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Disinfection By-products Formation after Coagulation of Algal Extracellular and Intracellular Organic Matters

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Graduate Program in Chemical and Biochemical Engineering
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Abstract

Algal organic matter (AOM) is found in high concentration during algal bloom season in drinking water systems, which is generally categorized into extracellular organic matter (EOM) and intracellular organic matter (IOM). These compounds are not well removed in traditional water treatment methods such as coagulation, and are the precursors of subsequent disinfection by-products (DBPs) during chlorination of water. In this study, EOM and IOM content of four different algae were quantified measuring dissolved organic carbon (DOC), UV absorbance at 254 nm and turbidity. Coagulation using alum ($\text{Al}_2(\text{SO}_4)_3 \cdot 16\text{H}_2\text{O}$) was used to remove the algal matters. UV radiation and post-UV chlorination were used to determine the DBPs formation potential of the algal matters. The DBPs such as trihalomethanes (THMs) and Haloacetic acids (HAAs) were analyzed after disinfection treatment. The DBPs formation decreased in coagulated algae. Compared with EOM, IOM produced more DBPs because of higher content of protein and aromatic organic matters.

Keywords

Algae, extracellular organic matter, intracellular organic matter, coagulation, disinfection by-products (DBPs), haloacetic acids (HAA), trihalomethanes (THMs)

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List of Acronyms

AOM	Algal Organic Matter
BDCM	Bromodichloromethane
BCAA	Bromochloroacetic Acid
CH	Chloral Hydrate
DBAA	Dibromochloromethane
DBCM	Dibromoacetic Acid
DBP	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
TBM	Bromoform
TCAA	Trichloroacetic Acid
TCM	Chloroform
TCNM	Trichloronitromethane
TOC	Total Organic Carbon
THMs	Trihalomethanes
USEPA	United States Environmental Protection Agency
UV ₂₅₄	Ultraviolet Absorbance at 254 nm
WTP	Water Treatment Plant

Chapter 1

1 Introduction

Algae are aquatic and photosynthetic microorganisms which utilize nitrogen, phosphorus, sunlight, carbon dioxide as well as water to produce biomass (Pivokonsky et al. 2015). The most commonly found algae in drinking water sources are green algae, blue-green algae, diatoms, euglenoids, dinoflagellates, cryptomonads, yellow-green algae, and golden algae (Knappe et al. 2004).

Algal bloom has been defined as a visible accumulation of algal biomass (Reynolds & Walsby 1975). Temperature, light exposure and trophic status of aquatic system are the three major factors that favor the formation of algal bloom (Merel et al. 2013). Since algae are primarily phototrophic microorganisms, groundwater resources are not as vulnerable to algal bloom as surface waters. Algal organic matter (AOM) is found in high concentration during algal bloom season in drinking water systems, and it affects the drinking water quality as one of the substantial contributors to natural organic matter concentration (NOM) (Pivokonsky et al. 2015). Algal organic matter (AOM) is generally categorized into extracellular organic matter (EOM) (Zheng et al. 2016), which is excreted to surrounding environment by living algae cells. Intracellular organic matter (IOM) is released mainly in stationary and declining growth phase, or during cell rupture and lysis (Pivokonsky et al. 2015). These organic substances are comprised of various compounds such as oligosaccharides, polysaccharides, proteins, peptides, amino acids, as well as other traceable organic acids (Pivokonsky et al. 2015). The composition of different algal matter varies with algae species (Hong et al. 2008).

Drinking water treatment began in the early 1900s, which mainly includes pretreatment and disinfection processes. The aim of pretreatment is to remove colloid contents and suspended solids, while the main purpose of disinfection is to kill microbial pathogens in water to prevent the spread and prevalence of waterborne infectious diseases (WorldChlorineCouncil 2008). The disinfection process not only kills pathogens, but also act as an oxidizing agent to remove taste, color, iron oxide and manganese of drinking

water, to improve coagulation and filtration efficiency, to prevent the growth of algae on the bottom of sedimentation tanks and filters as well as the regrowth of organisms in drinking water distribution systems (USEPA 1999a)(Wang et al. 2014)(SDWF 2012). During drinking water disinfection process, oxidation, addition and substitution reactions occur between disinfectant and natural organic matters (NOMs) such as humic and fulvic acids and algal matter, as well as bromide or iodide in source water, which produce disinfection by-products (DBPs). Since the time trihalomethanes (THMs) were found in the 1970s, more than 600 different DBPs have been identified. Most DBPs compounds have potential carcinogenicity, teratogenicity, and mutagenicity (Zhao et al. 2012; Hutzinger et al. 2011; Pan & Zhang 2013). After years of research, quantitative information of a few DBPs has been revealed.

AOM is rich in organic nitrogen and organic carbon compared with NOM, which causes the formation of more DBPs during disinfection treatment (Lui et al. 2012). The level of DBPs formation may vary considerably with algae species, the genus, the algal cells, the algal growth, biochemical composition and the applied conditions of disinfection treatment (Lv et al. 2014; Liang et al. 2012; Li et al. 2012; Lui et al. 2012; Lui et al. 2011; Huang et al. 2009). Up to now, the details of DBPs formation from IOM and EOM solutions for different algae are still very limited.

1.1 Objectives

- a) To determine the amount of EOM and IOM from four different commonly found species of algae commonly found in surface water.
- b) To determine the efficiency of a common coagulant in removing algal matter.
- c) To determine the impact of different drinking water treatment methods, such as UV radiation and post-UV chlorination on the formation of DBP for EOM and IOM.

1.2 Thesis Overview

There are five chapters in this thesis. A brief introduction of the research topic and objectives is provided in Chapter 1. Chapter 2 presents the literature review related to the pertinent research. The brief introduction of algae and algal organic matters, bloom problems from algae, drinking water treatment processes and DBP formation is presented in this chapter. Chapter 3 contains the experimental methods and analysis methods. The results and discussions are presented in Chapter 4. The conclusions and future directions are discussed in Chapter 5.

Chapter 2

2 Literature Review

2.1 Introduction of Algae

Algae are aquatic and photosynthetic microorganisms which utilize nitrogen, phosphorus, sunlight, carbon dioxide as well as water to produce biomass (Pivokonsky et al. 2015). Traditionally, algae are classified by the pigments and structure of their cells. For example, green algae are named after the grass-green shade while diatoms are brown in color (Fang, Ma, et al. 2010). The most commonly found algae in drinking water sources are green algae, blue-green algae, diatoms, euglenoids, dinoflagellates, cryptomonads, yellow-green algae, and golden algae (Knappe et al. 2004).

Table 2.1 List of common algae observed in drinking water sources

Algae species	Characteristics	Growth Condition	Typical genera
Blue-Green Algae	Prokaryotes Contains phycocyanin, allophycocyanin and chlorophyll a, which gives blue or blue-green color. Produce cyanotoxins, perform oxygenic photosynthesis	Warm, eutrophic water, above 25 °C	<i>Anabaena</i> , <i>Aphanizomenon</i> , <i>Microcystis</i> and <i>Oscillatoria</i>
Green Algae	Contains chlorophyll a and b, green color. Some genera are associated with taste and odor and filter clogging problems	Summer	<i>Ankistrodesmus</i> , <i>Chlamydomonas</i> , <i>Chlorella</i> , <i>Scenedesmus</i>
Euglenoids	Contains chlorophyll a and b, green color, capable of photosynthesis	Summer	
Dinoflagellates	Capable of photosynthesis and feeding on bacteria and small planktonic algae. Brownish color, some genera are commonly associated with taste and odor problems 90% are found in ocean.	Summer and fall	<i>Ceratium</i> , <i>Peridinium</i>
Cryptomonads	Contains chlorophyll a and c ₂ , and many pigments that mask the color of chlorophyll. May appear blue, blue-green, reddish, yellow-brown, olive-green. Light sensitive and prefer nutrient-enriched water.	Temperate climate throughout winter	<i>Cryptomonas</i> , <i>Chroomonas</i> , <i>Rhodomonas</i>
Yellow-Green Algae	Rarely present in large quantities Contains chlorophyll a β -carotene, and many pigments, appears yellow-green, bright green	Low temperature	<i>Tribonema</i>
Golden Algae	<i>Synura</i> , <i>Dinobryon</i> are commonly associated with taste and odor problems. Appears golden-brown Photosynthesis and feed on bacteria	Summer	<i>Synura</i> , <i>Dinobryon</i>
Diatom	Commonly associated with taste and odor and filter clogging problems. Appear in brown color Siliceous cell wall consists of polymerized silicic acid. Can perform oxygenic photosynthesis at water temperature of 5°C	Spring Optimum temperature at 10-20 °C	<i>Asterionella</i> , <i>Cyclotella</i> , <i>Fragilaria</i> <i>Melosira</i>

2.2 Algae Organic Matter

2.2.1 Introduction

Algal organic matter (AOM) is released into water as algal photosynthesis and secondary metabolism by-products. AOM is generally categorized into intracellular organic matter (IOM)(Pivokonsky et al. 2015) released mainly in stationary and declining growth phase, and extracellular organic matter (EOM) (Zheng et al. 2016) excreted to surrounding environment by living algae cells. These organic substances are comprised of various compounds such as oligosaccharides, polysaccharides, proteins, peptides, amino acids, as well as other traceable organic acids (Pivokonsky et al. 2015). Hong et al gave a general overview of major constituents of different algae species (Hong et al. 2008), which shows that the composition of different algal matter varies with algae species. Villacorte et al have studied different characteristics of three bloom-forming algae: growth, cell concentration and mechanism of AOM release (L O Villacorte et al. 2015).

Both EOM and IOM are hydrophilic with low SUVA (Pivokonsky et al. 2015). Compared with EOM, IOM is richer in proteins or peptide, more hydrophilic and have lower SUVA value. MW fractionation shows that both EOM and IOM of green algae and diatom contain large portions of low-MW (below 1 k Da) compounds and high-MW (over 100 k Da) polysaccharides (Pivokonsky et al. 2006). According to Fang et al, the MW of organic carbon in EOM and IOM is relatively lower compared with natural organic matters. EOM and IOM are both rich in organic nitrogen. IOM has a higher fraction of total organic nitrogen, higher fractions of free amino acids but lower fractions of aliphatic amines than EOM (Fang, Yang, et al. 2010).

2.2.2 Separation of IOM and EOM Solutions

All the methods to separate IOM and EOM are quite similar to each other. Basically, EOM remains in the solution after the filtration of algae solution, after which, some procedures such as freeze/thaw cycles and physically grinding are used to kill the algae to release IOM. Then filtration is used again to get EOM. However, the size of filtration film, centrifugal speed and time, as well as the times of freeze/thaw cycles sometimes vary with different algae species.

For a blue-green algae *M. aeruginosa* and a diatom *Cyclotella meneghiniana*, EOM can be extracted by first centrifuging the cells in growth phase at 10,000g for 10 min, then subsequently filtering the supernatant with 0.7 mm GF/F glass fiber filters (Whatman)(Zhou et al. 2014). Subsequently, the deposited algal cells are collected and washed 3 times with 100 mL Milli-Q water (Fang et al. 2010). To obtain IOM, freeze/thawing ($-18\text{ }^{\circ}\text{C}$ / $25\text{ }^{\circ}\text{C}$) cycles can be used to kill the cells to release the intracellular materials. After 3 cycles, ultrasonic treatments (500 W, 20 min, 2 s/2 s), centrifugation and filtration were conducted to extract the organic matter as IOM solution (Li et al. 2012; Li et al. 2014). Grinding is another way to kill algae cells to extract IOM. By physically grinding the cells with a mortar and pestle in Milli-Q water, IOM was extracted, which was also followed by filtration through a GF/F membrane (Fang et al. 2010). For another blue-green algae, *Anabaena flos-aquae*, a different 0.45mm membrane was used to separate EOM and algae cells (Huang et al. 2009). All samples need to be adjusted to $\text{pH } 7.0 \pm 0.1$ with KH_2PO_4 and Na_2HPO_4 (Liao et al. 2015).

2.2.3 Algal Problems

Algal bloom has been defined as a visible accumulation of algal biomass (Reynolds & Walsby 1975). Most algae prefer flowing water. Merel et al have summarized three major factors that favor the formation of algal bloom: Temperature, light exposure and trophic status of aquatic system (Merel et al. 2013). Since algae are primarily phototrophic microorganisms, groundwater resources are not as vulnerable to algal bloom as surface waters.

Over the years, big blooms have been observed via satellite in the lower Great Lakes since mid-1990s (Becker et al. 2009). In 2011, the western basin of Lake Erie experienced the largest blooms since 2002 (Bridgeman et al. 2013). The blooms, extending over 5,000 kilometer squares (Michalak et al. 2013), have led to the closure of beaches and drinking water advisories in both Canada and US (Pick 2016).

Blue-green algae, are one of the most problematic algae in drinking water system because of releasing algal toxins (Jančula & Maršálek 2011). Diatom species such as *Pseudo-nitzschia* are also very harmful. It was reported that the neurotoxin domoic acid produced

by *Pseudo-nitzschia* has killed at least three elderly people and led to at least over 100 illnesses in 1987 in North America (Lelong et al. 2012). Lelong et al. have published a critical review paper concerning a list of diatom species, their worldwide distribution, toxins produced and records of diatom blooms around the world (Lelong et al. 2012). A marine diatom species *Chaetoceros affinis*, has been used to investigate the mechanism and compositions of its releasing organic matters (L. O. Villacorte et al. 2015), and nano-mechanical properties (Gutierrez et al. 2016), as well as potential fouling and removal rate of its organic matters (Tabatabai et al. 2014).

Wang et al. have conducted both fields and laboratory experiments on diatoms to understand the mechanisms of blooms, the effects of varying phosphorus concentration and hydrodynamics on the growth (Wang et al. 2012). About 16 taxa were found to be dominant among various diatoms. Among them, *Cyclotella meneghiniana* was the predominant species (Ai et al. 2015). It is also reported that *Aulacoseira granulata*, *Asterionella formosa* and *Synedra spp.* co-dominated in succession with *C. meneghiniana* in winter and spring (Ying et al. 2015)(Ai et al. 2015).

Algal organic matter (AOM) is found in high concentration during algal bloom season in drinking water systems, and it affects the drinking water quality as one of the substantial contributors to natural organic matter concentration (NOM) (Pivokonsky et al. 2015). When compared to NOM, AOM appears to contain more organic nitrogen, more hydrophilic content, less aromatic carbon content and have much lower specific UV absorbance ($SUVA < 2L/mg/m$) (Fang, Yang, et al. 2010). It may be problematic when AOM enters into drinking water treatment systems, because it can increase coagulant demands, foul membranes, and produce disinfection by-product (DBPs) during chlorination. Traditional water treatment processes such as coagulation and filtration poorly remove the AOM (Cheng et al. 2015).

2.3 Drinking Water Treatment Process

The flow diagram for a typical drinking water treatment plant is shown below (Figure 2.1). In drinking water treatment processes, the main purpose of pretreatment is to remove the colloid contents, suspended solids, microorganism and heavy metals in the raw water, whilst disinfection is mainly used to remove viruses, bacteria and microorganisms (Environmental Protection Agency (Ireland) 2013; Bao et al. 2006; Jin et al. 2011).

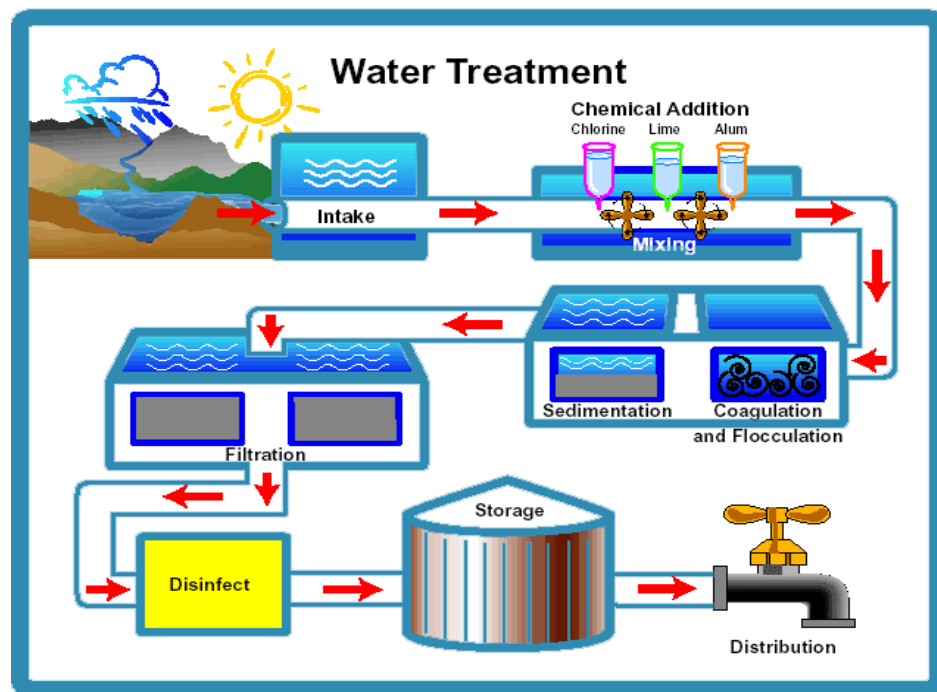


Figure 2.1 Typical flow of drinking water purification treatment process

(Figure Reference)

2.3.1 Pretreatment Process

Typically, the pretreatment process includes coagulation, flocculation, sedimentation and filtration. The objectives of pretreatment are mainly to remove suspended solids and colloidal impurities to decrease turbidity (Koohestanian et al. 2008). The size ranges of various suspended and colloidal particles are shown in Figure 2.2 (Koohestanian et al. 2008).

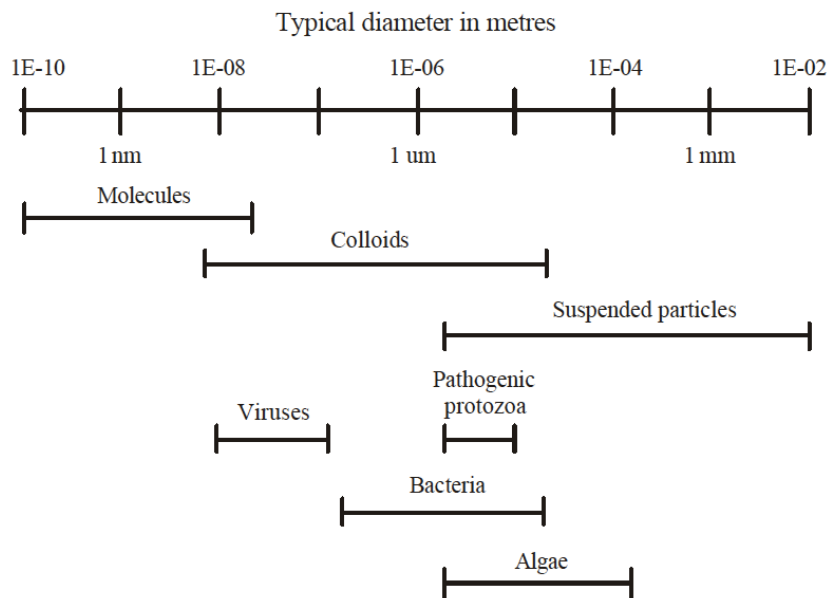


Figure 2.2 Size range of particles of concern in water treatment

Typical coagulants used in water treatment include metal salts and polymers class. Commonly used chemical coagulants are metal ions such as Al^{3+} , Fe^{3+} and Zn^{2+} (Gheraout et al. 2014; Alizadeh Tabatabai et al. 2014; Cheng et al. 2015). The coagulants are added into raw water to neutralize the negative charges of colloids preventing electrostatic repulsion between particles (Xie et al. 2016). The neutralized suspended particles and colloids tend to agglomerate and form bigger particles (Lin et al. 2015). These large particles are settled in the sedimentation tank by gravity separation. Filtration is mainly used after the coagulation and sedimentation process, to further reduce the turbidity of the water. The effective coagulation, sedimentation and filtration, are able to reduce the turbidity of water, to remove some of the organic matters, bacteria and virus in water (Tabatabai et al. 2014; Cheng et al. 2015).

Depending on the quality of the raw water, some other treatment methods may also be added or eliminated. For instance, sediment pre-sedimentation tank or sedimentation tank often needs to be used when dealing with high turbidity raw water. On the other hand, sedimentation tank even be spared if the turbidity of the raw water is very low, and in that case filtration can be directly used after the addition of coagulants. However, filtration is a very essential part in most drinking water treatment processes.

The surface of algae cells exhibits negative charge (Vandamme et al. 2013), so the positively charged coagulants can be strongly adsorbed on the surface of algae cells, neutralizing the surface charge and eliminate cell-based electrostatic effect. At some specific pH, the metal ions in coagulants can form insoluble substances such as $\text{Al}(\text{OH})_3(\text{s})$, $\text{Fe}(\text{OH})_3(\text{s})$ and $\text{Zn}(\text{OH})_2(\text{s})$. These insoluble substances can wrap the algae cells network to achieve coagulation. In addition, Al^{3+} , Fe^{3+} and other metal salts can form $[\text{Al}(\text{OH})_3]_n$, $[\text{Fe}(\text{OH})_3]_n$ and other polymers, which can connect two or more algae cells in the form of adsorption bridges to achieve coagulation. Chemical coagulation is successfully used on removing *Chlorella*, *Nannochloropsis*, *Neochloris* and *Phaeodactylum*. However, there are problems associated with coagulation such as high cost and secondary pollution. The metal ions in coagulants and high polymer residues in the water are extremely difficult to degrade, which may likely cause the secondary pollution of the environment. The advantages and disadvantages of various chemical coagulants are summarized in Table 2.2.

