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Harmful algae, algal toxin, taste and odor control and mitigation in public water system

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HARMFUL ALGAE, ALGAL TOXIN, TASTE AND ODOR CONTROL AND MITIGATION IN PUBLIC WATER SYSTEM

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ABSTRACT

Recent years, harmful algal blooms occurrence has increased quickly in the surface water worldwide, which has become a concern for drinking water plants due to the ability of toxic algae (cyanobacteria) to produce cyanotoxins including microcystins (MCs), mainly MC-LR, cylindrospermopsin (CYN), and taste and odor (T&O) compounds. Various types of chemicals are widely used in drinking water treatment plants as oxidants for treating source water challenged with harmful algal blooms. In this study, the release and degradation of intracellular MC-LR due to oxidation of *Microcystis aeruginosa* (*M. aeruginosa*), most common specie of cyanobacteria, was examined kinetically. Effect of water matrix and cell concentrations on the release and degradation of CYN as a result of chlorination of *Cylindrospermopsis raciborskii (C. raciborskii)* was examined in two lake water serving as drinking water resources. Furthermore, removal efficiencies of free chlorine, chlorine dioxide, permanganate, and peracetic acid (PAA) were compared for controlling *M. aeruginosa*, *C. raciborskii*, and related cyanotoxins, i.e., MC-LR and CYN. At the same time, the disinfection byproduct (DBPs) formation during oxidations of cyanobacteria and cyanotoxins were investigated. Furthermore, several T&O events occurred in Missouri drinking water systems were studied and the major cause of the T&O was 2,4,6-trichloroanisole (2,4,6-TCA), a compound with extremely low taste threshold (i.e., 0.3 ng/L). Thus, the resource/precursor(s) of 2,4,6-TCA and its formation and removal in drinking water treatment systems were investigated. These results provided essential information for utilities to select suitable chemicals and dosages to control harmful algal bloom, DBPs formation and T&O issues.

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1. INTRODUCTION

1.1. FORMATION AND TOXICITY OF CYANOBACTERIA AND CYANOTOXINS

Cyanobacteria (blue-green algae) were among the earliest organism on Earth, which are frequently associated with harmful algal blooms (HABs) (Merel et al., 2013). However, the increasing human-induced eutrophication of surface water and global warming lead to more and more occurrence of cyanobacterial blooms since 1960s (Miao and Tao, 2009; Huisman et al., 2018). HABs are a major environmental problem worldwide and has been founded in all 50 states based on data from the United States Environmental Protection Agency (US EPA). Cyanotoxins are metabolites produced by cyanobacteria which have more than 2600 cyanobacterial species been described (Westrick et al., 2010; Tokodi et al., 2018). Meanwhile, taste and odor (T&O) compounds producers have been identified from several cyanobacteria species (Jo et al., 2014; Suurnakki et al., 2015). There are three potential routes that human exposure to cyanotoxins including the ingestion of cyanobacteria-based food ingredients or shellfish containing bioaccumulated toxins through contaminated water, dermal contact and accidental inhalation/ingestion during recreational activities in waters subjected to a toxic algal bloom, and the ingestion of drinking water produced from a contaminated resource (Merel et al., 2013).

Microcystins (MCs), a family of cyclic heptapeptides, are the most prevalent class of cyanotoxins and strongly heptatoxic, causing liver damage, and initiate tumor-promoting activity because they could inhibit protein phosphatizing (Miao and Tao, 2009). MCs consist of several D-amino acids and two unusual amino acids namely the N-

methyldehydroalanine (Mdha) and hydrophobic b-aminoacid, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda), and the chemical structure of MCs is shown in Figure 1.1. X and Z are referred to as variable amino acids which multiple combinations make the difference between more than 240 variants of the toxin with a molecular weight of approximately 1000 g/mol (Merel et al., 2013; Minasyan et al., 2018). Among MCs, MC-LR is the most abundant cyanotoxin worldwide, which has leucine (initial L) and arginine (initial R). There are several species of cyanobacteria could produce MCs including *Microcystis*, *Anabaena, Planktothrix*, *Nostoc*, and *Anabaenopsis* etc (Westrick et al., 2010). During August 2104, the MCs concentration in drinking water supply for Toledo, OH, USA exceeded the WHO guideline value for safe drinking water resulting over 400,000 people being without tap water for more than 2 days (Huisman et al., 2018). More than 60% of the lakes in China have undergone cyanobacterial blooms in last two decades, and *Microcystis aeruginosa* (*M. aeruginosa)* is the dominant toxins producer (Sukenik et al., 2017).

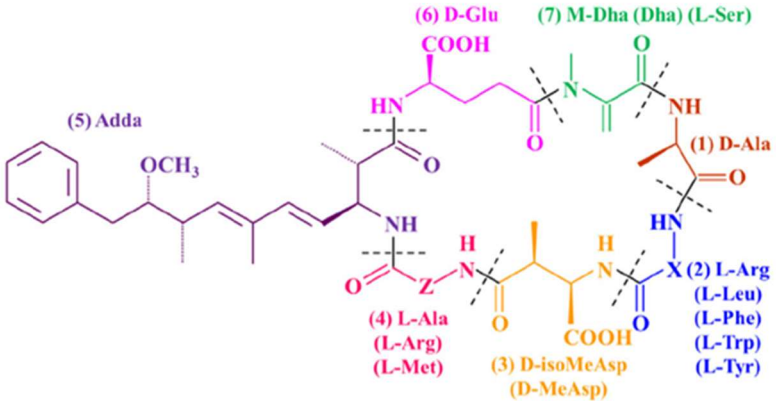

Figure 1.1. Chemical structure and molecular weight of microcystins.

Table 1.1. EPA advisory values for cyanotoxins.

Type of notice	Do not drink: children under 6 and sensitive population	Do not drink: Children 6 and older & adults	Do not use
Microcystins (MCs)	0.3 µg/L (EPA Health Advisory value)	1.6 µg/L (EPA Health Advisory value)	20 µg/L (Ohio EPA's Public Water System)
Cylindrospermopsin (CYN)	0.7 µg/L (EPA Health Advisory value)	3 µg/L (EPA Health Advisory value)	20 µg/L (Ohio EPA's Public Water System)

Cylindrospermopsin (CYN), an alkaloid hepatotoxin, is a problematic cyanotoxin containing a tricyclic guanidine moiety bridged to a hydroxymethyluracil group, which is considered to be the origin of toxicity (Yan et al., 2016). CYN was known as a tropical toxin which has been initially detected in Australia, New Zealand, and Thailand (Merel et al., 2013). However, recent studies have identified its occurrence in temperate climates including Germany, Italy, France, China, and United States (Graham et al., 2010; Vico et al., 2016; Yan et al., 2016). There are several species could produce CYN including *Cylindrospermopsis raciborskii (C. raciborskii)*, *Aphanizomenon ovalisporum*, *Raphidiopsis curvata* and *Umezakianatans* (Senogles et al., 2000; Rodriguez et al., 2007c). In Australia, more than 100 children were sent to the local hospital for gastroenteritis associated with the consumption of contaminated drinking water which was contaminated by CYN related HAB in 1979 (Merel et al., 2013). The acute and chronic toxicity of CYN

are associated with protein inhibition, DNA damage, and carcinogenic activity (de la Cruz et al., 2013).

The UA EPA has included MC-LR and CYN in the Contaminant Candidate List 3 (CCL 3) and current CCL4 list with guideline and recommendation levels (Table 1.1). Several states have implemented standards or guidelines that apply to cyanotoxins and cyanobacteria in drinking water using risk assessment methods. The world health organization (WHO) guidance values for the relative probability of acute health effects during recreational exposure to cyanobacteria and the probability of microcystins concentrations are shown in Table 1.2.

Table 1.2. The WHO guidance values for cyanobacteria.

Relative probability of acute health effects	Cyanobacteria (cells/mL)	Microcystin-LR (µg/L)	Chlorophyll-a (µg/L)
Low	<20,000	<10	<10
Moderate	20,000-100,000	10-20	10-50
High	100,000-10,000,000	20-2,000	50-5,000
Very High	>10,000,000	>2,000	>5,0000

1.2. CONTROL OF HARMFUL ALGAL BLOOMS

In general, various technologies include nutrient control and physical, biological and chemical strategies are currently used to treat cyanobacterial issues by drinking water treatment plant (DWTP) (Fan et al., 2016). These treatment technologies including coagulation, flocculation and filtration might be effective at removing intracellular toxins

with intact cells without causing additional release of intracellular toxins (Fan et al., 2013). However, these treatment processes are inefficient at removing extracellular toxins and T&O compounds. Various kinds of oxidants including chlorine (Cl_2), permanganate (MnO_4^-), chlorine oxide (ClO_2), monochloramine (NH_2Cl), and ozone (O_3), have been widely used as efficient disinfectants in DWTPs. They are also regarded as effective pre-oxidants and oxidants which could improve the overall removal of cyanobacterial cells and related cyanotoxins (Fan et al., 2016), but the reaction rates and dosage demands are depending on the disinfectant species and water matrix.

Chlorine as a commonly used oxidant in water treatment plants has shown the potential to lyse cyanobacterial cells and oxidation of cyanotoxins. The reaction between Cl_2 and MC-LR fit the second-order reaction with the rate constant at 20 ^oC varied from 475 $M^{-1}s^{-1}$ at pH 4.8 to 9.8 $M^{-1}s^{-1}$ at pH 8.8 (Acero et al., 2005). There was no accumulation of extracellular toxins during chlorine treatments which may be due to fast toxin oxidation rates (Fan et al., 2014). The oxidation of the MCs was related to the chlorine concentration with the ease of oxidation following the trend: MC-YR > MC-RR > MC-LR ≥ MC-LA (Ho et al., 2006). The reaction of MnO_4^- and MCs is second odor and the reaction rate constant for MC-LR, MC-RR and MC-YR at pH 7 and 20 ^oC was about 357, 418 and 405.9 $M^{-1}s^{-1}$, respectively (Rodriguez et al., 2007a). However, MnO_4^- did not have impact on cyanobacterial membrane integrity (Fan et al., 2014). There are two steps of MnO_4^- on *M. aeruginosa* inactivation: first, reacted with dissolved and cell-bound extracellular organic matter and resulted in a minor loss of cell viability and MC-LR release, then approached the inner layer of the cell wall and resulted in a rapid decrease of cell viability (Li et al.,

2014). Therefore, the MC-LR releasing rate was generally much slower than its degradation rate during MnO_4^- treatment.

Second-order rate constants measured in pure aqueous solutions could be used to predict the toxin oxidation efficiency of O_3 and ClO_2 for MC-LR and CYN (Rodriguez et al., 2007b), which are shown in Table 1.3. The carbon−carbon double bond in the uracil ring of CYN was found to be most susceptible to attack by ozone, and the ozonation products did not exhibit measurable cytotoxicity to human cells (Yan et al., 2016). O_3 and Cl_2 were determined to be highly effective for the inactivation of the cyanobacteria, *C. raciborskii*, but ClO_2, NH_2Cl, and MnO_4^- were only marginally effective (Cheng et al., 2009). Therefore, systemic studies are needed to better understanding the effect of water matrix on chlorine demand for cyanobacteria including *M. aeruginosa* and *C. raciborskii* in natural water system. With the exist of different level of ammonia or organic matters in source water for DWTPs, the releasing and degradation rates of MC-LR and CYN are still not very clear.

Treatment of source water containing toxic cyanobacterial cells with different oxidants might cause cell damage leading to the algal organic matters releasing out. These organic matters and natural organic matters could react with disinfectants and form disinfection byproducts (DBPs) (Fang et al., 2010). Not only carbonaceous disinfection byproducts (C-DBPs) such as trihalomethanes (THMs), halogen acetaldehyde (HAs), haloketone (HKs), and haloacetic acids (HAAs), but also nitrogenous disinfection byproducts (N-DBPs) such as haloacetonitriles (HANs), haloacetamides (HAcAms), and halogenated nitromethane (HNMs) were formed during *M. aeruginosa* chlorination (Qi et al., 2016). Both THMs and HAAs are regulated by US EPA for tap water of DWTPs with

a limit at 80 and 60 µg/L, respectively. Therefore, it is necessary to study the DBPs formation when disinfectants are applied for HAB control in DWTP, especially the DBPs formation and speciation with *C. raciborskii* which are still not clear.

Table 1.3. Kinetic database for the reaction of oxidants with cyanotoxins (Rodriguez et al., 2007b).

Oxidant	MC-LR			CYN	
	$k_T(m^{-1}s^{-1})$	pH 8	pH 8	pH 8	pH 8
		$k_{app}(m^{-1}s^{-1})$	$t_{1/2}$	$k_{app}(m^{-1}s^{-1})$	$t_{1/2}$
H^++HOCl	$2.07*10^7$	N/A	N/A	N/A	N/A
HOCl	116	33	24.8 min	490	1.7 min
OCl^-	6.78	N/A	N/A	N/A	N/A
NH_2Cl	N/A	<1	>14 h	<1	> 14h
O_3	$4.1*10^5$	$4.1*10^5$	0.08 s	$3.4*10^5$	0.10 s
·OH	$1.1*10^{10}$	$1.1*10^{10}$	5 min	$5.5*10^9$	10.5 min
MnO_4^-	357	357	5.2 min	0.3	4.2 d
ClO_2	N/A	1	13.1 h	0.9	14.4 h

Photodegradation promoted by halogen radicals has been investigated for MCs degradation in freshwater (Parker et al., 2016). MCs are relatively stable under sunlight illumination due to the low spectral overlap between their absorbance and solar radiation (Tsuji et al., 1994). The presence of photochemical sensitizers such as humic substances and algal pigments in water enhance the photodegradation of MCs (Song et al., 2007; Yan et al., 2014). Photodegradation of CYN is highly dependent on UV-A (400-320 nm) and was very low under natural conditions, which leads to the high extracellular CYN

concentration found in diverse waterbodies (Wormer et al., 2010). Advanced oxidation processes (AOPs) using H_2O_2, O_3/H_2O_2, O_3/Fe(II), TiO_2, and Fenton treatment were investigated for the degradation of cyanotoxins (Al Momani et al., 2008; Sharma et al., 2012). Conventional oxidants, such as chlorine, are selective as to which compounds they can degrade, whereas AOPs are able to react with a great variety of organic compounds. However the practical implementation of AOPs technology in water treatment plants is still limited and at lab scale (Matafonova and Batoev, 2018).

1.3. OCCURRENCE, FROMATIONTION AND CONTROL OF T&O COMPOUNDS

Most of the complaints of the consumers received by the water utilities are related to the bad T&O of the drinking water, which would let customers associated them with the quality and safety of drinking water (Antonopoulou et al., 2014). The treatment and control of T&O compounds has become a priority task for the DWTPs. Compounds from natural origin, disinfection byproducts formed during water treatment processes, chemicals leached from pipes, industrial and sewage effluents, and leachates from poor waste disposals could all leads to the T&O issues (Quintana et al., 2016).The presence of 2-Methylisoborneol (MIB) and geosmin (GEO) in public water supplies is the main cause of the earthy–musty taste and odor that renders drinking water unpalatable around the world (Burgos et al., 2014). MIB and GEO are normally produced by certain species of actinomyces, fungi and blue-green algae (Salto et al., 2008). Possibilities of mutagenicity and hepatotoxicity by MIB and GEO have been pointed out, but it is not enough to cause health concerns at the T&O threshold. The T&O compound 2,4,6-TCA has been rarely

reported in DWTPs (Bai et al., 2017; Zhang et al., 2018), which is known for causing cork taint in different wines (Marquez-Sillero et al., 2012). While, 2,4,6-TCA could be formed througth the reaction between sodium hypochloride and anisoles, and the formed chloroanisoles include 2,4-dichloroanisole (2,4-DCA) and 2,6-chloroanisole (2,6-DCA) (Zhang et al., 2016). However, the prodcution rate of 2,4,6-TCA was only about 0.4 % under its favorite formation conditions, i.e., low pH (pH=3), and long reaction time (24 hours) (Zhang et al., 2016). Previous researches demonstrated that 2,4,6-TCA are formed by microbiological methylation of halophenols during water treatment or during transport through the distribution system (Benanou et al., 2003; Fontana and Altamirano, 2010; Zhang et al., 2017b). It has been reported in drinking water distribution system, that the microbial O-methylation of precursor 2,4,6-trichlorophenol (2,4,6-TCP) in biofilms is the dominant mechanism of 2,4,6-TCA formation, which would be affected by many water distribution factors, e.g., pipe material, temperature, flow velocity, and residual chlorine (Wang et al., 2014; Zhang et al., 2017b).

