, CT) for sonication for 2 min at 47 kHz.

Potential Suspending Solutions

The impact of the suspending solution on the removal of biofilm cells was also examined. Solutions investigated were: deionized water, phosphate buffer, HEPES buffer, ethylenediaminetetraacetic acid (EDTA), and saline.

Deionized water was prepared from a Milli-Q® water purification system (Millipore Corp.; Bedford, MA) and autoclaved (121°C, 15 psi) for an exposure time of 15 min. All other buffers were prepared by dilution with deionized water and subsequent filter sterilization through a 0.22-μm polycarbonate filter.

The phosphate buffer consisted of a mixture of 5.0 mM KH_2PO_4 and 4.76 mM K_2HPO_4. HEPES buffer was examined at a concentration of 5 mM and 0.5 mM. Biofilm samples were also buffered with 0.05 % EDTA. Finally, physiological saline at concentrations of 0.85 % and 0.085 % was also evaluated as a potential buffering solution.

Evaluating Recovery of Biofilm Cells

After the homogenization step the samples were plated in duplicate on R2A agar, as described in Chapter 3. The plates were incubated for 7 days at room temperature (18 ± 2°C). Colonies were counted and reported as CFU/cm^2.

In addition to determining HPCs, direct microscopic cell counts were performed using acridine orange stained preparations, as described in Chapter 3. For the acridine orange direct counts (AODCs), 5 mL of the homogenized sample was diluted with 5 mL of filter sterilized deionized water and stored in sterile 16-mL culture tubes. The samples were preserved in 2 % formaldehyde and incubated at 4°C until enumeration.

RESULTS

Determining Removal and Mixing Conditions

Two independent experimental trials were performed to establish appropriate detachment and homogenization conditions for the removal and subsequent resuspension of biofilm cells. The first trial is a randomized block experiment comparing the four detachment methods and five mixing methods. The relative importance between detachment and resuspension of biofilm cells is also examined.

Figure 4.1 shows the amount of biofilm HPCs recovered from a specific detachment and mixing method. Each data point in Figure 4.1 represents an average of results from three coupons. Cells on the coupons were removed with one of the detachment methods and appropriate aliquots were then used for each of the resuspension methods.

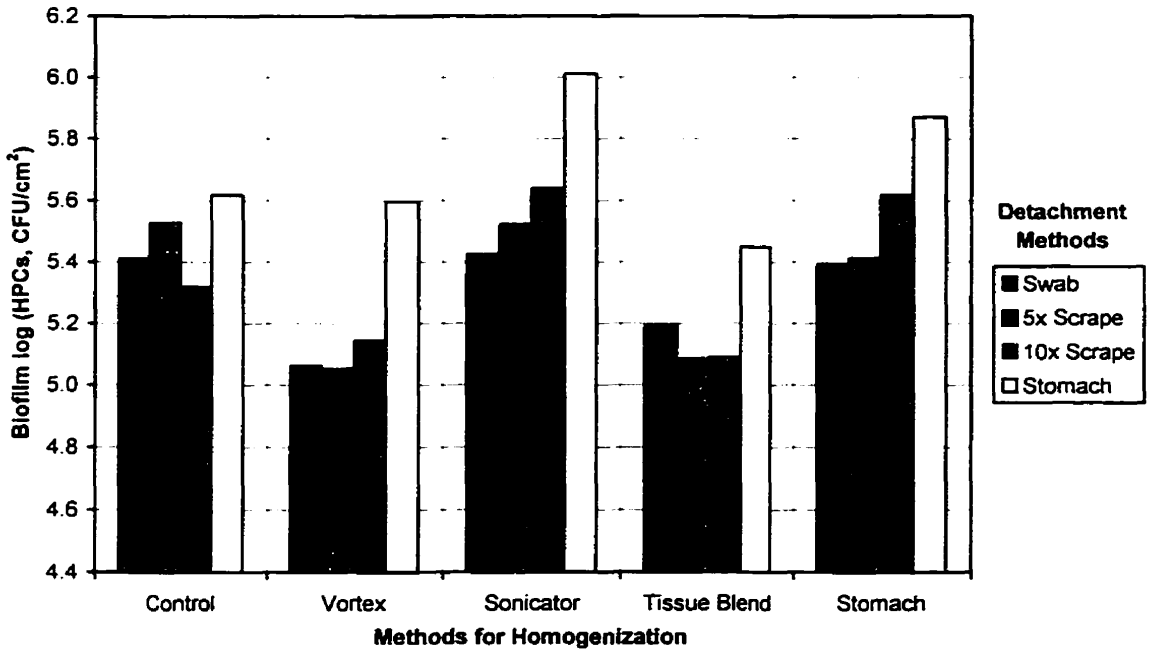

Figure 4.1: Recovery of biofilm HPCs using various detachment and homogenization methods.

The relative significance of detachment and resuspension were compared using an analysis of variance (ANOVA) test (Table 4.1). Both the removal and homogenization steps were significant (α = 0.05) for recovering biofilm HPCs from polycarbonate coupons. Comparing the means for the removal methods revealed that swabbing and scraping (regardless of frequency) were essentially the same. HPC recovery using the stomacher was significantly greater than all three of these methods, this is also qualitatively shown in Figure 4.1.

The tissue blender and vortex resulted in essentially the same number of biofilm HPCs and the lowest average HPC recovery. The greatest HPC numbers obtained from the homogenization used either the sonicator or the stomacher. Recovery from the control samples (e.g. little to no mixing) were not significantly different from either tissue

blender/vortex numbers (P value = 0.086) or the sonication/stomacher HPC numbers (P value = 0.22).

Table 4.1: ANOVA for Four Detachment Methods and Five Homogenization Methods

Source	Sum of Square	Mean Square	F value	p value
Detachment	1.683	0.561	12.89	< 0.0001
Homogenization	2.012	0.503	11.55	< 0.0001
Interaction	0.377	0.031	0.72	0.723
Residual	1.741	0.043	-	-

The effect of the described removal and mixing conditions on the total number of cells, as enumerated by acridine orange direct counts (AODCs), was generally similar to the effect on the HPC numbers (Figure 4.2). Both the detachment and homogenization methods were significant processes in the recovery of cells, having p values less than 0.01 % and 0.3 % respectively. Again the stomacher recovered the greatest number of biofilm cells from both a detachment and homogenization point of view. No significant differences ($\alpha = 0.05$) were calculated for the total cells enumerated from samples obtained from the tissue blender, stomacher or sonicator. However, the number of total cells was significantly greater after homogenization in either the tissue blender or stomacher as compared to numbers that received little mixing (control) or were resuspended using a vortex.

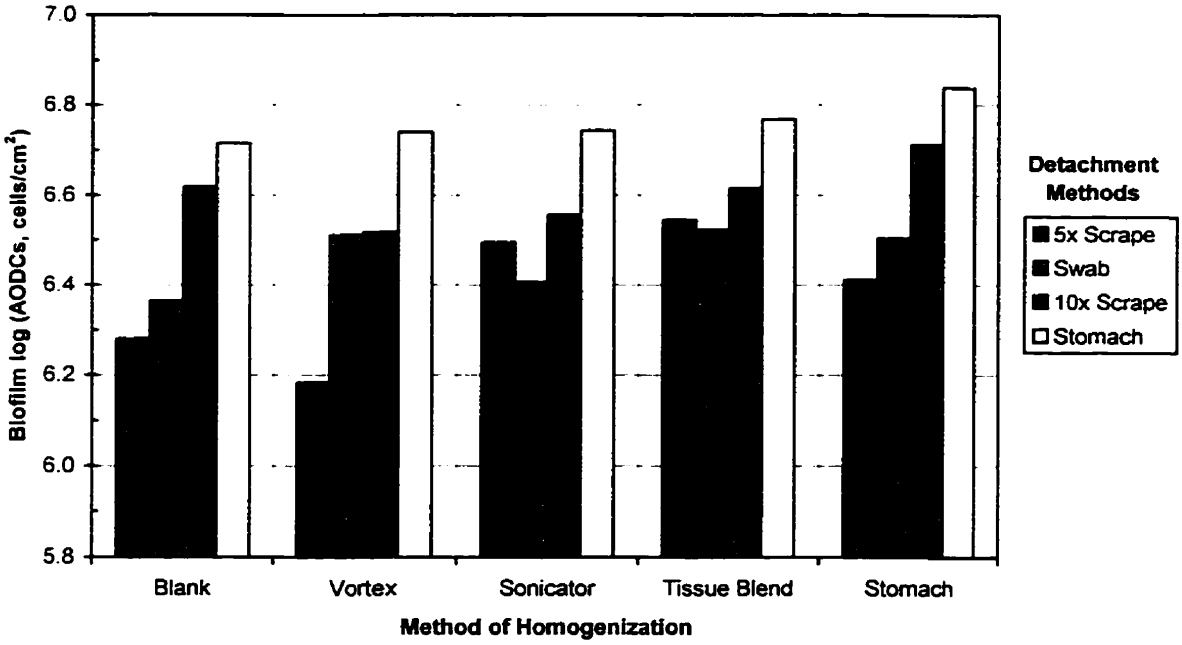

Figure 4.2: Recovery of biofilm AODCs using various detachment and homogenization methods.

Coupons that were either scraped or swabbed had cells removed from the side exposed to the bulk water in the annular reactor. Samples that were detached using the stomacher had cells removed from either the surface exposed to the bulk liquid or the surface mounted against the outer cylinder of the AR (backside). An additional experimental trial was performed to determine whether the high recovery observed using the stomacher for detaching cells was an artifact from the cells attached to the backside. For this experimental trial cells were detached from the coupon by either stomaching, scraping the side exposed to the water ten times, or swabbing the backside of the coupon and discarding these cells followed by stomaching. Also for this experimental trial samples were homogenized using either the stomacher, sonicator, or received no additional mixing.

For the second experimental trial, the greatest recovery was observed for cells detached with the stomacher (Figure 4.3). The recovery of cells that had the backside of the coupon swabbed resulted in greater recoveries than the coupons that were scraped. On average coupons that were stomached and not swabbed had a higher number of recovered cells then the swabbed coupons. However, the difference in recovered cells between swabbed and non-swabbed is less than one half-log. It is unlikely that swabbing the backside improves the recovery process.

Regardless of the technique used to homogenize the sample, the coupons that were scraped resulted in essentially the same number of biofilm HPCs. Similarly, the homogenization method marginally affected the recovery of cells for the two stomacher methods.

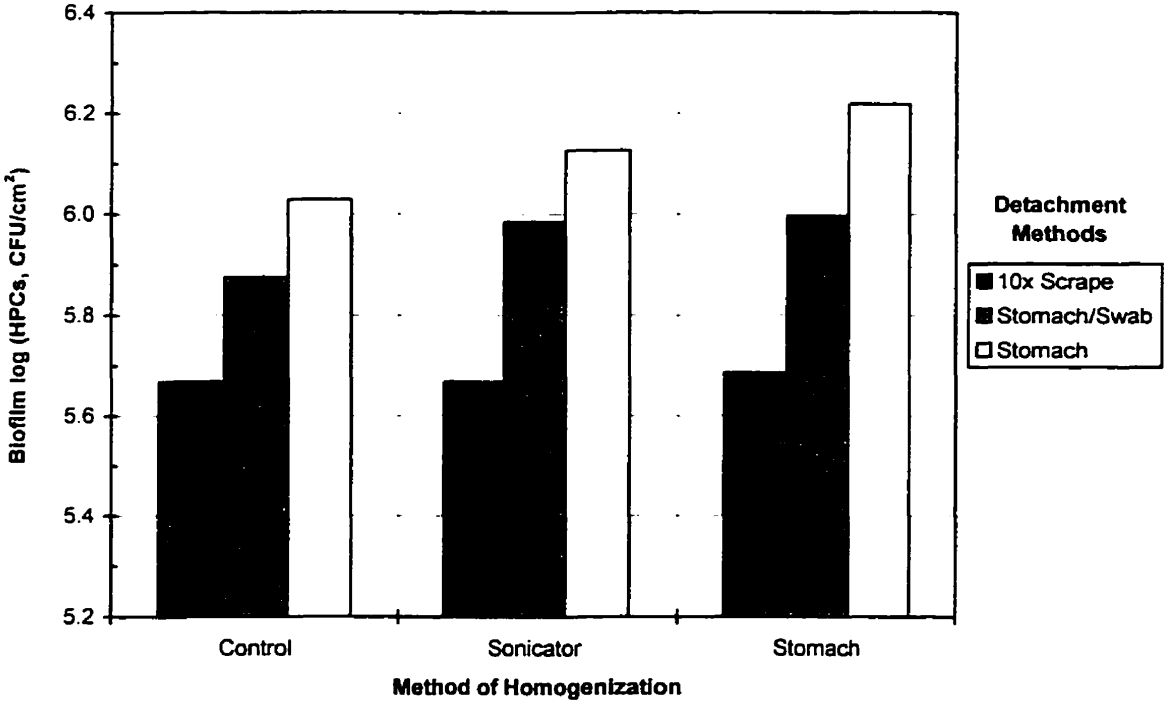

Figure 4.3: Recovery of biofilm HPCs after detachment from scraping, the stomacher and the stomacher with the backside swabbed, and a comparison of three mixing methods.

An ANOVA also showed that the detachment method significantly affected the recovery of biofilm HPCs (Table 4.2). Of these detachment methods, a comparison of the means revealed that the number of HPCs recovered from the stomacher was significantly greater than the number HPCs recovered after scraping. However, no statistical difference in the mean number of biofilm HPCs was observed for samples when shaken in the stomacher with the backside swabbed and without the backside swabbed.

Total direct cell counts for biofilm samples in the second experimental trial are shown in Figure 4.4. Similar to the HPC counts the homogenization technique had a minimal impact on the recovery of total cells (P value = 0.7479). The detachment method was highly significant for the recovery of total cell counts (P value < 0.0001). Each of the detachment methods resulted in significantly different total cell counts. However, unlike the HPC counts, the samples which had the backside swabbed prior to processing in the stomacher resulted in the highest total cell count.

Table 4.2: ANOVA for Three Detachment Methods and Three Homogenization Methods

Source	Sum of Square	Mean Square	F value	P value
Detachment	0.929	0.465	10.67	0.0009
Homogenization	0.055	0.027	0.63	0.544
Interaction	0.026	0.007	0.15	0.961
Residual	0.784	0.044	-	-

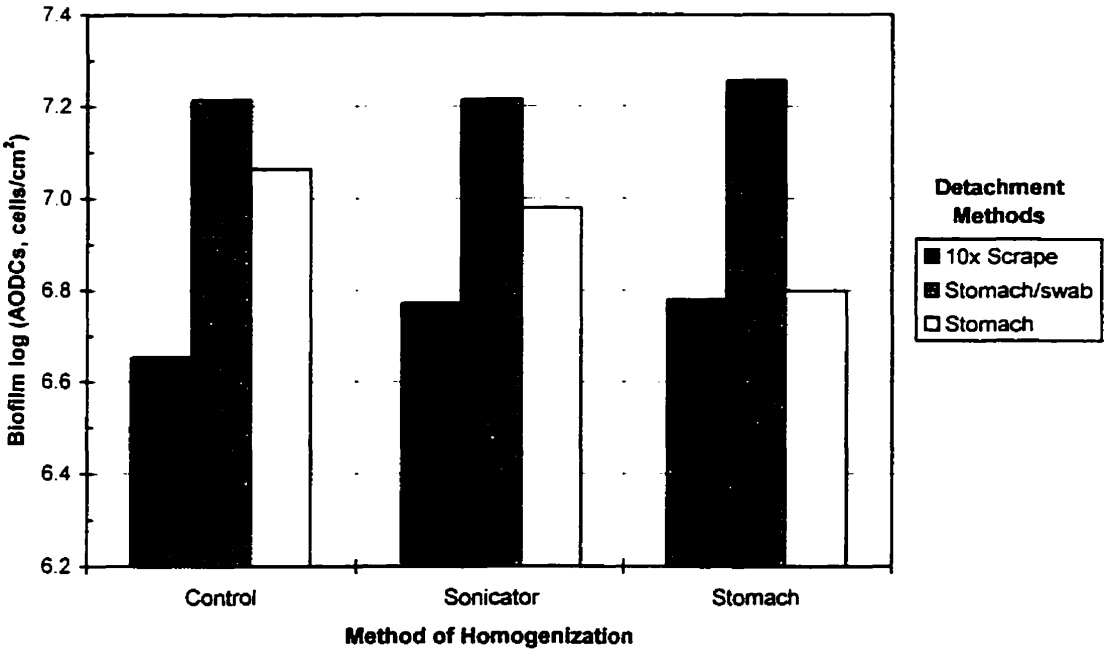

Figure 4.4: Recovery of biofilm AODCs after detachment from scraping, the stomacher and the stomacher with the backside swabbed, and a comparison of three mixing methods.

Evaluating Available Suspending Solutions

The buffering solutions were compared in three independent experimental trials (Table 4.3). Deionized water was used as a control in all of the trials. The greatest recovery of HPCs observed in the first trial was obtained using a phosphate buffer. The number of AODCs recovered from the first trial was essentially the same for the three suspending solutions examined.

In the second trial, the number of HPCs recovered from samples buffered with 5 mM HEPES and water were similar. However, in the third trial a greater number of biofilm HPCs were obtained from samples buffered 0.5 mM HEPES than those with water. Regardless of the saline concentration, the HPCs and AODCs were lowest in the samples buffered with saline (Table 4.3).

Table 4.3 A Comparison of Recovery of HPCs and Total Cells after Removal and Homogenization in the Stomacher with Different Buffering Solutions

Experimental Trial	Buffer	log (HPCs)	log (AODCs)
A	Water	6.22	6.64
	H_2PO_4/HPO_4	6.73	6.44
	EDTA	5.29	6.60
B	Water	6.47	7.30
	5 mM HEPES	6.56	7.00
	0.85 % Saline	4.84	6.48
C	Water	6.19	6.69
	0.5 mM HEPES	6.70	7.14
	0.085 % Saline	5.91	6.51

Stomacher Run Length

The duration of stomaching should be minimized to reduce possible cell damage, but sufficient to remove the majority of bacteria. Figure 4.5 shows the extent of HPC recovery for stomaching run lengths ranging from 0.5 to 4 min. Two suspending solutions used in this investigation were sterilized deionized water and 5 mM HEPES buffer. For both buffers the number of biofilm HPCs recovered gradually increased as the stomacher duration increased from 0.5 to 2 min. In a separate experimental trial the stomaching duration was extended to 8 min, however no additional HPCs were recovered beyond 2 min (data not shown).

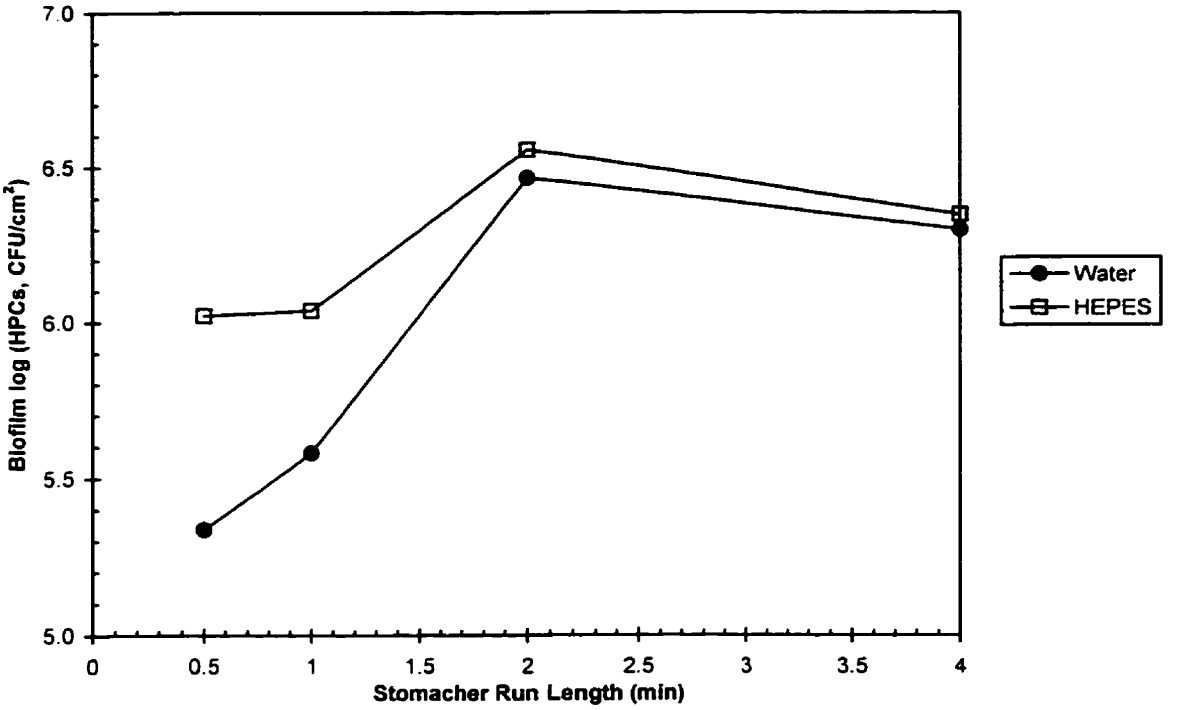

Figure 4.5: Effect of stomacher run time on the removal of biofilm HPCs attached to coupons submerged in water and 5 mM HEPES.

Over run lengths of 2 to 4 min, the total number of cells recovered was essentially independent of the stomacher duration. The number of AODCs recovered from the samples buffered with water had a geometric mean of 7.1 ± 0.3 (with 95 % confidence). Similarly, the number of AODCs recovered from the samples buffered with 5 mM HEPES had a geometric mean of 6.8 ± 0.3 (with 95 % confidence).

DISCUSSION

Of the detachment methods studied, the stomacher recovered the highest number of HPCs (Figure 4.1) and total cells (Figure 4.2). This was likely because the stomacher is a mechanical device having greater reproducibility than the manual methods. However,

the manual methods were consistent in that the frequency of scraping and swabbing were essentially similar in their ability to remove attached cells.

The stomacher combines the removal and homogenization step into one process, which may also account for the higher recoveries observed in this investigation. This quality is useful as subsequent experimentation revealed that the removal step is more critical than the resuspension step (Table 4.2). The stomacher was first introduced as non-destructive means for preparing bacterial suspensions from food products (Sharpe and Jackson, 1972) and is also used for the removal of epiphytic bacteria from plant leaves (Fry and Humphrey, 1978). Also stomaching causes minimal adverse effects on cell viability (Jacques and Morris, 1995) and can be easily used aseptically. However, similar to the other removal methods studied in this investigation, isolating cells by stomaching results in a loss of information regarding local biofilm characteristics (Morris *et al.*, 1997).

Sonication also has the capability to combine these two recovery steps, and was shown to be an efficient means for extracting bacteria from soil (Ramsay, 1984). For the conditions studied, sonication did not injure culturable cells during resuspension (Figure 4.1). However, in a comparative study on methods for liberating epiphytic cells, Donegan *et al.* (1991) found that stomacher blending was statistically more efficient ($\alpha = 0.05$) at recovering cells than sonication. Furthermore, recovering biofilm cells aseptically in a single step with sonication from polycarbonate coupons would be cumbersome and less practical for routine analysis.

Sterile water is often the buffer used to resuspend scraped biofilm cells in drinking water (Camper, 1996, Baribeau *et al.*, 1996). Of the buffers examined, 0.5 mM

HEPES appeared to have the greatest buffering capacity for recovering biofilm cells. However, assuming that the stomacher duration beyond 2 min does not improve removal efficacy, no statistical difference was observed between 0.5 mM HEPES and sterile deionized water. None of the buffers examined greatly improved the recovery of total or culturable cells than sterile water. Because the biofilm cells are grown under dilute conditions in filtered tap water, it is not surprising that sterile water does not cause osmotic shock in the resuspended cells.

Ideally the optimum duration of a stomaching run should be minimized to reduce possible cell damage, but sufficient to remove the majority of bacteria. Camper (1985) found that 90 % of the known bacterial population were recovered within 3 min of homogenizing GAC particles. An optimal stomacher run length of 5 min was found for removing epiphytic bacteria from aquatic plants (Fry *et al.*, 1985). For this investigation, no net gain in total or HPC numbers was observed for stomaching beyond two minutes.

CONCLUSIONS

Several methods for removing and resuspending biofilm cells from a polycarbonate surface were examined. The choice of the detachment method was found to be more critical than the resuspension technique. Consequently, removal of cells by the stomacher was selected as the ideal choice because it can remove and resuspend cells in a single step minimizing excessive sample preparation and maintaining aseptic conditions. The optimal stomaching duration was 2 min at normal speed. Sterile deionized water was found to be a suitable resuspension media for the growth conditions studied.

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CHAPTER 5: EFFECT OF OZONATION BY-PRODUCTS AND AMINO ACIDS ON BACTERIAL GROWTH IN AN ANNULAR REACTOR

INTRODUCTION

Water utilities may consider ozonation as a treatment step for various reasons. Recent interest in ozone has increased because it is a more powerful disinfectant than chlorine (Montgomery, 1985) and is likely more effective at controlling *Cryptosporidium* (Solo-Gabriele and Neumeister, 1996). During ozonation low molecular weight, easily biodegradable organic compounds, referred to as ozonation by-products, are formed by oxidizing natural organic matter (NOM) (Langlais *et al.*, 1991). In the absence of a biological filtration step, these ozonation by-products may be used as an energy source (e.g.,, electron donor) for bacterial growth in distribution systems. Compounds which have been identified as by-products include aldehydes (Najm and Krasner, 1995), oxo-acids (Xie and Reckhow, 1992), and carboxylic acids (Gagnon *et al.*, 1997).

Short-chain aldehydes, such as formaldehyde and acetaldehyde, can be effectively removed by biological filtration (Weinberg *et al.*, 1993), although differences in removal

rates have been observed at several temperature ranges (Coffey *et al.*, 1995). Studies have indicated that concentrations of carboxylic acids can be reduced during biological filtration (Urfer and Huck, 1997). However, a full-scale investigation has shown that complete removal of carboxylic acids does not occur during biological filtration, even at long retention times (Gagnon *et al.*, 1997).

In a pilot plant study which examined the efficacy of nanofiltration, Agbekodo *et al.* (1996) showed that the total amino acids represented approximately 63% of biodegradable dissolved organic carbon (BDOC) in the permeate. However during a full-scale investigation which studied the fate of BOM components, Anderson *et al.* (1997) inferred that the amino acids present were part of larger protein molecules, and free amino acids were either at or below the detection limit.

An objective of this chapter is to examine the impact on regrowth of biodegradable organic compounds at concentrations typical of treated waters. The relative importance of the presence of amino acids in drinking water will also be explored. Finally, relationships between biofilm accumulation, the number of planktonic bacteria and hydraulic retention time (HRT) will be discussed.

MATERIALS AND METHODS

Experimental Design

Three factors were examined for their influence on biofilm accumulation in an annular reactor (AR), namely: the influent BOM concentration, the fraction of amino acids in the BOM cocktail, and the hydraulic retention time (HRT). For a given pipe material, temperature, and disinfectant residual (in this case none), these factors were

expected to be the most significant. The levels for the influent BOM concentration were 50 and 500 μg C/L. Amino acids were either not included in the BOM cocktail or were present on a mass fraction basis of 0.34. Annular reactors were operated at an HRT of 2 or 4 h. Net biofilm accumulation, as measured by HPCs or AODCs, was used to determine the significant effects of these factors.

Physical Configuration

The experiments employed an AR (BioSurface Technologies Corp., Bozeman, MT) which was fed GAC filtered tap water and sterile nutrient cocktails (Figure 5.1). A detailed description of the physical characteristics of an AR was provided in Chapter 3. The general bench-scale set-up was similar to the configuration described in Chapter 3 with minor modifications (Figure 5.1). The BOM components were separated into sterile influent cocktails of amino acids, carboxylic acids, and aldehydes. To ensure the system was carbon limiting, ARs were also dosed with a separate cocktail of nitrogen ($NaNO_3$) and phosphorus (K_2HPO_4 and KH_2PO_4) containing compounds. The cocktails were contained in 4 L glass bottles and pumped into the ARs with a C:N:P ratio of 100:20:5 (molar basis).

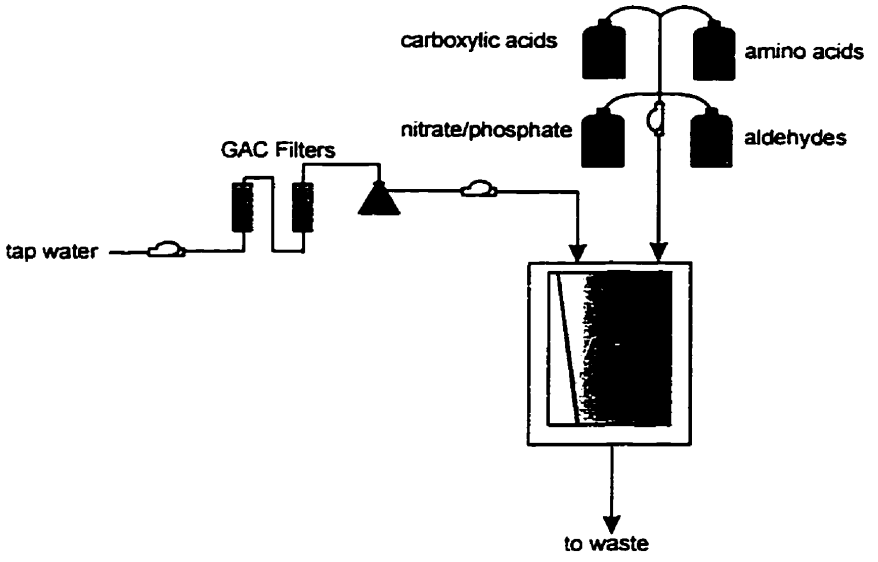

Figure 5.1: Schematic diagram of bench-scale system.

Aldehydes (formaldehyde, glyoxal) and carboxylic acids (acetate, formate) were always included in the BOM cocktail. Amino acids (L-aspartic acid, L-glutamic acid, and L-serine) were also included in the BOM cocktail depending on the experimental design. The theoretical oxygen demand (ThOD) of each component divided by the total calculated ThOD for all of the added synthetic compounds was used to determine the carbon mass ratio in the BOM cocktails (Table 5.1). A similar approach for adding synthetic compounds to a bio-reactor has been described elsewhere (e.g.,, Zhang and Huck, 1996).

The ThOD for all of the added amino acids was 68 % of the total influent BOM. It was felt that this fraction may be too high for many treated waters. Consequently, the amino acid to total BOM concentration ratio was set at 0.34 (mass basis) for all experiments.

Table 5.1: Summary of Biodegradable Organic Compounds Used in the Bench-Scale Experiments

Compound Type	Compound	Formula Weight (g/mol)	Percentage of Total Carbon by Weight	
			AA[†] = 0 %	AA = 34 %
Aldehyde	Formaldehyde	30	22.2	14.7
	Glyoxal	58	33.3	22.0
Carboxylic acid	Acetate	59	38.9	25.7
	Formate	45	5.6	3.7
Amino acid	Aspartate	131	0.0	10.2
	Glutamate	145	0.0	8.5
	Serine	116	0.0	15.3
Total			100.0	100.0

[†] AA = Amino Acids

The nutrient cocktails were prepared with autoclaved Milli-Q® (Millipore, Corp.; Bedford, MA) water and filter sterilized (0.2 μm) stock solutions. Stock solutions were kept refrigerated at 4°C and diluted with sterile water to create the cocktails.

Each AR was maintained at a fixed rotational speed of 50 rpm resulting in a calculated shear stress of 0.25 N/m^2 at the outer wall. This shear stress approximately corresponds to a flow of 0.30 m/s (1 fps) in a 100 mm (4 in.) diameter smooth pipe. All exposed surfaces (i.e., AR, tubing, feed tank) were covered with opaque plastic to reduce the potential of phototrophic growth in the bench-scale system. The experiments were conducted at ambient temperature resulting in a liquid phase temperature range of 19 ± 2°C.

Chemical Analyses

Details regarding the chemical analyses were provided in Chapter 3, and are briefly summarized in this Chapter. An ion chromatographic method (Peldszus *et al.*,

1996) was used to analyze the water samples for carboxylic acids. Precolumn derivatization with orthophthaldialdehyde (OPA) followed by HPLC analysis with fluorescence detection (Dossier-Berne *et al.* 1994) was used for measuring amino acids. The samples for this investigation were preserved in 0.1 % $CHCl_3$ and stored at 4°C immediately after sampling and measured within 2 weeks of their collection. Aldehydes were derivatized by oximation with σ-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine hydrochloride (PFBHA) and extracted with hexane as described by Sclimenti *et al.* (1990). NPOC was measured using a Xertex Dohrmann DC-180 Total Carbon Analyzer (Rosemount Analytical; Santa Clara, CA) using *Standard Methods* (Eaton *et al.*, 1995).

Microbiological Methods

Microbiological samples were taken from the influent and effluent of each AR. Biofilm coupons were aseptically sampled and placed into 100 mL of sterile deionized water. Biofilm was removed from the coupon and homogenized for two minutes in the diluent using a Stomacher 400 Lab Blender (Seward Ltd., London, UK), as described in Chapter 4.

Heterotrophic plate counts (HPCs) were determined by serially diluting the sample into sterile deionized water and spreading 100 μL of diluent onto R2A agar (Eaton *et al.*, 1995). Total direct microscopic counts were obtained by staining with acridine orange and enumerated using a Nikon Labophot 2A fluorescence microscope (Nikon Canada Inc.; Mississauga, ON) equipped with barrier filters. Cell numbers were reported as the mean count from 10 fields of view using a 10 by 10 microscopic graticule (Nikon Canada Inc.; Mississauga, ON) at 1000x magnification.

RESULTS

Analysis of Specific Experimental Trials

BOM components were measured in the influent and effluent of an AR during an experiment. BOM components were reported as the sum of the components on a mass carbon basis. Because of losses due to volatilization in the feed bottles, bacterial growth in the tubing, and error caused by diluting a concentrated stock into an AR, the measured influent concentrations were not necessarily the design BOM concentrations of 50 and 500 μg C/L. Consequently, the amino acid fraction was also variable between 0.3 and 0.7. A summary of several key operational parameters used in this investigation is provided in Table 5.2. Further details of each experimental trial are provided in Appendix A.

Table 5.2: Conditions for Each Experimental Trial

Trial	HRT (h)	Amino Acid Fraction	Influent BOM[†] (μg C/L)	Influent NPOC (mg/L)	Effluent BOM[†] (μg C/L)
5.1	2	0	52	1.44	16
5.2	4	0	110	1.32	9
5.3	2	0	200	1.15	64
5.4	4	0	265	1.32	13
5.5	2	0.54	45	1.43	10
5.6	4	0.30	110	1.32	12
5.7	2	0.69	300	1.36	22
5.8	4	0.32	350	1.43	33
5.9	1	0.35[††]	96	1.06	35
5.10	1	0.46[††]	540	1.68	58

[†] Calculated as the sum of the measurable BOM components on a mass carbon basis.
[††] Trials 5.9 and 5.10 included oxalate in the BOM cocktail.

Prior to data analysis, it was anticipated that the combined effect of BOM concentration and the presence of amino acids would be significant. At higher BOM levels, the influent BOM concentration was greater when amino acids were present (Table 5.2). The influent NPOC was measured with the combined influent BOM cocktail and the background organic matter in the filter effluent. Effluent (i.e., bulk) concentrations of the added BOM components were typically less than 70 μg C/L regardless of the influent BOM concentration (Table 5.2).

The duration for each experimental trial was approximately two weeks, which has been found to be a sufficient acclimation period for biofilm HPCs to achieve a steady-state number (Camper, 1996). The acclimation period for biofilm HPCs for experimental trial 5.8 is shown in Figure 5.2. Biofilm HPCs achieved a pseudo-steady-state number of 2.9×10^{6} CFU/cm^{2} (geometric mean) in about 5 days, which was a typical acclimation period for most of the experimental trials in this investigation. The effluent HPC numbers followed the same general trend as the biofilm HPCs for the initial sample points but were also strongly influenced by the influent number of HPCs. Under steady-state conditions (as defined by a relatively stable number of biofilm HPCs) the number of effluent HPCs was approximately 1-2 orders of magnitude greater than the number of influent HPCs.