Table 2.2 Comparison of different chemical coagulants

Chemical Coagulation (Dosage)	Algae (Cell density)	Features	Reference	
Inorganic coagulants	Al ₂ (SO ₄) ₃ (0.1 g/L) Fe ₂ (SO ₄) ₃ (1 g/L)	<i>Scenedesmus</i> sp. (0.23 g/L) <i>C. minutissima</i> (2.2 x 10 ⁸ /mL)	High efficiency, harmful to algal cells, secondary pollution	(Papazi et al. 2010; Chen et al. 2013)
	Aluminium nitrate sulphate (5.4 mg/L)	<i>N. salian</i> (15 or 20 g/L)	High efficiency, secondary pollution	(Rwehumbiza et al. 2012)
	Ammonia (38-120 mmol/L)	<i>N. oculata</i> (-*) <i>C. sorokiniana</i> (-) <i>Dunaliella</i> sp. (-)	High efficiency, long coagulating time, species dependent	(Chen et al. 2012)
Inorganic polymers	Poly aluminium chloride, polyacrylamide (0.1-0.2 g/L)	<i>Scenedesmus</i> sp. (~0.2-0.4 g/L) <i>C. vulgaris</i> (~0.4 g/L)	High efficiency, harmful to algal cells, risk of toxic acrylamide	(Lakaniemi et al. 2011; Chen et al. 2013; Beach et al. 2012)
Organic polymers	Chitosan (6-100 mg/L)	<i>Scenedesmus</i> sp. (~0.2-0.7 g/L) <i>Chlorella</i> sp. (0.5 g/L) <i>N. oleoabundans</i> (0.5 g/L)	High efficiency, high cost of coagulants	(Chen et al. 2013; Beach et al. 2012; Zheng et al. 2012)
	Cationic starch (30 mg/L)	<i>Parachlorella kessleri</i> (0.3 g/L)	High efficiency, pH dependent	(Vandamme & Foubert 2010)
	Poly g-glutamic acid (g-PGA) (~20 mg/L)	<i>C. protothecoides</i> (0.6 g/L) <i>N. oculata</i> (0.6 g/L) <i>P. tricornutum</i> (0.6 g/L)	High efficiency, salinity dependent	(Zheng et al. 2012)

* - data of concentration is unavailable.

2.3.2 Disinfection Treatment

Drinking water disinfection began in the early 1900s with the aim of killing microbial pathogens in water to prevent the spread and prevalence of waterborne infectious diseases (WorldChlorineCouncil 2008). At present, the commonly used methods of drinking water disinfectants are: chlorination, ultraviolet radiation, chloramine disinfection, chlorine dioxide disinfection and ozone disinfection. Disinfectant could not only kill pathogens, but also be as an oxidizing agent to remove taste, color, iron oxide and manganese of

drinking water, to improve coagulation and filtration efficiency, to prevent the growth of algae on the bottom of sedimentation tanks and filters as well as the regrowth of organisms in drinking water distribution systems (USEPA 1999a)(Wang et al. 2014)(SDWF 2012)

2.3.2.1 Ultraviolet Disinfection

2.3.2.1.1 UV Light

Ultraviolet (UV) rays are part of the sun light. Ultraviolet rays are divided into three zones of UV-A, UV-B, UV-C and vacuum ultraviolet rays according to the wavelength range: UV-A is in the range 320 - 400 nm, UV-B ranges from 275 - 320 nm, UV-C ranges from 200 - 275 nm and vacuum ultraviolet ray ranges from 100 - 200 nm (Arenas et al. 2016; Trang et al. 2014). The shortwave UV-C is used in water disinfection process (Canonica et al. 2008; Sommer et al. 2008).

2.3.2.1.2 UV Absorption

According to quantum theory, light is a special form of material and a grain of particles flow, which are not connected. Each of the 253.7 nm UV photons has energy of 4.9 eV (Liu et al. 2009; Xu et al. 2009b; Xu et al. 2009a; Canonica et al. 2008). On the other hand, nucleic acid is the basic material and life foundation of all living things. In essence, nucleic acid absorbs ultraviolet energy when microorganisms are radiated (Wenhai et al. 2016; Cui et al. 2016; Wang et al. 2015). Nucleic acid is divided into RNA (RNA) and deoxyribonucleic acid (DNA). The common point of DNA and RNA is the phosphodiester bond by purine and pyrimidine base pairing of the principle of linking the polynucleotide chain (Xu et al. 2009b; Cui et al. 2016; Wang et al. 2015; Bolton et al. 2003). Figure 2.3 and Figure 2.4 present the UV absorption spectra of DNA and RNA, respectively, ranging from 240 nm to 280 nm, with maximum absorption of UV at around 260 nm (Xu et al. 2009b; Xu et al. 2009a; Canonica et al. 2008; Sommer et al. 2008; Yuan et al. 2013; Wenhai et al. 2016; Cui et al. 2016; Wang et al. 2015; Bolton et al. 2003).

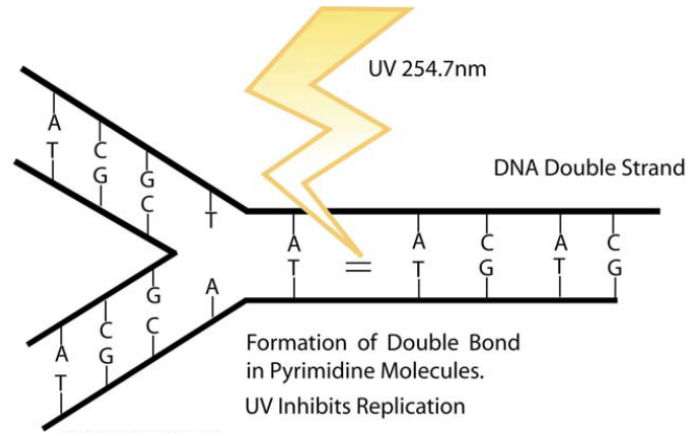


Figure 2.3 The chain of DNA and RNA attacked by UV

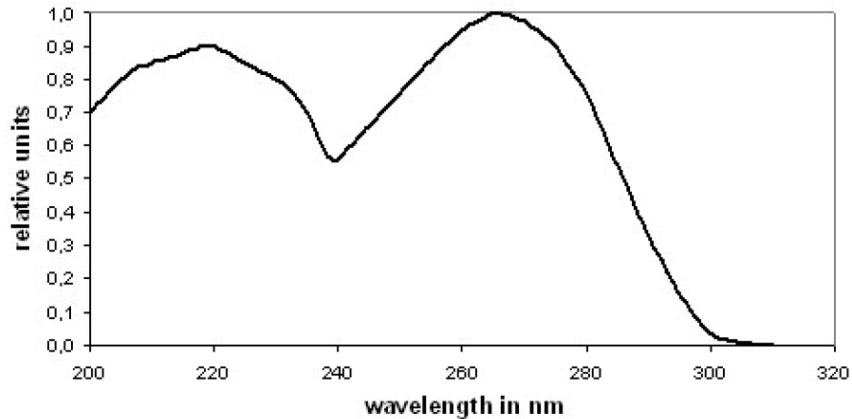


Figure 2.4 The UV absorption spectra of DNA and RNA

There are two most common forms of DNA damages, cyclobutane pyrimidine dimer (CPD) and pyrimidine-pyrimidone photoproducts (PP). The generation of free radicals can cause photoionization (Roccaro & Vagliasindi 2010; Roccaro & Vagliasindi 2010; Xu et al. 2010). Once the DNA is destroyed or becomes a dimer, organism cells like cryptosporidium are not able to perform routine cellular functions such as respiration, absorption of food, or replication. Once the cells become inactive, the organisms die quickly. UV is the only cost-effective disinfection option, and does not produce carcinogenic by-products to the environment.

2.3.2.1.3 Determination of UV Dosage

The bactericidal effect of ultraviolet light is determined by the intensity of ultraviolet radiation and the irradiation time. The key factor is irradiation dose, which is defined as:

UV Dose ($\mu\text{W}\cdot\text{sec}/\text{cm}^2$) = UV Intensity ($\mu\text{W}/\text{cm}^2$) * Exposure Time (sec) (Bolton & Linden 2003).

2.3.2.1.4 Types of UV lamps

In general, the central radiation wavelength of the UV lamp for water disinfection is 253.7 nm. In UV technology, there are two types of UV light currently: low pressure (LP) and medium pressure (MP) (Andrea 2009). The low-pressure UV lamp has a monochromatic UV spectral output (limited to 254 nm) while the medium-pressure lamp has a multicolored UV output (output wavelengths between 185 and 400 nm) (USEPA 2006).

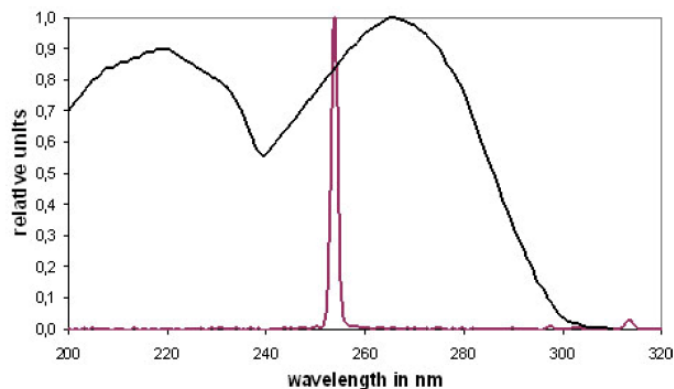


Figure 2.5 The output of low-pressure UV lamp

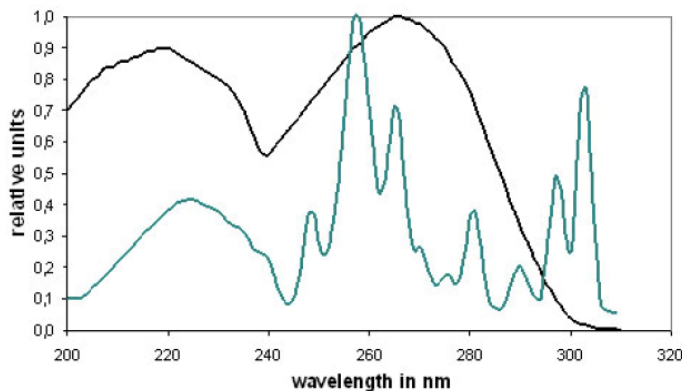


Figure 2.6 The output of high-pressure UV lamp

2.3.2.1.5 Irradiation UV Dosage of Microorganisms

UV inactivation efficiency is defined by how effective UV is at inactivating cells. Usually dose-response curves are used to measure the UV inactivation efficiency (Andrea 2009). Inactivation of microorganism in a disinfection process is determined as:

$$\text{Log Inactivation} = \log_{10} N_0/N \text{ (Andrea 2009)}$$

where N_0 = Concentration of microorganisms before exposure to UV light

where N = Concentration of microorganisms after exposure to UV light

The estimated radiation time to inactivate some common microorganisms with dosage of $30,000 \mu\text{W}\cdot\text{s}/\text{cm}^2$ at UV 254 nm can be found in Table 2.3 (Andrea 2009; ChesapeakeResearch 1982; Technologies 2006; AquaTreatmentService 2006; USEPA 1991).

Table 2.3 Estimated radiation time to inactivate some common microorganisms with a dosage of 30,000 $\mu\text{W}\cdot\text{s}/\text{cm}^2$ at UV 254 nm

Microorganisms	100% lethal Dosage	Microorganisms	100% lethal Dosage
Bacteria			
Dysentery Bacilli	0.15	Micrococcus Candidus	0.4-1.53
Leptospira SPP	0.2	Salmonella Paratyphi	0.41
Legionella Pneumophila	0.2	Mycobacterium Tuberculosis	0.41
Corynebacterium Diphtheriae	0.25	Streptococcus Haemolyticus	0.45
Shigella Dysenteriae	0.28	Salmonella Enteritidis	0.51
Bacillus Anthracis	0.3	Salmonella Typhimurium	0.53
Clostridium Tetani	0.33	Vibrio Cholerae	0.64
Escherichia coli	0.36	Clostridium Tetani	0.8
Pseudomonas Aeruginosa	0.37	Staphylococcus Albus	1.23
Virus			
Coxsackie Virus A9	0.08	Echovirus 1	0.73
Adenovirus 3	0.1	Hepatitis B Virus	0.73
Bacteiphage	0.2	Echovirus 11	0.75
Influenza	0.23	Poliovirus 1	0.8
Rotavirus SA 11	0.52	Tobacco Mosaic	16
Mucor Mucedo	0.23-4.67	Penicillium Roqueforti	9.87
Oospara Lactis	0.33	Penicillium Chrysogenum	2.93
Aspergillus Amstelodami	0.73-8.80	Aspergillus Niger	6.67
Penicillium Digitatum	0.87	Manure Fungi	8
Algae			
Chlorella Vulgaris	0.93	Protozoa	4-6.70
Green Algae	1.22	Paramecium	7.3
Nematode Eggs	3.4	Blue-Green Algae	10-40

In addition, it is found that ultraviolet disinfection technology has a good inactivation effect on pathogenic microorganisms such as Giardia and Cryptosporidium in recent

years. *Cryptosporidium* enters into the environment through human and animal feces (Xu et al. 2009b; Xu et al. 2009a; Canonica et al. 2008; Sommer et al. 2008; Yuan et al. 2013; Wenhai et al. 2016; Cui et al. 2016). They can survive in the environment for a long time. *Giardia* and *Cryptosporidium* can live much longer than other bacteria, which can cause multiple outbreaks of the disease. *Cryptosporidium*-causing disease is very dangerous, and the general symptoms are diarrhea, vomiting, fever, flu-like symptoms. For the patients with disabilities of immune, such as AIDS patients, the disease is even more dangerous, sometimes leading to death. The UV doses of 4-log inactivation for some common pathogens at 254 nm are shown in Table 2.4 (Andrea 2009; ChesapeakeResearch 1982; Technologies 2006; AquaTreatmentService 2006; USEPA 1991).

Table 2.4 UV Dose of 4-log Inactivation for some common pathogens at 254 nm

Pathogens	UV dose (mJ/cm ²) of 4-log inactivation
<i>Cryptosporidium parvum</i> oocysts	<10
<i>Giardia lamblia</i> cysts	<10
<i>Vibrio cholerae</i>	2.9
<i>Salmonella typhi</i>	8.2
<i>Shigella sonnei</i>	8.2
Hepatitis A virus	30
Poliovirus Type 1	30
Rotavirus SA11	36

As can be seen, for most pathogens, the UV doses (mJ/cm²) of 4-log inactivation are smaller than 40 mJ/cm², which is the most common UV dose used in real industries.

In the practical application of UV disinfection for drinking water, some other radiation loss and noise parameters such as distribution of water, lamps use during the radiation intensity changes, water quality, power characteristics, and environmental conditions.

2.3.2.1.6 Advantages and Disadvantages of UV Disinfection

UV disinfection is fast and highly efficient having small footprint, especially for *cryptosporidium* and *giardia* control. Compared with chlorination, it does not produce harmful disinfection byproducts such as trihalomethanes, etc. Also the inactivation of microbes by UV is independent of pH and temperature and without unpleasant taste or

odor. There is no transportation, storage or handling of chemicals. In addition, the UV equipment is easily installed within existing water treatment facilities with low capital and operating costs. The equipment is also very simple, and easy to operate and manage as well as to achieve automation, which highly minimize the hazard risk for operators. So in recent years, ultraviolet disinfection gradually has wide range of applications. For example, in some industries such as aquaculture and food industries, continuity of the chemical disinfectants should be avoided; otherwise it will result in killing aquatic organisms, odor in food, and other side effects due to the influence of chemicals.

The biggest drawback of ultraviolet sterilization is that it does not exhibit residual disinfection capacity and is vulnerable to secondary pollution.

2.3.2.2 Chlorination Disinfection

Chlorination is the oldest and most extensive disinfection technology. It played a major role since published in 1908 to prevent the spread of waterborne diseases. Chlorination includes two disinfectants: liquid chlorine and sodium hypochlorite.

At present, liquid chlorine disinfection is the most cost-effective, widely used method in drinking water disinfection process, since it is a mature technology with strong bactericidal capacity, long duration, low cost, etc. (WorldChlorineCouncil 2008). Approximately 99.5 percent of the drinking water plants in China are using chlorine disinfection process (US-EPA 2006; Standardization Administration of the People's Republic of China 2006). Sodium hypochlorite reduces the hazards of chlorine operation and technical requirements, but it is possible to introduce inorganic byproducts, such as chlorate (chlorate, ClO^{-3}), chlorite (chlorite, ClO^{-2}), and bromate (bromate, BrO^{-3}) (WorldChlorineCouncil 2008).

Due to a long history of application of chlorination, the studies of chlorine disinfection by-products are much deeper than others. Chlorination byproducts are mainly trihalomethane (THMs) and haloacetic acid (HAAs) (USEPA 2011b)(US-EPA 2006). Other chlorinated byproducts include: haloacetonitrile (HANs), cyanogen halide (XCNs),

halogenated acetaldehyde (HATs), halogenated phenol (HHBs), haloketones (HKs), halogen nitro methane (HNMs), furans, halogenated hydroxy (CHF_s) (Fang, Ma, et al. 2010; Zamyadi et al. 2011). In these chlorination DBPs, trihalomethanes (such as chloroform) have already been recognized as carcinogen. From America's drinking water safety regulations bromochloromethane, dichloroacetic acid, bromate, etc. are recognized as suspected carcinogens. Most of the other DBPs also have general toxicity, irritation or narcotic effect to human organs. Large number of epidemiological studies shows that long-term consumption of chlorinated drinking water can increase the digestive and urinary system cancer risk, which have a statistically significant correlation (Munch & Bassett 2004; Fang, Ma, et al. 2010; US-EPA 2006; USEPA 2011a).

2.3.2.3 Chloramine Disinfection

In 1930s, DBPs from chlorination process drew more and more attention. In order to control the concentration of THMs and HAAs in drinking water, many plants started to improve the disinfection process from chlorination to chloramine disinfection (Water & Centre 2007). Compared with chlorine, chloramine has higher penetration and stability, so it is better able to prevent the microbial growth in the distribution of drinking water supply system network; in addition, the chloramine disinfection also significantly improves the taste and smell of water bodies (WolrdChlorineCouncil 2008). However, due to the lower disinfection capacity of chloramine, it is often used as a secondary disinfectant, combined with other strong oxidizing disinfectants (such as chlorine, ozone).

The reactivity of organic compounds in water with chloramine is far below free chlorine. Under the same conditions, DBPs generated from chloramine, especially THMs are significantly lower than chlorinated production (WolrdChlorineCouncil 2008)(CDC 2008). However, recent studies have found that chloramine disinfection may have generated more potential hazardous nitrogenous disinfection byproducts (N-DBPs), such as cyanogen chloride (CCN), N-nitosodimethylamine (N-NDMA), halonitromethane (HNMs), haloacetamides (HAMs) (USEPA 2011a; Munch & Bassett 2004; Fang, Ma, et al. 2010).

2.3.2.4 Chlorine Dioxide Disinfection

ClO_2 sterilization method is an efficient, fast, long-lasting, safe drinking water disinfection method (USEPA 2006). ClO_2 has a strong oxidizing power, and it is a broad-spectrum disinfectant to effectively kill all waterborne pathogens. ClO_2 does not react with nitrogen and ammonia in water. Compared with chlorine, ClO_2 has better and faster sterilization effect with lower dosage and wider scope; the effect of pH and ammonia on the capacity of oxidation disinfection is small; and it can also significantly improve the color and taste of the water (WorldChlorineCouncil 2008). However, ClO_2 is a high cost disinfection technology, limiting its real application.

ClO_2 is a strong oxidant rather than chlorinating agent, which means during disinfection, small amounts of THMs are generated compared to chlorine or chloramine disinfection. However, ClO_2 disinfection process produces more HAAs (primarily DCAA, CBAA and DBAA). ClO_2 inorganic disinfection byproducts ClO^- , ClO^- and BrO^- have high potential toxicity at high-dose or high concentrations, wherein ClO^- can cause hemolytic anemia (Environmental Protection Agency (Ireland) 2013; USEPA 2011b).

2.3.2.5 Ozone Disinfection

As an alternative to chlorine disinfection, ozone disinfection in drinking water treatment is increasingly being used. Ozone sterilization effect is achieved through biochemical oxidation. Sterilization performance test showed that the ozone has significant inactivation on almost all bacteria, viruses, fungi and protozoa and oocytes (USEPA 2006). At room temperature (20°C), half-life of O_3 in water is only about 20 min (Eagleton 1999; Majewski 2012); therefore, chlorine, chloramines, chlorine dioxide as auxiliary disinfectants are needed in pipe network to maintain the disinfection ability. Ozone sterilization effect is stronger than chlorine and chlorine dioxide. However, ozone is extremely unstable, therefore it needs to be prepared on-site at the time of use, which increases the investment in equipment and disinfection costs (Gordon et al. 2008). At present, only a few companies in few countries are using ozone water disinfection process (USEPA 1999b; Eagleton 1999; Majewski 2012).

As a disinfectant, ozone will not produce the halogenated DBPs. The total amount of DBPs is also less than chlorination (WorldChlorineCouncil 2008). The higher concentrations of organic matter in source water, will result in a number of oxygen-containing compounds formation, such as aldehydes, carboxylic acids, ketones, phenols, bromate (when the source water contains higher concentrations of bromide) DBPs (Weinberg et al. 2002). Formaldehyde can cause human nasopharyngeal cancer, nasal sinus cancer, and cancer. Bromoacetate and chloro acetic acid have very stronger ability of DNA damage.

