Identification and correlation of disinfection byproducts and total organic halogen precursors in a biofilm matrix

Mohd Yahya Khan

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A Thesis

entitled

Identification and Correlation of Disinfection Byproducts and Total Organic Halogen Precursors in a Biofilm Matrix

by

Mohd Yahya Khan

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Master of Science Degree in Chemical Engineering

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The University of Toledo
August 2014
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An Abstract of
Identification and Correlation of Disinfection Byproducts and Total Organic Halogen Precursors in a Biofilm Matrix

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Mohd Yahya Khan

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Master of Science Degree in Chemical Engineering

The University of Toledo

August 2014

Disinfection by-products (DBP) formation in drinking water systems is a persistent issue for water utilities. Although DBP formation is complex due to the multitude of chemical and biological interactions that occur, unremoved natural organic matter (NOM) entering the water distribution system is generally regarded as the primary precursor for DBP formation. In addition, NOM also provides nutrients that support microbial growth and persistent biofilm formation. Biofilm formation is widespread within the water distribution system due to the continuous influx of unremoved NOM. Biofilm and its associated extracellular polymeric substances (EPS) provide a dynamic repository for organic matter accumulation, and can act as a DBP precursor. Trihalomethanes (THMs) and Haloacetic acids (HAAs) represent the major classes of regulated DBPs, yet there are several others that form due to the complex interaction between the organic matter and the disinfectants. The unknown total organic halogens (UTOX) is believed to contain toxicologically vital compounds. Until recently, there have been no reliable studies analyzing the relative contributions of biofilm and its associated DBP precursors to DBP formation and speciation, and how these different precursors contribute to the total organic halogen.
halogen (TOX) formation. This work seeks to abridge this knowledge gap by analyzing the DBP formation from chlorination of biofilms in simulated water distribution systems. The results of this study provide critical information about potential contributions of biofilms to the formation of DBPs and UTOX in the distribution systems and can help water utilities better control the levels of both regulated and unregulated DBPs while at the same time reducing health risks associated with DBPs. To help elucidate this interaction, heterotrophic plate counting (HPC) of bacterial colonies in different pipe materials under different chlorine residuals were conducted. Additionally, DBP and TOX formation tests were conducted and correlated with parallel factor analysis (PARAFAC) of fluorescent dissolved organic carbons.

The obtained results suggest that depending on the pipe material, the accumulation of organic matter in biofilm matrix contributes significantly towards DBP formation. Corrosion of iron pipes provides not only more opportunity for growth of biofilm, but also increased adsorption sites for humic substances, both of which lead to increased DBP and UTOX formation. Overall, strong evidence of biofilm contribution to DBP formation in drinking water distribution systems suggests that water utilities need to carefully consider biofilm eradication methods to minimize the subsequent formation of toxic compounds.
Acknowledgements

I express profound gratitude to my advisor, Dr. Youngwoo Seo, Associate Professor for the Chemical and Environmental Engineering Department at The University of Toledo in Toledo, Ohio, for his constant supervision and valuable guidance. This small endeavor would not have seen the light of the day without his constant scientific support and fruitful discussions in various capacities. I would also like to thank the National Science Foundation (award number: CBET1236433) for providing research funding for this work.

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I would also like to extend my whole-hearted thanks and gratitude to other members of laboratory: Zhikang Wang, who helped in the setup of the experiment, and Steven Cummins, for having provided invaluable help in conducting biofilm analysis. Also, I would like to thank Dr. Guanghui Hua (South Dakota State University) and his graduate student Mr. Shamimur Rahman for their untiring help in conducting TOX analysis.

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# Table of Contents

Abstract........................................................................................................................................... iii

Acknowledgements ......................................................................................................................... v

Table of Contents .......................................................................................................................... vi

List of Tables ................................................................................................................................... viii

List of Figures ............................................................................................................................... ix

List of Abbreviations ..................................................................................................................... x

List of Symbols ............................................................................................................................. xii

1 Literature review ........................................................................................................................ 1

1.1 Natural organic matter ........................................................................................................ 1

1.2 Disinfection byproducts ...................................................................................................... 2

1.3 Biofilm formation ................................................................................................................. 4

1.4 NOM Classification ............................................................................................................. 6

1.5 Fluorescence spectroscopy .................................................................................................. 7

1.6 PARAFAC analysis ................................................................................................................. 8

1.7 Total organic halogen (TOX) analysis .................................................................................. 9

1.8 Effects of biological activity and disinfectant residual on corrosion .................................. 10

1.9 Current understanding ......................................................................................................... 14

2 Introduction ................................................................................................................................ 16

3 Materials and methods ............................................................................................................. 20
3.1 Reactor operations ........................................................................................................20
3.2 Reactor sampling ..........................................................................................................24
3.3 Biofilm analysis ...........................................................................................................24
3.4 DBP analysis .................................................................................................................25
3.5 DBP formation and yield tests .....................................................................................26
3.6 Fluorescence spectroscopy and PARAFAC analysis .....................................................27
3.7 TOX analysis ...............................................................................................................27

4 Results and discussion .....................................................................................................28
4.1 HPC Results ................................................................................................................28
4.2 DBP formation upon chlorination in bulk phase ............................................................30
4.3 DBP Speciation ............................................................................................................38
4.4 DBP formation potential of biofilm and biofilm associated humic substances ..........43
4.5 Parafac analysis ..........................................................................................................49
4.6 TOX analysis ..............................................................................................................54

5 Conclusions ..................................................................................................................58

6 Future work ...................................................................................................................61

References .........................................................................................................................62
List of Tables

1.1 Common DBPs analyzed during the study ..........................................................3
1.2 Commonly reported excitation/emission NOM fluorophores ..........................8
3.1 Table of Reactor description ........................................................................22
4.1 Average and standard deviation values in log CFU .....................................30
4.2 Percentage DBP and unknown total organic halogen variation ..................55
List of Figures

1-1 Corrosion products and biofilm interaction.................................................................13
3-1 Experimental setup.................................................................................................22
3-2 Experimental setup.................................................................................................23
3-3 Coupon holders.......................................................................................................23
4-1 DBP formation results ..........................................................................................31
4-2 Humic substances DBP formation potential .........................................................34
4-3 THM Speciation .....................................................................................................39
4-4 HAA Speciation......................................................................................................40
4-5 HK and CH Speciation............................................................................................41
4-6 N-DBPs Speciation.................................................................................................42
4-7 DBP formation potential test ................................................................................45
4-8 DBP formation potential test ................................................................................46
4-9 Contour plots..........................................................................................................51
4-10 Percentage distribution using Fmax........................................................................51
List of Abbreviations

AOC ......................... Assimilable organic carbon
BDOC ....................... Biodegradable organic carbon

CDC ........................ Center for disease control
CDOM ....................... Colored dissolved organic matter
CF ............................ Chloroform
CFU .......................... Colony forming units
CH ............................ Chloral hydrate
CIC .......................... Chemical induced corrosion
CP ............................ Corrosion products

DBP .......................... Disinfection byproduct
DOC .......................... Dissolved organic carbon
DOM .......................... Dissolved organic matter

EEM .......................... Excitation and emission matrix
EPA .......................... Environmental protection agency
EPS .......................... Extracellular polymeric substance

FEEM ........................ Fluorescence excitation emission matrix
Fmax ........................ Maximum fluorescent intensity

GAC .......................... Granular activated carbon
GC .............................. Gas chromatography

HAA .......................... Haloacetic acid
HA .............................. Humic acid
HK ............................. Haloketone
HPC .......................... Heterotrophic plate counting

MCL .......................... Maximum Contaminant Level
MIC .......................... Microbial induced corrosion
MTBE ........................ Methyl tert-butyl ether

NDBP ........................ Nitrogenous disinfection byproduct
NOM ....................... Natural organic matter
PARAFAC .................. Parallel factor analysis
PVC .......................... Polyvinyl carbonate
THM ......................... Trihalomethane
TTHM ........................ Total trihalomethane
TOC .......................... Total organic carbon
TOX .......................... Total organic halogen
UTOX ........................ Unknown total organic halogen
List of Symbols

°C.................................Degree Celsius
dm ..............................decimeter
g .........................gram
µg ..............................microgram
L ..............................Liter
mg ..............................milligram
nm ..............................nanometer
% ..............................percentage
Chapter 1

Literature review

1.1 Natural organic matter (NOM)

NOM is a heterogeneous mixture of aromatic and aliphatic organic compounds containing oxygen, nitrogen, and sulfur functional groups (e.g., carboxyl, phenol, enol, alcohol, carbonyl, amine, and thiol). They are long-chain molecules having molecular weights ranging from 500 to 5,000 g mole$^{-1}$ [1]. Most of the organic matter is derived from both terrestrial and aquatic microorganism sources, however human activity is also a source of NOM [2].

NOM is a major concern in drinking water treatment since it causes adverse aesthetic qualities such as color, taste, and odor. Presence of NOM also affects water treatment steps such as granular activated carbon filtration and membrane filtration [3, 4].

The types of organics commonly found in finished water include humic and non-humic substances. Humic substances and humic-bound organics comprise the majority of organics found in treated water [5]. Humic substances are naturally occurring organic materials that result from the decomposition of vegetative material and residues [1, 4, 6]. The non-humic substances may include carbohydrates, proteins, and lipids [5].
1.2 Disinfection by-products (DBPs)

Despite numerous techniques and unit operations employed by drinking water utilities to remove NOM, some residual NOM survives and enters into the water distribution system. The concentration and quality of organic material entering water distribution systems are greatly impacted by water treatment processes, which yield a lower molecular weight biodegradable dissolved organic carbon (BDOC).

NOM reacts with disinfectants to produce undesirable DBPs [7, 8]. DBP formation in drinking water systems is a persistent issue for water utilities, as it raises public health concerns because these are highly carcinogenic and mutagenic to end users.