Most of the conventional treatment processes in DWTPs including coagulation, sedimentation and chlorination have been found to be ineffective for removal of T&O compoundss (Srinivasan and Sorial, 2011). Only a few water treatment technologies have been successfully applied to remove T&O compounds from water including the use of ozonating, granular activated carbon (GAC), powered activated carbon (PAC), biofiltration, and polymer (Rashmawi et al., 2008; Antonopoulou et al., 2014). There is a good linear relationship between the adsorption capacities for MIB and geosmin and the micropore volumes, but the relations with iodine number and methylene blue number wee insignificant (Yu et al., 2007). GEO was removed to a greater extent than MIB using GAC

(Zamyadi et al., 2015). Based on previous study, the biological activity on the surface of GAC enhanced the removal of T&O compounds (Zamyadi et al., 2015). It was also founded that adsorption of MIB decreased with the alum dose increasing since the flocs incorporating PAC into their structure, reducing the efficiency of mixing, and the bulk diffusion kinetics for the MIB molecule (Ho and Newcombe, 2005). During PAC absorption, the presence of NOM reduces the equilibrium capacity and kinetics of absorption for T&O compound (Newcombe et al., 2002). However, no publication has focused on the PAC absorption of 2,4,6-TCA in DWTPs for now.

1.4. RESEARCH OBJECTIVE

This work aims to improve the understanding about the releasing and degradation of cyanotoxins with related cyanobacteria using different oxidants and the occurrence, formation and removal of T&O compounds in drinking water systems. The whole work including three parts:

(1) Studying the effect of oxidant demand on the release and degradation of MC-LR from *Microcystis Aeruginosa* during oxidation, and kinetic releasing rate of MC-LR from *Microcystis Aeruginosa* in the water with and without addition of ammonia.

(2) Investigating the release and removal of CYN from *Cylindrospermopsis raciborskii* with various oxidants in different drinking water sources. Two different source water were used for the study and the releasing of CYN was investigated.

(3) Investigating the occurrence, formation pathway, and control with different species of powder activated carbons of T&O Causing Compounds 2,4,6-trichloroanisole in drinking water systems.

PAPER

I. EFFECT OF OXIDANT DEMAND ON THE RELEASE AND DEGRADATION OF MICROCYSTIN-LR FROM *MICROCYSTIS AERUGINOSA* DURING OXIDATION

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ABSTRACT

In this research, the release and degradation of intracellular microcystin-LR (MC-LR) due to oxidation of *Microcystis aeruginosa* (*M. aeruginosa*) was examined kinetically. Brief exposure to free chlorine with no measureable oxidant exposure was demonstrated to be sufficient to induce rapid release of intracellular MC-LR from *M. aeruginosa*. Thus,

in a water treatment plant, there is currently no level of prechlorination that can be assumed to be safe, since very low preoxidation prior to filtration and no measureable free chlorine residual may still observe the release and buildup of extracellular MC-LR. Higher chlorine dosages resulting in a measureable exposure or CT (concentration times contact time) cause more rapid release and oxidation of the intracellular toxins. Further, the rate of release of MC-LR with intermediate oxidant dosages were shown to be initially rapid, but then slowed to a lower release rate due to an as yet undetermined mechanism. While free chlorine was reactive with the extracellular MC-LR, the monochloramine resulting from the consumption of the free chlorine by ammonia was not. Consideration of the ammonia concentration and the chlorine dosage relative to the chlorination breakpoint dosages is important for utilities assessing the impact of prechlorination of water containing cyanobacteria. MC-LR, once released, was rapidly oxidized by permanganate resulting in only negligible buildup of extracellular toxins.

Keywords: *Microcystis aeruginosa,* Microcystin-LR, Chlorination, Toxin release rate, Permanganate, Cell lysis.

1. INTRODUCTION

Cyanotoxins in water sources provide a risk to the public upon entering water treatment plants in either extracellular or intracellular forms. Microcystins generated by *Microcystis aeruginosa* (*M. aeruginosa*) is the most abundant and common species (Westrick et al., 2010; Zamyadi et al., 2013a). Based on health advisories developed by United State Environmental Protection Agency (USEPA), microcystins concentration in drinking water should be lower than 0.3 µg/L for bottle-fed infants and pre-school children,

and lower than 1.6 µg/L for school-age children and adults (USEPA, 2015). Many water treatment plants require chemical preoxidation to achieve disinfection credits, manganese or iron removal, taste and odor control, or other objectives (Svrcek and Smith, 2004). At the same time, post-filtration oxidation is also used to remove extracellular toxins for water impacted by cyanobacteria (Ho et al., 2010; Zamyadi et al., 2012a; Merel et al., 2013).

A variety of studies have been conducted on the effects of oxidants releasing cyanotoxins from cyanobacterial cells via diffusion through, or actual rupture of, the cell membrane, though studies on brief non-measurable oxidant exposures have not been reported. Common oxidants used in water treatment that can cause release of toxins include free chlorine (HOCl, OCl^-) (Cheng et al., 2009; Ding et al., 2010; Ma et al., 2012; Zamyadi et al., 2013a), permanganate (MnO_4^-) (Fan et al., 2014; Li et al., 2014), ozone (O_3) (Fan et al., 2013), chlorine dioxide (ClO_2) (Ding et al., 2010; Zhou et al., 2014), and monochloramine ($NClH_2$) (Ding et al. 2010). Rates of oxidation of extracellular cyanotoxin range from very fast to recalcitrant depending on the oxidant and cyanotoxin type, as well as water quality conditions such as pH, temperature, and other factors (Zamyadi et al., 2013b; Stanford et al., 2016). Whether the concentration of intracellular cyanotoxin builds up in water phase upon release from a cell is dependent on the relative rates of toxin release from the cell versus the rate of oxidation of the extracellular toxin. While the rates of oxidation of extracellular toxins have been generally well characterized (Rodriguez et al., 2007b; Sharma et al., 2012), the effects of oxidants in relation to water quality parameters on the rate of cyanotoxin release from cells is not well understood.

Chlorine is a commonly used oxidant/disinfectant used in water treatment and undergoes a number of reactions including with ammonia, iron, manganese, natural organic

matter (including humic substances) and other substances (Zamyadi et al., 2013a). Chlorine decay in water treatment is often characterized by a fast demand that takes seconds or minutes followed by a slower often pseudo-first-order decay over minutes or hours (Hua et al., 1999). Rates of reaction with ammonia are especially rapid with half-lives on the order of seconds (Qiang and Adams, 2004). While free chlorine has been shown to cause *M. aeruginosa* cells to lyse (Ma et al., 2012; Zamyadi et al., 2013a), monochloramine on the other hand has less impact on cell lysing (Ding et al., 2010) under common exposure conditions. Therefore, if the oxidant is consumed rapidly (e.g., chlorine reaction with ammonia), then the oxidant reactive with the toxin may be replaced by a much slower oxidant (e.g., monochloramine), thus allowing the cell to release toxins that are not degraded, but will build up in aquatic environment.

Systemic studies are needed to better understanding the effect of water matrix on chlorine demand for cyanobacteria in natural water system. For free chlorine and ammonia, this hypothesis leads directly to consideration of the breakpoint chlorination chemistry such that chlorine dosages less than the breakpoint dosages (i.e., approximately 7.6 mg Cl_2/mg NH_3) will result in toxin release without subsequent rapid oxidation of the toxins. When chlorine dosages is higher than the breakpoint (including all rapid oxidant demand), it should result in a free chlorine residual and concurrent oxidation of the extracellular toxins. The purpose of this work was to test this hypothesis and study the importance of oxidant demand (specifically ammonia) on cell lysing, toxin release and degradation during oxidation.

2. MATERIALS AND METHODS

2.1. MATERIALS AND REAGENTS

Microcystin-LR (MC-LR) was purchased from Sigma-Aldrich Corporation (Milwaukee, WI, USA). Saline (0.9% NaCl) solution used for washing cells was prepared by dissolving sodium chloride (NaCl) in ultra-high purity water, which was produced with an ELIX-3 water purification system (Millipore, Billerica, MA, USA). The NaClO stock solution used for chlorination was prepared by dilution of 5% sodium hypochlorite solution (Fisher Scientific, Somerville, NJ, USA) and exact concentration was measured by *N,N*-diethyl-*p*-phenylendiamine (DPD) method (described below) before using in oxidation experiments. Ammonium hydroxide was purchased from Sigma-Aldrich (St. Louis, MO, USA). The permanganate stock solution was prepared by dissolving potassium permanganate (Fisher Scientific, Fair Lawn, NJ, USA) in ultra-high purity water.

2.2. CELL CULTURE AND PREPARATION

Microcystis aeruginosa (UTEX LB 2388) was purchased from Culture Collection of Algae at The University of Texas at Austin (Austin, TX, USA). The BG-11 growth media was purchased from Sigma-Aldrich (Saint Louis, MO, USA). Details on cell culture and saline washing procedures are provided in Ding et al., (2010). Cell concentrations were determined by direct counting with a hemacytometer (with an Olympus IX51 inverted microscope). The water sample for the oxidation tests was collected from Missouri River in Jefferson City, Missouri (USA) and filtered through 0.45 μm nylon membrane filter (GVS, Sanford, ME, USA) prior to use in experiments. All chlorination experiments were conducted in amber glass bottles at room temperature (24±1 ^{o}C).

Cell viability was monitored using the fluorescent assay agent Live/Dead SYTO 9 (Invitrogen Corporation, Carlsbad, California, USA) and observed using an Accuri C6 flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) by following the manufacture's instruction during the treatment processes. Green fluorescent in channel FL1 (530 nm) and red fluorescent in channel FL3 (630 nm) were measured and indicated viable and nonviable cells, respectively.

2.3. ANALYTICAL METHODS

Microcystins analysis - A Shimadzu ultra-fast liquid chromatography (UFLC) system (Columbia, MD, USA) including two pumps (LC-20 AD XR), an autosampler (SIL-20AC XR), an online degasser (DGU-30A3), and a column oven (CTO-20A) with a Synergi MAX-RP column (150×2.00 mm, 4 μm) was used for microcystins separation. The sample injection volume was 20 μL. Mobile phase A and B were ultra-high purity water and methanol with 2 mM ammonia formate and 3.6 mM formic acid, respectively. The elution flow rate was 0.2 mL/min with a gradient elution program as follows: 20% mobile phase B for 2 minutes, ramped to 60% B in 2 minutes, then increased to 17% over 8 minutes, hold at 90% B for 1 minute and then returned to 20% B in 0.1 minutes, and equilibrated at 20% B for 5 more minutes before next injection. A 4000Q Trap mass spectrometer (AB Sciex, Foster City, CA, USA) was used for quantitation. The method detection limit (MDL) for MC-LR was 20 ng/L which were determined as the lowest injected standard that gave a signal-to-noise (S/N) ratio at 3-5.

Water parameter monitoring – Dissolved organic carbon (DOC) measurements were performed using a Shimadzu Model TOC-L TOC analyzer (Shimadzu, Overland

Park, KS, USA). pH was measured on a ORION 3 STAR Benchtop pH meter (Thermo Electron Corporation, US) which was calibrated with pH 4, 7 and 10 standard buffers (RICCA Chemical Company, TX, US). Free ammonia concentration was tested by HACH TNTplus^{TM} 830 Ammonia Method 10205. Alkalinity and total hardness were determined with Hach Phenolphthalein and Total Alkalinity Method 8203, and titration using EDTA Method 8213, respectively. The water collected for this study had a DOC concentration of 3.47 mg/L, ammonia (NH_3-N) of 0.04 mg/L, alkalinity of 174 mg/L as $CaCO_3$, and total hardness of 328 mg/L as $CaCO_3$.

Oxidant measurement - Free chlorine, total chlorine and monochloramine (MCA) concentrations were determined using Hach USEPA DPD Method 8021 (Loveland, CO, USA), Hach USEPA DPD method 8167, and Hach Nitrogen, Free Ammonia and Chloramine (Mono) Method 10200, respectively.

Haloacetic acid (HAA5) and trihalomethanes (THM4) analyses – HAA5 concentrations were determined with a recent published high performance ion chromatography (HPIC)-MS/MS method (Runmiao Xue et al., 2016) using the same UFLC-MS/MS instrument for microcystins analysis. THM4 were analyzed by following the method developed by Shi and Adams (2012).

2.4. OXIDATION EXPERIMENTS

Prior to starting an experiment, specific concentrations of suspended cells were added into 250 mL of filtered Missouri River water to achieve a 2×10^6 cells/mL suspension (~ 40 µg/L total MC-LR). For the free chlorine treatment, a specific amount of chlorine stock solution was added into the cell suspensions and immediately tumbled to achieve

specific oxidant concentrations of 0, 0.5, 1.5, 4, 6 and 10 mg/L as Cl_2 for no-ammonia experiments and 0, 0.5, 1.5, 4, 6, 10, 14 and 17 mg/L as Cl_2 for ammonia-added experiments. For the permanganate experiments, a specific amount of potassium permanganate stock solution was added into the cell suspensions and immediately tumbled to achieve specific oxidant concentrations of 1.5, 4 and 8 mg/L as MnO_4^-. At preselected reaction times, samples were collected and immediately dosed with 10–20% excess of ascorbic acid stock solution to quench the oxidation reaction. Sub-samples for extracellular toxins analysis were filtered through 0.45 μm syringe filters prior to UFLC-MS/MS analysis. Sub-samples for total toxin analysis were lysed using the standard freeze-thawing method (Rodriguez et al., 2007) followed by filtration and UFLC-MS/MS analysis.

3. RESULTS AND DISCUSSIONS

3.1. FREE CHLORINE TREATMENT WITH NO ADDED AMMONIA

Chlorination of *Microcystis* was tested in the natural waters without ammonia addition and the results are shown in Figure 1. For the control test without oxidation treatment, no significant degradation of total MC-LR was observed over two-hour period, though a very slow release of intracellular MC-LR was observed. For the low chorine dosage (0.5 to 1.5 mg/L), all the dosed free chlorine was consumed very quickly (CT close to 0), causing MC-LR release from microcystis only, but without significant degradation (total MC-LR did not change significantly). Further increase of chlorine dosage (4 to 12 mg/L) resulted not only the release of MC-LR from Microcystis, but also degradation of the released MC-LR. With a chlorine dosage of 4 mg/L, a significant free chlorine CT of 30 mg·min/L was achieved with measurable free chlorine existing for about 40 minutes,

all of the intracellular toxin was released and nearly 80% of the total MC-LR was oxidized after 30 minutes of contact time. No additional removal was achieved through the end of the two-hour experiment as no chlorine remained to oxidize the toxin. A chlorine dosage of 6 mg/L resulted in a CT of about 100 mg·min/L and greater toxin removal, though the final concentration was still nearly four times the infant health advisory (HA) level of 0.3 µg/L (US EPA, 2015). A high chlorine dosage of 12 mg/L resulted in a CT of over 600 mg·min/L and a final MC-LR concentration of less than the 0.3 µg/L HA after 30 min. However, increasing chlorine dosages could also increase disinfection byproduct (DBP) concentrations (Fang et al., 2010) and increase health risk.

3.2. FREE CHLORINE TREATMENT WITH 0.5 MG/L ADDED AMMONIA

The same experiments as presented section 3.1 were conducted using the same natural water, but with the addition of 0.5 mg/L (as NH_3) of ammonia. This ammonia concentration resulted in a predicted breakpoint chlorine dosage of about 3.8 mg/L of chlorine (as Cl_2) plus any additional oxidant demand. Figure 2 shows the experiments results. The control test without chlorine treatment resulted in very slow release of intracellular MC-LR. With a 1.5 mg/L chlorine dosage, most of the chlorine was rapidly converted to MCA with only a free chlorine residual of 0.15 mg/L for less than an hour (and resulting in a free chlorine CT of about 7.5 mg·min/L). No significant total MC-LR reduction was observed, and much less intracellular toxin release was observed compared with the same treatment for the water without ammonia addition in Figure 1.