2.3.3 Disinfection By-Product (DBP) Analysis

During drinking water disinfection process, oxidation, addition and substitution reactions occur between disinfectant and natural organic matters (NOMs), such as humic acid, fulvic acid and algal matter, as well as bromide or iodide in source water, which produce new compounds: disinfection by-products.

Epidemiological studies have shown that long-term consumption of chlorinated drinking water and the incidence of bladder cancer are positively correlated, which may also cause early abortion and other side effects of reproductive system (International Agency for Cancer Research 2002). Since trihalomethanes (THMs) were found in the 1970s, more than 600 kinds of DBPs have been identified.

Most DBPs compounds have potential carcinogenicity, teratogenicity, and mutagenicity (Zhao et al. 2012; Hutzinger et al. 2011; Pan & Zhang 2013). After years of research, quantitative information of a few DBPs has been revealed. DBPs with extensive monitoring include: trihalomethanes (THMs), haloaceticacids (HAAs), haloacetonitriles, halo ketone, chloropicrin, trichloroacetaldehyde, cyanogen chloride, chlorite, chlorate, bromate, glyoxal, methylglyoxal, and other aldehydes class. Accordingly, the World Health Organization as well as relevant regulating bodies have developed limited standards of drinking water DBPs (Table 2.5, Table 2.6, Table 2.7) (Gordon et al. 2008; HealthCanada 2012; USEPA 2010; US-EPA 2006; Zerbe & Siepak 2001; Standardization Administration of the People's Republic of China 2006).

Table 2.5 Comparisons of Drinking Water Standards on DBPs (THMs)

All values are in units of mg/L	THMs ¹	TCM	BDCM	DBCM	TBM
Chinese Sanitary Standards for Drinking Water	The sum of the ratio of the concentration of each to its respective guideline value should not exceed 1	0.06	0.1	0.06	0.1
European Union Directives	0.1	*	*	*	*
National Primary Drinking Water Regulations (USA)	0.08	*	*	*	*
Guidelines for Drinking Water Quality (WHO)	The sum of the ratio of the concentration of each to its respective guideline value should not exceed 1	0.3	0.1	0.06	0.1
Guidelines for Canadian Drinking Water Quality	0.1	*	*	0.016	*
Risk Comments	Liver effects (fatty cysts) (chloroform classified as possible carcinogen); Kidney and colorectal cancers				

¹: Trihalomethanes – Total (THMs): chloroform (CHCl₃, TCM), dichlorobromomethane (CHCl₂Br, BDCM), dibromochloromethane (CHClBr₂, DBCM) and bromoform (CHBr₃, TBM) (US-EPA 2006)

*: None required

Table 2.6 Comparisons of Drinking Water Standards on DBPs (HAAs)

All values are in units of mg/L	HAAs ²	DCAA	TCAA
Chinese Sanitary Standards for Drinking Water	*	0.05	0.1
European Union Directives	*	*	*
National Primary Drinking Water Regulations (USA)	0.06	*	*
Guidelines for Drinking Water Quality (WHO)	*	0.05	0.2
Guidelines for Canadian Drinking Water Quality	0.08	*	*
Risk Comments	Liver and cancer (DCAA: DCAA is classified as probably carcinogenic to humans); Other organ cancers (DCAA, DBAA, TCAA); liver and (body, kidney and testes weights)		

²: Haloacetic acids – Total (HAAs): monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), bromoacetate (MBAA) and dibromoacetic acid (DBAA)(US-EPA 2006)

*: None required

Table 2.7 Comparisons of Drinking Water Standards on DBPs

All values are in units of mg/L	Bromate	Chlorite	Chlorate
Chinese Sanitary Standards for Drinking Water	0.01	0.7	0.7
European Union Directives	0.01	*	*
National Primary Drinking Water Regulations (USA)	0.01	*	*
Guidelines for Drinking Water Quality (WHO)	0.01	0.7	0.7
Guidelines for Canadian Drinking Water Quality	0.01	1	1
Risk Comments	Renal cell tumors (classified as probable carcinogen)	Neurobehavioral effects (lowered auditory startle amplitude, decreased exploratory activity), decreased absolute brain weight, altered liver weights	Thyroid gland effects (colloid depletion)

*: None required

However, 30 years of toxicology and risk assessments show that the DBPs in current drinking water standards are unlikely to cause very great health risk shown by epidemiology researches. It is still unclear that which contaminations are the occurrences of bladder cancer (Costet et al. 2011; Lee et al. 2013; Alija et al. 2016).

2.3.3.1 Trihalomethanes (THMs)

2.3.3.1.1 Introduction

THMs are the first series of DBPs found in chlorinated drinking water. There are 4 THMs often detected in drinking water, namely chloroform (CHCl_3 , TCM), dichlorobromomethane (CHCl_2Br , BDCM), dibromochloromethane (CHClBr_2 , DBCM) and bromoform (CHBr_3 , TBM), among which TCM is the main component. When the source water contains iodide, iodide THMs may also be generated with disinfectants (Zha et al. 2014; Bougeard et al. 2010; Grunwald et al. 2002).

THMs are a major component in the formation of DBPs in drinking water. THMs and HAAs make up to 25% of total halogenated DBPs (Stuart W Krasner et al. 2006). Concentrations of THMs generated in disinfection process depend on the type of disinfectant. Generally, THM formation follows the following order: chlorination > chloramine > ozone > chlorine dioxide (WorldChlorineCouncil 2008). Ozonation could produce higher concentration of brominated THMs if the source water contains high concentrations of bromide (Aljundi 2011; Moslemi et al. 2012).

2.3.3.1.2 Detection and Analysis

The concentration levels of iodide THMs in drinking water are mostly less than 1 ppb, but in some cases about 12-13 ppb can also be found with an average concentration of 10.2 ppb as found in 23 cities of United States and Canada (Weinberg et al. 2002; Stuart W Krasner et al. 2006).

There are many US EPA standard methods available for the detection of THMs in drinking water, such as EPA Method 502.2, 524.2, 551 and 551.1 which use mainly gas chromatography (GC) combined with photoionization detection, electronic capture detector (ECD) and mass spectrometry (MS) detector (EPA551 1995; EPA551.1 1995; EPA501.2 1996; EPA524.2 1995; EPA502.2 1995). Sample preparation methods such as purge & trap (P & T), headspace sampling techniques, liquid-liquid extraction (LLE), closed-loop stripping concentrate (CLSA), solid phase extraction (SPE) or solid phase micro extraction (SPME), etc. are all used. Headspace sampling techniques and GC/MS

method are the most effective analytical method for chlorinated and brominated THMs (including TCM, BDCM, DBCM and TBM). Low recovery (< 50 %) headspace sampling techniques or purge & trap (P & T) process cannot be used for accurate quantification of iodide THMs because of its low concentration level (< 1 ppb)(Jones et al. 2012). Thus, higher recovery LLE process (47 % - 94 %) is used for 6 iodide THMs, CHCl_2I , CHBrClI , CHBr_2I , CHClI_2 , CHBrI_2 and CHI_3 , with recovery of about 100 % with analysis in GC-ECD (detection limit of 0.01- 0.03 ppb, and the quantification limit of 0.1- 0.7 ppb)(Weinberg et al. 2002; Stuart W Krasner et al. 2006).

Over the last 3 decades, the studies on THMs are focused on their effects such as mutagenic activity (genetic or chromosomal mutation) and genotoxicity (mutagenicity and DNA damage). Large numbers of studies have shown that THMs have obvious mutagenicity or genotoxicity with accurate dose-response relationships. IARC and Richard SD, have reviewed the toxicity of TCMs and other THMs (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2010; Richardson et al. 2007). The cytotoxic order of THMs is: $\text{TBM} > \text{DBCM} > \text{TCM} > \text{BDCM}$; and the mutagenicity order follows: $\text{TBM} > \text{BDCM} > \text{DBCM} > \text{TCM}$ (Wang et al. 2014; Chang et al. 2010). US EPA indicates that THMs are carcinogenic for rats. TBM, DBCM and BDCM can cause intestinal, liver and kidney tumors in rats, respectively (IARC 1999; WHO 1991; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2010).

2.3.3.2 Haloacetic Acids (HAAs)

2.3.3.2.1 Introduction

1983, Christman etc. found haloacetic acids (HAAs) in chlorinated drinking water disinfection (Christman et al. 1983). HAAs caused high attention of US EPA due to its high boiling point, unable to be blown off, and the much higher unit cancer risk than THMs. There are 9 HAAs in total, the US EPA made five species defined: monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), bromoacetate (MBAA) and dibromoacetic acid (DBAA)(US-EPA 2006).

HAAs are formed during chlorine, chloramines, and chlorine dioxide and ozone disinfection process. But with different amounts of different disinfection methods, HAAs

are produced with various concentrations. Chlorination could form highest HAAs, which are often higher than the concentration limits specified in drinking water DBPs regulations (Zheng et al. 2016; Bougeard et al. 2010). Compared with chlorination, chloramine disinfection greatly reduces the HAAs formation (McGuire et al. 2002). HAAs from chlorine dioxide disinfection process are mainly DCAA, CBAA and DBAA (McKie et al. 2015; Richardson et al. 2007). Ozone disinfection greatly reduces the formation of THM and HAA, but when the concentration of bromide or iodide in source water is high, there would be the formation of bromo, iodo and mixed haloacetic acid, dibromoacetic acid, such as (DBAA), tribromoacetic acid, 1-iodoacetate (MIAA), 1-bromo acid chloride (CBAA), dichlorobromoacetic acid (DCMBAA), 1-chlorine-dibromoacetic acid, 1-bromine-iodine acid (BIAA) (Liu et al. 2013; Xie et al. 2010; Pan et al. 2015).

2.3.3.2.2 Detection and Analysis

There are many US EPA standard methods available for the detection of HAAs in drinking water, such as EPA Method 552.1, 552.2, 552.3 (EPA552 1990; EPA552.2 1995; Dell 1993; USEPA552.3 2003). Generally, methyl tert-butyl ether (MtBE) is used first for liquid-liquid extraction, then 1, 2- dibromopropane is added as an internal standard. Subsequently, acidified methanol or diazomethane is used for derivatization before analyzing in GC/ECD or GC/MS.

All 5 US EPA defined HAAs species have mutagenic activity. There are fewer genotoxicity studies for iodide, brominated, dibrominated, tribrominated and chlorinated acetic acids. Among them, the concentrations of chlorinated HAAs are much higher than brominated HAAs. Most commonly detected HAAs are DCAA and TCAA. Both of them have been identified as animal carcinogenicity, the cancer risks of which are about 50-fold and 100-fold of TCM. HAAs contribute more than 90% in total cancer risks among all DBPs; while HANs, HKs and other DBPs have relatively little cancer risk. Animal experiments have shown that, compared with chlorinated HAAs, brominated HAAs have stronger cell toxicity and genetic toxicity. The cytotoxic order of HAAs is: MBAA >> DBAA > MCAA > DCAA > TCAA; and the genotoxicity order follows: MBAA > MCAA > DBAA > TBAA, while DCAA and TCAA have no genotoxicity in

experiments. In addition, animal experiments also shown that iodide HAAs have 3 times greater cytotoxicity and genotoxicity than brominated HAAs (Xie et al. 2010; Hu & Hu 2013; Zhang et al. 2010; Richardson et al. 2007).

2.3.3.3 Effects of AOM on DBP Formation

The AOM from different algal species may contain different levels of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) (Lui et al. 2012). AOM is rich in organic nitrogen and lack of organic carbon compared to NOM, which results in more nitrogenous DBPs (N-DBPs) and less carbonaceous DBPs (C-DBPs) during disinfection treatment (Lui et al. 2012). However, C-DBP has drawn more attention from the researchers for its dominant occurrence and serious harm.

The level of DBPs formation may vary considerably with algae species, the genus, the algal cells, the algal growth, biochemical composition and the applied conditions of chlorination treatment (chlorination dose, pH and incubation time)(Lv et al. 2014; Liang et al. 2012; Li et al. 2012; Lui et al. 2012; Lui et al. 2011; Huang et al. 2009). The DBPs formation of nine different fresh water algal species were studied (three blue-green algae, three green algae, and three diatoms), and it showed that green algae and diatoms produced more DBPs than blue-green algae (Zhang et al. 2014). For green algae and blue-green algae, EOM produced less fraction in DBPs (except for trichloronitromethane) than IOM in chlorination treatment (Fang, Yang, et al. 2010) (Yang et al. 2011; Li et al. 2012). However, up to now, the details of C- DBPs and N-DBPs formation from IOM and EOM of diatoms are still very limited (Pivokonsky et al. 2014; Liang et al. 2012; Zhang et al. 2016).

2.4 The Importance of This Study

Algae are aquatic and photosynthetic microorganisms, which are widely living in lakes, reservoirs and surface waters. The most commonly found algae in drinking water sources are green algae, blue-green algae, diatoms, euglenoids, dinoflagellates, cryptomonads, yellow-green algae, and golden algae (Knappe et al. 2004). Algal bloom has been defined as a visible accumulation of algal biomass (Reynolds & Walsby 1975). Algal organic matter (AOM) is found in high concentration during algal bloom season in drinking water systems, and it affects the drinking water quality as one of the substantial contributors to natural organic matter concentration (NOM) (Pivokonsky et al. 2015). Algal organic matter (AOM) is generally categorized into extracellular organic matter (EOM) and intracellular organic matter (IOM).

Drinking water treatment mainly includes pretreatment and disinfection processes. The aim is to remove colloid contents and suspended solids as well as to kill microbial pathogens in water to prevent the spread and prevalence of waterborne infectious diseases (WorldChlorineCouncil 2008). Also, during drinking water treatment, the disinfectant also remove taste, color, iron oxide and manganese of drinking water (Wang et al. 2014)(SDWF 2012).

During drinking water disinfection process, oxidation, addition and substitution reactions occur between disinfectant and natural organic matters (NOMs) such as humic and fulvic acids and algal matter, as well as bromide or iodide in source water, which produce disinfection by-products (DBPs). Since the time trihalomethanes (THMs) were found in the 1970s, more than 600 different DBPs have been identified. Most DBPs compounds have potential carcinogenicity, teratogenicity, and mutagenicity (Zhao et al. 2012; Hutzinger et al. 2011; Pan & Zhang 2013). After years of research, quantitative information of a few DBPs has been revealed.

The bloom of algae causes a series of problems for drinking water treatment. It affects the efficiency of coagulation, causes bad taste and release algal toxins. What's more, AOM is rich in organic nitrogen and organic carbon compared with NOM, which causes the formation of more DBPs during disinfection treatment (Lui et al. 2012). Therefore, it is

very necessary to remove algae and AOM during drinking water treatment to avoid the problems described above.

In this study, two diatoms and two green algae were selected as the most common algae in natural water. The various dosages of coagulant were used for EOM and IOM to evaluate the efficiency of coagulation. UV and post-UV chlorination were used as the disinfection methods. Chlorination was a necessary part to provide the disinfectant residual which can maintain the disinfection effect. HAAs and THMs were determined after treatments to analyze the DBPs formation potential.

The specific objectives are:

- a) To determine and analyze the parameters of EOM and IOM from four different commonly found species of algae in surface water.
- b) To determine the efficiency of various dosages of common alum coagulant used in drinking water treatment plants for four different commonly found species of algae in surface water.
- c) To determine the impact of UV radiation and post-UV chlorination on the water parameters and DBPs formation potential for coagulated and not coagulated EOM and IOM.

Chapter 3

3 Materials and Methods

The experimental procedures adopted in this work are shown in the following schematic as shown in Figure 3.1.

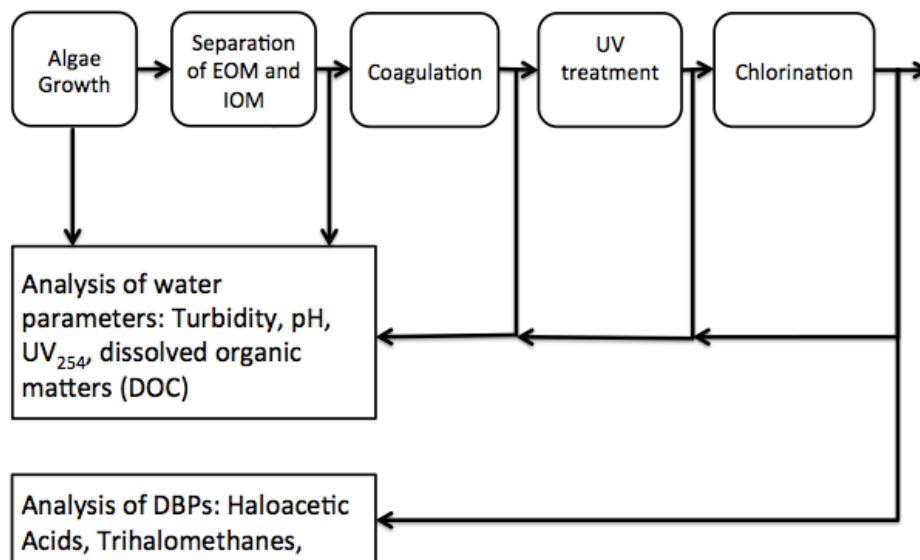


Figure 3.1 Experimental procedures

Test algal strains were grown in the laboratory in Western University. For each algae strain, AOM was separated into EOM and IOM raw solutions using freeze/thaw cycles as described later. Several water quality parameters such as turbidity, pH, DOC were determined after each experiment. Alum ($\text{Al}_2(\text{SO}_4)_3 \cdot 16\text{H}_2\text{O}$) was used as the coagulant for EOM and IOM removal from water. Before coagulation, pH of all the EOM and IOM solutions were adjusted to 8.0 ± 0.2 . The dosage of alum varied in the range from 20 - 60 mg/L. A bench-scale collimating beam apparatus supplied by Trojan Technologies was used for UV-disinfection experiments of water with algal matters with a fixed UV dosage of 40 mJ/cm^2 . The chlorination treatment was conducted based on the uniform formation conditions (UFC) (Summers 1996) for 24 h at room temperature. THMs (chloroform, bromoform, dibromochloromethane) and HAAs (MBAA, DCAA, TCAA) were

determined based on EPA methods (EPA334.0 2009; EPA551 1995; EPA330.5 1978; EPA552.2 1995; EPA502.2 1995) using a GC-ECD system.

3.1 Experimental Materials

3.1.1 Algae Suspensions

Two strains of diatom as well as two strains of green algae were used in this research. The two species of diatom are *Phaedactylum Tricornutum*, *Cyclotella Meneghiniana* and the two species of green algae are *Chlorella Vulgaris*, *Chlamydomonas Reinhardtii*. The two species of green algae were purchased from University of Texas at Austin (*C. vulgaris* strain no. UTEX 2714), Chlamydomonas Resource Center (*C. reinhardtii* strain no. CC-125). The two species of diatom (*P. tricornutum* strain no. CPCC 162 and *C. meneghiniana* strain no. CPCC710) were obtained from Canadian Phycological Culture Centre (Waterloo, ON, Canada). The characteristics of four strains are listed in Table 3.1 (Meza et al. 2015; Hernández-zamora et al. 2015; Harris & Coleman 2005; Yang & Li 2016; Rees & Victoria 2006).

Table 3.1 Characteristics of the four strains used in the experiments

Characteristics	<i>C. Vulgaris</i>	<i>C. Reinhardtii</i>	<i>P. Tricornutum</i>	<i>C. Meneghiniana</i>
Class	Green Algae		Diatom	
Geometric shape and dimensions	Spherical, 2-10 μm in diameter	Ellipsoidal, 10 μm in diameter	Fusiform, 10 μm length	Cylinder, 18-30 μm length
Typical bloom period	Summer or early fall		Spring	

3.1.2 Chemical Reagents

All the stock and experimental solutions were prepared from ACS reagent grade chemicals. The four mediums (Bold's Basal Medium, High Salt Minimal Media, F/2 Marine Medium and CHU-10 Medium) were purchased from Canadian Phycological Culture Centre and made based on standard methods (Robert R. L. Guilard 2014)(Rochaix 2002)(Robert R. L. Guilard 1962)(Abomohra & Wagner 2013). A stock solution of $\text{Al}_2(\text{SO}_4)_3 \cdot 16\text{H}_2\text{O}$ at 10 g/L was prepared by dissolving 10 g $\text{Al}_2(\text{SO}_4)_3 \cdot 16\text{H}_2\text{O}$ in 1 L Milli-Q water. A 5% commercial hypochlorite solution (NaOCl, ACROS, NJ, USA), DPD (N,N-diethyl-p-phenylene diamine) indicator as well as FAS solution (ferrous ammonium sulfate) were purchased from RICCA, Arlington, USA. 99.9%

Methyl tert-butyl ether (MTBE) was purchased from Sigma-Aldrich, USA. THMs (EPA 501 Trihalomethanes Mix) and HAAs (EPA 552 Methyl Esters Mix) standards were purchased from Fisher Scientific (ON, Canada).

3.2 Experimental and Analytical Methods

3.2.1 Algae Growth

The four strains were cultured in four different media in incubators maintained at 25°C, *Chlorella Vulgaris* (Bold's Basal Medium) (Robert R. L. Guilard 2014), *Chlamydomonas Reinhardtii* (High Salt Minimal Media) (Rochaix 2002), *Phaedactylum Tricornutum* (F/2 Marine Medium) (Robert R. L. Guilard 1962), *Cyclotella Meneghiniana* (CHU-10 Medium) (Abomohra & Wagner 2013). All media were autoclaved before use.

