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Formation Potential of Disinfection By-products after Coagulation of Algal Matters

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Abstract

Algal organic matters (AOM) are cellular materials releasing from algae species which can serve as precursors to form disinfection by-products (DBPs) after chlorination. AOM can be removed during drinking water treatment by coagulation. In this study, dissolved organic carbon (DOC) and UV$_{254}$ absorbance were used to assess the time-dependent release of algal matters for four algal species during coagulation using alum. A GC-ECD was employed to analyze the formation of DBPs at different background water qualities. Results showed that alum dose of 30 mg/L did not cause any damage to the algae cells, and they continued to release organic matters in treated water with time. Uniform formation condition (UFC) was used to determine the DBP formation potential, specifically haloacetic acids (HAA) from algal matter. DBP formation increased with increasing settling time. Empirical DBP formation models developed in this study showed good correlations between HAA formation and relevant water quality parameters.

Keywords

Algae, algal organic matter, coagulation, disinfection by-products (DBPs), haloacetic acids (HAAs)
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List of Acronyms

AOM  Algal Organic Matter
BCAA  Bromochloroacetic Acid
CH  Chloral Hydrate
DBAA  Dibromoacetic Acid
DBPs  Disinfection By-products
DCAA  Dichloroacetic Acid
DW  Deionized Water
DOC  Dissolved Organic Carbon
DPD  N,N-Diethyl-1,4-Phenylenediamine Sulfate
ECD  Electron Capture Detector
EEM  Excitation Emission Matrix
EOM  Extracellular Organic Matter
FAS  Ferrous Ammonium Sulfate
FTIR  Fourier Transform infrared spectrophotometer
GC  Gas Chromatography
HAAs  Haloacetic Acids
HANs  Haloacetonitriles
HK  Haloketones
IOM  Intracellular Organic Matter
LC  Liquid chromatography
MBAA  Monobromoacetic Acid
MCLs  Maximum Contaminant Levels
MS  Mass Spectrometry
MTBE  Methyl Tert-Butyl Ether
MW  Molecular Weight
NMR  Nuclear Magnetic Resonance
NOM  Natural Organic Matter
NTU  Nephelometric Turbidity Units
PHA  Polyhydroxyphenolic Acid
POC  Particulate Organic Carbon
SNWD  South to North Water Diversion
SUVA  Specific Ultraviolet Absorbance
TCAA  Trichloroacetic Acid
TCNM  Trichloronitromethane
TOC  Total Organic Carbon
THMs  Trihalomethanes
USEPA  United States Environmental Protection Agency
UV<sub>254</sub>  Ultraviolet Absorbance at 254 nm
WTP  Water Treatment Plant
Chapter 1

1 Introduction

Algae are ubiquitous in lakes, reservoirs, and surface waters. The most commonly found freshwater algae are diatoms, green algae, and blue algae (EPA, 2015). They grow drastically during algal bloom season in summer and can cause a series of problems including bad taste and odors, poor settling, clogging of filters, and release of algal toxins in drinking water facilities if not contained properly (Ghernaout et al., 2014).

Drinking water treatment processes are developed to remove dissolved and suspended contaminants from raw water and make water safe enough for human consumption without any adverse health effects. A typical treatment train of drinking water is the combination of many unit processes such as coagulation, flocculation, sedimentation, filtration, ion exchange, and disinfection. Coagulation is a key step in the conventional drinking water treatment process to remove the colloidal particles present in water. Coagulants are also used to facilitate the sedimentation of algae cells to the anoxic bottom layer of water, with no access to light and oxygen stopping their multiplication and eventual death (EPA, 2015). However, it is reported that some chemical coagulants may cause damage to algal cell integrity and lead to the release of intracellular toxins and taste and odor compounds to water (Chow et al., 1999; Peterson et al., 1995). There are also potentials for algae to release intracellular toxins to treated water if coagulated algae remain in the bottom of sedimentation basin and may cause disinfection by-product formation later when subjected to chlorination. A study has shown that it is necessary for cyanobacteria cells to be removed
from sedimentation basin within 6 days of coagulation to avoid the release of cyanotoxins to the treated water (Sun et al., 2012).

Chlorination of drinking water has been widely used as a disinfection method to control waterborne infections for ages and has been widely recognized among the most effective measures taken for public health (Raitt, 2000). However, the natural organic matter (NOM) can react with chlorine and form harmful halogenated disinfection by-products (DBPs). Many of the disinfection by-products are carcinogenic, so their formation should be avoided. Algae and their organic matters make up a portion of natural organic matter (NOM) in water, if left untreated before disinfection, there is a huge potential of formation of disinfection by-products (DBPs).

Algal organic matter (AOM) are metabolites releasing from algae species, which includes extracellular organic matter (EOM) and intracellular organic matter (IOM) (Fang et al., 2010). AOM is found in high concentration during algal bloom season in drinking water systems, and it affects the drinking water quality as it is the major contributor to the natural organic matter (NOM) concentration. Extracellular organic matter (EOM) are metabolites that excrete to the surrounding environment which are produced by living algae cells (Paralkar & Edzwald, 1996) and intracellular organic matter (IOM) are released after cell lysis which is generated during the population growth and decline (Thurman, 1985a). Algal organic matters can also serve as precursors to form disinfection by-products (DBPs) during and after chlorination (Fang et al., 2010). To avoid the effects of algae and algal organic matter on drinking water, removal of algae in the initial stage of water treatment is necessary. So far, few studies have focused on the potential risk of coagulated cell lysis over time in the coagulation basin warranting further research.
1.1 Objectives

- To determine the time-dependent release of algal matters subjected to common dosages used in drinking water treatment process using the commonly applied chemical coagulant, alum for four different species of algae suspensions.

- To investigate the impact of disinfection methods such as UV and post chlorination on filtered algae contaminated water samples.

- To determine the DBP formation potential for the different algal species in both pure water and natural water (Fanshawe Lake).

- To develop a disinfection model to predict the DBP formation from algal matter relating the dissolved organic carbon and dosage of disinfectant.

1.2 Thesis Overview

The thesis consists of five chapters. Chapter 1 gives a brief introduction to research topics and objectives. Chapter 2 contains a literature review relating to the research background, topics such as the introduction of common algae and algal organic matters, problems caused by algae, treatment methods dealing with algal matters are discussed. Materials and methods used in this research are described in Chapter 3, and the experimental results are discussed and analyzed in Chapter 4. The final Chapter (Chapter 5) presents the conclusions drawn from this work and recommendations for future work.
Chapter 2

2 Literature Review

2.1 Algae and Algal Organic Matter

Algae, a diverse group of eukaryotes, are photosynthetic microorganisms, containing organelles such as chloroplast and nucleus. They are able to utilize sunlight, carbon dioxide, water and other nutrients such as nitrogen and phosphorous and produce biomass (Raven & Giordano, 2014).

Algal organic matter (AOM) includes extracellular organic matter (EOM) and intracellular organic matter (IOM) (Fang, Yang, et al., 2010). It is found in high concentration during algal bloom season in drinking water systems, and it affects the drinking water quality as it is one of the major contributors to the natural organic matter concentration (NOM). Extracellular organic matter (EOM) are metabolites that excrete to the surrounding environment which are produced by living algae cells (Paralkar & Edzwald, 1996) and intracellular organic matter (IOM) are released after cell lysis, generated during the population growth and decline (Thurman, 1985b). EOM and IOM are rich in organic nitrogen, consisting of high (over 10 kDa) and low (70–1000 Da) molecular weight (MW) organic matter, whilst the MW of organic carbon in EOM and IOM is relatively lower. IOM has a higher fraction of total organic nitrogen, with larger proportions of higher MW and more hydrophobic contents than EOM. IOM also contains higher fractions of free amino acids but lower fractions of aliphatic amines than EOM.

Algae are commonly found in lakes, reservoirs, and surface waters, and they are abundant during bloom season. Factors that affect algae growth include temperature, light, mixed
layer depth, nutrients such as nitrogen and phosphorous, carbon dioxide and pH (Knappe et al., 2004). The factors that affect the optimum algae growth rate vary with algae class and genera. For instance, blue-green algae (cyanobacteria) with an optimum growth rate at above 25 °C (Chorus & Bartram, 1999). Cyanobacteria bloom during summer or throughout the year in a tropical environment, while many diatoms prefer cooler temperature climates (10 °C -20 °C) and are usually found in spring.

The most commonly found freshwater algae are diatoms, green algae and blue-green algae (EPA, 2015) although blue-green algae is not an eukaryote. Diatoms appear as yellow-brown or yellow-green algae, with siliceous wall (known as frustule) that mainly consists of polymerized silicic acid (Van Den Hoek, Mann, & Jahns, 1995), which gives diatoms several advantages over other algae (Horne & Goldman, 1994). Synthesis of diatoms’ frustule requires lower energy than cell wall synthesis of other algae species. Many diatoms have comparatively large density and can settle easily under quiescent conditions. Some of them have non-spherical shapes and grow spines to achieve higher surface area to volume ratio, and they might also produce oily metabolites or excrete heavy ions to regulate their density (Knappe et al., 2004).

Green algae are frequently found in the eutrophic water during summer, they are spherical colonies and some of them are filamentous. Green algae contains photosynthetic pigments chlorophylls $a$ and $b$, which are not masked by other pigments, so they appear to be bright green color. Approximately 8,000 species of green algae are known (Van Den Hoek et al., 1995), with common ones being *Chlorella, Chlamydomonas, Ankistrodesmus, Spirogyra Scenedesmus and volvox*. Some of them can cause problems such as causing bad taste and odor, and clogging of filters in water treatment plants.
Blue-green algae, also known as cyanobacteria, are prokaryotes because of lacking of the nucleus in cells. They are among the most problematic algae in drinking water system because of releasing algal toxins (Jančula & Maršálek, 2011). About 150 genera and 2,000 species of blue-green algae are known, typical genera are *Anabaena*, *Aphanizomenon*, *Microcystis*, and *Oscillatoria*. Among them, *Microcystis* has become the major harmful algal bloom (HAB) species since the mid-1990s (Brittain et al., 2000). Reports regarding to HAB are found in Lake Erie in North America throughout years (Michalak et al., 2013), Lake Taihu in China (Qin et al., 2010), Lake Victoria in Africa (Sitoki et al., 2012) and Lake Nieuwemeer in the Netherlands (Jöhnk et al., 2008) to cite a few. Harmful algal bloom toxins have been detected in countries including United States, Canada, China, Germany, Portugal, Spain, Poland, and Thailand (Hoeger et al., 2005).

### 2.1.1 Effects of Algae on Water Parameters

In presence of solar light, algae can utilize carbon dioxide from water during photosynthesis, increasing OH⁻ concentration and pH, whilst at night, photosynthesis is being replaced by cell respiration, when oxygen is consumed and carbon dioxide is produced, causing decrease in pH during night hours. Accordingly, pH keeps fluctuating in presence of algae.

Natural organic matter (NOM) refers to the complex matrix of organic compounds present in natural waters; dissolved NOM includes a wide variety of compounds such as proteins, amino acids, carbohydrates, amino sugars, carboxylic acid, etc. Total organic carbon/dissolved organic carbon (TOC/DOC) are surrogate parameters to evaluate the presence of NOM (Korshin et al., 2004). Algae themselves and organic matters they excrete make up a portion of total organic carbon (TOC) in water. Therefore, when the TOC/DOC
value is constant in background water, an increase of TOC/DOC might indicate the presence of algae (Knappe et al., 2004). The presence of algae cells can also increase the turbidity of water source. However, turbidity is also affected by many other factors, such as background water quality and storm events. As a result, raw water turbidity might of very limited use when determining the onset of algal blooms (Knappe et al., 2004).

