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COMPARISONS OF BACTERIAL INACTIVATION EFFICIENCY AND DISINFECTION BYPRODUCT FORMATION FROM FOUR DIFFERENT DISINFECTION PROCESSES

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Plant and Environmental Science

By
Binbin Li
August 2014

Accepted by:
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ABSTRACT

Formation of disinfection byproducts (DBPs) during *Escherichia coli* K12 and *Pseudomonas aeruginosa* PAO1 inactivation under four disinfection processes, including chlorination, chloramination, photo-Fenton reaction, and TiO$_2$/UV photocatalytic inactivation were examined. Factors of pH (5 or 8) and different disinfectant dosages were also investigated in order to balance the risks of DBP formation and bacterial cell viability in the four treatment processes. Chlorination had the fastest bacterial inactivation whereas chloramination, photo-Fenton, and TiO$_2$/UV requiring longer contact times to achieve the same inactivation efficiency. In spite of the lag phase in the treatment processes, both photo-Fenton and TiO$_2$/UV achieved 100% bacteria reduction after 80-min exposure, exceeding effectiveness of the chlorination (5.6 log reduction) and chloramination (4.4 log reduction). In addition, chlorination had the highest trihalomethanes (THMs) of 5.77 ± 0.21 μg/L and haloacetonitriles (HANs) of 1.02 ± 0.15 μg/L, followed by chloramination (with THMs of 1.37 ± 0.03 μg/L and HANs of 0.17 ± 0.04 μg/L). No THMs or HANs was detected in photo-Fenton or TiO$_2$/UV processes. In general, the risks of DBP and bacterial cells in finished water can be reduced through efficient operational conditions: Acidic pH condition for chlorination, chloramination, and TiO$_2$/UV photocatalytic inactivation. For photo-Fenton reaction, no DBPs formed with equal similar cell viability reduction observed under pH range of 5 – 8.
DEDICATION

To my parents, Límin Cái, Qínzhang Wáng.
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I would like to express my sincere gratitude to all the people who have helped me a lot during my master study at Clemson University and the preparation of this dissertation.

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1. CHAPTER ONE

INTRODUCTION

Chlorination is one of the most widely practiced disinfection methods in drinking water treatment because of its relatively high inactivation efficiency and low cost (Yang et al., 2005). However, chlorination is found to generate a variety of carcinogenic and mutagenic halogenated DBPs such as THMs and haloacetic acids (HAAs) (Hildesheim et al., 1998; Liang and Singer, 2003). DBPs in municipal water supplies are currently regulated by the US Stage 2 Disinfectants/Disinfection Byproducts Rule (D/DBPR) with the maximum contaminant level for THMs set to 80 μg/L and 60 μg/L for HAAs (USEPA 2006). In order to meet the regulation standard and minimize the DBPs in finished waters, alternative disinfectants such as chloramine and chlorine dioxide (ClO₂) have been employed in many public water utilities (Xie, 2004; Guay et al., 2005). Nevertheless, other types of DBPs can be generated from these disinfectants. For example, DBPs such as ketones and n-nitrosodimethylamine (NDMA) with potentially higher toxicity are detected in chloramination treatment (Choi and Valentine, 2002; Hua and Reckhow, 2007).

In addition to common disinfection processes, advanced oxidation processes (AOPs) involving powerful oxidative hydroxyl radicals (·OH) such as Fenton/Fenton like reactions have been tested for their efficiencies in bacterial inactivation and yields of DBP formation (Ribordy et al., 1997; Uyguner-Demirel and Bekbolet, 2011). Although its pH confinement is typically in acidic condition (i.e. 2.5-3.0), the photo-Fenton
reaction has been proved to inactivate *E. coli* under neutral condition (Rincón and Pulgarin, 2007; Spuhler et al., 2010). Similar to photo-Fenton reaction, TiO$_2$-mediated photocatalytic oxidation (PCO) with strong oxidative species (ROSs) including ·OH can also inactivate microbes. Significantly, degradation of various organic compounds including THMs is observed in both photo-Fenton and TiO$_2$/UV photocatalytic methods (Hsiao et al., 1983; Xu et al., 1988; Liu et al., 2008). Although concerns have been raised about the adverse effect by discharging nano-sized iron oxides or TiO$_2$ particles to the natural environment (Gottschalk et al., 2009; Wang et al., 2012a), and TiO$_2$/UV reaction causing other types of DBP formation (Richardson et al., 1996), AOP is generally considered as a promising technology in future drinking water treatment (Rosenfeldt and Linden, 2004; De Morais and Zamora, 2005, García-Fernández et al., 2012).

Breaking down the bacteria cell and releasing intracellular components such as protein, amino acids and DNA has been long recognized during different inactivation treatments (Knox et al., 1948; Patton et al., 1972; Haas and Engelbreche, 1980; Cho et al., 2010). Proteins and amino acids compose 30-70 percent of the total dry mass of microorganism (Cao et al., 1996). Because of their relatively lower carbon and nitrogen ratio (C/N) and smaller molecular size, their reactivity with disinfectants and disinfection byproduct formation potential (DBPFP) could differ from conventional DBP precursors such as humic substances. These nitrogen-enriched aromatic compounds have been reported to produce both regulated carbonaceous DBPs (C-DBPs) such as THMs as well as nitrogenous DBPs (N-DBPs) such as HANs (Trehy and Bieber, 1981; Hong et al., 2008; Fang et al., 2010). The DCAN can be formed after series of reactions between amino
groups and chlorine (Cl₂) (Trehy et al., 1986). In addition to chlorination, dichloroacetonitrile (DCAN) concentration can also be generated from amino acids during chloramination treatment (Yang et al., 2011). N-DBPs have been reported to be more carcinogenic and mutagenic than THMs and HAAs. For example, HANs is approximately two orders of magnitude more cytotoxic than HAAs (Muellner et al., 2007). Notably, HANs are unstable and subjected to decomposition through alkaline-based hydrolysis and oxidative reaction at pH higher than 7, producing THMs and HAAs as end products. Therefore, water quality during disinfection processes may affect the concentrations of DBPs in finished waters, and studies should focus on optimizing reaction conditions to reduce risks of both C-DBPs and N-DBPs contamination during microbe inactivation.

Reactions between disinfectants and biomolecules can also occur in other oxidative treatments including photo-Fenton reaction (Maness et al., 1999) and TiO₂/UV inactivation process (Sunada et al., 1998). Therefore, DBPFP of bacterial disinfection cannot be avoided. However, bacteria concentrations could be minimized though efficient disinfection processes. Previous DBP control studies have focused on precursors such as humic acid and fulvic acid (Chow et al., 2005; Hong et al., 2008). Very limited research has investigated the contribution of bacteria biomolecules themselves (Wang et al., 2013). To minimize the DBPs concentration in water supplies, the correlation between DBP formation and bacterial inactivation should be further investigated.
The hypothesis in this study is that DBP formation is inversely proportional to the bacterial inactivation but both are dependent on the contact time (i.e. concentration x reaction time) and water pH. The extensive contact time could cause a release of biomolecules from bacterial cells, resulting in an increase of DBP formation. Different disinfectants vary in their oxidative capability in inactivation efficiency as well as DBP formation or reaction pathway. Trade-off risk models could be optimized by controlling the reaction conditions such as pH and disinfectant dose during the treatment. In this study, both the inactivation efficiency and DBPFP from two bacteria, *Escherichia coli* K12 and *Pseudomonas aeruginosa* PAO1 were simultaneously evaluated. Chlorination, chloramination, photo-Fenton and TiO$_2$/UV oxidative reactions were the disinfection methods investigated. To identify optimal conditions, pH and disinfectant dose for each disinfection methods were evaluated. Fluorescence spectrum is detected and thus computed. A similar method was used by Yang et al. (2011) to illustrate correlation of the fluorescence and formation potential of DBPs from organic matter containing nitrogen. The aim of this study is to identify optimal disinfection conditions that minimize the formation of DBPs and prevent waterborne diseases in treated drinking water.
2. CHAPTER TWO
LITERATURE REVIEW

2.1 Water Scarcity and Waterborne Disease

Among all the water resource on planet, only 2.5% of which is fresh water and 69% of them are groundwater and icecaps that is hard for direct usage, and less than 0.3% of all fresh water stored in wetlands of lakes and rivers, and the atmosphere (Gleick et al., 1993). The difficulty of obtaining fresh water for use is a universal problem faced by a large number of people. Water shortage, water deficits and water stress is threatening human being’s lives. Every year there are two million diarrheal deaths related to unsafe water, hygiene, and sanitation. There are more than one billion people who have difficult access to clean water. Infectious diseases of typhoid, hepatitis A and E, diarrhea and cholera were found to cause 3,900 children’s deaths daily (Montgomery and Elimelech, 2007).