The stock algal suspensions were harvested during the exponential growth period, which was determined by counting the cells regularly. The experimental suspensions were prepared by diluting the stock algal suspensions using deionized water. The experimental suspensions were stained with methylene blue (MB) in 3% acetic acid, and incubated in dark at room temperature for 20 minutes (Imase et al. 2013). After that, a light microscope (ZEISS) with a hemocytometer (LW Scientific) was used to count the number of algae cells in the suspensions. Cells that were blue or pale blue color under the microscope were determined to be dead, while the living cells retained their own color (Imase et al. 2013). The cell counts of stock and experimental suspensions are shown in Table 3.2. The experimental suspensions were created for the next experimental step, which is the separation of IOM and EOM.

Table 3.2 Media and initial cell counts of the algae

Characteristics and parameters	<i>C. Vulgaris</i>	<i>C. Reinhardtii</i>	<i>P. Tricornutum</i>	<i>C. Meneghiniana</i>
Media	Bold's Basal Medium	High salt minimal media	F/2 Marine Medium	CHU-10 Medium
Stock suspension cell count (cells/mL)	$(8.1 \pm 0.3) \times 10^7$	$(5.6 \pm 0.4) \times 10^7$	$(9.4 \pm 0.2) \times 10^7$	$(8.8 \pm 0.3) \times 10^6$
Experimental suspension cell count (cells/mL)	$(5.1 \pm 0.2) \times 10^6$	$(8.3 \pm 0.3) \times 10^6$	$(5.5 \pm 0.3) \times 10^6$	$(4.0 \pm 0.2) \times 10^6$

3.2.2 Separation of IOM and EOM

As mentioned in the previous section, experimental algal suspensions were created by diluting the stock algal suspensions using deionized water. The algal suspensions were transferred into several 50 mL tubes and then centrifuged at $\sim 3000g$ for 20 min under 25 °C with a 6×50 mL rotor (Thermo Scientific Sorvall, Legend T Plus). The supernatant fractions were transferred into a 1L beaker, and then filtered through 0.45- μm sterilized 47 mm membranes (PALL life Sciences) into a 1 L flask by vacuum. The filtrate is referred to as experimental raw EOM solution. The organic matters content in EOM were determined by a TOC analyzer. The algae cells, which were separated in centrifugation at the bottom of the 50 mL tubes, were washed and followed by re-suspending in the same amount of Milli-Q water. The resulting cells suspensions were subjected into four freeze/thaw cycles (-15 °C/ 25 °C) to achieve the lysis of the algae cells. During the lysis of algae cells, IOM is released in water, which was subjected to similar centrifugation and filtration treatments as for the EOM solutions discussed above. The filtrate was referred to as experimental IOM solutions. Same water quality parameters such as turbidity, DOC and UV_{254} were determined for both EOM and IOM solutions.

Before coagulation, the pH of both EOM and IOM raw solutions was adjusted to 8.0 ± 0.1 using 0.1 M HCl and 0.2 M NaOH solution. The experimental procedures for EOM and IOM separation are shown in Figure 3.2.

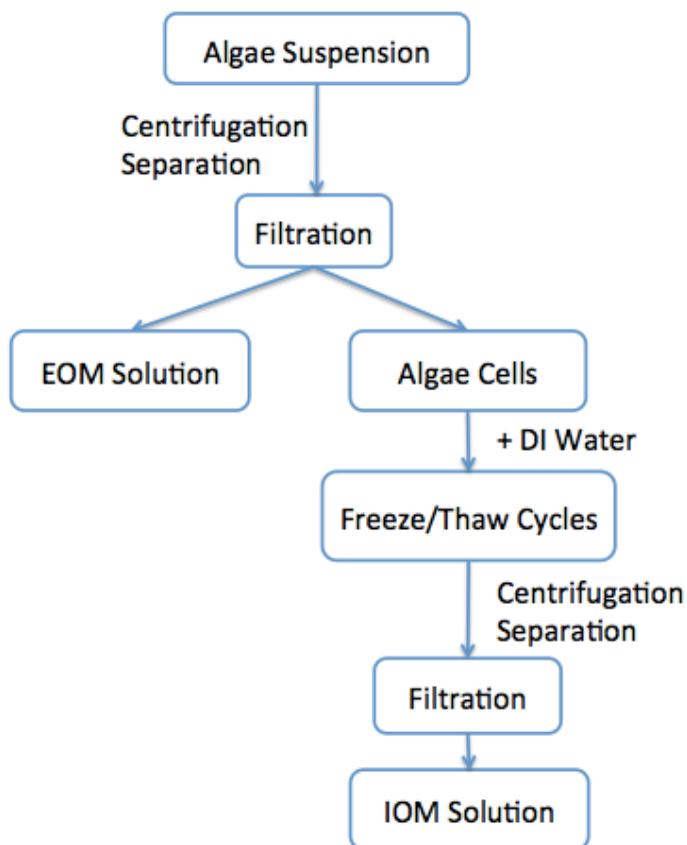


Figure 3.2 Experimental procedures of the separation of EOM and IOM

3.2.3 Pre-treatment: Coagulation

The coagulation setup (shown in Figure 3.3) consisted of a Phipps & Bird programmable jar tester (Model PB900) with six stainless steel mixing paddles, an LED illuminator fixed on the base and a chassis with powder coated steel frame. All the experiments were conducted at room temperature.

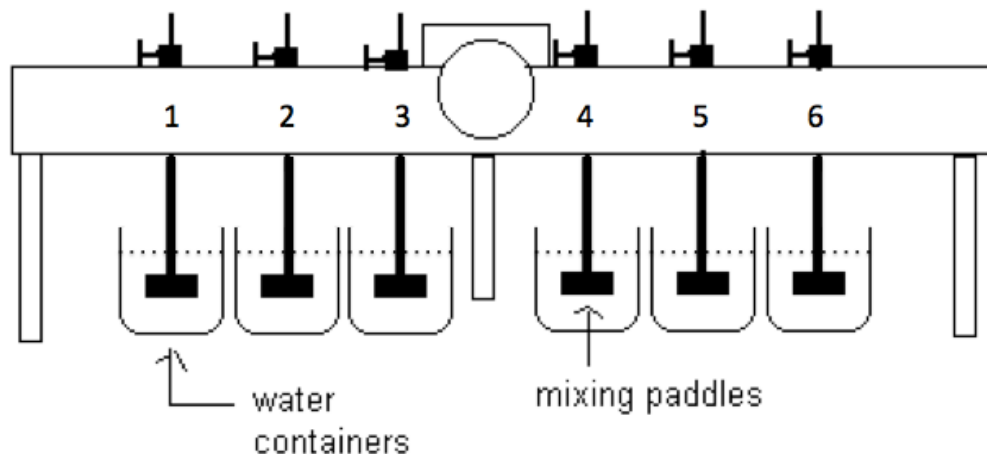


Figure 3.3 Coagulation setup

In this study, $\text{Al}_2(\text{SO}_4)_3 \cdot 16\text{H}_2\text{O}$ was used as a coagulant. The range of the added coagulant was between 20 to 60 mg/L. 500 ml sample of AOM in water was added into a clean 600 ml beaker, and then placed under the base with the mixing paddle inside of it. There were 6 groups of coagulation samples conducted at the same time at room temperature (shown in Appendix A-2). The first group was the control group without adding any coagulant, while groups 2-6 were with the coagulants at dosage of 20, 30, 40, 50, 60 mg/L respectively. Coagulation was conducted with the rapid mixing speed at 150 rpm for 2 min, followed by flocculation treatment at a slow mixing at 25 rpm for 20 min. After flocculation, all the six samples were allowed to settle for 2 hours.

After settling, the supernatant was collected for the analysis of turbidity, DOC and UV_{254} . For DOC determination, the supernatant needed to be filtered through 0.45- μm sterilized membranes with diameter of 47 mm (PALL life Sciences) into a 1 L flask by vacuum. The supernatants were buffered to $\text{pH } 8.0 \pm 0.1$ with 2 mL/L borate buffer (1.0 M boric acid and 0.26 M sodium hydroxide in ultrapure water) before disinfection treatment. 0.1 M HCl solution and 0.2 M NaOH solution were used to adjust the pH at 8.0 ± 0.1 of the supernatants if necessary. All the experimental data were obtained in triplicate.

3.2.4 Disinfection Treatment: UV Treatment

A bench-scale apparatus (shown in Appendix A-3) supplied by Trojan Technologies was used in this study. The apparatus (shown in Figure 3.4) consisted of a collimating tube, which provided the irradiation on the surface of water samples, using a low-pressure (monochromatic at 254 nm) UV lamp, and a magnetic stir plate with a small stir bar (shown in Appendix A-4), which was used to make a completely mixed water samples during the treatment.

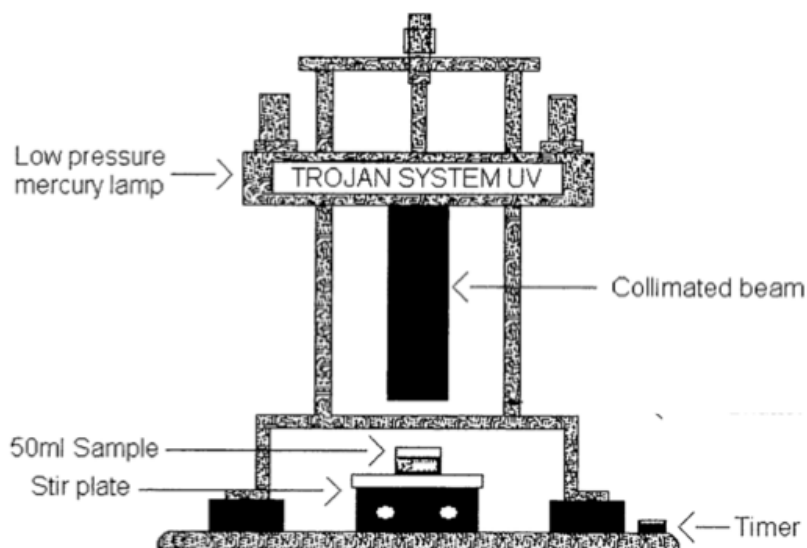


Figure 3.4 UV bench-scale apparatus

Before the UV radiation experiments, the UV lamp needed to be turned on for at least 4 hours to achieve a stable UV intensity. The calibrated radiometer (IL1400A, International Light, S/N 6976) equipped with a SEL 240 detector was used to determine the UV intensity. A new calibration was needed each time before treatment, which is described below.

The UV irradiation intensities were measured every 0.5 cm along both X and Y-axes from 0 to 3 cm to obtain the average irradiation intensity in the petri dish circle shown (Figure 3.5). The UV detector was placed at the same level of the water sample surface.

The average intensity in the petri dish was then calculated using the formulae shown in Appendix A-5.

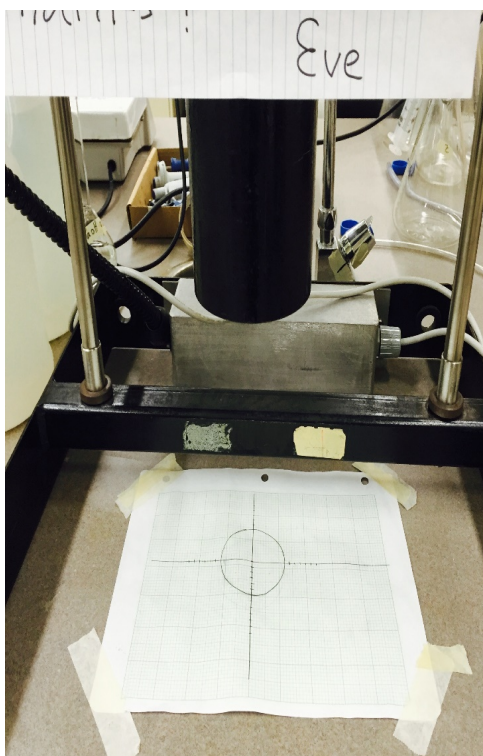


Figure 3.5 Collimated beam circle

In a collimated beam experiment, certain correction factors are needed to calculate the final irradiance of UV light. Bolton and Linden (Bolton et al. 2003) used 4 correction factors which are listed below.

a. Reflection Factor (R_f)

Light reflects off the interface between the media when it travels from one medium to the other (Bolton & Stefan 2002). Thus a constant reflection factor is necessary to correct the reflected UV light.

b. Petri Factor (P_f)

The Petri Factor is needed to account for the variance of irradiance over the surface of the sample (Andrea 2009). To get the Petri Factor, light intensity are measured from the

center of the Petri dish and divided by the center irradiance and an average of ratios known as the Petri Factor is calculated (Bolton et al. 2003).

c. Water Quality Factor (W_f)

A Water Quality Correction is needed to correct the energy absorbed by water body as UV light travels through it. Water Quality Correction is done by integrating the Beer-Lambert Law over sample depth and takes into account the water absorption coefficient (Bolton & Linden 2003). The equation of Water Factor is listed below (Bolton & Linden 2003):

$$\text{Water Factor} = \frac{1-10^{al}}{al \ln(10)}$$

where a = absorbance for a 1 cm path length

l = vertical path length (cm) of the water in the Petri dish

d. Divergence Factor (D_f)

Divergence Factor is very needed to correct the collimation of collimated (Bolton & Linden 2003). The equation of Divergence Factor is shown below (Bolton & Linden 2003):

$$\text{Divergence Factor} = \frac{L}{(L+l)}$$

where L = distance from the UV lamp to the surface of the cell suspension

l = vertical path length (cm) of the cell suspension in the Petri dish

Given all the 4 correctors, the final irradiance rate can be calculated by the following equation (Bolton & Linden 2003),

$$\text{Ave. final irradiance rate} = \text{UV beam irradiance rate} * P_f * R_f * W_f * D_f$$

As described above, the four correction factors of the collimated beam were determined from measured parameters such as UV transmittance, the depth of water sample, the

distance from UV lamp to the surface of water sample and were used in average intensity calculation. Among all these parameters, only UV transmittance and UV intensity were changed in different experiments while other parameters were constants. The irradiation time for a water sample is calculated based on the average intensity and the desired dosage (40 mJ/cm^2)(shown in Appendix A-6).

For UV radiation, only the algal solutions with 0 and 60 mg/L coagulants were treated. The samples without coagulants were the control groups and the samples with 60 mg/L coagulant were the experimental groups. Water samples were buffered at $\text{pH } 8.0 \pm 0.1$ with 2 mL/L borate buffer: (1.0 M boric acid and 0.26 M sodium hydroxide DI water). 0.1 M HCl and 0.2 M NaOH were used to adjust the pH at 8.0 ± 0.1 of the water samples when necessary. 50 ml samples were added into a clean petri dish, which contained a magnetic stir bar, and then placed under the collimating beam at room temperature. As used in many drinking water treatment plants, a UV dose of 40 mJ/cm^2 for 4-log inactivation was used in this study.

3.2.5 Disinfection Treatment: Chlorination

The chlorination treatment was conducted based on the uniform formation conditions (UFC)(Summers 1996). As above only the solutions with 0 and 60 mg/L coagulants were treated. The samples without coagulants were control groups and the samples with 60 mg/L were experimental groups. The basic uniform formation conditions include: $\text{pH } 8.0 \pm 0.2$, temperature $20.0 \pm 1.0 \text{ }^\circ\text{C}$, incubation time $24 \pm 1 \text{ h}$ and chlorine residual $1.0 \pm 0.4 \text{ mg/L}$ as free chlorine after 24 h (Summers 1996). The pH of all water samples were adjusted to 8.0 ± 0.2 before UV, so there was no need to add the buffer again. The chlorination solution is a combination of sodium hypochlorite (NaOCl) and a borate buffer. The combined hypochlorite-buffer solution was made by buffering the commercial 5% hypochlorite solution to pH 8.0 with pH 6.7 borate buffer (1.0 M boric acid and 0.11 M sodium hydroxide in DI water) (Summers 1996). The chlorine dosages in this study were adjusted to 1.8 times of the initial DOC values to achieve a 24 h chlorine residual of $1.0 \pm 0.4 \text{ mg/L}$. The bottles were then capped with PTFE lined caps and covered with aluminum foil and kept in dark at $20.0 \pm 1.0 \text{ }^\circ\text{C}$ for 24 h (shown in Appendix A-7). UV_{254} , pH, chlorine residual and DOC were determined after incubation.

3.2.6 Water Quality Parameters Analysis

The main water quality parameters measured in this study include pH, turbidity, DOC, UV_{254} and chlorine residual. A pH meter (Orion Model STAR A111) was used to determine the pH. The turbidity of water samples was measured by a Hach ratio turbid meter (Model 2100AN) in nephelometric turbidity units (NTU). A Shimadzu TOC- $V_{CPN}ASI-V$ analyzer was used to determine DOC of the water samples. Before determination, the water samples were filtered through 47 mm, 0.45- μm sterilized membranes (PALL life Sciences) by vacuum. UV_{254} is the absorbance of water samples at 254 nm, was determined by a dual-beam UV-VIS-NIR spectrophotometer (Shimadzu Model 3600). Chlorine residual was determined as free chlorine (hypochlorite ion, hypochlorous acid) based on DPD-FAS titration spectrophotometric method (EPA334.0 2009; EPA330.5 1978). The calibration was built up with Cl_2 concentration at a range of 0.05, 0.1, 0.5, 1, 2, 4 ppm. Standard ferrous ammonium sulfate (FAS) solution was used to titrate the standard $KMnO_4$ solution as well as water samples after disinfection. During the calibration, $KMnO_4$ standard solutions were used instead of chlorine solution because of the instability of chlorine in water. $KMnO_4$ as well as free chlorine can oxidize DPD (N,N-diethyl-p-phenylenediamine) to produce a red colored solution. The absorbance of the solution was spectrophotometrically determined at 515 nm (Public & Association 1992; ThermoFisher 2003; EPA334.0 2009; EPA330.5 1978).

3.2.7 DBP Analysis

THMs (chloroform, bromoform, dibromochloromethane) and HAAs (MBAA, DCAA, TCAA) were determined in this study based on EPA methods.

THMs determination was mainly based on EPA 551 and 551.1. A 50 mL sample aliquot was collected in a 65 mL vial. 0.833 g buffer/ dechlorinating agent powder was added to lower the pH as well as convert free chlorine to monochloramine (EPA551.1 1995; EPA502.2 1995; EPA524.2 1995; EPA501.2 1996). The phosphate buffer powder was a mixer of 1% sodium phosphate, dibasic (Na_2HPO_4) and 99% potassium phosphate, monobasic (KH_2PO_4) by weight (EPA551.1 1995). 1.2 g ammonium chloride (NH_4Cl) was added to the 200 g phosphate buffer powder as the dechlorinating agent to make the

buffer/ dechlorinating agent. The sample aliquot was then extracted with 3 mL of MTBE (Column et al. 1995). After shaking for 2 min, 20 g Na₂SO₄ was added to the aliquot. The vial was capped immediately and was shaken vigorously for 4 min, and then inverted to allow the MTBE and water phases to separate for another 5 min. 2 µL of the MTBE extract was then injected into a GC (Shimadzu GC-2014) equipped with a BPX5 column (0.25 mm ID × 30 m, 0.50 µm film thickness, 5% phenyl (equivalent) / 95% methyl polysilphenylene / siloxane phase) and electron capture detector (ECD) for separation and analysis (EPA551.1 1995). The temperature program of the column oven was as follows: an initial temperature of 45 °C was held for 5 min, then increased at a rate of 10 °C /min to 145 °C, 25 °C /min to 225 °C, 10 °C /min to 260 °C. The temperature of the injector and electron conductivity detector were set at 200 °C and 290 °C, respectively. Nitrogen and helium were used as the make-up gas and carrier gas, respectively. The calibration curves are shown in Appendix B-1, B-2, and B-3.

HAAs determination was mainly based on EPA 552 and 552.2. A 40 mL sample was collected in a 65 mL vial. 100 mg/L ammonium chloride (NH₄Cl) was added as the dechlorinating agent. About 2 mL of 95-98% sulfuric acid was then added into the vial to adjust pH < 0.5 (EPA552.2 1995; EPA552 1990; Dell 1993; USEPA552.3 2003). After 2 min of shaking, 16 g Na₂SO₄ was added to increase the ionic strength of the aqueous phase (EPA552.2 1995; EPA552 1990). 4 mL MTBE was then added as the extract solvent. The vial was recapped immediately, shaken vigorously for 4 min and then inverted to allow the MTBE and water phases to separate for another 5 min (EPA552.2 1995; EPA552 1990). About 3 mL of the MTBE extract was then transferred into a 15 mL glass vials and methylated using one mL 10% sulfuric acid / methanol solution (EPA552.2 1995; EPA552 1990). The tube was placed in a heating bath at 50 °C for two hours in dark. After heating, 4 mL of saturated sodium bicarbonate solution was added to remove the extra acid, which would damage the GC column. 2 µL of the MTBE extract was then injected into a GC (Shimadzu GC-2014) equipped with a BPX5 column (0.25 mm ID × 30 m, 0.50 µm film thickness, 5% phenyl (equivalent) / 95% methyl polysilphenylene / siloxane phase) and electron capture detector (ECD) for separation and analysis (EPA552.2 1995; EPA552 1990; Dell 1993). The temperature program of the column oven is as follows: an initial temperature of 42 °C was held for 8 min, then

increased at a rate of 15 °C /min to 100 °C and held for 5 min, 25 °C /min to 280 °C. The temperature of the injector and electron conductivity detector were set at 250 °C and 290 °C, respectively (EPA552.2 1995; EPA552 1990; Dell 1993). Nitrogen and Helium were used as the make-up gas and carrier gas, respectively (EPA552.2 1995; EPA552 1990; Dell 1993). The calibration curves are shown in Appendix B-4, B-5, and B-6.