The regulated DBP species for drinking water systems using chlorine as a disinfectant include four Trihalomethanes (THMs) and five Haloacetic acids (HAAs) species: THM4 [chloroform (CHCl₃), bromodichloromethane (DBCM), dibromochloromethane (DBCM) and bromoform (CHBr₃)], and HAA5 [monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), monobromoacetic acid (MBAA) and dibromoacetic acid (DBAA)].

In Stage 2 D/DBP Rule, the Maximum Contamination Level (MCL) for four THMs and five HAAs is set at 80 μgL⁻¹ and 60 μgL⁻¹ based on a locational running annual average [9]. The US EPA has also established a set of criteria for general precursor removal using TOC as the surrogate for DBP precursors [10]. In light of the regulation related to DBPs as well as the health risks associated with them, it is critical to understand the reactivity of NOM with disinfectants, such as chlorine, in order to best control the formation of
hazardous DBPs. Some of the DBPs which have been analyzed in this study have been listed in the Table 1-1.

Table 1-1: Common DBPs analyzed during the study

<table>
<thead>
<tr>
<th>DBPs categories</th>
<th>DBP class</th>
<th>DBP name</th>
<th>DBPs abbreviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trihalomethanes</td>
<td>Chloroform</td>
<td>Bromodichloromethane</td>
<td>TCM</td>
</tr>
<tr>
<td></td>
<td>Dibromochloromethane</td>
<td>DBCM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bromoform</td>
<td>Bromoform</td>
<td>BDCM</td>
</tr>
<tr>
<td></td>
<td>Monochloroacetic acid</td>
<td>MCAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dichloroacetic acid</td>
<td>DCAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Monobromoacetic acid</td>
<td>MBAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bromochloroacetic acid</td>
<td>BCAA</td>
<td></td>
</tr>
<tr>
<td>Regulated</td>
<td>Dibromoacetic acid</td>
<td>DBAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trichloroacetic acid</td>
<td>TCAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bromodichloroacteci acid</td>
<td>BDCAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dibromochloroacteci acid</td>
<td>DBCAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tribromoacetic acid</td>
<td>TBAA</td>
<td></td>
</tr>
<tr>
<td>Haloactetic acids</td>
<td>Dibromoacetic acid</td>
<td>DBAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trichloroacetic acid</td>
<td>TCAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bromodichloroacteci acid</td>
<td>BDCAA</td>
<td></td>
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<tr>
<td></td>
<td>Dibromochloroacteci acid</td>
<td>DBCAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tribromoacetic acid</td>
<td>TBAA</td>
<td></td>
</tr>
<tr>
<td>Haloacetonitrile</td>
<td>Dichloroacetonitrile</td>
<td>DCAN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trichloroacetonitrile</td>
<td>TCAN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bromochloroacetonitrile</td>
<td>BCAN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dibromoacetonitrile</td>
<td>DBAN</td>
<td></td>
</tr>
<tr>
<td>Halonitromethane</td>
<td>Trichloronitromethanes (Chloropicrin)</td>
<td>TCNM</td>
<td></td>
</tr>
<tr>
<td>Unregulated</td>
<td>1,1-dichloro-2-propanone</td>
<td>1,1-DCP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,1,1-trichloro-2-propanone</td>
<td>1,1,1-TCP</td>
<td></td>
</tr>
<tr>
<td>Haloaldehydes</td>
<td>Chloral-hydrate (trichloracetaldehyde)</td>
<td>CH</td>
<td></td>
</tr>
</tbody>
</table>
1.3 Biofilm

NOM may not only increase DBP formation through the reaction with a disinfectant, but also serve as nutrient sources for microbial biofilm growth. Biofilm is a unique system with many physical, chemical, and biological properties that aid microorganism to accumulate and colonize on the interior surfaces of drinking water pipes, thereby making themselves ubiquitous in the water distribution system. Once attached, they develop a physical and chemical structure that enables them to modify the microenvironment of the pipe surface in a manner that allows them to optimize their metabolism and become highly resistant to disinfectants [11, 12]. Biofilms can be home to various microorganisms that include heterotrophic bacteria, conforms, actinomyces, molds, fungi, nitrifying bacteria, iron oxidizing bacteria, sulfate reducing bacteria, and possibly act as a reservoir for Giardia cysts and Cryptosporidium oocysts [5].

The attachment and colonization of microorganisms on pipe surfaces are also problematic because many microorganisms produce metabolic by-products, and in many cases cause prolific occurrences of coliform bacteria in the tap water. These two problems are responsible for the leading consumer complaints (taste and odor) and the leading violation of the Safe Drinking Water Act (coliforms) [5]. Hence, it can be comfortably stated that biofilm formation and proliferation is ubiquitous in water distribution systems and poses a constant challenge to many water utilities. Biofilm consists of aggregated microbial cells embedded with a matrix of extracellular polymeric substances (EPS) [13, 14] composed of various biomolecules such as nucleic acids, proteins, polysaccharides,
and lipids [15]. Since biofilm EPS also is a reservoir of NOM and humic substances, it can serve as a DBP precursor [16].

EPS production will vary in composition and quantity depending on the bacteria present and may be influenced by environmental conditions. EPS and the accumulation of corrosion products and particulates exert a significant disinfectant demand and provides a shield that protects microorganisms from lethal levels of disinfectants [17-19].

Currently, a common biofilm control practice employed by water utilities include applying high doses of residual chlorine to maintain the biostability in water distribution systems [20]. However, given the rapid reaction between chlorine and biomass, continuous maintenance of disinfectant residuals may contribute to DBP formation exceeding the limits of US Environmental Protection Agency (EPA) DBPs regulations [21].

Although 90% of biomass in biofilm is contributed by EPS, which constitute organic composition and chemical structure similar to humic substance like DBP precursors [22, 23], the biological contributions to DBP formation in distribution systems have been largely overlooked, although previous studies reported increased DBP formation in the distribution system assuming organic deposits on pipe walls were the culprit [24-26].

Microbial related water quality issues have become an area of interest in recent years because the majority of water distribution pipelines are made up of iron and ferrous materials, which were historically found to support greater populations of microorganisms despite the presence of disinfectants [27].
1.4 NOM classification

NOM removal optimization in a drinking water treatment plant requires an understanding of its specific character which will likely determine its treatability [28].

It has been reported that NOM is a heterogeneous mixture of aliphatic and aromatic polymer [29], and can be operationally divided into two fractions: hydrophobic and hydrophilic. Hydrophobic fractions primarily consist of humic and fulvic acids [30]. Hydrophilic fractions are those comprised of carbohydrates with low molecular weight, proteins, and amino acids [31]. Hydrophobic NOM contains more aromatic carbon than hydrophilic NOM [32]. The reactivity of hydrophobic NOM with chlorine has been extensively studied. Comparatively few studies have been conducted to investigate the reactivity of hydrophilic NOM [33, 34].

Since both drinking water treatment process and water quality are affected by the quantity and characteristic of NOM, it is important to study the characteristic of NOM in order to design the efficient drinking water treatment. It is not realistic to characterize NOM on the basis of a thorough compilation of the individual compounds, considering the large number of individual compounds it has. Therefore, researchers have found it more practical to characterize NOM according to operationally defined chemical groups having similar properties [35, 36]. Dissolved organic material (DOM) in natural waters contains a group of light-absorbing components in the wavelength range of 200–800 nm, namely colored dissolved organic matter (CDOM) [37]. Its characterization can be achieved through the use of fluorescence spectroscopy [38, 39].
1.5 Fluorescence spectroscopy

Fluorescence spectroscopy can be used for characterizing the constituents of DOM according to the pattern and intensity of emitted light using fluorescence excitation emission matrices (FEEMs) coupled with parallel factor analysis (PARAFAC). FEEMs are generated by combining emission scans with excitations over a range of wavelengths [38, 40, 41].

The wavelength at which absorption (excitation) and emission occur is specific to the molecule [42]. Different compounds and compound groups exhibit unique fluorescence signatures that allow them to be distinguishable from one another [43].

In a study of sewage impacted rivers using FEEM spectroscopy, protein fluorescence was found to be a better indicator of sewage pollution than ultra-violet (UV) absorbance at 254 nm (UVA 254) [44].

Three fluorescent amino acids (tryptophan, tyrosine and phenylalanine) are indicative of proteins and peptides which provide evidence for a bacterial origin organic matter [45-47].

A limited number of studies have reported the use of fluorescence analysis for the assessment of engineered systems including drinking water treatment processes [48]. However in the aquatic systems, with the use of fluorescent spectroscopy, aromatic DOM has been found to form DBPs preferentially compared with microbial DOM [49]. Fluorescence characterization of NOM can be useful in determining its treatability.

Devices that measure fluorescence are called fluorimeters. Fluorescence technique is a simple, relatively inexpensive, and very sensitive tool that requires little or no sample
pre-treatment. Fluorescence spectroscopy involves a beam of light, usually ultraviolet light or xenon, that excites the electrons in molecules of certain compounds and causes them to emit light [3].

1.6 PARAFAC Analysis

“Parallel factor analysis (PARAFAC) is a multi-way data analysis which is applied to characterize DOM fluorescence properties by decomposing the fluorescence matrices into different independent fluorescent components.” [38, 40].

FEEM analysis is based on the realization that several components of NOM fractions exhibit unique fluorescence signatures that allow them to be distinguished from each other in a fluorescence spectrum [43]. Through FEEMs, typically humic acid, fulvic acid, and aromatic proteins (tryptophan or tyrosine-like) compounds are identifiable [50, 51]. Commonly reported excitation/emission fluorophores are which have been complied by Peleato et al. (2013) are presented in Table 1-2 [52].