### 2.1.2 Problems Caused by Algae

Algae grow drastically during algal bloom seasons and can cause a series of problems in water treatment plants (WTP) if not treated properly. The organic compounds in AOM are typically hydrophilic and comprise high molecular weight substances such as proteins and polysaccharides (Her et al., 2004), which have been proven to cause severe fouling for both low-pressure polymeric (Lee, Amy, & Croué, 2006) and ceramic membranes (Zhang, Fan, & Roddick, 2013). Problematic algae, species that do not settle or are not easily removed by water treatment processes, are common in many WTPs. Diatoms are common and other algae were not discernible in the flocs; long and needle-shaped algae are less likely to settle and more likely to be present in overflow water. Many diatom cells or colonies that are extremely deformed from spherical had high overflow rates. Species that overflow the basin clog the sand filters, leading to a need for repeated backwashing, thus limiting the production of clean water and economic loss.

It is widely known that serious algal bloom can lead to unpleasant odor and taste in water. For example, the earthy and musty odors mainly associate with (E)-1, 10-dimethyl-9-decalol (geosmin), and 2-methylisoborneol (MIB). Fishy, cucumber, rancid-cheesy, dirty socks sour smells are mainly produced by blue-green algae, golden algae, and some other kind (Knappe et al., 2004).
Algae, themselves are not harmful, however, can cause problems when present in large numbers and become dense in waters. Harmful algal bloom is also associated with acute morbidity and mortality in human and animals. Among them, the effects of the algal toxin are significant in terms of human health risk. For example, several species of cyanobacteria produce toxins with specific effects such as causing liver damage, neurotoxicity, and tumor promotion. Many acute symptoms also are reported which include fever, gastrointestinal disorders, and irritation of skin, ears, eyes, and respiratory tract (World Health Organization, 2011). In 1991, the Centers for Disease Control and Prevention reported that around 20% of food-related outbreaks attributed to the consumption of seafood, among half of which resulted from natural algal toxins (Teaching About Evolution and the Nature of Science, 1998). Six human poisoning syndromes are indicated as the result of exposure to water contaminated with algal bloom or volatile toxins and consuming contaminated seafood such as paralytic, neurotoxic, amnesic, diarrheic shellfish poisonings, ciguatera fish poisoning, and putative estuary-associated syndrome (Dolah, Roelke, & Greene, 2001). Most recently in 2014, a severe algal bloom in Lake Erie caused three-day water ban for Toledo, Ohio, when residents were ordered not to drink water from their taps for several days (Wilson, 2014). Throughout history, United States has had few outbreaks of algal bloom related to illness. It was reported in 1975, that 62% of Sewickley town, PA, was afflicted with gastrointestinal illness (Lippy & Erb, 1976). In China, a study has showed that 1,322 children aged 7-15 who received drinking water from a source contaminated with harmful algal bloom toxins in Gorge reservoir region had a higher level of liver enzymes in blood than other normal children (Li et al., 2011). Similarly, in Southern China,
high rate of primary liver cancer has been related to the long-term consumption of microsystin in drinking water (Ueno et al., 1996).

Algae and their metabolites can serve as precursors to form disinfection by-products (DBPs) after chlorination (Fang et al., 2010). It is required that algae are removed from drinking water, preferably during the initial stages (Wu et al., 2011). The United States Environmental Protection Agency (USEPA) requires the removal of NOM in terms of total organic carbon (TOC) from drinking water by enhanced coagulation (Ghernaout et al., 2014). It is found that exposure to DBPs might result in a decrease in birth rate, rectal and colon cancers, kidney and spleen disorders, and other problems such as immune system problem and neurotoxic effect (Richardson et al., 2007).

Drastic algae growths during algal bloom seasons have significant economic impact in dealing with public health issues, commercial fisheries, tourism and recreation, the remediation, management and controlling of ecological environment. The problems of poor settling, filter clogging and other equipment damage in water treatment plant lead to huge expenses spent on facilities maintenance, raising the cost of drinking water treatment.

2.2 Treatment Methods

![Figure 2.1 A typical configuration of water treatment plant (Shorney et al., 1999)]
Drinking water supply and disinfection systems are combinations of many unit processes such as coagulation, flocculation, sedimentation, filtration, ion exchange, and disinfection (USEPA, 2004). Different approaches are employed to reduce DBP precursors (NOM), turbidity, pathogens and other microorganisms. A typical configuration of water treatment plant is shown in Figure 2.1 (Shorney et al., 1999).

### 2.2.1 Coagulation

Coagulation is one of the main unit operations in drinking water treatment process and has been successfully implemented in several water facilities (EPA, 2015). It is applied to eliminate colloids and particles by coagulants such as alum and ferric chloride (Ghernaout et al., 2014). Coagulation as a conventional process in water treatment to reduce the effects of turbidity, alkalinity, UV$_{254}$, TOC and DBP precursors (Soh et al., 2008) is well studied. As well as the optimization of operating parameters (Rossini, Garrido, & Galluzzo, 1999) such as pH, coagulant dosage, and mixing rate have also been well studied (Wang et al., 2013). Issues dealing with the removal of natural organic matters (NOM) in drinking water due to coagulation have also been extensively discussed (Matilainen et al., 2010).

Coagulation has been employed in water treatment to lower color and turbidity, however optimum conditions for color or turbidity removal are not necessary the same as those for NOM removal. The coagulation conditions optimized for turbidity reduction are called baseline coagulation (Budd et al., 2004), while conditions of dose and pH especially optimized for organic matter removal are called optimized coagulation (Matilainen et al., 2010). Enhanced coagulation refers to conditions where excess coagulant is used than baseline coagulation, with alternations in pH, sequence of chemical addition, use of
alternative coagulant chemicals, and aim to achieve more efficiency in NOM removal (Budd et al., 2004).

The removal of NOM in conventional treatment process by the addition of coagulant has been demonstrated in laboratory research, pilot, demonstration and full-scale studies (USEPA, 1999). Many researchers have shown that total organic carbon in water shows wide range of responses to treatment with aluminum and iron salts (Z. K. Chowdhury, Roberson, & Owen, 1997; Edwards, 1997; White, Thompson, Harrington, & Singer, 1997). Most of these studies used regular and reagent grade alum ($\text{Al}_2\text{SO}_4$) as the coagulant. Other coagulant and coagulant aids such as polyaluminum chloride (PACl) and cationic polymers were also reported to be effective in removing DOC (USEPA, 1999).

When treating algal suspensions, coagulants are used to facilitate the sedimentation of algae cells to the anoxic bottom layer of water, denying algae to have access to the oxygen, light and nutrients, and accordingly causing the algae to fail to multiply and die. The surface charge of algae makes it possible their flocculation by conventional flocculants such as alum and polyelectrolytes, though flocculation of algae using alum is not always very effective. Ferric sulfate was found to be inferior in comparison with alum with respect to the optimal dose, pH and the quality of the harvested algal paste (Bare, Jones, & Joe, 1975; Moraine, Shelef, Sandbank, Bar-Moshe, & Shvartzburd, 1980). Chitosan, a cationic polyelectrolyte, was used to coagulate three freshwater algae, *Spirulina*, *Oscillatoria* and *Chlorella*, and one brackish alga, *Synechocystis* in the pH range 4 to 9, and a turbidity range of 10 -100 NTU in water (Divakaran & Pilla, 2002). Chitosan reduced the algal content effectively by flocculation and settling. The flocculation efficiency was very sensitive to pH, and reached a maximum at pH 7.0 for the freshwater species, but lower for the marine
species. The settled algal cells were intact and live, but were not re-dispersed by mechanical agitation.

Laboratory studies demonstrated that pretreatment with potassium ferrate enhanced the algae removal by coagulation with alum. Algae removal efficiency increased remarkably when the water was pretreated with ferrate (J. Ma & Liu, 2002). Pretreatment with ferrate resulted in a reduction of alum dosage required to cause an efficient coagulation for algae removal. Upon oxidation with ferrate, the cells were inactivated and some intracellular and extracellular components were released into the water, which was helpful to the coagulation by their bridging effect. The coagulation was also improved by increasing particle concentration in water, because of the formation of the intermediate forms of precipitant iron species during preoxidation. In addition, it was also observed that ferrate preoxidation caused algae to agglomerate before the addition of coagulant, the subsequent application of alum resulted in further coagulation.

To enhance the removal of toxic algae Microcystis aeruginosa, a sonication-coagulation method was used. The destruction of gas vacuoles during ultrasonic irradiation of algae cells occurred as the acoustic cavitation and collapse of bubbles resulting in the settlement of cyanobacteria. When applying sonication dosage at 47.2 W·cm⁻², the removal rate of M. aeruginosa was as high as 93.5% (Zhang et al., 2009).

Coagulation is an important step in the conventional drinking water treatment process, however, it is reported that chemical coagulants can cause damage to algal cells leading to the release of intracellular toxins and taste and odor compounds to water (Chow et al., 1999; Peterson et al., 1995). Sun et al. (2012) have concluded that M. aeruginosa cells should be disposed from the basin within 6 days of coagulation to avoid the release of MC-LR to the
treated water. So far, little studies have focused on the potential risk of coagulated cell lysis over time in the coagulation basin warranting further research (EPA, 2015).

2.2.2 Pre-Oxidation: Chlorination, Ozone, Permanganate, and Ferrate

Preoxidation is widely used in drinking water treatment systems and is said to be effective in promoting the coagulation of algae cells and algal organic matter. Preoxidation by chlorine, chlorine dioxide, ozone, permanganate, and ferrate have been well investigated (Henderson et al., 2008). The main mechanism involved in this process is the change in zeta potential and structure of algae cells and destruction of cells to promote aggregation (P. Xie, Ma, Fang, Guan, & Yue, 2013).

Among the preoxidation to remove algae cells, pre-chlorination is considered to be the most widely used and effective method. However, it is reported that pre-chlorination damages cell membrane and resulting in the release of intracellular metabolites such as algal toxins (Daly et al., 2007) although little change was found in the cell integrity by SEM (M. Ma, Liu, Liu, & Qu, 2012). The application of chlorine is limited by the potential of the direct disinfection by-product (DBP) formation (Wei et al., 2011) (Yang et al., 2011) (Fang et al., 2010).

Xie et al. (2013) have investigated the impacts of permanganate and preozonation on algae cells and their subsequent production of algal organic matter, the disinfection by-products (DBP) formed during chlorination of two treated waters. The results show that preoxidation by permanganate does not cause any damage to the cells, and accordingly, few disinfection by-products are formed. While preoxidation by ozonation actually destroyed algae cell
integrity, with only 2% of integrated cells left releasing intracellular organic matter (IOM), which lead to much higher amount of disinfection by-products (DBP) (P. Xie et al., 2013).

Studies were conducted by Zhou et al. (2014) to find the impact of potassium ferrate (VI) on algae cells integrity, the release of intracellular organic matter (IOM) and potential of disinfection by-product (DBP) formation (Zhou, Shao, Gao, Zhu, et al., 2014). The experimental results show that significant removal of *Microcystis aeruginosa* as well as decreased algal organic matter (AOM) occurred. According to their findings, the concentrations of THM and HAA were also reduced by 71% and 67%, respectively after applying potassium ferrate.