Waterborne disease such as bacterial infections, protozoal infections, and viral infections might be caused by pathogenic microorganisms transmitted in contaminated water. Bacteria are the most universal microorganism forms. Commonly seen bacteria included Escherichia coli and Pseudomonas aeruginosa, etc. Legionella sp., Salmonella typhi sp., Shigella sp., and Vibrio cholera are all key bacterial pathogens; protozoa such as Giardia and Cryptosporidium could secrete protective covering and form the cyst which is a resting stage and enable to protect the protozoa from environmental stress; Viruses
including hepatitis A and Norwalk virus are the one that have totally different reproduction pathway and might facilitate the water borne diseases in all over the world.
2.2 Disinfection Processes

Under the pressure for safe drinking water, disinfectants like Cl₂, ClO₂, chloramine, ozone (O₃), photo-catalytic oxidants are developed to inactivate the pathogens and prevent harmful effects of polluted water to human health. Disinfectants are used in (a) inactivate pathogens; (b) remove color and odor; (c) oxidize iron and manganese; (d) prevent algal from growing in sedimentation basins and filters; (e) prevent biological regrowth in the water distribution system (USEPA, 1999a). The oxidation potential of various disinfection species is present in Table 1.1 (Rice-Evans and Gopinathan, 1995):

2.2.1 Chlorination

Chlorination is by far the most common method for disinfecting wastewater effluents before their discharging into receiving streams, rivers or oceans. It is the primary disinfection methods chosen by the majority of surface water and ground water system, according to the report from 1995 Community Water System Survey.

The mechanism of chlorination inactivation is the release hypochlorite (HClO). The equilibrium between HCl and HClO when Cl₂ is present in the aqueous solution is shown in the following equation 1.1 and 1.2. HClO is a stronger oxidant than ClO⁻ for its ability to be in contact with bacteria cell. HClO is the major species in the acidic solution, and both HCl and ClO⁻ will be present at pH 7.3. While the in alkaline condition, ClO⁻ is the dominant species. Thus chlorine is a more effective disinfectant at lower pH solution.

\[
\text{Cl}_2 + \text{H}_2\text{O} \rightarrow \text{HClO} + \text{HCl} \quad \text{(equation 1.1)}
\]

\[
\text{HClO} \rightarrow \text{H}^+ + \text{ClO}^- \quad \text{(equation 1.2)}
\]
A wide range of pathogens like *E. coli* and *P. aeruginosa* (Xu et al., 1996) are found to be effectively inactivated by chlorine. In the past, a number of empirical formulations have been developed for the analysis of chlorine disinfection whether in the chlorine-demand or chlorine demand free systems. One of the most popularly utilized models is developed by Selleck and associates (Selleck et al., 1978), as illustrated below:

\[
\ln \left( \frac{N}{N_0} \right) = -n \ln (1 + bct) .
\]  \text{(equation 1.3)}

The curve of disinfection of chlorination follows a general tailing-off and biphasic mode of inactivation, i.e. a sharp reduce of bacterial cell density within 15 min followed by an extended phase, the disinfection curve shows little inactivating occurring after a contact time of 15 min (Lee and Nam, 2002)

The benefits of chlorination are universally acknowledged for the broad-spectrum germicidal potency and low cost of chlorine. But it is unavoidable that free chlorine reacts with organic matters presenting in the solution, mostly widely studies were humic acid and fulvic acid. The DBPs produced are mainly THMs and HAAs. Factors influencing the DBP formation potential are contact time, pH, temperature, disinfectants dosage, and DBP precursor characteristics (Geisser et al., 1979).

### 2.2.2 Chloramination

Chloramination is an alternative oxidation treatment used to prevent quick depletion throughout the distribution system (Seidel et al., 2005) and control the traditional detected disinfection byproducts (DBPs). It also maintains long-time inactivation effects by continuously releasing free chlorine compared to chlorination.
The following equations illustrate the formation process of chloramine. When adding NH₃ into the bulk solution, a series of reactions will take place and form chloramine, which include monochloramine (NH₂Cl), dichloramine (NHCl₂), and trichloramine (NCl₃) as shown in equation 3-7.

\[
\begin{align*}
\text{NH}_3 + \text{HOCl} & \leftrightarrow \text{NH}_2\text{Cl} + \text{H}_2\text{O} & \text{ (equation 1.4)} \\
\text{NH}_3 + \text{HOCl} & \leftrightarrow \text{NHCl}_2 + \text{H}_2\text{O} & \text{ (equation 1.5)} \\
\text{NHCl}_2 + \text{HOCl} & \leftrightarrow \text{NCl}_3 + \text{H}_2\text{O} & \text{ (equation 1.6)} \\
\text{HOCl} + \text{NH}_2\text{Cl} & \leftrightarrow \text{NHCl}_2 + \text{H}_2\text{O} & \text{ (equation 1.7)} \\
\text{NH}_2\text{Cl} + \text{NHCl}_2 & \leftrightarrow 3\text{H}^+ + 3\text{Cl}^- + \text{N}_2 \uparrow & \text{ (equation 1.8)}
\end{align*}
\]

2.2.3 Photo-Fenton Reaction

Photo-Fenton is one of the AOP, which is characterized by generating ·OH with the high oxidation potential of 2.8V. It is a much more powerful oxidative species than traditional chlorine and chloramine that could degrade organic components and bacteria constituents like polyunsaturated phospholipids component of cell membrane (Maness et al., 1999). Importantly, it is reported that artificially synthesized and natural compounds, which is resistant to degradation by other oxidation processes, could be fast oxidized and mineralized by AOP and yield CO₂ and other inorganic ions (Andreozzi et al., 1999).

In Photo-Fenton reaction, H₂O₂ serves as the main potential source of ·OH radicals. The formation of ·OH is highly catalyzed with the present of Fe (II) or Fe (III) and thus influenced by pH value. And the net reaction is shown in equation 9:
The above reaction could occur both in homogeneous and heterogeneous systems with dissolved ferrous iron (Fe$^{2+}$) or complex iron system. The ferric iron (Fe$^{3+}$) will keep react with Superoxide radical anion (•O$_2^-$) or peroxide and thus Fe$^{2+}$ forms. The reaction will come to an end until all the H$_2$O$_2$ is completed consumed. The equations are as follows:

$$\text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{2+} + \cdot\text{O}_2^- + 2\text{H}^+$$ \hspace{1cm} (equation 1.10)

$$\cdot\text{O}_2^- + \text{Fe}^{3+} \rightarrow \text{Fe}^{2+} + \text{O}_2$$ \hspace{1cm} (equation 1.11)

### 2.2.4 TiO$_2$/UV Photocatalytic Inactivation

TiO$_2$/UV photocatalytic inactivation is also a newly developed oxidation methods used in both degradation of organic matters and inactivation of microorganism. Titanium dioxide is an n-type semiconductor with a band gap of 3.2 eV. Titanium dioxide photo catalysis was first used for the remediation of environmental pollutants in 1977 when Frank and Bard reported the reduction of CN$^-$ in water (Frank and Bard, 1977).

The mechanism of PCO with TiO$_2$ under UV irradiation could be illustrated in figure 1.1. When the titanium dioxide particle receives light energy which is greater than the band gap of semiconductor ($\lambda$≤385nm), the absorption of a photon excites an electron to the conduction band (e$_\text{CB}^-$), which forms a positive hole in the valence band (h$_\text{VB}^+$) (Pelaez et al., 2012). Charge transports to the surface of the particle, and then leads to the generation of various catalytically active species such as electron (e-), •OH, H$_2$O$_2$, •O$_2^-$.
, ·OOH and other species. A series of oxidation reactions will later be triggered either on the surface of the particle or in the bulk solution. These combined species are found to successfully inactivate microorganism and degrade organic pollutant in environment.