Table 1-2: Commonly reported excitation/emission NOM fluorophores

<table>
<thead>
<tr>
<th>Compound group</th>
<th>Excitation/Emission wavelength (nm)</th>
<th>Description and source</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein-like</td>
<td>275/&lt;300</td>
<td>Amino acids, free or bound proteins</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td>275/310</td>
<td>Tyrosine-like</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td>275/340</td>
<td>Tryptophan-like</td>
<td>[38]</td>
</tr>
<tr>
<td>Humic-like</td>
<td>260/380-460</td>
<td>Terrestrial humic like</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td>350/420-480</td>
<td>Terrestrial humic like</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td>300-370/400-500</td>
<td>Humic-like</td>
<td>[88]</td>
</tr>
</tbody>
</table>
1.7 Total organic halogen (TOX)

There is ample evidence from toxicological and epidemiological studies that chlorination of drinking water produces halogenated compounds that are hazardous to consumers. It is now well accepted that ingestion of chlorinated drinking water has caused elevated levels of bladder cancer and adverse reproductive effects in the US population [53]. While many of the identified DBPs are known human carcinogens, none have been clearly identified as the source of the observed chronic or acute effects.

THMs and HAAs are the most abundant halogenated DBPs resulting from free chlorination, and are easily measured. However, most agree that they do not appear to be responsible for human toxic endpoints such as increased incidences of bladder cancer. For this reason, NOM may be viewed as surrogates for the DBPs of greatest health concern. There are several halogenated compounds that are formed apart from regulated DBPs, and these unknown halogenated compounds may be categorized as total organic halogen (TOX).

TOX measurement provides an estimate of the total amount of organically-bound (i.e., by covalent C-X bonds) chlorine, bromine, and iodine in a dilute water sample [54]. When chlorine is used, THM and HAA together account for roughly 50% of the TOX [55]. However the unknown TOX (UTOX) not attributed to prominent DBPs like THMs and HAAs) is also believed to contain toxicologically vital compounds. Despite their ubiquitous nature in the distribution systems, the contribution of biofilms to DBP and TOX formation is poorly characterized.
Hence it has been proposed that TOX would be a better surrogate for hazardous DBPs than the currently regulated compounds. Most agree that TOX encompasses the most toxic compounds along with many that are not of concern. Toxicologists have shown that some brominated, and iodinated DBPs are more toxic than their chlorinated counterparts. Partly for this reason, there has been an interest in developing a halogen-specific TOX method. Recognizing the issue of unknown toxic by-products, the US EPA has also established a set of criteria for the general precursor removal using TOC as the surrogate for DBP precursors [10]. Enhanced coagulation requirement in US EPA’s D/DBP rule is aimed at limiting the formation of UTOX, hence continuous monitoring of UTOX is required which is achieved by comparing the TOX values with the known DBPs.

It is clear that in this time of rapid changes in the US disinfection practice, there is a need for a better understanding of the importance of unidentified byproducts. Although TOX levels are not regulated, their determination represents a valuable measurement of global formation of known and unknown halogenated compounds. Association between TOX formation and PARAFAC components in the DOM pool should be studied in order to determine predictive capability of these parameters.

1.8 Effects of biological activity and disinfectant residual on corrosion

Bacterial communities in water distribution system is the single most significant issue confronted by water utilities due to a wide range of complications generated by it. Many
studies have reported widespread presence of microbes in water distribution system [56-59]. Along the same lines, there is widespread presence of corrosion products in the distribution system [56, 58]. These two factors can together interact in a number of ways and impact water distribution system.

Some authors have reported that the growth of a bacterial biofilm on a pipe wall may serve as a barrier to corrosion [60]. Corrosion can also be initiated by microbes by the creation of areas with differential concentrations of oxygen, minerals and metals. Even reactions associated with corrosion could be catalyzed by some microbes. The biofilm matrix develops a variety of niches that favor the metabolisms of aerobic, facultative, and anoxic microorganisms [5]. Thus, the role of biological activity in a water pipe can be mixed, but is generally considered to be detrimental to most aspects of iron corrosion.

Some bacteria like Gallionella have been reported to oxidize Fe(II) to a trivalent Fe(III) and can affect the structural characteristics of corrosion scales [61]. On the other hand some bacteria can affect iron speciation by reducing Fe$^{3+}$, while many others can oxidize Fe$^{2+}$ [62-64]. Some bacterial community can affect water characteristics like oxygen concentration which in itself can create corrosion problems [65], while other species of bacteria can cause pH variations, giving one other cause for corrosion [66] and finally some bacteria have been found to produce corrosive metabolites such as H$_2$S [66] or iron phosphide [67-71].

Microbial growth could also be supported by increased corrosion, where additional growth environment is provided by corrosion products. Corrosion products also reduce disinfectant efficiency by reacting with it [72, 73]. Corrosion product mass has direct influence on the biofilm growth that can be supported by a pipe surface at a given chlorine
residual [5]. From this it can be inferred that unlined iron pipes are typically more likely to have higher occurrences of biofilm associated problems.

The formation of corrosion products in water distribution systems results from the release of \( \text{Fe}^{2+} \) ions from the pipe surface that react with various electron acceptors such as carbonate, oxygen, and free chlorine in the bulk fluid. Dissolved iron (\( \text{Fe}^{2+} \)) can be released from the pipe by [74]:

(1) Microbiologically influenced corrosion (MIC), or by

(2) Chemical induced corrosion (CIC).

MIC is caused by the chemical gradient that develops between the pipe surface beneath a microbial colony and the bulk fluid. The oxygen gradient creates an electrochemical cell that causes the pipe to release a \( \text{Fe}^{2+} \) ion and two electrons [74, 75].

CIC is the result of an electrochemical potential differences between the ions present in the bulk fluid and the pipe surface, which promotes the release of the more thermodynamically stable form of iron (\( \text{Fe}^{2+} \)) and electrons from the pipe surface. \( \text{Fe}^{2+} \) ions from the pipe surface that can react with various electron acceptors such as carbonate, oxygen, and free chlorine in the bulk fluid to form precipitated corrosion products, or chelates with organic compounds in the bulk fluid or the biofilm matrix. A corrosion tubercle will typically have a layered structure [76, 77].

It has been reported that even at high disinfectant doses, iron materials have also been found to support biofilm growth [5]. This increase in biofilm density even with a chlorine residual can be attributed to: (a) Increased release of \( \text{Fe}^{2+} \) from the pipe surface resulting from increased chloride levels (b) Increased corrosion product formation associated with the presence of powerful oxidants such as free chlorine.
Another possible explanation could be that the presence of chlorine results in increased bioavailable organics in the finished water. That is large sized organics are broken down to smaller more easily available organic [78]. On iron pipe surfaces this can actually serve as food for bacterial community growth and can increase the biofilm densities [79-82]. Another interesting study [74] suggested that the terminal pathway of corrosion products in a distribution system will typically lead to the formation of goethite. When chlorine is added as a disinfectant, the pathways of goethite formation are significantly increased. The addition of chlorine, or other powerful disinfectants, is known to break down complex organics to a more bioavailable form of carbon. Hence, increasing the potential of biological activity. The increased bioavailability of carbon substrate resulting from disinfection, coupled with benefits of the physical and chemical (adsorption) properties of goethite, creates an environment that is well suited for biofilm survival. Figure 1-1 shows corrosion products and biofilm interactions in a typical submerged corroded pipe system.

![Figure 1-1: Corrosion products and biofilm interaction](image-url)
1.9 Current understanding

Generally, the DBP and TOX formation in the water distribution system is not well understood due to the complexity of biological and chemical interactions that are occurring. There has been a clear disconnect concerning the relationship between NOM, biofilm, DBP, and TOX in water distribution networks until recently. Research proves that biofilm may act as a DBP and TOX precursor, making the route from the water treatment plant to the public’s water faucet a much more complex area than previously considered. The relative contribution towards DBP and TOX formation and speciation as a result of the reaction between these precursors and disinfectant is still not very well understood, and hence is one of the focuses of this study.

Since it is important to create the best quality water exiting the water treatment complex, it becomes inevitably important to understand the mechanisms in play from plant to faucet. Arguably, considering the long residence times and increasingly poor quality of infrastructure in many water distribution networks, this study becomes extremely relevant. The more we understand about the interactions between biofilm and pipe surfaces in water distribution system, the better quality water could be provided to the end user.

Until recently, there have been no reliable studies analyzing the relative contributions of biofilm and its associated humic substances to DBP and TOX formation and speciation, and how these different precursors contribute to the unknown TOX formation. This work seeks to remedy this knowledge gap by analyzing the DBP and TOX formation from chlorination of bulk phase liquid and biofilms in simulated water.
distribution systems. At the same time, another aim was to characterize the organic precursors.
Chapter 2

Introduction

The primary purpose of disinfection in water treatment process is to kill or inactivate any pathogenic microorganisms during the water treatment. The second purpose is to provide a safe guard by suppressing microbial re-growth in the water distribution system, which makes disinfection critical to protect the public from diseases. However, disinfectants like chlorine also react with organic matter and produce various organic and inorganic by-products known as DBPs. DBPs have been reported to cause adverse health effects [83-85]. Significant efforts have been made to understand and predict DBP formation. The formation of DBPs is constantly monitored in an attempt to control the amount that forms [86, 87].

Apart from this, and despite the use of residual disinfectants, there have been reports from many water utilities in the US that have shown the survival of biofilm in water distribution systems. [88-91]. Injured or inactivated bacteria can re-grow and form persistent biofilm even under the presence of residual disinfectant, which is facilitated by biofilm EPS [92]. Biofilms in the water distribution system can adsorb and subsequently utilize available organic matter for its growth [93-96]. Depending on the biofilm structure (porosity and EPS composition), a large amount of organic precursors can accumulate on
the biofilm surface as well as on the internal structures [97, 98], increasing both disinfectant demand and biofilm growth. In addition to accumulated precursors on biofilm EPS, EPS themselves are composed of a significant concentration of proteins, polysaccharides, lipids, and nucleic acids [99-101].