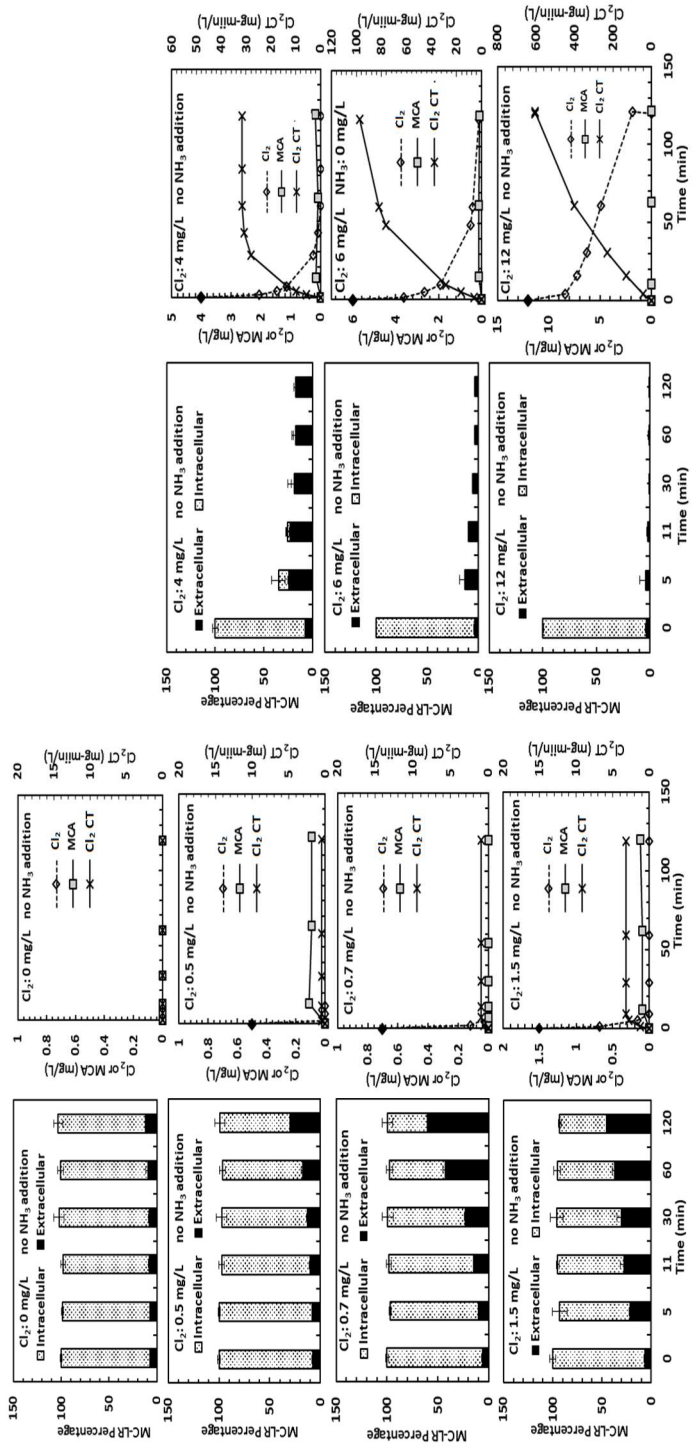

Figure 1. Intracellular and extracellular MC-LR for non-ammoniated natural waters spiked with *M. aeruginosa*, and corresponding free chlorine (FC) and monochloramine (MCA) concentrations, and free chlorine exposure (Cl_2 CT, mg·min/L).

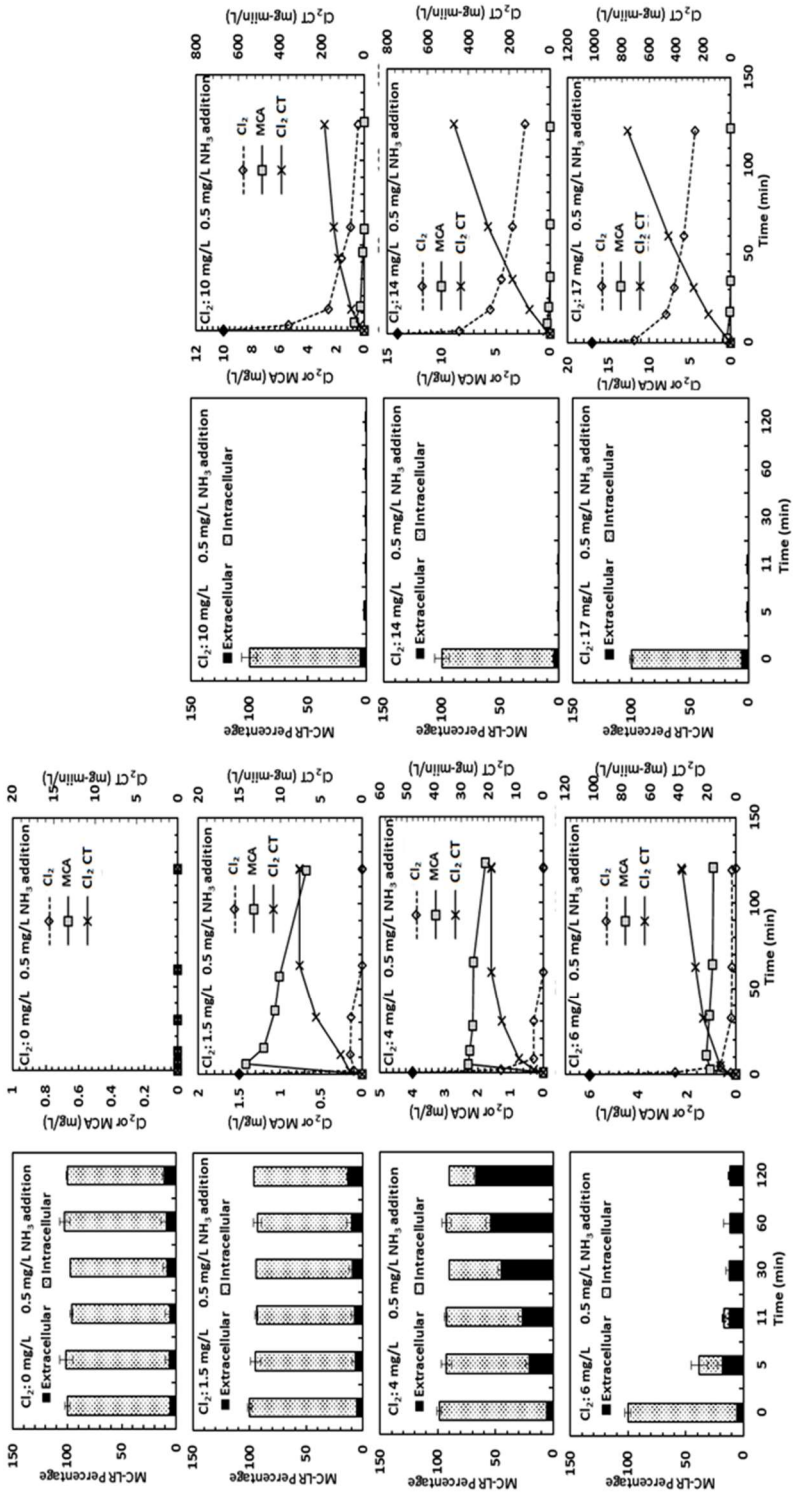

Figure 2. Intracellular and extracellular MC-LR for ammoniated natural waters spiked with *M. aeruginosa*, and corresponding free chlorine (FC) and monochloramine (MCA) concentrations, and free chlorine exposure (CT, mg·min/L).

With a 4 mg/L chlorine dosage, the effect of the 0.5 mg/L added ammonia was dramatic. A free chlorine CT of nearly 20 mg·min/L was achieved though most chlorine was rapidly converted to MCA. Final residual of total MC-LR for the ammoniated and non-ammoniated runs were 17% and 90%, respectively. With a 6 mg/L chlorine dosage, the chlorine dosage was approximately at the breakpoint, that is, about 3.8 mg/L for the ammonia demand, and approximately 1.5 mg/L for non-ammonia demand estimated from the non-ammoniated experiments. Free chlorine CT increased to about 40 mg·min/L, with nearly all the intracellular toxin released and subsequently oxidized to a final MC-LR residual of 12%. With higher dosages of 10, 14 and 17 mg/L chlorine, free chlorine exposures of about 200, 450 and 760 mg·min/L were observed driving MC-LR toxin concentrations to less than the 0.3 μg/L HA in less than 11, 5, and 5 minutes, respectively (Figure 2). Overall, ammonia has the effect of consuming the free chlorine at low oxidant dosages, thereby allowing increased buildup of extracellular toxin in solution. In the water with higher ammonia concentration, greater oxidant dosage are needed to achieve high removal efficiency.

3.3. MODELING INTRACELLULAR MC-LR RELEASE

Experimental results from these experiments were investigated using numerical modeling methods using MicroMath Scientist (Ver. 3.0) to solve the system of differential equations. A simple model was proposed that consisted of a first-order toxin release expression where intracellular toxin (I) is transported through a cell membrane to become extracellular toxin (E):

$$I \rightarrow E, \text{ or}$$

$$rate_I\ (mol/L \cdot s) = -\ k'_{IE}\ (s^{-1}) \cdot I\ (mol/L)$$

where k'_{IE} represents the effective first-order toxin-release rate constant. A second differential equation for E includes both the toxin release from intercellular toxin as well as the degradation of toxin by the oxidant:

$$I \rightarrow E \rightarrow \text{products, or}$$

$$rate_E = k'_{IE}\ (s^{-1}) \cdot I\ (mol/L) - k''_{EP}\ (L/mol \cdot s) \cdot E\ (mol/L) \cdot O\ (mol/L)$$

where k''_{EP} is the second-order rate constant for the oxidation of extracellular toxin, and O is the oxidant concentration (mol/L). The free chlorine concentration was modeled using first-order decay kinetics:

$$O = O_0\ e^{-k'_O \cdot t}$$

where k'_O is the chlorine decay constant. Second-order rate constants for the oxidation of the extracellular toxin in solution have been published in the literature and are found to be a function of both temperature and pH due primarily to the speciation of HOCl and OCl^- (pK_a~7.6). For example, Acero et al. (2005) found the rate constants of 116 and 6.78 L/(mol·s) for HOCl and OCl^-, respectively, at 20°C. The weighted effective rate constant at pH 8.3 and 22°C was, therefore, 22.3 L/(mol·s) which was used as a baseline rate constant in this modeling analysis.

For control samples (0 mg/L free chlorine adding) with and without ammonia added, release of intracellular toxin was exceeding slow with a k_{IE} of just 0.00001 s^{-1} estimated from the model (Table 1). For free chlorine doses of 0.5 and 0.7 mg/L with no ammonia added, estimated k_{IE}s were 0.00003 and 0.00011 s^{-1}, respectively (and no corresponding doses were conducted with ammonia addition). For a free chlorine dose of 1.5 mg/L, the initial few minutes of intracellular toxin release was modeled by a k_{IE}

approximately fifty times faster for no ammonia versus with 0.5 mg/L ammonia added. After the initial few minutes of toxin release, the k_{IE} then decreased dramatically for both the no-ammonia experiments to 0.00007 s^{-1}. Similarly, for a free chlorine dosage of 4 mg/L, the initial intracellular release rates for no-ammonia and 0.5 mg/L ammonia experiments were modeled by a k_{IE} of 0.006 and 0.0007 s^{-1}, respectively. For the 4 mg/L dose, the initial k_{IE} for the ammonia-added experiments dropped 70% to 0.00002 s^{-1} after approximately ten minutes (though a similar observation for the no-ammonia experiments was not possible due to nearly complete toxin release). For a free chlorine dosage of 6 mg/L, the initial intracellular release rates for no ammonia and 0.5 mg/L ammonia experiments were modeled by greater k_{IE} values of 0.010 s^{-1} and 0.004 s^{-1}. For the no-ammonia experiments, at a dosage of 12 mg/L free chlorine, the initial release rate constant increased further to 0.013 s^{-1}. For the ammonia-added experiments, at chlorine dosages of 10, 14 and 17 mg/L of free chlorine, the initial release rate constants increased further to greater than 0.015 s^{-1}.

These results showed that increased CT (per higher chlorine dosage) had a significant correlation with increasing initial toxin release rates (k_{IE}) for both no-ammonia ($r=0.95$; $r_{crit,0.05}=0.73$; $n=6$) and 0.5 mg/L ammonia-added ($r=0.99$; $r_{crit,0.05}=0.81$; $n=5$) experiments over the range for which constants could be accurately determined. Further, for the few experiments that had intermediate oxidant dosages such that significant release occurred though with measurable extracellular toxin concentration, a rapid initial toxin release rate appeared to be followed by a much slower release rate after the first few minutes of oxidant exposure.

Table 1. MC-LR releasing constants for chlorination treatment of *M. aeruginosa*.

No ammonia					Ammonia added (0.5 mg/L)				
Chlorine dose (mg/L)	Ultimate (2-hr) CT (mg·min/L)	Initial release k_{IE}' (1/s)	Final release k_{IE}' (1/s)	Percent decrease	Chlorine dose (mg/L)	Ultimate (2-hr) CT (mg·min/L)	Initial release k_{IE}' (1/s)	Final release k_{IE}' (1/s)	Percent decrease
0	0	0.00001	NC	- - -	0	0	0.00001	NC	- - -
0.25	0	0.00008	NC	- - -	1.5	8	0.00002	NC	- - -
0.5	0.4	0.00003	NC	- - -	4	18	0.0007	0.0002	71%
0.7	1.1	0.00011	NC	- - -	6	38	0.004	ND	ND
1.5	3.0	0.001	0.00007	93%	10	188	>0.015	ND	ND
4.0	30	0.006	ND	ND	14	473	>0.015	ND	ND
6.0	95	0.010	ND	ND	17	760	>0.015	ND	ND
12.0	600	>0.015	ND	ND					

Note: NC = No (discernable) change, ND = Not determinable (near complete release).

The mechanism of this fast release followed by slower release is not clear and warrants further detailed study as the impact on water treatment operations can be significant. Further, these results demonstrate the significant role of ammonia on the release (and subsequent buildup in solution) of intracellular cyanotoxins due to very rapid consumption of the applied free chlorine. With respect to oxidative removal of the extracellular toxin, effectively no oxidant exposure occurred for oxidant doses of 0.7 mg/L or less free chlorine, hence, no oxidation rate could be modeled. For a dosage of 1.5 mg/L, the 22.3 L/mol·s second order rate constant as determined from work by Acero et al. (2005) provided a reasonable fit of the experimental data. For higher oxidant exposures, a higher rate constant of approximately 100 L/mol·s generally provided a better fit of the data. The reason for this enhanced oxidation rate is not certain. However, work by Ho et al. (2006) also found significant enhancement of rate constants for the reaction of free chlorine with MC-LR when exposed to natural organic matter. For example, an enhancement of over four times was observed for oxidation in Myponga River water verses in laboratory water. Ho et al. (2006) hypothesized this enhancement was due to NOM-microcystin interactions or to formation of reactive quaternary chloramines on NOM moieties.

3.4. DISINFECTION BYPRODUCT FORMATION DURING CHLORINATION

Algal cells and extracellular organic matter may constitute a significant source of disinfection byproducts (Huang et al., 2009; Zamyadi et al., 2012). Therefore, both THM4 and HAA5 were analyzed for most oxidant dosages. THM4 was dominated by chloroform formation with the other three THM4 species at much lower concentrations (Figure 3). THM4 concentrations were well below the MCL of 80 µg/L even with the highest chlorine

dosage of 12 mg/L (and with no ammonia added). While, in ammoniated water, THMs concentrations were relatively less than non-ammoniated water. Specifically, in ammoniated samples, 17 mg/L Cl_2 after two hours contacting time generated about 20 µg/L TTHMs, 12 mg/L Cl_2 in non-ammoniated water could yield about 25 µg/L TTHMs correspondingly. In the no-ammonia added experiments, HAA5 did exceed the MCL of 60 µg/L at the highest chlorine dosage of 17 mg/L, but was only at about 25% of the MCL with 6 mg/L chlorine dosage. The addition of 0.5 mg/L of ammonia suppressed to some extent HAA5 formation such that the HAA5 MCL was not exceeded with a very high chlorine dosage of 14 mg/L.