### 2.2.3 Ultraviolet Irradiation (UV) and UV Based Processes

Ultraviolet irradiation, especially at 254 nm (UV-C), is widely used in water treatment plant to kill pathogens and inactivation of microorganisms in water. It is also reported to be highly effective for removal of cyanobacteria (Antoniou et al., 2009). According to Bjorn (2007), UV-induced damage in microorganisms is achieved by two pathways, direct photolysis and indirect oxidation by producing reactive oxygen species. Studies have found that the inactivation of cyanobacteria species can be achieved by UV-C irradiation for 30 s to 10 min depending on the intensity (Sakai et al., 2009). Tao et al. (2010) investigated the suppression and recovery effects of UV-C irradiation on cyanobacteria and green algae species and found that the sensitivity to UV-C irradiation of cyanobacteria is much higher than green algae species. UV based advanced oxidation processes such as UV/H₂O₂ have been considered as a possible means of pre-treatment for membrane processes as they can generate highly oxidizing hydroxyl radicals (•OH) which may degrade AOM as well as organic compounds derived from cyanobacteria in
drinking water, including geosmin, MIB and algal toxins such as microcystin (He et al., 2012).

2.3 Formation of Disinfection by-products (DBPs)

Chlorination has been widely used in drinking water treatment as disinfection method to control the waterborne infectious diseases for a long time and it is recognized as among the most effective measures undertaken to protect public health (Raitt, 2000). However, the risk of natural organic matter reacting with chlorine and forming halogen-related disinfection by-product (DBP) has set limits for the development of chlorination practice. The US Environmental Protection Agency (USEPA) D-DBP Rule of 1998 mandates that removal of predetermined concentration of total organic carbon (TOC) as means to reduce disinfection by-product (DBP) precursors (USEPA, 1999).

2.3.1 DBP Formation

Table 2.1 Name and Acronyms for HAAs

<table>
<thead>
<tr>
<th>Names</th>
<th>Formula</th>
<th>Acronyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haloacetic acid</td>
<td>-</td>
<td>HAA</td>
</tr>
<tr>
<td>Monochloroacetic acid</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CICOOH</td>
<td>MCAA</td>
</tr>
<tr>
<td>Monobromoacetic acid</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;BrCOOH</td>
<td>MBAA</td>
</tr>
<tr>
<td>Dichloroacetic acid</td>
<td>CHCl&lt;sub&gt;2&lt;/sub&gt;COOH</td>
<td>DCAA</td>
</tr>
<tr>
<td>Bromochloroacetic acid</td>
<td>CHBrCICOOH</td>
<td>BCAA</td>
</tr>
<tr>
<td>Dibromoacetic acid</td>
<td>CHBr&lt;sub&gt;2&lt;/sub&gt;COOH</td>
<td>DBAA</td>
</tr>
<tr>
<td>Trichloroacetic acid</td>
<td>CCl&lt;sub&gt;3&lt;/sub&gt;COOH</td>
<td>TCAA</td>
</tr>
<tr>
<td>Bromodichloroacetic acid</td>
<td>CBrCl&lt;sub&gt;2&lt;/sub&gt;COOH</td>
<td>BDCAA</td>
</tr>
<tr>
<td>Chlorodibromoacetic acid</td>
<td>CBr&lt;sub&gt;2&lt;/sub&gt;COOH</td>
<td>CDBAA</td>
</tr>
<tr>
<td>Tribromoacetic acid</td>
<td>CBr&lt;sub&gt;3&lt;/sub&gt;COOH</td>
<td>TBAA</td>
</tr>
</tbody>
</table>

The formation of disinfection by-product (DBPs) in drinking water is the result of reaction between natural organic matter (NOM) and mainly chlorine and/or other disinfectants.
Four groups of DBPs are regulated under the United States Environmental Protection Agency (USEPA) stage 1 Disinfectants and Disinfection Byproducts (D-DBP) rule (USEPA, 1999): they are trihalomethanes (THM), haloacetic acids (HAAs), chlorite and bromate (Y. Xie, 2013). The trihalomethanes viz. chloroform, dichlorobromomethane, dibromochloromethane and bromoforms are the major byproducts of chlorination. In addition to trihalomethanes, haloacetic acids and haloacetonitriles are the products of both chlorination and chloramination. Haloacetic acids (HAAs) are second major group of DBP following THMs. Chloramination also leads to the production of cyanogen chloride and N-organochloramine. There are nine common HAAs and they are listed in Table 2.1. By replacing hydrogen atoms with halogen atoms, partially or completely, a total of nine HAAs are obtained.

2.3.2 Effects of NOM on DBP Formation

The concentration of DBPs formed in drinking water is affected by many water quality parameters and operational conditions, including presence of natural organic matter (NOM), chlorine dosage, reaction time, inorganic compounds and pH.

![Figure 2.2 Natural organic matter (Garcia, 2005)](image-url)
Natural organic matter (NOM) exists in both surface and ground water, it is often divided into dissolved organic carbon (DOC) and particulate organic carbon (POC) based on filtration of water through 0.45 μm filter. NOM is generally produced by decaying vegetation, organic soils and biological activity (Garcia, 2005), and is composed of heterogeneous mixture of humic substances and non-humic substances (Figure 2.2). The amount of NOM is measured through surrogate parameters as total organic carbon (TOC) or dissolved organic carbon (DOC), or ultraviolet absorbance at 254 nm (UV$_{254}$). In measurement of drinking water qualities, UV$_{254}$ is reported to be a good surrogate for the concentration of activated aromatic groups in polyhydroxyphenolic acid (PHA) moieties, which are the reaction sites in humic species (Croué, Korshin, & Benjamin, 2000) (Korshin, Perry, & Ferguson, 1996) (Traina, Novak, & Smeck, 1990) and is usually better correlated than DOC with the yield of DBPs (Chellam & Krasner, 2001).

Algae and their organic matters make up a portion of natural organic matter (NOM) in water. Algal organic matter appears to contain more organic nitrogen and hydrophilic content, and less aromatic carbon content, with much lower specific UV absorbance (SUVA) values and higher heterogeneity as compared with natural organic matter (NOM) (Widrig, Kimberly, & McAuliffe, 1996) (Nguyen et al., 2005) (Her et al., 2004). Algal EOM and IOM, both contain biopolymers, such as proteins, peptides and amino acids, while the portion of protein in IOM is larger than that in EOM (Pivokonsky, Kloucek, & Pivokonska, 2006). However, less is known about the characteristics of AOM, such as molecular weight (MW) distribution, polarity distribution, and specific composition of org-N in IOM and EOM. Algogenic organic matter (AOM) from four algae species (*Chlorella vulgaris*, *Microcystis aeruginosa*, *Asterionella formosa* and *Melosira sp.*) were
characterized using DOC, SUVA, zeta potential, charge density, hydrophobicity, carbohydrate and protein, MW and fluorescence (Henderson et al., 2008). Henderson et al (2008) concluded that generally, AOM possesses very different characteristic from NOM.

Fang et al. investigated the formation of disinfection by-product (DBP) from *M. aeruginosa* under different conditions (Fang et al., 2010). Result shows that higher concentrations of N-DBPs (nitrogenous disinfection by-products), including haloacetonitriles (HANs) and trichloronitromethane (TCNM) and chloral hydrate (CH) were found after chlorination of algal cells than natural organic matter (NOM), while chlorination of natural organic matter produces more C-DBPs (carbonaceous disinfection by-product), which include trihalomethanes (THM), haloacetic (HAA), and haloketones (HKs). Fang et al. (2010) also found that the chlorination of extracellular organic matter (EOM) and intracellular organic matter IOM produce more nitrogenous DBPs and less carbonaceous DBPs than chlorination of natural organic matter (NOM). Factors that affect the formation of DBPs from chlorination of algal cells and organic matter include pH, temperature, ammonia concentrations, and algae growth stages (Fang et al., 2010).

Hong et al (Hong et al, 2008) investigated the behavior of three major biomolecules constructing algae cells: protein, carbohydrates, and lipid in DBP formation. Three model compounds: bovine serum albumin, starch, and fish oil were used for comparison. The results have shown that in general, the calculated chloroform formation from algae cells was consistent with the experimental data, and algae cells appear to have a higher potential of forming DCAA (diachloroacetic acid) after chlorination than humic substances like humic acids or fulvic acids (Hong et al., 2008).
Zamyadi et al. (2012) have investigated the chlorination of four blue-green algae species and three cyanotoxins and their analogues in both natural waters and bloom water samples. They found that pre-chlorination is an efficient way of treating natural waters that contain lower than 200,000 cells per mL of cyanobacteria cells. As well, they concluded that quality of background water is a key factor for DBP formation independent of cyanobacterial cells (Zamyadi et al., 2012). Merel et al. (2010) presented a study on cyanotoxins in water and the behavior during chlorination and concluded that chlorination efficiency of cyanotoxins depends on factors as pH, chlorine dose, and nature of oxidant. They also found out that Anatoxin-a resists chlorination (Merel et al., 2010).

2.3.3 Models for DBP Formation

Since the discovery of DBPs in drinking water in 1974, numerous studies have presented to developing models for predicting DBP formation in drinking water (S. Chowdhury, Champagne, & McLellan, 2009). For the past two decades, many investigations have been carried out by linking DBP concentrations with total organic carbon (TOC) or dissolved organic carbon (DOC), UV absorbance at 254 nm (UV254), chlorine dosage (D), reaction time, concentration of bromide ion (Br⁻), pH, and water temperature (Sadiq & Rodriguez, 2004). Studies have shown that higher disinfectant dosage and longer contact time contribute to higher DBP formation (W. Chen & Weisel, 1998; Sadiq, Rehan, Manuel, & Rodriguez, 2004).
Two types of models have been used to describe the DBP behavior: empirical models (statistical models) and chlorination decay models (mechanistic models) (Garcia, 2005). Empirical models (also called statistical models) are based on data from pilot studies and statistical approaches, they are commonly used to predict the formation of DBPs. Water quality parameters and operational parameters serve as independent variables and the disinfection by-product (DBP) precursors served as dependent variables. These empirical models enable THM and HAA concentrations to be estimated from more easily measured parameters, and are therefore of practical interest (G. Korshin et al., 2004a). A group of statistical equations that have been proposed to model DBP formation kinetics is presented in Table 2.2.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Eq. #</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>[THM] = 0.0035 \left[ \exp \left( \frac{4.297}{T} \right) \right] \text{pH} - 2.8 \</td>
<td></td>
<td>(2.7) Unano, Wada, and Takemasa (1983)</td>
</tr>
<tr>
<td>[THM] = 0.0031 \left( UV_{254} \right)^{0.85} \left( \text{DOC} \right)^{0.49} \left( \text{Cl}_2 \right)^{0.18} \left( \text{pH} - 2.6 \right)^{0.71} \left( \text{Br} \right)</td>
<td></td>
<td>(2.8) Amy, Chadik, and Chowdhury (1987)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.9) Harrington, Chowdhury, and Owen (1992)</td>
</tr>
<tr>
<td>[THM] = 0.0039 \left( \text{DOC} \right) \left( UV_{254} - 254 \right)^{0.40} \left( \text{Cl}_2 \right)^{0.88} \left( \text{pH} - 2.6 \right)^{0.71} \left( \text{Br} \right)</td>
<td></td>
<td>(2.10) Black, Harrington, and Singer (1996)</td>
</tr>
<tr>
<td>[THM] = 7.1 \left( UV_{254} \right)^{0.53} \left( \text{DOC} \right)^{0.66} \left( \text{Cl}_2 \right)^{-7.6} \left( \text{N} \right)</td>
<td></td>
<td>(2.11) Ruthburn (1996a,b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.12) Solarik et al. (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.13) Sung et al. (2000)</td>
</tr>
<tr>
<td>[THM] = 14.6 \left( UV_{247} \right)^{0.88} \left( \text{Cl}_2 \right)^{-2.0} \left( \text{pH} - 3.8 \right)</td>
<td></td>
<td>(2.14) Gofinopoulos and Ariondakis (2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.15)</td>
</tr>
</tbody>
</table>
Mechanistic models (chlorination decay models) are expected to be more robust and flexible than purely empirical models (G. Korshin et al., 2004a). This type of model can provide an insight into the treatment processes being modeled as it can reflect the features or chemistry of chlorine/NOM reactions. Development of mechanistic model for chlorine decay in natural waters started before 1950 (McClellan et al., 2000) and a number of studies have been conducted where chlorine decay has been modeled in the contexts of drinking water, waste water, and power plant cooling water (Garcia, 2005). Mechanistic models establish regressions that link concentrations of DBP to known reaction conditions (initial NOM and chlorine dosages, value of SUVA_{254}, pH, etc) at the start of chlorination process (G. Korshin et al., 2004a). Powell et al. (2000) have grouped models of chlorine consumption into the 6 categories shown in Table 2.3, however the model equations shown in the Table have been used with a reasonable level of success only for relatively long-term chlorine decay kinetics.