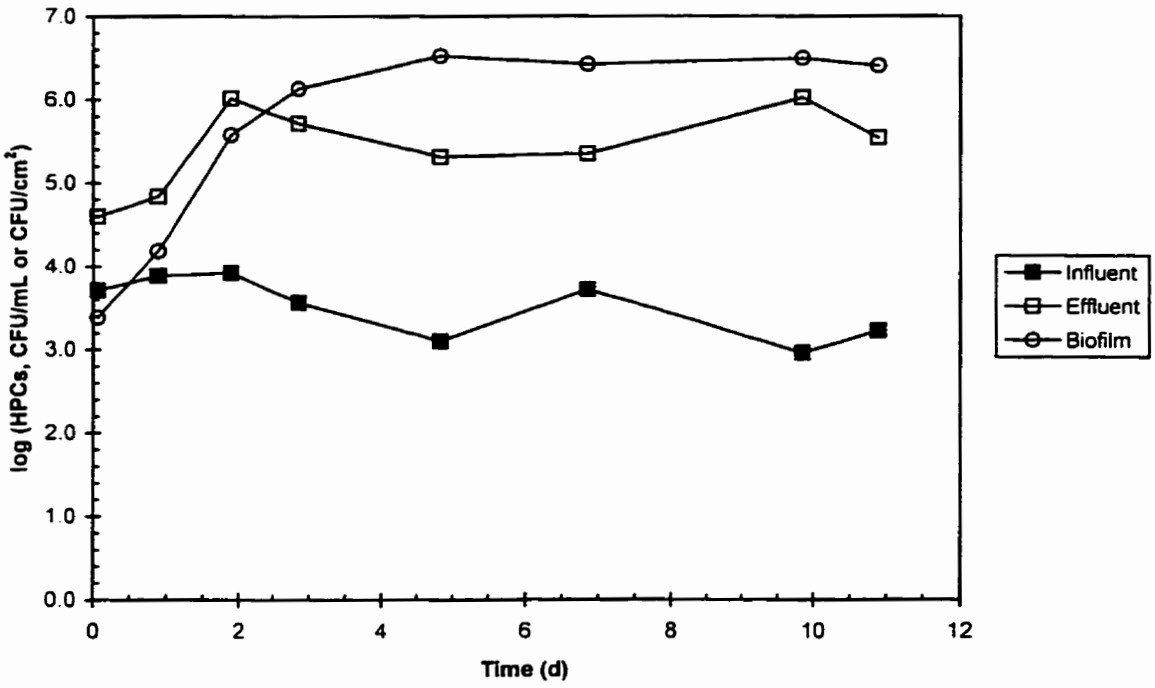

Figure 5.2: Accumulation of biofilm HPCs and net production of bulk HPCs for experimental trial 5.8.

In comparison to trial 5.8, an experimental trial was performed in the absence of any additional nutrients (i.e., ARs were fed only the GAC filtrate). The influent NPOC was 1.2 mg/L and the influent number of HPCs was 1.3×10^4 CFU/mL. The ARs were operated with a retention time of 2 h. The pseudo-steady-state number of biofilm and bulk HPCs were 2.1×10^5 CFU/cm^2 and 2.8×10^5 CFU/mL, respectively (data not shown). This number biofilm HPCs is approximately an order of magnitude less than the number of biofilm HPCs shown in Figure 5.2. Under pseudo-steady-state conditions, the number of bulk HPCs was essentially the same for either experimental condition (i.e., trial 5.8 or ARs only fed GAC-filtered water).

Under pseudo-steady-state conditions, a concentration profile of the components for the eighth experimental trial is shown in Figure 5.3. Complete removal for the three

amino acids was observed, which was typical for most experimental trials with amino acids. Formaldehyde was completely removed, whereas incomplete removal was observed for glyoxal. Greater than 80 % of the acetate was removed and essentially all of the formate was removed for trial 5.8.

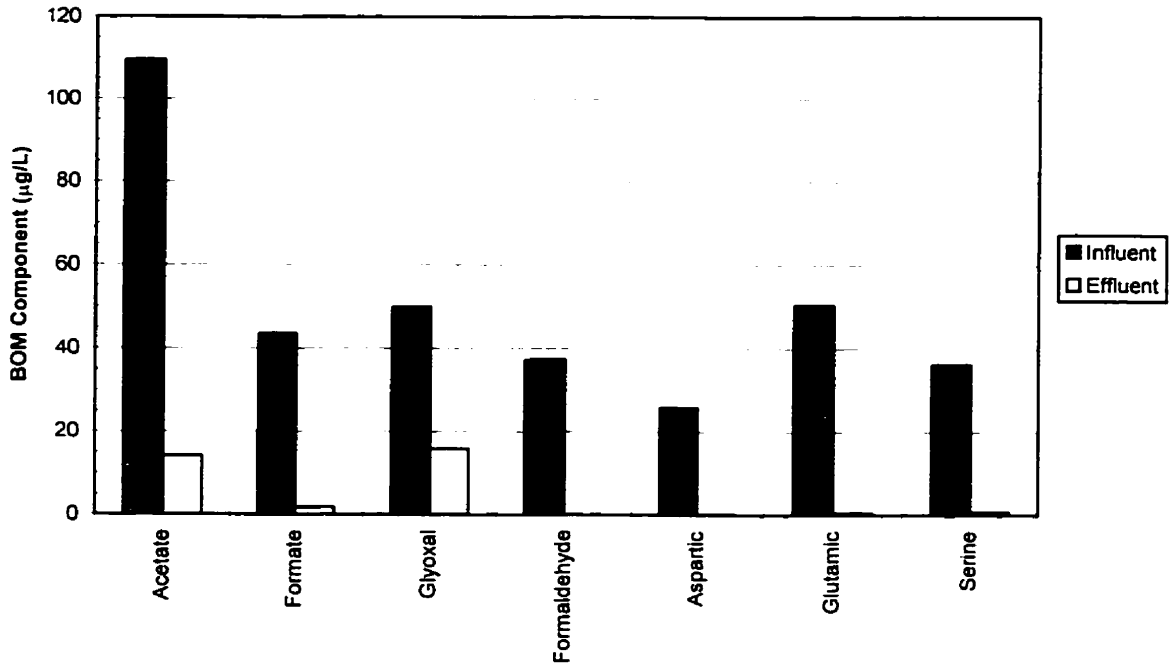

Figure 5.3: Steady-state influent and effluent concentrations for the organic compounds in trial 5.8.

Overall Effect on Bacterial Growth

For analysis purposes, all of the experimental factors were treated as qualitative parameters. The significance of the main effects on a given response parameter (e.g.,, number of biofilm HPCs) was determined using Yate's method, and a subsequent analysis of variance (ANOVA). All interactions, with the exception of the interaction between BOM concentration and the presence of amino acids, were assumed negligible

relative to the main effects. This assumption was checked for each response parameter using Bartlett's criterion at the 5 % level of significance (Davies, 1978).

Figure 5.4 shows the response of biofilm HPCs to the three input variables. In general, the number of biofilm HPCs for experimental trials which included amino acids in the influent cocktail were greater than the trials which did not include amino acids. With amino acids present in the influent, higher influent BOM concentrations resulted in an increased number of biofilm HPCs. However, for conditions which did not include amino acids, increasing the BOM concentration resulted in essentially the same number of biofilm HPCs. Retention time does not appear to have impacted the number biofilm HPCs as significantly as the other two input variables. For example, the retention time did not affect the number of biofilm HPCs for the two trials with amino acids and having a high influent BOM concentration (Figure 5.4).

An ANOVA confirmed the significant effect that BOM concentration (p value = 0.013) and amino acids (p value = 0.009) had on the number of biofilm HPCs (Table 5.3). Also the interaction between influent BOM concentration and the presence of amino acids had a significant effect on the number of biofilm HPCs (p value = 0.021). However the number of biofilm HPCs were not significantly impacted by the hydraulic retention time (p value = 0.056).

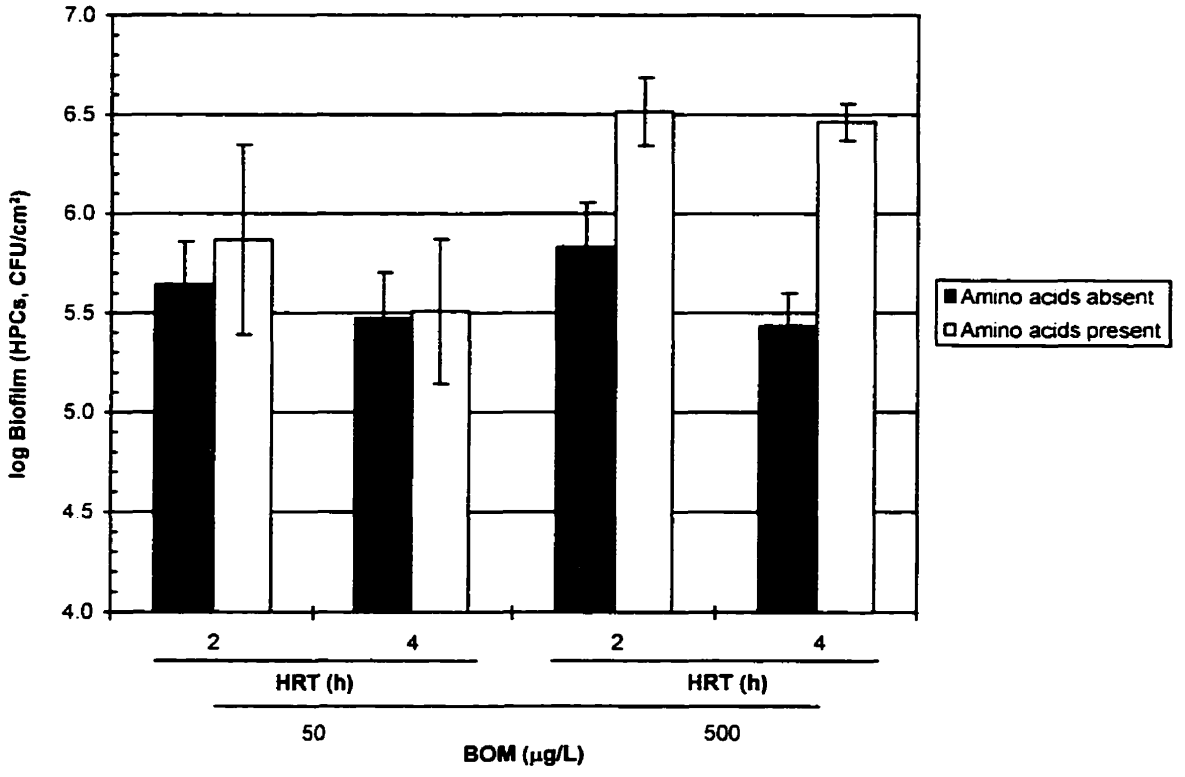

Figure 5.4: Influence of the presence of amino acids, BOM concentration and retention time on biofilm HPCs (error bars represent 95 % confidence intervals).

Table 5.3: ANOVA for the Effect of BOM Concentration, the Presence of Amino Acids and Hydraulic Retention Time on Biofilm HPCs

Source	Degrees of Freedom	Mean Square	F value	p value
HRT	1	0.121	9.175	0.056
BOM	1	0.382	29.06	0.013
Amino Acids	1	0.487	37.04	0.009
BOM / Amino	1	0.264	20.06	0.021
Residual	3	0.013	-	-

The response of the total number of biofilm cells, as measured by acridine orange direct counts (AODCs), to the eight experimental conditions (e.g.,, trial 5.1 to 5.8) is shown in Figure 5.5. At low retention times, the total number of cells was greater for higher BOM concentrations. However at a retention time of 4 h, a higher BOM

concentration either did not affect the number of AODCs or reduced the number of total biofilm cells (Figure 5.5). For experimental trials that had amino acids present in the BOM cocktail, the steady-state number of AODCs was not statistically different from each other. An ANOVA revealed that none of the three input variables significantly affected the number of biofilm AODCs at the 5 % level.

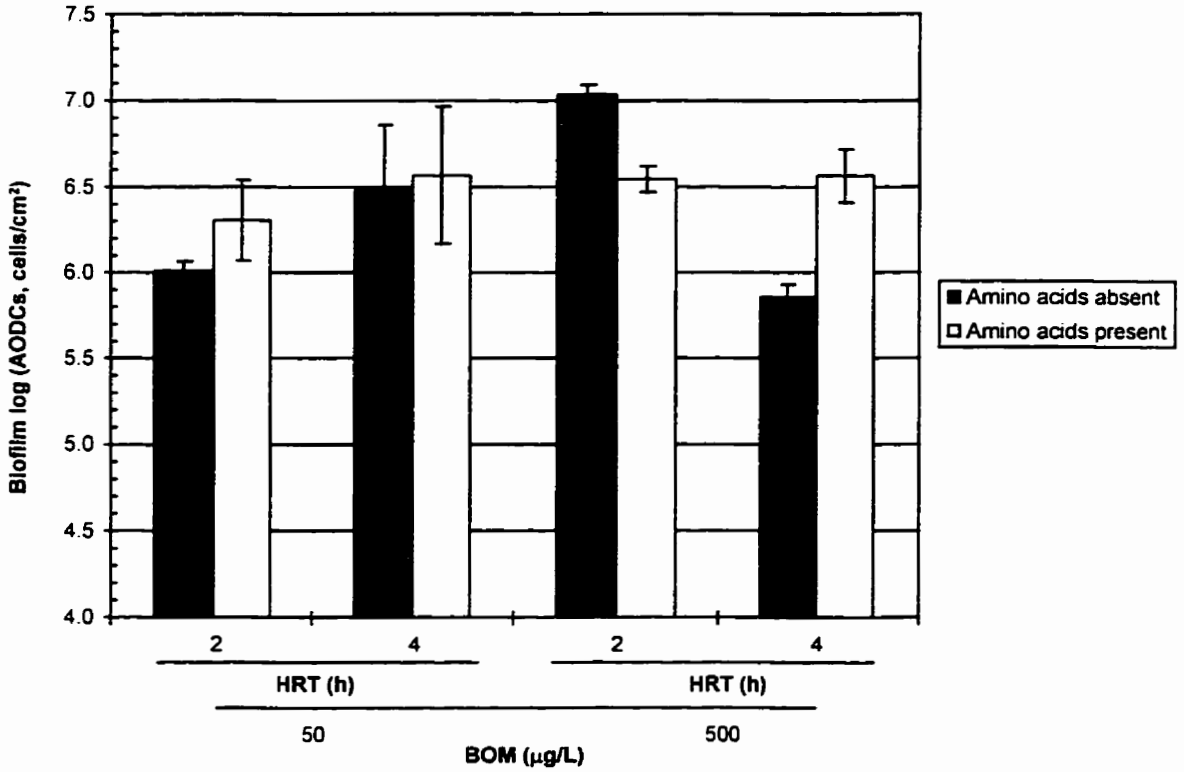

Figure 5.5: Influence of the presence of amino acids, BOM concentration and retention time on biofilm AODCs (error bars represent 95 % confidence intervals).

The total number biofilm cells were well correlated with the number of biofilm HPCs on a trial by trial basis. For the first experimental trial, the number of biofilm HPCs was positively correlated with the number of biofilm AODCs (Figure 5.6). Each data point in Figure 5.6 represents samples taken during the log growth phase and stationary phase of biofilm development. Linear relationships between the number of culturable and total cells for the other seven experimental trials were also calculated (Table 5.4). For all

trials a positive slope was observed, indicating that the number of culturable cells represented a portion of the total number of cells. In all but two cases the slopes were significantly different from zero.

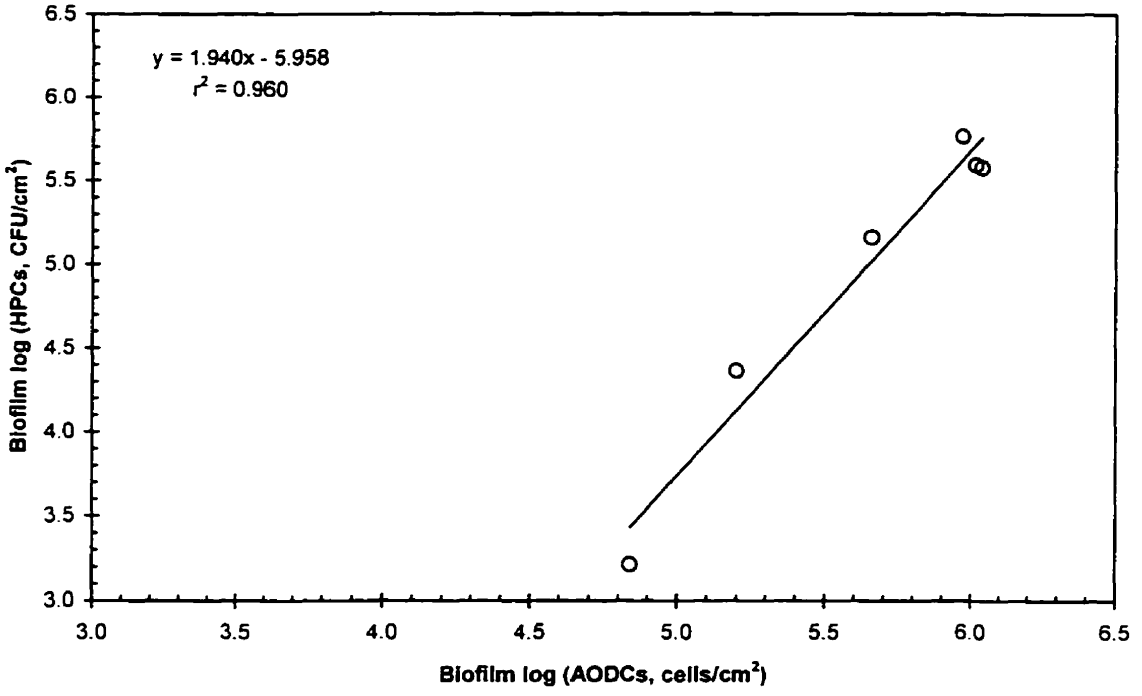

Figure 5.6: Linear relationship for biofilm AODCs and HPCs for trial 5.1 (HRT = 2 h. BOM = 50 μg C/L, and amino acids are not included in BOM cocktail).

Table 5.4: Linear Relationships for Biofilm Total Cell Counts and Biofilm HPCs

Trial No.	Linear Equation[a]	n	r^2	p value of Slope
5.1	$y = 1.940\,x - 5.958$	6	0.960	0.0006
5.2	$y = 1.084\,x - 1.359$	7	0.810	0.0057
5.3	$y = 0.518\,x + 2.277$	7	0.754	0.0112
5.4	$y = 1.645\,x - 4.214$	6	0.996	< 0.0001
5.5	$y = 1.228\,x - 1.886$	6	0.610	0.0667[b]
5.6	$y = 0.694\,x + 1.124$	7	0.783	0.0080
5.7	$y = 1.942\,x - 5.955$	6	0.882	0.0054
5.8	$y = 0.399\,x + 3.678$	6	0.723	0.0320[b]

[a] The independent and dependent variables for these regressions are biofilm log (AODCs) and biofilm log (HPCs), respectively.

[b] Slope not statistically different from zero at 5 % level of significance (two-sided test).

Net effluent HPCs and total cells were also analyzed as response parameters under the given experimental conditions (Figure 5.7). Net bulk cells were calculated as the difference between the log effluent and the log influent cell counts. Net values were analyzed instead of effluent HPC numbers because the influent HPC numbers were not constant over the experimental period.

In general longer retention times generally increased the number of net bulk HPCs (Figure 5.7). Surprisingly higher BOM concentrations generally decreased the number of net bulk HPCs. For conditions without amino acids, and at a retention time of 4 h the net number of bulk HPCs was lower for the higher influent BOM concentration, although a student t-test shows that this difference is not significant at the 5 % level. Amino acids did not appear to impact the net number of bulk HPCs.

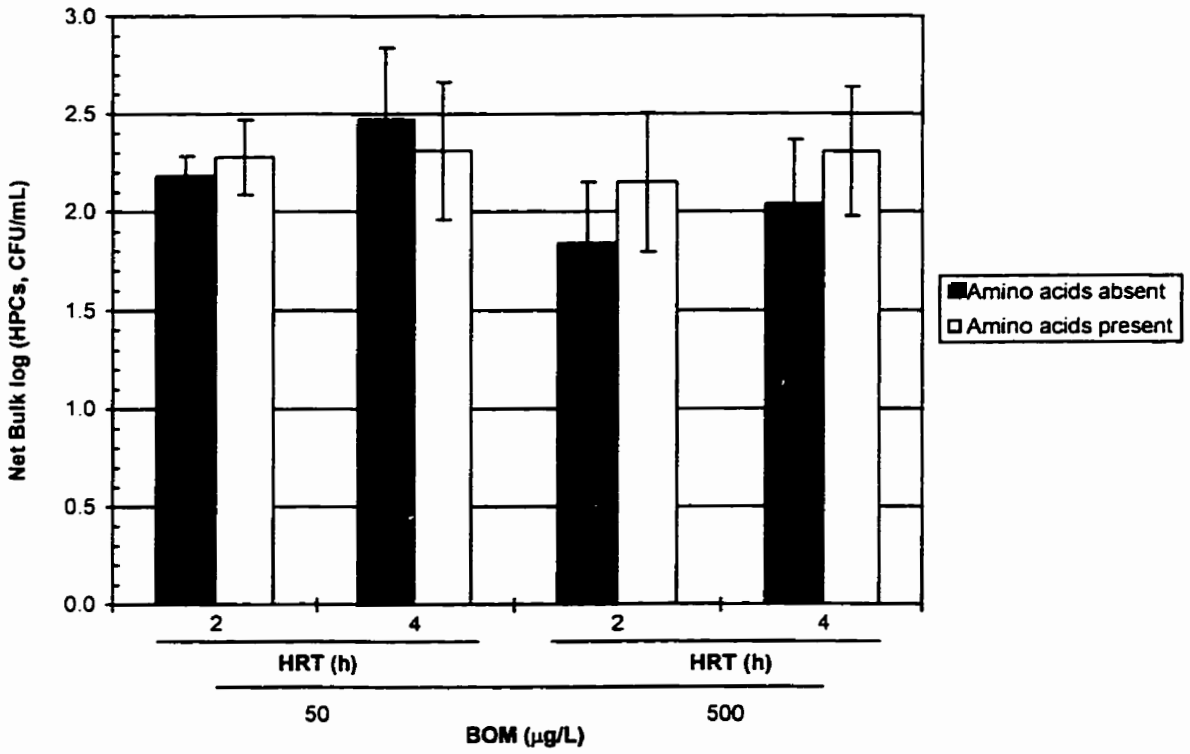

Figure 5.7: Influence of the presence of amino acids, BOM concentration and retention time on net bulk HPCs (error bars represent 95 % confidence intervals).

An ANOVA (Table 5.5) found that the only retention time (p value = 0.049) significantly increased the net number of bulk HPCs. The influent BOM concentration (p value = 0.023) negatively affected the net number of bulk HPCs. The interaction of BOM concentration and amino acids and amino acids as a main effect did not significantly affect the net number of bulk HPCs (Table 5.5).

Table 5.5: ANOVA for the Effect of BOM Concentration, the Presence of Amino Acids and Hydraulic Retention Time on Net Bulk HPCs

Source	Degrees of Freedom	Mean Square	F value	p value
HRT	1	0.057	10.34	0.049
BOM	1	0.104	18.70	0.023
Amino Acids	1	0.033	5.950	0.093
BOM / Amino	1	0.051	9.283	0.056
Residual	3	0.006	-	-

The effect of the three input variables on the net number of bulk AODCs is shown in Figure 5.8. With the exception of one experimental trial, longer retention times tended to increase the net number of bulk AODCs. One experimental trial (trial 5.7) had a significantly different value from the other seven trials. It is unclear whether the bulk AODC results obtained for trial 5.7 are anomalous or whether the combined effect of these three conditions on bulk AODCs is "real". Ignoring trial 5.7, when BOM concentration was increased and the net number of bulk AODCs was decreased. With the exception of trial 5.7, amino acids did not significantly impact the net number of bulk AODCs (Figure 5.8). Overall, the effect each input variable did not significantly influence the net number of bulk AODCs at the 5 % level.

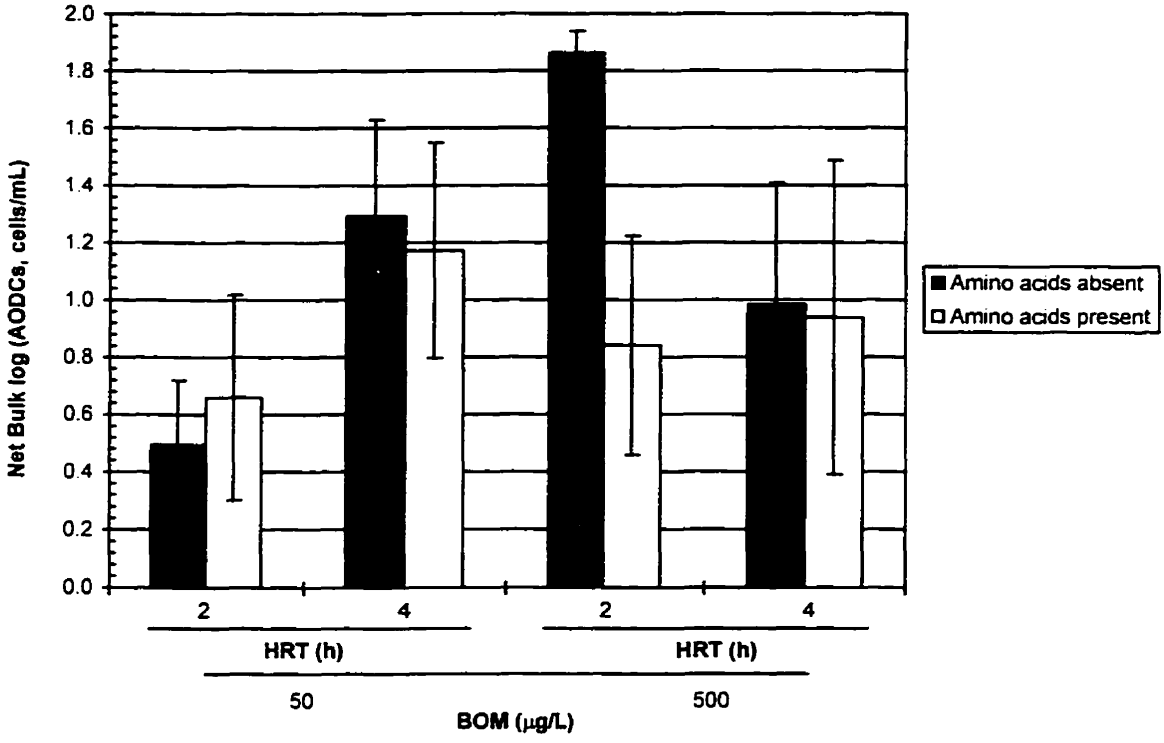

Figure 5.8: Influence of the presence of amino acids, BOM concentration and retention time on net bulk AODCs (error bars represent 95 % confidence intervals).

The relative numbers of biofilm HPCs and bulk HPCs were compared to determine if one phase (e.g.,, biofilm vs. bulk liquid) dominated in terms of relative number of colonies. The number of biofilm HPCs per reactor was calculated by multiplying the arithmetic mean number of biofilm HPCs per coupon (e.g., CFU/cm^2) by the reactor surface area (i.e.,, 0.18 m^2). Similarly, the number of bulk HPCs per reactor was calculated by multiplying the arithmetic mean number of bulk HPCs per millilitre (e.g., CFU/mL) by the reactor volume (i.e., 670 mL). The ratio of biofilm HPCs to bulk HPCs was then calculated on a per reactor basis.

Figure 5.9 shows the relative number of biofilm to bulk HPCs for the eight experimental trials (i.e., experimental trials 5.1 - 5.8). For all of the trials the number of biofilm HPCs is greater than the number of bulk HPCs (i.e., the ratio exceeds unity). In

all but three experimental trials the biofilm to bulk ratio was greater than 10. Two of the lower ratio values had long retention times and low influent BOM concentrations. The biofilm to bulk ratio exceeded 10 for conditions of short retention times. Amino acids generally increased the biofilm to bulk ratio. Also higher influent BOM concentrations increased the bulk to biofilm ratio for all but one of the experimental trials.

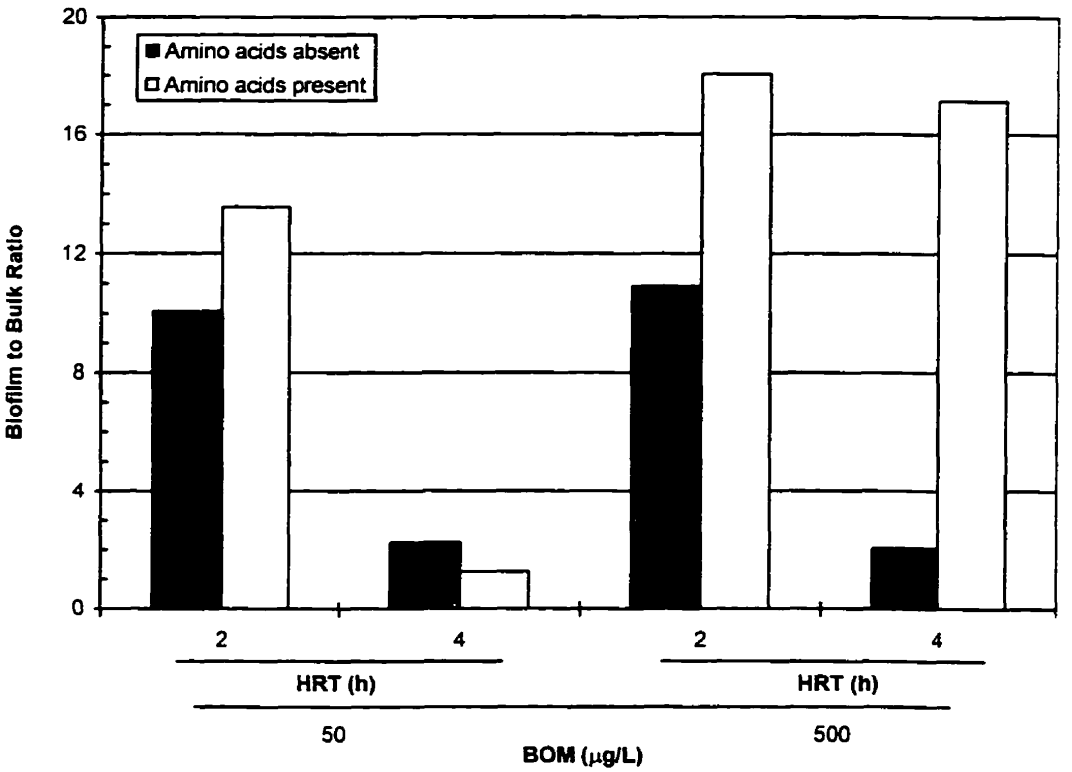

Figure 5.9: Relative number of biofilm to bulk HPCs for the 2^3 factorial experiment.

Subsequent experiments (trial 5.9 and 5.10) were performed to examine the extent to which HRT and BOM concentration together affected bacterial regrowth. In these two trials, amino acids were included in the BOM cocktails to eliminate the interaction between BOM concentration and amino acids, as amino acids had a positive effect on biofilm HPCs, and essentially no effect on net bulk HPCs.

Experimental trials 5.5 through 5.10 (Table 5.2) were used as a basis for a second experimental design. The second experimental set was analyzed as a separate experiment, with all factors being qualitative. A qualitative interpretation was necessary as hydraulic retention time has three non-orthogonal levels, 1, 2, and 4 hours, and BOM concentration had two levels, high and low. The high and low BOM levels were defined by a measured influent concentration greater than 300 μg C/L and less than 100 μg C/L respectively (Table 5.2). Also trials 5.9 and 5.10 included oxalate in the influent cocktail. The amino acid fraction was not constant for these six experimental trials.

The effect of BOM concentration and HRT on the number of biofilm HPCs is shown in Figure 5.10. The influent BOM concentration positively affected the number of biofilm HPCs, as shown previously. Retention time had no impact on the number of biofilm HPCs at low influent BOM concentrations. At the high influent BOM concentration, a retention time of 1 h resulted in a number of biofilm HPCs that was significantly greater than the number calculated for retention times of 2 and 4 h.

In contrast, the number of net bulk HPCs at retention times of 2 and 4 h were significantly greater than the net number of bulk HPCs at 1 h (Figure 5.11). For the three retention times, influent BOM concentration did not significantly impact the net number of bulk HPCs.

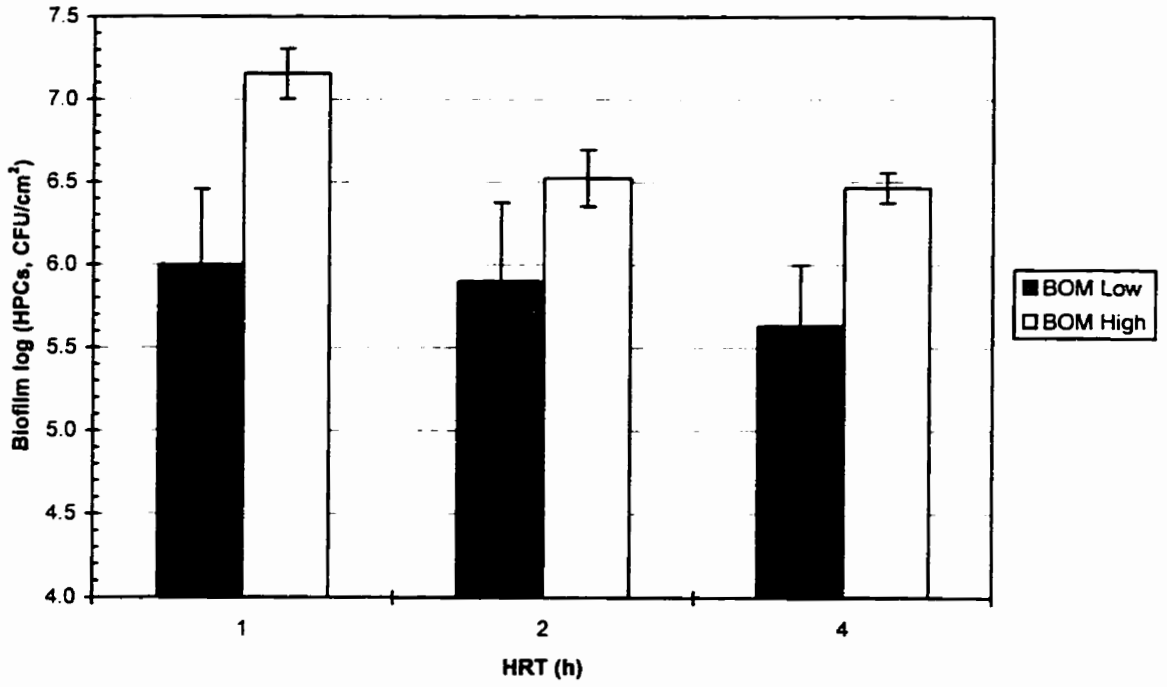

Figure 5.10: Influence of BOM concentration and retention time on biofilm accumulation for experimental trials which included amino acids (error bars represent 95 % confidence intervals).