It is assumed that the stronger oxidative species like ·OH generated in the surface of TiO\(_2\) particles has a short half-life and reacts rapidly with components closed to it. Therefore it will inactivate bacteria if when the bacteria reaches to the TiO\(_2\) particle surface, or it could be consumed by other organic or inorganic compounds closer. While other less oxidative species of H\(_2\)O\(_2\), make it possible to diffuse into the bacteria through plasma membrane. Thus the inactivation process is the combined effects of all the ROS species.
2.3 Bacteria

2.3.1 Escherichia coli

Both *Escherichia coli* and *Pseudomonas aeruginosa* are Gram-negative and rod-shaped bacterium.

*Escherichia coli* belong to the family *Enterobacteriaceae* and are commonly seen in soil, water, animals, humans and sewage. Its size is 1.1-1.5 um (diameter) × 2.0-6.0 um (long). The normal temperature range and pH for *Escherichia coli K12* is 21-37°C and 4.4-9.0 respectively, with optimum pH of 6.0-7.0. Below is the composition analysis of *E. coli* cell (Neidhardt et al., 1996) (Table 1.2) and the colony appearance (Figure 1.2) and transmission electron microscope (TEM) image (Figure 1.3) of *E. coli* (Kim et al., 2008).

2.3.2 Pseudomonas aeruginosa

The size of *Pseudomonas aeruginosa* is 0.5-1.0 um (diameter) × 1.0-5.0 um (long).

*Pseudomonas aeruginosa* is also very commonly seen in soil, water, animals, humans and sewage. Many species are capable of building up Polyhydroxybutyrate to be its carbon reserve material (Palleroni, 1984) under condition with limited nitrogen. *P. aeruginosa*’s growth occurs at temperature between 4-43°C and pH range for it is from 5.6 to 8.0, with the optimum pH is 6.6-7.0. Below is the colony appearance (Figure 1.4) and transmission electron microscope (TEM) image (Figure 1.5) of *P. aeruginosa* (Zaborina et al., 2008).

Both *E. coli* and *P. aeruginosa* are gram-negative bacteria. 10-20% of the gram–negative cell wall compositions are peptidoglycan, phospholipids, lipoproteins,
lipopolysaccharides, and proteins composed of the 7 nm thick outer membrane of their cell wall.

One of the most important structures of bacteria is outer membrane as it shows in Figure 1.6. The outer membrane contains two kinds of lipids, lipopolysaccharides and phospholipids. Outer membrane is a unique constituent of the bacterial outer membrane with three parts: the proximal, hydrophobic lipid region; the distal, hydrophilic region; and the core oligosaccharide region that connects the two. The outer surface of the outer membrane constitutes the outermost area of the cell; its characters influence the reactivity of bacteria to other components. These properties include the presence of hydrophilic, usually negatively charged carbohydrate chains.

Although both E. coli and P. aeruginosa are all Gram-negative bacteria, the two strains demonstrate some other distinctive characteristics. E. coli is a facultative anaerobe microorganism, while P. aeruginosa is strictly aerobic strain. And the outer membrane of the Gram-negative cell wall is inserted with proteins on the cell surface that differ between the strain and species of the bacterium. J.D.Stinnett (Stinnett et al., 1973) had treated cell envelopes of P. aeruginosa with N, N′-dimethylformamide or with ethylenediaminetetraacetate, and found out the major protein were glycoproteins with molecular masses of 43,000 and 16,500 daltons. While Carl A. Schnaitman (Schnaitman, 1970) reported the major component (70%) of protein on E. coli cell envelope is the protein with a molecular weight of 44,000 daltons. Vesicles prepared with the outer membrane of P. aeruginosa showed it could retain the saccharides with molecular weight
larger than 9000 daltons, which is much higher than that of *E. coli* to retain 550-650 daltons of molecular weight.

### 2.3.3 Bacterial Defense Mechanism towards Oxidative Stresses

Leung (Leung, 2008) used chlorine dose of 1.5, 2.5 and 3.5 mg/L Cl$_2$ to inactivate *E. coli K12* and investigated the inactivation mechanism by TEM. It was shown that the cell structure was slight changed if using low concentration of 1.5 mg/L Cl$_2$ with 5 min’s period. There was an electron translucent region occurred at the peripheral region of the cell and the region became larger and more clear if adding higher dose of chlorine or waiting for longer reaction time. The dark region electron only appeared clear if 5-log inactivation effects were reached. Cho (Cho et al., 2010) also compared inactivation mechanism of various disinfectants of chlorine, chlorine dioxide and UV. It was also reported the appearance of net-like structure inside the cytoplasmic region and the preservation of the cell wall during chlorination. Thus chlorine is observed to attack both intercellular components and outer cell membrane constituents, causing protein release, lipid peroxidation and cell permeability change when reaching 1 log cell viability reduction. In contrast, other stronger oxidants like ozone and hydroxyl radicals cause clearer damage on the cell surface structure. The cell morphology was expected to be fully distorted with a longer reaction time or with higher concentration of disinfectants. Except for UV irradiation, it showed very little surface damage for UV light could directly induce damage to the intracellular components of DNA (Cho et al., 2010).
2.4 Disinfection Byproducts (DBPs)

2.4.1 Carbonaceous DBPs (C-DBPs) and Nitrogenous DBPs (N-DBPs)

There are hundreds of DBPs have been identified and some of them are carcinogenic and mutagenic. DBP formation is one of the most important issues for water engineers and scientists.

C-DBPs include THMs, HAAs, haloketones (HKs), and CHs. Recently more and more N-DBPs have been reported and are receiving increasingly interests. N-DBPs include haloacetonitriles (HANs), NDMA, and trichloronitromethane (TCNM), etc. N-DBPs such as DCAN, trichloroacetonitrile (TCAN) and NDMA are more of health concern than C-DBPs (Bull and Robinson, 1986). In addition, TCNM has been reported to be some orders of magnitude higher than C-DBPs in toxicity (Richardson et al., 2007).

2.4.2 DBP Precursors

2.4.2.1 Humic Substance as DBP Formation Precursors

DBPs are formed from the reaction between disinfectants and natural organic matter (NOM) and/or inorganic matter during disinfection process. For example, chlorine might react with humic substances to form a wide range of unwanted halogenated organic compounds.

The generalized DBP formation equation is as follow:

\[ \text{HOCl} + \text{Br}^- + \text{NOM} \rightarrow \text{THMs} + \text{Other Halogenated DBPs} \quad \text{(equation 1.12)} \]
Two of the most commonly studied known DBPs from humic substances sources are THMs and HAAs during chlorination of natural water (Singer, 1994). These are traditional carbonaceous DBPs that has been identified and studied for years.

2.4.2.2 Algal cells as DBP precursor

Previously, attentions have been focus on DBPs formed from humic acid and fulvic acid, nowadays increasingly interests have been focused on N-DBPs generated from algae cells with lower aromatic carbon content, more hydrophilic content, or organic nitrogen (org-N) (Hua and Reckhow, 2008; Yang et al., 2011; Li et al., 2012).

Algal and its cellular debris of microbial products contain macromolecules like protein (nitrogen-enriched) and polysaccharide (Namkung and Rittmann, 1986; Barker et al., 1999; Krasner et al., 2009). Those compounds present different DBP formation potential compared to humic substances. N-organic compounds react with chlorine to form organic chloramines, some of which will be decomposed to DCAN and might be further hydrolyzed to dichloroacetic acid (DCAA) and chloroform (Peters et al., 1990). Algal cells are reported to have high formation potential of C-DBPs and N-DBPs (Kwon et al., 2005).

2.4.2.3 Isolated nitrogen organic matter as DBP precursors

Except for study DBPs from algal cells, nitrogen enriched dissolved organic matter/organic compounds has also been separated to demonstrate its DBP formation potential (Dotson et al., 2009). Hong (Hong et al., 2008) investigated the Disinfection
byproduct formation potential (DBPFP) of BSA and fish oil, which represents algal cellular protein and lipid, and found that they presented higher DBPFP than starch (surrogate for carbohydrate). Trehy et al (1986) found the chloral hydrate (CH) concentration was significantly increased if the pH was raised from pH 7 to 8.