EPS can also serve as DBP precursors due to similarity in structural and functionality to humic substances. Biomolecules derived from aquatic organisms may pose a greater risk for DBP formation than traditionally investigated NOM due to their higher contribution to toxic nitrogenous DBP (N-DBP) formation [102, 103]. This increase in organic matter concentration through biofilm presents a challenge to utilities as chlorine, the most common disinfectant, reacts with accumulated organic matter on microbial biofilms to form additional DBPs, result of which is that utilities are unable to meet DBP regulations. Therefore, biofilm may contribute the highest concentration of DBP precursors, and biofilm control practices may significantly enhance DBP formation in the water distribution system. However, biological contributions to DBP formation in distribution systems have been largely overlooked, and detailed information regarding the relative DBP contribution by biofilm is not understood.

Also, a large fraction of halogenated compounds being produced in the water distribution system remains undetected because of limitations in available detection techniques. The different DBP precursors could produce a variety of unidentified halogenated DBPs. For these unidentified DBPs, an additional technique, TOX, has been used as a collective measure to represent nearly all halogenated organic compounds in water with quantitative information on unidentified DBPs. Some of these compounds are
responsible for elevated levels of bladder cancer and adverse reproductive effects in the US population [53].

Rossman et al. (2001) hypothesized that the increased halogenated organic compounds concentration in the distribution system was due to a reservoir of organic materials (organic precursors and biofilm) deposited on the pipe [24]. Many older drinking water distribution system pipes are composed of unlined steel, cast, or ductile iron pipes that are subjected to corrosion in the drinking water environment [17, 72]. Iron corrosion can change the pipe surface roughness and can consume residual disinfectant. Iron also enhances bacterial biofilm formation and proliferation by providing additional surface area in distribution systems [83, 84].

Adsorption of humic substances to iron oxides may promote biofilm growth, resulting in an increase of coliform and heterotrophic plate count (HPC) bacteria in drinking water. Therefore a better understanding about the interactions between pipe materials, disinfectants, and natural organic matter, how these factors influence distribution biofilms, and how to determine the best techniques to limit DBP and TOX formation in the water distribution system is needed.

The primary objective of this research was to monitor the influence of biofilm and biofilm associated humic substances grown on common water distribution system pipeline materials on both DBP and UTOX formation.

In this study, annular reactors with removable iron and polycarbonate coupons were operated for four months to establish a confluent biofilm followed by six months of experimental operation under chlorine doses of 0.5, 2, and 4 mg L⁻¹ for two months each. Bacterial activity was measured in both the bulk and biofilm phases using HPC technique.
DBP and TOX formation in bulk, and formation potentials of biofilm and biofilm associated humic substances, were determined to correlate the relative variation of known and unknown DBPs under different chlorine dose over a period of time in the presence of different pipe materials. A wide array of DBPs were analyzed for this study including: trihalomethanes (THM), haloacetic acids (HAA), haloketones (HK), haloacetonitriles (HAN), halonitromethanes (HNM), haloacetaldehydes, and total organic halogen (TOX).

The biofilm’s characteristics were studied with fluorescence spectroscopy, coupled with PARAFAC analysis. DBP formation from the extracted biofilm coupons and measureable DBPs in the bulk were obtained. Also, TOX analysis was conducted to monitor unknown TOX formation. Then, the obtained results were correlated to discover relative contributions of humic and non-humic sources to UTOX and DBP formation.
Chapter 3

Materials and methods

3.1 Reactor operations

Four parallel continuous annular reactors (CDC reactor, Bozeman, MT) were inoculated with mixed species biofilm from a water treatment facility and operated over a ten month period. Two reactors were equipped with iron coupons, and another two with polycarbonate coupons. One of each was disinfected and the others were used as controls. The disinfected reactors were of the gas-tight variety and were operated with no headspace to minimize DBP volatilization. Chlorine was used as a disinfectant. Figures 3-1 and 3-2 show the experimental set-up for a simulated water distribution system. Each reactor was equipped with 21 removable coupons for various analysis (Figure 3-3). The city of Toledo tap water was used as the basis for the feed solution. After treatment in a granular activated carbon (GAC) filter column to remove existing DBPs and residual disinfectant, the solution was combined with humic acid (Sigma Aldrich, MO, USA). Further to simulate the conventional water treatment processes (coagulation, flocculation, sedimentation, and preozonation) jar tests were used to remove the organic matter before using a lab scale ozonator (OZOTECH, CA, USA). Aluminum sulfate (Al$_2$(SO$_4$)$_3$.18H$_2$O) was the coagulant used, and 2 mgL$^{-1}$ of ozone was applied to supernatant from jar tests for 5 minutes using a lab scale ozonator. Total organic carbon (TOC) was maintained
around 2.5-3 mgL\(^{-1}\). Two peristaltic pumps (Masterflux, Cole-Parmer, IL) were used to deliver water to two reactors each at 1 mL min\(^{-1}\). This was designed to create a 12 hour retention time. To try to limit headspace in the reactors, tubing was applied to effluent spouts in such a way to increase the volume of the reactors.

The description of the reactors can be found in Table 3-1. Cl\(_2\) stock solutions were prepared by adding Clorox bleach (Oakland, CA) in deionized (DI) water (18.2 M\(\Omega\cdot\text{cm}\)). Disinfectant stock solutions were stored in separate syringes and delivered to reactors by a syringe pump (Fisher Scientific, USA). Concentrations of chlorine were determined by DPD methods via a spectrophotometer (DR 2800, HACH, USA).

After obtaining confluent biofilm formation for four months, reactors were operated over 6 months under three different disinfectant doses. For the first experimental phase, reactors were operated around 0.5 mgL\(^{-1}\) of residual disinfectant (both Cl\(_2\)) for two months. For the second phase, higher disinfectant residual (2 mgL\(^{-1}\) of Cl\(_2\)) was applied for two months to simulate commonly applied disinfectant residual concentration in water distribution systems. During the third phase, considering biofilm eradication practices with the maximum allowable residual in water distribution system, 4 mgL\(^{-1}\) of Cl\(_2\) was maintained for the last two months [104, 105].
Table 3-1: Table of Reactor description

<table>
<thead>
<tr>
<th>Reactor No.</th>
<th>Operation condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactor 1</td>
<td>Reactor with iron coupons (control)</td>
</tr>
<tr>
<td>Reactor 2</td>
<td>Reactor with iron coupons (chlorinated)</td>
</tr>
<tr>
<td>Reactor 3</td>
<td>Reactor with polycarbonate coupons (control)</td>
</tr>
<tr>
<td>Reactor 4</td>
<td>Reactor with polycarbonate coupons (chlorinated)</td>
</tr>
</tbody>
</table>

Figure 3-1: Experimental setup
Figure 3-2: Experimental setup

Figure 3-3: Coupon holder with iron and polycarbonate coupons
3.2 Reactor sampling

To monitor the influence of biofilm formation on DBP and TOX formation, influent, effluent, and biofilm (from reactor coupons) samples were periodically collected for various analyses during the study. Heterotrophic plate counting (HPC), PARAFAC analysis, total organic carbon (TOC), pH, and disinfectant residuals were measured for reactor effluents. For the DOC analyses, samples were filtered by 0.45 μm nitrocellulose membranes and analyzed using a TOC analyzer (TOCVSH, Shimadzu, Japan).

3.3 Biofilm analysis

HPC was used for biofilm analysis because the presence or absence of coliform bacteria is the primary measurement parameter currently used in the US to predict microbial safety of drinking water in the distribution system [106, 107]. Biofilm samples were taken every two weeks during the reactor operation.

HPC was conducted by immersing the extracted coupon in 10 mL of phosphate buffer, vortex mixing for 2 minutes to remove all biofilm and corrosion products from the coupon into the suspension, and then using 30 seconds of homogenization using a Tissue Tearor (Bio Spec, USA) to make sure any extracted liquid volume is an average representation of the suspension. After serial dilution, R2A agar was plated using spread plate technique and incubated for 2-3 days at 37°C, which was derived from standard plating methods [108].
For every other collection, the extracted coupon would be scrapped off around once a month using a sterilized scalpel. The material being collected underwent the procedure for HPC, DBP formation, and TOX tests.

### 3.4 DBP analysis method

The following DBPs were analyzed in this study: THM4 [chloroform (CF), bromodichloromethane (BDCM), dibromochloromethane (DBCM) and bromoform (BF)], HAA9 [monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), bromochloroacetic acid (BCAA), trichloroacetic acid (TCAA), bromodichloroacetic acid (BDCAA), dibromoacetic acid (DBAA), chlorodibromoacetic acid (CDBAA), and tribromoacetic acid (TBAA)], HAN4 [trichloroactetonitrile (TCAN), dichloroacetonitrile (DCAN) bromochloroacetonitrile (BCAN), and dibromoacetonitrile (DBAN)], HK2 [1,1-dichloro-2-propanone (1,1-DCP) and 1,1,1-trichloropropanone (1,1,1-TCP)], TCNM [Trichloronitromethane] and CH [Chloral hydrate].

Analysis was carried out using a gas chromatograph (GC) (Shimadzu, Japan, GC-2010 Plus) with dual electron-capture detectors (ECD). THM4/HAN4/HK2/TCNM extraction followed EPA method 551.1,[109] where DBPs were extracted from water samples by methyl tert-butyl ether (MTBE), and the organic phase was analyzed. HAA9 were recovered by liquid-liquid extraction with MTBE, followed by methylation with acidic methanol based on EPA method 552.2 with small modifications [110, 111]. For quality assurance, average spike recoveries and method detection limits (MDL) were monitored [21].
3.5 DBP formation and yield test

To monitor the DBP formation and speciation in reactors, samples were collected from reactor effluents every 3-4 days and analyzed for DBP formation. Before DBP analysis, Cl₂ residuals in each sample were quenched, and the samples were analyzed according to the US EPA method [109-111]. To examine DBP formation from biofilm as well as biofilm associated HA, DBP yield (formation potential) tests were also conducted with biofilm samples grown on removable coupons from the reactors. Biofilm samples were firstly removed from coupons using sterilized surgical blades, then a high speed vortex and a mechanical homogenizer were applied to detach biofilm samples from coupons in a certain amount of phosphate buffer (pH=7). Then, homogenized biofilm solution was transferred and mixed with 10 mL phosphate buffer (pH=7) in amber glass vials. Finally, DI water was added to make head space free to minimize volatilization. Samples were exposed to excessive Cl₂ doses following the standard method 5710B [112] and incubated in the dark at room temperature 22 ±2 ºC for seven days. After that, free chlorine concentrations were examined, and then residuals were quenched by NH₄Cl before the samples were ready for DBP analysis.