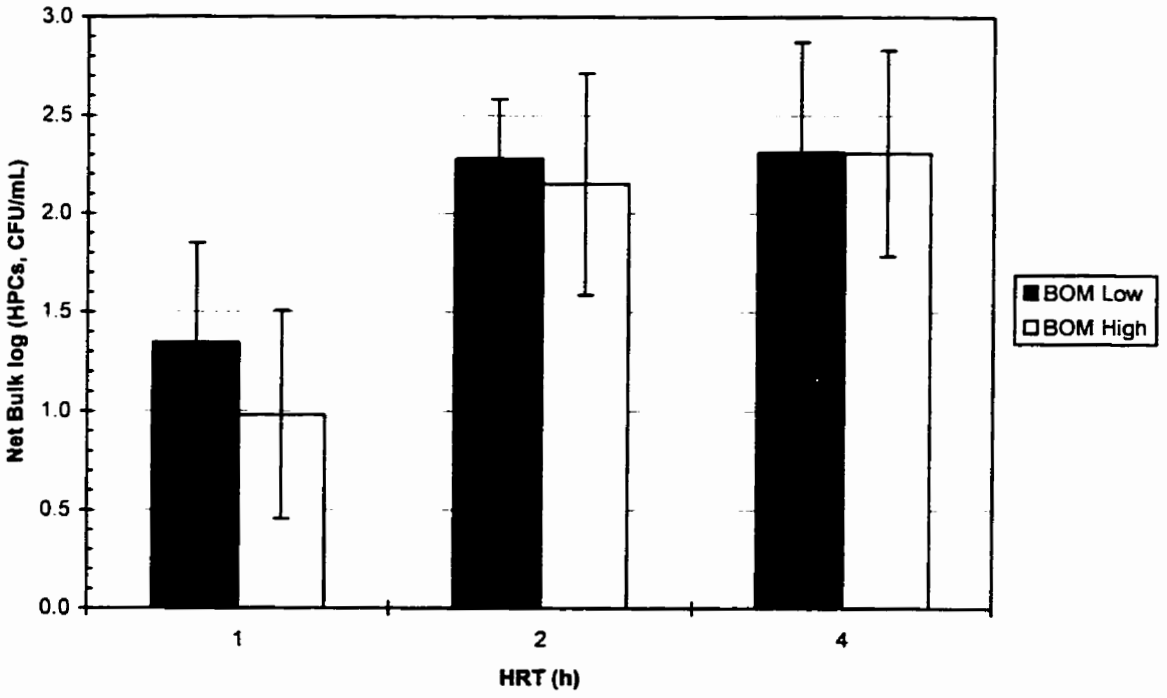

Figure 5.11: Influence of BOM concentration and retention time on net bulk HPCs for experimental trials which included amino acids (error bars represent 95 % confidence intervals).

DISCUSSION

Response Variables

The impact of the three main effects on bacterial regrowth was measured using four response parameters, the number of: biofilm HPCs, net bulk HPCs, biofilm AODCs, and net bulk AODCs. In order to interpret the extent to which each experimental input variable affected bacterial regrowth, an understanding of the meaning and the limitations of the response variables must be established.

Plate counting measures the number of cells that can be cultured on a given media. For drinking water R2A agar has been shown to be an optimal medium for colonizing heterotrophic bacteria (Reasoner and Geldreich, 1985). One of the major limitations of this assay is that nonculturable but viable (NCBV) cells are not enumerated. Possible explanations for NCBV cells in a stressed environment are (Morgan and Winstanley, 1996): 1) some cells are simply not culturable on a given media; 2) severely starved cells cannot be recovered by culturing but remain metabolically active; 3) NCBV may be a lifecycle state for organisms which are required to survive in the environment until subsequent transfer to a new plant or animal host; and 4) stressed populations which have been injured or damaged by environmental conditions such as a disinfectant residual may enter a NCBV state. It is assumed that R2A media can consistently isolate a large majority of cells in drinking water systems. In this investigation no chlorine or other disinfectant has been added to inhibit growth. It is likely that the cells present in this system are not injured and are able to be recovered on R2A. In terms of using the plate counts as a surrogate for viability, the primary limitation on the results presented in this study may be points 2 and 3 as discussed by Morgan and Winstanley (1996).

Total counts with acridine orange are used to represent the amount of non-viable and viable bacterial populations in this micro-environment. Total counts were generally greater than the HPC counts and were well correlated on a trial by trial basis (Table 5.4). AODCs were also well correlated to HPC counts (P value = 0.001) at 10 drinking water sample locations in Southern California (McCoy and Olson, 1985). In the present investigation, net bulk liquid and biofilm samples were not sensitive to the extremities of the three input variables. In the presence of amino acids, biofilm HPCs ranged from 7.2×10^5 CFU/cm^2 to 1.1×10^7 CFU/cm^2. However, the number of biofilm AODCs was essentially independent of retention time and influent BOM concentration with an average number of 3.1×10^6 cells/cm^2 (Figure 5.4). This suggests that the total number of cells is constant relative to the available surface area, whereas the number of viable cells may be changing in response to varying environmental conditions.

It is widely believed that the majority of bacterial growth in distribution systems occurs attached to the pipe surface, consequently accounting for this variable was critical to this study. Under steady-state conditions the rate of biofilm detachment is essentially equal to the growth rate of biofilm cells (Stewart, 1993). Bulk cells are assumed to be a result of detached biofilm cells rather than bulk growth (Camper, 1996). Figure 5.9 shows that the number of biofilm HPCs are at least an order of magnitude greater than the number of bulk HPCs for high loading rates (e.g., short retention times and high influent BOM concentrations). Therefore at higher loading rates the data presented in Figure 5.9 would support the negligible bulk cell growth (relative to biofilm accumulation).

For low loading rates (e.g., long retention time and low influent BOM concentrations), Figure 5.9 shows that the ratio of biofilm to bulk HPC numbers ranges

between 1 and 3. In general, lower loading rates will lead to lower biofilm accumulation rates and thus the detachment rate will also be reduced accordingly. These data generally lack the physiological information to compare the relative rates of bulk growth and biofilm detachment. However, the data presented in Figure 5.9 would suggest that negligible bulk cell growth (relative to biofilm accumulation) is a reasonable assumption for higher BOM loading rates, but is less conservative at lower loading rates.

BOM Concentration

It is well established in the literature that lower BOM concentrations will decrease the likelihood of bacterial regrowth in distribution systems (Servais *et al.*, 1995; van der Kooij, 1992; LeChevallier *et al.*, 1991). Similarly, Camper (1996) found that increased substrate concentrations increased the number of biofilm HPCs, which concurs with the data presented in this investigation (Table 5.3).

Camper (1996) also found that higher BOM concentrations significantly increased the number of bulk HPCs. However, in this study the number of net bulk HPCs significantly decreased for higher BOM concentrations. The discrepancy in bulk numbers calculated for this study and for Camper (1996) could be due to the different methods used for accounting for bulk cells (e.g., net bulk HPCs vs. bulk HPCs). Net bulk cells were used because the number of influent bulk cells was not constant over the experimental period. Nonetheless, having a lower number of net bulk HPCs for higher BOM concentrations may appear counterintuitive.

A possible explanation for the result obtained for the number of net bulk HPCs could be an increased death rate at higher BOM levels. The inoculum for these experiments was taken from tap water which had been passed through two GAC filters. The background NPOC for the filtered tap water was generally low, having a concentration range between 1.0 - 1.5 mg/L for most of the experimental period. BOM components were generally not detectable in the effluent of GAC filters. Consequently, by spiking the filtered tap water with higher levels of BOM some of the bulk cells may have died due to shock. Koch (1997) suggested that oligotrophs which are able to grow under low nutrient conditions may not be able to grow at higher nutrient levels. Because biofilm cells tend to be more resistant, to stresses such as chlor(am)ination (LeChevallier *et al.*, 1990), they were also able to survive at higher BOM levels.

Presence of Amino Acids

Assuming that BOM utilization was by the biofilm, it is not surprising that amino acids positively affected the number of biofilm HPCs (Figure 5.3) and did not affect the number of bulk HPCs (Figure 5.4). It could be argued that amino acids had a positive effect on the number of biofilm HPCs because their presence coincided with higher influent BOM concentrations in some trials (Table 5.2). The BOM/amino acid interaction also significantly increased the number of biofilm HPCs (Table 5.3). Although the effect of amino acids on bacterial growth is confounded by higher BOM concentrations, studies reported in the literature suggest that the presence of amino acids may enhance biofilm accumulation.

Similar to many amino acids, aspartate, glutamate, and serine are actively transported into the bacterial cell. Active transport of amino acids is characterized by enzyme systems having a high affinity for substrates and strict substrate specificity (Anraku, 1978). Michaelis constants (K_m) for aspartate, glutamate and serine in *Escherichia coli* have been reported as 3.7 μM (high affinity system), 0.7 μM (in the presence of 40 mM NaCl) to 10 μM (in the absence of Na^+), and 1.4 μM, respectively (Anraku, 1978). Alternatively, K_m values of 36 μM for acetate have been measured with *E. coli* (Kay, 1978). Because direct metabolic pathways for formaldehyde and glyoxal are limited it is assumed that these compounds are oxidized into carboxylic acids prior to being utilized by the cell, which could explain an apparent lag period in the biodegradation reported for some aldehydes (Booth *et al.*, 1996).

van der Kooij and Hijnen (1988) found that the growth of *Aeromonas hydrophila* in drinking water was enhanced in mixtures containing 21 amino acids. A carboxylic acid mixture of 18 compounds had a limited effect on the growth of *A. hydrophila* at an individual component concentration of 1 μg C/L, whereas the amino acid cocktail promoted growth even at an individual component concentration of 0.1 μg C/L. The apparent half velocity constants for the amino acid and carboxylic acid mixtures were 8.0 and 209 μg C/L, respectively.

The study with *A. hydrophila* and investigations with *E. coli* suggest that bacteria have a greater affinity for amino acids than carboxylic acids. This could offer an additional explanation for the higher HPC numbers observed in the presence of amino acids (Figure 5.3).

Hydraulic Retention Time

Camper (1996) connected four ARs in series with individual retention times of 2, 4, 8, and 16 h, respectively. The AR system for that study was operated at 20°C and the first AR in the train was fed an organic carbon cocktail of 500 μg/L. Similar to the present investigation, Camper (1996) found that hydraulic retention time had no effect on the number of biofilm HPCs. A statistically significant increase (P value = 0.0001) in the number of bulk HPCs through the AR system was also observed by Camper (1996). Similarly pilot scale (Holden *et al.*, 1995) and full-scale (LeChevallier *et al.*, 1987) investigations have reported an increased number of bulk HPCs for longer retention times. However those results were often confounded by a decrease in chlorine residual.

The present investigation showed that the number of net bulk HPCs significantly increased with longer retention times (Figure 5.11). Because hydraulic retention time is the reciprocal of the dilution rate, it is not surprising that a relationship between net bulk cells and dilution rate (i.e., decreasing retention times) can be obtained (Figure 5.12). In a chemostat it is proposed that at high dilution rates the chemostat becomes unstable and any increase in the dilution rate will cause the bulk cells to wash out (Ingraham *et al.*, 1983). Figure 5.12 shows a similar behaviour to a chemostat, however it is unlikely that the bulk cells will be washed out, as the biofilm cells are the dominant population in an AR.

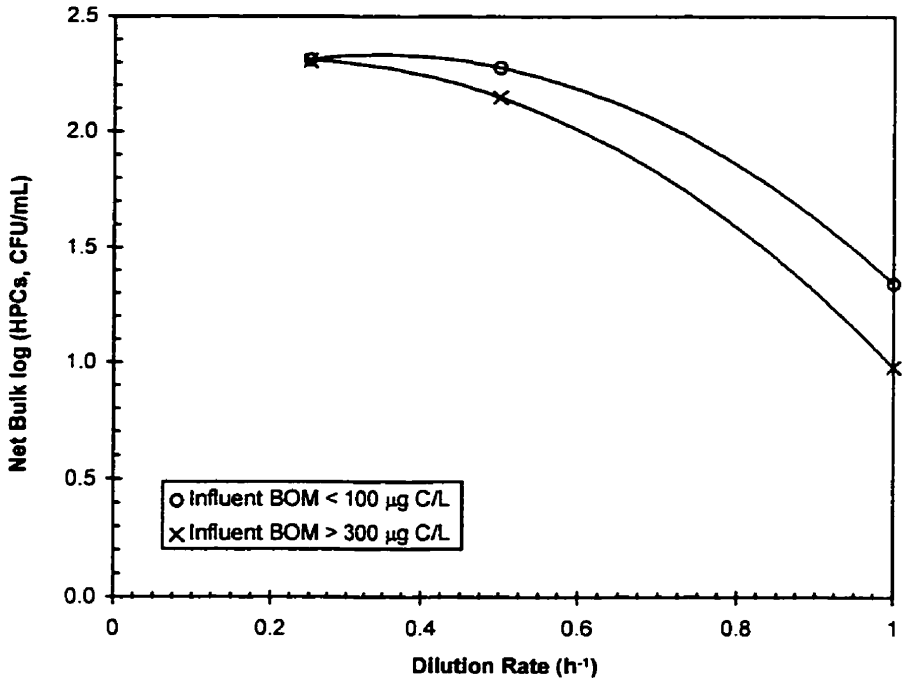

Figure 5.12: Relationship between net bulk HPCs from experimental trials 5.5 to 5.10 and AR dilution rate.

For investigations with bulk growth in a chemostat, it is anticipated that the bulk concentration of the limiting nutrient will have a non-linear relationship with the reactor dilution rate. For the conditions of low influent BOM concentration (e.g., BOM_{in} < 100 µg C/L), the effluent BOM concentration had a non-linear relationship with the dilution rate (Figure 5.13). It is tempting to interpret Figure 5.13 as evidence of bulk growth with Monod kinetics. However, the previous discussion showed that higher influent BOM concentrations increased the number of biofilm HPCs, whereas the number of net bulk HPCs were found to decrease. In addition, Figure 5.10 suggests that the amount of biofilm accumulation is related to the BOM loading rate, and thereby intrinsically associated with the substrate (BOM) utilization rate. Thus, it is likely that bulk growth occurs, however the extent of bulk growth is more noticeable at longer retention times

because of favourable dilution rates relative to the bulk cell doubling time and the BOM flux to the biofilm is also reduced.

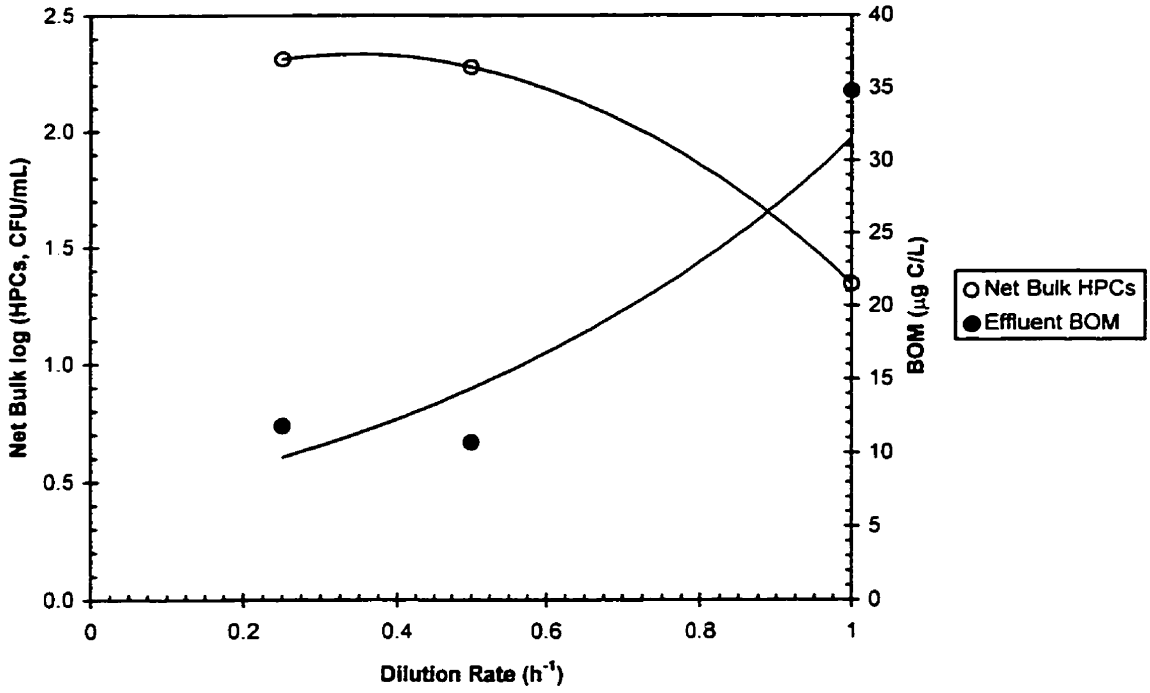

Figure 5.13: Bio-kinetic relationships for experimental trials, which had influent BOM concentrations less than 100 µg C/L and amino acids present in the BOM cocktail.

CONCLUSIONS

The effect BOM concentration, the presence of amino acids and the hydraulic retention time on bacterial regrowth in a model distribution system was examined. Elevated numbers of biofilm HPCs were obtained for increased BOM concentrations. Shorter retention times significantly increased the number of biofilm HPCs. Therefore, higher BOM loading rates increased the number of biofilm HPCs. Consequently, BOM consumption was predominantly by the biofilm.

The presence of amino acids increased the number of biofilm HPCs. One explanation for this increase in biofilm HPCs was the higher BOM levels present for the trials that included amino acids. Also, the utilization rates for amino acids have been reported to be higher than the other BOM components, which could explain the elevated biofilm numbers observed for the BOM cocktails containing amino acids.

Retention time had a positive effect on the number of net bulk HPCs. Conversely, BOM concentration had a negative impact on the number of net bulk HPCs. It is proposed that the growth rate of bulk cells was slower than the higher dilution rates (i.e., retention times of 1 to 2 h). At higher BOM concentrations the bulk cells were likely shocked by the sudden increase in substrate concentration.

Kinetic behaviour similar to chemostat operations was observed. BOM utilization was primarily by the biofilm at higher dilution rates. Net bulk cells had a low growth rate and were more relevant (in terms of numbers of colonies) at a retention time of 4 h.

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CHAPTER 6: QUANTIFYING THE RATE OF BOM UTILIZATION AND BIOFILM ACCUMULATION IN AN ANNULAR REACTOR

INTRODUCTION

Biofilm models consist of numerous parameters that are often difficult to estimate under drinking water conditions (Gagnon *et al.*, 1997a). Modeling biofilm accumulation in drinking water distribution systems may require simplifying assumptions, thereby reducing the number of estimated parameters. Two models currently available to model bacterial growth in distribution systems are SANCHO (Laurent *et al.*, 1997; Servais *et al.* 1995; Servais *et al.*, 1992) and BAM (Camper, 1996). SANCHO models the utilization of biodegradable dissolved organic carbon (BDOC), the decay of chlorine, and the growth of free and fixed biomass in distribution systems. Two major assumptions are: BDOC is divided into three portions (easily and slowly hydrolyzable BDOC and monomeric substrates) and mass transfer to the biofilm does not limit the removal rate of BDOC (Servais *et al.*, 1995).

BAM is based on the multispecies model BIOSIM (Wanner, 1994; Wanner and Gujer, 1986) which is a general biofilm model that has been used for modeling wastewater applications (Wanner and Gujer, 1986) and porous media (Wanner *et al.*, 1995). Because of the generalities of BIOSIM, BAM requires estimates for numerous parameters that may be difficult to determine (e.g., effective diffusivity, detachment coefficient). Camper (1996) found that the critical parameters of BAM for modeling biofilm accumulation in a drinking water pipe are: the yield coefficient, the biofilm detachment coefficient, the liquid boundary layer thickness, and the maximum growth rate. Detachment is the biofilm process responsible for maintaining a steady-state biofilm thickness by balancing bacterial loss with biofilm expansion due to growth (Stewart, 1993). The biofilm maximum growth rate is strongly related to the flux of BOM to the biofilm.

Huck and co-workers (Gagnon *et al.*, 1997; Huck *et al.*, 1994; Huck and Anderson, 1992) have shown that BOM removal during biological filtration can be approximated by empirical first-order models. In these models, a linear relationship between the amount of BOM removed and the influent BOM concentration is established for a specific empty-bed contact time. Linear models have been established for many BOM parameters (e.g., carboxylic acids, AOC, BDOC). Although this empirical approach can be useful from a process optimization perspective, it does not address some major modeling issues such as mass transfer to and within the biofilm.

The purpose of this chapter is to examine: the degree to which mass transfer limits BOM removal in drinking water biofilms, the utilization rate of BOM (on a combined

mass basis), the fraction of BOM components removed by the biofilm and the factors influencing biofilm detachment in distribution systems.

BACKGROUND

For this chapter a steady-state biofilm is idealized as a matrix of cells and organic material having a uniform thickness. This simplification is necessary for evaluating the utilization of BOM by the biofilm for the entire reactor. A uniform biofilm thickness is a common and often necessary assumption for evaluating biodegradation kinetics of substrate by biofilms (Zhang and Huck, 1996a; Rittmann *et al.*, 1986; Wanner and Gujer, 1986; Rittmann and McCarty, 1980). Figure 6.1 shows the idealized biofilm having a uniform biofilm thickness (L_f) and density (X_f).

In Chapter 5 it was shown that BOM utilization was primarily by the biofilm. Consequently, this chapter focuses on the flux of organic material into the biofilm. Thus the substrate concentration (i.e., the BOM components) is assumed to change only in the z-direction, or the direction perpendicular to the biofilm surface. Because the annular reactor is a continuously stirred tank reactor (CSTR), the concentration of BOM in the bulk liquid (S_b) is assumed to be constant. This is a reasonable assumption as the mixing time for ARs operating under shear conditions relevant to the present research is less than 60 s (Gjaltema *et al.*, 1994).

From the bulk liquid, substrate is transferred through a uniform liquid boundary layer (L_l) according to Fick's first law of diffusion. Depending on the rate of external mass transfer (i.e., substrate diffusion through the liquid boundary layer), relative to the

substrate utilization rate, the substrate concentration at the surface of the biofilm (S_s) will be less than or equal to S_b.

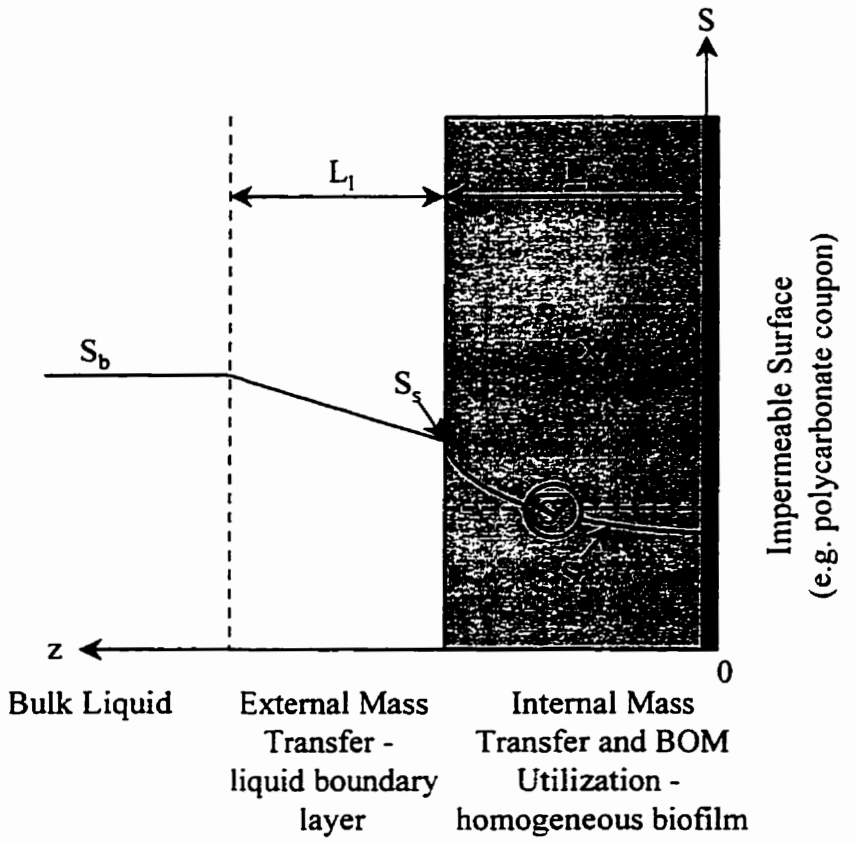

Figure 6.1: Conceptual model of substrate utilization by a steady-state biofilm having a uniform thickness.

The substrate at the surface of the biofilm is transferred through the biofilm by diffusion, according to Fick's second law of diffusion. The diffusion of substrate in the biofilm (S_f) is balanced with the utilization of substrate by the biofilm cells. For conditions where the biofilm is thin and S_s is low (i.e., conditions relevant to drinking water), it can be argued that the change in S_f with respect to the z-direction is relatively small. Therefore, S_f could be approximated with an average biofilm substrate concentration $\left(\overline{S_f}\right)$.

MATERIALS AND METHODS

All of the experimental data presented in this Chapter were either presented previously in Chapter 5 or collected from annular reactors which were operated in a manner similar to that in Chapter 5. All of the ARs were fed a BOM cocktail which consisted of carboxylic acids, aldehydes, and amino acids. The specific organic compounds are similar to those used in Chapter 5. A general summary of the eight experimental trials used for analysis in this chapter is presented in Table 6.1 and is shown in detail in Appendix A.

Table 6.1: Summary of the Steady-State Values from the Experimental Trials used for Kinetic Evaluation.

Experimental Trial	HRT (h)	Influent BOM (μg C/L)	Influent HPCs[a] (CFU/mL)	Effluent BOM (μg C/L)	Effluent HPCs (CFU/mL)	Biofilm HPCs[a] (CFU/cm^2)
5.5	2	45	8.8×10^2	10	1.8×10^5	7.9×10^5
5.6	4	110	1.5×10^3	12	6.3×10^5	4.3×10^5
5.7	2	300	3.1×10^3	22	5.0×10^5	3.3×10^6
5.8	4	350	2.3×10^3	33	4.6×10^5	2.9×10^6
5.9a	1	96	4.3×10^4	35	4.0×10^5	9.0×10^5
5.10	1	540	6.5×10^4	58	1.2×10^6	1.5×10^7
6.1a	1	2000	6.3×10^4	210	1.7×10^6	2.8×10^7
6.2	1	900	4.8×10^4	96	6.7×10^5	4.4×10^7

[a] HPC values are reported as arithmetic means.

MATHEMATICAL MODELING

Each AR is modeled as a CSTR with bulk liquid and biofilm compartments. Mass balances on substrate and biomass yielded equations 6.1 through 6.3. The major substrate in this analysis is the aggregate BOM (or simply BOM), which consists of a sum of all of the measurable BOM components on a mass carbon basis.

Bulk Liquid BOM

$$V\frac{dS_b}{dt} = Q\,(S_i - S_b) - \frac{r_{g,b}}{Y_{x/s}}V - J\,A \qquad (6.1)$$

where, S_b is the bulk liquid concentration of BOM, $M_S\,L^{-3}$
V is the volume of the AR, L^3
S_i is the influent concentration of BOM, $M_S\,L^{-3}$
Q is the volumetric flow rate, $L^3\,T^{-1}$
$r_{g,b}$ is the growth rate of planktonic HPCs, $M_X\,L^{-3}\,T^{-1}$
$Y_{x,s}$ is the yield coefficient, M_X/M_S
J is the flux of BOM to the biofilm, $M_S\,L^{-2}\,T^{-1}$
A is the surface area of the reactor, L^2

Heterotrophic Bacteria in the Bulk Liquid

$$V\frac{dX_b}{dt} = Q\,(X_i - X_b) + r_{g,b}V + r_d\,A \qquad (6.2)$$

where, X_b is the bulk liquid concentration of heterotrophic bacteria, $M_X\,L^{-3}$
X_i is the influent concentration of heterotrophic bacteria, $M_X\,L^{-3}$
r_d is the biofilm detachment rate, $M_X\,L^{-2}\,T^{-1}$

Heterotrophic Bacteria in the Biofilm

$$\frac{dX_f}{dt} = r_{g,f} - r_d \qquad (6.3)$$

where, $r_{g,f}$ is the growth rate of biofilm HPCs, $M_X\,L^{-2}\,T^{-1}$

Model Assumptions for Data Analysis

Experimental pseudo-steady-state conditions are evaluated, thus temporal changes in the state variables given in equations 6.1 through 6.3 are assumed to be negligible (e.g., $dS/dt = 0$). Heterotrophic growth rate in the bulk liquid is assumed negligible, as discussed in Chapter 5. Although a more rigorous approach could be employed (e.g., finite difference method), evaluating steady-state conditions is generally more relevant to distribution systems.

The biofilm is idealized such that the cell density (X_f) is assumed to be uniformly distributed across the substratum. It is recognized that bacterial populations will not exist as a uniform layer biofilm, rather the fixed population is more likely to form micro-environments consisting of cellular clusters (Costerton *et al.*, 1994). However, this simplification is necessary to model the overall observed reactions within the context of an annular reactor, and not reactions of bacterial micro-environments which may exist in the reactor.

Under steady-state conditions and negligible growth rate of suspended cells, equations 6.1 through 6.3 are simplified to the following three equations.

$$\frac{(S_i - S_b)}{\tau} = J a \qquad (6.4)$$

$$\frac{(X_b - X_i)}{\tau} = r_d a \qquad (6.5)$$

$$r_{g,f} = r_d \qquad (6.6)$$

where, τ is the hydraulic retention time, T
a is the specific surface area, L^{-1}

BOM Utilization Rate

The influent BOM concentration has more of an effect on the number of biofilm HPCs than it does on the net bulk HPCs (Chapter 5). Consequently, it is assumed that BOM is predominately utilized by biofilm cells. Therefore, the mass flux of BOM is to the biofilm and the change in BOM concentration is normal to the biofilm surface as shown in Figure 6.1.

Rittmann and McCarty (1980a) assumed that the only method for substrate transport in a biofilm is by molecular diffusion. However experimental evidence has suggested that advective transport may also be a factor (de Beer *et al.*, 1994). For lack of conclusive evidence within the context of the experiments in the present research, diffusion will be assumed as the major substrate transport mechanism.

Rittmann and McCarty (1980a) also assumed that substrate utilization in the biofilm can be modeled with Monod kinetics. Monod kinetics will initially be assumed for modeling the rate of BOM utilization in the present research.

Thus the mass balance of BOM in the biofilm (S_f) is given by:

$$\frac{\partial S_f}{\partial t} = D_f \frac{\partial^2 S_f}{\partial z^2} - \frac{k_{max}\, X_{f,V}\, S_f}{K_s + S_f} \qquad (6.7)$$

where, D_f is the molecular diffusivity in the biofilm, $L^2\ T^{-1}$
k_{max} is the maximum specific rate of substrate utilization, $M_S\ M_X^{-1}\ T^{-1}$
$X_{f,V}$ is the bacterial density, $M_X\ L^{-3}$
K_S is the half-velocity constant, $M_S\ L^{-3}$

Rittmann and McCarty (1980a) showed that under steady-state conditions the flux of substrate into the biofilm is balanced by the substrate utilization rate. The steady-state relationship for substrate in a biofilm is reduced to:

$$D_f \frac{d^2 S_f}{dz^2} = \frac{k_{max} X_{f,V} S_f}{K_s + S_f} \qquad (6.8)$$

For the BOM flux to be approximately equal to the rate of BOM utilization within the biofilm (i.e., right-hand side of equation 6.8) two conditions must be satisfied. The rate of external mass transfer to the biofilm must not be limiting and the rate of internal mass transfer within the biofilm must not limit substrate utilization.

The Damköhler number (Da) relates the utilization rate of substrate to the mass transfer rate for immobilized cells, as given by (Bailey and Ollis, 1986):

$$Da = \frac{v_{max}}{k_L S_b} \qquad (6.9)$$

where, v_{max} is the maximum reaction rate for enzyme catalysis with Michaelis-Menton kinetics, $M_S L^{-2} T^{-1}$
k_L is the mass transfer coefficient, $L T^{-1}$

If the Da is much less than unity (mass transfer rate is larger than the reaction rate), then the enzymatic catalyzed process is reaction limited. When the Da is much greater than unity, then the process is limited by the rate of diffusion.

A limitation of the Damköhler number is that it requires prior knowledge of the kinetic parameters associated with the rate of utilization (i.e., v_{max}). Thus, an observable external mass transfer modulus (Ω) has been suggested (Karel *et al.*, 1985):

$$\Omega = \frac{R_{obs} L_c}{k_L S_b} \qquad (6.10)$$

where, R_{obs} is the observed reaction rate, $M_S L^{-3} T^{-1}$
L_c is the characteristic length, L

For this analysis the observed reaction rate is equal to the left-hand side of equation 6.4, the observed BOM removal rate.

Similarly, the Thiele modulus can be used for comparing the BOM removal rate with mass transfer within the biofilm (Bailey and Ollis, 1986). The Thiele modulus also requires that the reaction rate parameters are known. To circumvent this problem an observable modulus can also be used (Weisz, 1973):

$$\Phi = \frac{R_{obs} L_c^2}{D_f S_b} \tag{6.11}$$

If the observed Thiele modulus (Φ) is less than 0.3 then diffusion is fast compared to the BOM removal rate and the biofilm is fully penetrated with respect to substrate (Weisz, 1973).

Detachment Rate

Stewart (1993) developed a conceptual detachment model on the basis of a local detachment frequency. The local detachment frequency can vary across the depth of the biofilm and is dependent of the physiological state of cells in the biofilm. With a spatial coordinate z, Stewart (1993) expressed the rate of detachment as:

$$r_d = \int_0^{L_f} f_d(z)(L_f - z) A_d \rho(z) dz \tag{6.12}$$

where, L_f is the biofilm thickness, L
$f_d(z)$ is the local detachment frequency
A_d is the detached particle area, L^2
$\rho(z)$ is the local biofilm density, $M_x L^{-3}$

By assuming a form for $f_d(z)$ and integrating equation (6.12) across the biofilm depth, various detachment rate expressions can be obtained. If detachment is assumed constant throughout the biofilm, then:

$$f_d(z) = \frac{k_d}{A_d} \tag{6.13}$$

where, k_d is the detachment coefficient, $T^{-1} L^{-1}$

After substituting (6.13) into (6.12) and integrating, the following rate expression can be obtained:

$$r_d = \tfrac{1}{2} k_d \rho L_f^2 \qquad (6.14)$$

Equation (6.14) shows a second-order dependence of detachment rate on biofilm thickness and would suggest that detachment is non-growth related. This detachment model is also commonly assumed by others (e.g., Wanner and Gujer, 1986).

Another detachment expression developed by Stewart (1993) is given by:

$$r_d = k_{d1} \rho \int_0^{L_f} \mu(z)(L_f - z)\,dz + \tfrac{1}{2} k_{d2} \rho L_f^2 \qquad (6.15)$$

where, $\mu(z)$ describes the biofilm growth rate as function of biofilm depth, T^{-1}
k_{d1} is the growth related detachment coefficient, L^{-1}
k_{d2} is the non-growth related detachment coefficient, $L^{-1}\ T^{-1}$

Analytical solutions for the integral in equation (6.15) are generally not available, however Stewart (1993) solved the integral for several special cases.

The general detachment expression (equation 6.15) proposed by Stewart (1993) combines two fundamentally different processes: growth related detachment (first term of equation 6.15) and non-growth related detachment (second term of equation 6.15). Growth related detachment is influenced by the substrate loading rate and biofilm growth rate. Non-growth related detachment is a function of shear stress, biofilm areal density and biofilm thickness.

Empirical detachment models have been developed in numerous investigations (Peyton and Characklis, 1993; Speitel and DiGiano, 1987; Rittmann, 1982). One detachment model proposed by Peyton and Characklis (1993), was based on experimental work with *Pseudomonas aeruginosa* biofilms attached to polycarbonate coupons in ARs.

In their model the steady-state detachment rate is proportional to the substrate utilization rate, as given by:

$$r_d = k_d \frac{Q}{A}(S_i - S) Y_{x/s} L_f \qquad (6.16)$$

where, k_d is the detachment coefficient, L^{-1}

Equation (6.16) suggests that as more biomass is produced, detachment will increase. If biofilm detachment is predominately growth associated and the growth rate in the biofilm is relatively constant (*e.g.*, substrate diffusion is relatively faster than the growth rate) then the empirical model developed by Peyton and Characklis (1993) approximates the general detachment model (Stewart, 1993).

Another empirical model developed by Rittmann (1982) assumes that the detachment rate is predominately non-growth associated and is a function of the shear stress (τ_s):

$$r_d = k_d \rho_f L_f \tau_s^{0.58} \qquad (6.17)$$

RESULTS

Mass Transfer Limitations

The observable external and internal mass transfer moduli were calculated for the described experimental conditions to determine the extent to which mass transfer limits substrate utilization. The observed external mass transfer was calculated for the removal rates of the AR experiments (Figure 6.2).

The biofilm thickness was used as the characteristic length for calculating the external observable mass transfer modulus (Ω) and the observable Thiele modulus (Φ).

Biofilm thickness was selected as the characteristic length because it represents the length substrate diffuses across. Biofilm expansion will be primarily due to increased microbial growth, which is closely related to the substrate utilization rate and the availability of substrate.