Amino acids are ubiquitous in natural environment and usually range between 20 and 10,000 ug/L which consists of 2-13% of dissolved organic carbon in natural water body (Thurman, 1985), thus its DBPFP has been received attention. It was reported that amino acid (Trehy and Bieber, 1981) and nucleic acids (Young and Uden, 1994) form higher amount of DCAN than that from humic substances. Chlorination (oxidation) of amino acids can result in the formation of aldehydes and nitriles, with subsequent or concomitant chlorine substitution to form CH and DCAN, respectively (Hong et al., 2008). Amino acids with higher aromatic ones produced more THMs than those with less aromatic or nonaromatic ones (Hong et al., 2009). In addition, Yang discovered that (Yang et al., 2012) amino acid, such as tryptophan, tyrosine, asparagine, and alanine, produce similarly high DCAN concentration from chlorination and chloramination.

2.4.2.4 Bacteria as DBP precursors

These bacterial biomolecules such as amino acid, protein, lipid, and polysaccharides released from bacteria cells as they breaks down might produce more N-DBPs with higher toxicity compared to humic acid or fulvic acid. It is assumed that the yield and species of DBP formation might increase as more microorganisms are inactivated. Thus our study hypothesis is a trade-off risk model between the waterborne diseases and the
yield DBPs produced from cellular materials of microorganisms. We might define the optimal water condition of bacterial inactivation that achieves the disinfection efficacy while minimizing production of excessive DBPs.

2.4.3 DBP Formation from Disinfection Methods

2.4.3.1 Chlorination and chloramination

THMs and HAAs are two most common DBPs found when using chlorine in natural water and THMs formation was correlated to HAAs. Chloramination generates significantly less THMs and HAAs than chlorination (Hua and Reckhow, 2007, comparison), (Guay et al., 2005; Bougeard et al., 2010). However, even the DHAAs and total organic halogen (TOX) was reduced by using chloramination, more than 70% of the TOX generated from chloramination are failed to be identified.

Additionally, although chloramination might produce higher percentage of HANs of all DBPs formed than chlorination, only 93% and 81% of total HANs produced by switching from chlorination to chloramination (Hua and Reckhow, 2007; Bougeard et al., 2010).

Considering the HKs formation, 1,1,1-trichloropropanone (1,1,1-TCP) was the major HKs generated during chlorination of natural water. While chloramination reduce total HKs by 70%, and no 1,1,1-TCP was detected, the reason might be NH₂Cl is unable to provide enough free chlorine to substitute atoms in 1,1-dichloropropanone (1,1-DCP) (Yang et al., 2007).
2.4.3.2 Photo-Fenton reaction and TiO\textsubscript{2}/UV photocatalytic process

Photo-Fenton reaction and other AOP processes were found to generate much less DBPs than other conventional disinfection treatment due to the strong oxidative capability of OH radicals (Toor and Mohseni, 2007) to reduce the levels of NOM (DBP precursors). Specifically, OH radicals are able to remove hydrogen atoms (Beltran et al., 1993; Murray and Parsn, 2004; Moncayo-Lasso et al., 2008) or add electrophiles to the double bonds of the compounds. It was also reported that artificially synthesized and natural compounds, which is resistant to degradation by other oxidation processes such as THMs, could be rapidly oxidized and mineralized by AOP (Zepp et al., 1992). For TiO\textsubscript{2}/UV inactivation, NOM were also reported absorbed by titanium dioxide particles (Eggins et al., 1997)

2.4.4 DBP Toxicity

DBPs could be absorbed into human body by drinking, bathing and inhaling. DBPs are reported to cause reproductive and other adverse health effects including cancers of bladder, colon and rectal (Cantor et al., 1998; Hildesheim et al., 1998). Various cancers have been linked to exposure to DBPs in drinking water by epidemiology studies.

Table 1.3 (Sadiq and Rodriquez, 2004) shed lights on the adverse effects of common DBPs. In addition to that, THMs is the mostly studied DBP species and its carcinogenic potential in rodents is widely investigated. Halogenated acetonitrile have also been reported to be genotoxic and possible carcinogenic (Bull and Robinson, 1986), and it is also found to cause DNA strand breaks in lab cultured human lymphocytes (Ballmaier
and Epe, 1995). Mutagenic effects were observed in Salmonella species using DBCN (Mortelmans et al., 1986). Limited information of dermal exposure of HKs has been revealed although HKs are very common species of DBPs (Steven et al., 1990) and Bull and Robinson had discovered HK’s carcinogenic and mutagenic properties in mice (Bull and Robinson, 1984). It is also found that it takes only a few minutes for the penetration of HKs into human skin (Xu and Weisel, 2005). CH has been demonstrated genotoxic and carcinogenic in animal studies (Haselkorn et al., 2006). But the clearer causation of CH to human cancer is still not fully developed.

2.4.5 DBP Regulation

Some of identified cytotoxic and genotoxic DBPs were regulated by the US Environmental Protection Agency (USEPA) such as THMs and HAAs (U.S. EPA, 2006) with limitation of 80 ug/L and 60 ug/L respectively. Nowadays, 11 of DBPs are currently regulated by the U.S. and 74 DBPs are named emerging DBPs with their limited concentration in treatment facilities (Richardson et al., 2007). DBP regulations and guidelines of USEPA (Table 1.4) (2006) are shown as follows:

2.4.6 DBP Controlling

There are a series of methods for controlling DBPs in water distribution system including:

(a) Reduce DBP precursors of NOM/inorganic substance in water (Nikolaou et al., 2004);
(b) Replace tradition chlorination to alternative disinfection process such as ozone, photocatalytic reaction, chlorine dioxide; (c) Optimize react condition (pH, contact time) for less DBP formation; and (d) Remove organic contaminants and DBPs after the treatment
Among all, removing NOM/inorganic substance remained to be a very effective method to control DBP concentration in water, and methods of coagulation, flocculation, traditional filtration and granular activated carbon GAC filtration have also be used for removing.

2.4.7 Effects Influencing DBP Formation/trade-off Model

Formation of DBPs is influenced by various factors such as pH, contact time, chemical concentration, temperature, etc. (Liang and Singer, 2003; Hua and Reckhow, 2008). Some factors will be detailed in following sections.

2.4.7.1 The pH effect

The effect of pH differed for the various DBP groups: Stable C-DBPs such as THMs is observed higher at alkaline condition (Hansen et al., 2012). It is generally accepted that base-catalyzed reactions play a significant role in THM formation (Peters et al., 1980; Reckhow et al., 1990).

In contrast, DHANs are unstable in aqueous solution and can be decomposed fast by residual chlorine at pH higher than 7.0 (Krasner et al., 1989). It was discovered that when the pH increased from 8 to 10, all three DHAN species degraded with increasing time and the DHAN concentrations were close to 0 at the end of 72 h incubation time (Hua and Reckhow, 2012). Other DBPs like DCAN and 1, 1-DCP was also observed decomposed due to base-catalysis.
The pH effect on DBP formation was different for chloramination compared to chlorination. (Croue and Reckhow, 1989; Xie and Reckhow, 1993; Yang et al., 2007) reported the different pH effect on DBPFP during chloramination. Maximum DCAN, chloroform, 1, 1-DCP were produced during pH from 5-6, and less were generated at pH 8. Reasons might be: lower pH increase hydrolysis rate of NH₂Cl to free chlorine and thus lead to higher DBP formation.

2.4.7.2 Temperature effect

Generally Ct (concentration x reaction time) required for inactivating microorganisms is lower in warm water than in cold water (Richardson and Postigo, 2012) to reach the same level of inactivation. However, it is reported microorganism activities are higher in warm water rather in cold water in water distribution system. In addition, disinfectants such as chlorine are easier to deplete under higher temperature, hence it requires higher chlorine concentration in warmer water to maintain inactivation effects and prevent microorganism regrowth.