Chapter 4

4 Results and Discussions

4.1 Initial Water Parameters of EOM and IOM solutions

The stock algal suspensions were harvested during the exponential growth. The experimental cultures were obtained by diluting the stock algal suspensions using deionized water. As mentioned, the four strains of algae were cultured in four different media in incubators maintained at 25 °C. The final algal densities in the suspensions were higher than the natural water to simulate the natural algal bloom condition. The final experimental densities of the two green algae, *Chlorella Vulgaris* and *Chlamydomonas Reinhardtii* were $(5.1 \pm 0.2) \times 10^6$ cells/mL and $(8.3 \pm 0.3) \times 10^6$ cells/mL, respectively. The experimental suspensions of two diatoms, *Phaedactylum Tricornutum* and *Cyclotella Meneghiniana*, were created with the initial cell counts of $(5.5 \pm 0.3) \times 10^6$ and $(4.0 \pm 0.2) \times 10^6$, respectively. The main water quality parameters, UV_{254} (cm^{-1}), DOC (mg/L), turbidity (NTU) of the algal solutions are shown in Table 4.1 and Table 4.2.

Table 4.1 Initial Water Parameters of EOM and IOM Solution (Green Algae)

Parameters	<i>Chlorella Vulgaris</i>		<i>Chlamydomonas Reinhardtii</i>	
Type	Green Algae			
Experimental suspension cell count (cells/mL)	$(5.1 \pm 0.2) \times 10^6$		$(8.3 \pm 0.3) \times 10^6$	
	EOM	IOM	EOM	IOM
UV_{254} (cm^{-1})	0.017 ± 0.002	0.012 ± 0.001	0.028 ± 0.002	0.027 ± 0.002
DOC (mg/L)	8.62 ± 1.5	11.98 ± 1.5	12.08 ± 1.8	15.36 ± 1.8
Turbidity (NTU)	4.16 ± 0.8	7.32 ± 0.9	7.77 ± 0.9	8.97 ± 1.0

Table 4.2 Initial Water Parameters of EOM and IOM Solution (Diatom)

Parameters	<i>Phaedactylum Tricornutum</i>		<i>Cyclotella Meneghiniana</i>	
Type	Diatom			
Experimental suspension cell count (cells/mL)	$(5.5 \pm 0.3) \times 10^6$		$(4.0 \pm 0.2) \times 10^6$	
	EOM	IOM	EOM	IOM
UV ₂₅₄ (cm ⁻¹)	0.026 ± 0.002	0.032 ± 0.001	0.012 ± 0.001	0.019 ± 0.002
DOC (mg/L)	19.71 ± 1.8	17.29 ± 1.9	14.93 ± 0.8	12.99 ± 1.3
Turbidity (NTU)	11.01 ± 1.7	12.33 ± 1.5	17.49 ± 1.8	16.49 ± 1.9

Turbidity is the ratio of intensities of the incident light intensity and the light scattered by the solutions (Karanfil et al. 2005; Mark 2002; Chln et al. 1994; Cheng et al. 2005). For the suspensions with algae cells, Beer-Lambert law was followed. The turbidity is proportional to the concentration of the cells and was slightly affected by the size and the shape of the algae cells (Karanfil et al. 2005; Mark 2002; Chln et al. 1994; Cheng et al. 2005). In this study, the initial turbidity of EOM solution was much lower than IOM solution for *Chlorella Vulgaris*, while all other three algae had very similar values for both EOM and IOM.

It has been widely reported that DOC contains several types of unsaturated bonds. These unsaturated bonds can absorb light over a wide range of wavelengths (Karanfil et al. 2005). The strong correlations between UV absorbance of humics and fulvic isolates and the aromatic carbon content in DOC have been reported by many researchers (Karanfil et al. 2005; Mark 2002; Chln et al. 1994). As a result, aromatic structures may primarily cause the absorbance of natural water at UV wavelengths near 250 nm (from 240 to 280) (Karanfil et al. 2005; Mark 2002; Chln et al. 1994). The UV absorbance at 254 nm has been chosen to be an important water quality parameter for most researches because of the following reasons: (i) it is an easy and reliable method to determine the absorbance at 254 nm of organic matter, (ii) it has minimal or even non-existent interference due to other inorganic compounds (especially at concentrations of most natural waters), (iii) a low-pressure mercury lamp can produce very strong irradiation (Cheng et al. 2005; Chen et al. 2008; Altmann et al. 2016), (iv) it has been reported by many researchers that there

are very strong correlations between DOC value and absorbance at 254 nm for natural water body (Chen et al. 2008; Altmann et al. 2016; Roccaro et al. 2015). However, saturated aliphatic acids, alcohols and some other organic compounds cannot be measured by UV absorbance at 254 nm as they do not absorb UV light at 254 nm. Therefore, a sample may actually contain larger organic matters even if it has a very small value of UV absorbance at 254 nm.

The comparisons UV absorbance at 254 nm and DOC for the four different algae cultures are shown in Figure 4.1 and Figure 4.2. Overall, diatoms have larger values than green algae both for UV absorbance and DOC. For green algae (*Chlorella Vulgaris* and *Chlamydomonas Reinhardtii*), the UV_{254} values of EOM solutions were higher than IOM solutions, which indicate that EOM in green algae contains more aromatic organic matters especially for *Chlorella Vulgaris*. Diatoms had the opposite results with the UV_{254} values of IOM solutions were higher than the EOM solutions. The initial DOC concentrations of IOM solutions for the green algae were higher than EOM solutions, with the value of 11.98 ± 1.5 mg/L for IOM and 8.62 ± 1.5 mg/L for EOM for *Chlorella Vulgaris* as well as 15.36 ± 1.8 mg/L for IOM and 12.08 ± 1.8 mg/L for EOM for *Chlamydomonas Reinhardtii*. It needs to be mentioned that the cell counts of *Chlamydomonas Reinhardtii* was almost twice of *Chlorella Vulgaris*. For diatoms, the initial DOC concentrations of EOM solutions were slightly higher than IOM solutions with the similar cell counts of both *Phaedactylum Tricornutum* and *Cyclotella Meneghiniana*.

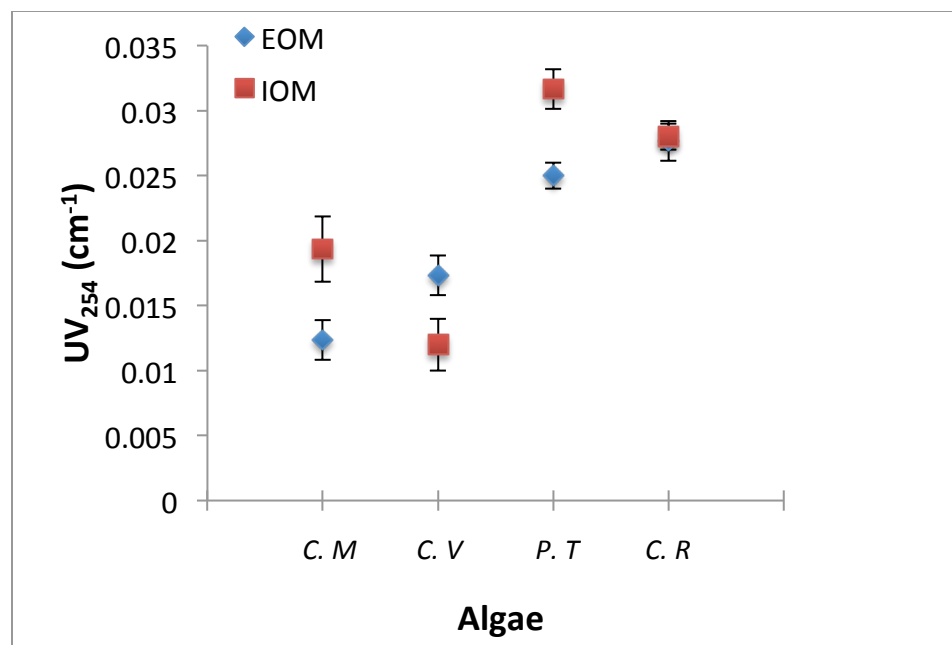


Figure 4.1 The comparison of UV absorbance (cm^{-1}) at 254 nm for the four different algae cultures

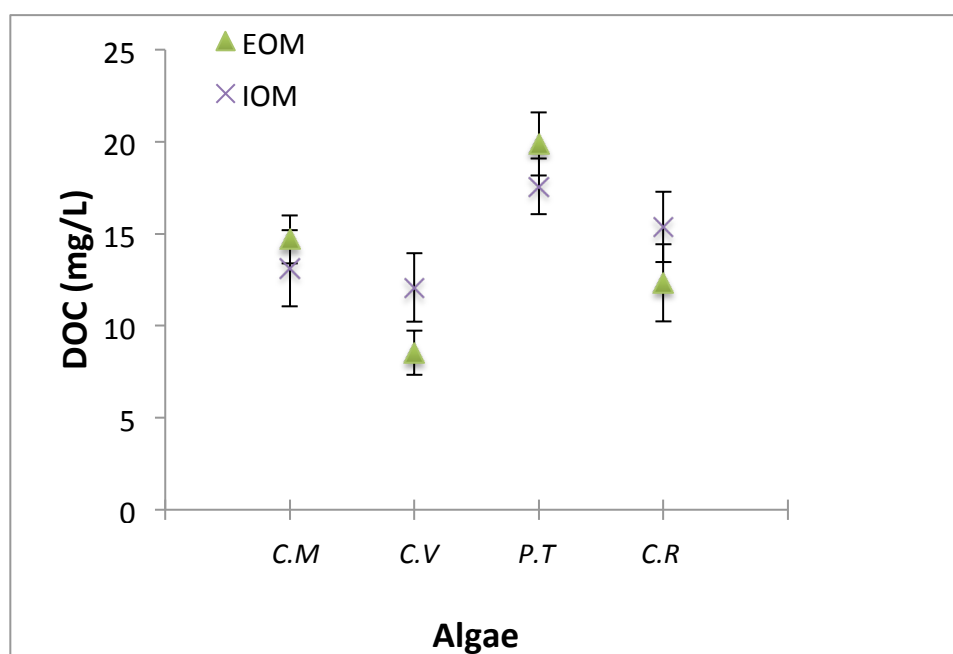


Figure 4.2 The comparison of DOC (mg/L) for the four different algae cultures

4.2 Coagulation Effects

4.2.1 Coagulation Effects on Turbidity

The turbidity variations (NTU) of EOM and IOM solutions for four algae cultures after coagulation with the coagulants range from 0 – 60 mg/L and settling time of 2 h at room temperature (20 ± 2 °C) is shown in Figure 4.3. The initial turbidities of diatoms were higher than green algae for both EOM and IOM solutions. It was easier to coagulate diatoms than green algae. The turbidity removals of the EOM solutions for *Phaedactylum Tricornutum* and *Cyclotella Meneghiniana* (diatoms) were 59.2% and 75.0% while for *Chlorella Vulgaris* and *Chlamydomonas Reinhardtii* (green algae) were 24.0% and 30.0%, respectively. Over all, the turbidity of both EOM and IOM solutions declined after coagulation, while the effect on EOM solutions was more significant than IOM solutions. However, for *Chlamydomonas Reinhardtii* (green algae) the turbidity removal of IOM (41.0% in NTU) solution was somehow higher than EOM solution (30.0% in NTU). The most significant decline occurred to the EOM solution of *Phaedactylum Tricornutum* (diatom) with turbidity removal of 75.0% (from 11.01 ± 1.2 NTU to 2.75 ± 0.3 NTU), while *Chlorella Vulgaris* (green algae) has the minimum decline in IOM solution with turbidity removal of 19.5% (from 4.16 ± 0.8 NTU to 3.16 ± 1.1 NTU). Most turbidity values remained at the same level after the addition of coagulant of 40 mg/L, especially for IOM solutions.

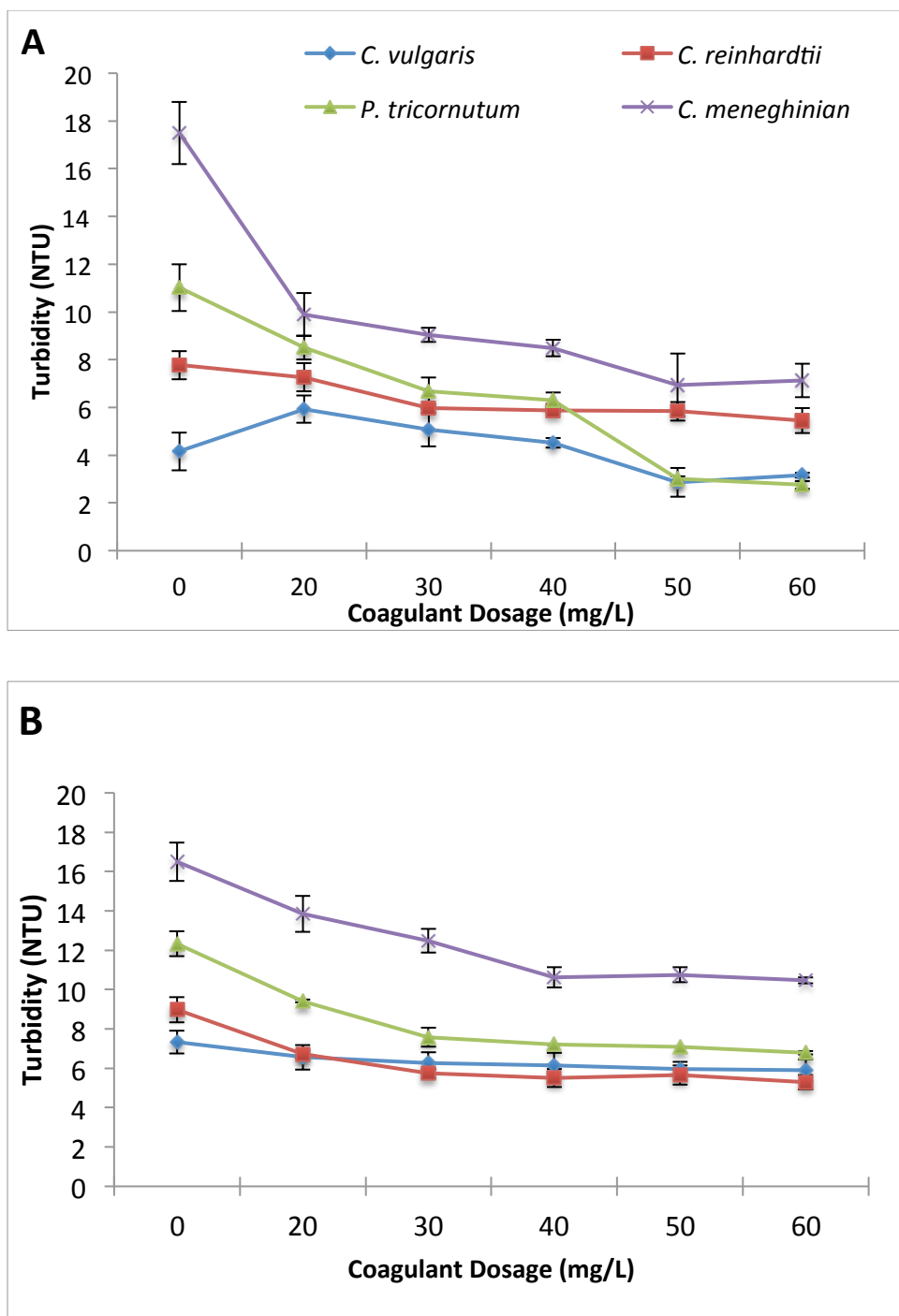


Figure 4.3 Turbidity (NTU) variations of EOM (A) and IOM (B) for four algae cultures after coagulation with the coagulants range from 0 – 60 mg/L and settling time of 2 h at room temperature (20 ± 2 °C). (Standard deviations of triplicate experiments are represented by the error bars.)

4.2.2 Coagulation Effects on UV_{254} and DOC

The AOM from different algal species may contain different levels of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) (Lui et al. 2012). In this study, the amount of IOM and EOM were measured as DOC in water samples. DOC is a very important precursor of carbonaceous DBPs (C-DBPs) formation potential.

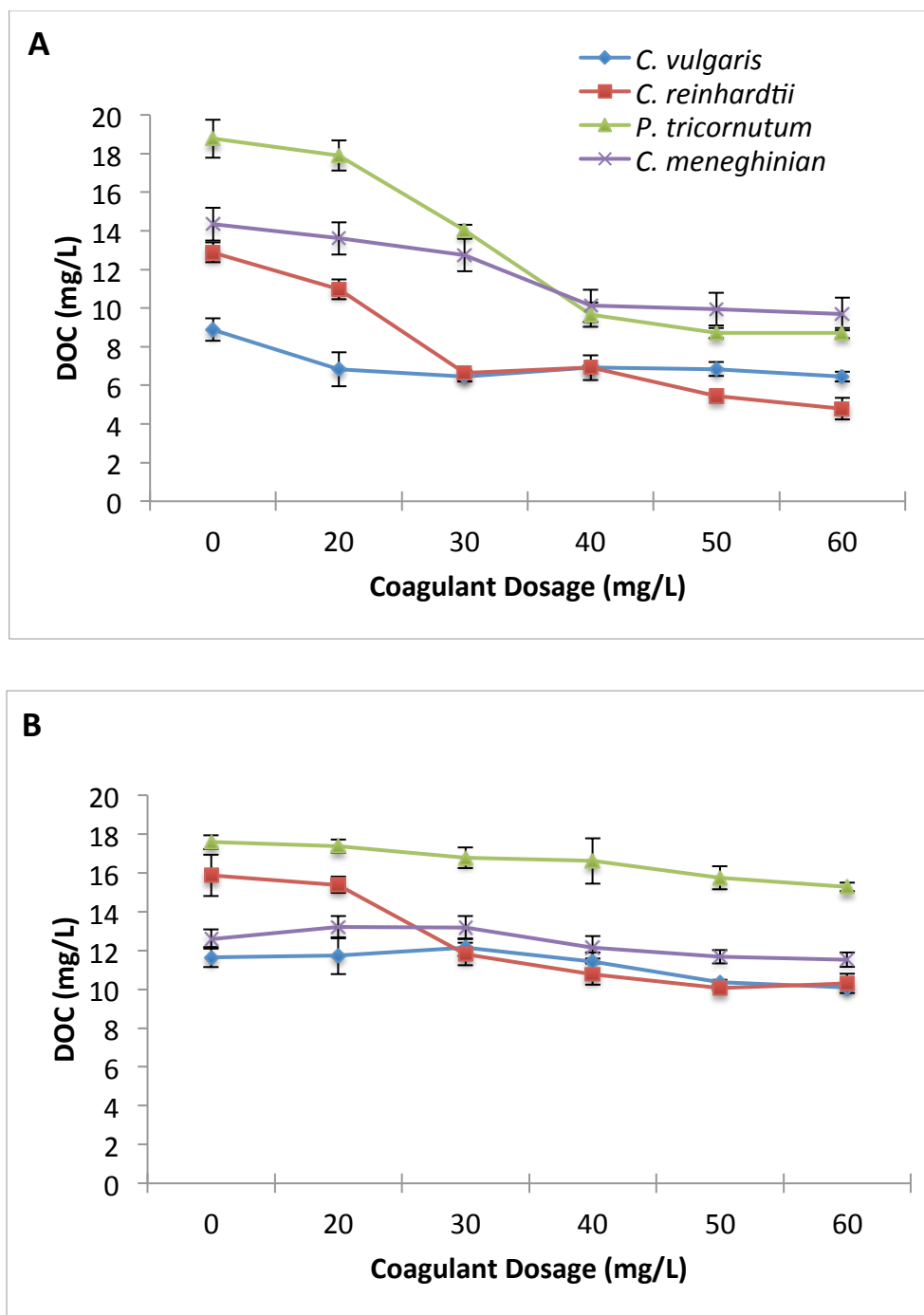


Figure 4.4 DOC (mg/L) variations of EOM (A) and IOM (B) for four algae cultures after coagulation with the coagulants range from 0 – 60 mg/L and settling time of 2 h at room temperature (20 ± 2 °C). (Standard deviations of triplicate experiments are represented by the error bars.)