Peyton (1992) dosed ARs with glucose at loading rates between 10 and 92 $mg/m^2.h$, which resulted in a steady-state biofilm thickness, for a *Pseudomonas aeruginosa* biofilm, of 6 and 32 μm, respectively. Similarly, Seibel and Characklis (1991) measured a steady-state biofilm thickness of 40 μm (± 10 μm) for a binary biofilm (*Klebsiella pneumoniae* and *P. aeruginosa*) which received a glucose loading rate of 120 $mg/m^2.h$. In the AR experiments of this investigation a mixed biofilm population received a BOM loading rates ranging between 0.083 to 7.6 mg $C/m^2.h$. An average biofilm thickness of 10 μm was assumed for all experimental trials for calculating the observable external modulus and the Thiele modulus.

Mass transfer coefficients were obtained from experimental work by Yang and Lewandowski (1995). In their investigations biofilms were cultured in a small flow cell reactor (2 cm deep × 4 cm wide × 75 cm long). Using microelectrodes to measure mass transfer coefficients, it was found that mass transfer coefficients range from 10^{-5} (within the biofilm) to 10^{-4} at the top a cell cluster (Yang and Lewandowski, 1995). External mass transfer was proportional to the bulk fluid velocity, which ranged between 0.4 - 1.6 cm/s. The maximum Reynold's number (Re) in the experiments performed by Yang and Lewandowski (1995) was approximately 160. The Re for the AR experiments described in Chapter 5 was approximately 2500. It is recognized that the mass transfer coefficient

would be greater for the AR experiments, because of increased velocities. A mass transfer coefficient of 10^{-5} m/s was assumed for these calculations.

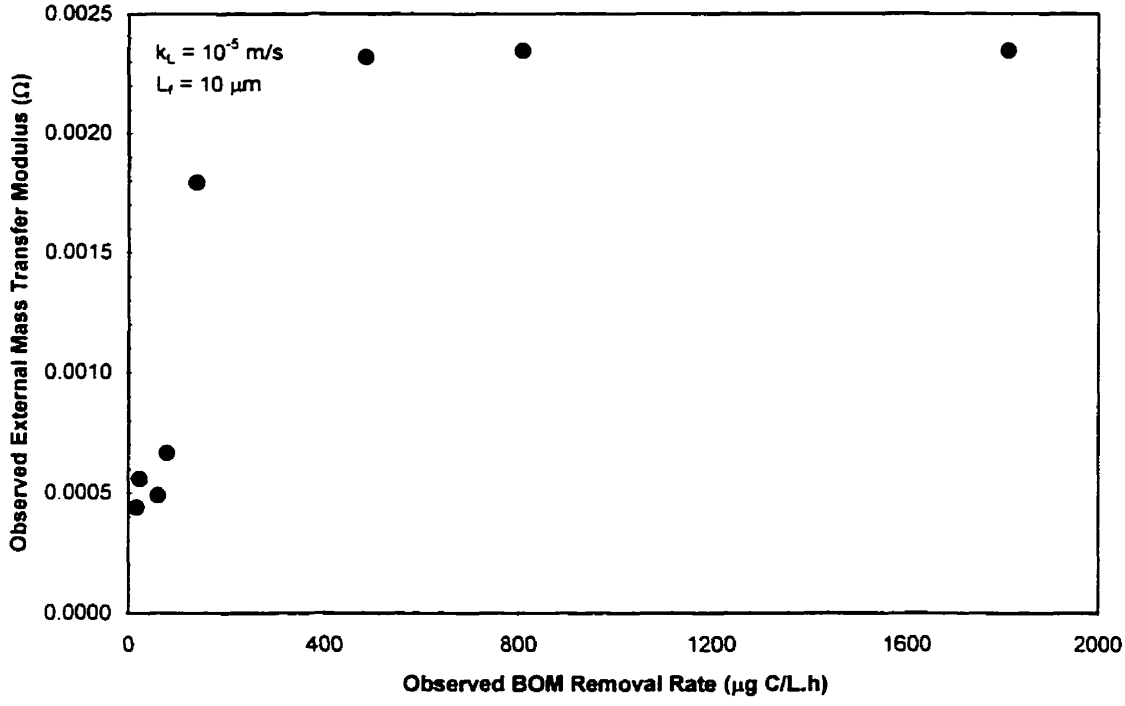

Figure 6.2: Observed external mass transfer modulus calculated for an external mass transfer coefficient of 10^{-5} m/s.

The diffusion coefficient of BOM in biofilm is another parameter that needed to be estimated in order to calculate the observable Thiele modulus. Diffusion coefficients that have been reported in the literature are summarized in Table 6.2. A diffusion coefficient of 10^{-10} m^2/s was assumed for all experimental conditions. Given the conservative estimates for the biofilm thickness and the diffusion coefficient the observable Thiele modulus for all experimental conditions was calculated (Figure 6.3).

Table 6.2: Biofilm Diffusion Coefficients Reported in the Literature

Source	D_f (m^2/s)	Compound	Method of Determination
1	1.01×10^{-9}	Acetate	Calculated
2	1.67×10^{-9}	AOC	Calculated
3	6.0×10^{-10}	Fluorescein	Measured

1. Rittmann and McCarty, 1980b
2. Zhang and Huck, 1996
3. de Beer *et al.*, 1997

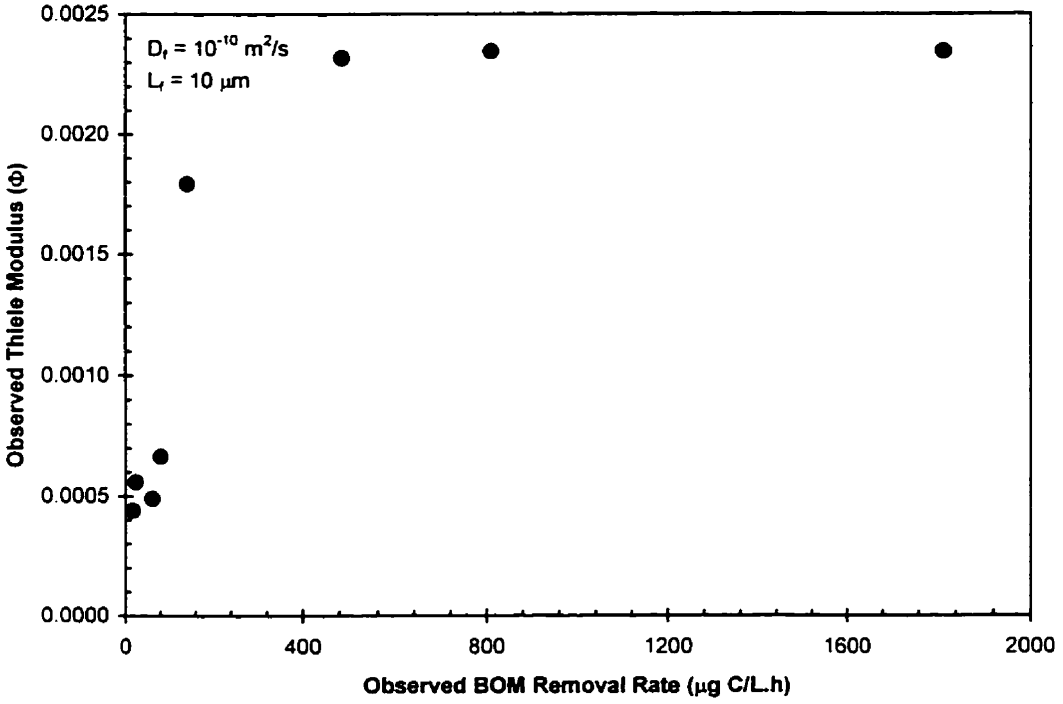

Figure 6.3: Observed internal mass transfer (Thiele) modulus for the eight experimental conditions, calculated with conservative parameter estimates.

BOM Utilization

Assuming that mass transfer does not limit the reaction rate of BOM, then the BOM flux is approximately equivalent to the rate of substrate utilization.

$$\frac{(S_i - S_b)}{\tau a} \cong \frac{k_{max} X_{f,V} L_f S_f}{K_s + S_f} \qquad (6.20)$$

In equation (6.20) the biofilm thickness (L_f) is introduced to complete the mass balance on substrate utilization. In this research, the product of $X_{f,V}$ and L_f is approximated by the number of biofilm HPCs per unit area.

If external mass transfer limitations are relatively minor compared to the rate of substrate utilization then the bulk BOM concentration (S_b) will be approximately equal to the BOM concentration at the surface of the biofilm (S_s). For biofilms which are thin, and the BOM mass transfer rate in the biofilm is low (relative to the BOM utilization rate) the change in concentration in S_f across the depth of biofilm may be low in absolute terms. Therefore, the biofilm substrate concentration can be approximated with an average substrate concentration $\left(\overline{S_f}\right)$. Finally, if the bulk BOM concentration is low then $S_b \cong S_s \cong \overline{S_f}$. More sophisticated modeling may show that $S_b > \overline{S_f}$, however to evaluate reactor performance in its entirety, assuming $S_b \cong \overline{S_f}$ is reasonable for the conditions studied in the present research. Given these simplifications an approximate reaction rate can be determined by plotting the BOM removal rate against the bulk BOM concentration (Figure 6.4).

The linear relationship in Figure 6.4 suggests that BOM removal is a first-order process or the conditions studied are in the linear range of a Monod kinetic expression, which has a maximum utilization rate greater than the measured BOM removal rates. Figure 6.4 assumes that the biofilm density is relatively constant with respect to the observed removal rate. Interestingly, a linear relationship was found using AOC data from bench-scale glass bead recirculating filters as reported by Zhang (1996). A summary

of the linear models calculated for this data set and the data reported by Zhang (1996) is shown in Table 6.3.

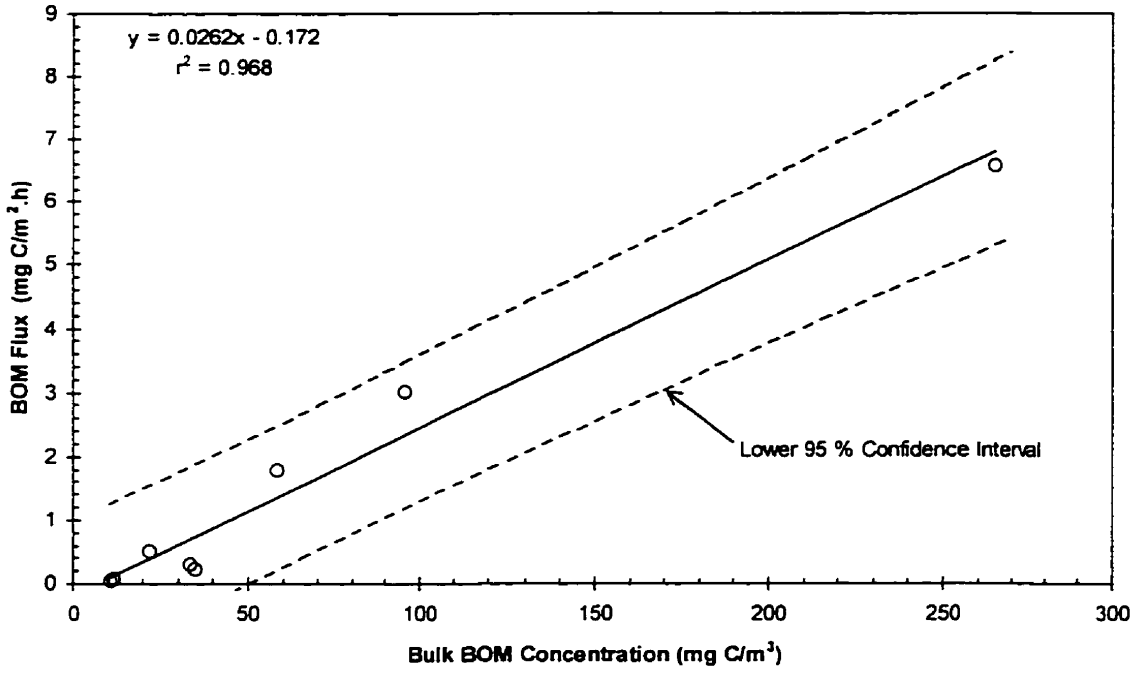

Figure 6.4: BOM reaction rate for AR experiments, assuming no mass transfer limitations.

Table 6.3: Linear Models for the Relationship between the BOM Flux and the Bulk BOM Concentration.

Data Source	Linear Model	r^2	Comments
Table 6.1	$y = 0.0262\,x - 0.172$[a]	0.984	• BOM based on component analysis and reported on carbon basis.
Zhang (1996)	$y = 0.0314\,x - 0.485$[a]	0.946	• Ozonated raw water as nutrient source. • BOM measured as AOC
Zhang (1996)	$y = 0.0656\,x + 0.0368$[a]	0.961	• Nutrient source was synthetic ozone by-products in deionized water.

[a] Slope is significant at the 1 % level.

The slope calculated in Figure 6.4 is equivalent to $\frac{k_{max} X_{f,V} L_f}{K_s}$ for high K_S values, several orders of magnitude greater than S_b, and a constant biofilm density. Zhang and Huck (1996b) have reported K_S values for AOC greater than 10 mg acetate/L, therefore in this research it is likely that $S_b << K_S$. However, Figure 6.5 shows that the number of biofilm HPCs is non-linearly related to the BOM flux, indicating that the amount of biofilm per unit area $\left(X_{f,V} L_f\right)$ was not constant for the eight experimental trials.

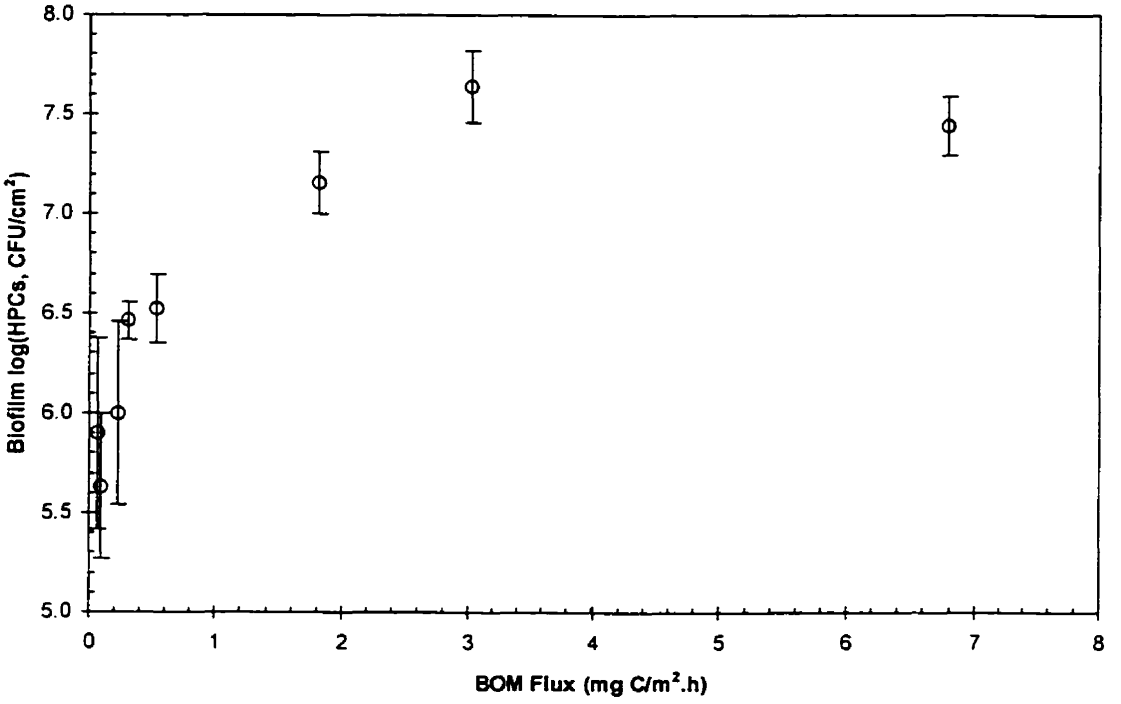

Figure 6.5: Relationship between the number of biofilm HPCs and the BOM flux (error bars indicate 95 % confidence intervals).

To calculate a pseudo-first-order rate coefficient, the number of biofilm HPCs for each experimental trial is used to normalize the BOM flux. A linear regression was calculated for the normalized BOM flux and the bulk BOM concentration, however the

slope of the line was not significant for this relationship. Therefore, an alternate method for accounting for the number of biofilm HPCs was used.

A linear relationship was determined between the BOM flux and the product of the number of biofilm HPCs and the effluent BOM concentration (Figure 6.6). Although this line is representative of essentially four data groupings, the slope of the line was found to be significant (p value = 0.0001). Thus, the pseudo-first-order coefficient for easily biodegradable components was 8.0×10^{-14} m^3/CFU.h.

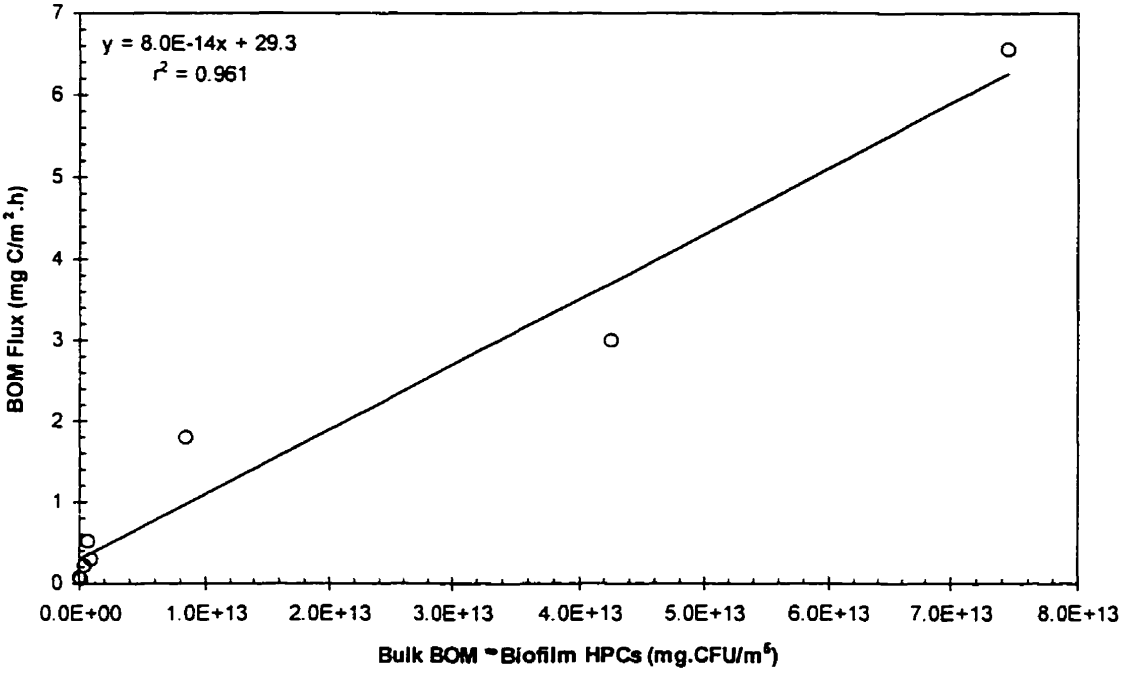

Figure 6.6: First-order removal of BOM accounting for the amount of biomass.

A similar analysis was performed on each of the seven added organic compounds. The specific pseudo-first-order rate coefficients are presented in Table 6.4. Regressions for the three amino acids were not possible because the effluent amino acid concentration was essentially zero for 7 of the 8 trials. Regressions were possible for the other four compounds, however only two of the linear models were significant at the 5 % level. The

relative rate of biodegradability can be directly compared using the specific pseudo-first-order rate coefficient. Formate has the highest rate of biodegradability and glyoxal has the lowest (Table 6.4).

Table 6.4: Pseudo-First-Order Rate Coefficients for Four BOM Components

BOM Component	Pseudo-First-Order Rate Coefficient 10^{-14} m^3/CFU.h	95 % Confidence Interval		r^2
		Upper	Lower	
Acetate	1.2	2.6	-0.18	0.433
Formate	9.4†	12	7.1	0.953
Formaldehyde	2.3	5.7	-1.1	0.316
Glyoxal	0.96†	1.6	0.28	0.665

† Coefficient is significant at 5 % level.

In biological filters an apparent linear relationship between the amount of substrate removed and the influent substrate concentration has been observed for carboxylic acids (Gagnon *et al.*, 1997), AOC (Huck *et al.*, 1994) and other indices of BOM (Huck and Anderson, 1992). This linear relationship provided empirical insight into the fraction of BOM removed (slope) and the minimal achievable concentration (C_{MA}) removed during biological filtration (x-intercept). The linear relationship for the amount of BOM removed can be obtained from a biological filter operating at a specific empty bed contact time (EBCT). The experiments of this investigation had different hydraulic retention times, consequently a different expression was sought.

The fraction removed for a specific BOM component was calculated through a linear relationship involving the organic flux (J) and the organic loading rate (R_L). The loading rate is calculated by multiplying the influent concentration of the BOM

component by the volumetric flow rate and dividing by the reactor surface area. A linear relationship for the removal of formate was calculated (Figure 6.7). The slope of the line for formate is 1.0, indicating essentially complete removal of formate. The C_{MA} value for formate cannot be obtained directly from Figure 6.7 because the x-intercept is the minimal surface loading rate. Linear models involving organic flux and loading rate for all of the BOM components in the influent stock solutions are summarized in Table 6.5.

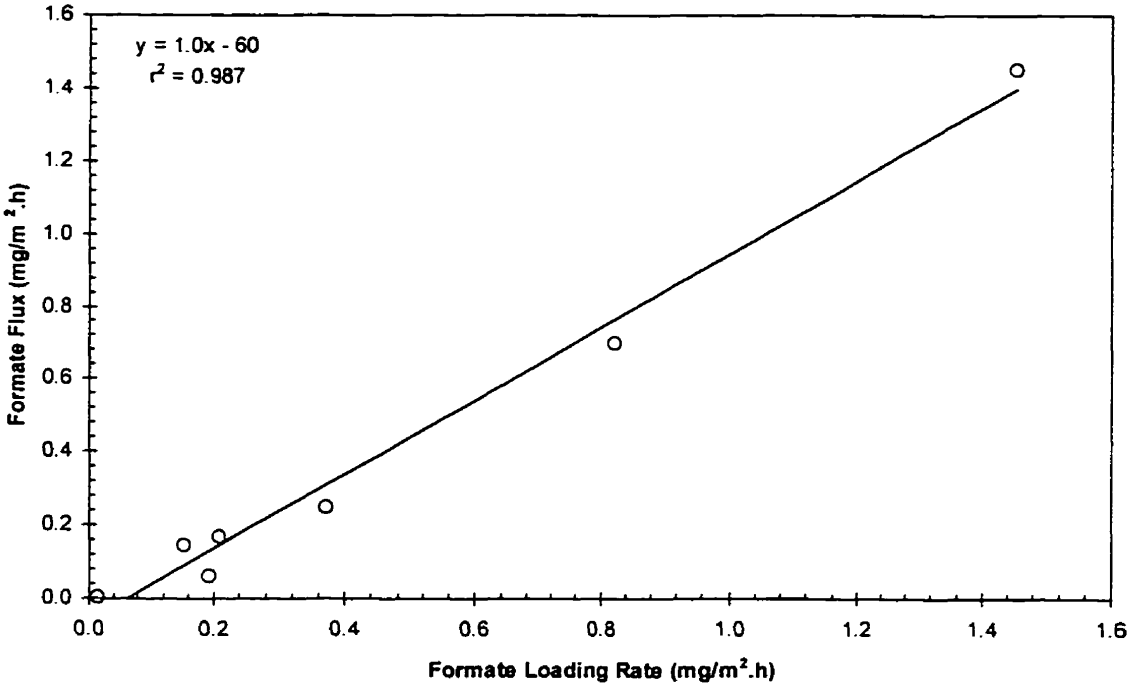

Figure 6.7: Formate flux to the biofilm is linearly related to the formate loading rate.

Table 6.5: Linear Relationships between Organic Flux and Loading Rate for the BOM Components

BOM Component	Equation	r^2	F value
Acetate	$J = 0.62\ R_L + 80$	0.867	39.1
Formate	$J = 1.0\ R_L - 60$	0.987	457
Formaldehyde	$J = 0.87\ R_L + 4.8$	0.979	281
Glyoxal	$J = 0.52\ R_L + 2.3$	0.946	106
Aspartate	$J = 1.0\ R_L - 6.4$	0.999	106000
Glutamate	$J = 1.0\ R_L - 3.7$	0.999	35900
Serine	$J = 0.96\ R_L + 8.3$	0.999	71600

Biofilm Detachment

Detachment was evaluated as growth-related and non-growth-related phenomena. A relationship was established between the observed detachment rate (equation 6.5) and the BOM flux to the biofilm (Figure 6.8). Although the linear relationship may be strongly influenced by the point at the highest BOM flux, the slope of the line was significant at the 5 % level of significance (p value = 0.0019).

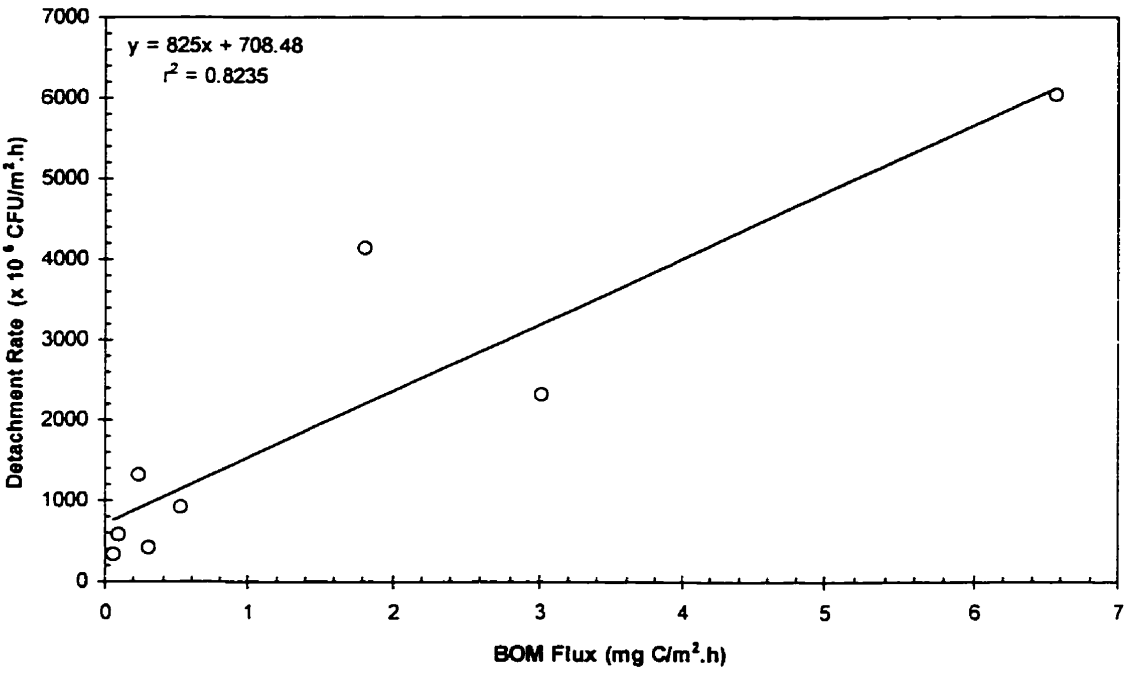

Figure 6.8: Growth-related detachment of HPCs.

Two additional experiments were performed in parallel with experiments 5.10a and 6.1a. The replicate ARs received similar BOM constituents and concentration, but were operated at a rotational speed of 350 rpm. The increased rotational speed would increase the shear stress, thereby increasing the opportunity for non-growth-related detachment. Biofilm detachment was relatively unaffected by increased shear stress at influent concentrations of 250 μg C/L (Figure 6.9). An analysis of variance showed that BOM was the only significant parameter (p value = 0.0005) influencing detachment. Both the rotational speed (p value = 0.295) and the interaction between rotational speed and BOM (p value = 0.295) were not significant factors.

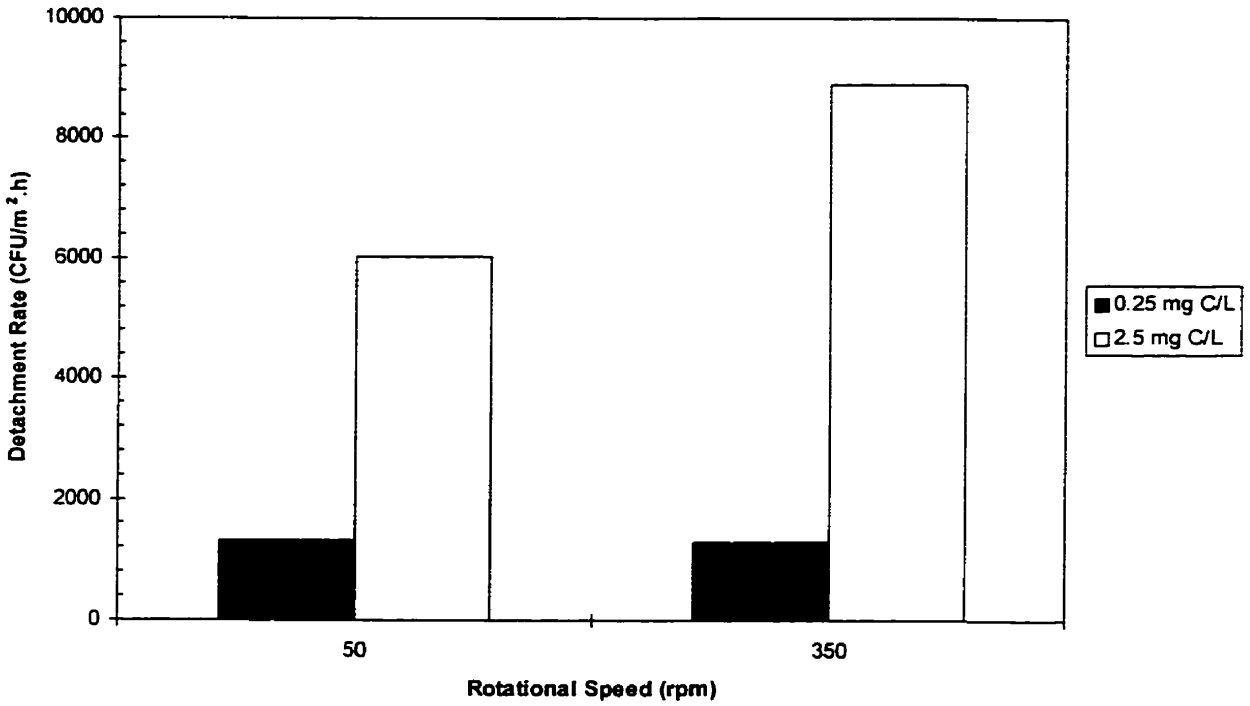

Figure 6.9: Non-growth-related detachment experiments.

DISCUSSION

Mass Transfer Limitations

The analysis of mass transfer properties of BOM under conditions relevant to drinking water was not rigorous, in the sense that mass transfer coefficients were not calculated. However, the analysis of mass transfer provides useful insights into the relative importance of diffusion and bio-reaction of BOM in drinking water biofilms. For the assumed conditions, the rate of both external and internal mass transfer does not appear to limit BOM removal.

Wang (1995) found that external mass transfer did not limit the removal of NOM in a biofilter, for mass transfer coefficients as low as 10^{-5} m/s. In Figure 6.2, a mass transfer coefficient of 10^{-5} m/s was found not to limit the removal rate of BOM. Because the surface velocities employed in the described AR experiments are greater than those in

a biofilter (e.g., 0.3 m/s vs. 5-20 m/h), it is unlikely that an average mass transfer coefficient of 10^{-5} m/s would be realized in an AR operated under conditions described in the present research.

The internal mass transfer of BOM for the assumed conditions was shown not to limit BOM removal for the described experimental conditions (Figure 6.3). The observable Thiele modulus is proportional to the square of the assumed biofilm thickness, and thus the Thiele modulus is highly sensitive to this parameter. If a value of 100 μm was assumed for the biofilm thickness, the Thiele modulus may approach the critical value of 0.3 for the higher BOM removal rates.

Biofilms in drinking water distribution systems are typically thin, and measured thicknesses are rarely reported in the literature. For different experimental trials during the course of the present research, polycarbonate coupons were placed under microscope (Nikon Labophot 2A fluorescence microscope; Nikon Canada Inc., Mississauga, ON) to measure biofilm thickness. Biofilm thickness was measured according to the method described by Bakke and Olsson (1986). After the coupon was prepared for viewing at the proper magnification, cells appeared in clusters scattered across the coupon surface. The thickness of any given cluster was typically less than 10 μm. Because of the randomness of these clusters and the fact that the clusters were very thin (to the point of having an unreliable measurement), biofilm thickness was not a reported measured parameter for the present research.

In other biofilm investigations for which ARs were operated under conditions more favourable to biofilm accumulation (e.g., Peyton, 1992; Siebel and Characklis, 1991), measured biofilm thicknesses rarely exceeded 100 μm. Therefore, it is highly

unlikely that the average biofilm thickness would equal or exceed a value of 100 μm in the present research. Thus, it would be expected that mass transfer to the biofilm would not limit the rate of substrate utilization.

The calculations show that mass transfer does not limit BOM removal in a distribution system biofilm attached to smooth surface (e.g., PVC), further calculations would be necessary to examine the relative importance of mass transfer on other surfaces (e.g., cast-iron pipe).

BOM Utilization

BOM removal by the biofilm in the ARs followed an apparent first-order reaction (Figure 6.4). This is not surprising given the high K_S values that have been reported in the literature for mixed biofilms growing in oligotrophic environments. Zhang and Huck (1996b) reported K_S values for AOC of 17 and 55 mg acetate C/L for a mixed heterotrophic biofilm attached to glass bead columns utilizing synthetic and ozonated waters, respectively. The reported K_S values are at least 2-3 orders of magnitude greater than BOM effluent concentrations measured in this investigation.

Similarly, the regression models developed from the data of Zhang (1996) indicate a first-order removal process for AOC (Table 6.3). The slope from the ozonated was essentially the same as the data presented in this investigation. The synthetic water slope was approximately two times greater than the slope from this investigation. The synthetic cocktail in Zhang (1996) did not include amino acids and the BOM composition of the ozonated water was not determined. It is likely, however, that some proteins were hydrolyzed through ozonation resulting in the formation of free amino acids.

The biofilm column reactor employed by Zhang (1996) had a specific surface area of 1280 m^{-1}, which is approximately 5 times greater than the specific surface area for the ARs (270 m^{-1}). By performing regressions with the BOM flux the specific surface area of each respective reactor is accounted for, thus comparisons in removal rates can be compared directly. The significance of surface area to volume ratios in biofilm reactors is discussed in greater detail in Chapter 8.

Figure 6.5 shows that amount of biomass was proportional to the BOM flux. Figure 6.6 considers this proportionality by performing a regression on BOM flux and the product of $(X_{f,V} L_f)^* S_b$. The slope of this line represents a pseudo-first-order rate constant and can be used for modeling BOM removal in drinking water for situations where both the number of biofilm HPCs and BOM concentration are known.

From a practical perspective, a first-order rate expression could potentially be useful for modeling the fate of BOM in full-scale distribution systems. In general hydraulic models, such as EPANET, allow users to model contaminants which have either zero-order or first-order rate kinetics. Pseudo-first-order rate constants were calculated for four of the BOM components used in this research (Table 6.4). The rate constants are especially useful for comparing the relative rates of biodegradability.

Another method for comparing the utilization of BOM components is by comparing their per cent removals, as given by the slopes in Table 6.5. The fraction of acetate removed was less than the fraction of formate removed (Table 6.5). Similarly, the pseudo-first-order rate constant for formate is almost an order of magnitude greater than the constant for acetate (Table 6.4). Urfer and Huck (1997) found that formate was easily removed through a biological filter, whereas the removal of acetate was more complex. It

was found that the concentration of acetate increased from the influent value at the top portion of the filter and then was steadily removed in the bottom portion. Measurable quantities of both formate and acetate (approximately 50 - 100 μg/L) were detected in the filter effluent (Urfer and Huck, 1997). The fraction of carboxylic acids removed in this study was also in agreement with a full-scale biofiltration study, which found that on average formate was more readily removed than acetate (Gagnon *et al.*, 1997b).