2.4.7.3 Contact time effect

In chlorination, THMs was reported goes up with increasing incubation time (Nikolaou et al., 2004; Hua and Reckhow, 2008). Yang (Yang and Shang, 2004) found the THM concentration was about 3 times higher at 24 hours than that at 1 min. Haloacetonitiriles and HKs generated rapidly but then are hydrolyzed as further reaction with residual chlorine, hence lead to the reduction concentration along the disinfection process.
2.4.7.4 Bromide effect

During chlorination, hypochlorous acid (HOCl) quickly oxidizes bromide to form HOBr as bromide is present in water system. As a result, chloro-bromo substitution components will be formed when HOCl and HOBr react with NOM in water. Bromide often increases DBP formation potential and shifts DBP formation to brominated ones (Hua and Reckhow, 2007). Incorporation of bromide increases the total THM, HAA formation during chlorination (Yang and Shang, 2004).
Figure 2.1 Schematic photoexcitation in a TiO$_2$ semiconductor particle

Figure 2.2 The colony appearance of Escherichia coli
Figure 2.3 The transmission electron microscope image of Escherichia coli

Figure 2.4 The colony appearance of *Pseudomonas aeruginosa*
Figure 2.5 The transmission electron microscope image of *Pseudomonas aeruginosa*

Figure 2.6 Structure of a gram-negative cell wall
<table>
<thead>
<tr>
<th>Species</th>
<th>Oxidation potential (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoride (F⁻)</td>
<td>3.03</td>
</tr>
<tr>
<td>Hydroxyl radical (·OH)</td>
<td>2.80</td>
</tr>
<tr>
<td>Ozone (O₃)</td>
<td>2.07</td>
</tr>
<tr>
<td>Hydrogen peroxide (H₂O₂)</td>
<td>1.78</td>
</tr>
<tr>
<td>Perhydroxyl radical (·OOH)</td>
<td>1.70</td>
</tr>
<tr>
<td>Permanganate (MnO₄⁻)</td>
<td>1.68</td>
</tr>
<tr>
<td>Hypobromous acids (HOBr)</td>
<td>1.59</td>
</tr>
<tr>
<td>Chlorine dioxide (ClO₂)</td>
<td>1.57</td>
</tr>
<tr>
<td>Hypochlorous acid (HOCl)</td>
<td>1.49</td>
</tr>
<tr>
<td>Chlorine (Cl₂)</td>
<td>1.36</td>
</tr>
</tbody>
</table>
Table 2.2 Chemical compositions of an average *Escherichia coli*

<table>
<thead>
<tr>
<th>Components</th>
<th>% Total dry wt</th>
<th>No. of different kinds molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>55.0</td>
<td>1,850</td>
</tr>
<tr>
<td>RNA</td>
<td>20.5</td>
<td>1</td>
</tr>
<tr>
<td>DNA</td>
<td>3.1</td>
<td>1</td>
</tr>
<tr>
<td>Lipid</td>
<td>9.1</td>
<td>1</td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>3.4</td>
<td>1</td>
</tr>
<tr>
<td>Peptidoglycan</td>
<td>2.5</td>
<td>1</td>
</tr>
<tr>
<td>Glycogen</td>
<td>2.5</td>
<td>1</td>
</tr>
<tr>
<td>Polyamines</td>
<td>0.4</td>
<td>1</td>
</tr>
<tr>
<td>Metabolites, cofactors, ions</td>
<td>3.5</td>
<td>800+</td>
</tr>
</tbody>
</table>

(Calculated for an average cell in a population of *Escherichia coli* B/r in balanced growth at 37 °C in an aerobic glucose minimal medium with a mass doubling time of 40 min)
<table>
<thead>
<tr>
<th>Class of DBPs</th>
<th>Compound</th>
<th>Rating</th>
<th>Detrimental effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trihalomethanes</td>
<td>Trichloromethane</td>
<td>B2</td>
<td>Cancer, liver, kidney, and reproductive effects</td>
</tr>
<tr>
<td></td>
<td>Dibromochloromethane</td>
<td>C</td>
<td>Nervous system, liver, kidney and reproductive effects</td>
</tr>
<tr>
<td></td>
<td>Bromodichloromethane</td>
<td>B2</td>
<td>Cancer, liver, kidney, and reproductive effects</td>
</tr>
<tr>
<td></td>
<td>Tribromomethane</td>
<td>B2</td>
<td>Cancer, nervous system, liver and kidney effects</td>
</tr>
<tr>
<td>Haloacetonitriles</td>
<td>Trichloroacetonitrile</td>
<td>C</td>
<td>Cancer, mutagenic and clastogenic effects</td>
</tr>
<tr>
<td>Halogenated aldehydes and ketones</td>
<td>Formaldehyde</td>
<td>B1</td>
<td>Mutagenic</td>
</tr>
</tbody>
</table>

B1: Probable human carcinogen (with some epidemiological evidence); B2: Probable human carcinogen (sufficient laboratory evidence); C: Possible human carcinogen;
Table 2.4 US Environmental Protection Agency regulations of disinfection by products

<table>
<thead>
<tr>
<th>Disinfection byproducts</th>
<th>Maximum contaminant level (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total trihalomethanes</td>
<td>0.080</td>
</tr>
<tr>
<td>Five haloacetic acids</td>
<td>0.060</td>
</tr>
<tr>
<td>Bromate</td>
<td>0.010</td>
</tr>
<tr>
<td>Chlorite</td>
<td>1.0</td>
</tr>
</tbody>
</table>
3.1 Bacterial Strain

*E. coli* K12 and *P. aeruginosa* PAO1 (ATCC, USA) were cultured in nutrient broth (Biolife, Milano, Italy) at 37 °C in incubator (Figure 3.1) to reach the stationary stage (10⁹ colony forming unit (cfu)/mL) (Gao et al., 2012). The cultures were then aseptically centrifuged and washed three times with 9% sodium chloride (NaCl) solution (≥99.5%; Sigma-Aldrich). Finally, the cell concentrations were adjusted to 1.5×10⁷ cfu/mL in each reaction mixture for the four inactivation methods. To prevent contamination, all Milli-Q water (Millipore, Bedford, MA, USA), NaCl/PBS solution, and pipette tips were autoclaved (121 °C for 20 min) before use.
3.2 Disinfection Processes

Bacterial cells were suspended in 60 ml phosphate buffered saline (PBS) solution containing sodium hypochlorite (NaOCl) (0, 0.5, 1.5 mg-C1/L) or NHCl₂ (0, 0.5, 1.0 mg/L). The initial pH of PBS solution was then adjusted to 5 and 8 with the addition of 0.1 M sulfuric acid (H₂SO₄) and 0.1 M sodium hydroxide (NaOH) with pH meter (Figure 3.2) for further inactivation reaction. Inactivation procedure was carried out in air-free bottles fixed in a shaker (70 rpm) under dark condition. For chlorination, NaOCl solutions were made from diluting 3 g-C1/L NaOCl solution (Aldrich). For chloramination, NH₂Cl stock solution was prepared by adding 3 g-C1/L NaOCl to 0.53 g/L ammonium chloride (NH₄Cl) (Cl₂/N mass ratio of 3.5:1) (Hu et al., 2010) solution (Aldrich) and then rapidly stirring for 30 min with pH buffered at 8.5 ± 0.2 with NaOH. Both chlorine and NH₂Cl diluted in PBS solution and transferred in 60 ml chlorine demand free glass bottles with no air space for inactivation. The final chlorinated compounds were all measured and standardized with the DPD ferrous titrimetric method (Clesceri et al., 1989).

The photocatalytic inactivation procedure followed the method found in Zhang et al. (2010). Both photo-Fenton reaction (0, 0.1 mM Fe²⁺/2.5 mM H₂O₂, 0.1 mM Fe²⁺/5.0 mM H₂O₂) and TiO₂/UV oxidation (0, 100, 250 mg/L TiO₂) were conducted in air-free glass flasks with 100 ml mixture of PBS, diluted disinfectant, and bacterial cells. The Fenton reagent was made by diluting 500 mM ferrous sulfate solution (Sigma-Aldrich) and 30% w/v H₂O₂ solution (Sigma-Aldrich). The TiO₂ solution was prepared by TiO₂ powder (P-25 Degussa Corporation, Germany) which was in crystalline form (70%
anatase and 30% rutile). The light intensity of Xeon lamp (Cole-Parmer, Vernon Hills, USA) for photo-Fenton reaction and UV-A lamp (Cole-Parmer, Vernon Hills, USA) in TiO₂/UV photocatalytic inactivation were fixed at 250 mW/m² and 0.22 mW/cm² respectively prior to experiment. Light control experiments (with no Fenton reagents or TiO₂) were also conducted to exclude the light effect on inactivation.