For iron coupon solution, a magnetic separation technique was used to remove large iron corrosion products in order to prevent any interference to GC operation. To quantify DBP formation from biofilm, DBP formation tests with feed solution (humic solution) were also conducted using different residual disinfectant concentrations (0.5, 2, and 4 mgL⁻¹) for each experimental phase. The results were compared to DBP formations from bulk solutions.
3.6 Fluorescence spectroscopy and PARAFAC analysis

Fluorescence spectroscopy coupled with PARAFAC was used to determine the DBP precursor composition. To minimize fluorescence quenching resulting from the relatively high concentrations of dissolved organic carbon (DOC) (inner filter effects), the prefILTERED samples were diluted to a DOC concentration of 1 mg TOC L⁻¹ [3].

Fluorescence intensities were measured at room temperature (27°C). EEMs were generated for each sample by scanning over excitation wavelengths between 250 and 400 nm at intervals of 10 nm, and emission wavelengths between 260 and 540 nm at intervals of 2 nm. Fluorescence EEM spectra were recorded on a Spectra Max M5 microplate reader [Molecular Devices, USA, diffraction-grating monochromator for fully adjustable excitation (300-900nm) and emission (200-900nm) wavelength settings], with a xenon lamp as the excitation source. To partially account for Raleigh scattering, the fluorometer’s response to a milli-q water was subtracted from the fluorescence spectra recorded for samples containing DOC [113, 114]. The PARAFAC modeling was performed using MATLAB 7.0 (Mathworks, Natick, MA, USA) with the DOM Fluor Toolbox (http://www.models.life.ku.dk).

3.7 TOX analysis

TOX was determined using an adsorption-pyrolysis-titration method and a Mitsubishi TOX-100 Analyzer (Cosa Xentaur Inc., Norwood, NJ). The method was based on standard method 5320 B [115] with minor modifications.
Chapter 4

Results and discussion

4.1 HPC Results

HPC was used to monitor biofilm formation on the corroded pipe surface. It has been reported that the concentration of organic substances is substantially higher on corrosion products than in the bulk fluid and biofilm commonly found in distribution systems could utilize the humic substances [5]. The microbial growth was substantial after 2nd and 3rd phase for each reactor containing iron coupon reactors and less in the polycarbonate coupon reactors (no corrosion products present). The results are shown in Table 4-1. These results indicate that microorganisms could readily utilize humic substance as sole carbon source.

Reported values for the untreated reactors achieved a steady state at just above $10^5$ CFUcm$^{-2}$, which fall within values for pipe wall biofilm bacteria [116]. These results are similar to previous studies, where it was observed that surface qualities such as roughness have a significant effect in early forming stages but are indistinguishable in the long-term [117]. There have been results that indicate higher bacterial activity on iron materials [118], which could explain the small increase in counts.

There were significant differences in the two disinfected reactors. Throughout the experiment, while identical free chlorine residuals were applied, the iron coupon reactor
maintained a 1.5 magnitude greater amount of colony forming units (CFUs). Corrosion tubercles make up a significant amount of the biofilm sample taken from the reactors, and it is likely there is a protective effect that this electrochemical structure provides. In addition, due to the effects of the iron corrosion, microbial makeup may be different in the iron coupon biofilm, promoting the protective effects to bacteria in biofilm matrix [119]. According to the previous studies, the use of disinfectants can actually increase the bioavailability of organics in the bulk fluid [5]. In the biofilm/corrosion product matrix, the presence of corrosion products is capable of adsorbing organics humic substances from the bulk fluid [76, 77]. It is hypothesized that these adsorbed organics exert a substantial chlorine demand along with increasing the bioavailability of carbon substrates for microorganisms [1, 76, 78]. As a result, the BDOC (biodegradable dissolved organic carbon) and AOC (assimilable organic carbon) levels increase, perhaps leading to elevated microbial growth in the distribution system [120]. These results concur with previous studies which reported that adsorption of humic substances to iron oxides may create a nutrient-rich environment that promotes biofilm growth, resulting in an increase in heterotrophic and coliform bacteria in drinking water [107].

In either case, at 4 mgL⁻¹, the maximum allowed level of chlorine residual per the EPA disinfection rules, both reactors still exhibited bacterial activity and showing persistence of biofilm even at high chlorine dose. The iron coupon reactor maintained a level of around 10⁴ CFU cm⁻² and the PC coupon reactor maintained 10³ CFU cm⁻². Planktonic bacteria in both reactors also eradicated in early stages due to disinfectant activity because microorganisms that are suspended in the bulk fluid are exposed to disinfectants from every possible angle.
The first key result from HPC was that even at maximum allowed level of chlorine residual per the EPA disinfection rules, both reactors still exhibited bacterial activity showing persistence of biofilm on pipe material even at high chlorine dose. The second key result was that consistently higher bacterial activity was observed on iron materials.

**Table 4-1: Average and standard deviation values in log CFU cm\(^{-2}\) of the four reactors in each stage of chlorination.**

<table>
<thead>
<tr>
<th>Log CFU/cm(^2)</th>
<th>Reactor 1</th>
<th>Reactor 2</th>
<th>Reactor 3</th>
<th>Reactor 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>5.5</td>
<td>5.3</td>
<td>5.3</td>
<td>3.3</td>
</tr>
<tr>
<td>±0.3</td>
<td>±0.6</td>
<td>±0.4</td>
<td>±1.5</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>5.9</td>
<td>4.4</td>
<td>5.5</td>
<td>2.9</td>
</tr>
<tr>
<td>±0.4</td>
<td>±0.5</td>
<td>±0.2</td>
<td>±0.2</td>
<td></td>
</tr>
<tr>
<td>Stage 3</td>
<td>5.9</td>
<td>4.1</td>
<td>5.8</td>
<td>3.2</td>
</tr>
<tr>
<td>±0.3</td>
<td>±0.3</td>
<td>±0.3</td>
<td>±0.5</td>
<td></td>
</tr>
</tbody>
</table>

* Reactor 1 (Iron, control); Reactor 2 (Iron, chlorinated); Reactor 3 (polycarbonate, control); Reactor 4 (polycarbonate, chlorinated).

**4.2 DBP formation upon chlorination in bulk phase**

Figure 4-1 shows the formation of bulk phase DBPs during Cl\(_2\) disinfection for Reactor 2 (chlorinated iron) and Reactor 4 (chlorinated polycarbonate). Throughout the study, it was difficult to manage chlorine residual (especially during the early period of the phase 1 in the iron reactor because of the high reactivity of chlorine with iron tubercles. Unlined metals resulted in significantly greater residual consumption, because it is well established that material on the pipe wall, such as corrosion products and biofilm slime, can exert a significant chlorine demand [24, 121, 122] (Data not shown).
Figure 4-1: DBP formation results (a) Reactor 2 (Iron, chlorinated) (b) Reactor 4 (polycarbonate, chlorinated).

Overall, Reactor 2 upon chlorination showed the higher DBP yields compared to those of Reactor 4. Previous studies have also reported that iron tubercles contains organic
material that includes DBP precursors resulting in higher DBPs [123, 124]. On an average, HAA9 in Reactor 2 were 62.6% higher than HAA9 in Reactor 4, and THM4 were 15.6% higher in Reactor 2 in comparison to Reactor 4. Also, emerging DBPs like HKs were 106.3% higher in Reactor 2 and CH formation in Reactor 2 was 64.2% higher than Reactor 4. This can be explained on the basis that Reactor 2 also consistently demonstrated higher biofilm formation, where it is providing higher amount of organic carbon in terms of biofilm EPS and associated humic substances. Hence, it can be assumed that higher biofilm quantity is contributing towards higher DBP formation in Reactor 2. Previous studies involving several full-scale distribution systems also found that unlined iron pipes with higher bacteria count number [17, 72, 125].

During the entire operation, there was small difference in formation of THM4 between the two reactors as compared to HAA9. This could be explained by the fact that despite the use of gas tight reactors, some of the DBPs might have volatilized during the analysis. Previous studies reported the volatilization of THM, HAN, HK, CH and TCNM [126, 127], which may explain the low difference in THM and HK formation.

Overall, the DBP values found during the experiment were higher than the actual distribution systems because previous studies have reported that due to the high surface area to volume ratio, the annular reactors are highly biased towards surface interactions associated with microbial attachment/detachment (biofilms) and chemical reactions. As a result, annular reactors will have higher disinfectant demands and higher microbial counts in the bulk fluid than an actual distribution system [5].

During the first phase (0.5 mgL⁻¹ of free Cl₂ residual), comparatively higher average THMs (~45.0 μgL⁻¹) and average HAAs (~97.0 μgL⁻¹) were detected in Reactor 4
as compared to Reactor 2, where average THM4 and HAAs were ~12.5 μgL⁻¹ and ~58.3 μgL⁻¹ respectively. This trend is different from the DBP formation trend in phase 2 and 3. Even the emerging DBPs had higher concentrations in Reactor 4 than Reactor 2. HK and CH in Reactor 2 were ~5.7 μgL⁻¹ and ~2.9 μgL⁻¹ as compared to ~11.1 μgL⁻¹ and ~16.5 μgL⁻¹ in Reactor 4. Average N-DBPs (HAN4+TCNM) were also higher for R4 (~ 9.4 μgL⁻¹) than those of reactor 2 (~1.7 μgL⁻¹). The reason for having low DBPs in Reactor 2 in comparison to Reactor 4 at low chlorine dose may be explained by the fact that the reaction between chlorine and iron oxide corrosion products can reduce chlorine concentration in drinking water while also affecting the efficacy of chlorine to act on biofilm [107, 128]. One other explanation could be the fact that chlorine is a strong oxidant which results in accelerated corrosion that provides a safe environment for the biofilm proliferation. At the same time, chlorine was not sufficient enough to affect the biofilm. Whereas in Reactor 4, there is no diffusion limitation offered by corrosion products. The chlorine dose may directly interact with biofilm and will result in biofilm disinfection and detachment, thus increasing the formation of DBPs in Reactor 4.