Aldehydes have been shown to be easily removed during biological filtration (Weinberg *et al.*, 1993; Krasner *et al.*, 1993), although, the percentage removal of glyoxal is consistently lower than formaldehyde for similar media types and hydraulic loadings (Krasner *et al.*, 1993). Similarly, this study found that the fraction of glyoxal removed, as indicated by the slopes reported in Table 6.5 is lower than the fraction of formaldehyde and the other BOM components in the AR influent. The rate of biodegradability for formaldehyde was found to be at least twice that of glyoxal (Table 6.4).

The fraction of aspartate, glutamate, and serine removed was at or near unity indicating that complete removal of these compounds was achieved for most of the studied loading rates. Consequently, pseudo-first-order rate constants were not obtainable. Van der Kooij *et al.* (1988) reported high utilization rates for amino acids by drinking water isolates of *Aeromonas hydrophila*. The mechanisms associated with the transport and utilization of amino acids in drinking water are discussed in further detail in Chapter 5.

Biofilm Detachment

A limited number of data suggest that in drinking water biofilms, detachment may be related to the BOM flux to the biofilm (Figure 6.8). Although at low levels of BOM flux more typical of treated waters (< 1000 μg $C/m^2.h$) a zero order detachment rate with flux may be reasonable. For BOM fluxes between 1000 and 10000 μg $C/m^2.h$ further investigations would be necessary to confirm a first-order relationship between detachment and BOM flux, as the model presented in this study is dependent on three groupings of data.

The data also suggest that shear stress does not significantly impact the amount of biofilm detachment in an AR, which concurs with findings presented elsewhere (e.g., Peyton and Characklis, 1993). However these results are limited to conditions of constant shear stress over a smooth surface. For situations having intense particle-particle interaction (e.g., backwashing a biological filter with air scour), studies have demonstrated (Tijhuis et al., 1994) that the shear stress is more relevant to biofilm detachment than substrate loading rate.

CONCLUSIONS

The rate of mass transfer, either internal or external, to the biofilm, was found not to limit BOM removal. Because mass transfer does not limit BOM utilization, it was assumed that bulk BOM concentration approximately equals the average concentration of BOM in the biofilm. This assumption was useful for obtaining the rate of BOM removal.

A linear regression between BOM flux and bulk BOM concentration showed that BOM removal can be approximated by a first-order rate expression. This linear model was found to be significant for the present research and for a kinetic evaluation of AOC performed by Zhang (1996). Because the biofilm density was proportional to the BOM flux a linear regression involving BOM flux and the product of $(X_{f,v}L_f)^*S_b$ was performed. The slope of this second regression represents a pseudo-first-order rate constant for BOM. Implicit in this first-order rate coefficient is specific maximum growth rate and the half velocity coefficient.

By performing a similar approach for the individual compounds, pseudo-first-order constants were obtained for four the BOM components studied. However, only the constants for formate and glyoxal were significant at the 5 % level. The rate constants indicate the relative rate of biodegradability for each compound. The rate of biodegradability of each component can be ranked accordingly as (highest to lowest): formate, formaldehyde, acetate, and glyoxal.

Linear models involving the BOM flux and the BOM loading rate for each of the studied BOM components. This offered another method for comparing the relative removals. The slopes for these linear models indicate average per cent removal for each compound. As indicated by a high slope, amino acids and formate were found to be BOM components that are easily removed. Of the components studied, glyoxal had the lowest slope. Acetate also had a lower slope, however acetate is an intermediate in many metabolic reactions. Consequently, a simplified linear model may adequately estimate the removal of amino acids and aldehydes, but may be less appropriate for organic acids such as acetate.

The BOM utilization rate was found to have a stronger relationship with biofilm detachment than shear stress for polycarbonate surfaces. Under low nutrient conditions, (BOM < 100 μg/L) the biofilm detachment rate may have a zero order dependence on the BOM removal rate, at higher BOM loading rates detachment may be a first-order process (with respect to BOM removal rate).

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CHAPTER 7: FORMATION OF SOLUBLE MICROBIAL PRODUCTS IN A MODEL DISTRIBUTION SYSTEM

INTRODUCTION

The majority of drinking water research concerning biodegradable organic matter (BOM) has focused on the quantification, removal and fate of many easily biodegradable constituents (e.g., Cipparone *et al.*, 1997; Gagnon *et al.*, 1997; Paode *et al.*, 1997; van der Kooij *et al.*, 1995). Many of the easily biodegradable components are at low concentrations (less than 100 μg/L) after either conventional or biological treatment (LeChevallier *et al.*, 1996; Kaplan *et al.*, 1994). At low disinfectant concentrations these easily biodegradable compounds may be utilized by bacteria within the initial portions of distribution systems. Under these circumstances the persistence of biofilms in mid to latter portions of distribution systems would suggest that energy sources alternative to these easily biodegradable components are available in sufficient quantities to support regrowth.

In treated waters which are chloraminated and/or have naturally higher concentrations of nitrogen species (e.g., ammonia), nitrogen may be used as a major energy source for autotrophic or chemolithotrophic growth in the extremities of the distribution system (Wilczak *et al.*, 1996; Wolfe *et al.*, 1990). For areas having longer retetnion times which favor the growth of heterotrophic bacteria (i.e., carbon is considered to be the limiting nutrient) soluble microbial products (SMPs) may be responsible for supporting bacterial growth.

The purpose of this chapter is to describe experiments involving a model distribution system. In this investigation, the removal of easily biodegradable organic compounds and formation of other organic compounds is examined. The effect chlorine and the composition of the influent BOM (e.g., the presence of amino acids) have on bacterial growth and SMP formation will also be evaluated. The extent SMPs may influence the formation of unwanted organohalides is also examined.

BACKGROUND

In treated waters, natural organic matter (NOM) has been observed to change during distribution (Reckhow *et al.*, 1996). The evolution of NOM during distribution may in part be influence by the formation of refractory organic matter (ROM), which is a stable by-product resulting from bacterial decomposition (Zumstien and Buffle, 1989). Several properties of ROM include: molecular weights which are greater than a few hundred daltons but less than a few thousand daltons, a relatively large degree of aromaticity, and a low nitrogen to carbon ratio (Krasner *et al.*, 1996). In natural waters, ROM can be divided into two categories: those originating from soils (pedogenic,

PROM) and those originating from aquatic media (aquagenic, AROM) (Buffle *et al.*, 1982). PROM can be measured as UV absorbance (λ = 285 nm, UV_{285}). During the distribution of treated waters the formation of UV_{285} will simply be referred to as ROM.

In biological systems ROM may be closely related to the formation of soluble microbial products (SMPs). Characteristics common to SMPs are a relatively high molecular weight (usually greater than the original substrate), high degree of aromaticity, and slow bio-reaction kinetics (Rittmann *et al.*, 1987). Some of the SMP constituents which have been reported are (as summarized by Rittmann *et al.*, 1987): humic and fulvic acids, polysaccharides, proteins, nucleic acids, organic acids, amino acids, enzymes and other organic material of microbial origin. SMPs have been divided into utilization-associated products, whose formation is controlled by the specific utilization rate of the original substrate, and biomass associated products, whose formation is controlled by the cell density within the biofilm (Namkung and Rittmann, 1986). In drinking water biofilters Carlson *et al.* (1996) have shown that SMPs increased with filter depth, however, the amount of SMPs in the filter effluent was relatively minor in comparison to the pool of dissolved organic carbon.

MATERIALS AND METHODS

Experimental Design

Two factors studied in this investigation were the chlorine dose and the fraction of amino acids present in the influent BOM cocktail. A total chlorine residual of 0.2 mg/L or zero was maintained in the influent of AR_1. Amino acids were present in the influent BOM cocktail at a fraction of 0.0 or 0.34 (mass carbon basis).

The experimental design would suggest that the experiments were operated as a standard 2^2 factorial experiment (e.g., Box *et al.*, 1978). However, in the experiments involving chlorine, it was added after a biofilm had been established. The experimental trial with amino acids and no chlorine was operated for approximately 2 weeks, which was sufficient time to establish a steady-state biofilm. Chlorine was then added and the ARs were operated for an additional 2 weeks. The latter 2 weeks represented the amino acid high level and chlorine dose high level for data analysis purposes. The ARs were cleaned prior to operation for two trials operated without amino acids in the influent BOM. The model distribution system for the remaining two trials was chlorinated after two weeks as described previously.

Physical Configuration

Three ARs (Model 920LJ; BioSurfaces Technologies Corp., Bozeman, MT) were connected in series to simulate a model distribution system. BOM nutrients, chlorine, and GAC-filtered tap water were pumped into the first AR (Figure 7.1). The first AR had a hydraulic retention time of 1 h. Approximately half of the effluent from the first AR was pumped into the second AR, such that AR_2 had a retention time of 2 h. Similarly AR_3 had a retention time of 4 h. Preparation of the reactors and the nutrient cocktails followed the same general outline as described in Chapter 3.

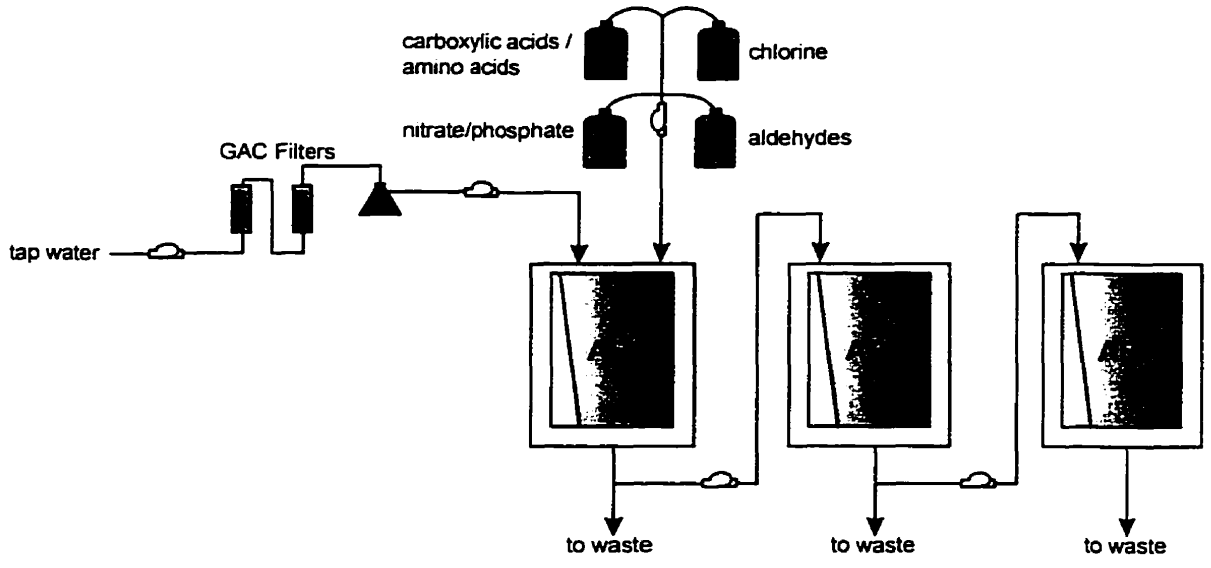

Figure 7.1: Physical configuration of model distribution system.

Experimental Measurements

Microbial Enumeration. Samples from the bulk water and from the surface of the biofilm coupon were analyzed for heterotrophic plate counts (HPCs) as outlined in Chapter 3. Samples were plated in duplicate on R2A agar and stored at room temperature (18 ± 2°C).

BOM Components. Carboxylic acids, aldehydes, and amino acids were measured in samples taken from the influent to AR_1 and the effluent of reactors 1 through 3. Carboxylic acids were analyzed using ion chromatography (Peldszus *et al.*, 1996). Aldehydes were extracted in hexane and analyzed with a GC/ECD (Sclimenti *et al.*, 1990). Amino acids were derivatized with OPA and measured using HPLC (Dossier Berne *et al.*, 1994). Details of these methods are discussed in Chapter 3.

UV Absorbance. Samples were analyzed in triplicate on an HP 8453 UV-visible spectrophotometer (Hewlett-Packard; Sunnyvale, CA) at wavelengths of 254 nm and 285 nm in a 5 cm cell. The samples were stored in 0.01 % chloroform at 4°C for a maximum

period of two weeks. Prior to analysis the spectrophotometer was zeroed with a blank (deionized water and 0.01 % chloroform). The 5 cm cell was rinsed with deionized water between readings. The outer edges of the cell were dried with tissue prior to analysis. Samples measured at UV_{254} and UV_{285} had sample coefficient of variations ranging from 0.5 % to 99.7 % and from 0.05 % to 2.5 %, respectively. The 5-cm cell was made of glass, consequently this resulted in a variability in UV_{254}. The glass cell had less of an affect on the measurement of UV_{285}, however comparisons between the two parameters not established.

Chlorine. Total and free chlorine were measured using the colorimetric version of the N,N-diethyl-p-phenylenediamine (DPD) method (Eaton *et al.*, 1995). Chlorine was measured at a wavelength of 530 nm using a DR-200 UV-visible spectrophotometer (HACH, Co.; Loveland, CO).

Trihalomethane Formation Potential (THMFP). The uniform formation conditions outlined by Summers *et al.* (1996) were used to determine the THMFP in all of the samples. The uniform formation conditions are: incubation at a pH of 8.0 (± 0.2) and a temperature of 20°C (± 1.0°C), for a period of 24 h (± 1 h), with a final chlorine residual of 1.0 (± 0.4) mg/L. The samples were stored in chlorine demand-free glassware. Chlorine demand free glassware was prepared by washing the all glassware and placing 20 mg/L chlorine solution in the glassware for at least 24 h. The glassware was then rinsed 5 times with deionized water and air dried overnight. Chlorine dosing followed the procedure described by Summers *et al.* (1996). A chlorine to TOC ratio of approximately 2.0:1 was used for dosing the hypochlorite-borate buffer solution. The samples were incubated in the dark for 24 h. After incubation the samples were measured for free

chlorine residual, pH, and trihalomethanes (THMs). THMFP was reported for only those samples which were in the target range for residual chlorine and pH.

Trihalomethanes (THMs). THMs were analyzed using Standard Method 6232 (Eaton et al., 1995) with minor modifications. The samples were preserved with 32.5 mg of $HgCl_2$ and NH_4Cl_2 and stored in 20 mL vials at 4°C for a maximum of 30 days. After incubation, 5 g of Na_2SO_4 and 4 mL of pentane containing an internal standard (dibromopropane) were added to the sample. Samples were homogenized for approximately 5 min and were allowed to stand for phase separation. The pentane layer was analyzed by gas chromatography equipped with electron capture detection (HP 5890 series II, Hewlett-Packard; Sunnyvale, CA). The samples were analyzed in duplicate, and standards containing the four major THM compounds were prepared prior to analysis.

MATHEMATICAL MODELING

A mass balance expression for BOM reaction in an AR can be expressed by:

$$V\frac{dS_b}{dt} = Q(S_i - S_b) + R_{obs}V \qquad (7.1)$$

where, V is the volume of the AR, L
S_b is the bulk BOM concentration, M L^{-3}
S_i is the influent BOM concentration, M L^{-3}
Q is the volumetric flow rate, L^3 T^{-1}
R_{obs} is observed BOM reaction rate, M L^{-3} T^{-1}

Under steady state conditions equation 1 can be reduced to:

$$-R_{obs}\tau = S_i - S_b \qquad (7.2)$$

where, τ is the hydraulic retention time, T

In Chapter 6, it was hypothesized that BOM removal may be written as a first-order rate expression. Substituting a first-order rate expression into equation 7.2, and expressing the effluent concentration as a fraction of the influent, the following can be obtained:

$$\frac{S_b}{S_i} = \frac{1}{1+\tau k} \tag{7.3}$$

where, k is the first-order rate coefficient, T^{-1}

For a single CSTR the fractional BOM removal can be written as:

$$\frac{S_i - S_b}{S_i} = \frac{\tau k}{1+\tau k} \tag{7.4}$$

In general, for n CSTRs in series the fractional BOM removal is approximated by:

$$\frac{S_i - S_{b,j}}{S_i} = \frac{k\sum_{j=1}^{n}\tau_j}{1 + k\sum_{j=1}^{n}\tau_j} \qquad j = 1,2,...n \tag{7.5}$$

A first-order rate coefficient was calculated by fitting the non-linear model developed in equation 7.3. Regressions were performed with SYSTAT® (SPSS Inc.; Chicago, IL). SYSTAT estimates the parameters using the Guassian iterations, which is similar to the linearization technique outlined by Draper and Smith (1981). Where appropriate SYSTAT allows the user to implement the Marquardt method. In the present research, the Marquardt method was primarily used to improve model convergence.

RESULTS

Bacterial Growth

The number of biofilm HPCs is shown for each AR under the four experimental conditions in Figure 7.2. Amino acids and chlorine had the greatest effect on biofilm HPCs in the first AR, which is in agreement with the results presented in Chapter 5. In the presence of chlorine the steady-state number of biofilm HPCs decreased by at least an order of magnitude in AR_1. The impact of either chlorine or amino acids on the number of biofilm HPCs was less in the downstream ARs.

An analysis of variance (ANOVA) was performed to quantify the effects of each factor on the number of steady-state biofilm HPCs in each AR. Rather than showing each ANOVA table, the p-values calculated for each factor are shown in Table 7.1. Both experimental factors and their interaction significantly affected the number of biofilm HPCs in AR_1. However, the fraction of amino acids did not influence the number of biofilm HPCs in any other reactor. Chlorine did not affect the number of biofilm HPCs in AR_2, but significantly affected the number of biofilm HPCs in AR_3. A similar trend was observed between chlorine as chlorine as a main effect and the interaction of chlorine and the amino acid fraction, suggesting that chlorine may have a greater impact on the number of biofilm HPCs than the amino acid fraction.

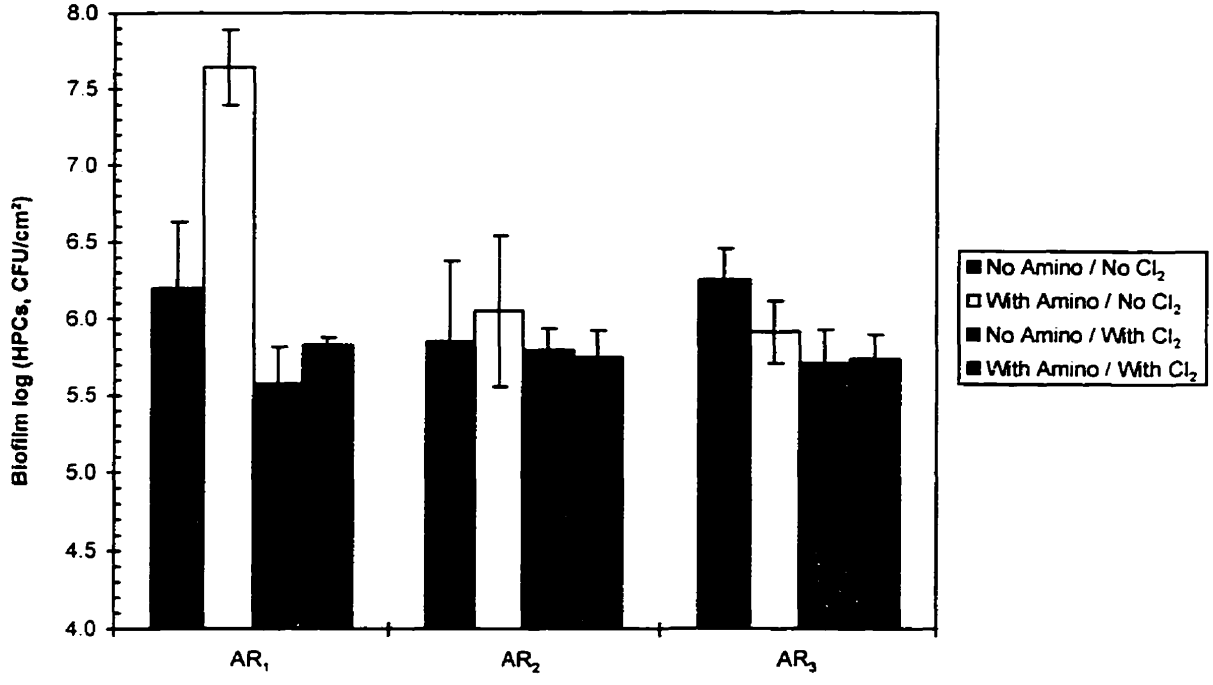

Figure 7.2: Changes in the number of biofilm HPCs through the model distribution system (error bars are 95 % confidence intervals).

Table 7.1: p values for the Number of Biofilm HPCs

Factor	Influent	AR_1	AR_2	AR_3
Amino fraction	-	< 0.0001	0.6799	0.1118
Chlorine	-	< 0.0001	0.3082	0.0009
Amino × Chlorine	-	0.0005	0.4696	0.0180

Essentially the same number of bulk HPCs was present in the influent to the model distribution system for all experimental conditions (Figure 7.3). In the absence of chlorine the number of bulk HPCs in the effluent of AR_1 increased by at least an order of magnitude and remained relatively constant through the remaining two ARs. In the presence of chlorine the number of bulk HPCs was reduced substantially in the first AR.

However, differences between the number of bulk HPCs in the effluent of AR_2 and AR_3 were relatively small regardless of the experimental factors.

The p-values from ANOVA tests for the number of bulk HPCs are presented in Table 7.2. In agreement with the observations made from Figure 7.3, chlorine affected the number of bulk HPCs in AR_1. The chlorine amino acid interaction also significantly impacted the number of bulk HPCs in AR_1. However, the two main effects did not significantly impact the number of bulk HPCs at any other sample location.

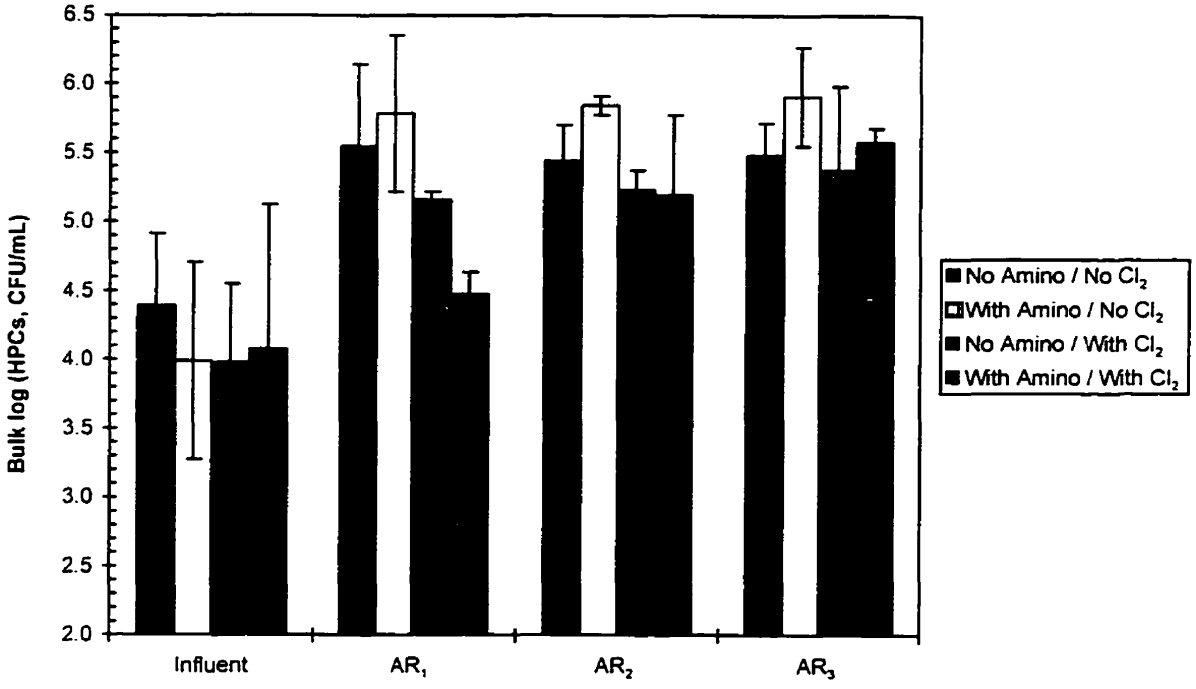

Figure 7.3: Changes in the number of bulk HPCs through the model distribution system (error bars are 95 % confidence intervals).

Table 7.2: p values for the Number of Bulk HPCs

Factor	Influent	AR_1	AR_2	AR_3
Amino fraction	0.4388	0.2679	0.3335	0.8490
Chlorine	0.4261	0.0010	0.1129	0.1457
Amino × Chlorine	0.0640	0.0352	0.4528	0.2220

Removal of BOM Components

The removal of BOM components was relatively similar regardless of whether chlorine was dosed into the model distribution system. The removal of carboxylic acids and aldehydes also appeared to be independent of the fraction of amino acids present in the influent stock.

Figure 7.4 shows the steady-state concentration of all BOM components in the influent to AR_1 and the effluent of the three ARs. The three carboxylic acids were steadily removed through the ARs. Formaldehyde is completely removed in AR_1, whereas glyoxal was marginally removed through the entire system (i.e., was essentially not removed). Most of the amino acids were utilized in the first AR, the free amino acid concentration in either AR_2 and AR_3 was less than 10 μg C/L.

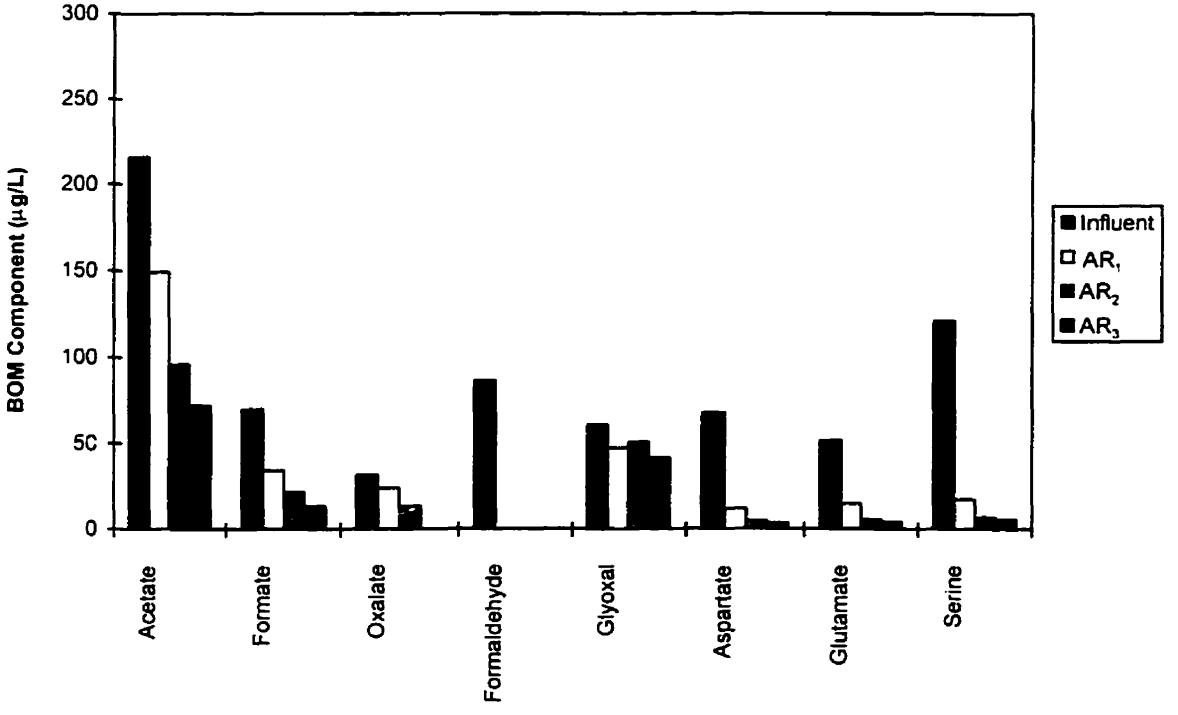

Figure 7.4: Removal of BOM components through the model distribution system (no chlorine in the influent).

An aggregate BOM term was calculated by summing all of the measured easily biodegradable components present in the model system on a mass carbon basis. The aggregate BOM was calculated for each experimental condition, as shown in Table 7.3. In general, the greatest BOM removal was achieved by the biofilm in the first AR. It is suspected that amino acids reacted with chlorine in the trial with chlorine and amino acids, which may contribute to the high BOM removal rate for this trial.

Table 7.3: Removal of Aggregate BOM through Model Distribution System

Experimental Condition		Steady-State BOM Concentration, μg C/L			
Amino Acid	Chlorine	Influent	AR_1	AR_2	AR_3
0.0	0.0	180 ± 33[a]	96 ± 22	63 ± 8	36 ± 14
0.34	0.0	220 ± 60	110 ± 6	75 ± 20	54 ± 22
0.0	0.2	200 ± 14	110 ± 13	52 ± 12	26 ± 8
0.34	0.2	190 ± 18	60 ± 8	54 ± 23	39 ± 18

[a] Average BOM concentration ± standard deviation.

The fractional removal through the model distribution system for each experimental trial was calculated. A first-order rate coefficient was estimated with equation 7.5. Figure 7.5 shows the fractional removal of BOM through the three ARs for the experimental trial that did not have amino acids, but was dosed with chlorine. Although the regression is based on only three data points (i.e., retention times of three ARs), the model shows in a general sense that BOM removal can be fitted to a first-order expression.

A summary of all of the relationships obtained for each experimental trial is given in Table 7.4. The rate coefficients ranged between 0.6 and 0.9 h^{-1} for all experimental conditions. A non-linear model could not be obtained for the experimental trial which

included both amino acids and chlorine because the amino acids likely reacted with the free chlorine. Confidence intervals (α = 0.05) for the first-order coefficient are also provided in Table 7.4. For one of the models the rate coefficient was not significantly different form zero, although the confidence region would likely be reduced with a greater number of data.

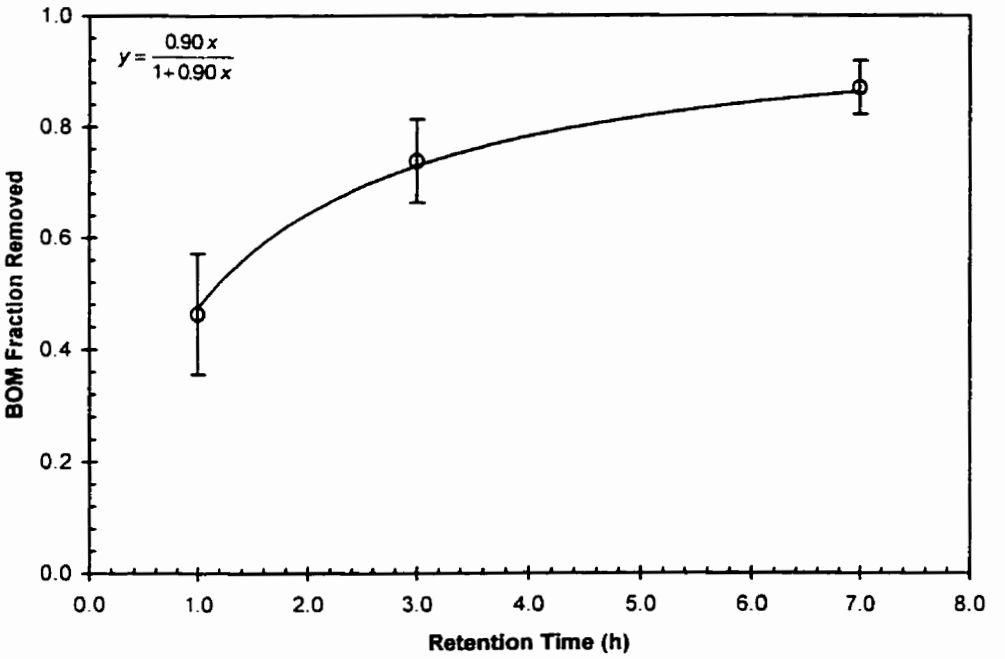

Figure 7.5: First-order removal of aggregate BOM through the model distribution system for the experimental trial that did not include amino acids in the BOM cocktail and was dosed with chlorine (standard deviations shown with error bars).

Table 7.4: Non-linear Models Calculated for the Four Experimental Conditions

Experimental Condition		Model	95 % Confidence Interval for k	
Amino Acids	Chlorine		Lower	Upper
0.0	0.0	$y=\frac{0.68x}{1+0.68x}$	0.30	1.1
0.34	0.0	$y=\frac{0.79x}{1+0.79x}$	-0.08	1.7
0.0	0.2	$y=\frac{0.90x}{1+0.90x}$	0.78	1.0
0.34	0.2	did not converge	-	-

Chlorine Residual and Chlorinated By-Products

The total chlorine residual concentration entering AR_1 averaged greater than 0.2 mg/L regardless of whether amino acids were present in the influent BOM cocktail (Table 7.5). The free chlorine residual in the influent of AR_1 for trials with amino acids and for trials without amino acids was 0.08 and 0.22 mg/L, respectively. The free chlorine residual was essentially zero in the effluent of AR_1 regardless of the presence or absence of amino acids. However, the total chlorine residual concentration was approximately 0.10 mg/L (Table 7.5). Under steady-state conditions, detectable amounts of total chlorine were present in AR_2, but AR_3 rarely had a total chlorine residual.

The concentration of total trihalomethanes (TTHMs) in the model system is provided in Table 7.5. In general chloroform was the most predominant THM constituent throughout the system, responsible for greater than 90 % of TTHM concentration (molar basis).

Table 7.5: Residual Chlorine and Trihalomethanes in the Model Distribution System

	Influent	AR_1	AR_2	AR_3
Absence of Amino Acids				
Total Chlorine, mg/L	0.22	0.07	0.05	0.00
TTHMs, μg $CHCl_3$/L	12.1	12.4	7.8	7.7
Presence of Amino Acids				
Total Chlorine, mg/L	0.24	0.09	0.05	0.00
TTHMs, μg $CHCl_3$/L	15.1	11.3	10.2	9.9

It had been intended that the influent free chlorine residual to AR_1 be 0.2 mg/L in all trials. As discussed above, this was the case for the experimental trial without amino acids, however this was not achieved for the trial that included amino acids.

The chlorine demand of the three amino acids studied in the present research, as measured by Hureiki *et al* (1994), was used to calculate the chlorine consumed by each amino acid (Table 7.6). In Table 7.6, the calculated chlorine consumed was obtained by multiplying the average amino acid concentration (in mg C/L) by the chlorine demand (in mg/mg C). The total chlorine consumed by the three amino acids was calculated as 0.23 mg/L. However, the difference between total and free chlorine in the influent was 0.16 mg/L, which is 70% of the calculated chlorine consumption. This is reasonable considering that the coefficient of variation for the amino acid concentration for this experimental trial was 30%.

The reaction pathway for amino acids with one amine group and chlorine, as described Hureiki *et al.* (1994), shows that 2 mol of chlorine are consumed in the first step to form organic monochloramine and dichloramine. Further reactions lead to the formation of nitriles and aldehydes. The formation of odorous aldehydes after

chlorination of amino acids was demonstrated by Bruchet *et al.* (1992). In the present research, an increase in the concentration of formaldehyde and glyoxal was not observed for the trials with amino acids and chlorine. A detectable concentration of other short-chained aldehydes (e.g., acetaldehyde) was also not observed. However, it is possible that the aldehydes produced by this reaction may have either undergone further oxidation or were consumed by the biofilm.

Table 7.6: Calculated Chlorine Consumption of Free Amino Acids

Amino Acid	Cl_2 Demand[†]		Amino Acid Concentration		Cl_2 Consumed
	mol/mol	mg/mg C	mg/L	mg C/L	mg/L
Aspartate	5.3	10.4	0.025	0.0091	0.095
Glutamate	5.5	8.1	0.026	0.011	0.086
Serine	2.4	2.8	0.056	0.017	0.049

[†] Measured by Hureiki *et al.* (1994).