Table 2.3 Chlorine decay kinetic models (Powell et al., 2000)

<table>
<thead>
<tr>
<th>Classification of model</th>
<th>Analytical expression for chlorine consumption</th>
<th>Eq. #</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>First order Cl₂ decay</td>
<td>$C = C_0 e^{-kt}$</td>
<td>(2.1)</td>
<td>Simplest and most widely used model</td>
</tr>
<tr>
<td>Limited first order</td>
<td>$C = C^* + (C_0 - C^*) e^{-kt}$</td>
<td>(2.2)</td>
<td>First order decay approaching a finite, minimum Cl₂ residual at long $t$</td>
</tr>
<tr>
<td>Parallel first order</td>
<td>$C = C_0 e^{-kt} + C_b (1 - e^{-kt})$</td>
<td>(2.3)</td>
<td>Considers two, kinetically distinct Cl₂ consumption processes</td>
</tr>
<tr>
<td>First order with respect to Cl₂ and another reactant</td>
<td>$C = \frac{U - C_0}{C_0} \exp\left(\frac{W(U - C_0)x - 1}{U - C_0}\right)$</td>
<td>(2.4)</td>
<td>$U$ and $W$ relate to initial concentration of second reactant, reaction stoichiometry, and rate constant (see original article for details)</td>
</tr>
<tr>
<td>Second order Cl₂ decay</td>
<td>$C = \frac{C_0}{1 + C_0 k^2 t}$</td>
<td>(2.5)</td>
<td>Predicts steeper initial decline in Cl₂ concentration than first order model, as is often observed experimentally</td>
</tr>
<tr>
<td>$n$th order Cl₂ decay</td>
<td>$C = \left(k_n^{(n-1)} t (n-1) + C_0^{(n-1)}\right)^{\frac{1}{n-1}}$</td>
<td>(2.6)</td>
<td></td>
</tr>
</tbody>
</table>
2.4 Importance of This Study

Algae are ubiquitous in lakes, reservoirs and surface waters. The most commonly found freshwater algae are diatoms, green algae and blue algae (EPA), and they grow drastically during algal bloom season. In real water treatment practice, they can cause a series of problems if not treated properly, which includes causing bad taste and odors, poor settling, clogging filters, and release of algal toxins (Ghernaout et al., 2014). In addition, algae can also serve as precursors to form disinfection by-products (DBPs) after chlorination (Fang, Ma, et al., 2010). As a result, to avoid the effects of algae and algal organic matter on drinking water, removal of algae in the initial stage of water treatment is necessary.

Coagulation is frequently used in water treatment facilities to remove colloidal and suspended particles and DOC in water. Among all the methods of removal of algae, compared to other pretreatment methods such as pre-oxidation, coagulation has the lowest risk of damaging algal cells causing the release of DBP precursors. Although, many studies have investigated the optimum condition of coagulation in water treatment, and optimization of coagulation operating parameters such as pH, temperature, the dosage of coagulants etc., however, no studies were conducted to determine if coagulation can cause algae cell damage and subsequent release of algal organic matters over time, and subsequent DBP formation. This study is a part of a larger study on providing safe water for the South to North water diversion project for Beijing, China. The project was launched by Chinese government to solve shortage of water because of disparity in water resource availability in China. The global objective of the research being conducted in our laboratory is to determine the optimum UV-chlorine dosage for minimum DBP formation and maximum pathogen reduction for different influent water quality parameters relevant to
China including the presence of various algal species. The objective of this project is to determine the release of algal matter and subsequent DBP formation due to chemical coagulation, which has never been reported in literature. Diatom and green algae were selected in this study to investigate the time dependent release of algal matters subjected to chemical coagulant treatment due to the following reasons: (i) diatom and green algae are reported as the most (48.4 %) and the third (19.3 %) most, respectively algae species detected in Danjiangkou reservoir, China, which served as the major water source for South to North Water Diversion (SNWD) project, and ii) secondly, numerous studies were based on one algae species: cyanobacteria (blue-green algae), and not much research has been carried out on other common algae species such as green algae and diatom subjected to coagulation. Alum is selected as the model coagulant as alum is the most commonly used coagulant in drinking water treatment.

The specific objectives of this study are: (1) to determine the time dependent release of algal matters subjected to common alum dosage used in drinking water treatment process for four different species of algae suspensions; (2) to determine the DBP formation potential for the different algal species in both pure water and natural water (local water from Fanshawe Lake); (3) to develop simple statistical models to predict DBP formation for different water qualities (mainly different DOC values).
Chapter 3

3 Experimental Materials and Methods

DBP formations at various conditions were determined in bench-scale experiments conducted in this research. Different algae cultures were grown at the laboratory at Western. While most of the experiments were conducted using deionized water, a few experiments were also conducted in water samples collected from Fanshawe Lake. For coagulation experiments, algae cultures were spiked in the water (either in deionized water or lake water) to make algae suspensions to simulate an algal bloom condition. Jar tests were used to perform laboratory coagulation experiments. Lake water was filtered within 24 hours of collection and the water quality parameters were determined. Temperature, pH, turbidity, $\text{UV}_{254}$, and DOC of water were analyzed before and after coagulation. Coagulated water was filtered before disinfection using UV and post-chlorination, after that, a GC-ECD was used to analyze disinfection by-products from the treatment of water. The flow chart below describes the experimental process adopted in this work.
3.1 Materials

3.1.1 Chemical Reagents

Solutions were prepared from ACS reagent grade chemicals or stock solutions. A free chlorine stock solution (2707 mg/L as Cl₂) was prepared from 5% sodium hypochlorite (NaOCl, ACROS, New Jersey, USA) and periodically standardized by DPD/FAS titration (EPA, 1978). N,N- diethyl-1,4-phenylenediamine sulfate (DPD) indicator and ferrous ammonium sulfate (FAS) were purchased from (RICCA, Arlington, USA). Haloacetic acid (HAA) calibration standards (EPA 522 Methyl Esters Mix, Supelco, St Louis, USA), 99.9% Methyl tert-butyl ether (MTBE, Sigma-Aldrich, St Louis, USA) were purchased from Sigma-Aldrich. Al₂(SO₄)₃ coagulant stock solution was prepared by dissolving 5 g Al₂(SO₄)₃·16H₂O (EMD, Gibbstown, USA) in 500 mL MiliQ water. Alum dose was kept constant at 30 mg/L of Al₂(SO₄)₃·16H₂O.
3.1.2 Algae Suspension

Figure 3.2 Algal species used for experiment. (a) *Chlorella vulgaris* (b) *Phaeodactylum tricornutum* (c) *Chlamydomonas reinhardtii* and (d) *Cyclotella meneghiniana*.

Four different kinds of algae shown in Figure 3.2 were used in this research. Four species: green algae *Chlorella vulgaris, Chlamydomonas reinhardtii* and diatom *Phaeodactylum tricornutum, Cyclotella meneghiniana* were used for this study. The characteristics of four algal species are listed in Table 3.1. Four strains were originally supplied by University of Texas at Austin and Chlamydomonas Resource Center (*C. vulgaris* strain no. UTEX 2714 and *C. reinhardtii* strain no. CC 125) and Canadian Phycological Culture Centre (*P. tricornutum* strain no. CPCC 162 and *C. meneghiniana* strain no. CPCC 710).

---

1 Figure references: *Chlorella vulgaris* (UTEX 2714) https://utex.org/products/utex-2714
*Chlamydomonas reinhardtii*
http://protist.i.hosei.ac.jp/pdb/images/chlorophyta/chlamydomonas/Euchlamydomonas/reinhardtii/sp_10.html
*Phaeodactylum tricornutum* (UTEX 0646) https://utex.org/products/utex-0646
*Cyclotella meneghiniana* :https://utex.org/collections/freshwater-diatom-collection/products/utex-lb-fd-0257
Table 3.1 Characteristics of algae used in this study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Chlorella vulgaris</th>
<th>Chlamydomonas reinhardtii</th>
<th>Phaeodactylum tricornutum</th>
<th>Cyclotella meneghiniana</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
<td>Green algae</td>
<td>Diatom</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Geometric shape</strong></td>
<td>Spherical</td>
<td>Ellipsoidal</td>
<td>Fusiform</td>
<td>Cylinder</td>
</tr>
<tr>
<td><strong>Typical dimensions</strong></td>
<td>2-10 μm in diameter</td>
<td>10 μm in diameter</td>
<td>10 μm in length</td>
<td>10-35 μm in diameter, 8.4-28 μm in height</td>
</tr>
<tr>
<td><strong>Typical bloom period</strong></td>
<td>Summer/early fall</td>
<td></td>
<td></td>
<td>Spring</td>
</tr>
</tbody>
</table>

The algae culture used for making an algae suspensions of *C. vulgaris*, *C. reinhardtii*, *P. Tricornutum* and *C. meneghiniana* were grown in Bold’s Basal Medium (Andersen, Robert, 2014), high salt minimal media (HSM) (Sueoka, Chiang, & Kates, 1967), f/2 Marine Media (Guillard & Ryther, 1962) and CHU-10 medium (Stein, 1973). The number of algal cells in suspension was counted using a light microscope (ZEISS) with a hemocytometer (LW Scientific); each sample was counted 3 times. Stock algae cultures were obtained at cell yield of $2.5 \times 10^7$ cells/mL and $2.4 \times 10^6$ cells/mL for *C. vulgaris* and *P. Tricornutum*, $4.4 \times 10^5$ cells/mL and $3.0 \times 10^4$ cells/mL for *C. reinhardtii* and *C. meneghiniana*, respectively.