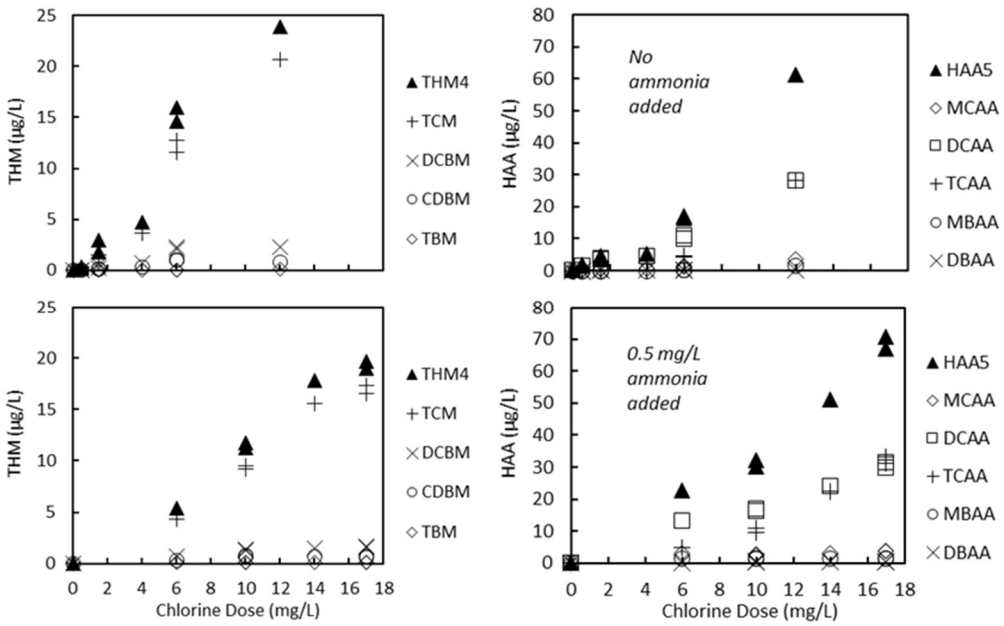

Figure 3. Trihalomethane (THM4) and Haloacetic acid (HAA5) formation from chlorination without and with 0.5 mg/L of ammonia addition.

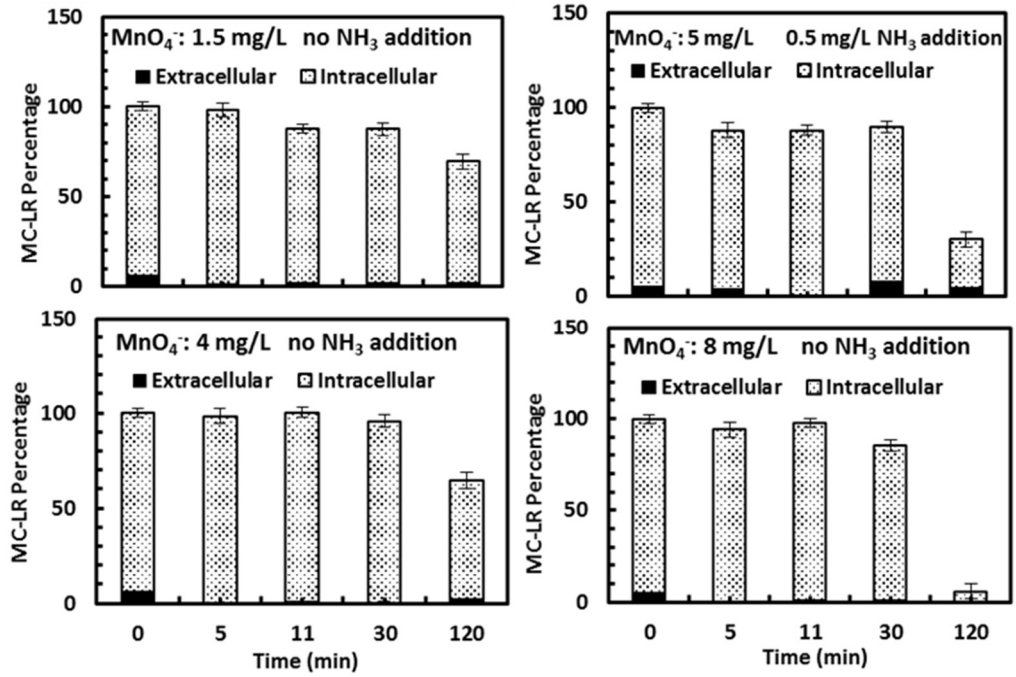

Figure 4. Intracellular and extracellular MC-LR levels by permanganate treatment (doses of 1.5 through 8 mg/L as MnO_4^-) in non-ammoniated and ammoniated natural waters spiked with *M. aeruginosa*.

3.5. PERMANGANATE OXIDATION RESULTS

For comparison of oxidants with the same cell culture, experiments were conducted at 1.5, 4, and 8 mg/L as MnO_4^- (or 0.89, 2.4 and 4.8 mg/L as Cl_2) with non-ammoniated water, and 5 mg/L as MnO_4^- (or 3.0 mg/L as Cl_2) with ammoniated water. Over the first 30 minutes of oxidation by permanganate, only limited intracellular toxin was released from the *M. aeruginosa* cells (Figure 4). At 120 minutes, however, increasing amounts of intracellular toxin was released with increasing permanganate dose. Compared with chlorination in which toxin releasing and oxidation rates are faster in the first several minutes and slowed after a short period of time, permanganate treatment needs more time to reach the same effect. Due to the rapid oxidation rate of MC-LR by permanganate,

relatively little extracellular toxin was observed at any oxidant level. Ammonia concentration in water has no significant effect on toxin releasing and oxidation, different from the effect of chlorine treatment (Figures 1 and 2).

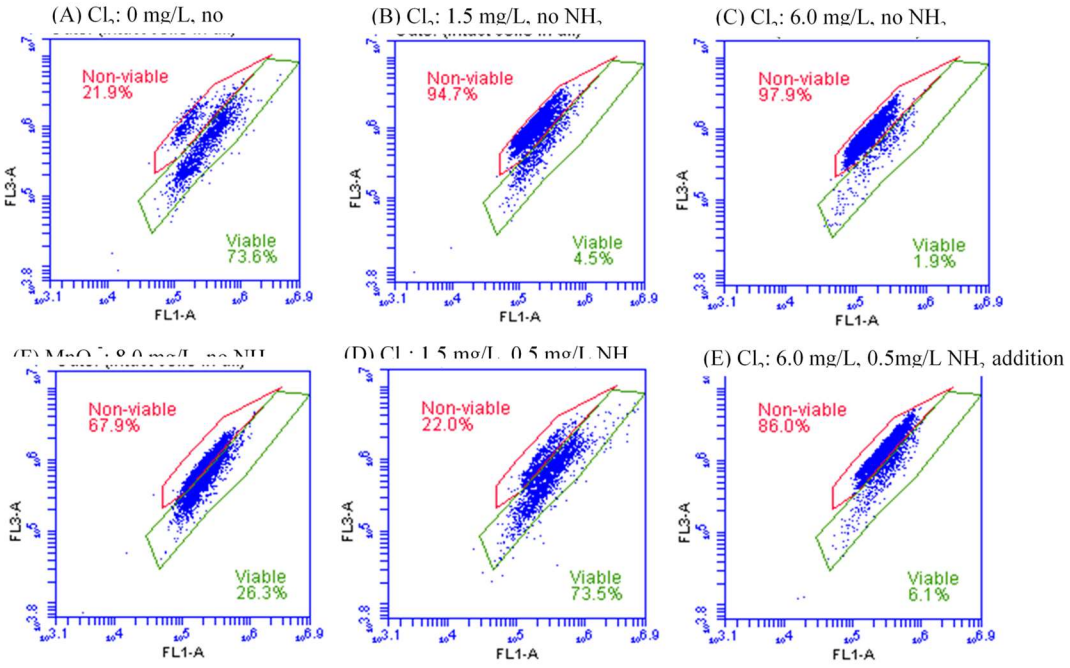

Figure 5. Flow cytometry results of Microcystis aeruginosa cells under different oxidant concentrations after 2 hours treatment.

3.6. CELL VIABILITY EVALUATION WITH FLOW CYTOMETRY

Recently, flow cytometry has been adopted in the evaluation of algal cell viability and membrane integrity resulting from oxidation processes (Wert et al., 2013; Liu et al., 2015). With the help of nucleic acid stains, a flow cytometer can quickly perform numerous quantitative and sensitive measurements on each individual cyanobacteria cell (Daly et al., 2007). The results showed that the population of damaged cells increased with increased

oxidant dosages, specifically from 21.9% to 94.7% to 97.9% with 0, 1.5, and 6.0 mg/L free chlorine (as Cl_2), respectively, for non-ammoniated natural water after two hours of treatment (Figure 5 (A)-(C)). Comparing these *M. aeruginosa* cell "damage" results with toxin release (Figure 1) shows that even with 94.7% damage at 1.5 mg/L free chlorine, only 50% toxin release observed. For ammoniated natural water, comparing the control with the 1.5 mg/L free chlorine treatment, the percentage of damaged cells had almost no difference. This is consistent with results by Ding et al. (2010) that reported the lack of cell damage with monochloramine for *M. aeruginosa*. Permanganate was seen to have much less effect on cell integrity than free chlorine (Figure 5). Specifically, 8 mg/L of permanganate (4.8 mg/L as Cl_2) resulted in just 67.9% non-viable cells (Figure 5 (F)).

4. CONCLUSIONS

This work demonstrated that low dosages of chlorine with no measureable free chlorine exposure can cause the majority of intracellular toxin to be released from the cyanobacterial cells with little destruction of the total toxin present. There is likely no sufficiently low level of prechlorination that can currently be assumed to be safe from the release of microcystins from cyanobacterial cells. The role of even very low levels of ammonia and other oxidant demand in the raw water is important due to its effect on the rapid conversion of the reactive free chlorine to non-reactive species. The releasing rates of intracellular toxins with ammonia adding were much lower than without ammonia. Increasing oxidant exposure was shown to cause increased rates for toxin release from cells. The results also showed that the effect of chlorine exposure on the release of MC-LR from *M. aeruginosa* cells was much greater than for permanganate exposure. These results

are important in the developing treatment strategies for utilities that must continue preoxidation to achieve disinfection (and disinfection compliance) and other simultaneous objective during HAB events.

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II. RELEASE AND REMOVAL OF CYLINDROSPERMOPSIN FROM *CYLINDROSPERMOPSIS RACIBORSKII* WITH CHLORINE AND OTHER OXIDANTS IN DIFFERENT DRINKING WATER SOURCES

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ABSTRACT

Various types of chemicals are widely used in drinking water treatment plants as a pre- or post-oxidant for treating source water challenged with harmful algal blooms. In this research, effect of water matrix and cell concentrations on the release and degradation of cylindrospermopsin (CYN) as a result of chlorination of *Cylindrospermopsis raciborskii (C. raciborskii)* was examined in two lake water serving as drinking water resources. The results showed that cell concentration and organic content greatly increased the chlorine demand to destroy *C. raciborskii* cells and CYN in natural surface water. Most of the removal of *C. raciborskii* cells and CYN oxidation happened in the first five minutes, and

the reaction rates became much slower in the later stage. Formation of chlorinated disinfection byproducts (DBPs) during chlorination was also evaluated. The increase of algae cell density resulted slightly different on DBPs formation with same concentration of chlorine treatment. Furthermore, removal efficiencies of free chlorine, chlorine dioxide, permanganate, and peracetic acid (PAA) were compared. The release and removal of CYN from *C. raciborskii* cells were in the following order: chlorine > > chlorine dioxide > PAA > permanganate. These results provided novel information for utilities to select suitable chemical and dosages to control *Cylindrospermopsis* bloom and DBPs formation. Chlorine is the most effective oxidant to inactivate *C. raciborskii* cells and degrade CYN when *C. raciborskii* bloom happens.

Keywords *Cylindrospermopsis raciborskii;* cylindrospermopsin; harmful algal bloom; disinfectants; disinfection by-product; water matrix

1. INTRODUCTION

Global climate change and anthropogenic eutrophication in water system have led to the widespread occurrence of harmful algal (cyanobacteria) bloom (de la Cruz et al., 2013; May et al., 2018, Rodriguez et al., 2007a). The algal bloom enhances occurrences of cyanobacteria in the source water for many drinking water systems (McQuaid et al., 2011; Zamyadi et al., 2012), which has become a concern for drinking water plants due to the ability of toxic algae to synthesize toxic and odorous metabolites (Bakheet et al., 2018). Cyanotoxins have been identified to be toxic to livestock and human beings through direct digestion and display tumour promoting, and even carcinogenic properties (Merel et al., 2010; Smith et al., 2008). United State Environmental Protection Agency (US EPA) has

set health advisory level of cylindrospermopsin (CYN) concentration to be 0.7 µg/L in drinking water for bottle-fed infants and pre-school children, and 3 µg/L for school-age children and adults (US EPA, 2015).

CYN is one of the most commonly occurring algal toxins, which is associated with blooms of *Cylindrospermopsis*, *Anabaena*, and *Aphanizomenon* (Rodriguez et al., 2007a; Vico et al., 2016). Cyanotoxins in cells (intracellular) can be released to outside of cells (extracellular) by excretion and cell lysis etc. To ensure water safety for human consumption, it is necessary to remove both cyanobacteria and cyanotoxins that have already been released or would be released during the treatment (Fan et al., 2018; Senogles et al., 2000). Cares should be taken that intracellular toxins could be released into water during damage or inactivation of cyanobacteria with different methods like oxidation. A variety of studies have been conducted to investigate the removal of CYN kinetically, and its potential pathway with different oxidants (Rodriguez et al., 2007a; Rodriguez et al., 2007b; Onstad et al., 2007; Song et al., 2012). However, very limited researches have focused on removal of CYN related to inactivation and lysis of cyanobacteria i.e., *Cylindrospermopsis racuborskii* (*C. raciborskii*) (Cheng et al., 2009; Zamyadi et al., 2012b) from natural source drinking water with different water matrix. However, the releasing raters of intracellular CYN from *C. raciborskii* cells with different amount of chlorine has not been well investigated. The cell concentration in previous study was relatively low, and 1 mg/L could removal all the *C. raciborskii* cells in every short time (Cheng et al., 2009), which is not representative in real aquatic environment. Comparing with *Microcystis aeruginosa*, which is another ubiquitous cyanbacteria and has been well studied in different water matrix (Fang et al., 2010, Zhang et al., 2017), *C. raciborskii* has

not been studied systematically through different oxidations in different water matrixes, though its blooms have been increasingly reported. More investigations should be conducted for the competition between *C. raciborskii* cells and organic matters in water to react with chlorine.

Mitigating harmful algal bloom and removal of related cyanotoxins from the water using various chemicals are popular and economic techniques among drinking water systems (de la Cruz et al., 2013). Chlorine disinfection is widely used in water treatment plants and free chlorine can react with inorganic ions, natural organic matter, and other substances (Zamyadi et al., 2013). Cell lysis caused by chlorine may also lead to release of cell-bound matter components. Therefore, all of these cell-related ions and organic matters combined with natural occurring matter lead to a significant chlorine demand and contribute to the formation of DBPs (Fang et al., 2010; Zamyadi et al., 2012a). The effects of water quality and cell concentrations on chlorine demand, *C. raciborskii* cell lysis rate, and CYN removal efficiency in drinking water systems are not well understood currently. To control the formation of DBPs (i.e., trihalomethanes (THMs) and haloacetic acids (HAAs)) in drinking water treatment processes, alternative oxidants are commonly used to replace chlorine as disinfectant pre-oxidant, such as chlorine dioxide (ClO2), and permanganate (KMnO4) (Rodriguez et al., 2007a). In recent years, peracetic acid (PAA) has been applied in North America and Europe for wastewater disinfection and ballast water treatment (Antonelli et al., 2009; Gehr et al., 2003a). Commercially available PAA (also known as ethaneperoxoic acid or peroxyacetic acid) is an aqueous mixture of acetic acid, hydrogen peroxide, peracetic acid in water could inactivate many bacteria, fungi, and crustaceans effectively in various water matrix (Antonelli et al., 2009). Previous study.

Therefore, it is very necessary to study the efficiency of PAA on controlling *C. raciborskii* cells since it has been demonstrated that in wastewater, without comprising disinfection, PAA produced much less DBPs than chlorine disinfection (Shah et al., 2015; Veschetti et al., 2003).

This study investigated the effects of water matrix and *C. raciborskii* cell concentration on chlorine demand. The release and removal of CYN from *C. raciborskii* cell at different contact time in two Oklahoma lake water which were served as drinking water resources were studied. Furthermore, different disinfectants (i.e., Cl_2, ClO_2, $KMnO_4$, and PAA) were compared for their ability to lysis of *C. raciborskii* cells and oxidize CYN. At the same time, DBPs formations were determined when Cl_2 was used.