Formation of ROM

The UV absorbance measured at 285 nm is shown for different locations in the system in Figure 7.7. The UV_{285} in the influent was greater for the two trials that did not have amino acids present in the influent BOM cocktail. The difference in UV absorbance is likely due to a slight change in the background water matrix rather than the presence of amino acids. The amino acid trials were performed in the late winter months, whereas the non-amino acid trials were performed in the early spring. Regardless of the influent conditions, absorbance under steady-state conditions was relatively similar in AR_1. In general, for each experimental trial the UV_{285} gradually increased from the influent of AR_1 to the effluent of AR_3 (Figure 7.6)

Similar to the HPC counts, the effect of each experimental factor on UV_{285} was quantified with an ANOVA test (Table 7.7). The influent UV_{285} was significantly impacted by the presence of amino acids. Chlorine also impacted the influent UV_{285}. Amino acids had a significant effect on UV_{285} in AR_3, however, the ANOVA was primarily influenced by an unexpected low value for one experimental trial. The main effects did not influence the absorbance in AR_1 and AR_2, however, the amino/chlorine interaction significantly affected the UV absorbance in AR_2.

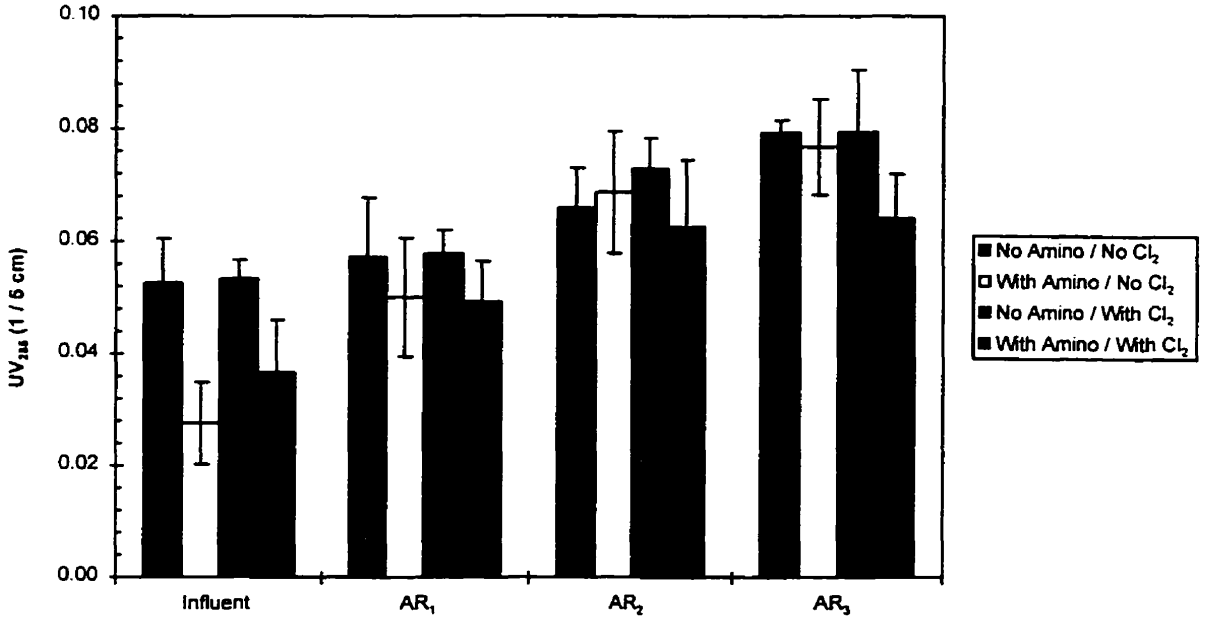

Figure 7.6: Evolution of increased UV_{285} absorbance through the model distribution system (error bars are 95 % confidence intervals).

Table 7.7: p values for the Absorbance at UV_{285}

Factor	Influent	AR_1	AR_2	AR_3
Amino fraction	0.0017	0.1840	0.1544	0.0458
Chlorine	0.0303	0.5893	0.5267	0.2858
Amino × Chlorine	0.0122	0.5022	0.0145	0.2768

Paired t-tests were performed on all of the UV_{285} data for each reactor. The absorbance was significantly greater in AR_2 than AR_1 (p value < 0.0001). Similarly, the absorbance was significantly greater in AR_3 than AR_2 (p value = 0.0012). No statistical difference in the concentration of NPOC was observed through the model distribution system. NPOC was also independent of the experimental factors having a typical concentration of 1.1 mg/L with a coefficient of variation of 20 % through the system.

Although THMs themselves decreased from AR_1 to AR_3, trihalomethane formation potential (THMFP) generally followed a similar trend as the UV_{285} (Figure 7.7). The concentration of THMFP increased through the model distribution system. Chlorine had a significant effect on the THMFP concentration for the samples collected in the effluent of AR_1 and AR_2. However, the difference in THMFP could have been due to the chlorination by-products that were present in the chlorinated reactors, and not the residual chlorine concentration in the reactor. For all THMFP measurements the chlorine was dosed such that a free chlorine residual of 1.0 ● 0.4 mg/L was obtained after a 24 h incubation period (Summers *et al.*, 1996). The presence of amino acids did not affect the THMFP concentration in the effluent of any of the reactors. A paired t-test on all of the reactor effluent data showed that the increases in THMFP from AR_1 to AR_2 and from AR_2 to AR_3 were statistically significant at 5 % level.

Values taken from the effluent of AR_1 to AR_3 for all experimental trials were used to generate a linear model between THMFP and UV_{285} (Figure 7.8). The influent values were not used to form the model because of the variability THMFP and UV_{285} exhibited for the different influent conditions. The slope of the model was significant at the 5 % level and no systemic trends were observed for the model residuals.

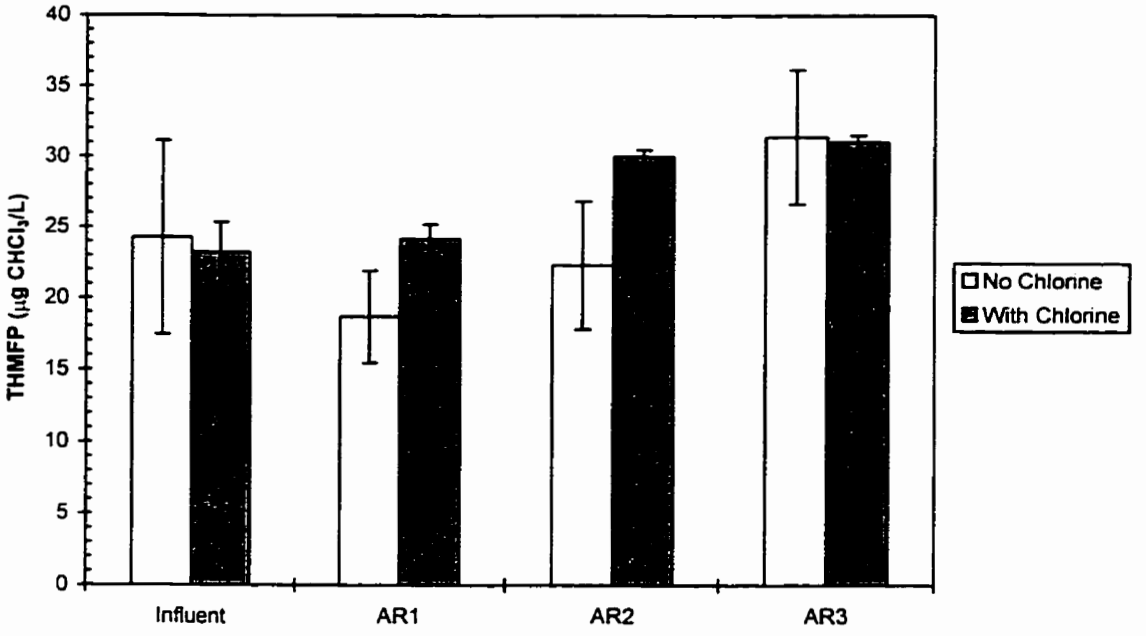

Figure 7.7: A comparison of THMFP values through the model distribution system for experimental trials which did not have amino acids in the influent BOM cocktail (error bars are 95 % confidence intervals).

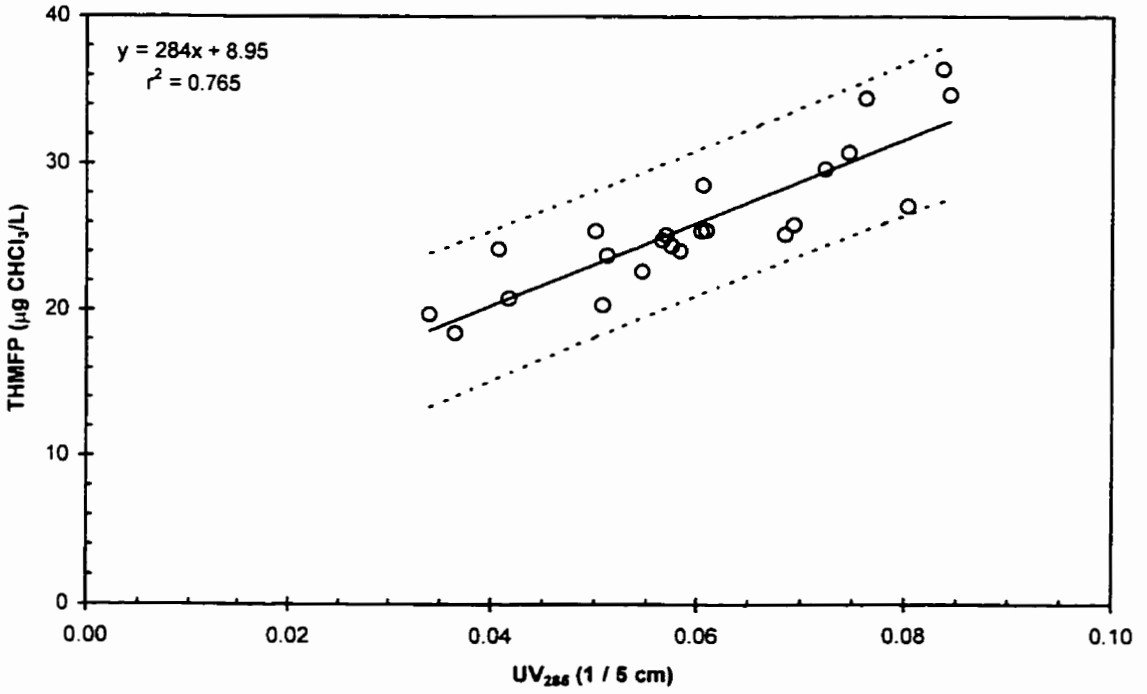

Figure 7.8: Regression between THMFP and UV_{285} for all AR effluent data.

Modeling SMP Formation

Namkung and Rittmann (1986) modeled SMP formation as a two part process which can be expressed by:

$$P = \left(k_1 YJ + k_2 X_f L_f\right) \tau \, a \qquad (1)$$

where, P is the amount of SMP formed ($M_S L^{-3}$)
k_1 is the utilization-associated SMP formation constant ($M_S M_X^{-1}$)
Y is the yield coefficient ($M_X M_S^{-1}$)
k_2 is the biomass-associated SMP formation constant ($M_S M_X^{-1} T^{-1}$)

In words, equation (1) shows that SMPs are the result of formation from utilized-associated products (UAP) and/or the formation from biomass-associated products (BAP).

Using UV_{285} as a surrogate for SMPs, regressions involving the influence of BOM removal rate (UAP) and the geometric mean number of biofilm HPCs (BAP) on SMPs formed were performed. For these regressions the removal of SMPs was assumed negligible. The regression between BOM removal rate through the model distribution system and the normalized amount of ROM was significant (p value = 0.0019), with a coefficient of determination of 0.635 (Figure 7.9). BOM removal rate was calculated as the difference between influent and effluent BOM (mass carbon) divided by the reactor retention time. The formation rate of ROM was calculated by dividing the measured UV absorbance at 285 nm (measured in a 5 cm cell) by the AR retention time. The correlation between the number of biofilm HPCs and the UV_{285} absorbance was not significant at the 5 % level.

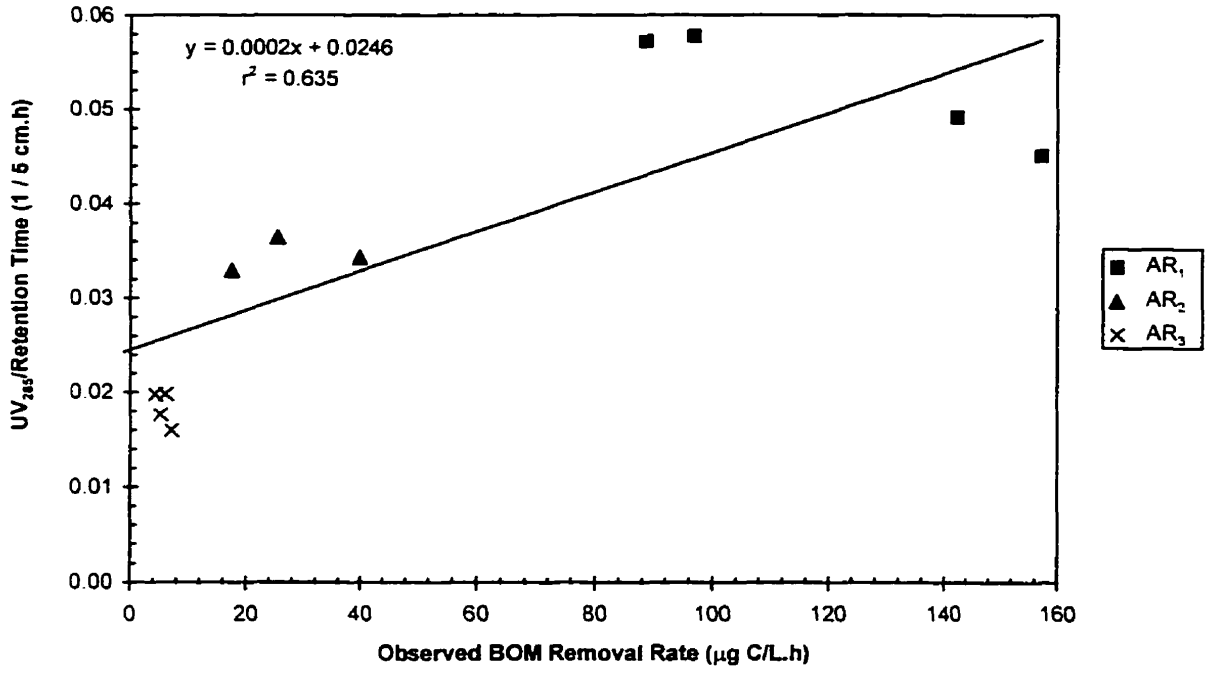

Figure 7.9: Formation of utilization-associated SMPs.

DISCUSSION

The presence of amino acids and chlorine had an important role in the accumulation of biofilm HPCs in AR_1, however their impact on the number of biofilm HPCs in subsequent reactors was limited. It is not surprising that free amino acids had a marginal effect on biofilm accumulation in AR_2 and AR_3, as greater than 80 % of the free amino acids were removed by the biofilm in AR_1 (mass basis). In agreement with the results presented in Chapter 5, the presence of amino acids substantially increased the number of biofilm HPCs in AR_1.

The effect of amino acids on biofilm accumulation in AR_1 was minimized in the presence of chlorine residual (Figure 7.2). It is unlikely that the decreased number of biofilm HPCs was solely due to disinfection. It is considered that reactions with free

chlorine and amino acids played a role in reducing the number of biofilm HPCs as the amount of available substrate was likely reduced.

The calculated chlorine demand for aspartate, glutamate, and serine (Table 7.6) shows that it is possible that the amino acids completely reacted with the available chlorine. Thus, the actual BOM concentration available for utilization was approximately 150 μg C/L, which corresponds to a BOM flux of 0.83 mg C/m^2.h in AR_1. In comparison, trial 5.3 (Table 5.2) had no amino acids and no chlorine in the influent. The BOM flux for trial 5.3 was 0.55 mg C/m^2.h. The number of biofilm HPCs for trial 5.3 and for AR_1 in the trial with amino acids and chlorine in the present chapter was 8.0×10^5 and 6.7×10^5 CFU/cm^2, respectively. Therefore, for the trial with amino acids, chlorine had a minimal disinfection capacity in AR_1. Chlorine primarily reacted with the free amino acids, thereby reducing the amount of available substrate. The reduction in amino acids available for utilization resulted in a decreased the number of biofilm HPCs (Figure 7.2). These results would concur with the results presented in Chapter 5.

Chlorine significantly reduced the number of bulk and biofilm HPCs in AR_1. However, the number of bulk and biofilm HPCs in AR_3 recovered to values similar to those runs that did not have chlorine. The extent to which bacterial regrowth is reduced may be overestimated with HPC numbers (Saby *et al.*, 1997) because cells injured through disinfection may not be isolated on R2A agar. However, the results of the present research concur with experimental findings reported elsewhere (LeChevallier *et al.*, 1990; van der Wende *et al.*, 1989). Similarly, the recovery of HPCs in AR_3 are in general agreement with modeling predictions by SANCHO, which suggest that chlorination

delays bacterial regrowth to sections further downstream in the distribution system (Laurent *et al.*, 1997).

BOM removal through the model distribution system was modeled with a first-order rate expression. The first-order rate coefficient was highest for trials that were dosed with chlorine. The BOM components may have reacted with chlorine increasing the overall removal of BOM. Thus, distribution systems having higher BOM concentrations could be more vulnerable to regrowth because an increased amount of available nutrients and the chlorine demand of BOM reduce the disinfection efficacy of chlorine.

Removal of BOM components through the model distribution system followed similar trends to the quantitative analysis performed in Chapter 6. Acetate was more slowly removed than formate. Formaldehyde was completely removed and glyoxal was poorly removed. Amino acids were easily removed and enhanced the number of biofilm HPCs (Figure 7.2). Also in agreement with data presented in Chapter 6, a first-order expression was able to define the removal of BOM through the model distribution system. Further discussion regarding the impact of amino acids on biofilm accumulation and the removal rates of these specific compounds is presented in Chapters 5 and 6, respectively.

The concentration of total trihalomethanes (TTHMs) was found to decrease through the model distribution system, being about 30 % lower in the effluent of AR_3 than in the influent to AR_1. This contrasts with results for the simulated distribution system (SDS) test, where the concentration of THMs has been shown to increase with increasing incubation time (Summers *et al.*, 1996; Koch *et al.*, 1991). In a SDS test

chlorine is typically dosed at higher concentrations or dosed to achieve a target residual concentration (e.g., 1.0 mg/L after 24 h). The model distribution system in this study was dosed at an influent free chlorine concentration of 0.2 mg/L and the total chlorine residual in the final AR was essentially 0.0 mg/L (Table 7.2). THMs were formed in the influent to AR_1 and were removed through the system. The removal of THMs has occurred by consumption within the biofilm, although further experimentation would be necessary to confirm these reactions. Previous studies have found that chloroform was not biologically removed as a secondary substrate by aerobic biofilms (Bouwer and McCarty, 1985).

Refractory organic matter (ROM), as measured by UV_{285}, was found to increase through the model distribution system (Figure 7.6). The UV absorbance in the effluent of each annular reactor was statistically independent for the all experimental trials, therefore the effluent absorbance was independent of the influent conditions. This would suggest that the ROM present was related to microbiological processes occurring within each individual AR.

UV absorbance at 285 nm was found to have a reasonable correlation with the amount of trihalomethane formation potential (Figure 7.8). This was anticipated as treated waters measured at UV_{254} were found to be a good indicator of trihalomethane formation potential (Najm *et al.*, 1994; Edzwald *et al.*, 1985).

A linear model developed by Namkung and Rittmann (1986) was used to understand the mechanisms associated with the formation of SMPs. In Figure 7.9 a correlation was found between the amount of normalized ROM and the observable BOM removal rate, indicating that utilized-associated products may be important to the SMPs measured in the model distribution system. However, biologically-associated products

(BAP) were not found to be a significant component of the SMPs measured. The apparent lack of fit for BAP formation could be due to either a low amount of biomass or because of the method for determining cell density (i.e., number of biofilm HPCs).

It is recognized that the SMP model developed by Namkung and Rittmann (1986) was developed for a single continuously stirred tank reactor (CSTR). The data used for the regression shown in Figure 7.9 were collected from 3 CSTRs in series. To obtain a linear model, the measured absorbance from each AR was assumed to be statistically independent. Consequently, Figure 7.9 should be regarded as an empirical fit rather than a mechanistic evaluation as presented by Namkung and Rittmann (1986).

CONCLUSIONS

This chapter reported investigations with a model distribution system consisting of three annular reactors in series. The experimental design for this investigation was a 2^2 factorial design, the two factors were chlorine and amino acids. The results showed that, in the absence of chlorine, amino acids increased the number of biofilm HPCs in the AR_1. Chlorine reduced the number of biofilm HPCs in AR_1, although chlorine reacted with the amino acids when they were present in the BOM cocktail. The number of biofilm HPCs in the subsequent ARs appeared to be independent of the influent conditions.

BOM removal followed a first-order removal process through the model distribution system. The highest removal was observed in AR_1. Approximately 20 % of the original BOM remained in the effluent AR_3, of which acetate was the primary BOM component. Similar to Chapter 6 the free amino acids and formaldehyde had the highest per cent removals, whereas glyoxal had the lowest per cent removal.

The amount of refractory organic matter (ROM) increased through the distribution system and was independent of influent conditions. A relationship between microbial activity and ROM likely exists, however the reaction kinetics of ROM was not established. ROM as measured by UV_{285}, was linearly related to the amount of THMs formed through chlorination. The practical implication of this relationship is that the ROM produced by bacterial activity may also impact upon chlorination strategies within distribution systems.

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CHAPTER 8: AN APPROACH TO RELATE MODEL REACTORS TO FULL-SCALE DISTRIBUTION SYSTEMS

INTRODUCTION

The water industry has become increasingly more aware of the deleterious effects distribution systems can have on water quality. Consequently, numerous model reactors have been designed to predict changes in water quality during distribution. Model reactors are typically employed so that researchers can control operational (e.g., flow rate) and environmental (e.g., temperature) parameters common to distribution systems, which is typically not possible in most full-scale situations. To that end model reactors have been used for examining the effect of corrosion inhibitors (Rompré *et al.*, 1996), evaluating biocide (e.g., chlorine) efficacy (Camper, 1996), and assessing biofilm accumulation/bacterial regrowth for various NOM sources (Butterfield *et al.*, 1996).

The objectives of this chapter are: 1) to present and critically assess model reactors available for evaluating biofilm processes under conditions relevant to drinking

water distribution systems, and 2) to present a simple modeling approach which can be used to apply results obtained from model reactors to full-scale situations.

MODEL REACTORS

Several reactor designs have been developed to experimentally model microbial processes in a distribution system. Although chemical reactions such as trihalomethane formation in a distribution system can be approximated with a simulated distribution system (SDS) test (Eaton *et al.*, 1995), this test is limited in other capacities, such as hydrodynamics, facilitating and evaluating heterotrophic biofilm growth. Reactors more useful for evaluating substrate kinetics and other microbial processes in a distribution system include: biofilm column reactors, annular reactors, and pipe loop systems.

Biofilm Column

Although not designed specifically for distribution systems, the biofilm columns have been used to evaluate kinetics for the removal of xenobiotics (Rittmann *et al.*, 1986) and have operated under drinking water conditions to determine kinetics of total organic carbon (TOC) removal (Wang, 1995) and assimilable organic carbon (AOC) removal (Zhang and Huck 1996). The biofilm column typically consists of a glass column, 25 mm in diameter and 100 - 200 mm in length, packed tightly with inert media (e.g., glass beads). The columns are inoculated with bacteria indigenous to a water treatment environment and fed a controlled source of nutrients at a low influent flow rate (Q). The columns operate in an upflow mode with a portion of the reactor effluent being recycled. The recycle flow rate (Q_r) is high such that the system essentially behaves as a

continuously stirred tank reactor (CSTR) with a Q/Q_r ratio of approximately 20-25 (Rittmann *et al.*, 1986). This CSTR behaviour facilitates estimation of kinetic parameters.

The biofilm column has been subsequently modified by van der Kooij *et al.* (1995) such that *in situ* sampling of biofilm is available. The column designed by van der Kooij *et al.* consists of a column (length 60 cm, diameter 2.5 cm) containing about 40 cylinders of glass (or Teflon) placed vertically adjacent to each other. Each cylinder has length of 1.5 cm and a diameter of 1.8 cm, for a total exposed surface area of 17.4 cm^2. Water flows downward through the column contacting the inner and outer surface of the cylinders. The superficial velocities typically used in these reactors are similar to velocities of distribution systems in the Netherlands (e.g., 0.2 m/s).

Biofilm is aseptically sampled by removing the top cylinder (without replacement) during an experiment and placing the cylinder in an aliquot of sterile water for subsequent sonication. Because of the high flow rates it is assumed that the reactor is well mixed. However, it is likely that vertical gradients would exist, both in terms of biomass and biodegradable organic matter (BOM), as the column is essentially operated as a plug flow reactor (PFR).

Annular Reactor

Annular reactors (ARs) are completely mixed reactors which allow for *in situ* sampling and analysis of accumulated biofilm. Two commonly used ARs in the water industry are Propella™ (Parent *et al.*, 1996) and models developed by BioSurface Technologies Corporation (Bozeman, MT). Because a greater database exists for the ARs manufactured by BioSurface Technologies Corp., this discussion will focus on these

reactors. Both ARs are similar in principle in that water flows through an annular gap which is mixed by an inner rotating drum. Shear stress is controlled by the rotational speed of the inner drum and hydraulic retention time (HRT) is controlled independently by the volumetric flow rate. Biofilm accumulates on removable coupons which are flush mounted to the reactor surface.

In most bench-scale experiments ARs are assumed to approximate a finite section of a distribution system. In the lab, a common set-up for drinking water experiments consists of pumping tap water, which may have been treated to remove background chlorine and organic material, into an AR. Additional nutrients, disinfectants, and/or corrosion inhibitors are also pumped into the AR depending on the experimental objectives (Butterfield *et al.*, 1996; Rompré *et al.*, 1996). For evaluating biological stability during treatment, ARs would receive the effluent of a specific process (Baribeau *et al.*, 1996).

Pipe Loop

Although annular reactors can provide the shear forces present in a distribution system, the amount of pressure applied to the system is limited. Industrial pilot-scale distribution systems, or pipe loops are frequently used to facilitate this requirement. The two basic pipe loop designs are recirculating and flow-through. Similar to the annular reactors, recirculating pipe loops allow the researcher to assume uniform conditions throughout the reactor. This is a significant advantage for examining growth-promoting conditions in distribution systems. Flow-through pipe loops do not possess this quality. However, pipe loops are often constructed as flow-through systems because most

distribution systems are of this nature. Research associated with evaluating corrosion diagnostics has frequently employed flow-through pipe loops (Levin and Schock, 1991). Camper (1995) used recirculating pipe loops to investigate environmental (e.g., temperature) and operational (e.g., chlorine residual, hydraulic retention time) factors influencing coliform regrowth (Table 8.1). Recirculating pipe loops have also been used for monitoring biofilm accumulation under drinking water conditions at Lyonnaise des Eaux (Piriou and Lévi, 1994) and at the International Water Research Center at Nancy, France (Mathieu *et al.*, 1993). The American Water Works Service Company (AWWSCo) constructed a flow-through pipe loop to examine biofilm growth in relation to the type of piping material and concentration of disinfectant (LeChevallier *et al.*, 1990).

Table 8.1: Comparison of Pilot-Scale Distribution Systems

Pipe Loop Property	Montana State University	AWWSCo	Lyonaise des Eaux	Nancy
Loop configuration	CSTR	PFR	CSTR	CSTR
Pipe Material	mild steel	iron, galvanized steel, copper, PVC	cast iron	cement lined cast iron
Pipe Diameter	100 mm	12 mm	100-150 mm	100 mm
Number of Loops	5	1	3	3
Loop Length	12 m	22 m	15 and 20 m	31 m
Total System Length	60 m	22 m	50 m	93 m

Summary of Available Reactors

The three different reactor designs presented in this chapter are the biofilm column, annular reactor, and pipe loop (Table 8.2). The biofilm column has the advantage of being relatively inexpensive and easy to operate. The major difficulties with these types of reactors are: it is difficult to sample biofilm without disrupting an experimental trial, gradients in substrate may exist in the direction of flow (although this is generally minimal for the recirculating column design), and it is difficult to relate these reactors to a distribution system on a hydraulic basis.

Biofilm sampling in ARs can be performed easily and aseptically. ARs are reasonably priced, approximately $5000 (US). The major advantage of an AR is that shear stress can be controlled independently of HRT, thus the hydraulic conditions can be well defined.

Recirculating pipe loops offer the greatest flexibility, in that the designs are manufactured to fulfill a specific need by the researcher. Consequently, the capital costs are high and operational costs may also be high, covering electrical, water and operator expenses (Camper, 1995). Nevertheless, pipe loops can offer the most realistic snapshot of a full-scale distribution system.

Table 8.2: Comparison of Biofilm Reactors Available for Distribution Systems

Reactor	Biofilm Sampling	Mixing	Cost (Operational and Capital)	Relationship to Full-Scale
Biofilm Column	not convenient	poor; possible vertical gradients	minimal	hydraulically difficult to define
Annular Reactor	convenient	CSTR	intermediate	shear stress can be controlled
Pipe Loop	difficult, but possible	CSTR or PFR	expensive	Similar pressures and specific surface area; once though allows for plug flow behaviour

MODELING APPROACH

The most direct way to interpret data obtained from bench-scale experiments is to compare the hydraulic retention time (HRT) set in a bench-scale experiment with an equivalent length of pipe. A calculated shear stress which corresponds to the preset rotational speed on the AR can be related to the shear stresses observed for water flowing with a specific velocity for a given diameter. This direct approach is straightforward, however it could lead to erroneous predictions regarding the full-scale system.

Another parameter which could also be used is the BOM flux (J). This parameter can be readily calculated from most bench-scale experiments. Under steady-state conditions and assuming negligible microbial growth in the bulk fluid, the BOM flux is given by:

$$J = \frac{(S_i - S_b)}{\tau \cdot a} \tag{8.1}$$

BOM flux is a convenient parameter because it is normalized by the dilution rate and the reactor specific surface area. The surface area available differs for ARs and pipes of various diameters. BOM flux is also convenient because it does not require prior knowledge of specific parameters related to BOM utilization, relying solely on the observed amount of BOM removed through the AR.

By using substrate flux as a scale-up parameter, it is assumed that the rate of substrate utilization in an AR is approximately similar to an equivalent length of pipe. Because several CSTRs in series can approximate a PFR a general expression for BOM flux in a pipe (J_p) can be written as:

$$J_p = \frac{S_i - S_{b,n}}{2\sum_{i=1}^{n}\left(\frac{L}{v \cdot D}\right)_i} \qquad i = 1, 2, \ldots n \qquad (8.2)$$

where, L is the equivalent length of pipe for the i^{th} reactor in series, L
v is the linear velocity for the i^{th} reactor in series, $L\ T^{-1}$
D is the pipe diameter for the i^{th} reactor in series, L

Equation (8.2) is a general expression, which allows an AR to be put into the context of a full-scale system. It assumes that the bulk concentration of the n^{th} reactor in series is equal to the measured bulk concentration in the AR. The numerator of equation (8.2) is determined by the biological stability of a given treated water and the denominator can be specific to the physical characteristics of the pipe network.

Case Studies

Several case studies will be used to demonstrate how BOM flux can be used as an indicator to scale-up AR experiments to an equivalent length of pipe. For convenience, a 100 mm (4 in.) diameter pipe is chosen for this chapter. The specific case studies are:

- two experimental trials from Chapter 5,
- experiments conducted at the Metropolitan Water District of Southern California (MWD) by Baribeau *et al.* (1996), and
- an investigation which compared the reactor performance of an AR to a 100 mm (4 in.) diameter pipe loop (Camper, 1996).

The two laboratory experiments, trial 8.1 and trial 8.2, had measured BOM influent concentrations of 300 μg C/L and 45 μg C/L. These experimental trials corresponded to trial 5.7 and trial 5.5 (Table 5.2). The specific objectives and details are discussed in greater detail in Chapter 5. BOM concentrations were calculated by adding the concentrations of individual compounds (e.g., formaldehyde, formate) on a mass carbon basis. ARs in both experimental trials had hydraulic retention times of 2 h. The BOM flux for trial 8.1 and 8.2 were 520 μg $C/m^2 \cdot h$ and 63 μg $C/m^2 \cdot h$, respectively. The influence of BOM flux on the number of heterotrophic bacteria, as measured by heterotrophic plate counts (HPCs), is shown in Figure 8.1. Experiments with higher BOM loading rates (trial 8.1) had greater bulk HPC numbers, however, the net log difference (bulk or effluent less influent) was essentially the same for both trials (Figure 8.1). Biofilm HPC numbers in trial 8.1 were an order of magnitude greater than trial 8.2.

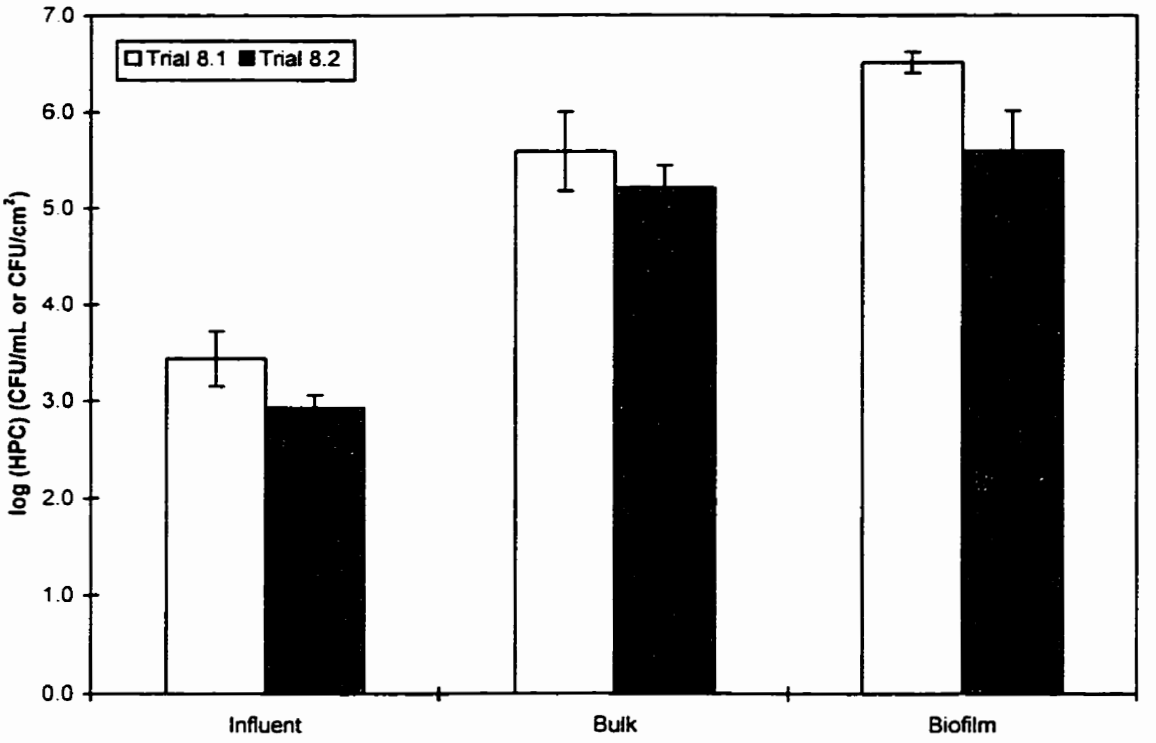

Figure 8.1: Steady-state results from two laboratory trials (standard deviations are shown with error bars).

In order to relate this bench-scale result to a full-scale application, a graphical approach is proposed (Figure 8.2). In Figure 8.2, BOM flux is plotted as a function of pipe length, assuming perfect mixing (axial and longitudinal) in a given pipe length, no consumption in the bulk phase, and no generation of substrate. Although treating a length of pipe as a CSTR represents a considerable simplification, it provides a point of departure for this analysis. Substrate flux was calculated based on a 100 mm (4 in.) pipe having a water velocity of 0.3 m/s (1 fps). These conditions were approximately similar to the shear conditions of the described experiments.