For coagulation experiments, two levels (high cell density and low cell density) of experimental suspension were created: higher density group was created in order to simulate an algal bloom condition. The suspensions of *C. vulgaris* and *P. tricornutum* in deionized water contained approximately $1.5 \times 10^6$ cells/mL and $1.1 \times 10^6$ cells/mL, respectively. Lower density group was prepared by filtering *C. reinhardtii* and *C. meneghiniana* through 0.45 μm membrane filter and re-suspending algae cells in deionized water. Final counts of $4.3 \times 10^4$ cells/mL and $2.3 \times 10^4$ cells/mL were obtained for *C. reinhardtii* and *C. meneghiniana*, respectively.
3.1.3 Background Water

The stock algae suspension was spiked into two background waters, deionized water, and lake water. Deionized water was obtained directly from the tap in the lab, whereas lake water sample was collected in the fall, 2015 from Beach Pavilion of Fanshawe Lake, Fanshawe Conservation Area Authority. This place was selected as natural water source as it is known to support algal growth (seasonal algae grows in the lake) and it was a representative of local water quality. Several 1L amber bottles were used for collecting water samples. Each water sample was filtered through 0.45-μm sterilized membrane filter (PALL life Sciences) within 24 hours of sampling, the water was kept in the amber bottle in 4°C. Prior to use, water was filtered through a 0.2 μm PTFE membrane filter (Chromspec) to remove natural algae and other microorganisms. Water quality parameters measured for deionized water (DW) and lake water (LW) are shown in Table 3.2.

<table>
<thead>
<tr>
<th>Source water</th>
<th>Turbidity NTU</th>
<th>pH</th>
<th>DOC (mg/L)</th>
<th>UV254</th>
<th>Alkalinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW</td>
<td>0.263±0.01</td>
<td>6.50±0.2</td>
<td>0</td>
<td>0.002±0.001</td>
<td>6.4±0.2</td>
</tr>
<tr>
<td>LW</td>
<td>0.205±0.50</td>
<td>8.65±0.2</td>
<td>72.6±0.5</td>
<td>0.129±0.003</td>
<td>208±1.0</td>
</tr>
</tbody>
</table>

The stock suspension was diluted with deionized water or lake water to make experimental suspensions to certain algae density as mentioned above.

---

2 Values are averages of 3 measurements.
3.2 Coagulation Experiment

3.2.1 Jar Test

For all coagulation experiments, dosage of alum was fixed at 30 mg/L (reported as Al₂(SO₄)₃·16H₂O). This dosage was selected based on the common dosages that are used in drinking water treatment facilities ranging between 20 - 30 mg/L of Al₂(SO₄)₃·14 H₂O, depending on the turbidity of raw water sources (Garcia & Moreno, 2006). Jar test was conducted using a Phipps & Bird programmable jar tester (Model PB900) with six stainless steel 1" x 3" paddles, an LED floc illuminator built into the base and a powder-coated steel uni-frame chassis. All tests were conducted at room temperature (~24 °C). 500 mL water samples were placed on the illuminator base with six samples tested at the same time. Experiment group samples were dosed with 30 mg/L of Al₂(SO₄)₃ from a stock solution. The programmable jar testing apparatus was used with following procedure: coagulant added followed by rapid mixing at 150 rpm, after 2 min the speed was reduced to 25 rpm for 20 min flocculation. Following flocculation, samples were allowed to settle for 30 min, 2 hours, 24 hours, 5 days, and 9 days. The supernatant was collected for pH, turbidity, DOC and UV₂₅₄ analysis after the settling. After 9 days, the algae suspensions were filtered through a 0.45 μm membrane filter and the filtrates were collected, and the algal organic matter was determined as DOC. The filtrate was then adjusted to pH 8 using a borate buffer (1.0 M boric acid and 0.26 M sodium hydroxide in distilled water) for disinfection experiments.
3.2.2 Water Quality Parameters Measurement

A Hach ratio turbidimeter (model 2100AN) was used to give a direct reading of the turbidity of the samples in nephelometric turbidity units (NTU). UV absorbance at 254 nm (UV$_{254}$) was measured using a dual-beam UV-VIS-NIR spectrophotometer (Shimadzu Model 3600). Dissolved organic carbon (DOC) samples were analyzed using a Shimadzu TOC-VC$_{PN}$ ASI-V analyzer. Samples for UV$_{254}$ and DOC analysis were filtered through a 0.45 μm membrane filter. Temperature and pH were measured using a pH meter (Orion Model STAR A111).

3.2.3 Viability Test

Cell viability was examined shortly after coagulation (for coagulated algae cells) by using a microscope employing methylene blue. To remove culture component from cell suspension, the cell suspension was centrifuged (3500 rpm) for 5 min and the algae were collected. The cells were re-suspended in DW with a vortex mixer. Both control and coagulated cells were stained with methylene blue (MB) in 3% acetic acid, and incubated in dark at room temperature for 20 minutes. After that, algal cells in suspension were examined using a light microscope (ZEISS) with a hemocytometer (LW Scientific). Cells
that stained blue or pale blue with MB were considered to be dead under the microscope, while living cells retained their own color (Imase, Ohko, Takeuchi, & Hanada, 2013).

3.3 Disinfection Experiment

3.3.1 Photolysis Experiment

The irradiation was performed with a bench scale collimated beam enclosing a low pressure (LP) UV lamp and a collimated tube with a non-reflective inner surface (Trojan Technologies). The lamp emits monochromatic light at $\lambda = 254$ nm. UV irradiation was measured with ILT1400 radiometer (International Technologies) and SED 240SEL detector. The Collimated beam irradiation equipment consists of a collimated beam apparatus, a radiometer and SED detector, 254 nm Photometer, 60 $\times$ 35 crystallizing petri dishes, small magnetic stir bars, and a magnetic stir plate.

![Figure 3.4 Bench-scale UV collimated beam for UV experiments (Bolton & Linden, 2003)](image)

Before treatment, the UV lamp was allowed to warm up for at least one hour and wait for it to be stable. UV intensities along the X and Y axes were measured, other parameters regarding water samples and collimated beam such as absorbance of the water sample, the total volume of the petri dish, distance from UV lamp to top of water surface were also
measured and inserted into a spreadsheet (shown in Appendix) to determine correction factors and exposure time. The UV dose can be calculated by the relationship: UV dose (J/cm$^2$) = UV Intensity (W/cm$^2$) x Time (s) (D=I x T). UV dose in the experiments was adjusted by changing the time of radiation instead of changing power output.

At room temperature, 50 mL of field water or DI water was added to a petri dish and placed under the UV lamp under constant stirring and temperature conditions. The initial pH of 8 was adjusted with 0.01 M H$_2$SO$_4$ or 0.1 M NaOH and borate buffer. A UV dose of 40 mJ/cm$^2$ typically used for 4-log reduction of pathogen in drinking water, is maintained throughout the process. Each experimental series was carried out in triplicate.

### 3.3.2 Post-Chlorination Experiment

Chlorination experiment was carried out according to the uniform formation conditions (UFC), (Summers, Hooper, Shukairy, Solarik, & Owen, 1996). The pH of samples was kept as 8.0 ± 0.2 with a borate buffer solution, subsequently combined hypochlorite-buffer dosing solution was added to the water samples. Thereafter, all the samples were kept in dark at room temperature for 24 hours. After 24 hours of incubation, ammonium chloride was added to quench the free residual chlorine in water.

A free chlorine stock solution was prepared by the dilution of sodium hypochlorite reagent with deionized water. Chlorine residual concentrations were determined using DPD-FAS titration method (EPA, 1978). Chlorination was conducted by mixing irradiated water samples with chlorine at the desired dose in 65ml clear Boston round glass bottles (with PTFE lined caps). Sample bottles were covered with aluminum foil and placed in darkness at room temperature to avoid photodegradation. Preliminary studies were performed using
a series of three dosages based on Cl\textsubscript{2}: DOC ratio in order to get a suitable chlorination dose which can lead to recommended 1.0 ± 0.4 mg/L free chlorine as chlorine residual after 24 hours incubation in dark at room temperature.

DPD spectrophotometric method (EPA method 330.5) was used to measure the free and total residual chlorine after 24-h incubation. Calibration curve of DPD titration was built at a range of 0, 0.1, 0.5, 1, 2, 4 ppm Cl\textsubscript{2} concentration and shown in the appendix. Series of KMnO\textsubscript{4} standards covering the equivalent chlorine range of 0.1 to 4 ppm were prepared and titrated with standard ferrous ammonium sulfate (FAS). The UV absorbance of the titrated solution was recorded at 515 nm. 17 points were collected to build a curve.

3.4 DBP Sample Analysis

Haloacetic acids (5HAAs including MBAA, DCAA, TCAA, BCAA, DBAA,) were determined using USEPA method 552.2. Briefly, 40 mL aliquots of chlorinated water sample were collected and 95-98% sulfuric acid (36N) was added to adjust pH less than 0.5; approximately 16 g of sodium sulfate was added to increase the ionic strength of the aqueous phase, and 4 mL of MTBE was added to the solution. Thereafter, the HAAs partitioned into the MTBE phase (upper layer) were transferred to a 15 mL tube and methylated by 1 mL of 10% sulfuric acid in methanol. After 2 hours of heating at 50 °C, 4 mL of saturated sodium bicarbonate solution was added to each tube before being analyzed by a GC-ECD system. Gas chromatography (Shimadzu GC-2014) with an electron captures detector and a BPX5 fused silica capillary column (30 m × 0.25 mm i.d., 0.50 μm film thickness) was used for the analysis. The oven temperature was programmed as follows: an initial temperature of 40 °C was held for 10 min, then increased at a rate of 10
°C/min to 95 °C, 25 °C/min to 200 °C, 35 °C/min to 280 °C. The temperature of the injector and electron conductivity detector were set at 250 °C and 300 °C, respectively. Nitrogen and Helium were used as the make-up gas and carrier gas, respectively.
Chapter 4

4 Results and Discussions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Chlorella vulgaris</th>
<th>Chlamydomonas reinhardtii</th>
<th>Phaeodactylum tricornutum</th>
<th>Cyclotella meneghiniana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Green algae</td>
<td>Diatom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stock cell count (cells/mL)</td>
<td>$1.5 \pm 0.6 \times 10^7$</td>
<td>$4.4 \pm 0.6 \times 10^5$</td>
<td>$2.4 \pm 0.2 \times 10^6$</td>
<td>$3.0 \pm 0.7 \times 10^4$</td>
</tr>
<tr>
<td>Experimental suspension cell count (cells/mL)</td>
<td>$1.5 \pm 0.6 \times 10^6$</td>
<td>$4.3 \pm 0.6 \times 10^4$</td>
<td>$1.1 \pm 0.4 \times 10^6$</td>
<td>$2.3 \pm 0.1 \times 10^4$</td>
</tr>
<tr>
<td>Initial turbidity (NTU)</td>
<td>$9.6 \pm 0.4$</td>
<td>$6.6 \pm 0.02$</td>
<td>$23.6 \pm 0.6$</td>
<td>$32.2 \pm 4.6$</td>
</tr>
<tr>
<td>Initial DOC (mg/L)</td>
<td>$4.6 \pm 0.6$</td>
<td>$3.4 \pm 0.6$</td>
<td>$16.3 \pm 1.6$</td>
<td>$1.6 \pm 0.5$</td>
</tr>
</tbody>
</table>

Stock algae cultures were obtained in their stationary growth phase in media and experimental suspensions were created by diluting the stock suspensions using deionized water or lake water. As mentioned in Chapter 3, two levels of experimental suspensions were created: higher density group was created in order to simulate an algal bloom condition. By mixing stock culture of *C. vulgaris* and *P. tricornutum* individually with deionized water, final cell counts of $(1.5 \pm 0.6) \times 10^6$ cells/mL and $(1.1 \pm 0.4) \times 10^6$ cells/mL, respectively were obtained. Lower density group was created by filtering *C. reinhardtii* and *C. meneghiniana* through 0.45 µm membrane filter and re-suspending algae cells in deionized water. Final counts of $(4.3 \pm 0.6) \times 10^4$ cells/mL and $(2.3 \pm 0.1) \times 10^4$ cells/mL were obtained for *C. reinhardtii* and *C. meneghiniana*, respectively. The corresponding turbidity and initial DOC are presented in Table 4.1. Turbidity is the ratio...
of intensities of the incident light intensity and the light scattered by the suspended algae. At higher cell densities it follows Beer's law, i.e. turbidity is proportional to the cell concentration, which was seen for green algae as turbidity increased with cell count. However, for diatom, *Cyclotella meneghiniana* at lower cell density, initial NTU was higher than that of *Phaedactylum tricornutum*. The range of proportionality of turbidity to number of cells depends on the size and shape of the algae. *Cyclotella meneghiniana*, a cylindrical shape algae has the highest length between 10 - 30 μm compared to all other species. In addition, morphology and structural features of the algae also play an important and complex role in their light-scattering behavior. In particular, internal cell structures such as gas vacuoles alter the scattering patterns of the phytoplankton species considerably (Konopka, Jacco, & Mur, 1987).