2. MATERIALS AND METHODS

2.1. MATERIALS AND ANALYTICAL METHODS

CYN standard was purchased from Sigma-Aldrich Corporation (St Louis, MO, USA). Saline (0.9% NaCl) solution was prepared by dissolving sodium chloride in ultra-high purity water, which was produced with an ELIX-3 water purification system (Millipore, Billerica, MA, USA). A Shimadzu ultra-fast liquid chromatography (UFLC) system (Columbia, MD, USA) coupled with a 4000Q Trap mass spectrometer (MS/MS) (AB Sciex, Foster City, CA, USA) was used for CYN detection with our previous developed HPLC-MS/MS method (Zhang et al., 2017a).

The sodium hypochlorite (NaClO) stock solution used for chlorination was prepared by dilution of 5% sodium hypochlorite solution (Sigma-Aldrich, St Louis, MO, USA) in ultrapure water. Hach *N,N*-diethyl-*p*-phenylendiamine (DPD) Method 8021 and

8167 (Loveland, CO, USA) were used to determine free chlorine and total chlorine concentrations, respectively. The permanganate (MnO_4^-) stock solution was prepared by dissolving potassium permanganate in ultrapure water. Permanganate concentration was determined using the Hach DPD method 8167 (Ding et al., 2010) during treatment experiments. Chlorine dioxide stock solution was generated onsite at one drinking water treatment plant of Oklahoma, USA. Its concentration was determined using the Hach DPD method 8167 as well. PAA was purchased from Solvay Chemicals Inc. (Vandalia, IL, USA) which contained 12.0-12.8% of PAA, 18.5-20.0% of hydrogen peroxide, and 20-25% of acetic acid (by weight), and this mixture is named as PAA in this study. The PAA concentration was determined with a method from HACH application note (Determination of PAA and Hydrogen Peroxide (H_2O_2) in Water, HACH, 2014). HAAs concentrations were determined with a recently published high performance ion chromatography (HPIC)-MS/MS method (Xue et al., 2016). THMs were analyzed by following a solid-phase micro-extraction-gas chromatography method (Shi and Adams, 2012)(Shi and Adams, 2012) .

Dissolved organic carbon (DOC) concentrations were detected by a Shimadzu Model TOC-L TOC analyzer (Shimadzu, Overland Park, KS, USA). An ORION 3 STAR pH meter (Thermo Electron Corporation, US) was used to measure pH. HACH TNT plus™ 830 Ammonia Method 10205, Phenolphthalein and Total Alkalinity Method 8203, and Titration Method using EDTA Method 8213 were used to measure free ammonia, alkalinity and total hardness, respectively.

2.2. CELL CULTURING AND OXIDATION EXPERIMENTS

C. raciborskii CS-505 was purchased from Australian National Algae Supply Service (Hobart, Tasmania, Australia). The MLA growth media was prepared by following the protocols from the supplier (http://www.marine.csiro.au/microalgae/methods/). Details on cell culturing, saline washing for experiment, and cell viability tests are provided in Cheng et al. (2009). Cell concentrations were determined by direct counting with a hemocytometer (with an Olympus IX51 inverted microscope, Center Valley, PA, USA). Two lake water, which are source waters for two drinking water treatment plants of Oklahoma, USA, were collected and used for treatment experiments. One of them had more complex water matrix and relatively high DOC concentration (HD Water), and another one had relatively less natural organic compounds (LD Water). The basic water parameters for collected source waters were shown in Table 1. The water was filtered through a 0.45 μm nylon membrane filter before used in experiments. All experiments were conducted in amber glass bottles at room temperature (20±2 ^{o}C).

Similar to the World Health Organization guidance for Miscorcystin-LR, in this study when CYN concentration was lower than 10 μg/L, the relative probability of acute health effects was supposed to be relatively low; when CYN concentration is higher than higher than 20 μg/L, the relative probability of acute health effects was supposed to be relatively high. Therefore, the cell concentrations which could produce 10 and 40 μg/L of total CYN were set as low and high *C. raciborskii* concentration, respectively. Prior to starting an experiment, specific amount of suspended cells were added into 250 mL of filtered water samples to achieve a high cell concentration (~40 μg CYN/L, 4×10^5 cells/mL) or a low cell concentration (~10 μg CYN/L, 8×10^4 cells/mL). For the oxidation

experiments, a desired amount of chlorine stock solution was spiked into the water dosed with appropriate amount of cell suspensions. The tumbler was then started immediately to mix chlorine and the water to achieve desired dosage. At preselected reaction times, samples were collected and immediately quenched with 20% excess of an ascorbic acid solution. Aliquot of sample for extracellular toxins analysis was filtered through 0.22 μm nylon syringe filters prior to analysis. Aliquot of sample for total toxin analysis was lysed using a freeze-thaw method (Rodriguez et al., 2007a) followed by filtration and the HPLC-MS/MS analysis.

Table 1. Source water parameters of two drinking water facilities.

Source water	NH_3-N (mg N/L)	Total Hardness (mg/L as $CaCO_3$)	DOC (mg/L)	pH	TDS (mg/L)	Turbidity (NTU)
HD	0.072	157	4.99	7.92	153.1	14.5
LD	0.086	103	2.78	7.73	109.3	12.4

3. RESULTS AND DISCUSSIONS

3.1. FREE CHLORINE TREATMENT FOR HIGH CONCENTRATION ALGAL WATER

High cell concentration (4×10^5 cells/mL) of *C. raciborskii* were treated with free chlorine in both HD and LD waters and the results are shown in Figures 1 and 2. Lysis for high algal concentration water could produce approximately 40 ug/L of total CYN (sum of intracellular and extracellular CYN). For source water with high DOC concentration (HD

water), there was no significant degradation of CYN and releasing of CYN from *C. raciborskii* after 4 hours in the control sample. When treated with 0.5 mg/L chlorine, the oxidant was consumed very quickly and no noticeable removal of *C. raciborskii* nor CYN was observed. When the chlorine dosage was increased to 2 mg/L, there was obvious degradation of total CYN concentration in 5 minutes, especially the intracellular CYN. After 5 minutes, no more free chlorine residual was left in the samples and total CYN concentration did not change afterwards. Further increasing of chlorine concentration (4-10 mg/L chlorine) resulted in both releasing of intracellular CYN from *C. raciborskii* cells and degrading of total CYN. Most of degradation of total CYN was happened in the first 5 minutes when the chlorine dosages were relatively higher (i.e. greater than 2 mg/L). Specifically, when chlorine concentrations were 4, 6, and 10 mg/L, 87 percent, 93 percent, and 97 percent of total CYN were oxidized after 5 minutes of contact time, respectively (Figure 1). At 4 mg/L chlorine, after 4 hours reaction, the total CYN concentration was about 2.5 µg/L (~94 percent removal and a CT of 27 mg•min/L), which was still about four times of the EPA infant health advisory level (i.e., 0.7 µg/L) (USEPA 2015). When the chlorine concentration was increased to 6 mg/L, only 0.5 µg/L CYN left after 4 hours of contact time (~99 Percent removal and a CT of 50 mg•min/L). When the chlorine concentration was further increased to 10 mg/L, CYN concentration was below the EPA infant health advisory level in 11 minutes of contact time (~99 percent removal and an equivalent CT of 65 mg•min/L).

For low DOC concentration source water (LD water), the oxidant demand was much less than the HD water with same *C. raciborskii* cell concentration, as expected. As shown in Figure 2, no significant total CYN concentration removal was observed at 0.5

mg/L of chlorine dosage. When treated with 1.5 mg/L chlorine, 60% of total CYN was removed after 5 minutes of contact time, which was similar to 2.0 mg/L chlorine dosage in HD water. When the chlorine dosages were increased to 3 and 6 mg/L, total CYN concentration dropped to only 1.4 and 0.3 μg/L after 5 minutes of contact time, respectively. The CT reached to over 70 and 300 mg•min/L at 6 and 10 mg/L after 4 hours contact, which are higher than corresponding in HD water. The DOC concentration had no significant effect on the rates of *C. raciborskii* lysis and CYN degradation, which were mostly occurred in the first five minutes.

3.2. FREE CHLORINE TREATMENT FOR LOW CONCENTRATION ALGAL WATER

Similar experiments as described in section 3.1 were conducted, but spiked with lower *C. raciborskii* algal concentration (8×10^4 cells/mL). The low algal concentration produced approximately 10 μg/L of total CYN in the water. The results are shown in Figures 3 and 4. No significant degradation of total CYN and release of intracellular CYN were observed over 4 hours contact time in the control sample. In HD water, at 0.5 mg/L chlorine dosage, small amount of intracellular CYN was released and oxidized. A chlorine concentration of 2 mg/L led to about 80 percent of total CYN oxidized in the first 5 minutes and more than 90% of intracellular CYN released. Comparing with results of 2 mg/L of chlorine for high algal concentration in HD water as discussed in section 3.1, much more intracellular CYN were released out from cells and total CYN were removed. After 5 minutes, no obvious chlorine residual was detected in the samples and no significant changes of intracellular and extracellular CYN concentrations were observed. At higher

chlorine concentration (i.e., 4 and 6 mg/L), the intracellular CYN releasing rate from *C. raciborskii* and total CYN degradation rate in the first 5 minutes were rapid. More releasing and degradation of CYN continued after 5 minutes since chlorine residual was still present, but the rate was relatively low. After 4 hours reaction, the total CYN was almost degraded completely at concentrations of 0.3 μg/L (for 4 mg/L chlorine) and 0.03 μg/L (for 6 mg/L chlorine), which were below the EPA HA level for infants.

In LD water, no apparent total CYN change was observed in the control sample. At a chlorine dosage of 0.5 mg/L, small amount of total CYN were oxidized after four hours. The removal efficiency of total CYN reached 97% after 5 minutes of contact time with 1.5 mg/L of chlorine, which was much higher than that in the sample spiked with high algal concentration (about 60% removal) treated with 2.0 mg/L chlorine (Figure 1). After 4 hours contact, the chlorine exposure (about 30 mg•min/L) of low cell concentration with 1.5 mg/L chlorine was also slightly higher than the high concentration algal water (about 20 mg•min/L), which was because that less cells in the water samples less chlorine demand, and therefore higher chlorine residual. When the chlorine dosage was increased to 3 mg/L, the total CYN concentration was only 0.05 μg/L left after four hours of contact, and chlorine exposure was over 100 mg•min/L. As expect, when the *C. raciborskii* algal concentration was low, the chlorine demand in LD water was relatively lower than in HD water. The cell concentration in this section was 8×10^4 cells/mL, which was about 5 times less than that in section 3.1. However, the removal efficiencies of chlorine in same water with low cell concentration were not 5 times higher than it in high cell concentration, but less than 5 times. Specifically, in HD water with high and low *C. raciborskii* cell concentration, the removal of 4.0 mg/L free chlorine after 4 hours contacting were 87 %

and 98 %, respectively. It may be because of the contributions of natural organic compounds and ions, which will also consume the chlorine.

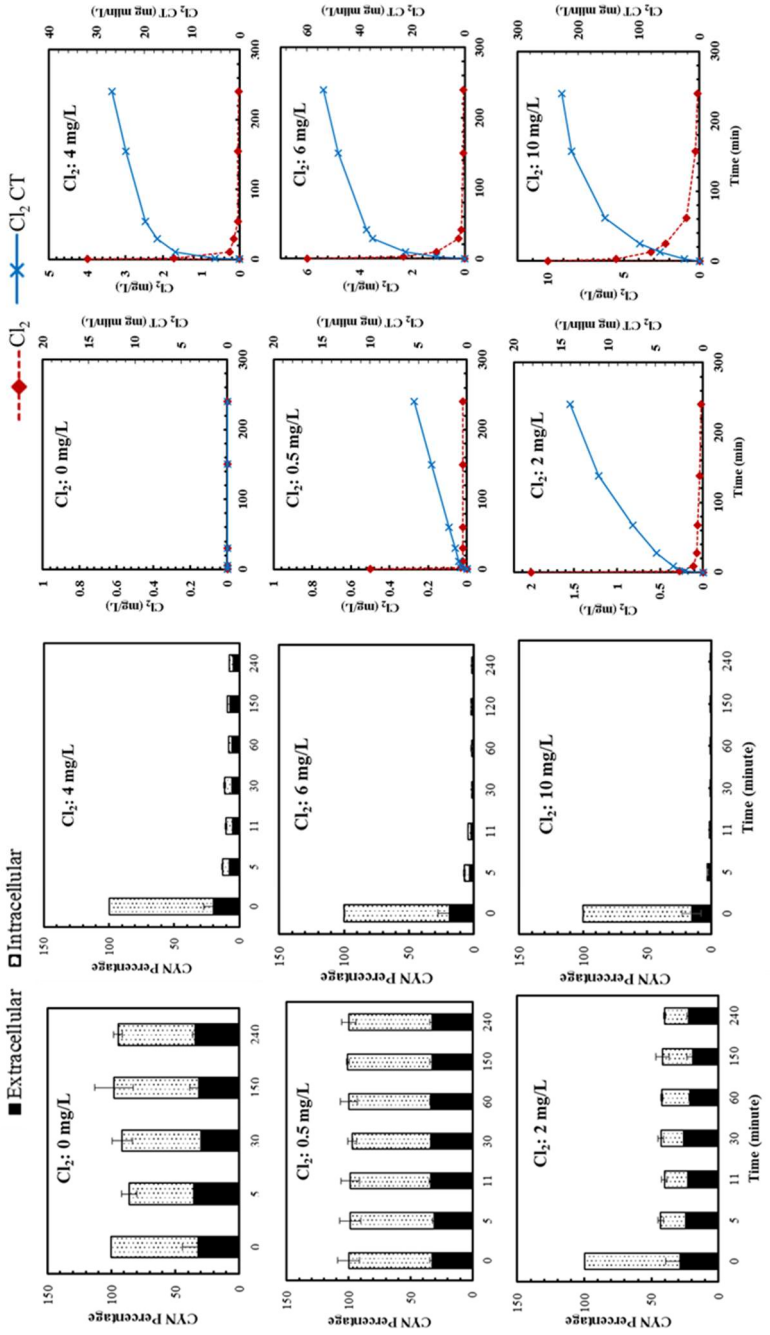

Figure 1. The concentration profiles of extracellular and intracellular CYN, free chlorine and free chlorine exposure (CT, mg.min/L) in HD water with high concentration (~ 40 µg/L CYN) of *C. raciborskii* treated with 0, 0.5, 2, 4, 6, and 10 mg/L of free chlorine at different contact times.