The DOC (mg/L) variations of EOM and IOM solutions for four algae cultures after coagulation with dosage ranging from 0 – 60 mg/L and settling time of 2 h at room temperature (20 ± 2 °C) are shown in Figure 4.4. Both EOM and IOM are more hydrophilic than DOC in natural waters. Compared with EOM, IOM is richer in proteins or peptide, more hydrophilic and tends to have lower SUVA value. MW fractionation shows that both EOM and IOM of green algae and diatom contain large portions of low-MW (below 1 k Da) compounds and high-MW (over 100 k Da) polysaccharides (Å et al. 2006). After coagulation, DOC values of both the EOM and IOM solutions for all four algae declined initially, but remained constant at a larger dose. The decrease was more for EOM solutions because of higher hydrophobicity. For EOM solutions, the DOC removal of *Chlamydomonas Reinhardtii* (green algae) and *Phaedactylum Tricornutum* declined very fast with the DOC values dropping from 12.88 to 4.79 mg/L and 18.77 to 8.70 mg/L, respectively. For IOM solutions, the significant drop only occurred to *Chlamydomonas Reinhardtii* (green algae) from 15.86 to 10.30 mg/L, while all other three algae showed marginal decline in DOC with coagulation. The DOC values start to maintain at a same level from a coagulant dose of 20 mg/L, which indicates that large dose of coagulant, is not very necessary for AOM coagulation. However, larger dosage of coagulant is widely used in drinking water plants because of the much higher turbidity and particles in natural water body. Figure 4.5 shows the DOC removal (%) (with a coagulant dose of 20 mg/L) and average DOC removal (%) (with a coagulant dose of 30 mg/L, 40 mg/L, 50 mg/L, and 60 mg/L) of EOM and IOM for four different algae after coagulation and settling time of 2 h at room temperature (20 ± 2 °C). Among all the four algae, coagulation had the best effect on *Chlamydomonas Reinhardtii* (green algae) (with 62.8% DOC removal for EOM and 35.1% DOC removal for IOM) and worst on *Chlorella Vulgaris* (green algae) (with 27.4% for EOM and 13.4% for IOM).

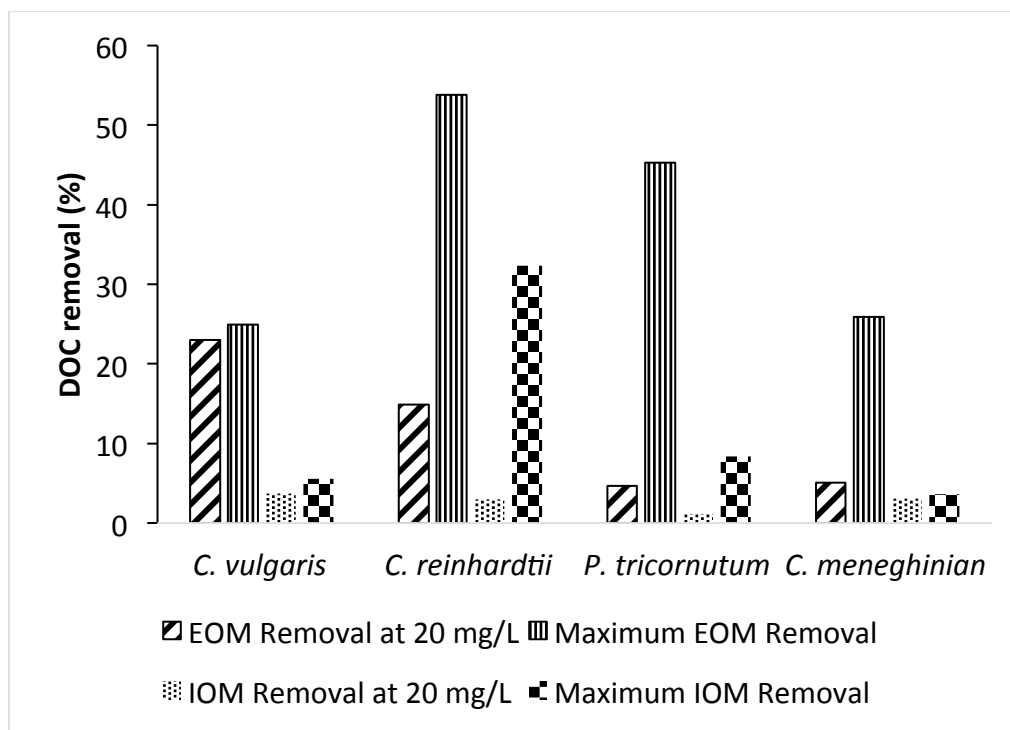


Figure 4.5 DOC removal (%) (with a coagulant dose of 20 mg/L) and average DOC removal (%) (with a coagulant dose of 30 mg/L, 40 mg/L, 50 mg/L, and 60 mg/L) of EOM and IOM for four algae cultures after coagulation with settling time of 2 h at room temperature (20 ± 2 °C). (Standard deviations of triplicate experiments are represented by the error bars.)

As mentioned before, it has been reported by many researchers that there are very strong correlations between DOC value and absorbance at 254 nm for natural water body, because the aromatic compounds in DOC primarily cause the UV absorbance of natural water at 254 nm. The UV_{254} variations (mg/L) of EOM and IOM solutions for four algae cultures after coagulation with the coagulants range from 0 – 60 mg/L and settling time of 2 h at room temperature (20 ± 2 °C) is shown in Figure 4.6. Only very small changes in the UV_{254} values were obtained for both EOM and IOM. For *Chlorella Vulgaris* and *Chlamydomonas Reinhardtii* (green algae), the UV_{254} values slightly dropped for both EOM and IOM while for *Cyclotella Meneghiniana* (diatom) the values slightly increased possible due to experimental error.

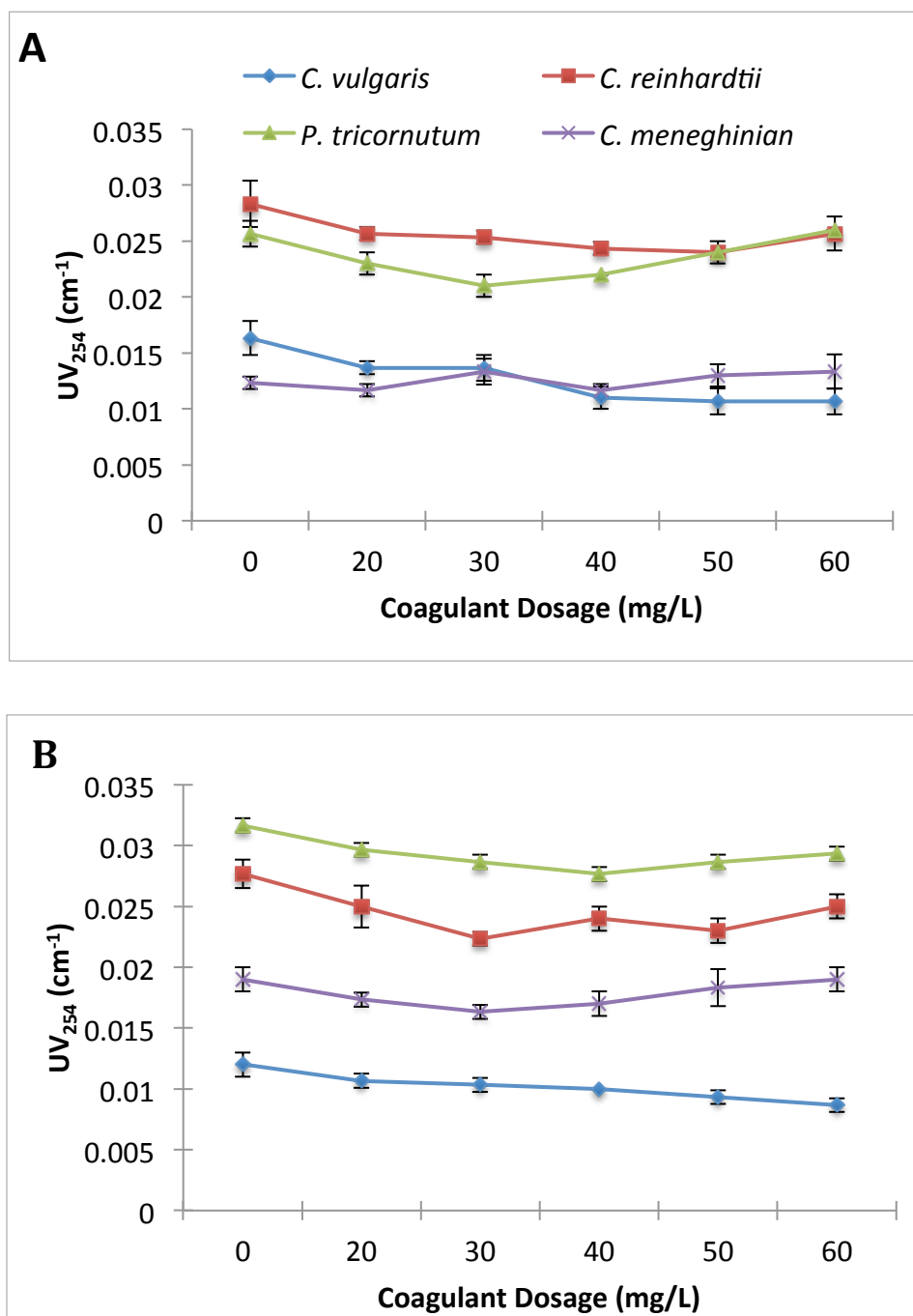
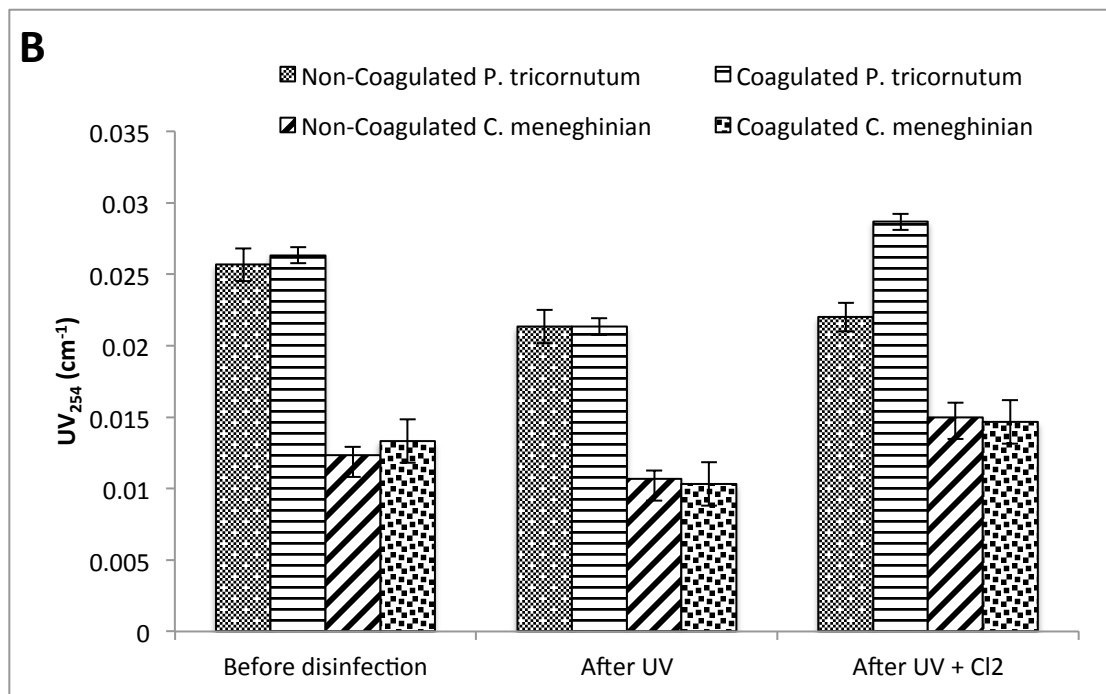
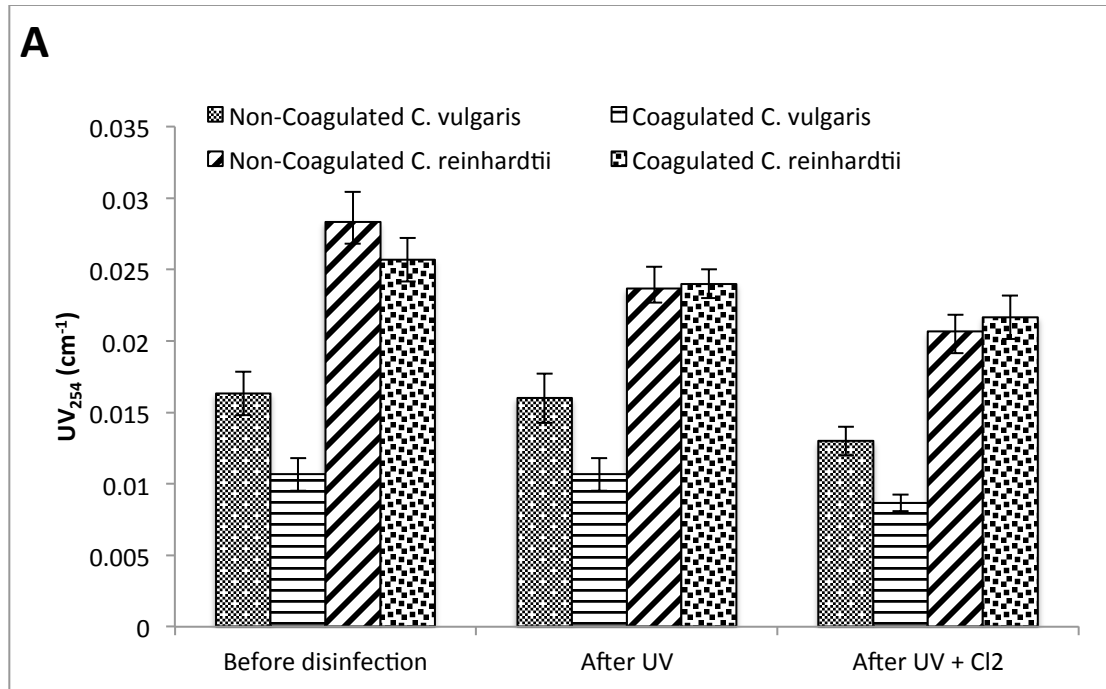


Figure 4.6 UV_{254} (cm⁻¹) variations of EOM (A) and IOM (B) for four algae cultures after coagulation with the coagulants range from 0 – 60 mg/L and settling time of 2 h at room temperature (20 ± 2 °C). (Standard deviations of triplicate experiments are represented by error bars.)

4.3 Effects of Disinfection on EOM and IOM

4.3.1 Effects on UV_{254} and DOC

Figure 4.7 shows the UV_{254} (cm^{-1}) variations of coagulated and not coagulated EOM and IOM for the four types of algae after UV and chlorine disinfection processes.



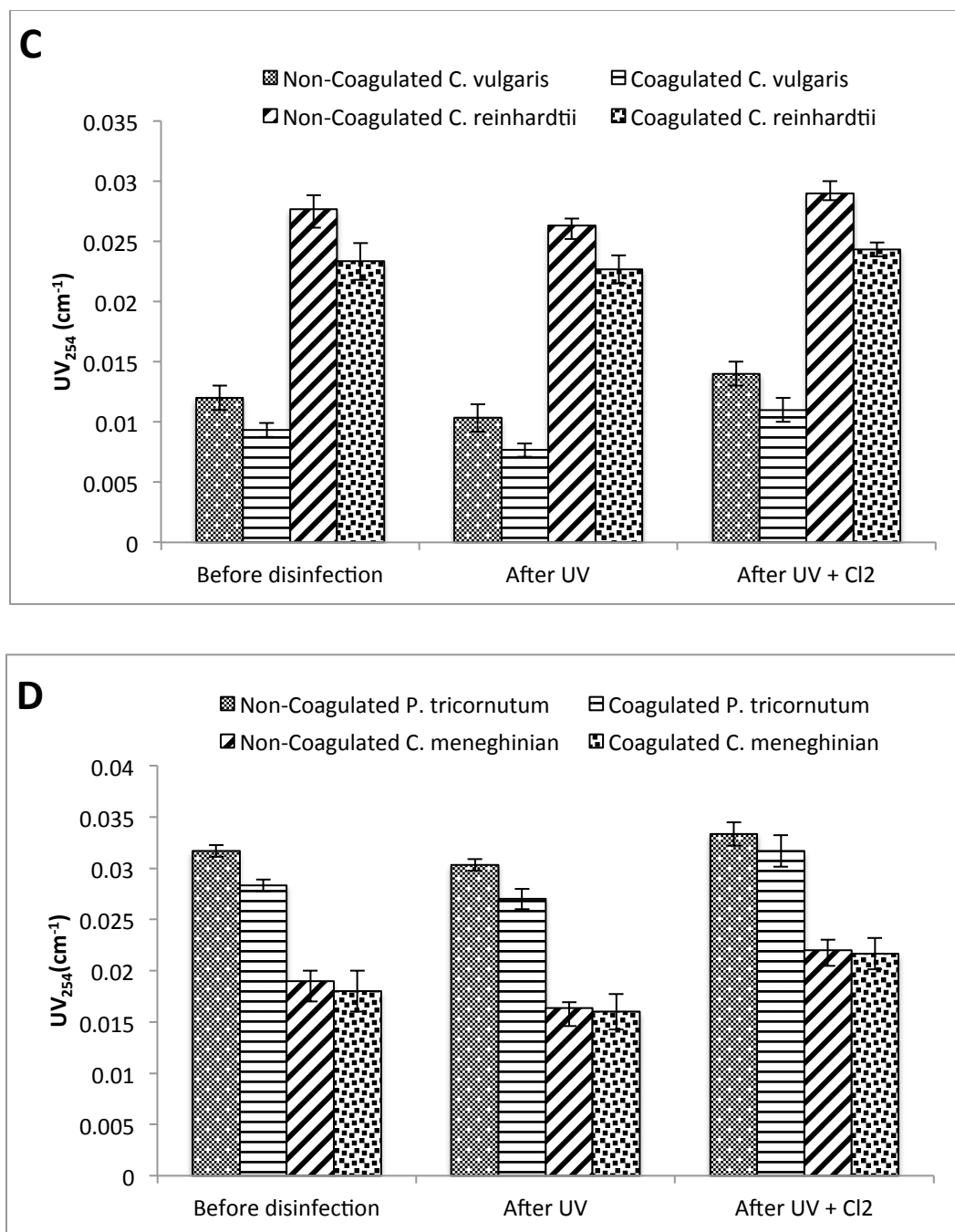
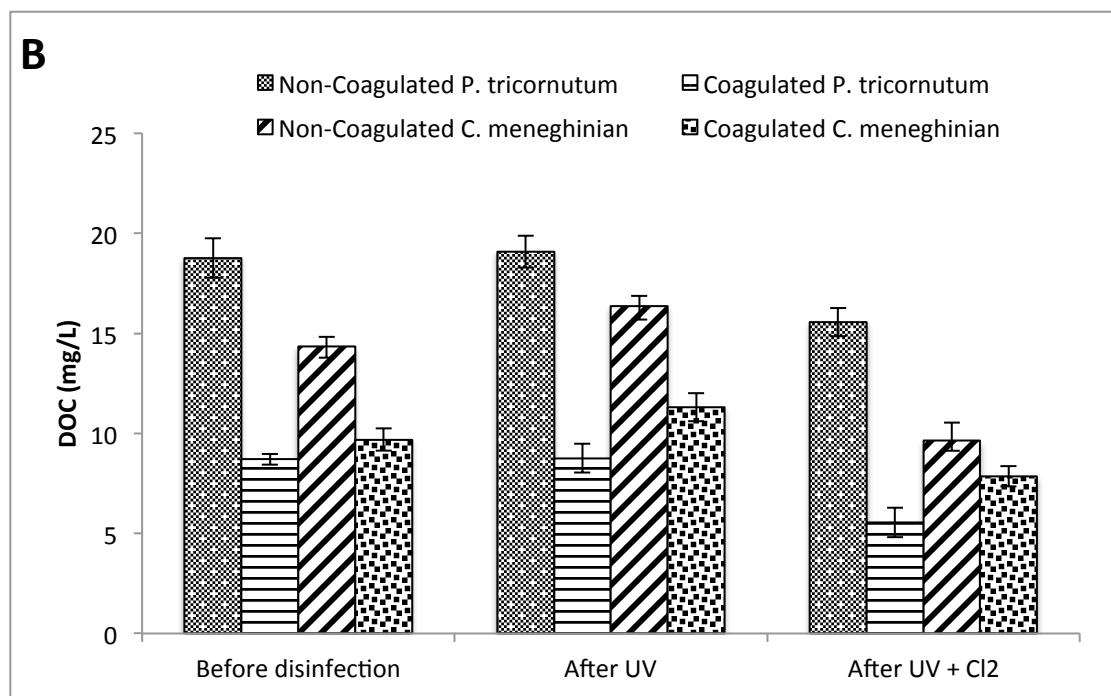
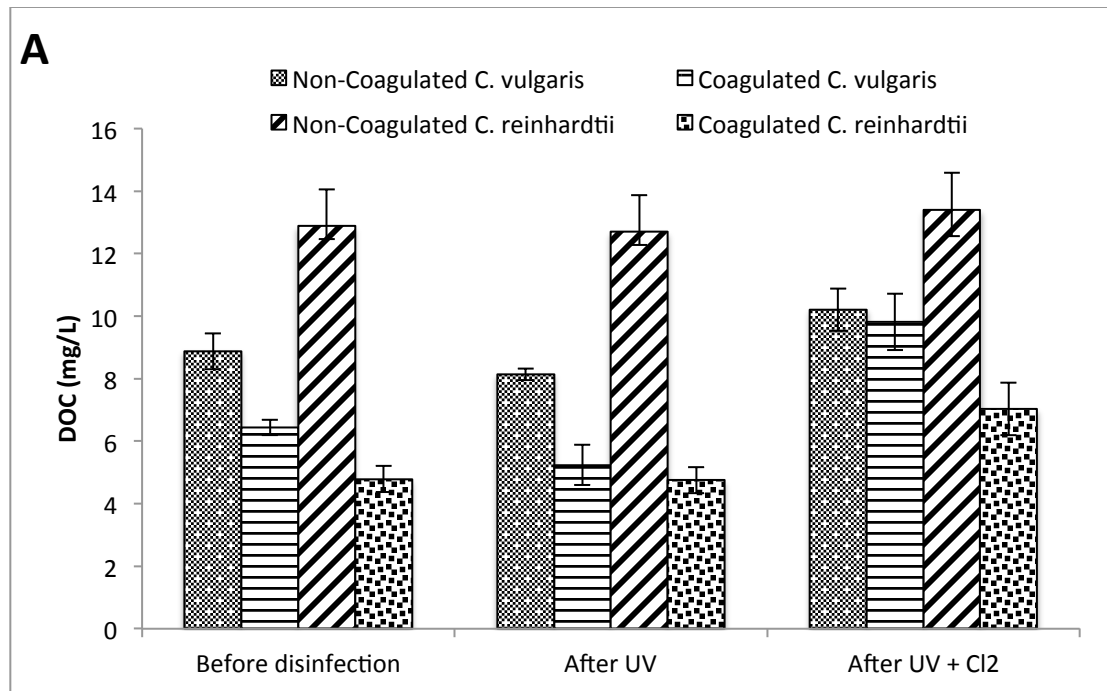


Figure 4.7 UV₂₅₄ (cm⁻¹) variations of EOM (A) and (B), as well as IOM (C) and (D) for four types of algae before and after disinfection with and without coagulation. coagulant dosage = 60 mg/L, pH = 8.0 ± 0.2, UV dose: 40 mJ/cm², chlorine dose: Cl₂: DOC = 1.8, temperature: 20 ± 2 °C, incubation time: 24 h. (Standard deviations of triplicate experiments are represented by the error bars.)