However, initial DOC concentration followed the trend with initial cell count within a specific group of algae, i.e., initial DOC increased with increase in cell count. Since the DOC of DI beyond the detection limit, initial DOC values in algal suspension are probably due to release of algal matter.

In all cases, three replicates of control and three replicates of experiments with coagulation were conducted, and the averages of the three replicates are presented in the following figures. For all the coagulation experiments, dosage of alum was fixed at 30 mg/L (reported as Al₂(SO₄)₃·16 H₂O). This dosage was selected based on the common dosages that are used in drinking water treatment facilities ranging between 20 - 30 mg/L of Al₂(SO₄)₃·14 H₂O, depending on the turbidity of raw water sources (Garcia & Moreno, 2006). The higher dosage was selected to determine the worst case scenario in case of algal DOC release.
Coagulation causes particulate matters to aggregate and thus lowers the turbidity by settling of the coagulated and flocculated particles. Figure 4.1 shows gentle mixing of four algae suspensions with and without the addition of coagulant. When $\text{Al}_2(\text{SO}_4)_3$ is added to water, aluminum sulfate dissociates and release positive aluminum ions to water forming free and Al-hydroxyl complexes such as $\text{Al}^{+3}$, $\text{AlOH}^{+2}$, $\text{Al(OH)}_2^+$, $\text{Al(OH)}_3^+$, $\text{Al(OH)}_4^-$ (Howe & Clark, 2002). The highly charged positive ions neutralize negatively charged algae cells and particles, allowing suspended small particles to stick together and form microflocs. As shown in Figure 4.1, particle size was increased as a result of continuing collisions in gentle mixing stage, slower mixing speed allows flocs to grow and prevent them from tearing apart. It can be seen from Figure 4.1 that four different species of algae were coagulated well using alum.
4.1 Effect of Coagulation on Turbidity and pH

Figure 4.2: Turbidity variation of (a) control and (b) coagulated algae suspensions of four algae species with settling time, temperature: 22 ± 2°C. Error bars represent the standard deviation of triplicate experiments.

The turbidity of supernatant of control and coagulated algae suspensions with different settling time is shown in Figure 4.2a and Figure 4.2b, respectively. It can be seen in Figure 4.2b that the turbidity declined significantly by coagulation for all four algal spices within 30 min, although both species of diatom (*P. tricornutum* and *C. meneghiniana*) with higher natural settling velocities showed higher removal of turbidity without coagulation. It was somewhat difficult to coagulate *C. vulgaris*. However, a decline of 82.3% in NTU occurred after 9 days for *C. vulgaris* (green algae), and 92.6% for *P. tricornutum* (diatom) by natural gravitational settling. After applying coagulant aluminum sulfate, turbidity decreased rapidly for *P. tricornutum* with 93.2% removal (from 33.6 ± 0.3 NTU to 2.3 ± 0.3 NTU) in 30 minutes, while only 24.4% removal (from 8.4 ± 1.3 NTU to 6.3 ± 2.0 NTU) in 30 minutes occurred for *C. vulgaris* (green algae). Even for algae suspensions with lower cell density, the turbidity removal rate was 75.6% for *C. reinhardtii* (green algae) and 83.1% for *C. meneghiniana* (diatom), respectively. Because of their siliceous cell wall, diatoms
have a relatively large density and they can settle under quiescent conditions (Knappe et al., 2004).

Figure 4.3: pH variation of (a) control and (b) coagulated algae suspensions of four algae species with settling time, temperature: 22 ± 2°C. Error bars represent the standard deviation of triplicate experiments.

Although, the uniform formation condition (UFC) (described in Chapter 3) stipulates the experimental pH for DBP formation reaction to be 8.0 ± 0.2, it is important to determine the variation of pH of algal solution due to coagulation as disinfection by-product formation below pH 7 is found to be higher in amount as well as in toxicity (G. Korshin et al., 2004a).

The pH variation for both control and coagulated algae is shown in Figure 4.3a and Figure 4.3b, respectively. The initial pH of four species of algal suspension varied from 6 – 9, and depended on the initial concentration of the algae. Higher pH of algae suspensions could be explained by the uptake of CO₂ by algae resulting in increase of pH and OH⁻ concentration during photosynthesis (Knappe et al., 2004). Maberly (1996) mentioned that pH ranging from 7.1 to 10.3 have been observed in natural waters. Bicarbonate and carbonate ions are depleted in water to make up for the extracted CO₂ according to the equations shown below (Knappe et al., 2004):

\[
\text{CO}_2 + H_2O \rightarrow \text{H}_2\text{CO}_3 \\
\text{H}_2\text{CO}_3 \rightarrow \text{H}_2O + \text{CO}_2
\]
\[2\text{HCO}_3^- \rightarrow \text{CO}_2 + \text{CO}_3^{2-} + \text{H}_2\text{O}\]  \hspace{1cm} (Equation 4.1)

\[\text{CO}_3^{2-} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{OH}^-\]  \hspace{1cm} (Equation 4.2)

The initial pH also depends on the initial concentration of the algae, as higher cell density produces higher initial pH with the highest pH 9.1, occurred for \textit{P. tricornutum}, which is due to higher extent of photosynthesis for \textit{P. tricornutum} due to higher concentration of the algae. Alum is acidic and the addition of it as a coagulant will lower the pH and alkalinity of water. According to Figure 4.3b, for both high and low cell density algae, adding aluminum sulfate (at the dosage of 30 mg/L), pH was dropped approximately by 2 units.

Algal suspensions are electronegative in charge, and the coagulation of algae is achieved by charge neutralization (Ives, 1959). The composition of algae cell walls offers some insights to the electrophoretic properties of algae. The charge of proteins on the cell wall is dictated by the acid/base characteristics of carboxylic acid (-COOH) and amino (-NH\textsubscript{2}) groups. By increasing pH, resulting protein become negatively charged as -COOH groups dissociate to COO\textsuperscript{-} and H\textsuperscript{+}, and –NH\textsubscript{3}\textsuperscript{+} to –NH\textsubscript{2} and H\textsuperscript{+}) in water (Knappe et al., 2004). Surface charge neutralization could happen when cations, like aluminum adsorb on the surfaces of algae that are negatively charged (Dentel, Thomas, & Kingery, 1989). Thereafter, pH remained mostly constant in all cases except for, \textit{C.vulgaris} (green algae) and \textit{P. tricornutum} (diatom), when pH declined slightly after 24-hours of settling, (but the pH increased again slightly). However, these changes are not significant and are within the experimental errors. Cell viability tests of the algal cells immediately after coagulation indicated that the algae species were alive, and similar findings can be found in literature
Therefore the slight variation in pH may be due to the metabolic functions of the algae.

4.2 Effect of Coagulation on Organic Matter

Many studies have been carried out in order to determine the correlations between water quality parameters and DBP formation. The most commonly used surrogate parameters are UV absorbance at 254 (UV254/A254), dissolved organic carbon (DOC), and specific ultraviolet absorbance at 254 nm (SUVA254) (Ates, Kitis, & Yetis, 2007). Specific UV absorbance (SUVA) is defined as the UV absorbance of a water sample at a given wavelength normalized for dissolved organic carbon (DOC) concentration.

Extracellular organic matters (EOM) primarily consist of sugars, lipids and amino acids and algae can release these organic matters during all phases of their growth (Bernhardt, Hoyer, Lusse, & Schell, 1987). It is also reported that carbohydrates, especially polysaccharides, can comprise 80 % to 90 % of the total EOM released at times (Myklestad, 1995). The quantity and composition of EOM released per unit biomass are different from species to species. It has also been speculated that algae may release additional EOM due to natural and inflicted stress conditions, coagulation can be one such condition. (EPA, 2015). Algae and their metabolites constitute a portion of natural organic matter (NOM) in natural water sources. As the organic carbon content of water increases, the coagulant demand, chlorine demand and DBP formation potential are likely to increase. To assess how algae affects the DOC in water and to determine if coagulation process will result in increase of EOM, the DOC contributions of 4 algae: *C.vulgaris*, *P. tricornutum*, *C. reinhardtii* and *C. meneghiniana* were studied. The EOM present in algae suspensions was measured by filtering algae suspensions and analyzing the filtrate for DOC.
Figure 4.4: DOC variation of (a) control and (b) coagulated algae suspensions of four algae species with settling time, temperature: 22 ± 2°C. Error bars represent the standard deviation of triplicate experiments.

Figure 4.4a and Figure 4.4b show DOC variation of the control and coagulated algae suspensions with settling time, respectively. Figure 4.4a shows DOC value of control group *C. vulgaris* (green algae) and *P. tricornutum* (diatom) at high cell density of ≈10^6 cells/mL while *C. reinhardtii* (green algae) and *C. meneghiniana* (diatom) at low cell density of ≈10^4 cells/mL. DOC values of *C. vulgaris* (green algae) and *P. tricornutum* (diatom) suspension are 4.6 ± 0.6 mg/L and 16.3 ± 1.6 mg/L, respectively, whereas DOC value of *C. reinhardtii* (green algae) and *C. meneghiniana* (diatom) are 3.4 ± 0.6 mg/L and 1.6 ± 0.5 mg/L, respectively. There was no change in DOC in initial 2 hours, but interestingly the DOC content increased significantly for diatom *P. tricornutum* after 2 hours: the DOC value for *P. tricornutum* (diatom) without coagulation increased from 18.6 ± 0.6 mg/L to 32.9 ± 0.9 mg/L. All other algal species showed slight increase in DOC with time, after 9 days of settling, the DOC of *C. vulgaris* (green algae), *C. reinhardtii* (green algae) and *C. meneghiniana* (diatom) in control group were 7.4 ± 0.4 mg/L (59.9% increase from initial value), 2.1 ± 0.6 mg/L (38% increase from initial value) and 2.0 ± 0.1 mg/L (24% increase from initial value). Figure 4.4b shows DOC variation for 4 algal species after coagulation.
The DOC value decreased to 0 mg/L after coagulation for lower density algae: *C. reinhardtii* (green algae) and *C. meneghiniana* (diatom), the DOC of which was below 5 mg/L initially. While DOC of *P. tricornutum* (diatom) increased by 51% from 16.0 ± 0.6 mg/L to 27.7 ± 0.8 mg/L for the coagulated sample. Although, algal DOC is difficult to remove by coagulation, some removal occurs due to the combination of charge neutralization, entrapment, adsorption and complexation with coagulant metal ions (Matilainen et al., 2010).