During the inactivation, water samples were taken from the reaction mixture at fixed time intervals (0, 5, 20, 40, 60, 80 min). Chlorination and chloramination were stopped with a quencher of 50 μL of 5% NH₂Cl and 5 μL of 10 Na₂SO₃, respectively. Water samples were then immediately diluted with different volume of sterilized saline to three levels (1, 1×10², and 1×10⁴), spread on nutrient agar plates and incubated at 37 °C for 24 h at which time viable bacteria were counted. The DBPs formed at 80 min reactions were extracted by methyl tert-butyl ether and then quantified by GC-ECD. Additionally, water samples at 80 min were also treated with H₂SO₄ and measured with fluorescence spectrophotometer (F-7000, Hitachi, Japan).
3.3 DBP Quantification

The DBP standards (Sigma-Aldrich) were four THMs (including trichloromethane (TCM), bromodichloromethane (BDCM), dibromochloromethane (DBCM) and bromoform (TBM)), four HANs (including TCAN, DCAN, bromochloro-acetonitrile (BCAN) and dibromo-acetonitrile (DBAN)), two HKs (including 1,1-DCP and 1,1,1-TCP), chloropicrin (CHP) and CH. GC-ECD (Agilent 6890) with column (A - 0.25 mm ID x 30 m fused silica capillary) was used to quantify the DBP concentration according to U.S. EPA Method 551.1 (Munch and Hautman, 1995). The temperature program was slightly modified as follows: held at 30 °C for 7 min then increased to 100 °C at 20 °C /min and held for 3 min, and then increased to 260 °C at 30 °C /min and held for 2 min. The injector and detector temperature were set at 250 °C. The DBP retention time is shown in table 3.1.
3.4 Fluorescence

To indicate the relationship of the fluorescence dissolved organic matter (FDOM) property of bacterial solution and their DBP formation potential, an F-7000 Fluorescence Spectrophotometer (Hiachi, USA) (Figure 3.3) was used for fluorescence excitation-emission matrix (EEM) measurement. Slits of the spectrometer were set to 10 nm for both excitation and emission. The excitation wavelengths were increased from 200 to 400 nm at 5 nm steps; and the emission wavelengths from 290 to 500 nm at 5 nm steps. First- and second-order Raman scattering was removed by accounting for the water blank samples. The EEM figures were drawn with Matlab software.

Fluorescence region integration was evaluated the percentage distribution of five dissolved organic matter fractions (Zhou et al., 2013): Region I: aromatic protein I (200 nm < excitation wavelength ($\lambda_{\text{ex}}$) < 250 nm, 280 nm < emission wavelength ($\lambda_{\text{em}}$) < 330 nm); Region II: aromatic protein II (200 nm < $\lambda_{\text{ex}}$ < 250 nm, 330 nm < $\lambda_{\text{em}}$ < 380 nm); Region III: fulvic acid-like (200 nm < $\lambda_{\text{ex}}$ < 250 nm, 380 nm < $\lambda_{\text{em}}$ < 500 nm); Region IV: soluble microbial byproduct-like (250 nm < $\lambda_{\text{ex}}$ < 400 nm, 280 nm < $\lambda_{\text{em}}$ < 380 nm), and region V: humic acid-like (250 nm < $\lambda_{\text{ex}}$ < 400 nm, 380 nm < $\lambda_{\text{em}}$ < 500 nm).
3.5 Statistical Analysis

A three-factor factorial statistical analysis was conducted. Three factors analyzed including four levels of disinfectant (free chlorine, monochloramine, Fenton reagent, TiO$_2$), two levels of pH (5 and 8), and two levels of dose (low and high for each disinfectant: 0.5/1.5 mg/L Cl$_2$ in chlorination; 0.5/1.0 mg/L NH$_2$Cl in chloramination; 2.5/5.0 mM Fe$^{2+}$ in photo-Fenton reaction; 100/250 mg/L TiO$_2$ in TiO$_2$/UV photocatalytic inactivation). Each combination of pH, dose, and disinfectant were randomly assigned to a batch and replicated three times. Bacteria cell viability and DBP concentration were then measured. Average value and standard deviation were computed. In addition, three main effects (pH, dose, and disinfectant), three two-way interactions (pH and disinfectant, dose and disinfectant, pH and dose), and the three-way interaction between pH, dose and disinfectant were investigated. Main effects were examined when the interaction effects were not significant. Follow-up analyses were conducted with Tukey’s Honestly Significant Difference (HSD) adjustment for multiple comparisons when relevant. A significance level of 0.05 was used for all hypothesis tests. For ease of readability, dose and pH will be discussed for each disinfectant separately.
Figure 3.1 Shaking incubator for culturing bacteria

Figure 3.2 pH meter (Thermo Orioin, USA)
Table 3.1 Retention time of disinfection byproducts (DBPs) detected

<table>
<thead>
<tr>
<th>DBPs</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trihalomethanes</strong></td>
<td></td>
</tr>
<tr>
<td>Trichloromethane</td>
<td>6.210</td>
</tr>
<tr>
<td>Bromodichloromethane</td>
<td>9.668</td>
</tr>
<tr>
<td>Dibromochloromethane</td>
<td>17.764</td>
</tr>
<tr>
<td>Bromoform</td>
<td>26.786</td>
</tr>
<tr>
<td><strong>Haloacetonitriles</strong></td>
<td></td>
</tr>
<tr>
<td>Trichloroacetonitrile</td>
<td>8.227</td>
</tr>
<tr>
<td>Dichloroacetonitrile</td>
<td>10.714</td>
</tr>
<tr>
<td>Bromochloroacetonitrile</td>
<td>24.568</td>
</tr>
<tr>
<td>Dibromoacetonitrile</td>
<td>28.339</td>
</tr>
<tr>
<td><strong>Haloketones</strong></td>
<td></td>
</tr>
<tr>
<td>1,1-dichloropropanone</td>
<td>11.648</td>
</tr>
<tr>
<td>1,1,1-trichloropropanone</td>
<td>21.936</td>
</tr>
<tr>
<td><strong>Chloropicrin</strong></td>
<td></td>
</tr>
<tr>
<td>Chloropicrin</td>
<td>16.240</td>
</tr>
<tr>
<td><strong>Chlora hydrate</strong></td>
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<td>Chlora hydrate</td>
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</tr>
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</table>
4. CHAPTER FOUR

RESULTS

4.1 Chlorination

4.1.1 Bacterial Inactivation

Both *E. coli* K12 and *P. aeruginosa* PAO1 were effectively inactivated by free chlorine. The viability of both bacteria sharply decreased in the first five min, reaching 99.9% (3 log of cell viability reduction) of inactivation rate (Figure 4.1a and b). A constant extended phase followed with 99.999% (5 log of cell viability reduction) of bacteria inactivation rate observed after 80 min of chlorination. The pH effect was significant on inactivation rate of *E. coli* (Figure 4.1a) but not *P. aeruginosa* (Figure 4.1b). The cell viability of *E. coli* after 80 min was significantly higher at pH 8 than pH 5 (p = 0.0080). Increasing the chlorine dose from low to high promoted the inactivation rate for *E. coli* (p = 0.0210). For *P. aeruginosa*, a significant three-way interaction effect between pH, dose and disinfectant was detected with the higher chlorine dose resulting in a higher inactivation rate than the lower chlorine dose for pH 5 and 8 (p = 0.0152, p = 0.0117, respectively).