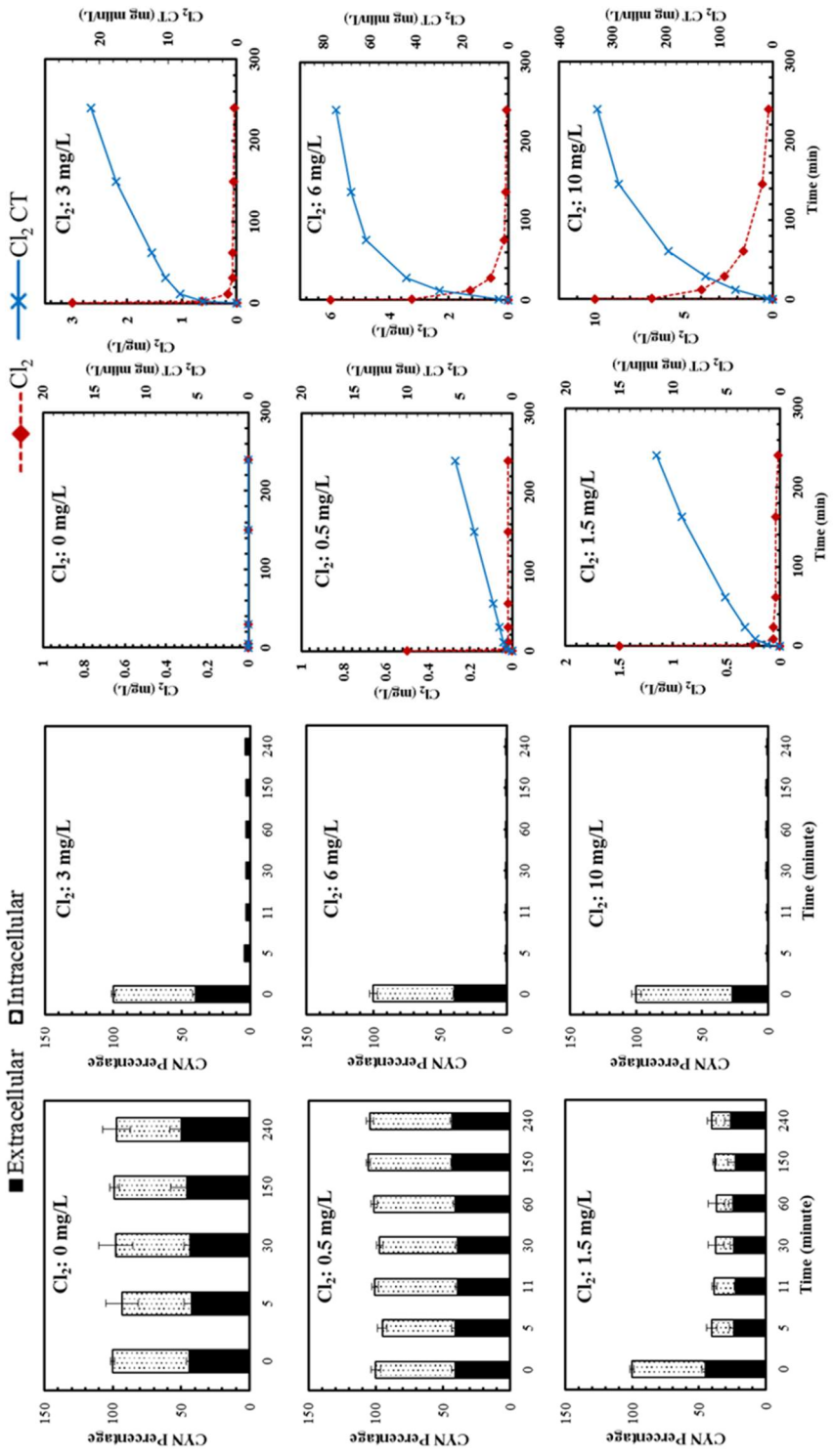

Figure 2. The concentration profiles of extracellular and intracellular CYN, free chlorine and free chlorine exposure (CT, mg.min/L) in LD water with high concentration (~ 40 µg/L CYN) of *C. raciborskii* treated with 0, 0.5, 1.5, 3, 6, and 10 mg/L of free chlorine at different contact times.

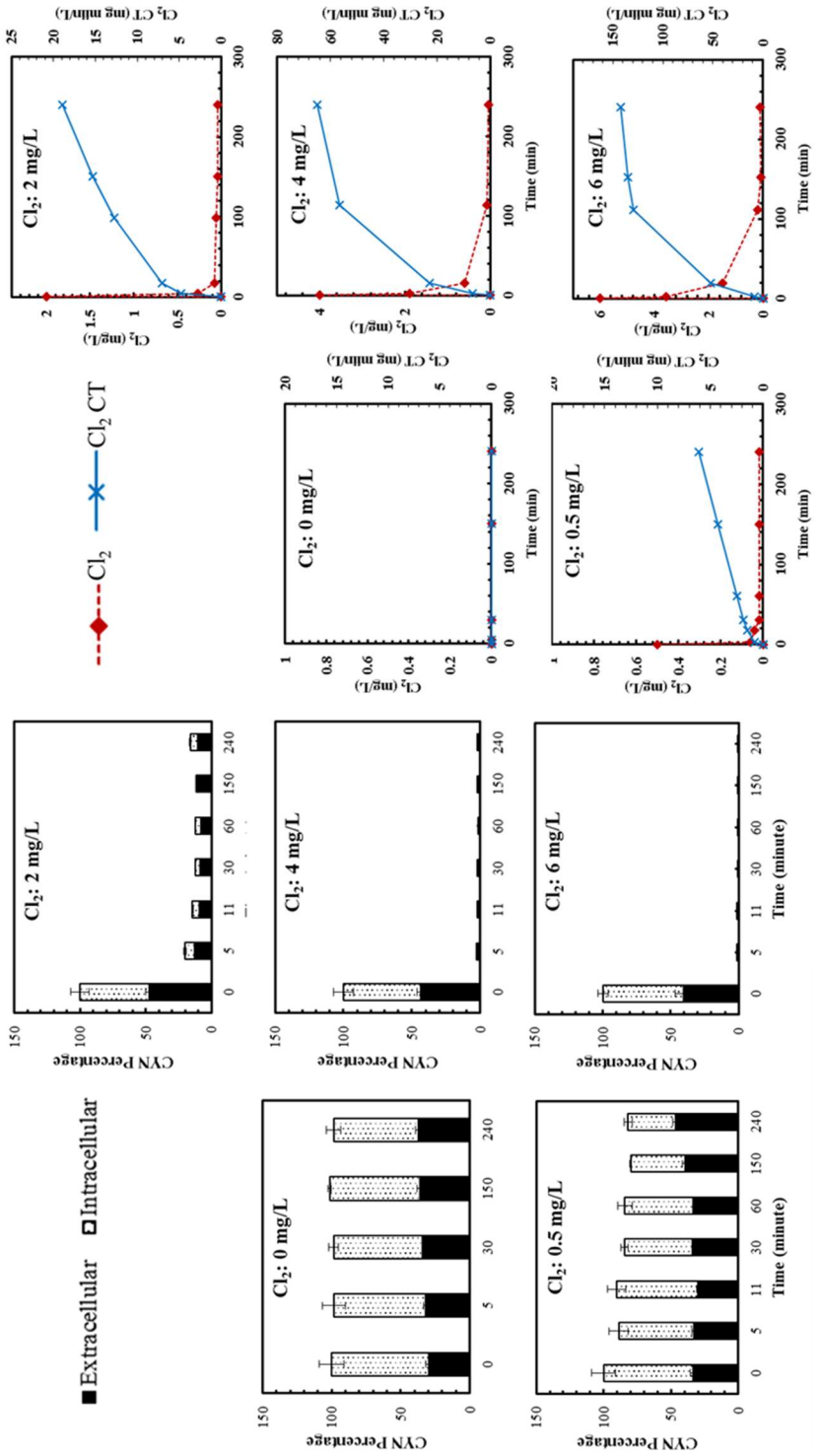

Figure 3. The concentration profiles of extracellular and intracellular CYN, free chlorine and free chlorine exposure (CT, mg.min/L) in HD water with high concentration (~ 10 µg/L CYN) of *C. raciborskii* treated with 0, 0.5, 2, 4, and 6 mg/L of free chlorine at different contact times.

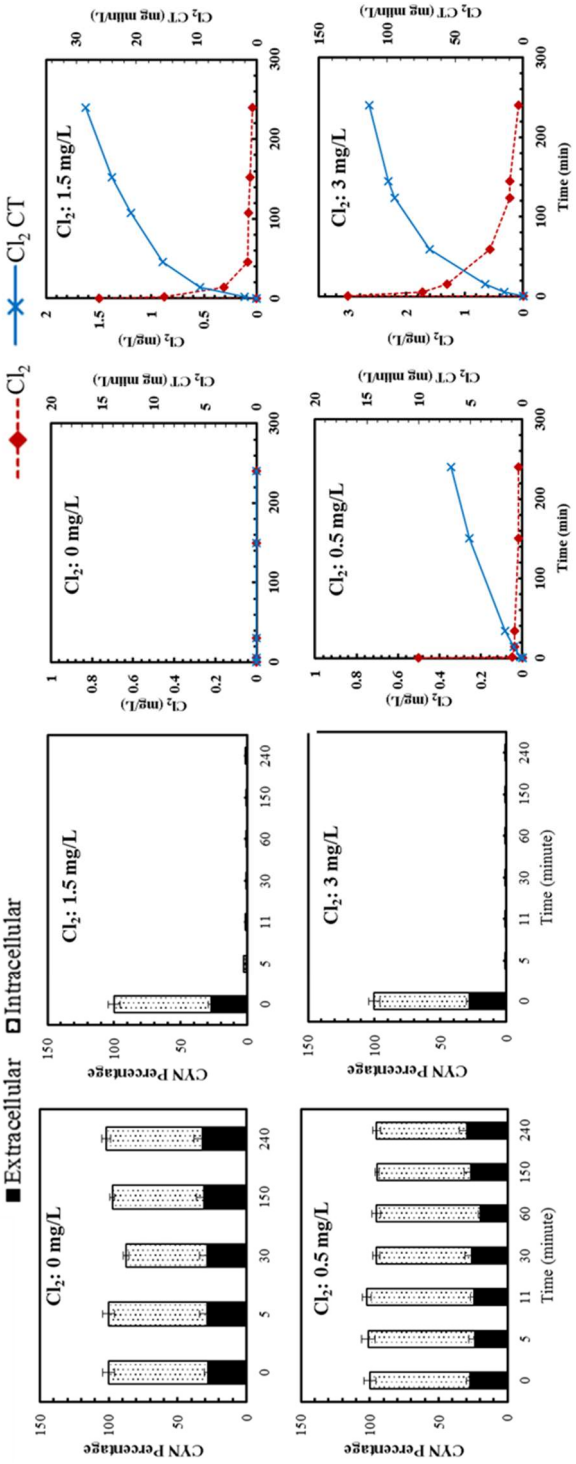

Figure 4. The concentration profiles of extracellular and intracellular CYN, free chlorine and free chlorine exposure (CT, mg.min/L) in LD water with low concentration (~ 10 µg/L CYN) of *C. raciborskii* treated with 0, 0.5, 1.5, and 3 mg/L of free chlorine at different contact times.

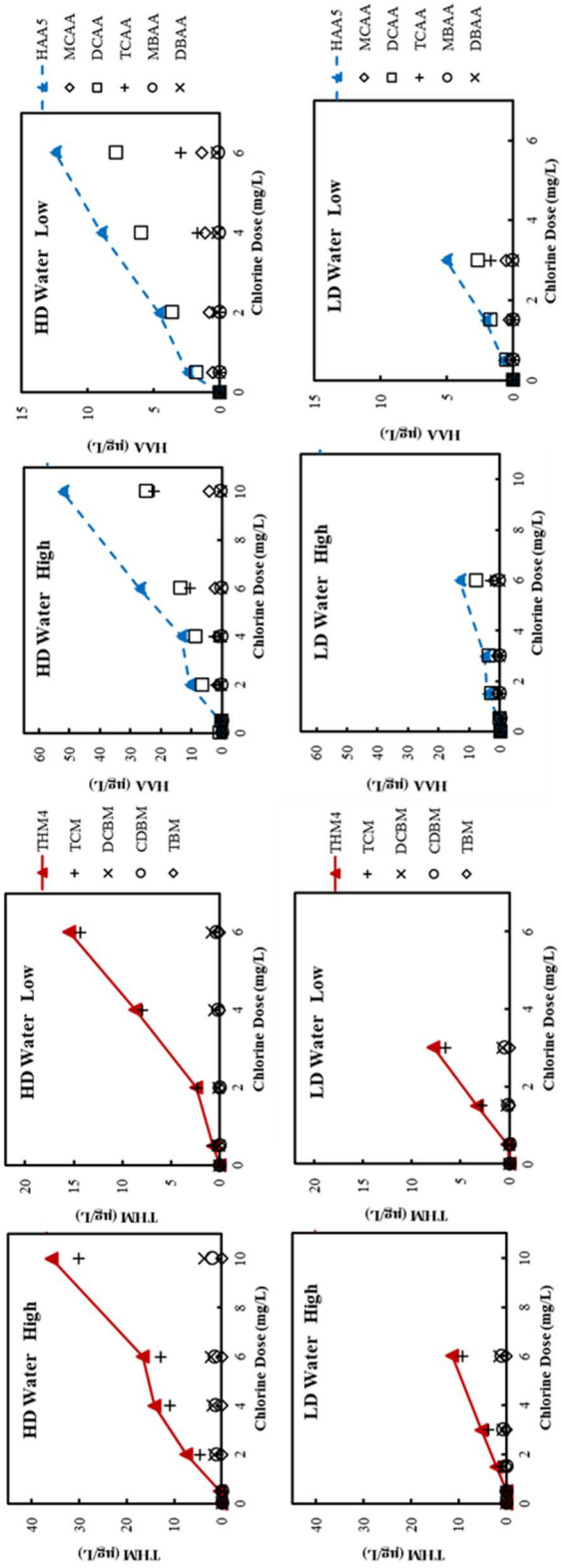

Figure 5. THMs and HAAs generated during chlorination treatment.

3.3. DISINFECTION BYPRODUCT FORMATION

Based on the results from Sections 3.1 and 3.2, chlorination could release and removal *C. raciborskii* and CYN if the dosage is sufficient. However, with the application of chlorine in water with *C. raciborskii* bloom, not only the CYN will be released out, but also the cell related organic matter. Chlorine could attack these cells related organic matter, naturally occurring organic matters and bromide to form DBPs (Zhang et al., 2015). The DBPs formed through the reaction between free chlorine and intracellular and extracellular organic matters of Microcysts aeruginosa have been well studied (Li et al., 2012; Wert and Rosario-Ortiz, 2013). However, the investigation about DBPs concentration and speciation after chlorination of *C. raciborskii* was very rare (Zamyadi et al., 2012). Both of THMs and HAAs are most common DBPs in chlorinated water and both groups of chemicals are known carcinogens. THMs and HAA5 are currently regulated by USEPA as TTHMs and HAA5 at maximum contaminant levels of 80 and 60 µg/L, respectively. Therefore, THM4 and HAA5 concentrations were also monitored after 4 hours of treatment. From the results in Figure 5, an increased chlorine dosage and a high algae concentration enhances formation of the tested DBPs drastically. Trichloromethane (TCM) contributed the most for THM4, from 60 percent to 100 percent. For example, 0.39 µg/L TCM was formed when 0.5 mg/L chlorine was applied in HD water with high algae concentration, and others (i.e., dichlorobromomethane (DCBM), chlorodibromomethane (CDBM), and tribromomethane (TBM)) were all blow our detection limits. For HAA5, the most abundant specie was dichloroacetic acid (DCAA) followed by trichloroacetic acid (TCAA). In HD water, with high cell concentration, more HAA5 (~52.3 µg/L) was generated than THM4 (~35.8 µg/L); however, at the low cell concentration, the difference between HAA5 and THM4 was

negligible. The competition between NOM and algal cells to react with chlorine may result in the different types of DBPs species. Nevertheless, the chlorination of high cell suspensions (4×10^5 cells/mL) in these two types of natural waters using sufficiently higher amounts of chlorine to remove cyanobacteria and cyanotoxins resulted in pretty high concentrations of THM4 and HAA5. The difference of THM4 and HAA5 formation between HD and LD waters, with same amount of *C. raciborskii* cells by same free chlorine treatment, was negligible. If taking into the potential formation of THM4 and HAA5 in the distribution systems, the DBPs concentrations in the tap water might higher than US EPA regulations. Therefore, more studied should be performed for DBPs formation in drinking water distribution systems during harmful algal blooms.

3.4. OTHER OXIDANTS

Chlorine dioxide and permanganate are also frequently used in drinking water treatment processes. Unlike chlorine, chlorine dioxide and permanganate do not promote the formation of chlorinated or brominated THMs and HAAs (Rodriguez et al., 2007d; Qiang et al., 2015). PAA is often used in hospitals, food and beverage industries, and wastewater treatment plants, since it is an efficient organic bactericide, sporicide, fungicide, and algaecide (Shah et al., 2015; Dunkin et al., 2017). It has been proved that DBPs formed by PAA have less mutagenicity, carcinogenicity, and genotoxicity than halogenated DBPs produced from chlorination (Dell'Erba et al., 2007; Dunkin et al., 2017). A similar experiment was conducted in the LD water with low cell concentration to compare the removal efficiencies of 6 mg/L chlorine, chlorine dioxide, PAA, and permanganate. From the results shown in Figure 6, with 6 mg/L of chlorine, almost all of

the *C. raciborskii* cells and CYN were removed in 5 minutes. However, even after 4 hours of contact, chlorine dioxide, PAA, and permanganate cannot remove all of the *C. raciborskii* cells and CYN. The removal efficiencies of chlorine dioxide, PAA, and permanganate with 4-hr of contact time were 40%, 34%, and 26%, respectively. The removal rate of chlorine dioxide was faster than that of permanganate which coincided with the kinetic rate for the degradation of CYN (Rodriguez et al., 2007b). In the study of (Rodriguez et al., 2007b), the kinetic degradation rates of CYN by ClO_2 and MnO_4^- were about 1,000 times lower than free chlorine. When PAA was used as the disinfectant in wastewater, researches have shown that it was quite selective and it might need assistance from UV radiation to improve its performance, which may explain why it has little effect on inactivating the *C. raciborskii* (Gehr et al., 2003; Formisano et al., 2016. Therefore, for natural water, chlorine dioxide, PAA, and permanganate may not be the effective ways to control the *C. raciborskii* algal bloom, at least cannot reach the same removal efficiency as chlorine. Consequently, when different disinfectants are used in different drinking water treatment plants, attentions should be given to provide adequate disinfectant to guarantee the efficient oxidation and removal of cyanobacteria and cyanotoxins during harmful algal bloom events. Chlorine is a good choice to be applied during this type of algal bloom if the formation of THMs and HAAs could be controlled under US EPA regulation limits.