To confirm potential DBP biodegradation and volatilization, batch experiments were conducted with treated humic acid at the initial Cl₂ dose (0.5 mgL⁻¹) for 12 hours (hydraulic retention time in this study). The average concentrations for the compounds were determined as follows: THM4 were~24.64 μgL⁻¹, HAA9 were ~32.04 μgL⁻¹, HKs were ~2.25 μgL⁻¹, CH were ~ 0.11 μgL⁻¹, and N-DBPs (HAN4 and TCNM) at ~4.27 μgL⁻¹. These results are shown in Figure 4-2.
Average values of DBPs in Reactor 2 were very close to the DBP formation potentials of humic substance as compared to those of Reactor 4 despite having higher biofilm formation, attesting that in Reactor 2, chlorine was being consumed due to a reaction with iron tubercles. Also, there is a diffusion limitation being offered by iron tubercles, thereby reducing chlorine reactivity with biofilm. In Reactor 4, chlorine was directly interacting with biofilm. As a result, detachment of biofilm from the pipe material may occur and additional DBPs could be produced consequently. Laboratory and pilot-scale studies have demonstrated that coliform bacteria in drinking water can result from detachment of organisms from biofilm on pipe surfaces [129]. These detached coliform bacteria could be another contributing factor towards increased DBP formation.

However in the second phase, the chlorine dose was increased in order for residuals to reach ~2 mgL\(^{-1}\). The average THM4 for Reactor 2 was ~74.66 \(\mu\)gL\(^{-1}\)and HAA9s were
~228.46 μg L\(^{-1}\), whereas in Reactor 4, THM4 was ~53.73 μg L\(^{-1}\) and HAAs were ~110.46 μg L\(^{-1}\), showing trend which is different from phase 1, (Reactor 2 is now producing more DBPs). This could be explained by the fact that in Reactor 2, higher Cl\(_2\) residual was able to overcome diffusion limitation created by corrosion tubercles and now could react with heavily grown biofilm and biofilm associated humic substances. Also in Reactor 4, an increased chlorine dose is reacting with biofilm and thereby producing increased amounts of DBPs than its phase 1 concentration. In Reactor 2, HAA9 increased 291.6% and THM4 increased 495.7 % from phase 1 whereas in Reactor 4, HAA9 increased 13.8% and THM 4 increased 19.9% from phase 1.

After sharp increases in DBP formation in the second phase for Reactors 2 and 4 (Figures 4-1 & 4-2), a decrease in DBP levels in reactor effluent were observed in both the reactors, and HPC plate counting numbers representing biofilm in reactor coupons in Reactor 2 and 4 also stayed significantly low, with Reactor 4 even lower as compared to Reactor 2, thus implying that biofilm begins to get eradicated by high Cl\(_2\) residual and losing its potential to provide reaction sites for DBP formation [130]. On the other hand in Reactor 2, the DBP formation remained high representing that chlorine at high dose was reacting with biofilm on iron pipe material or detached biofilm in the media solution along with the humic substances, in both cases representing persistent biofilm on iron pipe material. Due to the presence of organic content in the bulk solution, there was continuous formation of DBPs. HPC numbers also decreased in Reactor 2 from phase 1, yet remained higher than Reactor 4. This can be correlated with biofilm and inference could be drawn that reduced DBP formation is in both the reactors is related to biofilm eradication.
In addition, the levels of HKs and CH exhibited comparatively similar trends to those of the THMs and HAAs in Reactor 2, with the 276.1% rise in HK (~21.3 μgL⁻¹) and 793.1% rise in CH (~26.2 μgL⁻¹) from phase 1 in Reactor 2. Also, in case of N-DBPs (~11.5 μgL⁻¹), there was an increase of about 584% from first phase. However, as expected, rise in DBPs in the Reactor 4 was less than that of Reactor 2, because of already reacted biofilm with chlorine in phase 1. Higher levels of N-DBP formation were also observed in Reactor 2 than Reactor 4 (47.4% high), during the second phase, which can also suggest that the biofilm contributed to N-DBP formation. It has been reported that nitrogenous precursors from algae or its effluent organic matter have been related to N-DBP formation, especially HANs [131]. Again, it can be correlated with HPC results, that Reactor 2 produces higher amount of N-DBPs than Reactor 4 along with consistently maintaining higher amount of biofilm count number. Since biofilm itself includes organic nitrogen content and adsorbs humic substances, the increase of regulated and N-DBPs further indicate that biofilm and biofilm associated HA could enhance DBP levels in the water distribution system.

Also in the second phase, both Reactors 2 and 4 were producing higher DBPs as compared to the DBP formation potential of humic substances for the same amount of organic carbon and chlorine dose residual values, proving that high chlorine residual interacted with biofilms and its associated organic matter producing high amount of DBPs. Again at this time, Reactor 2 showed a higher difference of DBPs from HA than Reactor 4, thus showing that the additional biofilm observed in HPC results (Table 4-1) is directly related to increased DBP formation in Reactor 2. This confirms observation for biofilm contribution to DBP formation in the beginning of second phase.
However during the third phase, constant DBP formation was observed even under the presence of 4 mgL$^{-1}$ Cl$_2$ residual especially in the case of Reactor 2. Reactor 4 also showed consistent DBP formation, which was lower than Reactor 2. During the third phase, average HAAs were ~159.37 μgL$^{-1}$ and THMs were ~84.76 μgL$^{-1}$ in Reactor 2, which was significantly higher for high chlorine dose like 4 mgL$^{-1}$. The reason, as previously discussed, could be persistent biofilm on iron pipe material. HKs values were ~12.15 μgL$^{-1}$ and CH was ~23.83 μgL$^{-1}$. N-DBPs were ~4.67 μgL$^{-1}$. On the other hand, Reactor 4 had HAAs ~78.86 μgL$^{-1}$ and THMs ~49.32 μgL$^{-1}$ which is higher than its phase 1 concentration but much less than Reactor 2’s DBP concentration in the same phase. Also HKs values were ~6.31 μgL$^{-1}$ and CH was ~6.35 μgL$^{-1}$. N-DBPs were ~3.96 μgL$^{-1}$. From the beginning to the end of third phase, the total HPC number remained low for Reactor 4 as compared to Reactor 2. This shows that biofilm removal on Reactor 4 was more significant. Therefore, the DBP formation was lowered losing potential reaction sites. DBP formation potential of humic solution were in the range of DBPs in the Reactor 4 suggesting that humic substance in the feed solution is a major contributor towards DBP formation in the presence of 4 mgL$^{-1}$ Cl$_2$ residual. On the other hand in Reactor 2, the continuous high DBP formation signifies persistence of biofilm even under high chlorine dose. As can be seen in HPC coupon data (Table 4-1), the HPC results did not get changed significantly for Reactor 2 as compared to those of Reactor 4, again showing persistence of biofilm even under high chlorine dose and at the same time its contribution to DBP formation.
4.3 DBP Speciation

Detailed DBP speciation results for Reactor 2 (chlorinated iron) and Reactor 4 (chlorinated polycarbonate) are summarized in Figures 4-3 through 4-6. In the Figure 4-3 for THMs speciation, CF on average had the highest formation during the entire operation compared to other THMs for both Reactor 2 (averaging ~50.70 μgL⁻¹) and Reactor 4 (averaging ~43.96 μgL⁻¹). Approximately 15.3% higher CF was observed in Reactor 2 as compared to Reactor 4. For HAAs speciation (Figure 4-4), DCAA was the dominant species formed in both types of reactors (averaging ~95.94 μgL⁻¹ in Reactor 2 and ~56.72 μgL⁻¹ in Reactor 4) during the entire study. Other HAA in significant quantity was TCAA (averages of ~30.91 μgL⁻¹ in Reactor 2 and ~21.89 μgL⁻¹ in Reactor 4) during the entire study. Average MCAA concentrations were ~17.03 μgL⁻¹ in Reactor 2 and ~11.46 μgL⁻¹ in Reactor 4. The lower concentrations of MCAA formed may be partially attributed to biodegradation, since one halogen HAA species have been reported to be utilized by bacteria for growth [132, 133]. For HKs, 1, 1, 1-TCP was dominant species in both reactors (Figure 4-5). Also significant concentrations of CH were observed. For other NDBPs, DCAN was the dominate species of HANs in the presence of higher chlorine residual (averaging ~ 5-6 μgL⁻¹) in both reactors (Figure 4-6).
Figure 4-3: THM Speciation (a) Reactor 2 (Iron, chlorinated) (b) Reactor 4 (polycarbonate, chlorinated).
Figure 4-4: HAA Speciation (a) Reactor 2 (Iron, chlorinated) (b) Reactor 4 (polycarbonate, chlorinated).
Figure 4-5: HK and CH Speciation (a) Reactor 2 (Iron, chlorinated) (b) Reactor 4 (polycarbonate, chlorinated).
Figure 4-6: N-DBPs Speciation (a) Reactor 2 (Iron, chlorinated) (b) Reactor 4 (polycarbonate, chlorinated).
Changes in pH are also known to affect the DBP speciation [134]. However, during the entire study, the pH levels were maintained constant (~pH 7.1) (data not shown). In addition to chlorinated DBPs, brominated DBPs were also observed in this study, since a trace amount of bromide might be present in the feed solution. Overall, Reactor 2 showed 31.0% more regulated brominated DBPs than Reactor 4, average regulated brominated DBPs throughout the study period in Reactor 2 was ~25.71 μgL⁻¹, and in Reactor 3 it was ~19.62 μgL⁻¹. Previous studies reported that the formation and speciation of brominated THMs and HAAs were affected by bromide concentration in water [135, 136]. Unregulated brominated DBPs were also observed in this study with Reactor 2 having ~0.75 μgL⁻¹ and Reactor 3 having ~0.91 μgL⁻¹ average DBPs over the course of experiment.