![Graph showing percentage change of DOC with settling time for *P. tricornutum*.](image)

**Figure 4.5: Percentage change of DOC with settling time for *P. tricornutum*.**

The changes in DOC for these algae were not significant as the initial cell counts of these algae were also low compared to *P. tricornutum*. It is indicated by the experimental results that organic matters continued to be released by algae species with settling time, while no extra release of organic matter due to coagulation occurred as the DOC values of coagulation group remained to be lower than the controlled group (group without
coagulation). This was also the case for *P. tricornutum*, which produced the most amount of DOC as can be seen in Figure 4.5.

**Table 4.2 Mass of initial algal biomass in solution**

<table>
<thead>
<tr>
<th></th>
<th><em>C. vulgaris</em></th>
<th><em>C. reinhardtii</em></th>
<th><em>P. tricornutum</em></th>
<th><em>C. meneghinian</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell count (cells/mL)</td>
<td>1.53×10⁶</td>
<td>4.25×10⁴</td>
<td>1.08×10⁶</td>
<td>2.25×10⁴</td>
</tr>
<tr>
<td>Diameter (µm)</td>
<td>6.00</td>
<td>10.00</td>
<td>-</td>
<td>22.50</td>
</tr>
<tr>
<td>Volume (µm³)</td>
<td>113.10</td>
<td>523.60</td>
<td>80.00</td>
<td>7236.46*</td>
</tr>
<tr>
<td>Mass of algae cells in</td>
<td>1.73×10⁻⁴</td>
<td>2.23×10⁻⁵</td>
<td>8.64×10⁻⁵</td>
<td>1.63×10⁻⁵</td>
</tr>
<tr>
<td>1mL of algae suspension (g/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass of algae cells in</td>
<td>173.04</td>
<td>22.25</td>
<td>86.40</td>
<td>162.82</td>
</tr>
<tr>
<td>1mL of algae suspension (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon content**</td>
<td>92.06</td>
<td>11.84</td>
<td>45.96</td>
<td>86.62</td>
</tr>
<tr>
<td>(mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*V = \frac{4}{3} \pi \left(\frac{D}{2}\right)^3* was used for calculating biovolume of *C. vulgaris* and *C. reinhardtii*. \(V = \frac{\pi}{4} D^2 h\) was used to calculate biovolume for *C. meneghiniana* (Average height = 18.2 µm)

**Carbon content was calculated using biomass formula C₅H₇NO₂ (Droste R, 1997).**

In order to determine whether the DOC produced commensurate with the amount of algal biomass present in water, a simple mass balance calculation was conducted based on the physical size of the algae and presented in Table 4.2. It can be seen that the amount of biomass initially present in water is always about one order of magnitude higher than the DOC release.
Dissolved NOM in water source comprises several types of compounds, aside from algal organic matters, the precursors for most DBPs are thought to be humic species. (Larson & Weber, 1994) (Croué et al., 2000) It has been reported that the absorbance of ultraviolet light at 254 nm (UV$_{254}$/A$_{254}$) is a good surrogate parameter for DOC in water as chromophores such as aromatic structures and some unsaturated bonds absorb UV light (Schnitzer & Khan, 1972).

Figure 4.6 shows ultraviolet absorbance at 254 nm increases with settling time for *C. vulgaris* (green algae) and *P. tricornutum* (diatom) suspension indicating the possible presence of aromatic compounds in the supernatant. The UV$_{254}$ value of three other algae is much lower when compared to the UV$_{254}$ value of *P. tricornutum* (diatom). The initial DOC values for these algae also were low. The relationships between DOC and UV$_{254}$ absorbance for different algae, individually and combined, both are shown in Figure 4.7 a-e. It can be seen better linear correlation ($R^2$ around 0.65) was obtained for the two algae *C. vulgaris* and *P. tricornutum* producing greater amount of DOC, possibly due to analytical error. Higher correlation indicates the presence of chromophores in DOC.
Figure 4.7. Correlation for DOC and UV$_{254}$ for (a) C. vulgaris (b) P. tricornutum (c) C. reinhardtii and (d) C. meneghiniana, (e) all species

Figure 4.7 also indicates that EOM of algae contains aromatic or unsaturated functional groups, and DOC increased with increasing UV$_{254}$ values for all algae species except C. meneghiniana. Comparing the slopes of regression lines of two green algae; C. vulgaris and C. reinhardtii, it seems the aromaticity of EOM for two green algae is similar. While
EOM of *P. tricornutum* (diatom) exhibited much higher aromatic features than green algae as indicated by larger slope of regression line of *P. tricornutum*.

In order to determine the effect of background water quality on the coagulation process, *C. vulgaris* (green algae) cultures were mixed with Fanshawe lake water to achieve a cell density of $2.3 \times 10^6$ cells/ml. Lake water was filtered through a 0.2 μm PTFE membrane filter to remove natural algae and other microorganisms, before spiking *C. vulgaris*. These experiments were carried out in same condition as deionized water groups. Coagulation did not remove the background DOC significantly, only about 15% decline in initial DOC occurred. As with the experiments in DI water, DOC increased in both coagulated and control groups with time in lake water indicating similar behavior. Due to time limitation, these experiments were conducted with only *C. vulgaris*. 
Figure 4.8: (a) UV$_{254}$ (b) DOC (c) pH and (d) Turbidity variations of coagulated and non-coagulated algae spiked lake water with settling time, temperature: 23 ± 2°C. Error bars represent the standard deviation of triplicate measurements.

As shown in Figure 4.8a and 4.8b, the value UV$_{254}$ and DOC were higher than deionized group, mainly attributed to the background lake water quality. In general, variations of UV$_{254}$ and DOC in lake water followed similar pattern as in deionized water: coagulation results in decline of UV$_{254}$ and DOC values, as UV$_{254}$ decreased from 0.128 ± 0.001 cm$^{-1}$ to 0.095 ± 0.006 cm$^{-1}$, DOC decreased from 70.2 ± 4.0 mg/L to 63.5 ± 4.0 mg/L. However, DOC increases gradually with time, from 63.5 ± 4.0 mg/L to 74.3 ± 0.3 mg/L, from 70.2 ± 4.0 mg/L to 79.2 ± 0.3 mg/L with and without coagulation, respectively. This might be attributed to releasing of EOM by *C. vulgaris* as well as change of organic matter in lake water during settling process.

Figure 4.8c shows different pattern of pH variations in lake water, the pH slightly dropped from 9.1 to 8.5 after adding coagulant, and followed by steady increase to pH 9 again over
settling time, due to the buffering capacity of lake water. While DOC removal was marginal, alum removed turbidity of lake water significantly. After 30 min settling, turbidity decreased by 54% from $12.4 \pm 2.6$ NTU to $5.7 \pm 0.3$ NTU.

### 4.3 Effect of Disinfection on DOC and UV$_{254}$

![Graph showing variation in DOC and UV$_{254}$](image)

Figure 4.9: Variation of (a) DOC and (b) UV$_{254}$ before and after disinfection of supernatant of *C. vulgaris* and *P. tricornutum* spiked deionized water with and without coagulation. Settling time: 9 days, pH= 8.0 ± 0.2, chlorine dosage: Cl$_2$: DOC=1.8, UV dosage: 40 mJ·cm$^{-2}$, temperature: 22 ± 2°C, 24-hrs incubation. Error bars represent the standard deviation of triplicate experiments.

As it is shown in Figure 4.9a, little variations of DOC can be observed before and after disinfection of supernatant of algae suspensions. Disinfection using UVC seemed has little effect in removal of DOC for *C. vulgaris* and *P. tricornutum*, while a small decline of DOC
was observed for \textit{P. tricornutum} after chlorination. It is interesting to see that UVC$_{254}$ decreased slightly (about 12.5\%) due to UV radiation as the chromophores like aromatics may absorb and break down. This decline is not reflected in DOC as the intermediates produced due to UV photolysis do not break down completely. Similar pattern was found for the coagulation group indicating the presence of similar types of organics in both groups. Figure 4.9 b shows that after chlorination, the value of UV$_{254}$ for control \textit{P. tricornutum} increased from 0.07 cm$^{-1}$ to 0.11 cm$^{-1}$, a 57\% increase. Chlorinated compounds typically absorb more UV radiation compared to corresponding non-chlorinated compounds. For example, peak absorbance of chlorobenzene shifts to 310 nm compared to benzene at 295 nm ("Tables of Physical & Chemical Constants (16th edition 1995). 3.8.7 UV-visible spectroscopy," 2005)

Similar results were obtained with algae in lake water (Figure 4.10 b) where the changes were more pronounced (almost 3.5 time increase) due to the presence of higher amount background DOC.
Figure 4.10: Variation of (a) DOC and (b) UV_{254} before and after disinfection of *C. vulgaris* spiked lake water with and without coagulation. Settling time: 9 days, pH=8.0 ± 0.2, chlorine dosage: Cl₂: DOC=1.8, UV dosage: 40mJ·cm⁻², temperature: 22 ± 2°C, 24-hrs incubation. Error bars represent the standard deviation of triplicate measurements.

Figure 4.10a and 4.10b showed DOC and UV_{254} variations after disinfection for *C. vulgaris* in lake water. The changes in DOC in lake water declined from 74.3 ±0.3 mg/L to 66.5 ± 2.7 mg/L after UV radiation, showing possible photo-oxidation of background DOC in UVC, which was not the case in DI. Some experiments were also conducted with only chlorination of algal matter in DI water with and without coagulation, and the results are presented in 4.11.
Figure 4.11: Variation of (a) DOC and (b) UV\textsubscript{254} before and after chlorination of supernatant of algae spiked deionized water with and without coagulation. Settling time: 5 days, pH=8.0 ± 0.2, chlorine dosage: Cl\textsubscript{2}: DOC=1.8, temperature: 22 ± 2°C, 24-hrs incubation. Error bars represent the standard deviation of triplicate measurements.

In experiment groups of \textit{C. reinhardtii} (green algae) and \textit{C. meneghiniana} (diatom), lower cell density of algae suspensions were chlorinated after 5 days of settling after coagulation. Figure 4.11a shows that DOC increased slightly after chlorination for both coagulation and control groups. For \textit{C. reinhardtii} (green algae) and \textit{C. meneghiniana} (diatom), DOC value increased from 1.2 ± 0.2 mg/L to 1.7 ± 0.3 mg/L and 0.65 ± 0.2 mg/L to 1.6 ± 0.2 mg/L, respectively after 24-hours chlorination. Although, there seems to be an increasing trend in DOC after chlorination, it is difficult to explain these results. In water quality determination, the existence of Cl\textsuperscript{-} seems causing an increase in the TOC determination due to interference (W. Wang, 2016). Since, the DOC values in these cases are almost at the instrument detection limit (1 mg/L), the effects seem to be more magnified. After coagulation, DOC of both species declined to 0 mg/L, as a result no DOC values after coagulation were showed on the graph. Figure 4.11b illustrated the increase of UV\textsubscript{254} as a result of chlorination, which was seen earlier as well. In control groups, UV\textsubscript{254} increased by 52\% for green algae, while only marginal changes occurred for \textit{C. meneghiniana} (diatom). With
coagulation, UV$_{254}$ value of *C. reinhardtii* (green algae) also increased but the absolute value was lower than that of control group. Overall, chlorination increased the UV$_{254}$ in almost all cases.