Figure 4.8 show the DOC variations (mg/L) of EOM and IOM for the four algae cultures before and after disinfection with and without coagulation, respectively. Overall, the control groups had higher UV_{254} and DOC values than the coagulated groups. The UV_{254} value slightly decreased after UV disinfection and increased after chlorination. The reason of the decline after UV radiation is due to the breakdown or photodegradation of some organic compounds like aromatics, which may absorb UV radiation and followed by photolysis to smaller compounds. On the other hand, UV_{254} absorbance increased slightly after chlorination due to the possible formation of chlorinated compounds with the intermediates formed during photolysis. For the IOM solutions of *Cyclotella Meneghiniana* (diatom), both the decline and increase were quite obvious from 0.028 to 0.031 cm^{-1} . Compared with non-chlorinated compounds, the corresponding chlorinated compounds could absorb more UV radiation, which results in a higher UV_{254} value after chlorination. It is interesting to see that the UV_{254} decreased after the disinfection experiments for the EOM of green algae, indicating lower formation of chlorinated compounds from the EOM. Figures 4.8 A-D present the variations in DOC concentration after coagulation and disinfection experiments. It can be seen that coagulation removed more EOM compared to IOM decreasing the value of DOC for all four algae. DOC remained almost constant after UV radiation, the reason of which is because the total amount of organic carbon should not change even if some compounds break into smaller organic matters due to photolysis. DOC values increased slightly after chlorination, probably due to the interference of Cl^- for the determination of DOC.



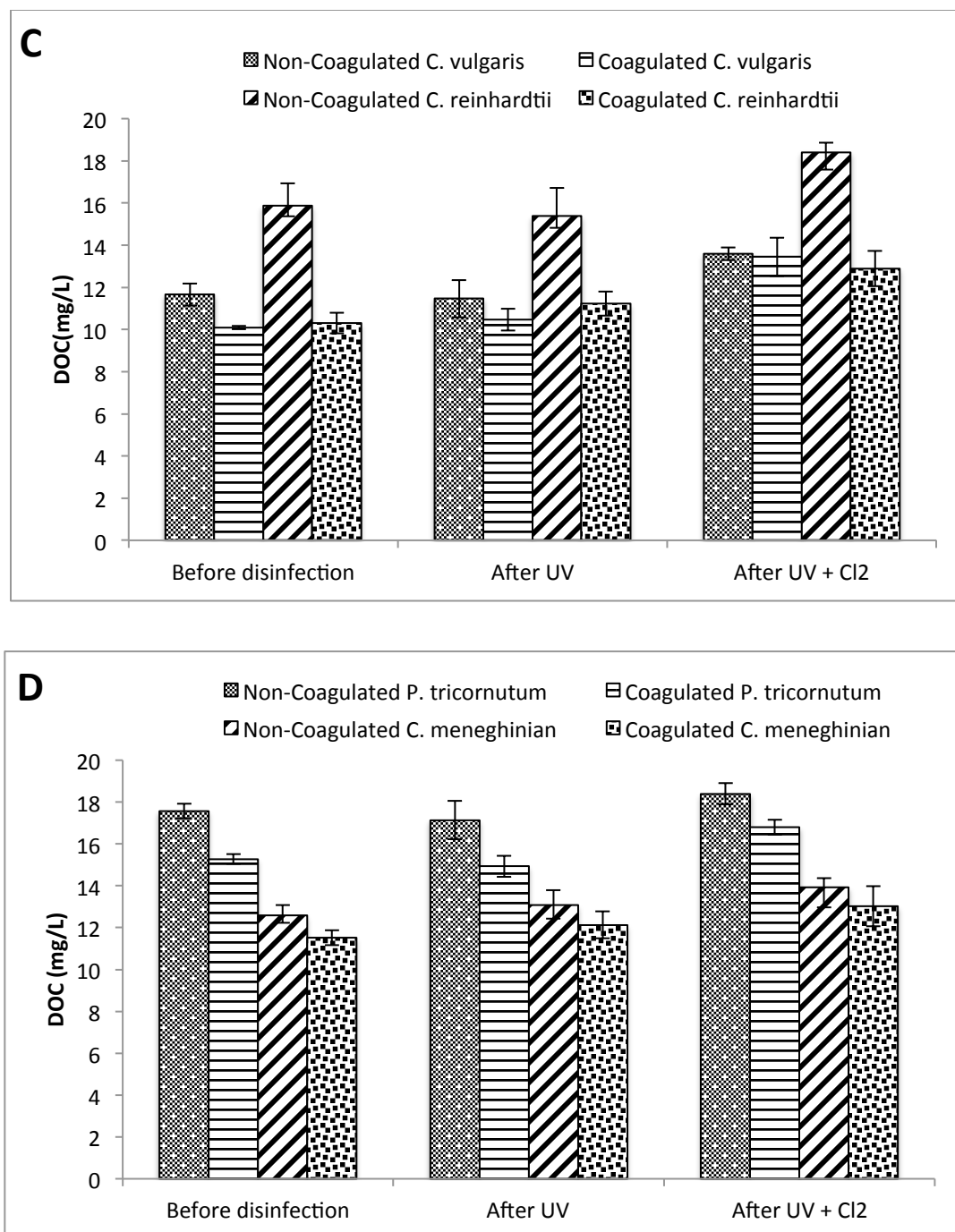


Figure 4.8 DOC (mg/L) variations of EOM (A) and (B), as well as IOM (C) and (D) for four types of algae before and after disinfection with and without coagulation. coagulant dosage = 60 mg/L, pH = 8.0 ± 0.2, UV dose: 40 mJ/cm², chlorine dose: Cl₂: DOC = 1.8, temperature: 20 ± 2 °C, incubation time: 24 h. (Standard deviations of triplicate experiments are represented by the error bars.)

4.3.2 DBPs Formation Potential

4.3.2.1 Possible Pathways of DBPs Formation

The EOM and IOM from different algal species may contain different levels of dissolved organic carbon (DOC) (Lui et al. 2012). AOM is rich in organic nitrogen and organic carbon compared with NOM, which results in that there are more DBPs forming during disinfection treatment (Lui et al. 2012). The maximum contaminant level (MCL) of THMs and HAAs are 80 and 60 ppb, respectively (National Primary Drinking Water Regulations). The results of DBPs formations for *C. Vulgaris* (green algae) and *P. Tricomutum* (diatom) are shown in this study. For THMs, TCM (Chloroform), TBM (Bromoform) and BDCM (Bromodichloromethane) were determined. For HAAs, MBAA (Monobromoacetic Acid), DCAA (Dichloroacetic Acid) and BCAA (Bromochloroacetic Acid) were determined. The cell density of *C. Vulgaris* (green algae) and *P. Tricomutum* (diatom) were $(5.1 \pm 0.2) \times 10^6$ and $(5.5 \pm 0.3) \times 10^6$, respectively.

For organic matters, the activated aromatic groups, namely polyhydroxyphenolic acid (PHA) moieties are considered as the predominantly reaction sites. Some other organic matters such as esters and ketones, are also considered as sources of to the formation of DBPs with less contribution than PHAs (Huang et al. 2009; Korshin et al. 2004; Lyon et al. 2014; Lui et al. 2012; Stuart W. Krasner et al. 2006; Cumming & Jolley 1993).

However, the reactivity of organic compounds is not completely understood. Even for some pure aromatic compounds, such as phenol, resorcinol and hydroxybenzoic acids, the incorporation of chlorine includes multi-step and some other branching reactions. The incorporations of organic compounds are much more complex and varied (Lui et al. 2012; Stuart W. Krasner et al. 2006; Cumming & Jolley 1993). Also, the molar concentration, distribution, structure and chemical properties of most chemical sites are not available for the reactions during chlorination process.

There are two main sites that can be attacked by chlorine in the sequences of reactions for the formation of almost all DBPs, which are activated aromatic units (PHAs moiety) and ketone groups (Larson et al. 1994; Tretyakova et al. 1994). The formation of THMs from a ketone site via the classic haloform reaction is described in Figure 4.9.

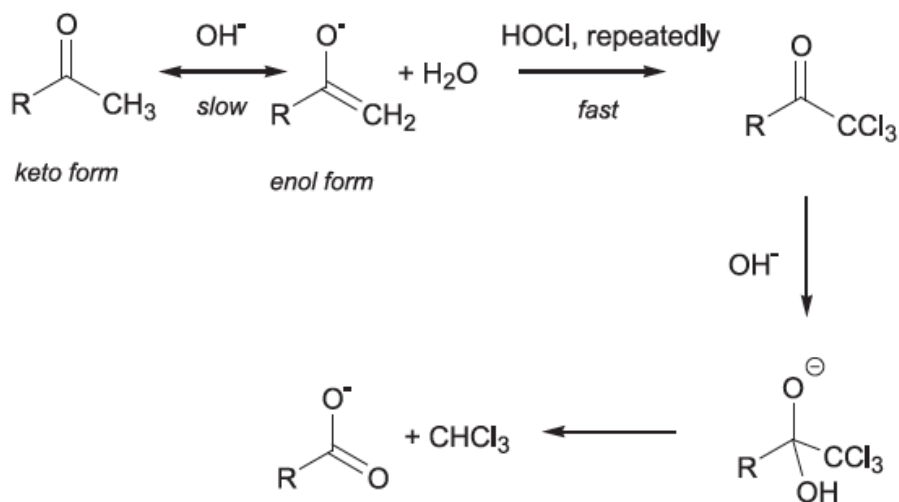


Figure 4.9 Pathway of chloroform formation via haloform reaction (ketoenol functional groups)

The reactions of aromatic sites are much more complex. The reaction starts from the multiple attacks by chlorine on the aromatic rings, then the intermediates are generated, which are cyclic but non-aromatic chlorinated compounds. The process is shown in Figure 4.10. A series of transformations following the ring opening occurs, which lead to more halogens being incorporated into the products, and finally smaller products are formed. Eventually smaller products such as THMs and HAAs (one- and two-carbon molecules), as well as some larger unidentified DBPs are formed (Huang et al. 2009; Korshin et al. 2004; Lyon et al. 2014; Lui et al. 2012; Stuart W. Krasner et al. 2006; Cumming & Jolley 1993).

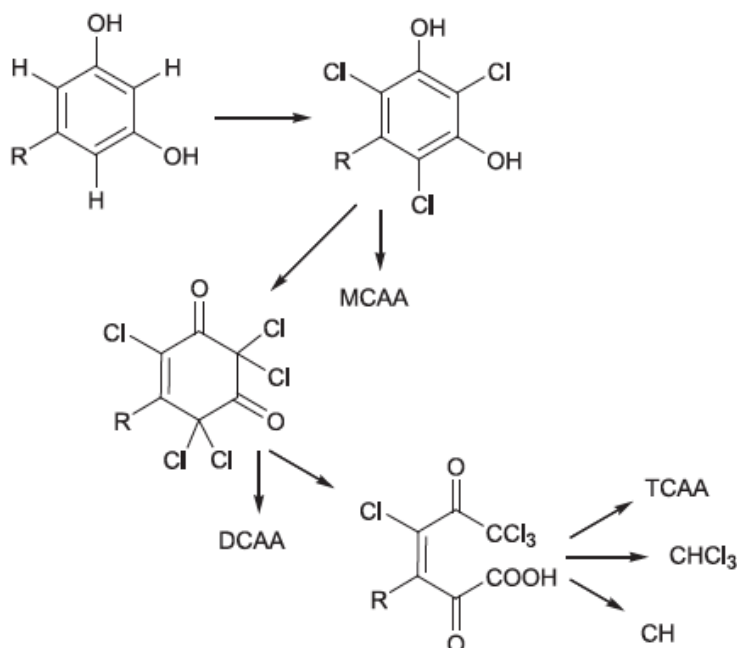


Figure 4.10 Possible pathways of chloroform and HAAs formation via haloform reaction (activated aromatic ring)

4.3.2.2 DBPs Formation Potential Analysis

For THMs formation, there was no significant difference between *C. Vulgaris* (green algae) and *P. Tricomutum* (diatom), which is shown in Figure 4.11. For *C. Vulgaris* (green algae), the THMs formation of the non-coagulated groups was higher than the coagulated groups, which indicated the efficiency of coagulation. Similar results could also be observed for IOM solutions of *P. Tricomutum* (diatom). The most significant difference happened to TBM formation. The formation of TBM for *P. Tricomutum* (diatom) was significantly higher than *C. Vulgaris* (green algae), which resulted in a higher amount of total THMs.

The HAAs formation is shown in Figure 4.12. Overall, the non-coagulated groups had more HAAs formation potential. For EOM solutions, there was no significant difference between *C. Vulgaris* (green algae) and *P. Tricomutum* (diatom). The formation of MBAA was much lower than DCAA and TCAA. It was interesting that for IOM solutions, the formation of MBAA was much higher compared to EOM solutions, especially for *C.*

Vulgaris (green algae). The increase of MBAA formation mainly contributed to higher total HAAs for IOM solutions even with a lower TCAA formation. IOM contains up to 90% of polysaccharides, which are attributed to the formation of low HAA species like MBAA.

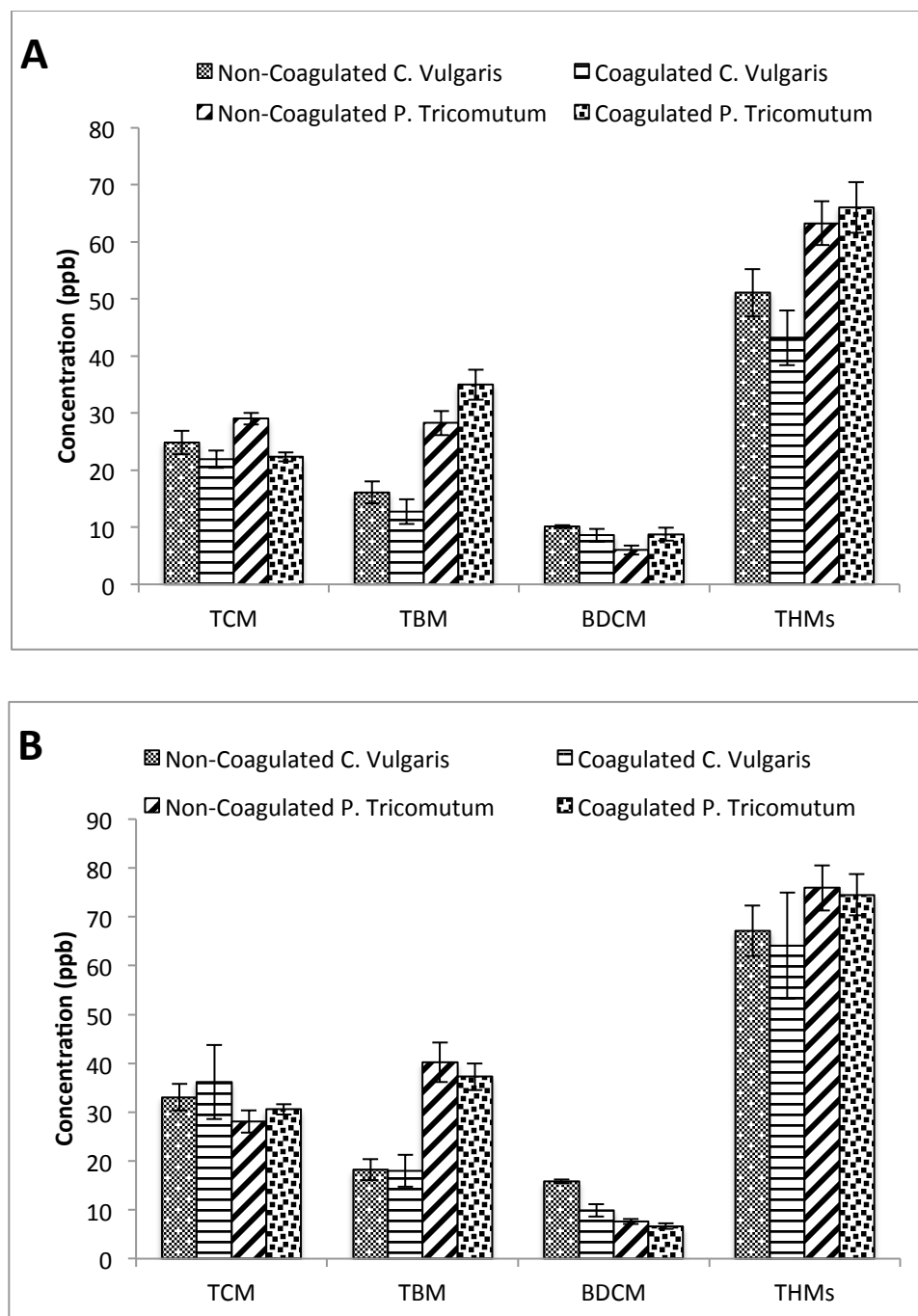


Figure 4.11 THMs formation (ppb) of EOM (A) and IOM (B) for *C. Vulgaris* and *P. Tricomutum* after disinfection with and without coagulation. coagulant dosage = 60 mg/L, pH = 8.0 ± 0.2 , UV dose: 40 mJ/cm^2 , chlorine dose: Cl_2 : DOC = 1.8, temperature: $20 \pm 2 \text{ }^\circ\text{C}$, incubation time: 24 h. (Standard deviations of triplicate experiments are represented by the error bars.)

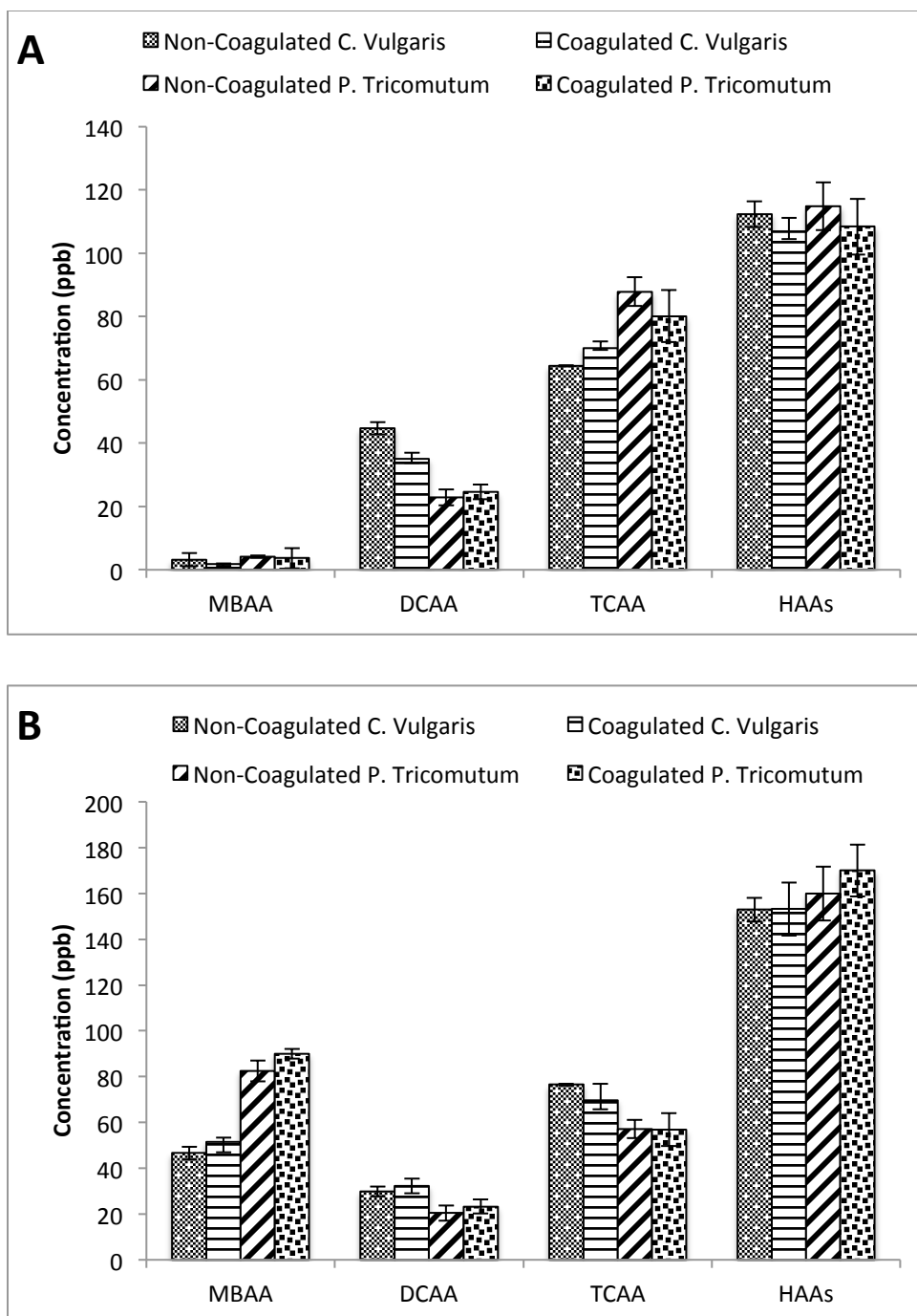


Figure 4.12 HAAs formation (ppb) of EOM (A) and IOM (B) for *C. Vulgaris* and *P. Tricomutum* after disinfection with and without coagulation. coagulant dosage = 60 mg/L, pH = 8.0 ± 0.2, UV dose: 40 mJ/cm², chlorine dose: Cl₂: DOC = 1.8, temperature: 20 ± 2 °C, incubation time: 24 h. (Standard deviations of triplicate experiments are represented by the error bars.)