The first key results from the study was that in Reactor 2, the iron coupons in which higher biofilm count numbers were observed, consistently produced higher DBPs than Polycarbonate reactors. Secondly, observed DBPs were higher than DBPs from humic substances. Both of these results give strong indication of the role biofilm is playing towards DBP formation and probable unknown TOX formation.

4.4 DBP Formation Potential of Biofilm and Biofilm Associated Humic substances

DBP yield results of biofilm and biofilm associated humic substances from all four reactors upon chlorination are shown in Figures 4-7 and 4-8. In order to quantify DBP formation from biofilm samples associated with humic substances, DBP yield results were presented as μg dm⁻² of surface of pipe material. Chlorine can be highly efficient at
inactivating microorganisms in the bulk fluid, but have been found to be several times less efficient at inactivating distribution biofilms [19, 81].

Figures 4-7 show the DBP formation potential for Reactors 3 and 4. The DBP yields for Reactor 3 increased continuously during the study. For Reactor 3, during the first and second phases, the average CF was ~1690.3 μg dm⁻² and HAA3 were ~2965.0 μg dm⁻², however in the third phase the concentration increased to 4471.4 μg dm⁻² for CF and 4919.8 μg dm⁻² for HAA3.

Before starting the experiments, all reactors were operated for four months to obtained confluent biofilm. However, while not appearing any different, biofilm grew continuously, in Reactor 3 according to biofilm analysis data (Table 4-1), which would explain the increase of DBP yields. For the other emerging DBPs, the increase was much less significant than that of regulated DBPs.
Figure 4-7: DBP formation potential test results (a) Reactor 3 (Polycarbonate, control) (b) Reactor 4 (Polycarbonate, chlorinated), DBPs analyzed: Chloroform [ ], HAA3 (Monohaloacetic acid, Dihaloacetic acid, Trichaloacetic acid) [ ], Dichloroacetonitril (DCAN) [ ], HK2 (1,1-Dichloropropanone, 1,1,1-Trichloropropanone) [ ], Trichloronitromethane [ ]
Figure 4-8: DBP formation potential test results (a) Reactor 1 (Iron, control) (b) Reactor 2 (Iron, chlorinated), DBPs analyzed: Chloroform, HAA3 (Monohaloacetic acid, Dihaloacetic acid, Trichaloacetic acid), Dichloroacetonitril (DCAN), HK2 (1,1-Dichloropropanone, 1,1,1-Trichloropropanone), Trichloronitromethane

In Reactor 4, in comparison to Reactor 3, both regulated and emerging DBP yields stayed low during the operation, which may be attributed to decreased amount of biofilm as well as pre-occurred interactions between Cl₂ residuals and biofilm/humic substance. From the first and second phase biofilm samples, the yields of CF and HAA3 were around...
959.9 μg dm\(^{-2}\) and 1816.7 μg dm\(^{-2}\), respectively. However, both CF and HAA3 yields of biofilm and biofilm associated HA decreased to around 496.3 μg dm\(^{-2}\) and 772.0 μg dm\(^{-2}\) during the third phase, which may suggest that more DBP precursors (biofilm and biofilm associated humics) on coupons were already reacted under increased Cl\(_2\) residual (2 mg L\(^{-1}\)). In addition, based on the biofilm analysis data, increased Cl\(_2\) residual affected biofilm structure and began to eradicate preformed biofilm under applied shear stresses in the reactor. This confirms our hypothesis that decreased DBP formation was correlated to reduced biofilm formation (Table 4-1).

Figures 4-8 shows the DBP yields of biofilm and biofilm associated humic substances in Reactor 1 (control iron) and Reactor 2 (chlorinated iron) upon chlorination. In Reactor 1, similar trends for both regulated and emerging DBPs were comparable to observations of R3, but significantly higher CF and HAA3 yields were found in Reactor 1 as compared to Reactor 3 [~11.9% higher CF and ~20.1% higher HAA3]. Similarly higher percentage of non-regulated DBPs were also observed. According to previous studies, due to the presence of corrosion products there is large surface area available for microbial attachment and substantially higher biofilm densities [80, 81]. Based on elevated biofilm colonies according to our HPC results in Table 4-1, it can be hypothesized that humic substance adsorption by iron corrosion products can support biofilm growth and as a result will produce higher amount of DBPs. The combination of humic substances and corrosion products (CPs) could lead to an increase in biofilm biomass (HPC results) when free chlorine was not present, similar to conditions that could occur at the dead ends of water distribution systems [107]. Previous studies have also reported that bacteria has been found to be more abundant in the presence of ferrous metal CPs [129] and in corrosion
tubercles [118]. Humic substances are a primary source of organic carbon found in drinking water [137] and will be readily adsorbed to iron oxides similar to those formed during ferrous pipe corrosion [76]. This eventually results in increased DBP precursors in biofilm.

In the case of Reactor 2 as shown in Figure 4-8, DBP formation potential in the initial phase 1 was higher than Reactor 1 because chlorine residual (0.5 mgL⁻¹) was not high enough to eradicate or inactivate the biofilm. As previously reported, the reaction between chlorine and iron oxide Corrosion Products (CPs) can reduce chlorine concentration in drinking water while also affecting the ability of chlorine to act on biofilm [107, 128]. However being a strong oxidant, it leads to the production of more corrosion products. Previous studies have reported that the reactivity disinfectants such as chlorine with corrosion products and other matter creates increased chloride levels, which have been shown to accelerate corrosion [75].

However in phase 2 and phase 3 when chlorine residual was increased to 2 mgL⁻¹ and 4 mgL⁻¹, respectively, DBP formation potential started decreasing. This can be attributed to the fact that although chlorine is a good oxidant and can contribute to corrosion, the chlorine dose begins to eradicate or inactivate the biofilm. As reported by Abernathy et al. (1997, 1998), generally once the chlorine residual exceeds approximately 0.75 mgL⁻¹, the biofilm densities begin to decrease since chlorine is able to overcome diffusion limitation by corrosion products [5, 74]. This chlorine residual, which results in a decrease in biofilm density, can be defined as the threshold residual. The threshold residual will vary from site-to-site and will likely change with the physical and chemical properties of the water.
In conclusion, the results from this study revealed that biofilm could facilitate DBP formation in drinking water distribution systems upon chlorination. Based on the results it can also be concluded that iron pipe materials provide habitats for microbial growth because corrosion can support microbial colonies even in the presence of disinfectants. If corrosion products are present, it is important to maintain disinfectant levels above the threshold residual level if a reduction in biofilm density is desired.

4.5 PARAFAC Analysis

After quantifying the potential DBP formation on pipe materials, determining the contribution of bacterial or humic substances can help further establish role of biofilm towards DBP formation. Hence, fluorescent spectroscopy and PARAFAC analysis was conducted to determine the source of organic matter. FEEM represent fluorescence intensities at various excitation-emission pairs. Because NOM may contain thousands of different chemical constituents, it is not realistic to characterize it on the basis of a thorough compilation of the individual compounds [138]. The components extracted by PARAFAC are more likely to represent groups of organic compounds with similar fluorescence properties. In this study, the maximum fluorescence intensity (Fmax) for each component was obtained and used to illustrate the quantitative and qualitative differences among samples [3].

A series of PARAFAC models consisting of 2-4 components were generated using the DOMfluor toolbox [139]. A dataset of F-EEMs for 57 samples collected from coupons of four reactors were used to develop the PARAFAC model. In this study, the Fmax for
each component was obtained and used to illustrate the quantitative and qualitative differences between samples [3].

Two fluorescent components were successfully identified from the reactor and the effluent DOMs by EEM- PARAFAC modeling. Figure 4-9 shows contour plots of each component identified by a two-component PARAFAC model. Figure 4-10 represents the relative concentration of the two PARAFAC components over time in the reactors.

Chen et al. (2003) reported that peaks at longer excitation wavelengths (250-340 nm) and longer emission wavelengths (>380 nm-540 nm) are related to humic acid-like organics. Component 1 represents humic like substance. Peaks at shorter emission wavelengths (<380 nm) and shorter excitation wavelengths between 250-340 nm were reported to be related to be protein like organic matter. Component 2 represent microbial origin NOM [7].
Figure 4-9: Contour plots showing two groups of species dominant in the biofilm and its associated humic substances. Component 1 represents humic-like substances and component 2 represents protein-like substance [7]

Figure 4-10: % distribution using Fmax values obtained using PARAFAC analysis, humic-like (■) and Protein-like (○) (a) Reactor 1 (b) Reactor 2 (c) Reactor 3 (d) Reactor 4
These results can be correlated with HPC plate counting for coupons, as can be seen in Reactors 1 and 2. There is higher bacterial count during phase 1 in Reactor 2 (chlorinated iron), which is, as previously explained, may be due to high corrosion and accumulation of more colonies in corrosion product. Through PARAFAC results, it can also be seen that there is a high concentration of microbial origin organic matter in comparison to humic substances during phase 1 of Reactor 2 than Reactor 1. This fortifies the perspective that corrosion product provides high surface area for biofilm proliferation, assuming chlorine provided increased corrosion. DBP formation also during initial phases in Reactor 2 is high as compared to Reactor 1, which concurs with previous studies. In Reactor 1, Fmax values oscillate at a roughly equal accumulation of humics and protein- based DOM without the presence of chlorine. HPC results suggest constant (if steadily increasing) bacterial activity; the humic component must vary considerably.