4. CONCLUSIONS

This study indicates that the chlorine dosage necessary to oxidize and remove *C. raciborskii* cells and CYN in natural water was highly dependent on the cell and organic matters concentrations in the water, as expected. It is apparent that the CYN releasing rates

of chlorine decreased with the increase of *C. raciborskii* cell density. The rates of cell lysis in high organic content water were relatively slower than that of LD water under equivalent conditions, which was most likely attributed to the presence of high amount of NOM in HD water. While comparing with chlorine dioxide, PAA, and permanganate, chlorine released and oxidized CYN from *C. raciborskii* cells more effectively in both water matrix. The removal was in the order of chlorine > > chlorine dioxide > PAA > permanganate. Therefore, attention should be given to provide an adequate disinfectant to guarantee the efficient oxidation and removal of cyanobacteria and cyanotoxins for different disinfectants used during water treatment. Source water matrix can also significantly affects the treatment efficiency by oxidation. These results provide useful information for utilities to select an appropriate oxidizer to control algal bloom, cyanotoxin, and the formation of DBPs.

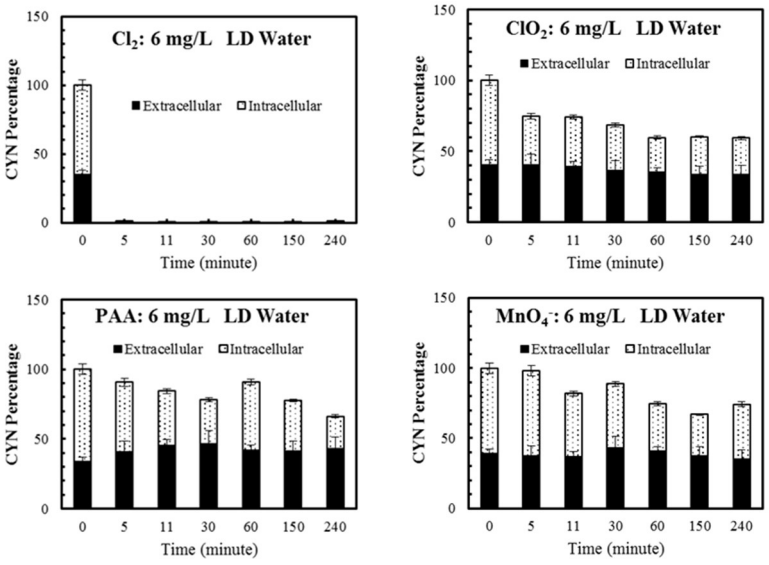

Figure 6. Comparing free chlorine treatment with other oxidants.

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III. OCCURRENCE, FORMATION, AND CONTROL OF TASTE AND ODOR CAUSING COMPOUNDS 2,4,6-TRICHLOROANISOLE IN DRINKING WATER SYSTEMS

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ABSTRACT

Drinking water taste and odor (T&O) is a common problem for many drinking water treatment plants (DWTP) treating surface water. The most well-known chemicals causing T&O problems in drinking water are geosmin and 2-methylisoborneol. Our study for several T&O events occurring in Missouri drinking water systems discovered that the major cause of the T&O was 2,4,6-trichloroanisole (2,4,6-TCA), a compound with extremely low taste threshold (i.e., 0.3 ng/L). Thus, the resource/precursor(s) of 2,4,6-TCA

and its formation and removal in drinking water treatment systems were investigated. The results showed that the 2,4,6-TCA formation in drinking water treatment plants needs not only its chemical precursor (i.e., 2,4,6-trichlorophenol), but also corresponding bacteria and/or other conditions. Fifteen different types of powder activated carbon (PAC) with a variety of brands and materials were used to remove 2,4,6-TCA and its precursor. The absorptive removal rates were increased with increasing dosage of PAC and contact time. After reaching to 5 mg/L and 4 hours contacting, there was no more significant increasing with further increasing dosage and/or contact time. Therefore, when T&O issues occur, attentions should be paid by the DWTPs to not only traditional T&O compounds but also 2,4,6-TCA. PAC could be applied to provide quality tap water for the customers, but the amount depends on the brands and materials of PAC and contact time in each DWTP.

Keywords: taste and odor, 2,4,6-trichloroanisole, formation pathway, powdered activated carbon, drinking water

1. INTRODUCTION

Drinking water taste and odor (T&O) is a common problem for many drinking water systems treating surface water throughout the world. The majority of daily complaints received by drinking water suppliers are related to bad T&O of tap water. The presence of these T&O compounds in water is often linked by the consumer not only to unacceptable quality but also to an unsafe product, even though most of time they are harmless.[1] The most well-known chemicals causing T&O problems in drinking water treatment plant (DWTP) are geosmin (GEO) and 2-methylisoborneol (MIB) which impart earthy and musty flavors into the water.[2] Other compounds, including hexanal, dimethyl

disulfide (DMDS), dimethyl trisulfide (DMTS), cis-3-hexen-1-ol (C3H), cis-3-hexenyl acetate (C3HA), 2,4-heptadienal (24H), 2,4-decadienal (24D), 3-chloroanisole (3CA), trans-2,cis-6-nonadienal (T2C6N), 2,3-benzopyrrole (indole, 23B), and 2,4,6-trichloroanisole (2,4,6-TCA), are also reported to cause T&O problems in drinking water, but are relatively less frequent.[3]

T&O issues may have several sources, including natural microorganisms, high chlorine residue in tap water, products formed during water treatment processes, chemical leaching out of the pipe.[2, 4] Moldy, musty, earthy, grassy, or fishy odors are generally caused by fungi, bacteria and algae growing or from organic matters such as plants and animals.[5] When these bacteria die, they release T&O chemicals. Several cyanobacterial species, including *Anabaena*, *Aphanizomenon*, *Planktothrix*, *Planktolyngbya*, and *Pseudanabaena* etc., are potent producers of T&O compounds and algal toxins.[6] GEO and MIB are normally produced by blue-green algae, actinomycetes, and other bacteria.[7-9] The conditions favorable to algae and bacteria blooms are seasonal, but the T&O events were not confined to hot season[10]. The massive amount of rainfall or snowfall in the watershed could also cause the earthy-musty T&O issues. Biological activity in surface waters can produce 24H and 24D,[11] and C3H (grassy-green/sharp) and C3HA (grassy-fresh, sweet) are two grassy compounds that have been confirmed in drinking water supplies[12]. When grass was allowed to decay in water, the first compound to be released in the water was acetate [13]. The T&O thresholds and other basic properties of some T&O compounds are shown in Table 1.

Table 1. Chemical and physical characters of T&O causing compounds.

Compound	CAS number	Boiling point	Taste/Odor Threshold	Flavor Note
1-hexanal	66-25-1	127.9±3.0	9.18 μg/L[14]	Immature, greenish
Dimethyl disulfide	624-92-0	109.7	120 μg/L[15]	Cauliflower, garlic
Dimethyl trisulfide	3658-80-8	183.1±23	0.1-2.5 μg/L[15]	Alliaceous, meaty
Cis-3-hexen-1-ol	928-96-1	156.5	5.8 mg/L[16]	Grassy
Cis-3-hexenyl acetate	3681-71-8	174.2±19.0	1-2 mg/L[17]	Grassy
2,4-heptadienal	4323-03-5	177.4±9.0	5 μg/L[18]	Fishy
3-chloroanisole	108-42-9	95-96	34 ng/L	Medical, sweet
Trans-2, cis-6-nonadienal	557-48-2	203.3	5 μg/L	Rancid fishy
2-methylisoborneol	2371-42-8	208.7	18 ng/L[9]	Musty, earthy
2,3-benzopyrrole (indole)	120-72-9	125.30±9.0	0.1 ng/L[19]	Fecal
2,4-decadienal	25152-84-5	244.6	0.07 μg/L[20]	Fatty, earthy
2,4,6-trichloroanisole	87-40-1	60-62	0.03-10 ng/L[21]	Musty, earthy
Geosmin	19700-21-1	270.5±1.0	16 ng/L[9]	Musty, earthy

The T&O compound 2,4,6-TCA has been rarely reported in DWTPs [22, 23], which is also known for causing cork taint in different wines [24]. Normally, 2,4,6-TCA could be formed through the reaction between sodium hypochloride and anisoles, and the formed chloroanisoles include 2,4-dichloroanisole (2,4-DCA) and 2,6-chloroanisole (2,6-DCA).[25] However, the prodcution rate of 2,4,6-TCA was only about 0.4% under its favorite formation conditions, i.e., low pH (pH=3), and long reaction time (24 hours).[25]. The pH values of water in DWTPs are always higher than 7.0 to avoid the corrosive of pipes in the

following distribution systems, especially when lime softening is applied. Another possible formation pathway for 2,4,6-TCA is microbiological methylation of halophenols during water treatment or during transport through the distribution system.[3, 26, 27] It has been reported that the microbe O-methylation of precursor 2,4,6-trichlorophenol (2,4,6-TCP) in biofilms is the dominant mechanism of 2,4,6-TCA formation in drinking water distribution systems, which would be affected by many water distribution factors (e.g., pipe material, temperature, flow velocity, and residual chlorine).[27, 28]. To minimize the formation of 2,4,6-TCA in finished water, the formation pathway in the DWTPS must be determined, which is not very clear for now.

Removing T&O compounds from drinking water is a significant challenge for water facilities worldwide since conventional treatment process (coagulation, chlorination and sand filtration) can hardly remove them,[29] and their presence in the distribution systems, even at low ng/L levels, could result in consumer complaints. Powdered activated carbon (PAC) is often used in treatment plants for the mitigation of T&O issues as it is relatively simple, inexpensive and can be applied only when required [30]. However, only a few studies have focused on removal of 2,4,6-TCA, and the removal method includes bauxite as a catalyst combined with ozonation [31], tight ultrafiltration membranes [32], UV activated persulfate [33], which are either relatively expensive or difficult to be practically applied in DWTPs comparing with PAC. Therefore, the removal of 2,4,6-TCA and its precursor (2,4,6-TCP) with PAC should be well studied to help the utilities improve customer satisfaction and limit health risks of the drinking water at low cost.

The aim of this study is to investigate the origin of the T&O events in source and treated water of several DWTPs (Missouri, USA) in 2015−2017. Additionally, the

formation pathway of detected T&O compound (i.e., 2,4,6-TCA) was investigated with surface water. The effect of microorganisms during T&O event on 2,4,6-TCA formation was studied. To control the T&O issues, PAC was applied for the removal of 2,4,6-TCA and its precursor (i.e., 2,4,6-TCP), and the effects of PAC type, dosage amount, and contact time were investigated.

2. EXPERIMENTAL SECTION

2.1. MATERIALS AND ANALYTICAL METHODS

The T&O compounds including GEO, MIB, 1H, dimethyl disulfide (DMDF), dimethyl trisulfide (DMTF), cis-3-hexen-1-ol (C3H), cis-3-hexenyl acetate (C3HA), 2,4-heptadienal (24H), 3-chloroanisole (3CA), trans-2, cis-6-nonadienal (TCN), 2,3-benzopyrrole (indole, 23B), 2,4-decadienal (24D), 2,4,6-trichloroanisole (2,4,6-TCA), and 2,4,6-trichlorophenol (2,4,6-TCP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol used for preparing standards and sodium sulfate used for improving the fiber extract efficiency were purchased from Fisher (Fisher Scientific, Fair Lawn, NJ, USA).

The T&O compounds were analyzed by a solid phase microextraction–gas chromatography-mass spectrometry (SPME-GC-MS) method. The method was modified and validated for these compounds' analysis [34]. Ten mL sample was placed in a 20 mL glass headspace SPME sampling vial with 3 g sodium sulfate and sealed tightly, and then shaken for 30 minutes to let the sodium sulfate dissolve. The headspace microextraction was performed by using a SPME fiber coated with 75 μm polydimethylsiloxane-Carboxen (Supelco, Bellefonte, PA, USA) for 30 min at 60 °C in an incubator. Then desorption was

performed on a GC injection port with a Merlin Microseal High Pressure Septum (Merlin Instrument Company, Half Moon Bay, CA, USA) manual SPME device at 250 ºC for 4 minutes in the splitless mode. GC-MS analysis was performed using an Agilent 6890N GC with a 5973 inert MSD detector (Agilent Technologies, Inc., Santa Clara, CA, USA). An Agilent HP-5ms column (30 m × 250 μm ID, 0.25 μm film thickness) was used for separation. The column oven temperature program was 35 °C for 23 min, increased to 139 °C at 4 °C/min, and then increased to 250 °C at 20 °C/min. The total run time was 55 minutes. The carrier gas (helium) flow rate was 1.5 mL/min. The MS quad temperature was set at 150 ºC, and the MS source temperature was 230 ºC. The chemical molecular weights, chromatographic retention times, mass detection quantification ion pairs, and confirmation ion pairs are shown in Table 2.

Several cyanobacteria species have been identified as the producer of T&O compounds.[35, 36] Therefore, nine cyanobacterial toxins (algal toxins) including cylindrospermopsin, saxitoxin, neo-saxitoxin, decarbamoyl-saxitoxin, microcystin-LR (MC-LR), MC-RR, MC-YR, MC-LA, and MC-LF have been analyzed in the water samples collected during T&O events to test if the cyanobacteria bloom involved for the T&O. The cyanotoxins were analyzed by using a method developed previously in our lab [37]. The water eutrophication process by phosphorus (P) and nitrogen promotes cyanobacteria blooms which lead to the generation and release of T&O compounds in the environment.[38, 39] The total P concentrations were analyzed for the samples collected during T&O events by a PerkinElmer (Shelton, CT, USA) NexION 300D inductively coupled plasma-mass spectrometry (ICP-MS). All the samples were acidified with trace metal level HNO_3 to 1% HNO_3 immediately after collecting.

Table 2. Optimized MS parameters for selected taste and odor compounds.

Compound	Abbreviation	Quantification Ion	Confirm Ions	Retention time (min)	MDL (ng/L)
1-hexanal	1H	56	44/82	5.84	20
Dimethyl disulfide	DMDF	94	79	21.18	10
Dimethyl trisulfide	DMTF	126	79	25.58	2
Cis-3-hexen-1-ol	C3H	41	67/82	28.84	5
Cis-3-hexenyl acetate	CSHA	43	67/84	28.85	10
2,4-heptadienal	24H	81	110	28.95	5
3-chloroanisole	3CA	142	112/99	34.24	1
Trans-2, cis-6-nonadienal	TCN	41	69/70	37.45	5
2-methylisoborneol	MIB	95	108	38.33	0.2
2,3-benzopyrrole (indole)	23B	117	90	42.93	20
2,4-decadienal	24D	81	41/67	43.99	10
2,4,6-trichloroanisole	2,4,6-TCA	195	197	44.08	1
Geosmin	GEO	112	97	46.52	0.5

2.2. SAMPLE COLLECTIONS AND TREATMENTS

T&O Events: The first T&O event is one DWTP (Facility C&R) located on the Mississippi River (St. Louis, MO, USA). Water samples were collected from the malfunctioning basin (basin 1) and the finished water. The second T&O event is happened in another DWTP (Facility AW) using the Meramec River as source water (St. Louis, MO, USA). The water treatment schematic of facility AW and water collection spots including source water, after chlorination, and finished water were shown in Figure 1. All the collected water samples were shipped to the laboratory in an ice-cold cooler within 2 hours and were analyzed immediately after arriving. When samples were stored for stability test, all the samples bottles were totally full and there was no headspace existed.

To investigate the formation pathway of the T&O compounds, 5 mg/L of chlorine and specific amount of chemical (TCP or phenol) were added into ultra-pure water, Missouri river water with T&O issue, and Missouri river water without T&O issue. After 4 hours contacting, L-ascorbic acid was added into samples to quench the oxidants. Then, the samples were analyzed with the SPME-GC-MS method.