Inferences regarding a full-scale distribution system can be established using the experimental data presented and the curve in Figure 8.2. Considering the BOM flux for trial 1 and a hypothetical distribution system which consists of a pipe with a diameter of

100 mm (4 in.) some approximate relationships can be realized. For a treated water which has an influent BOM concentration of 300 μg C/L (component basis), and a BOM flux of 520 μg C/m^2.h (e.g., experimental trial 8.1), the following modeling approach is proposed.

1. Using Figure 8.2, find the BOM flux (e.g., 520 μg C/m^2·h) from a specific bench-scale experiment.
2. Locate the removal contour which corresponds to the amount of BOM removed through an AR (e.g., 280 μg C/L) during an experiment.
3. Find the point of intersection for the removal contour and observed removal rate and calculate the corresponding 100 mm (4 in.) pipe distance (e.g., 15 km)
4. Estimate the resulting pipe retention time for a 100 mm pipe (e.g., 13.7 h).

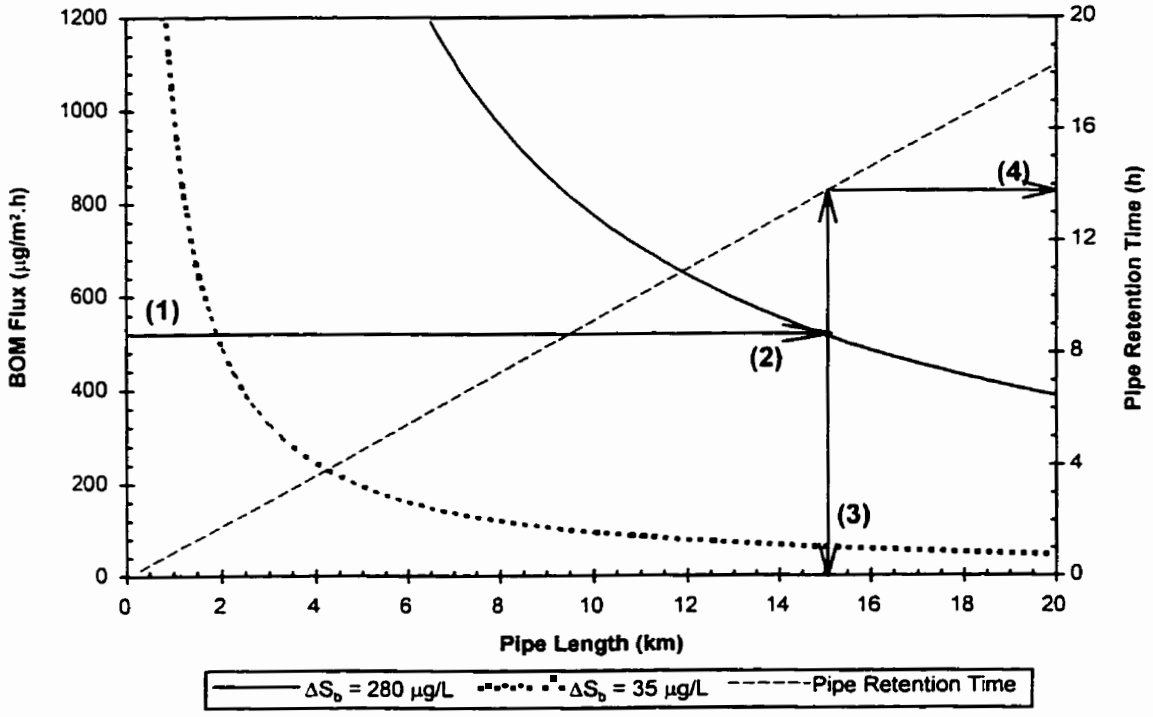

Figure 8.2: Empirical approach to relating AR experiments to an equivalent length of 100 mm (4 in.) pipe with a velocity of 0.3 m/s (1 fps).

For experimental trial 8.2 (BOM flux of 63 μg C/m^2·h) the proposed modeling approach gives a similar pipe distance and retention time of 15 km and 13.7 h,

respectively. In both cases the predicted equivalent pipe retention time was 6.8 times greater than the HRT used for the AR experiment. The same approach could be made more rigorous (and complicated) by assuming PFR behaviour for a pipe section. An approximation of this, obtained by treating a length of pipe as several CSTRs in series, gave generally similar results. For trial 8.2, if three CSTRs were placed in series the total HRT is 13.7 h, however, the HRT of each reactor is approximately 4.6 h or one third of the total HRT.

Regrowth investigations conducted in the field at the MWD demonstration plant in La Verne, California (Baribeau *et al.*, 1996) are used for trial 8.3. Baribeau *et al.* (1996) evaluated the biological stability of water filtered through anthracite/sand and GAC/sand by connecting an AR to the effluent of each filter. The HRT in each AR was 12 h. The influent BOM concentration, as measured by assimilable organic carbon (AOC), was approximately 100 μg/L (Coffey *et al.*, 1996). The effluent AOC concentration was not provided, therefore it was assumed to be 25 μg/L for the purposes of this paper. Consequently, the observed AOC removal rate under these conditions is 25 $\mu g/m^2.h$, which for a ΔBOM of 75 μg/L corresponds to approximately 90 km of 100 mm pipe. The calculated HRT for a 100 mm pipe at 0.1 m/s was 82 h (greater than 3 d), which is 6.8 times greater than the HRT used for the AR (i.e., it is the ratio of specific surface areas for an AR and 100 mm pipe). If a different effluent BOM concentration (e.g., 50 μg/L) were assumed the corresponding equivalent pipe retention time and distance would still be 82 h and 90 km, respectively.

Camper (1996) compared the reactor performance of a 100 mm pipe to an AR. It was observed that the number of biofilm HPCs were approximately 10 times greater in

the AR than in the pipe loop (100 mm diameter). In keeping with the proposed modeling approach, the difference in HPC numbers could be explained by a larger specific surface area in the AR (270 m^{-1}) than in the pipe loop (40 m^{-1}). The resulting specific surface ratio for the AR to pipe loop is approximately 7 to 1.

Table 8.3 compares the surface area to volume ratio of an AR and the ratios for a specific diameter of pipe. For each of the described experiments the calculated 100 mm pipe retention time was greater than the specified HRT in the AR by a factor of 6.8. The specific surface area ratio between an AR and a 100 mm pipe is 6.7. Thus, it is proposed that the retention time from an AR is multiplied by the specific surface area ratio of an AR and a specific diameter of pipe to obtain an approximate equivalent pipe retention time (Table 8.3).

Table 8.3: Comparison of the Specific Surface Areas for ARs and Pipes of Various Diameters

	Pipe Diameter (mm)			
	25	100	300	600
Pipe Specific Surface Area, m^{-1}	160	40	13	6.7
AR:Pipe Specific Area Ratio	1.7	6.7	20	40

Although simple, the described approach is intended to make bench-scale testing with ARs more useful for utilities. Both small and large utilities would be able to operate affordable bench-scale tests under different plant conditions and relate the results qualitatively or semi-quantitatively to different sections of their distribution system.

CONCLUSIONS

An evaluation of several reactors available for evaluating biofilm growth in distribution systems was presented. Biofilm column reactors were shown to be useful for predicting *in situ* bio-kinetic parameters, but are not ideal reactors hydraulically. Annular reactors were also discussed, and several experiments employing ARs were presented. Pipe loops may be ideal from a practical sense, however, the capital and operating costs associated with these greatly exceed the costs associated with an AR.

Results from two laboratory experiments were presented. It was found that the net difference between the bulk HPCs numbers was essentially the same for low and high substrate loading rates, whereas the biofilm HPC differed by an order of magnitude. Consequently, the BOM flux was understood to be a result of substrate utilization by the biofilm.

A modeling approach was developed for relating retention times in an AR to retention times of an equivalent length of pipe. By using observed BOM fluxes from several experiments, it was shown that the ratio of pipe to AR retention time was 6.8, which is similar to the AR to pipe specific surface area ratio for the 100 mm pipe diameter assumed. Thus multiplying the AR retention time by the specific surface area ratio is proposed as a rule of thumb for relating bench-scale experiments to full-scale situations.

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CHAPTER 9: CONCLUSIONS AND RECOMMENDATIONS

This investigation used annular reactors (ARs) at bench-scale to study the biochemical processes of treated waters during distribution. Maintaining a long-term objective of producing biologically stable water, the research takes a fundamental approach to understanding problems which are of practical relevance to the water industry.

CONCLUSIONS

Prior to investigating factors which contribute to biological instability, several methods for removing (e.g., swabbing, scraping, stomaching) and re-suspending (e.g., sonication, tissue blending, stomaching) biofilm cells from a polycarbonate surface were examined. The method for detaching cells from the coupon was found to be of greater importance than the re-suspension technique. The stomacher was selected as the ideal choice because it can remove cells and re-suspend cells in a single step minimizing

excessive sample preparation and maintaining aseptic conditions. The optimal stomaching duration was established at 2 min on normal speed (230 rpm ± 5 %).

The effects biodegradable organic matter (BOM) concentration, the presence of amino acids and hydraulic retention time had on bacterial regrowth in an annular reactor were examined. The number of biofilm HPCs increased at higher BOM loading rates (i.e., higher BOM concentrations and lower retention times). It was shown that BOM consumption was primarily by the heterotrophic cells in the biofilm.

Amino acids present as part of the BOM cocktail also increased the number of biofilm HPCs. By comparing Michaelis-Menten parameters for carboxylic acids and amino acids, faster utilization rates for amino acids were proposed as an explanation for the elevated biofilm numbers observed for BOM cocktails containing amino acids.

Higher BOM concentration decreased the net number of bulk HPCs. It was proposed that higher BOM levels may have shocked the culturable cells in the bulk liquid.

Increased retention time positively affected the number of net bulk HPCs. Because the retention time is the reciprocal of the dilution rate, it was proposed that bulk cells were being washed out of the AR at higher dilution rates (lower retention times).

It was found that the utilization of BOM by a drinking water biofilm could be represented as a first-order process. Under the conditions studied, mass transfer rate was faster than the BOM removal rate. Consequently, under the conditions studied, mass transfer does not limit the removal of BOM.

For a specific BOM component, the organic flux was found to be linearly related to the influent loading rate. The slope of this relationship was used to indicate the fraction

removed. As indicated by a high slope, amino acids and formate were found to be BOM components that are easily removed. Of the components studied, glyoxal had the lowest slope. Acetate also had a lower slope, however the amount of measured acetate in the effluent may have been the consequence of incomplete removal and/or its production as a metabolic intermediate.

For the conditions studied, biofilm detachment was found to be more strongly related to the substrate utilization rate than the shear stress. Under low nutrient conditions (BOM concentrations less than 100 μg/L), the biofilm detachment rate may have a zero order dependence on the utilization rate. Biofilm detachment was found to have a linear relationship with BOM removal for higher influent loading rates.

A model distribution system was constructed by connecting three ARs in series. The number of bacteria in the biofilm in the downstream portion of the model distribution system were similar (expressed as areal cell density) and appeared to be independent of the influent conditions in the subsequent ARs. These two factors imply that other energy sources may be available for cell maintenance in the biofilm in the distant portions of the distribution systems, and that other indicators of regrowth should be sought.

The amount of refractory organic matter (ROM) increased throughout the model distribution system and was independent of influent conditions. ROM, as measured by ultra violet absorbance at 285 nm (UV_{285}), was linearly related to the concentration of trihalomethane formation potential. In the model distribution system, a relationship between the ROM production rate and the observed BOM removal rate was calculated.

A modeling approach was developed for relating retention times in an AR to retention times of an equivalent length of pipe. By using observed BOM fluxes from

several experiments, it was shown that the ratio of pipe to AR retention time was 6.8, which is similar to the AR to pipe specific surface area ratio for the 100 mm pipe diameter assumed. Thus, multiplying the AR retention time by the specific surface area ratio is proposed as a rule of thumb for relating bench-scale experiments to full-scale situations.

RECOMMENDATIONS

Results from this investigation could be expanded in scope for future experimental projects. Specifically, recommended studies for future research include:

- The stomacher should be tested for removing attached cells from other media (e.g., filter particles, pipe tubercles) to test its efficacy. Ultimately, a reliable method for removing cells from different pipe materials should be developed.
- The relationships between biofilm and bulk cell numbers in drinking water should be examined more closely. In this study, it was shown that bulk cells are the result of both bulk growth (more relevant at longer retention times) and biofilm detachment (more relevant at higher BOM loading rates). However, the relative rate of detachment and bulk growth was not established from a quantitative perspective. The relevance of biofilm detachment in drinking water distribution systems could be examined by either improved analytical techniques (e.g., bio-markers) or experimental designs.
- Because BOM utilization rates by biofilms can be approximated by a first-order removal rate, simplified rate expressions could be used in hydraulic models (e.g., EPANET) for modeling the fate of BOM in distribution systems. The model

predictions could be verified using either a pilot-scale distribution system, or a hydraulically well defined full-scale network.

- A major response parameter in this study was the number of HPCs either in the biofilm or in the bulk liquid. Other reliable methods for measuring microbial accumulation should be used to confirm these results. Specifically, the number of biofilm HPCs remained relatively constant through the model distribution system (e.g., three ARs in series). It would be useful to examine whether relationships between cell growth and the production of refractory organic matter (ROM) exist.
- The importance of ROM in distributions systems could be studied in greater depth. Possible investigations include: measuring trends in ROM at full-scale; other methods available for measuring ROM (e.g., fluorescence, characterization with XAD resins) utilization rates of ROM for different treated waters; relationship between ROM and the amount of protein-bound amino acids and their involvement with nitrification in distribution systems; and the role of ROM in the production of organohalides (e.g., trihalomethanes, haloacetic acids) in full-scale systems.
- Biological stability in drinking water is typically characterized by the amount of available substrate (e.g., BOM). For waters where carbon is not limiting, or where BOM levels are reasonably low (e.g., less than 100 μg C/L) but excessive bacterial growth still occurs in the distribution system, measuring the amount of BOM may not be a sensitive indicator of regrowth. A standardized method that directly measures the biofilm accumulation rate could be developed for field assessment of biological stability.

APPENDIX A

EXPERIMENTAL TRIALS: 5.x & 6.x

Experiment 5.1

HRT = 2 hr
BOM = 50 µg/L
Amino Acids = 0.0
Trial Date: April 1996

HPCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.04	1.3E+04	3.3E+03	1.0E+00
0.79	7.0E+03	8.5E+03	1.6E+03
1.88	1.6E+04	7.7E+04	2.3E+04
3.04	7.3E+03	1.1E+05	1.5E+05
4.83	1.3E+03	1.5E+05	3.9E+05
6.98	8.3E+02	1.3E+05	5.8E+05
8.81	6.0E+02	1.2E+05	4.4E+05
9.88	8.4E+02	1.2E+05	3.8E+05

AODCs

Time	Influent	Effluent	Biofilm
d	cells/mL	cells/mL	cells/cm^2
0.04	6.7E+04	8.4E+04	1.2E+05
0.79	6.7E+04	3.8E+04	6.9E+04
1.88	1.2E+05	1.5E+05	1.6E+05
3.04	1.5E+05	3.5E+05	4.6E+05
4.83	5.8E+04	2.7E+06	1.0E+06
6.98	1.3E+05	3.5E+05	9.3E+05
8.81	-	-	-
9.88	1.3E+05	2.5E+05	1.1E+06

Average Influent BOM Components, µg C/L

Carboxylic Acids		Aldehydes		Amino Acids			Total
Acetate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
22.4	4.5	13.3	11.8	-	-	-	51.9

Effluent BOM Components, µg C/L

Time	Carboxylic Acids		Aldehydes		Amino Acids			Total
d	Acetate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
0.00	40.2	15.7	1.9	2.5	-	-	-	60.3
0.77	36.2	14.2	0.0	0.0	-	-	-	50.4
1.13	44.2	17.2	1.7	0.0	-	-	-	63.1
1.83	24.6	9.3	2.9	0.0	-	-	-	36.8
3.02	8.4	12.2	2.3	0.0	-	-	-	22.9
4.81	8.1	13.7	0.0	0.0	-	-	-	21.8
6.94	8.9	2.2	3.8	0.0	-	-	-	14.9
8.77	7.7	2.1	3.9	0.0	-	-	-	13.7
9.81	7.9	6.6	0.0	0.0	-	-	-	14.5

Experiment 5.2

HRT = 4 hr
BOM = 50 µg/L
Amino Acids = 0.0
Trial Date: November, 1995

HPCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.04	1.4E+03	9.7E+03	1.0E+00
0.83	6.0E+02	2.9E+05	1.0E+04
1.85	2.5E+03	4.8E+05	1.8E+05
2.88	2.3E+03	1.8E+05	2.2E+05
4.81	7.2E+02	1.3E+06	2.6E+05
6.90	6.4E+02	7.5E+04	4.8E+05
8.85	1.0E+03	5.4E+05	2.8E+05
10.85	2.0E+03	8.0E+05	4.7E+06

AODCs

Time	Influent	Effluent	Biofilm
d	cells/mL	cells/mL	cells/cm^2
0.04	1.7E+05	3.4E+05	3.9E+04
0.83	1.2E+05	9.6E+05	1.8E+05
1.85	1.0E+05	1.1E+06	9.2E+05
2.88	7.4E+03	5.9E+05	5.6E+05
4.81	7.0E+04	2.8E+06	2.1E+06
6.90	1.8E+05	2.2E+06	2.4E+06
8.85	6.6E+04	1.5E+06	5.7E+06
10.85	1.4E+05	2.8E+06	1.7E+07

Average Influent BOM Components, µg C/L

Carboxylic Acids		Aldehydes		Amino Acids			Total
Acetate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
101.7	3.5	4.5	2.3	-	-	-	112.0

Effluent BOM Components, µg C/L

Time	Carboxylic Acids		Aldehydes		Amino Acids			Total
d	Acetate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
0.00	185.1	34.9	6.3	9.0	-	-	-	235.4
0.17	181.0	34.9	7.1	2.5	-	-	-	225.6
0.38	189.2	20.0	7.2	0.0	-	-	-	216.4
0.79	16.7	8.5	7.7	0.0	-	-	-	32.9
1.13	22.0	4.0	7.8	0.0	-	-	-	33.8
1.42	1.6	0.0	7.6	0.0	-	-	-	9.2
1.81	38.6	0.0	4.6	0.0	-	-	-	43.3
2.35	130.6	0.0	4.6	0.0	-	-	-	135.2
2.88	7.3	0.0	7.1	0.0	-	-	-	14.4
4.81	4.5	0.0	4.6	0.0	-	-	-	9.1
6.85	4.1	0.0	4.6	0.0	-	-	-	8.7
8.81	3.7	1.1	7.6	0.0	-	-	-	12.3
10.85	12.2	2.7	4.6	0.0	-	-	-	19.5

Experiment 5.3a (replicate)

HRT = 2 hr
BOM = 500 μg/L
Amino Acids = 0.0
Trial Date: January 1996

HPCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.04	2.6E+03	3.7E+05	1.9E+04
1.04	1.2E+03	9.4E+04	7.4E+04
2.04	2.4E+03	2.6E+05	2.9E+05
3.13	8.9E+02	1.5E+05	6.5E+05
4.96	1.1E+03	7.7E+04	3.5E+05
7.00	5.3E+03	1.7E+05	8.3E+05
8.33	1.3E+03	1.9E+05	1.1E+06
11.21	3.5E+04	3.9E+05	4.4E+05

AODCs

Time	Influent	Effluent	Biofilm
d	cells/mL	cells/mL	cells/cm^2
0.04	7.6E+04	6.7E+05	1.1E+05
1.04	8.1E+04	2.5E+05	1.7E+05
2.04	4.7E+04	3.3E+05	6.3E+05
3.13	4.6E+04	2.1E+05	8.6E+05
4.96	4.1E+04	2.1E+06	7.0E+06
7.00	5.1E+04	3.0E+06	9.0E+06
8.33	3.5E+04	3.8E+06	1.1E+07
11.21	4.3E+04	3.6E+06	1.2E+07

Average Influent BOM Components, μg C/L

Carboxylic Acids		Aldehydes		Amino Acids			Total
Acetate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
79.7	36.5	48.1	31.8	-	-	-	196.1

Effluent BOM Components, μg C/L

Time	Carboxylic Acids		Aldehydes		Amino Acids			Total
d	Acetate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
0.00	22.0	20.3	2.7	0.0	-	-	-	45.0
0.21	44.9	10.7	42.9	41.2	-	-	-	139.6
0.44	49.9	33.9	16.3	36.8	-	-	-	136.8
0.85	50.5	38.1	67.5	38.5	-	-	-	194.7
1.19	49.5	35.5	10.5	38.8	-	-	-	134.2
1.42	48.9	33.1	81.6	49.7	-	-	-	213.3
1.83	48.5	19.5	6.7	49.6	-	-	-	124.2
2.17	31.0	10.4	18.9	46.8	-	-	-	107.1
3.08	47.9	2.7	7.2	9.0	-	-	-	66.8
4.02	49.8	1.1	9.3	3.7	-	-	-	63.8
6.13	41.5	0.3	19.2	0.0	-	-	-	61.0
7.94	45.1	0.0	33.9	0.0	-	-	-	79.0
10.06	48.4	0.0	29.4	0.0	-	-	-	77.8

Experiment 5.3b (replicate)

HRT = 2 hr
BOM = 500 µg/L
Amino Acids = 0.0
Trial Date: January 1996

HPCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.04	2.6E+03	5.0E+04	3.6E+03
1.04	1.2E+03	9.4E+04	2.4E+04
2.04	2.4E+03	1.2E+05	1.3E+05
3.13	8.9E+02	3.0E+05	2.4E+05
4.96	1.1E+03	2.6E+05	3.2E+05
7.00	5.3E+03	4.0E+04	1.5E+06
8.33	1.3E+03	2.0E+05	4.7E+05
11.21	3.5E+04	2.5E+05	1.4E+06

AODCs

Time	Influent	Effluent	Biofilm
d	cells/mL	cells/mL	cells/cm^2
0.04	7.6E+04	1.1E+05	1.8E+05
1.04	8.1E+04	1.0E+05	1.3E+05
2.04	4.7E+04	2.5E+05	3.2E+05
3.13	4.6E+04	3.9E+05	6.0E+05
4.96	4.1E+04	2.3E+06	8.0E+06
7.00	5.1E+04	3.3E+06	8.7E+06
8.33	3.5E+04	3.3E+06	1.2E+07
11.21	4.3E+04	4.0E+06	1.3E+07

Average Influent BOM Components, µg C/L

Carboxylic Acids		Aldehydes		Amino Acids			Total
Acetate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
79.7	36.3	48.1	31.8	-	-	-	195.9

Effluent BOM Components, µg C/L

Time	Carboxylic Acids		Aldehydes		Amino Acids			Total
d	Acetate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
0.00	31.7	27.7	2.8	7.8	-	-	-	70.0
0.21	44.9	12.3	23.3	48.9	-	-	-	129.3
0.44	49.9	45.6	8.5	24.2	-	-	-	128.2
0.85	50.5	33.9	68.9	45.0	-	-	-	198.3
1.19	49.5	34.4	21.2	45.8	-	-	-	150.9
1.42	54.5	31.5	11.7	25.3	-	-	-	122.9
1.83	53.3	29.9	15.1	47.0	-	-	-	145.3
2.17	35.7	11.2	6.5	37.7	-	-	-	91.1
3.08	53.1	12.0	6.0	0.0	-	-	-	71.1
4.02	37.3	0.0	22.8	0.0	-	-	-	60.1
6.13	42.5	0.0	18.5	0.0	-	-	-	61.0
7.94	45.2	0.0	16.6	0.0	-	-	-	61.8
10.06	48.4	0.0	23.5	0.0	-	-	-	71.9

Experiment 5.4

HRT = 4 hr
BOM = 500 µg/L
Amino Acids = 0.0
Trial Date: March 1996

HPCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.04	2.0E+03	9.3E+03	1.0E+00
0.92	3.6E+03	1.6E+04	6.2E+03
1.88	2.0E+03	7.9E+04	2.7E+04
2.90	3.7E+03	1.1E+06	1.8E+05
4.81	3.7E+03	5.5E+05	2.6E+05
6.88	4.9E+03	4.6E+05	2.2E+05
9.83	1.1E+03	8.7E+04	2.4E+05
11.04	2.9E+03	3.7E+05	3.8E+05

AODCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.04	4.8E+04	1.5E+05	9.8E+04
0.92	4.8E+04	1.7E+05	7.2E+04
1.88	9.8E+04	2.2E+05	1.8E+05
2.90	-	-	-
4.81	1.5E+05	1.1E+06	7.5E+05
6.88	2.2E+05	1.7E+06	6.9E+05
9.83	5.5E+04	5.3E+05	6.7E+05
11.04	5.5E+04	6.7E+05	8.1E+05

Average Influent BOM Components, µg C/L

Carboxylic Acids		Aldehydes		Amino Acids			Total
Acetate	Formate	Formald	Glyoxal	Aspartic	Glutamic	Serine	BOM
130.7	76.8	26.6	30.2	-	-	-	264.3

Effluent BOM Components, µg C/L

Time	Carboxylic Acids		Aldehydes		Amino Acids			Total
d	Acetate	Formate	Formald	Glyoxal	Aspartic	Glutamic	Serine	BOM
0.00	208.9	23.8	2.9	2.1	-	-	-	237.6
0.92	90.6	76.6	0.0	5.1	-	-	-	172.3
1.83	2.8	17.8	0.0	21.9	-	-	-	42.5
2.88	1.3	4.8	0.0	20.2	-	-	-	26.3
4.79	7.6	7.9	0.0	18.1	-	-	-	33.6
6.83	2.5	1.6	0.0	1.0	-	-	-	5.1
9.79	1.4	0.8	0.0	1.0	-	-	-	3.2
11.00	0.3	0.0	0.0	10.1	-	-	-	10.4

Experiment 5.5

HRT = 2 hr
BOM = 50 μg/L
Amino Acids = 0.34
Trial Date: April 1996

HPCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.04	1.3E+04	2.7E+03	1.0E+00
0.79	7.0E+03	9.8E+03	2.1E+03
1.88	1.6E+04	1.7E+05	7.7E+04
3.04	7.3E+03	2.9E+05	5.0E+05
4.83	1.3E+03	1.5E+05	1.6E+06
6.98	8.3E+02	1.8E+05	1.1E+06
8.81	6.0E+02	8.4E+04	3.5E+05
9.88	8.4E+02	3.1E+05	1.6E+05

AODCs

Time	Influent	Effluent	Biofilm
d	cells/mL	cells/mL	cells/cm^2
0.04	6.7E+04	1.6E+05	1.6E+04
0.79	6.7E+04	6.4E+04	8.4E+04
1.88	1.2E+05	2.4E+05	9.8E+04
3.04	1.5E+05	3.0E+05	8.1E+05
4.83	5.8E+04	2.4E+05	2.1E+06
6.98	1.3E+05	7.8E+05	1.4E+06
8.81	-	-	-
9.88	1.3E+05	5.2E+05	2.8E+06

Average Influent BOM Components, μg C/L

Carboxylic Acids		Aldehydes		Amino Acids			Total
Acetate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
8.8	4.5	5.2	4.6	6.9	10.4	6.8	47.3

Effluent BOM Components, μg C/L

Time	Carboxylic Acids		Aldehydes		Amino Acids			Total
d	Acetate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
0.00	27.5	12.5	0.0	0.0	10.4	13.4	9.1	72.8
0.77	27.2	13.3	2.3	0.0	7.7	11.0	5.8	67.4
1.13	27.7	11.6	2.3	0.0	6.0	8.4	4.5	60.5
1.83	4.8	1.1	2.0	0.0	0.7	0.7	0.1	9.4
3.02	2.3	0.0	1.7	0.0	0.0	0.2	0.0	4.3
4.81	1.1	11.3	1.6	0.0	0.0	0.0	0.0	14.1
6.94	4.2	4.5	0.0	0.0	0.0	0.0	0.0	8.9
8.77	2.9	2.5	1.8	0.0	0.0	0.0	0.0	7.3
9.81	3.6	8.3	0.0	0.0	0.0	0.0	0.0	12.0

Experiment 5.6

HRT = 4 hr
BOM = 50 µg/L
Amino Acids = 0.34
Trial Date: November, 1995

HPCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.04	1.4E+03	1.8E+04	6.6E+02
0.83	6.0E+02	4.2E+04	1.5E+04
1.85	2.5E+03	4.7E+05	1.3E+05
2.88	2.3E+03	2.8E+05	6.9E+05
4.81	7.2E+02	1.7E+06	1.9E+05
6.90	6.4E+02	9.9E+04	5.4E+05
8.85	1.0E+03	3.8E+05	3.3E+05
10.85	2.0E+03	9.0E+05	6.6E+05

AODCs

Time	Influent	Effluent	Biofilm
d	cells/mL	cells/mL	cells/cm^2
0.04	1.7E+05	6.4E+05	4.1E+03
0.83	1.2E+05	9.7E+04	5.8E+04
1.85	1.0E+05	7.8E+05	2.2E+05
2.88	7.4E+03	8.2E+05	1.5E+06
4.81	7.0E+04	1.7E+06	1.7E+06
6.90	1.8E+05	6.1E+06	3.5E+06
8.85	6.6E+04	1.0E+06	6.1E+06
10.85	1.4E+05	2.3E+06	5.3E+06

Average Influent BOM Components, µg C/L

Carboxylic Acids		Aldehydes		Amino Acids			Total
Acetate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
66.3	3.5	3.7	1.9	9.0[a]	14.0[a]	9.0[a]	107.4

[a] Assumed values, amino acids not measured.

Effluent BOM Components, µg C/L

Time	Carboxylic Acids		Aldehydes		Amino Acids			Total
d	Acetate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
0.00	183.1	26.7	6.3	8.6	-	-	-	224.6
0.17	173.3	23.7	6.5	0.0	-	-	-	203.6
0.38	76.5	11.5	7.5	0.0	-	-	-	95.4
0.79	30.5	5.1	7.5	0.0	-	-	-	43.1
1.13	16.7	2.9	7.5	0.0	-	-	-	27.2
1.42	5.3	0.0	6.8	0.0	-	-	-	12.1
1.81	30.5	0.0	5.3	0.0	-	-	-	35.8
2.35	2.0	0.0	6.7	0.0	-	-	-	8.8
2.88	35.8	0.0	6.3	0.0	-	-	-	42.1
4.81	0.0	0.0	4.6	0.0	-	-	-	4.6
6.85	5.7	0.0	5.7	0.0	-	-	-	11.4
8.81	19.9	10.7	5.3	0.0	-	-	-	35.9
10.85	56.9	0.0	6.3	0.0	-	-	-	63.2

Amino acids not measured complete removal was assumed for steady-state conditions.

Experiment 5.7

HRT = 4 hr
BOM = 500 µg/L
Amino Acids = 0.34
Trial Date: March 1996

HPCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.04	2.0E+03	1.4E+03	1.0E+00
0.92	3.6E+03	1.5E+03	1.3E+03
1.88	2.0E+03	6.6E+04	1.2E+05
2.90	3.7E+03	5.0E+05	5.8E+05
4.81	3.7E+03	8.5E+05	2.3E+06
6.88	4.9E+03	4.5E+05	3.4E+06
9.83	1.1E+03	6.0E+05	4.1E+06
11.04	2.9E+03	9.8E+04	3.5E+06

AODCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.04	4.8E+04	1.4E+05	1.4E+05
0.92	4.8E+04	1.4E+05	9.2E+04
1.88	9.8E+04	1.4E+05	2.8E+05
2.90	-	-	-
4.81	1.5E+05	3.7E+06	9.7E+05
6.88	2.2E+05	1.1E+06	3.2E+06
9.83	5.5E+04	4.1E+05	4.0E+06
11.04	5.5E+04	5.1E+05	3.4E+06

Average Influent BOM Components, µg C/L

Carboxylic Acids		Aldehydes		Amino Acids			Total
Acetate	Formate	Formald	Glyoxal	Aspartic	Glutamic	Serine	BOM
46.8	29.5	8.5	9.7	55.4	92.2	61.3	303.5

Effluent BOM Components, µg C/L

Time	Carboxylic Acids		Aldehydes		Amino Acids			Total
d	Acetate	Formate	Formald	Glyoxal	Aspartic	Glutamic	Serine	BOM
0.00	9.1	7.9	0.0	9.9	0.0	0.0	0.0	27.0
0.92	62.7	46.2	0.0	5.1	75.7	118.5	73.9	382.0
1.83	98.2	19.8	0.0	6.8	41.6	55.5	55.7	277.5
2.88	5.3	0.0	0.0	11.6	1.6	2.5	0.6	21.6
4.79	17.5	14.7	0.0	12.8	0.1	0.0	0.0	45.1
6.83	21.0	7.7	0.0	1.5	0.4	0.9	0.0	31.6
9.79	0.0	0.0	0.0	1.6	0.0	0.0	0.0	1.7
11.00	6.2	0.0	0.0	1.6	0.0	0.5	0.0	8.3

Experiment 5.8

HRT = 4 hr
BOM = 500 µg/L
Amino Acids = 0.34
Trial Date: February 1996

HPCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.06	5.2E+03	3.9E+04	2.4E+03
0.90	7.7E+03	6.9E+04	1.5E+04
1.90	8.4E+03	1.0E+06	3.7E+05
2.85	3.7E+03	5.2E+05	1.4E+06
4.83	1.3E+03	2.0E+05	3.3E+06
6.85	5.2E+03	2.2E+05	2.6E+06
9.85	9.0E+02	1.1E+06	3.1E+06
10.90	1.7E+03	3.5E+05	2.5E+06

AODCs

Time	Influent	Effluent	Biofilm
d	cells/mL	cells/mL	cells/cm^2
0.06	1.7E+05	5.3E+05	1.7E+05
0.90	1.5E+05	2.5E+05	1.8E+05
1.90	6.9E+04	1.0E+06	1.9E+05
2.85	7.4E+03	3.4E+05	1.9E+06
4.83	8.0E+04	4.4E+05	5.0E+06
6.85	7.3E+04	5.7E+05	3.6E+06
9.85	7.7E+04	1.2E+06	2.9E+06
10.90	7.6E+04	1.0E+06	3.5E+06

Average Influent BOM Components, µg C/L

Carboxylic Acids		Aldehydes		Amino Acids			Total
Acetate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
109.5	43.5	49.9	37.4	25.9	50.4	36.3	352.9

Effluent BOM Components, µg C/L

Time	Carboxylic Acids		Aldehydes		Amino Acids			Total
d	Acetate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
0.00	232.3	54.7	43.1	54.1	33.1	47.2	68.5	533.0
0.17	272.9	46.7	1.3	34.6	38.8	83.8	67.1	545.2
0.35	202.2	38.8	47.2	25.1	36.8	87.1	61.1	498.2
0.85	171.3	21.8	2.2	10.3	36.2	79.6	61.6	382.9
1.15	168.0	13.7	41.0	10.9	34.7	71.0	74.2	413.5
1.46	115.5	11.8	42.0	0.0	10.6	12.8	55.5	248.2
1.88	3.3	0.0	0.0	0.0	0.0	0.0	0.0	3.4
2.81	32.1	1.2	3.8	0.0	0.0	0.0	0.0	37.2
4.83	29.7	7.0	21.7	0.0	0.1	0.2	0.7	59.4
6.85	4.4	0.0	25.8	0.0	0.3	0.6	1.6	32.8
9.85	22.4	0.0	74.9	0.0	0.5	1.0	1.0	99.7
10.90	0.0	0.0	84.9	0.0	0.0	0.2	0.0	85.2

Experiment 5.9a (rotational speed: 50 rpm)

HRT = 1 hr
BOM = 50 μg/L
Amino Acids = 0.34
Trial Date: July 1996

HPCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.04	4.0E+03	9.2E+03	1.0E+00
1.08	1.7E+03	4.1E+04	9.3E+03
2.00	3.6E+03	1.8E+05	1.2E+05
3.75	1.9E+04	9.2E+04	7.7E+05
4.98	1.1E+04	2.9E+05	4.2E+05
6.98	5.0E+03	4.3E+05	7.2E+05
10.92	1.4E+05	7.8E+05	2.1E+06

AODCs

Time	Influent	Effluent	Biofilm
d	cells/mL	cells/mL	cells/cm^2
0.04	8.8E+04	2.3E+05	4.3E+04
1.08	7.8E+04	2.2E+05	4.8E+05
2.00	3.1E+05	5.3E+05	8.9E+05
3.75	5.0E+04	6.3E+05	2.1E+06
4.98	4.4E+05	1.8E+06	3.1E+06
6.98	3.9E+05	7.8E+05	4.0E+06
10.92	3.4E+05	1.1E+06	4.4E+06