However in the middle and later phases, as the chlorine concentration rises to 2 and 4 mgL⁻¹, Reactor 2 decreases its percentage of protein-like organics concentration as well as DBP formation. An inference can be drawn that biofilm and its EPS are being eradicated, and at the same time precursors for DBP formation are reducing. Corresponding DBP formation potential results also show very high DBP concentration. Chlorine may preferentially interact with protein-like organic matter, leaving mainly humic-like organic matter for steady-state chlorinated systems to react with. In Reactor 1, there is equal accumulation of humics and organics from microbial origins because there is no chlorination. Consequently there is increased growth of biofilm, and at the same time, there is heavy built up of the corrosion products as a result of which there is more organic matter.
accumulation, which might be responsible for high humic content and corresponding high DBP formation.

Reactors 3 and 4 show similar results with microbial origin organic matter dominating the concentration in Reactor 3 (control polycarbonate). In Reactor 3, there is equally high accumulations of humics and protein-based DOM because there is no chlorination. Without chlorination, significant growth of biofilm can occur in addition to organic matter accumulation. Also, with no change in the reactor conditions, the relative ratio of humics and protein-based DOM stay very similar. Whereas in Reactor 4 (chlorinated polycarbonate) under chlorination, the microbial origin organic matter concentration is less than Reactor 3 especially in the second and third phase which concurs with the previous results showing less biofilm formation and at the same time much less DBP formation was observed.

Comparing Reactor 2 with Reactor 4, it appears that the microbial component increases in the early section of phase 1 of Reactor 2, with Reactor 4 staying in roughly the same ratio. This correlates with some of the HPC data where Reactor 4 is affected by the weak concentration of chlorination to a much higher degree. More microbial DOM measured in Reactor 2 also correlates to the higher DBP formation during the initial phases in the reactor.

During the rest of the experiments, however, it appears that Reactor 2 is comprised of more humic DOM compared to Reactor 4. This is contrary to HPC data and anecdotal data looking at the biofilm left on Reactor 3’s coupons, which indicated little biological activity. It does however track with other studies reporting humic interactions with iron corrosion products. Due to the PARAFAC results being relative compositions, it may be
that humics increased under chlorination in stages 2 and 3 while the microbial content stayed the same, as suggested by HPC results.

Although both humic substances and protein-based DOM contribute to DBP formation, it appears that depending on the conditions in water distribution networks, humic substances are still the primary DBP precursor. However at long residence times, biofilm DOM will play a larger role than previously considered. More attention should be paid to this protein-based DOM in source waters since this fraction is not amenable to the removal by water treatment processes but still can produce a certain amount of DBPs, in addition to more toxic NDBPs.

### 4.6 TOX analysis

DBP contribution towards unknown TOX was also analyzed and results in Table 4-2 show TOX and DBP variation in the four reactors in three different phases. In Reactor 1 (control iron), UTOX percentage increased over time suggesting that, due to a high amount of biofilm and its associated humic substance accumulation over a longer period of time, organic matter concentration increased producing high concentration of halogenated organic compounds. In first phase it averages out around 40.4 %, however it increases to 71.4 % in second phase and in third phase it increased to 86.5%.
Table 4-2: Percentage DBP and unknown total organic halogen variation

(a) Reactor 1 (b) Reactor 2 (c) Reactor 3 (d) Reactor 4

<table>
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<tr>
<th></th>
<th>% conc.</th>
<th>THM</th>
<th>HAN</th>
<th>HK</th>
<th>CP</th>
<th>MHAA</th>
<th>DHAA</th>
<th>THAA</th>
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<td>0.6</td>
<td>0.0</td>
<td>14.4</td>
<td>18.4</td>
<td>10.8</td>
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<td>0.5</td>
<td>0.1</td>
<td>0.9</td>
<td>6.8</td>
<td>5.3</td>
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<td>0.7</td>
<td>0.1</td>
<td>0.7</td>
<td>3.6</td>
<td>1.9</td>
<td>86.5</td>
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</tr>
<tr>
<td>R2 Phase 1</td>
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<td>0.3</td>
<td>0.1</td>
<td>1.2</td>
<td>5.7</td>
<td>3.1</td>
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<td>0.6</td>
<td>0.1</td>
<td>1.1</td>
<td>6.1</td>
<td>4.4</td>
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<td>0.0</td>
<td>2.0</td>
<td>9.8</td>
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<td>4.1</td>
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<td>4.6</td>
<td>2.8</td>
<td>86.6</td>
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<tr>
<td>R4 Phase 3</td>
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<td>3.8</td>
<td>0.6</td>
<td>7.2</td>
<td>18.9</td>
<td>9.8</td>
<td>54.1</td>
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</table>

In Reactor 3 (control polycarbonate), UTOX % remained high over time. In the first phase, it averaged out around 73.4%, however it reduced to 56.3% in the second phase and spiked again to 66.9% in the third phase.

Results from HPC also suggests that in the two control reactors biofilm density remained high, which is further confirmed by PARAFAC results suggesting that, both humic and microbial origin organic compounds remained high and further increased during study. Hence it can be assumed that due to high biofilm density as well as continuous accumulation of humic substances in the biofilm EPS throughout the experimental phase,
organic matter concentration continued to increase and as a result was producing high concentration of UTOX over time. Previous studies by Hua et al. (2007) have also shown that hydrophobic and high molecular weight compounds produced a greater amount of unidentifiable organohalides [140]. Since previous studies have reported that 90% of biomass in a biofilm EPS is composed of components very similar in structure to humic substance organic matter, hence high formation of UTOX can be expected due to biofilm growth [22, 23].

In Reactor 2 (chlorinated iron) UTOX % decreases over time suggesting that, due to increasing amount of chlorine dose, biofilm is being eradicated and along with it humic substances associated with it is also eradicating and consequently decrease in TOX concentration was observed. In first phase it averages out around 81.7%, however it decreases to 76.9% in second phase and in third phase it further reduced and average 50.7%. Reactor 4 (chlorinated polycarbonate) also showed similar trend with UTOX in first phase it averages out around 74.2%, however it increased slightly to 86.6% in second phase and in third phase it reduced significantly and average 54.1%.

TOX analysis was also conducted for organic matter present in the bulk solution where approximately equal percentage of unknown TOX was detected in both chlorinated reactors. Reactor 2, had unknown TOX ~54% with standard deviation of ± 10.1% and in Reactor 4 unknown TOX was ~55% with standard deviation of ± 8.7%.

Previous studies have also reported that both humic fraction [141, 142] and non-humic fraction [34, 143] contribute towards TOX and with increase in biofilm density over time, not only DBP concentrations increases but also TOX values also increases over time. Therefore biofilm eradication practices must carefully be considered in order to reduce
unregulated toxicologically important compounds formed in drinking water distribution system.
Chapter 5

Conclusions

Specific results from the study are as follows:

a) The results from this study have revealed that biofilm concentration varies significantly from iron and polycarbonate pipe material, with iron pipe material having approximately 1.5 times higher biofilm activity as compared to pipe material made of non-corrosive polymer substance.

b) DBP experiments on both pipe materials have fortified the idea of biofilm contribution towards DBP as well as unknown TOX formation. On an average, HAA9 in Reactor 2 were 62.6% higher than HAA9 in Reactor 4, and THM4 were 15.6% higher in Reactor 2 in comparison to Reactor 4. Also, emerging DBPs like HKs were 106.3% higher in Reactor 2 and CH formation in Reactor 2 was 64.2% higher than Reactor 4. In addition regulated and unregulated brominated DBPs were ~31% and ~75% respectively higher in Reactors 2 in comparison to Reactor 4.

c) Higher DBP formation potential results from biofilm and its associated humic substances in reactors with iron coupons in comparison to polycarbonate coupons reactors revealed that biofilm could facilitate DBP formation in drinking water distribution systems upon chlorination. Based on the results it can also be concluded that iron pipe materials provide habitats for microbial growth because corrosion can
support microbial colonies even in the presence of disinfectants. If corrosion products are present, it is important to maintain disinfectant levels above the threshold residual level if a reduction in biofilm density is desired.

d) From PARAFAC and TOX analysis, it was observed that with increase in biofilm density TOX formation also increased. From these results it can be concluded that at long residence times, biofilm DOM will play a larger role than previously considered. More attention should be paid to this protein-based DOM in source waters since this fraction is not amenable to the removal by water treatment processes but still can produce a certain amount of DBPs, in addition to more toxic NDBPs.

Based on the obtained results and considering huge network of water distribution pipelines majority of which are composed of iron, biofilm control practices in water distribution system must be applied in order to prevent production of toxic halogenated chlorine compounds. For effective biofilm eradication, some important recommended techniques like hydraulic flushing coupled with high disinfectant dose could be used. High disinfectant dose will vary, depending upon the pipe material and type of disinfectant being used. Disinfectants like chlorine react with iron pipe materials to accelerate corrosion [75]. Hence the objective of an effective biofilm disinfection scheme should be to provide a lethal concentration of disinfectant high enough to overcome diffusion limitations created by biofilm EPS and disinfectant consumption by corrosion products in case of iron pipes to inactivate attached microorganisms. One approach to overcome these diffusion/reaction limitations is to use a disinfectant that is not as reactive with EPS, organics, and corrosion products like chloramine. Also use of non-ferrous pipe materials should be encouraged.
because they were found supporting fewer microorganisms and consequently lower DBP formation was also observed.

Overall results of this study provide critical information about potential contributions of biofilms accumulation on pipe materials and detached coliform bacteria detached from biofilm to the formation of DBPs as well as unknown TOX in different pipe materials under different chlorine doses over a duration of time in the distribution systems and can help water utilities better control the levels of both regulated and unregulated DBPs, at the same time reducing health risks associated with chlorinated DBPs.
Chapter 6

Future work

Based on the obtained results from this study, followings are suggestions for future study.

1. Pipe material using X-ray diffraction over a period of time under different chlorine dose.
2. Bacteria community analysis on different pipe material under different chlorine dose over a duration of time using pyrosequencing DNA analysis.
3. Analysis of abiotic and biotic degradation of disinfection by products.
4. Disinfection byproduct formation due to algal influenced source water.
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