### 4.4 DBP Formation Potential

Generally, the concentration of disinfection-by-product (DBP) increases with dissolved carbon (DOC) in the water, chlorine dosage and reaction time. The mass of DBP formed per unit mass of natural organic matters (NOM), as well as the kinetics of their release are complex functions of water quality parameters (G. Korshin et al., 2004a).

Figure 4.12 Haloacetic acids (HAAs) produced by (a) chlorination of lake water and (b) deionized water, and (c) tap water. Chlorine dosage for lake water: Cl$_2$: DOC=1.8, Error bars represent the standard deviation of triplicate measurements.
Initially, presence of DBP in tap water and deionized water was tested using the standard protocol of extraction and esterification as described in Chapter 3 followed by the analysis in GC-ECD. Thereafter, DBP experiments were carried out in lake water and deionized water (without algae). Lake water was filtered through 0.2 µm filter and chlorinated (using UFC as mentioned in Chapter 3). Subsequently, haloacetic acids (HAA) formation was determined using GC-ECD. The formation of HAAs is shown in Figures 4.12a and 4.12b. Much higher concentration of HAA was detected in untreated lake water (653.21 ppb) than deionized water (4.63 ppb) and tap water (47.09 ppb). The DI and tap water values are below the maximum contaminant level (MCL) of 60 ppb for HAAs set by USEPA (Paull & Barron, 2004). Similar range of HAAs concentration (29.2-45.9 ppb) was found in water supply in several locations in US (Savitz et al., 2006) (C. Chen, Chang, & Wang, 2009). In a study of 12 drinking water treatment plants in the US, Krasner et al. found the concentrations of the nine HAAs ranged 3–18 µg/L (3-18 ppb). In Canada, except at plants using chlorine as the disinfectant, concentrations were found to be lower than 50 µg/L for most HAAs Analyzing tap water from 10 locations in Taiwan, Hsu found the individual concentrations of six HAAs to range from 1–13 µg/L and total concentrations to range from 5–33 µg/L. The types of HAAs formed in three water samples were different. No dibromoacetic acid (DBAA) was found in deionized water, while 157.26 ppb DBAA was found in lake water. High concentration of HAA found in lake water is due to the high NOM content in water with DOC of 72.62 mg/L.
Haloacetic acids (HAAs) production by chlorination of two algae species: *C. vulgaris* (Green algae) and *P. tricornutum* (diatom) with cell density of $1.5 \times 10^6$ cells/mL and $1.1 \times 10^6$ cells/mL, respectively is shown in Figure 4.13. There is no significant difference in DBP formation by two groups (controlled vs. coagulated); coagulation did not affect the release of AOM significantly, although there seem to be slight increase in HAA production for *C. vulgaris*. Differences were observed in the formation of individual HAAs for green algae versus diatom. Although with similar cell density algae suspensions ($\approx 10^6$ cells/mL), diatom produced higher total HAA yields (499.2 ± 48.1 ppb) than green algae (136.2 ± 2.1 ppb). No dibromoacetic acid (DBAA) was observed in green algae samples. The difference in HAA distribution between green algae and diatom may be attributed to the dissimilarity of the chemical composition of algal organic matter (AOM). EOM excreted by algae is comprised of up to 90 percent of polysaccharides and small portion of nitrogenous organic matter such as protein, amino acids (Myklestad, 1995), and polysaccharides are responsible for the production of low halogenated HAA species such as MCAA and MBAA (Huang et al., 2009). Huang et al also found out that an increase in tri-HAA in algae *Microcystis*
samples during algae growth phase, and they suggested that intracellular organic matter from decayed cells can be a dominant precursor for tri-HAA. It is suggested that organic nitrogenous compounds contain active sites for di-HAA formation (Croue et al, 2000).

Figure 4.14 Haloacetic acids (HAAs) produced by chlorination of *C. vulgaris* (Green algae) EOM in lake water (LW) with and without coagulation. Settling time: 9 days, chlorine dosage: Cl2: DOC=1.8, Error bars represent the standard deviation of triplicate measurements.

Figure 4.14 shows haloacetic acids (HAAs) yields by chlorination of *C. vulgaris* (Green algae) EOM in lake water (LW) with and without coagulation. 614.7 ± 47.8 ppb total HAA was detected for control group, which attributed to higher DOC level of *C. vulgaris* suspensions in lake water. Unlike *C. vulgaris* (Green algae) in deionized water, dibromoacetic acid (DBAA) was observed in lake water samples. Also, coagulation assisted in some NOM removal, which helped to reduce the total HAA formation in the coagulated group.
Figure 4.15 Haloacetic acids (HAAs) produced by chlorination of (a) *C. reinhardtii* (Green algae) and (b) *C. meneghiniana* (diatom) EOM in deionized water (DW) with and without coagulation. Settling time: 5 days, chlorine dosage: Cl2: DOC=1.8, Error bars represent the standard deviation of triplicate measurements.

Figure 4.15 presents haloacetic acids (HAAs) yields by chlorination of two algae species: *C. reinhardtii* (Green algae) and (b) *C. meneghiniana* (diatom) at lower cell density of $4.3 \times 10^4$ cells/mL and $2.3 \times 10^4$ cells/mL respectively. The total HAA for *C. reinhardtii* (Green algae) and (b) *C. meneghiniana* (diatom) are almost at the same level with 25.5 ppb and 18.9 ppb, respectively.

Figure 4.16 Haloacetic acids (HAAs) formation after settling of 5 days and 9 days for (a) *C. reinhardtii* (Green algae) and (b) *C. meneghiniana* (diatom). Error bars represent the standard deviation of triplicate measurements.

Figure 4.16 demonstrates the formation of HAA after 5 days and 9 days of settling. HAA significantly increased for both *C. reinhardtii* (Green algae) and *C. meneghiniana* (diatom)
with time. This may be attributed to the increase of DOC during additional 4 days of settling. So it is suggested that coagulated algae cells to be disposed of from settling basin as soon as possible in order to avoid additional release of organic matters to treated water, which contribute to higher HAA formation in water.

4.5 Modeling of DBP Formation

The use of mathematical models to predict the effects of water quality and operational parameters on DBP formation is potentially important to water utilities, regulators and epidemiologists. In recent years, some attempts have been made to develop predictive models for the formation of DBPs in water, either from data generated in full-scale studies at operational water plants or at laboratory-scale using controlled chlorination condition. Most of the work reported has been focused on the formation of THMs, and a few studies have considered HAAs. In this work, an attempt has been made to develop general empirical models using multiple and nonlinear regression approach to predict total and individual HAA formation for different water qualities.

Concentrations of individual HAAs and total HAAs were designated as the dependent variable, and the water quality parameters such as pH, DOC, UV$_{254}$, SUVA$_{254}$ and chlorine dosage were defined as independent variables. The predictive model development was based on the sample data collected from the experimental cases mentioned above. Some of the derived models are shown in the following section with their associated coefficient of determination (R$^2$).
$y = 563.19 - 4411.59 \times (UV_{254}) + 135.47 \times (DOC) - 72.94 \times (SUVA_{254})$
$- 69.67 \times (Cl_2 \text{ Dosage}) - 61.90 \times (pH)$

$R^2 = 0.997$

**Figure 4.17 (a) Modeling of MBAA+DCAA Concentration (Control)**

\[
\ln y = 13.63 + 3.29 \times \ln(\text{UV}_{254}) - 5.05 \times \ln(\text{DOC}) - 4.71 \times (\text{SUVA}_{254}) + 1.49 \\
\times \ln(\text{Dosage}) + 2.64 \times \ln(\text{pH})
\]

$R^2 = 0.943$

**Figure 4.17 (b) Modeling of Total HAAs Concentration (Control)**
It can be seen that the developed models for individual HAAs fitted the experimental data better than the total HAAs correlating the important experimental parameters such as
chlorine and UV dosage, DOC, and pH. However, the predictive capability of the models has not been tested.
Chapter 5

5 Conclusions

The results obtained from experiments presented in this thesis give a better understanding of the behavior of four algae species: *C. vulgaris*, *P. tricornutum*, *C. reinhardtii*, and *C. meneghiniana* during coagulation and settling process. The impact of UV and chlorine on algal organic matters during disinfection, and the formation potential of disinfection by-products from organic matters of different algae species in both pure and natural water have been evaluated. Empirical models have been developed to predict total and individual HAA formation for different water quality.

The results of this study showed that alum dose at 30 mg/L in coagulation, as well as settling did not cause lysis of algae cells *C. vulgaris*, *P. tricornutum*, *C. reinhardtii*, and *C. meneghiniana*. As indicated by the experimental results that organic matters continued to be released by algae species with settling time, while no additional release of organic matter to water due to coagulation occurred. At dosage of 30 mg/L, alum is efficient in removing turbidity of algae suspensions, and better removal rates were observed for diatom than green algae, however, the removal of algal organic matter is relatively difficult by coagulation.

Experimental results also indicated that during water treatment, algae species continued to release organic matters with time even though the changes in organic matters for algae *C. reinhardtii*, and *C. meneghiniana* were not as significant as for *C. vulgaris*, *P. tricornutum*, as the initial cell counts of these algae were also low. Therefore, it is suggested that the
coagulated algae cells to be disposed from settling basin soon after coagulation in order to avoid additional release of organic matters to treated water.

It is also confirmed by experimental results that algal organic matter serves as important DBP precursor during drinking water treatment process, higher the algal organic matter in water, higher is the DBP formation potential. Attempts have been made to develop empirical models using multiple and nonlinear regression approach, in order to predict total and individual HAA formation for different water quality. Good correlations were obtained between predicted value and actual value through these statistical models.

5.1 Future Work

Further work can be done in the following aspects:

(1) In coagulation process, different kinds of coagulants (chemical coagulant and biological coagulant), the dosage of coagulants, conditions of coagulation such as mixing speed, pH, may be optimized especially for algal organic matters to achieve better removal efficiency of DOC and control of HAA concentration levels. Addition of coagulant aids may be needed when necessary.

(2) In disinfection process, optimization of the different process parameters (UV dose and chlorine dosage) based on different water qualities is needed.

(3) UV-visible absorbance, Fourier transform infrared (FTIR) spectrophotometer, solid state $^{13}$C NMR, fluorescence excitation emission matrix (EEM), liquid chromatography with mass spectroscopy detection (LC/MS) can be utilized for characterization/identification of algal organic matter before and after disinfection process.
(4) Toxicity tests may be carried out for algal organic matters and haloacetic acids to analyze the risk of these compounds in drinking water.
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Appendices

Appendix B: Experimental Apparatus

Figure A-1 Jar test setup

Figure A-2 Filtration Apparatus
Figure A-2 UV Disinfection Setup

Figure A-4 Collimated beam UV calibration spreadsheets-step 1
Figure A-5 Collimated beam UV calibration spreadsheets-step 2
Appendix B:

Figure B-1 Chemistry of DPD-FAS Titration

Figure B-2 GC-ECD output for HAA standards
Appendix C: Calibration Curves

Figure C-1 Calibration Curve for MBAA+DCAA

Figure C-2 Calibration Curve for TCAA

Figure C-3 Calibration Curve for BCAA
**Figure C-4** Calibration Curve for DBAA

**Figure C-5** Calibration Curve for Residual Chlorine
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