4.1.2 DBP Formation

The highest total DBPs (THMs, HANs, CH, 1,1-DCP) produced were observed in 1.5 mg/L Cl₂ at pH 8 from both *E. coli* and *P. aeruginosa* (9.63 ± 1.15 μg/L and 7.99 ± 0.08 μg/L respectively) (Figure 4.2a and b). All THMs were detected and were generally the dominant species, especially for *P. aeruginosa*. CH concentration was found to be the
second highest species for *E. coli* (0.19 – 2.91 μg/L) and *P. aeruginosa* (0.07 – 1.34 μg/L). The concentration of CH exceeded THMs only in 1.5 mg/L Cl₂ at pH 5 in *E. coli* solution. Even though bacteria cell component had relatively higher N/C ratio than humic acid because of a high N content, very limited amount of BCAN (0.07 – 1.02 μg/L) was measured. Although BCAN was the only HANs detected, it’s presence is important since it has a higher genotoxicity than other HANs like trichloroacetonitrile (TCAN) and DCAN (Plewa et al., 2007) and is regulated by the US Federal Drinking Water Guide (20 μg/L) (Plewa et al., 2007).

Regardless of dose, increasing pH also increased formation potential of THMs and CH of chlorinating *E. coli* (all p < 0.0001, Figure 4.2a). Water pH did not influence HAN formation potential (HANFP) of *E. coli* when dosing with 0.5 mg/L Cl₂ (p = 1.0000), however pH 8 significantly increased HANFP in 1.5 mg/L Cl₂ solution as compared to pH of 5 (p < 0.0001). Trace amounts of 1,1-DCP (0.26 ± 0.06 g/L) was measured from *E. coli* only in 1.5 mg/L Cl₂ solution at pH 5 (Figure 4.2a). The pH effect on THMs and CH formation potential of *P. aeruginosa* was similar as *E. coli* (Figure 4.2b).

We observed the chlorine demand (with initial chlorine dosage of 1.5 mg/L Cl₂) was significantly higher at pH 8 than pH 5 for both *E. coli* and *P. aeruginosa* (Table 4.1). The higher chlorine consumption under alkaline condition might contribute to the reaction with intermediates (Helbling and VanBriesen, 2007; Huang et al., 2013) generated from bacteria inactivation and thus produced DBPs. As shown in Figure 4.2, a significant three-way interaction effect between pH, dose, and disinfectant was detected in HAN and
CH formation, and residual dose favored formation of HANs and CH from *E. coli* at each of pH 5 (p = 0.0239, p < 0.0001, respectively) and pH 8 (p < 0.0001, p < 0.0001, respectively). Higher dosage promoted THM formation potential (THMFP) of *E. coli* at pH 8 (p < 0.0001) but not at pH 5 (p = 0.7334). For *P. aeruginosa*, higher chlorine dosage led to higher HAN and CH concentration at each of pH 5 (p = 0.0001, p < 0.0001, respectively) and pH 8 (p < 0.0001, p < 0.0001, respectively). Higher dosage also promoted THMFP of *P. aeruginosa* only at pH 8 (p < 0.0001) but not at pH 5 (p = 0.6445).
4.2 Chloramination

4.2.1 Bacterial Inactivation

Five log cell viability reductions were achieved for both bacteria (Figure 4.1c and d). *P. aeruginosa* tended to be more resistant to NH$_2$Cl than *E. coli*. No regrowth was observed within time frame for the two bacteria. PH 5 and pH 8 resulted in similar inactivation rate of *E. coli* ($p = 0.9992$). For *P. aeruginosa*, a significant three-way interaction effect between pH, dose, and disinfectant was detected, but no pH effect was found in either dosage of 0.5 mg/L NH$_2$Cl ($p = 0.9513$) or 1.0 mg/L NH$_2$Cl ($p = 0.8534$). Dosing also did not influence the inactivation rate of *E. coli* ($p = 0.9918$) or of *P. aeruginosa* ($p = 1.0000$).

4.2.2 DBP Formation

The DBPFP was low with no DBPs formed at pH 5. At pH 8, only THMFP of *E. coli* and THM and HAN formation potential of *P. aeruginosa* were detected, with no differences determined from dosing ($p = 1.0000$ for all comparisons).
4.3 Photo-Fenton Reaction

4.3.1 Bacterial Inactivation

Complete inactivation rate was measured for both bacteria after 1 hour of reaction under pH 5 with 99.999\% (5 log of cell viability reduction) of inactivation achieved at pH 8 (Figure 4.1e and f). The cell viability was reduced slowly for the first 20 min followed by an exponential increase in inactivation rate over the following 20 min (Figure 4.1e and f). Neither pH (p = 1.000 in 2.5 mM Fe$^{2+}$ and p = 0.6457 in 5.0 mM Fe$^{2+}$) nor dose (p = 0.2162 at pH 5 and p = 0.9923 at pH 8) influenced inactivation rate of \textit{P. aeruginosa} at the end of 80 min. pH did not influence inactivation rate of \textit{E. coli} (p = 0.1327), however, \textit{E. coli} cell viability in 5.0 mM Fe$^{2+}$/0.1 mM H$_2$O$_2$ was significantly higher than that in 2.5 mM Fe$^{2+}$/0.1 mM H$_2$O$_2$ at 80 min (p = 0.0002).

4.3.2 DBP Formation

No DBPs were detected from \textit{E. coli} and \textit{P. aeruginosa} solutions.
4.4 TiO$_2$/UV Photocatalytic Inactivation

4.4.1 Bacterial Inactivation

There was only 1 log of cell viability reduction of *E. coli* at pH 8, with significantly higher inactivation rate achieved with pH 5 (p < 0.0001) (Figure 4.1g). The inactivation rate of *E. coli* was not influenced by dose (p = 0.2875) at 80 min. Inactivation kinetics of *P. aeruginosa* with TiO$_2$/UV photocatalysis were similar as the photo-Fenton reaction, with the initial lagging phase of 20 min followed by 100% inactivation rate achieved by 80 min (Figure 4.1h). Regardless of dose, the pH 8 had a lower inactivation rate of *P. aeruginosa* that at pH 5 (p < 0.0001). The higher TiO$_2$ dose promoted greater inactivation of *P. aeruginosa* at pH 8 (p < 0.0001) compared to pH 5 (p = 1.0000), however, the best inactivation rate was achieved with pH 5 (Figure 4.1h).

4.4.2 DBP Formation

No DBPs were detected from *E. coli* and *P. aeruginosa* solutions.
4.5 Fluorescence Excitation-Emission Matrix

The fluorescence intensity of *E. coli* in water solution was relatively high in the aromatic protein regions, and soluble microbial byproduct-like region (Figure 4.3a). No obvious peaks were observed after chlorination (Figure 4.3b). Chloramination eliminated the aromatic protein peaks but the soluble-microbial byproduct like region remained (Figure 4.3c). Both peaks were remained after the photo-Fenton treatments but the intensities were lower than untreated samples (Figure 4.3d). TiO$_2$/UV eliminated the soluble microbial byproduct-like region and only aromatic protein peaks remained (Figure 4.3e).
Figure 4.1 Inactivation efficiency of *Escherichia coli* K12 and *Pseudomonas aeruginosa* PAO1 during 80 min in four different inactivation methods
Figure 4.2 Disinfection byproducts formation potential of (a) *Escherichia coli* K12 and (b) *Pseudomonas aeruginosa* PAO1 at 80 min in chlorination. (DBPFP: disinfection byproduct formation potential; THMs: trihalomethanes; HANs: haloacetonitriles; CH: chloral hydrate; DCP: 1,1-dichloro-2-propanone)
Figure 4.3 Fluorescence image of water samples with *Escherichia coli* K12 (a) in water and after 80 min treatments: (b) chlorination (c) chloramination (d) photo-Fenton and (e) TiO$_2$/UV
### Table 4.1 Residual chlorine at 80 min in chlorination

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<th>pH 8</th>
<th>pH 5</th>
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5. CHAPTER FIVE

DISCUSSION

5.1 Types of Disinfectants

These experiments demonstrated that the types of disinfectants, their dosage and solution pH can affect the rates of bacterial inactivation. Chlorination has the fastest inactivation and reached 99.9% inactivation for *E. coli* and *P. aeruginosa* in less than five minutes. Although chloramination is generally considered a weaker disinfectant (Chaurasia and Verma, 1995), it also reached 99.9% disinfectant inactivation in a relatively short time of 20 min. However, as AOP, photo-Fenton reaction and TiO$_2$/UV required longer contact times, with no or very low inactivation occurring in the first 20 min of the treatments (Fig. 1e, f, g and h). Inactivation slowly proceeded but did reach 100% by 80 min., which was better than chlorination and chloramination. In general, the rate of cell viability reduction was greatest in chlorination, then chloramination, then Photo-Fenton, and with TiO$_2$ being the slowest.