Based on the experimental results in this study, IOM of diatoms produces more bromine by-products. Since there was no background bromine in water AOM is the source of bromine in the experimental solutions. It has been widely known that diatoms are producers of reactive bromine and iodine (primarily hypobromous acid [HOBr] and hypoiodous acid [HOI], respectively), and polybromomethanes (Nguvava et al. 2016; Leblanc et al. 2014; Kurihara et al. 2012). Most of the HOBr released by diatoms may react with dissolved organic matters to form nonvolatile bromine organics. Some of the produced HOBr and HOI may also form volatile Br₂ and I₂ (Nguvava et al. 2016; Leblanc et al. 2014; Kurihara et al. 2012).

The formations of HAAs were much higher than THMs. IOM produced more DBPs than EOM. As mentioned before, during chlorination, the predominant reaction sites are the activated aromatic groups (mainly polyhydroxyphenolic acid (PHA) moieties), although some other organic matters such as esters and ketones also contribute to the formation of DBPs. AOM especially, IOM contains more proteins, a higher fraction of aromatic organic matters, total organic nitrogen and free amino acids compared to natural organic matters. Higher DBP formation by IOM was found by other researchers (Fang, Yang, et al. 2010) (Yang et al. 2011; Li et al. 2012).

According to the USEPA regulation mentioned in Chapter 2, the limit of THMs is 80 ppb and HAAs is 60 ppb. In our work, we have used higher initial algal concentration to simulate the algal bloom condition, and also to have better analytical accuracy, which is difficult at the trace concentration of organics in ppb level.

Chapter 5

5 Conclusions and Future Directions

5.1 Conclusions

The evaluation of coagulation and disinfections of EOM and IOM solutions for four different algae cultures has been presented in this thesis. The four algae used were *Chlorella Vulgaris*, *Chlamydomonas Reinhardtii*, *Phaedactylum Tricornutum* and *Cyclotella Meneghiniana*. The disinfection treatments include UV radiation and chlorination.

The coagulant dosages were from 0 to 60 mg/L with settling time of 2 h. It was easier to coagulate diatoms cells than green algae cells. The turbidity and DOC of both EOM and IOM solutions declined after coagulation especially for turbidity, while the effect of coagulation on EOM was more significant than IOM. A coagulant dose more than 40 mg/L did not bring any additional benefit in terms of turbidity and DOC removal.

The UV radiation dose was fixed at 40 mJ/cm², as this is the typical dosage in water treatment industry. The chlorination dose was fixed at Cl₂: DOC = 1.8, with the incubation time of 24 h. Overall, experimental results indicated that higher AOM resulted in higher DBP formation potential, especially the formation of HAAs. EOM solutions showed the lower concentration of DBP compared with IOM solutions. Coagulation slightly reduced the formation of DBPs, as DOC removal was not very significant due to coagulation.

5.2 Future Directions

Some future directions are presented.

a) Different conditions can be used for coagulation. Some other coagulants and longer settling time are recommended. Coagulation modeling may be developed based on the various conditions for better removal of DOC and lower DBP formations.

b) For disinfection process, chlorination may be used as a pretreatment to remove DOC

before UV radiation. A better understanding of the correlation between DOC and UV absorbance at 254 nm should be obtained.

c) The analysis of organic matters of EOM and IOM is necessary to get a better understanding of the DBP formation. Determination of N-DBPs is highly recommended.

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Appendices

Appendix A:



Figure A-1 Growth of four different algae at Western University

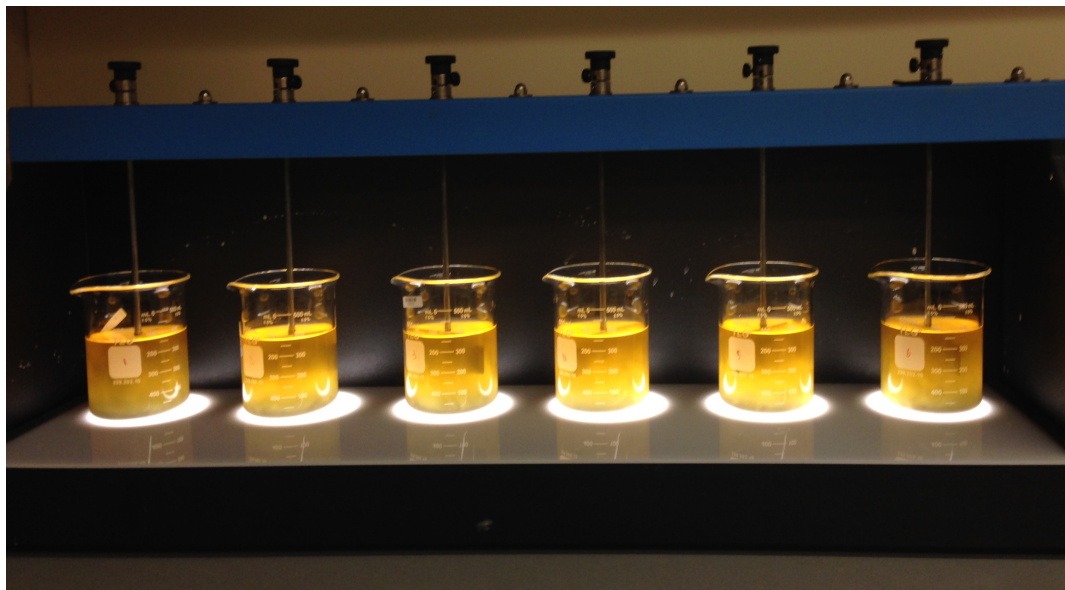


Figure A-2 Coagulation jar test setup



Figure A-3 UV bench-scale apparatus

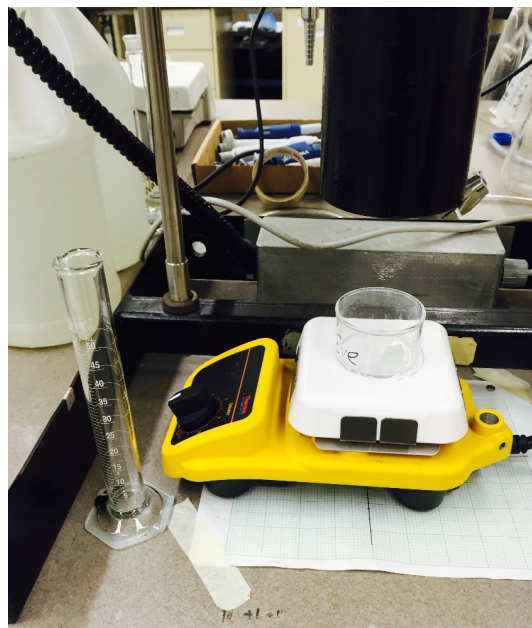


Figure A-4 Water sample exposed under collimated beam

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1																				
2																				
3																				
4																				
5																				
6																				
7																				
8																				
9																				
10																				
11		x	y	Meter Reading μW/cm²	Ratio	x	y	Meter Reading μW/cm²	Ratio											
12																				
13				y			x													
14	0	-6.0		0.000		-6.0	0	0.000												
15	0	-5.5		0.000		-5.5	0	0.000												
16	0	-5.0		0.000		-5.0	0	0.000												
17	0	-4.5		0.000		-4.5	0	0.000												
18	0	-4.0		0.000		-4.0	0	0.000												
19	0	-3.5		0.000		-3.5	0	0.000												
20	0	-3.0		0.000		-3.0	0	0.000												
21	0	-2.5	147.70	0.950		-2.5	0	148.30	0.954											
22	0	-2.0	150.20	0.966		-2.0	0	152.90	0.983											
23	0	-1.5	152.10	0.978		-1.5	0	155.30	0.999											
24	0	-1.0	153.90	0.990		-1.0	0	158.00	1.003											
25	0	-0.5	155.20	0.998		-0.5	0	155.80	1.002											
26	0	0.0	155.50	1.000		0.0	0	155.50	1.000											
27	0	0.5	152.00	0.977		0.5	0	154.20	0.992											
28	0	1.0	149.80	0.963		1.0	0	152.40	0.980											
29	0	1.5	147.10	0.946		1.5	0	148.20	0.953											
30	0	2.0	144.40	0.929		2.0	0	143.20	0.921											
31	0	2.5	141.20	0.908		2.5	0	132.90	0.855											
32	0	3.0	136.60	0.878		3.0	0	111.90	0.720											
33	0	3.5		0.000		3.5	0		0.000											
34	0	4.0		0.000		4.0	0		0.000											
35	0	4.5		0.000		4.5	0		0.000											
36	0	5.0		0.000		5.0	0		0.000											
37	0	5.5		0.000		5.5	0		0.000											
38	0	6.0		0.000		6.0	0		0.000											
39																				
40																				
41						Petri Dish Inner Diameter =	5.6	cm		Average Petri factor =	0.9465			PASS		Petri factor should be greater than 0.9				
42																				

Figure A-5 Collimated beam UV calibration spreadsheets-step (a)

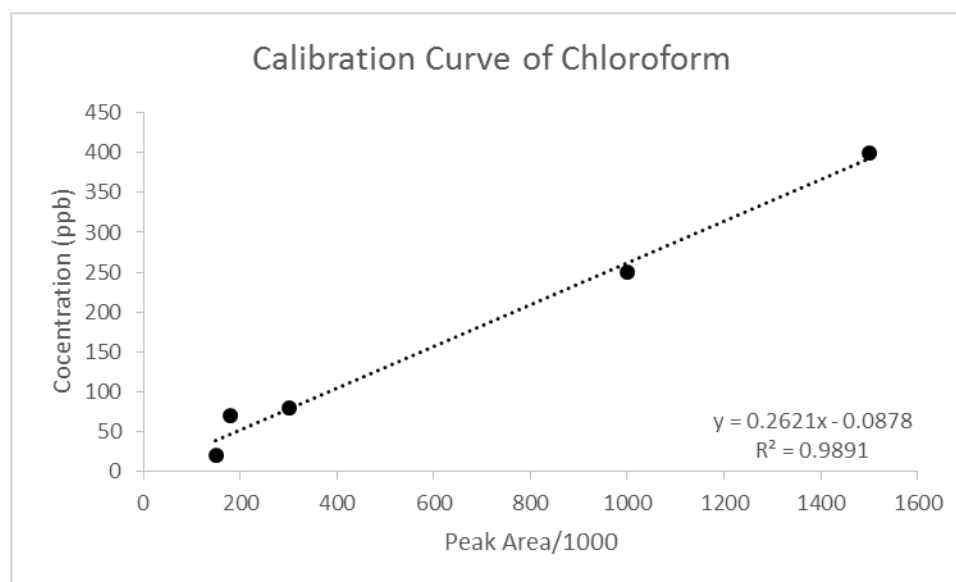
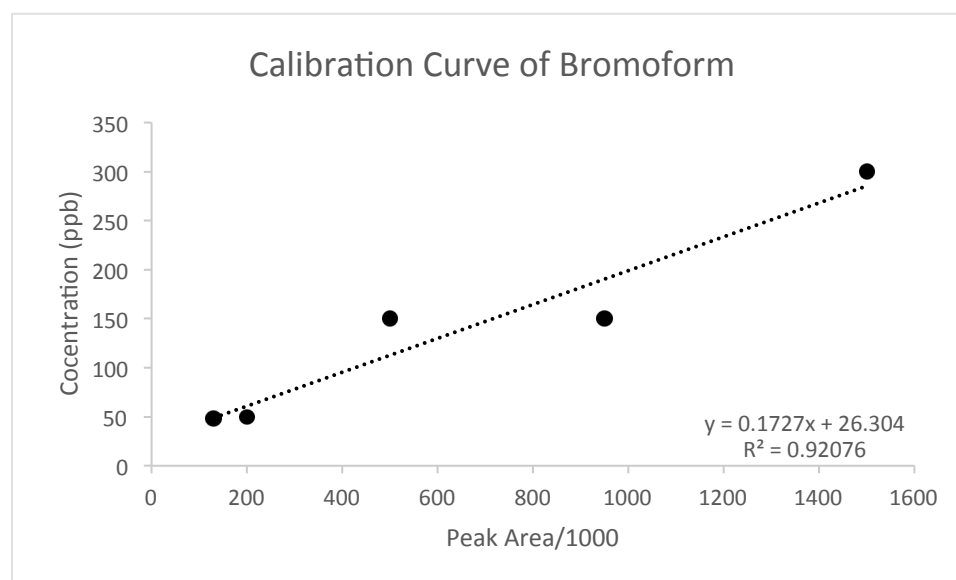
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Figure A-6 Collimated beam UV calibration spreadsheets-step (b)



Figure A-7 Chlorination bottle with PTFE cap and aluminum foil

Appendix B:

**Figure B-1 Calibration Curve of Chloroform****Figure B-2 Calibration Curve of Bromoform**

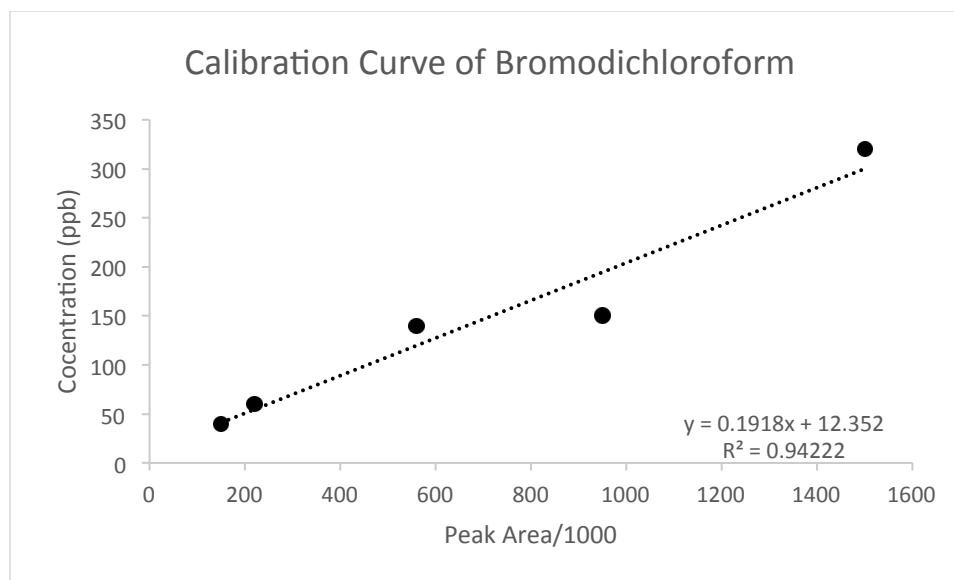


Figure B-3 Calibration Curve of Bromodichloroform

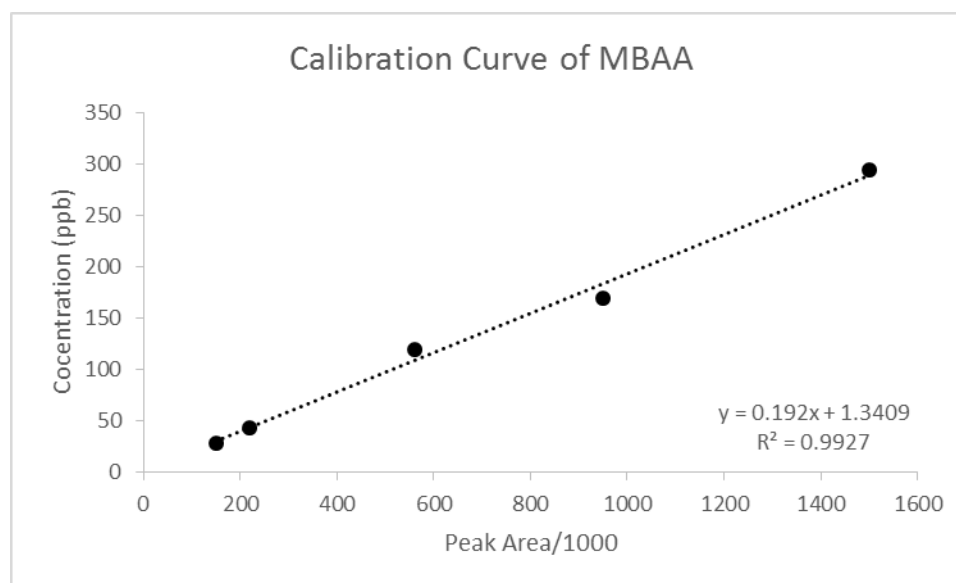


Figure B-4 Calibration Curve of MBAA

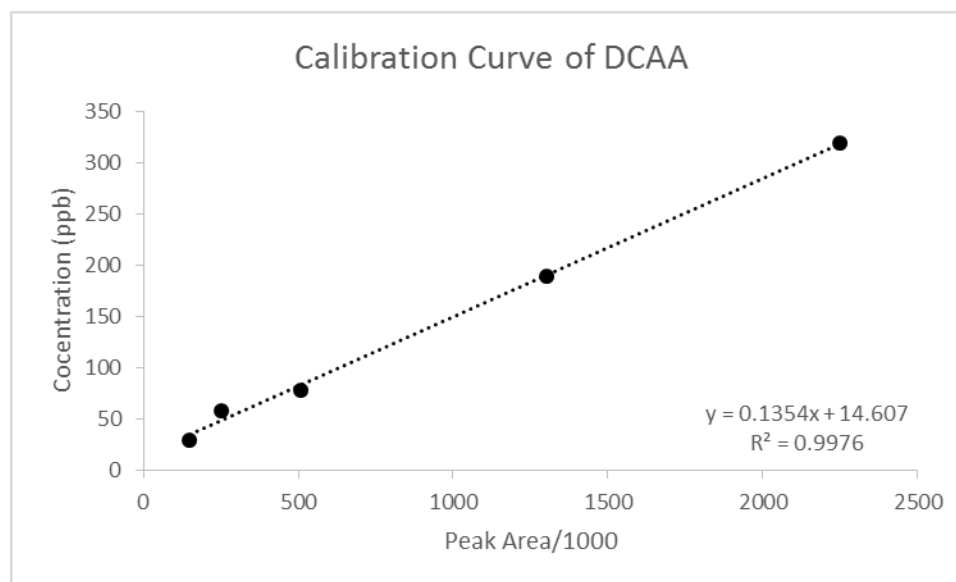


Figure B-5 Calibration Curve of DCAA

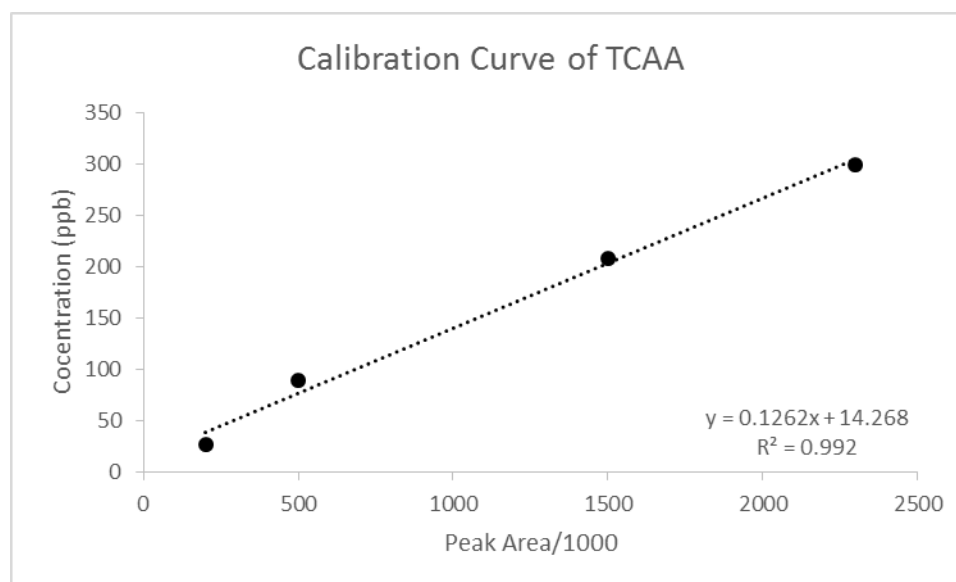


Figure B-6 Calibration Curve of TCAA

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