Average Influent BOM Components, μg C/L

Carboxylic Acids			Aldehydes		Amino Acids			Total
Acetate	Oxalate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
37.6	4.5	13.7	3.4	3.6	9.7	8.7	15.0	96.2

Effluent BOM Components, μg C/L

Time	Carboxylic Acids			Aldehydes		Amino Acids			Total
d	Acetate	Oxalate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
0.00	201.0	14.5	61.8	8.6	8.8	17.5	14.4	22.3	349.0
0.29	80.0	12.3	10.6	0.0	9.0	0.6	0.0	0.0	112.6
0.94	29.7	14.9	40.9	54.7	2.0	23.9	19.1	29.5	214.7
1.21	38.6	11.5	11.8	6.0	49.4	0.1	0.1	0.3	117.8
1.48	75.7	11.1	2.5	0.0	2.8	0.2	0.3	0.1	92.7
1.94	20.4	7.5	3.8	0.0	6.0	1.2	0.5	1.7	41.2
3.69	25.1	4.5	5.0	0.0	4.1	0.0	0.0	0.0	38.7
4.85	32.6	4.5	24.2	0.0	4.6	0.0	0.0	0.0	65.8
6.90	2.0	4.0	0.0	0.0	4.0	0.0	0.0	0.0	10.0
10.88	10.4	6.5	7.7	0.0	0.0	0.0	0.0	0.0	24.6

Experiment 5.9b (rotational speed: 350 rpm)

HRT = 1 hr
BOM = 50 μg/L
Amino Acids = 0.34
Trial Date: July 1996

HPCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.04	4.0E+03	6.0E+02	1.0E+00
1.08	1.7E+03	5.0E+04	9.7E+04
2.00	3.6E+03	1.1E+05	1.5E+05
3.75	1.9E+04	3.9E+05	7.8E+05
4.98	1.1E+04	1.8E+05	7.3E+05
6.98	5.0E+03	5.5E+05	8.3E+05
10.92	1.4E+05	4.6E+05	1.9E+06

AODCs

Time	Influent	Effluent	Biofilm
d	cells/mL	cells/mL	cells/cm^2
0.04	8.8E+04	1.3E+05	1.2E+05
1.08	7.8E+04	2.6E+05	7.0E+05
2.00	3.1E+05	7.9E+05	7.4E+05
3.75	5.0E+04	4.2E+05	2.0E+06
4.98	4.4E+05	9.7E+05	2.9E+06
6.98	3.9E+05	1.2E+06	4.0E+06
10.92	3.4E+05	1.3E+06	3.9E+06

Average Influent BOM Components, μg C/L

Carboxylic Acids			Aldehydes		Amino Acids			Total
Acetate	Oxalate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
37.3	4.2	13.0	3.7	3.0	6.3	4.1	15.3	86.9

Effluent BOM Components, μg C/L

Time	Carboxylic Acids			Aldehydes		Amino Acids			Total
d	Acetate	Oxalate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
0.00	104.7	2.2	42.8	9.8	7.3	1.0	1.0	19.3	188.0
0.29	30.1	0.6	8.1	0.0	7.4	19.6	16.1	24.1	105.9
0.94	0.0	1.2	2.1	65.6	1.9	24.0	19.1	29.5	143.3
1.21	11.4	7.9	0.0	0.0	1.6	0.3	0.1	0.0	21.3
1.48	36.3	1.9	10.1	8.2	2.8	0.1	0.1	0.0	59.5
1.94	0.0	5.9	0.0	0.0	6.1	0.2	0.3	0.0	12.5
3.69	7.3	4.2	1.0	0.0	5.1	0.0	0.0	0.0	17.6
4.85	17.9	3.1	6.6	10.1	4.2	0.0	0.0	0.0	41.9
6.90	2.1	1.5	0.0	0.0	1.3	0.0	0.0	0.0	4.9
10.88	3.7	9.3	4.2	4.5	0.0	0.0	0.0	0.0	21.7

Experiment 5.10

HRT = 1 hr
BOM = 500 μg/L
Amino Acids = 0.34
Trial Date: October 1996

HPCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.06	6.2E+04	3.1E+04	3.4E+03
1.00	5.5E+04	7.7E+04	9.8E+04
1.79	1.0E+05	1.3E+06	1.9E+06
2.85	8.0E+02	4.1E+05	3.3E+06
3.98	1.2E+05	2.5E+06	1.5E+07
5.96	1.0E+05	1.5E+06	1.9E+07
7.77	8.1E+04	4.2E+05	1.4E+07
13.85	5.5E+04	3.0E+05	1.1E+07

AODCs

Time	Influent	Effluent	Biofilm
d	cells/mL	cells/mL	cells/cm^2
0.06	3.5E+05	-	4.0E+05
1.00	2.8E+05	-	5.8E+05
1.79	-	-	-
2.85	2.6E+05	-	3.3E+06
3.98	-	-	4.7E+06
5.96	3.1E+05	-	6.8E+06
7.77	-	-	8.9E+06
13.85	-	-	9.7E+06

Average Influent BOM Components, μg C/L

Carboxylic Acids			Aldehydes		Amino Acids			Total
Acetate	Oxalate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
128.7	25.4	26.4	40.3	73.2	117.9	57.5	72.9	542.4

Effluent BOM Components, μg C/L

Time	Carboxylic Acids			Aldehydes		Amino Acids			Total
d	Acetate	Oxalate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
0.00	57.8	16.0	22.4	33.8	78.9	135.4	82.2	71.4	497.8
0.25	29.6	6.1	6.3	69.7	72.6	127.8	59.9	70.0	442.0
0.79	54.1	10.3	10.2	66.2	77.7	72.4	31.9	68.6	391.5
1.15	16.9	11.5	8.9	64.0	64.6	0.0	0.0	7.3	173.1
2.00	20.9	3.7	4.0	29.0	59.8	0.0	0.0	0.0	117.6
2.79	34.5	22.6	6.1	34.6	26.6	0.0	0.0	0.0	124.3
4.94	22.9	4.5	13.3	10.7	3.4	0.0	0.0	0.0	54.8
6.96	20.3	6.1	2.7	3.2	1.9	0.0	0.0	0.0	34.3
8.79	29.8	4.6	12.2	50.8	0.0	0.0	0.0	0.0	97.4
15.06	23.8	12.9	6.8	2.1	0.0	0.0	0.0	0.0	45.6

Experiment 6.1a (rotational speed: 50 rpm)

HRT = 1 hr
BOM = 2000 μg/L
Amino Acids = 0.34
Trial Date: July 1996

HPCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.08	7.9E+03	9.3E+03	5.3E+02
0.94	6.3E+02	8.0E+03	1.8E+02
1.92	1.0E+03	3.8E+04	3.3E+03
2.90	1.9E+05	4.8E+05	1.8E+05
4.15	1.5E+05	2.5E+06	1.5E+06
5.94	6.0E+04	1.8E+06	3.5E+07
7.15	1.0E+05	3.1E+06	2.4E+07
7.98	8.5E+04	1.5E+06	2.4E+07
9.96	5.8E+03	3.6E+05	9.3E+06

AODCs

Time	Influent	Effluent	Biofilm
d	cells/mL	cells/mL	cells/cm^2
0.08	2.0E+05	1.4E+05	8.6E+04
0.94	1.4E+05	4.5E+04	1.3E+05
1.92	1.5E+05	1.5E+05	7.6E+04
2.90	-	-	3.0E+06
4.15	4.2E+05	4.3E+06	8.5E+06
5.94	-	4.9E+06	4.8E+07
7.15	-	-	5.6E+07
7.98	-	-	-
9.96	2.6E+05	3.0E+06	-

Average Influent BOM Components, μg C/L

Carboxylic Acids			Aldehydes		Amino Acids			Total
Acetate	Oxalate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
330.1	143.3	103.5	37.5	124.3	671.3	308.7	310.1	2028.9

Effluent BOM Components, μg C/L

Time	Carboxylic Acids			Aldehydes		Amino Acids			Total
d	Acetate	Oxalate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
0.00	185.5	2.0	70.7	1.3	0.0	721.4	388.1	322.9	1691.7
0.44	427.4	182.5	101.0	11.7	95.6	854.6	446.9	395.1	2514.8
0.90	390.8	144.7	136.1	1.1	0.0	797.6	413.7	368.9	2253.0
1.13	394.6	145.4	119.8	3.7	87.1	734.2	383.7	337.5	2206.1
1.88	299.8	87.0	120.0	8.1	61.4	428.2	227.7	198.3	1430.4
2.13	429.2	146.8	110.5	11.1	94.8	738.7	391.8	324.3	2247.1
2.88	379.1	149.5	76.9	1.1	0.0	579.0	293.0	282.5	1761.1
4.13	388.1	126.3	0.0	80.1	38.7	0.0	0.0	9.2	642.4
5.88	308.7	26.3	0.0	59.7	5.5	0.0	0.0	11.5	411.8
7.13	180.5	9.4	0.0	3.3	5.2	0.0	0.0	0.0	198.4
7.92	268.2	8.8	0.0	24.8	5.9	0.0	0.0	21.6	329.2
9.92	92.6	7.7	0.0	2.6	5.4	0.0	0.0	13.2	121.6

Experiment 6.1b (rotational speed: 350 rpm)

HRT = 1 hr
BOM = 2000 μg/L
Amino Acids = 0.34
Trial Date: August 1996

HPCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.08	7.9E+03	9.5E+03	1.8E+02
0.94	6.3E+02	1.5E+04	2.0E+03
1.92	1.0E+03	2.6E+04	1.4E+05
2.90	1.9E+05	1.5E+06	3.7E+06
4.15	1.5E+05	4.9E+07	1.7E+07
5.94	6.0E+04	1.4E+06	4.5E+07
7.15	1.0E+05	2.2E+06	5.7E+07
7.98	8.5E+04	3.1E+06	7.7E+07
9.96	5.8E+03	3.2E+06	9.3E+07

AODCs

Time	Influent	Effluent	Biofilm
d	cells/mL	cells/mL	cells/cm^2
0.08	2.0E+05	1.9E+05	1.9E+04
0.94	1.4E+05	6.8E+04	1.3E+05
1.92	1.5E+05	1.6E+05	5.4E+05
2.90	-	-	7.1E+06
4.15	4.2E+05	1.9E+07	1.5E+07
5.94	-	3.5E+06	-
7.15	-	-	3.7E+07
7.98	-	2.4E+06	-
9.96	2.6E+05	5.2E+06	7.0E+07

Average Influent BOM Components, μg C/L

Carboxylic Acids			Aldehydes		Amino Acids			Total
Acetate	Oxalate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
310.8	157.2	89.2	37.5	100.6	660.8	303.9	310.0	1970.1

Effluent BOM Components, μg C/L

Time	Carboxylic Acids			Aldehydes		Amino Acids			Total
d	Acetate	Oxalate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
0.00	121.5	1.9	28.1	3.4	58.6	33.6	29.2	24.9	301.1
0.44	194.6	15.6	48.4	3.2	9.0	32.2	14.8	26.0	343.7
0.90	424.7	133.4	117.3	12.6	98.9	626.8	331.7	268.5	2013.9
1.13	436.5	164.9	126.0	7.2	105.2	671.5	354.5	293.8	2159.5
1.88	439.3	157.2	116.3	12.8	17.2	433.9	209.2	254.5	1640.4
2.13	406.2	162.5	108.4	20.5	96.6	103.0	40.1	192.5	1129.8
2.88	172.7	128.5	0.2	66.7	89.8	0.0	0.0	4.5	462.4
4.13	401.1	144.1	0.0	1.1	0.0	0.0	0.0	0.0	546.3
5.88	291.7	13.9	0.0	22.9	6.8	0.0	0.0	0.0	335.3
7.13	178.7	12.7	0.0	4.2	3.8	0.0	0.0	0.0	199.4
7.92	347.7	13.2	0.0	75.9	4.1	0.0	0.0	0.0	440.9
9.92	113.8	12.2	0.0	4.5	1.0	0.0	0.0	0.0	131.4

Experiment 6.2

HRT = 1 hr
BOM = 900 µg/L
Amino Acids = 0.34
Trial Date: December 1996

HPCs

Time	Influent	Effluent	Biofilm
d	CFU/mL	CFU/mL	CFU/cm^2
0.96	7.5E+02	1.9E+03	6.6E+02
2.00	1.6E+04	1.4E+05	4.2E+05
3.21	5.2E+04	8.7E+04	8.0E+05
6.21	6.6E+04	1.0E+06	3.0E+07
8.04	5.0E+04	5.5E+05	4.4E+07
10.08	3.8E+04	8.7E+05	5.5E+07
12.21	3.8E+04	2.5E+05	4.9E+07

AODCs
- did not measure parameter

Average Influent BOM Components, µg C/L

Carboxylic Acids			Aldehydes		Amino Acids			Total
Acetate	Oxalate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
222.1	54.3	58.5	42.1	125.0	125.6	108.1	168.2	904.0

Effluent BOM Components, µg C/L

Time	Carboxylic Acids			Aldehydes		Amino Acids			Total
d	Acetate	Oxalate	Formate	Glyoxal	Formald	Aspartic	Glutamic	Serine	BOM
0.00	227.2	14.7	51.9	13.8	45.5	-	-	-	353.1
1.00	225.1	11.3	57.7	8.9	51.0	0.0	0.0	0.0	354.0
1.33	179.1	8.2	47.0	13.5	93.9	-	-	-	341.7
2.04	180.6	9.5	31.9	8.5	60.5	45.3	35.3	83.1	454.7
3.04	19.5	53.8	6.4	0.0	0.0	1.4	1.1	2.7	84.9
6.17	24.9	0.0	7.3	15.5	28.4	4.8	4.6	6.6	92.1
8.00	15.8	4.1	12.9	2.3	40.9	10.6	7.6	14.3	108.4
9.06	16.3	0.0	8.3	35.7	18.5	2.7	2.2	3.1	86.7

APPENDIX B

EXPERIMENTAL TRIALS OF CHAPTER 7

HRT - AR1. 1 h
HRT - AR2. 2 h
HRT - AR3. 4 h
Trial Date: March, 1997

HPCs (Bulk Cells, CFU/mL and Biofilm Cells, CFU/cm^2)

Time		AR_1		AR_2		AR_3	
d	Influent	Biofilm	Bulk	Biofilm	Bulk	Biofilm	Bulk
0.00	8.3E+02	1.6E+02	9.5E+02	1.3E+02	0.0E+00	0.0E+00	0.0E+00
0.94	2.9E+03	1.5E+04	1.5E+04	1.1E+03	4.5E+03	4.0E+03	1.0E+03
2.04	1.9E+03	1.6E+04	1.9E+04	1.6E+04	6.3E+03	2.0E+05	5.2E+05
3.85	5.3E+04	6.9E+06	2.3E+04	2.6E+06	9.0E+05	2.7E+05	3.0E+06
6.06	6.9E+04	2.4E+07	1.0E+06	4.3E+05	7.7E+05	6.7E+05	1.4E+06
7.98	3.8E+04	4.8E+07	2.1E+06	3.6E+05	8.3E+05	5.6E+05	1.5E+06
11.00	4.7E+04	6.1E+07	9.2E+05	1.6E+06	6.8E+05	1.5E+06	8.3E+05
13.85	8.2E+04	7.8E+07	1.1E+05	2.9E+06	6.6E+05	9.6E+05	8.7E+05
18.17	3.5E+02	3.0E+07	3.8E+05	2.4E+06	5.8E+05	6.4E+05	2.5E+05
			Chlorine Dosing Begins on Day 21				
22.08	5.0E+04	2.7E+07	4.9E+05	4.1E+06	4.9E+06	2.3E+06	5.2E+05
22.92	8.5E+02	1.8E+06	6.3E+04	6.7E+05	5.3E+04	6.0E+05	6.9E+04
26.02	4.5E+02	1.6E+06	8.3E+03	5.9E+05	8.4E+04	3.9E+05	4.1E+05
28.15	6.1E+04	6.7E+05	2.6E+04	3.6E+05	7.0E+04	7.2E+05	1.9E+03
29.83	4.1E+04	7.2E+05	4.1E+04	6.7E+05	5.8E+05	6.1E+05	3.0E+05
33.52	6.0E+04	6.1E+05	2.6E+04	6.7E+05	1.8E+05	5.1E+05	4.4E+05

Carboxylic Acids, µg/L

Time	Acetate				Formate				Oxalate			
d	AR_{1in}	AR_1	AR_2	AR_3	AR_{1in}	AR_1	AR_2	AR_3	AR_{1in}	AR_1	AR_2	AR_3
0.08	0.0	0.0	0.0	0.0	53.9	80.6	100.0	136.0	0.0	0.0	0.0	0.0
2.08	226.2	177.2	148.3	63.6	70.0	99.0	53.0	14.1	52.6	45.5	25.6	0.0
2.69	202.6	104.2	187.0	161.4	180.0	75.0	9.3	0.3	50.8	53.5	56.0	51.1
2.96	110.8	152.1	154.7	166.3	93.8	41.7	5.8	0.0	21.1	36.7	35.7	42.5
3.77	-	466.1	375.2	-	-	50.0	61.3	-	-	58.7	34.7	-
6.65	-	-	368.6	-	-	-	67.9	-	-	-	59.0	-
8.65	-	334.1	-	-	-	27.5	-	-	-	21.2	-	-
12.56	388.7	316.2	91.8	54.1	53.5	21.6	28.5	7.5	66.0	18.8	0.0	0.0
14.81	152.0	326.8	46.8	104.1	18.4	41.2	32.8	20.2	9.1	24.7	21.7	0.0
16.73	98.5	124.3	148.6	57.7	19.0	40.2	3.5	12.7	21.9	28.5	18.6	0.0
					Chlorine Dosing Begins on Day 21							
21.46	250.5	260.8	100.8	69.4	35.5	22.2	8.9	27.1	27.7	6.9	18.7	0.0
24.65	168.2	139.4	134.6	108.2	65.7	23.7	31.5	69.4	19.2	14.5	0.0	0.0
26.77	211.0	55.4	317.4	30.3	50.9	15.6	7.1	4.0	24.9	24.0	11.0	0.0
28.81	205.7	86.0	106.3	73.9	44.8	66.8	33.5	24.5	18.0	13.9	16.0	0.0
29.81	265.5	89.8	36.6	68.6	37.5	19.8	20.8	34.4	28.8	13.8	19.9	0.0
32.79	208.0	34.0	65.8	20.7	53.5	17.1	37.0	19.2	23.8	6.6	0.0	0.0

Aldehydes, µg/L

Time (d)	Formaldehyde				Glyoxal			
	AR_{1in}	AR_1	AR_2	AR_3	AR_{1in}	AR_1	AR_2	AR_3
2.69	89.3	114.0	95.9	82.9	14.6	11.0	13.0	7.3
2.96	91.8	93.6	96.0	-	95.1	55.7	95.1	2.6
6.65	99.3	15.3	4.9	0.0	82.6	8.9	11.6	10.5
8.65	150.6	6.3	0.0	n.d.	85.8	21.6	85.8	2.6
12.56	106.8	0.0	0.0	0.0	65.4	52.1	65.4	49.4
14.81	86.1	0.0	0.0	0.0	48.6	48.6	42.5	40.1
16.73	49.0	0.0	0.0	n.d.	66.7	66.7	8.6	74.8
			Chlorine Dosing Begins on Day 21					
21.46	39.6	0.0	0.0	0.0	46.8	18.1	17.6	46.8
24.65	69.6	0.0	0.0	0.0	9.7	7.4	10.6	4.9
32.79	83.1	6.6	0.0	n.d.	92.4	66.5	77.5	6.3

n.d. - non-detectible peak

Amino Acids, µg/L

Time	Aspartic Acid				Glutamic Acid				Serine			
d	$AR_{1\,in}$	$AR_{1\,out}$	$AR_{2\,out}$	$AR_{3\,out}$	$AR_{1\,in}$	$AR_{1\,out}$	$AR_{2\,out}$	$AR_{3\,out}$	$AR_{1\,in}$	$AR_{1\,out}$	$AR_{2\,out}$	$AR_{3\,out}$
0.08	2.1	12.7	3.1	4.6	2.1	12.1	5.8	15.3	9.4	47.5	14.7	16.7
1.08	1.2	10.7	0.6	3.3	0.6	7.9	0.3	9.7	5.5	24.3	2.8	13.3
2.08	79.3	50.9	36.1	3.6	58.5	38.9	27.4	0.4	167.2	93.2	76.7	15.1
2.69	60.4	28.3	15.7	1.4	47.8	19.7	8.0	1.1	113.9	69.1	66.1	6.9
2.96	36.3	7.0	0.7	1.7	28.3	6.5	0.6	1.8	71.4	16.1	6.9	7.2
3.77	61.5	24.6	10.9	16.1	50.9	18.5	12.0	14.1	131.1	64.3	33.5	40.5
4.71	46.3	8.8	3.4	8.1	37.8	13.5	7.0	15.1	87.6	18.0	6.4	1.3
6.65	52.6	13.9	1.6	40.3	43.5	15.2	1.9	0.0	109.6	36.8	7.5	0.0
8.65	157.0	11.2	3.6	1.2	102.3	14.5	2.6	1.8	278.2	17.9	10.6	4.0
12.56	83.2	17.3	4.4	5.3	58.4	13.1	5.8	8.0	121.1	34.4	2.1	2.5
14.81	40.9	4.5	10.1	0.0	30.3	10.4	10.5	0.0	56.0	0.2	14.4	17.1
16.73	60.1	14.1	1.1	3.1	58.5	20.7	2.8	6.4	78.0	16.8	0.0	0.0
20.75	22.8	5.7	4.6	n.d.	20.8	8.0	7.0	5.8	39.1	21.9	6.0	n.d.
Chlorine Dosing Begins on Day 21												
21.46	35.3	12.3	-	-	31.5	9.0	-	-	64.3	22.9	-	-
24.65	30.6	3.5	24.3	7.6	28.7	6.5	20.9	13.8	62.2	14.1	73.8	1.6
26.77	30.1	2.2	-	7.2	28.4	5.0	-	12.2	62.3	12.5	-	1.6
28.81	2.0	1.9	3.2	5.3	8.9	3.4	6.4	9.4	28.6	1.5	1.3	0.0
29.81	24.7	2.7	9.2	3.3	26.7	5.1	12.1	6.3	75.3	13.8	10.6	20.3
32.79	14.0	1.5	6.7	6.0	18.5	4.1	12.6	8.7	58.1	4.6	1.0	11.3

UV Absorbance, 5 cm cell

Time	UV_{254}				UV_{285}			
d	$AR_{1\,in}$	$AR_{1\,out}$	$AR_{2\,out}$	$AR_{3\,out}$	$AR_{1\,in}$	$AR_{1\,out}$	$AR_{2\,out}$	$AR_{3\,out}$
0.08	0.032	0.018	0.000	0.061	0.031	0.049	0.072	0.265
1.00	0.039	0.000	0.000	0.141	0.020	0.034	0.028	0.239
2.08	0.051	0.022	0.027	0.018	0.032	0.019	0.036	0.036
2.69	0.010	0.000	0.036	0.014	0.026	0.025	0.027	0.033
2.96	-	0.054	0.065	0.059	-	0.026	0.027	0.042
3.77	-	0.021	0.042	0.129	-	0.044	0.069	0.113
4.71	0.006	0.058	0.068	0.025	0.038	0.068	0.073	0.084
6.65	0.017	0.025	0.043	-	0.020	0.044	0.081	0.084
8.65	0.017	0.015	0.037	-	0.027	0.048	0.067	0.057
12.56	0.036	0.015	0.080	0.033	0.032	0.057	0.059	0.072
14.81	0.058	0.089	0.051	0.072	0.027	0.051	0.063	0.056
16.73	0.074	0.106	0.049	0.081	0.026	0.051	0.041	0.060
20.75	0.066	0.084	0.061	0.110	0.037	0.034	0.042	0.050
Chlorine Dosing Begins on Day 21								
21.46	0.059	0.116	0.035	0.413	0.036	0.045	0.072	0.415
24.65	0.160	0.078	0.072	0.087	0.061	0.055	0.063	0.058
26.77	0.066	0.238	0.070	0.129	0.036	0.227	0.057	0.061
28.81	0.074	0.074	0.100	0.115	0.030	0.036	0.051	0.057
29.81	0.125	0.079	0.145	0.112	0.044	0.046	0.066	0.137
32.79	0.118	0.131	0.101	0.151	0.056	0.060	0.076	0.080

Chapter 7 Experimental Trials without Amino Acids

HRT - AR1: 1 h
HRT - AR2: 2 h
HRT - AR3: 4 h
Trial Date: May, 1997

HPCs (Bulk Cells, CFU/mL and Biofilm Cells, CFU/cm^2)

Time		AR_1		AR_2		AR_3	
d	Influent	Biofilm	Bulk	Biofilm	Bulk	Biofilm	Bulk
0.65	4.1E+03	1.1E+03	7.2E+02	1.3E+03	3.0E+05	1.9E+03	0.0E+00
1.71	3.9E+03	1.2E+04	7.2E+04	9.0E+04	3.0E+05	1.9E+03	3.0E+05
3.90	4.4E+04	5.0E+05	8.7E+04	3.8E+06	9.1E+05	5.7E+05	2.2E+06
5.58	3.0E+04	6.8E+05	6.2E+05	4.0E+05	2.5E+04	2.2E+06	1.0E+06
6.65	4.9E+04	1.0E+06	4.5E+05	4.6E+05	4.7E+05	1.1E+06	2.1E+04
8.77	8.2E+04	2.0E+06	9.5E+04	2.3E+06	5.0E+05	2.2E+06	4.8E+05
11.65	7.1E+04	4.3E+06	5.9E+05	5.8E+05	1.0E+06	1.8E+06	8.2E+05
		Chlorine Dosing Begins on Day 14					
14.81	2.4E+03	6.3E+05	1.3E+05	4.7E+05	4.3E+05	3.4E+06	1.2E+06
15.54	6.8E+04	7.4E+05	1.2E+05	6.4E+05	1.2E+05	5.9E+05	4.4E+05
18.75	4.2E+04	9.5E+04	9.2E+04	4.0E+05	9.3E+04	3.6E+05	4.7E+05
21.60	7.6E+03	2.7E+05	1.3E+05	7.4E+05	2.1E+05	6.9E+05	1.0E+05
22.58	2.1E+03	4.0E+05	1.5E+05	6.1E+05	1.7E+05	4.7E+05	3.2E+05
23.63	1.3E+04	4.9E+05	1.6E+05	5.3E+05	1.4E+05	4.0E+05	4.2E+05

Carboxylic Acids, µg/L

Time	Acetate				Formate				Oxalate			
d	$AR_{1,in}$	AR_1	AR_2	AR_3	$AR_{1,in}$	AR_1	AR_2	AR_3	$AR_{1,in}$	AR_1	AR_2	AR_3
0.00	263.2	213.7	263.1	248.7	97.7	93.6	113.7	113.7	76.6	87.3	89.0	66.6
0.56	329.9	220.3	195.6	248.7	62.0	54.5	84.1	23.2	64.8	92.7	67.1	84.3
1.63	125.6	158.4	229.2	163.1	40.1	92.5	59.5	21.0	11.7	45.0	3.1	5.7
2.83	89.6	147.5	209.5	163.9	48.3	48.3	33.3	12.5	40.0	68.2	65.9	86.6
5.50	149.2	85.0	88.0	190.2	48.4	68.4	58.9	105.2	53.7	6.3	0.0	0.0
6.52	224.3	209.4	145.3	80.8	72.8	32.7	11.7	20.3	53.7	39.4	0.0	0.0
8.81	276.3	217.9	137.4	70.8	68.5	27.0	9.6	28.4	46.1	0.0	0.0	11.7
11.63	179.8	210.9	108.0	2.3	79.5	35.9	9.3	2.5	76.0	0.0	16.5	24.8
					Chlorine Dosing Begins on Day 14							
14.67	196.6	193.4	113.9	93.2	64.3	32.8	10.0	7.2	83.5	22.2	0.0	0.0
15.52	240.7	180.8	113.3	100.3	59.0	12.4	11.3	4.1	32.3	4.0	0.0	0.0
18.75	195.7	188.5	109.0	90.6	87.6	76.0	16.9	3.3	84.1	8.2	12.8	6.2
20.75	190.9	219.2	141.7	52.0	86.7	43.7	9.9	6.7	82.8	14.8	0.0	9.3
21.60	247.2	209.0	99.9	40.2	80.6	21.3	9.9	5.6	52.1	9.2	0.0	0.0
22.65	248.2	205.9	120.5	28.9	77.4	3.9	5.0	16.5	97.8	0.0	0.0	0.0
23.65	242.5	167.5	85.9	28.4	91.8	0.0	1.5	0.0	75.1	0.0	0.0	0.0

Aldehydes, µg/L

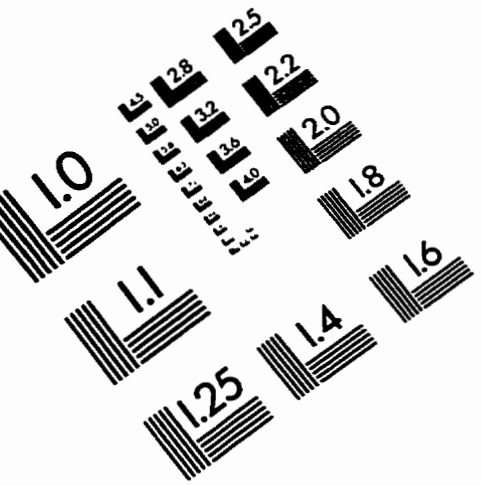
Time (d)	Formaldehyde				Glyoxal			
	$AR_{1\,in}$	AR_1	AR_2	AR_3	$AR_{1\,in}$	AR_1	AR_2	AR_3
0.00	146.8	137.6	121.1	213.5	133.8	15.2	133.8	49.1
0.56	162.7	133.1	129.2	170.1	61.6	20.3	61.6	16.8
1.63	133.1	100.4	54.2	35.1	18.8	12.2	10.0	29.3
2.83	99.6	29.6	0.7	0.0	3.8	4.3	15.7	2.6
5.50	100.0	13.9	0.0	0.0	13.3	10.6	20.2	20.3
6.52	145.4	11.9	0.0	0.0	23.9	18.1	23.9	22.5
8.81	132.5	12.8	0.0	0.0	32.1	6.2	21.6	3.3
11.63	131.3	3.5	0.0	0.0	6.1	8.9	6.5	16.4
Chlorine Dosing Begins on Day 14								
14.67	130.1	17.0	0.0	0.0	18.8	15.0	73.2	17.9
18.75	132.5	20.6	0.0	0.0	114.5	32.0	14.8	66.6
20.75	129.6	14.3	0.0	0.0	16.1	31.4	16.1	21.6
22.65	138.6	37.8	8.6	0.0	7.5	17.2	6.0	28.2

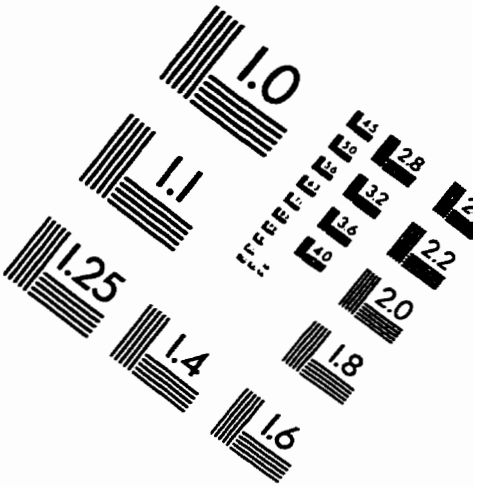
Amino Acids, µg/L - not present in the influent feed stock

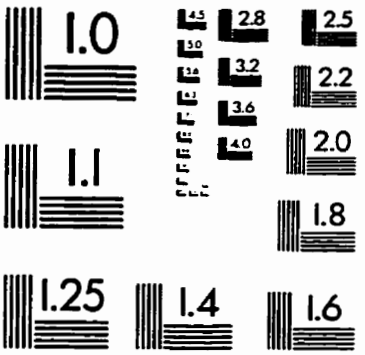
UV Absorbance, 5 cm cell

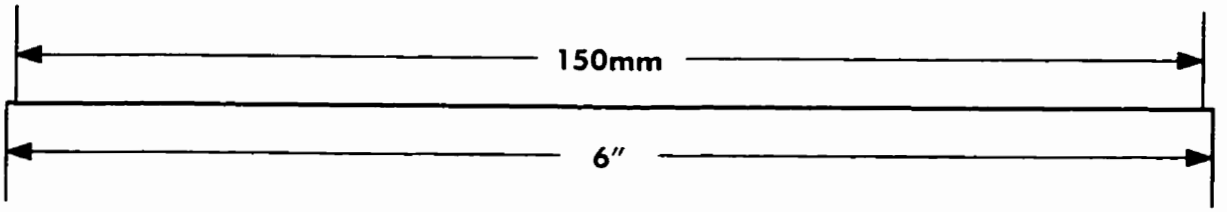
Time	UV_{254}				UV_{285}			
d	$AR_{1\,in}$	$AR_{1\,out}$	$AR_{2\,out}$	$AR_{3\,out}$	$AR_{1\,in}$	$AR_{1\,out}$	$AR_{2\,out}$	$AR_{3\,out}$
0.00	0.048	0.047	0.112	0.238	0.050	0.065	0.148	0.476
0.56	0.044	0.043	0.058	0.198	0.048	0.052	0.068	0.375
1.63	0.034	0.031	0.084	0.065	0.047	0.047	0.053	0.063
2.83	0.031	0.055	0.063	0.043	0.055	0.049	0.061	0.063
5.50	0.044	0.029	0.049	0.078	0.054	0.048	0.062	0.079
6.52	0.041	0.030	0.021	0.075	0.049	0.060	0.063	0.077
8.81	0.051	0.062	0.077	0.079	0.060	0.058	0.067	0.080
11.63	0.035	0.054	0.065	0.088	0.057	0.063	0.072	0.080
Chlorine Dosing Begins on Day 14								
14.67	0.077	0.060	0.043	0.048	0.056	0.058	0.081	0.086
15.52	0.135	0.050	0.085	0.084	0.144	0.065	0.080	0.093
18.75	0.053	0.084	0.075	0.043	0.054	0.068	0.077	0.078
20.75	0.046	0.054	0.051	0.081	0.056	0.055	0.068	0.069
21.60	0.079	0.086	0.060	0.093	0.054	0.058	0.072	0.080
22.65	0.065	0.068	0.071	0.071	0.050	0.057	0.075	0.084
23.65	0.068	0.083	0.090	0.089	0.053	0.061	0.076	0.084

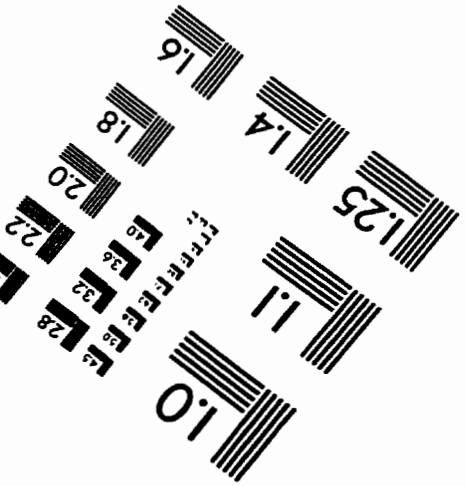
IMAGE EVALUATION TEST TARGET (QA-3)

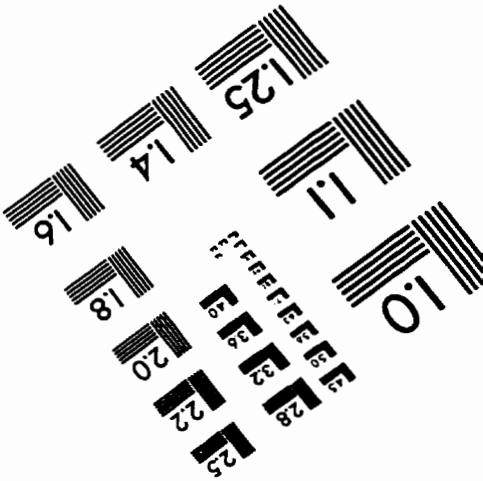












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