The differences in inactivation kinetics may be due to different types of reactive species involved in the inactivation. In chlorination, hypochlorite (OCl$^-$ and HClO) are the reactive species in inactivation. Fast inactivation rate of bacteria was often measured when adding free chlorine in the water solution (Haas et al., 1980). The damage of components of both the outer and inner membranes was observed in chlorination (Jacangelo et al., 1987; Young and Setlow, 2003) within a few minutes after the reaction began. Chloramine is thought to be a weaker bactericide and produce a more stable
residual than free chlorine (Worley et al., 1983; Zhang and DiGiano, 2002). For AOP, ·OH is considered the major reactive species in both photo-Fenton and TiO₂ photoa
talytic inactivation, which is a stronger oxidant than HClO. However, lag phase is always observed partly because of the shortage of catalyze Fe (II) or Fe (III) involving in ·OH generation (Kavitha et al., 2004). The lag phase phenomenon also results in bacterial inactivation delay. Notably, ·OH is a strong oxidative species with oxidation potential of 2.80 (eV) and it favors reaction with cell wall components before penetrating through the protective barrier (Rice-Evans and Gopinathan, 1995). The diffusion of free chlorine into bacteria cells is less retarded by oxidation of the cell wall and membrane constituents (Cho et al., 2010). Hence not until the integrity of the cell wall is compromised, will the intracellular component be targeted by ·OH. In addition, Huang et al., (2000) documented that pure cell wall damage could be repaired during subculture of cell on agar plates and thus does not necessarily mean the reduction of microbe viability. Therefore, even though cell all damage occurs immediately in photocatalytic treatment, it was mediated by the recovery ability of microorganism (Huang et al., 2000; Dunlop et al., 2002) which potentially lead to the appearance of the lag phase in photo-Fenton reaction and TiO₂/UV photocatalytic inactivation.
5.2 Dosages of Disinfectants

An increase of disinfectant dosage generally increases the inactivation efficiencies in chlorination, photo-Fenton reaction but not in chloramination and TiO$_2$/UV inactivation. However, excess dosage should be avoided because the operational cost is proportional to the amount of disinfectants applied. In addition, protein release, lipid peroxidation and cell permeability change all occurs during chlorination of bacteria (Cho et al., 2010) and thus excess disinfectants can increase the formation of DBPs in finished water, particularly in chlorination. Extra disinfectants of chlorine and photo-Fenton reagent were assumed to further breakdown the cellular structures, releasing intracellular materials into water. Cellular materials have been known a DBP precursor (Wang et al., 2013). Excess chlorine can react with organic matter to form a variety of DBPs. In particular, organic matters with a low C-N ratio are precursors of more toxic nitrogenous DBPs such as NDMA (Wang et al., 2012b). Some DBPs such as 3-methyl-2,4-hexanedione and dihydro-4, 5-dichloro-2(3H) furanone are identified in TiO$_2$/UV treatments (Richardson et al., 1996), but overall the risks on DBPs are much lower than chlorination and chloramination. In general, the risks of DBP formation from a descending order are Photo-Fenton > TiO$_2$ > chloramination > chlorination.
5.3 The pH Effect

In the present experiment a greater inactivation was observed in pH 5 rather than 8 in all tested disinfection processes. For chlorination, higher inactivation rate is the result of dominant of HClO species under acidic pH. HClO is about 80 to 100 times more effective at killing *E. coli* than is OCl⁻ which is dominant at higher pH (Scherson et al., 2008). The pH affects the photo-Fenton reaction by ·OH formation with the presence of Fe (II) or Fe (III) under acidic pH. In addition, the decrease of Fe (II) and Fe (III) in water through precipitation at alkaline pH prolongs the lag phase during photo-Fenton reaction (Kavitha et al., 2004).

The interaction of bacteria cells and UV-illuminated TiO₂ particles was correlated with pH in the solution. The TiO₂ particles were at the point of zero charge, named isoelectric point (Hoffmann et al., 1995; Ku and Jung, 2001) at pH range 6.2-7.5. Hence the particles surface became electronegative and repulses the bacteria particles (negative charged) when the pH was higher than the range. While at pH lower than the range, there is an attraction between TiO₂ and bacteria particles. Notably, this mechanism is still under debate. Some supported (Watts et al., 1995) no pH effect while other studies (Herrera et al., 2000; Nadtochenko et al., 2005) detected the higher inactivation rate at lower pH. It is assume that under our experiment conditions, surface-bound ·OH (Cho et al., 2004) played a critical role in bacteria inactivation rather than free ·OH in the solution. Thus the higher pH remarkably hinders the inactivation of *E. coli* and *P. aeruginosa*. 
For DBP formation, pH affects chlorination in several ways: (1) the inactivation efficiency (2) halogenation ability of chlorine species in reaction with organic precursors (Hua and Reckhow, 2007; Fang et al., 2010; Wang et al., 2012b); (3) the formation and decomposition rate of DBPs (Hua and Reckhow, 2008). Protein release, lipid peroxidation and cell permeability change all occurs during chlorination of bacteria (Cho et al., 2010) and the material can react with chlorine contributing the DBP formation (Wang et al., 2013). We also observed the chlorine demand (with the same initial chlorine dosage) was significantly higher at pH 8.0 than pH 5.0 (Tab. 4.1). The higher chlorine consumption under alkaline condition might contribute to the reaction with intermediates (Helbling and VanBriesen, 2007; Huang et al., 2013) generated from bacteria inactivation and thus produced DBPs. Stable C-DBPs of THMs and CH generating from both DOC and DON source were reported to be accumulated under alkaline environment rather than in acidic condition (Hua and Reckhow, 2007; Hua and Reckhow, 2008; Fang et al., 2010; Hansen et al., 2012). The pH effect on HAN formation potential was relatively complex due to the balance of the formation and alkaline based-hydrolysis formation (Reckhow et al., 2001; Hua and Reckhow, 2007; Fang et al., 2010), together with the formation reaction from both organic and inorganic nitrogen (Lee et al. 2007). Hua and Reckhow (2008) found the HAN decomposition speed exceeded formation rate after 30 minute of chlorination at pH 10. Besides chlorination, the pH can also alter speciation of chloramines (Yang et al., 2007): with the dominant species of NHCl₂ at pH 4-7.5 and NH₂Cl at pH 7.5-9.0, respectively. The DBPF of each species is distinctively dependent on pH.
Among the four disinfection methods, chlorine was a rapid and effective inactivation method but a variety of DBPs including THMs, HANs, and CH were generated from reaction between microbe organic precursors even no other precursors such as humic acid existed in water. Chloramination is a good alternative disinfection method because five log cell viability reductions were observed in relatively short time with only limited amount of THMs and HANs formed. However, further studies are needed to investigate other emerging DBPs such as NDMA. In spite of the lag phase in the treatment processes, both photo-Fenton reaction and TiO$_2$/UV photocatalytic inactivation (except at pH 8) present high rate in complete microbe inactivation and no DBPs detected at 80 min. Although the photo-Fenton reaction has been less studied in inactivation rate due to its dependence on water parameters; it was successfully proved in this experiment to guarantee microorganism (E. coli K12 and P. aeruginosa PAO1) safety and prevent DBPs under pH range of 5-8. The heterogeneous TiO$_2$/UV photocatalytic process is regarded as a promising inactivation method and is frequently used in lowering microorganisms risks. However, pH 8 significantly hinders inactivation efficiency in TiO$_2$/UV treatment. Besides, there potential adverse health effect related to TiO$_2$ nanoparticles applied in finished water which requires further exploration.
7. CHAPTER SEVEN

REFERENCES