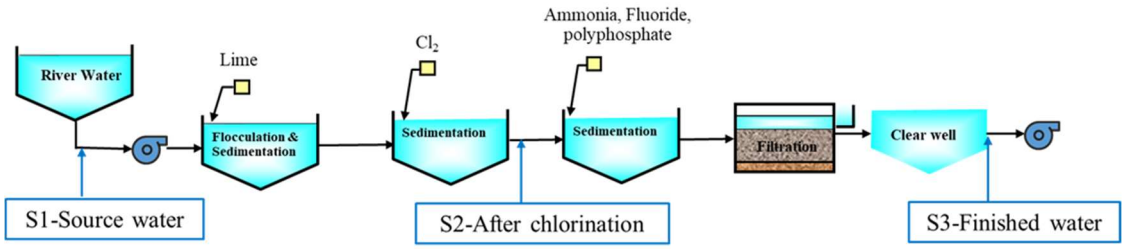

Figure 1. Water treatment schematic and samples collection spot of Facility AW.

2.3. 2,4,6-TCA REMOVAL STUDY

Water samples before adding PAC from one representative DWTP were collected to perform the T&O compounds removal study. The study started with a screening test with 16 types of PACs that were made from different types of materials by different manufacturers, and the information of PACs were shown in Table 3.

Based on the results of the screening tests, dosage tests were conducted with a similar experimental procedure. Different dosages of nine selected top performance PACs were added to water sample with 100 ng/L 2,4,6-TCA and TCP, including 0, 0.5, 2, 5, and 10 mg/L. The reaction time was 4 hours. Then, the contact time test was conducted with 5

mg/L of PAC, and samples were collected at specific contact time (i.e., 5 minutes, 30 minutes, 2 hours, 4 hours and 24 hours). Samples were filtered and analyzed as described in the screening study.

Table 3. Basic information on 15 types of tested powdered activated carbons.

Carbon #	**Manufacturer**	**Made from**	**Iodine number, mg/g**	**Surface area, m^2/g**	**Total pore volume, mL/g**
1	NA	Lignite coal	N/A	550-650	1.0 mL/g
2		Lignite coal	500 min	550-650	
3		Lignite coal	500 min	550-650	
4		Bituminous coal	800 min	900	0.79 mL/g
5	CC	Bituminous coal	800 min	N/A	
6		Bituminous coal	500 min		
7		Sub-bituminous coal	800 min		
8		Sub-bituminous coal	1000 min		
9		Bituminous coal	1000 min		
10		wood	900 min		
11	MW	Wood	900 min	1700	1.3 ml/g
12		Wood	900 min	1650	1.25 ml/g
13	SP	Bituminous coal	800	850	0.39 mL/g
14		Lignite	N/A	>450	0.25 mL/g
15	CA	coal	N/A	N/A	N/A

Note: N/A means “not available” because the manufacturers did not provide the related information.

A programmable six gang stirrer (Phipps & Bird, Richmond, VA) was used to sequentially simulate PAC sorption in drinking water treatment facility with three finally selected best performance PACs (i.e., #2, #8, and #14). T&O compounds were added to 2 L of water at 100 ng/L and mixed well by stirring for 1 min at 100 rpm before a sample was taken. In Step 1 for the PAC absorption test, 0.6 mg/L F^-, 6.8 mg/L ferric sulfate, 0.4 mg/L polymer, sodium hypochlorite, and PAC were added and then the water was mixed rapidly at 300 rpm for 30 seconds, mixed slowly to form floc for 10 min each at 58, 42, and 28 rpm, and allowed to settle for 140 min before samples were taken for analysis. Sodium hypochlorite solution was added to obtain a residual concentration of 2–3 mg/L as Cl_2. In Step 2, the treated water was decanted into a new 2 L square beaker, leaving only solids behind, and then was settled for another 21.6 hours before another sample was taken for analysis to simulate the secondary sedimentation in the drinking water treatment facility. All the chemicals added in the simulation study were received from the selected DWTP, and the dosage amount and contact time were same as the treatment processes in the DWTP.

3. RESULTS AND DISCUSSIONS

3.1. IDENTIFICATION OF T&O COMPOUNDS

Event I: The treatment process of facility C&R is a multi-stage, conventional water treatment process consisting of coagulation, flocculation, sedimentation, disinfection, and filtration. The water in lime softening basin (Basin 1) changed to a greenish color (looking like algal bloom). Bad T&O (mainly earthy and musty) was encountered in the finished drinking water.

Table 4. T&O compounds concentrations in the 1st and 2nd T&O issues in Missouri.

Events	Sample Location	Analyzation Date	T&O (ng/L)			
			3CA	MIB	TCA	GEO
1st (Facility C&R)	Basin 1	Event day	9.9	8.0	67.4	14.4
	Finished water		37.8	26.9	172.6	62.0
	Finished water	3 days later*	30.9	7.7	93.9	8.5
	Basin 1	7 days later*	6.9	<MDL	35.2	6.3
	Finished water		25.1	<MDL	80.8	8.2
2nd (Facility AW)	S1-Source water	Event day	<MDL	1.1	3.3	<MDL
	S2-After chlorination		<MDL	1.1	11.2	0.8
	S3-Finished water		<MDL	1.2	5.8	1.3

Note: *samples were collected on the event day, but stored in refrigerator and analyzed at different time

Four of the thirteen detected T&O compounds were found in the water samples, including 2,4,6-TCA, 3CA, and GEO, and their concentrations are listed in Table 4. The other nine compounds were all below method detection limits. As described in Table 1, the taste threshold of 3CA is around 5 μg/L, therefore it was not the compounds causing T&O during this event. The highest concentrations of these compounds were in the finished drinking water. High concentrations of 2,4,6-TCA and GEO are the major cause of the T&O of the drinking samples. The concentration of 2,4,6-TCA increased sharply from 30 ng/L to 173 ng/L after chlorination. This 2,4,6-TCA increase in tap water was caused by the formation of 2,4,6-TCA during chlorine disinfection and subsequent methylation[40].The

concentration range of 2,4,6-TCA were reported from 0.03-122.3 ng/L worldwide.[5, 21] The stabilities of the compounds were also tested by analyzing at different time intervals. The concentrations of all these compounds decreased with time, indicating either they were degraded or volatized during storage. This possibility is evidenced by quick decreasing at the beginning, and the decrease slows down with the time in the DWTPs. The results of total P and other basic water parameters are shown in Table 5. The total P concentration in Basin 1 is much lower than under normal conditions (~150 μg/L). The algae and other microorganism that existed consumed P,[41] leading to less P that could be detected in Basin 1.

Upon investigation, it was determined that the likely source of the T&O issues originated due to an appearing microorganism bloom after the disinfection process conveyed through the plant and into the finished drinking water. The chemical analysis confirmed that the high concentrations of 2,4,6-TCA and GEO should be the major cause of the T&O.

Event II: Three of the thirteen tested T&O compounds were found in the water samples from facility AW, including 2,4,6-TCA, MIB, and GEO, and their concentrations are listed in Table 4. The other 10 compounds were all below detection limits or T&O threshold. The highest concentrations of these compounds were in the finished drinking water. MIB and GEO concentrations were below their T&O threshold in all samples; therefore, high concentrations of 2,4,6-TCA should be the major cause of the T&O of the drinking samples. After chlorination, the concentration of 2,4,6-TCA increased from 3.3 ng/L to 11.2 ng/L. A similar trend had been discovered in Facility C&R. Since the water treatment steps after chlorination (i.e., adding polyphosphate, rapid mix, and sand

filtration) might lead to the decomposition and evaporation of 2,4,6-TCA, the concentration of 2,4,6-TCA in finished water (5.8 ng/L) was lower than in the water sample after chlorination. In a previous study, the concentration of 2,4,6-TCA was also founded to be increased after chlorination, which was suggested to be the methylation of 2,4,6-TCP[22].

The concentrations of 2,4,6-TCA in water samples we collected ranged from 3.3-11.2 ng/L. However, the taste threshold of it was reported to be 0.03-10.0 ng/L[21]. To confirm the concentrations of 2,4,6-TCA in the water were high enough to cause taste problem and to determine the taste threshold of 2,4,6-TCA, a panel taste examination was conducted. Different concentrations (0, 0.03, 0,1, 0.3, 1, and 5 ng/L) of 2,4,6-TCA were prepared in ultrapure water and transferred into different cups. A group of tasters was selected randomly to taste these samples. The detection method for taste threshold of 2,4,6-TCA was modified from Young et al[9]. The taste evaluations from different people are shown in Table 6. Almost all the tasters had threshold levels 0.1 ng/L to 0.3 ng/L and the taste was the same as the water samples collected from the water treatment facility. Thus, 2,4,6-TCA was the primary, if not all, compounds causing T&O problem in this event. The total P concentrations and other basic water parameters were shown in Table 5. All the basic water parameters were in normal range. Previous studies demonstrated that, in lake water, high concentrations of P and low N:P supply ratio (TN:TP<29:1 by mass) are favorable for the production of cyanobacteria blooms[42, 43]. The source water in here had a relatively high P concentration and low N:P ratio.

Table 5. Total P concentrations and other basic water parameters.

Events	Sample Name	Total P (µg/L)	pH	Conductivity (microohm)	UV254 (1/cm)	DOC (mg/L)	TN (mg/L)	SUVA (L/(mg m))
1st (Facility C&R)	Basin 1	50.22	9.67	486	0.0548	3.25	1.54	1.69
	Finished water	6.99	9.24	589	0.0560	2.58	2.37	2.17
2nd (Facility AW)	S1-Source water	277.8	7.74	261	0.0813	3.92	0.64	2.07
	S2-After chlorination	10.23	10.07	262	0.0449	3.03	0.53	1.48
	S3-Finished water	57.85	10.00	266.5	0.0579	2.65	1.11	2.19

Table 6. Results of taste threshold concentration of TCA from different people. “X”: no taste, “√”: obvious taste.

Taster	2,4,6-trichloroanisole concentration (ng/L or ppt)					
	0	0.05	0.1	0.3	1	5
A	X	X	X	uncertain	√	√
B	X	X	X	√	√	√
C	X	X	weak	√	√	√
D	X	X	weak	√	√	√
E	X	X	weak	√	√	√
F	X	√	√	√	√	√

Upon investigation, it was determined that the likely source of the 2nd T&O event was due to the massive amount of rainfall in the Meramec River watershed. The unpleasant T&O was primarily described as earthy-musty. Under favorable conditions, cyanobacteria can quickly reproduce to yield large algae blooms that may release toxins, some of which are known to cause T&O problems in addition to serious health effects to aquatic life, animals, and humans. The water in Basin 1 (Facility C&R), the 1st T&O event, showed greenish color and looks like algal bloom. Nine most commonly reported cyanobacterial toxins (algal toxins) were analyzed in all of the water samples from two DWTPs during the T&O events, and no detectable algal toxin was found. This result indicated there is no harmful algae, at least not well known algal toxin producing algae, involved in these T&O events.

3.2. THE FORMATION PATHWAY OF 2,4,6-TCA

These two T&O events demonstrated that a sparsely investigated compound (i.e., 2,4,6-TCA) caused the T&O problems. Thus, it is important to study the source, precursor,

and formation pathway of this chemical in public drinking water systems in order to solve the drinking water T&O problem. The microbiological methylation of halophenols during water treatment or during transport through the distribution system has been supposed to be the potential formation pathway of 2,4,6-TCA [3, 26, 27]. Therefore, 2,4,6-TCP and phenol had been added into the water samples as the precursors to check the probability of 2,4,6-TCA formation.

The results are shown in Table 7. In the water with T&O issue before any treatment, the GEO concentration (i.e., 3.8 ng/L), was lower than its T&O threshold. However, the 2,4,6-TCA concentration (1.5 g/L) was high enough to cause a T&O problem. After treating the water samples for 2 hours using 5 mg/L free chlorine, more 2,4,6-TCA was formed in the water (i.e., 1.7 ng/L MIB, 6.1 ng/L 2,4,6-TCP, 15.0 ng/L 2,4,6-TCA, and 3.6 ng/L GEO). Therefore, during the chlorination, T&O compounds could be formed through the reaction of precursors or leached out from the micoorganisms in the water. In one of the previous study, comparing with no chlorine added, the addition of free chlorine leads to the formation of 2,4,6-TCA in the distribution system decreased about 30% [23]. However, in the DWTP, the 2,4,6-TCA concentrations were found to either increased or decreased after chlorination[22]. Therefore, 2,4,6-TCA formation may be affected by the chlorine concentration, contact time, microorganism species, and water matrix etc. More investigations should be conducted to illustrate it clearly. Concentrations of 1 μg/L 2,4,6-TCP or 10 μg/L phenol were added into the water sample and treated with 5 mg/L chlorine for 2 hours. 2,4,6-TCA concentration increased from 15 ng/L to 22 ng/L when 1 μg/L 2,4,6-TCP was added, indicating that adding 2,4,6-TCP promoted the formation of 2,4,6-TCA. On the other hand, much higher concentration of phenol only led to a slight increase

of 2,4,6-TCP and 2,4,6-TCA. Both 2,4,6-TCP and phenol addition did not increase MIB and geosmin formation.

Table 7. T&O compounds test results for three selected water samples.

Sample	Treatment	Chemical Added	Phenol (ng/L)	MIB (ng/L)	TCP (ng/L)	TCA (ng/L)	GEO (ng/L)
Missouri River Water (with T&O issue)	No treatment	No	<MDL	<MDL	<MDL	1.5	3.8
	5 mg/L Cl_2	No	<MDL	1.7	6.1	15	3.6
		1 µg/L TCP	<MDL	1.6	1,000	22.4	3.0
		10 µg/L phenol	10,000	1.6	8.0	17.1	3.2
Missouri River Water (without T&O issue)	No treatment	No	<MDL	<MDL	<MDL	<MDL	1.8
	5 mg/L Cl_2		<MDL	<MDL	<MDL	<MDL	1.8
	No treatment	1 µg/L TCP	<MDL	<MDL	1000	<MDL	1.6
	5 mg/L Cl_2		<MDL	<MDL	1000	<MDL	1.8
Ultra-pure Water	No treatment	No	<MDL	<MDL	1000	<MDL	<MDL
	5 mg/L Cl_2	1 µg/L TCP	<MDL	<MDL	1000	<MDL	<MDL

In the water without a T&O issue, all T&O compounds concentrations were below their T&O threshold or detection limits (Table 4) before chlorination. After being treated with 5 mg/L free chlorine for 2 hours, no T&O compounds were detected. When 1 µg/L of 2,4,6-TCP was added to high purity water and the collected river water (without T&O issue) followed by treatment with chlorine, no detectable 2,4,6-TCA was generated, indicating that the 2,4,6-TCA formation was not from the direct reaction between chlorine

and 2,4,6-TCP. Therefore, fungi or other microorganisms, or other conditions in the water must play an important role in the formation of 2,4,6-TCP in DWTPs.

3.3. 2,4,6-TCA REMOVAL WITH PAC

Ozonation is a powerful tool capable of oxidizing most of the T&O compounds to more than 50% under typical drinking water treatment conditions. For ozone-resistant T&O compounds, the application of advanced oxidation processes may be appropriate[44]. Several studies were using tight ultrafitration membranes[32], catalyzed ozonation[45, 46], and UV activated persulfate[33] for 2,4,6-TAC removal. However, the catalysts, chemicals or light energy input limit the applicae of previous technology in DWTP. Studies have shown that PAC absortion was the effective way to treat the GEO and MIB[1]. There are no PAC absorption studies has been performed on 2,4,6-TCA and 2,4,6-TCP in DWTP, which is most frequently used in DWTPs worldwide.

Screen Study: A screen study with 15 differeent PACs was performed for 2,4,6-TCA and 2,4,6-TCP removal. As shown in Figure 2, most of the PACs removed all TCA and TCP (efficiencies reached to about 100%) after 4 hours contacting time with 5 mg/L PAC. However, the method detection limit of 2,4,6-TCA was 1 ng/L, and the taste threshold was 0.3 ng/L. Therefore, the concentration of 2,4,6-TCA might be still higher than its taste threshold. Based on the raw material of these selected PACs, bituminous coal, sub-bituminous coal, and wood-based PAC are more suitable to absorb T&O compounds. A higher iodine number would also increase the 2,4,6-TCA and 2,4,6-TCP removal efficiencies. Overall, PAC# 2, 5, 8, 9, 10, 11, 12, 13, and 14 were selected for following studies based on the performance and the range of application in DWTPs.

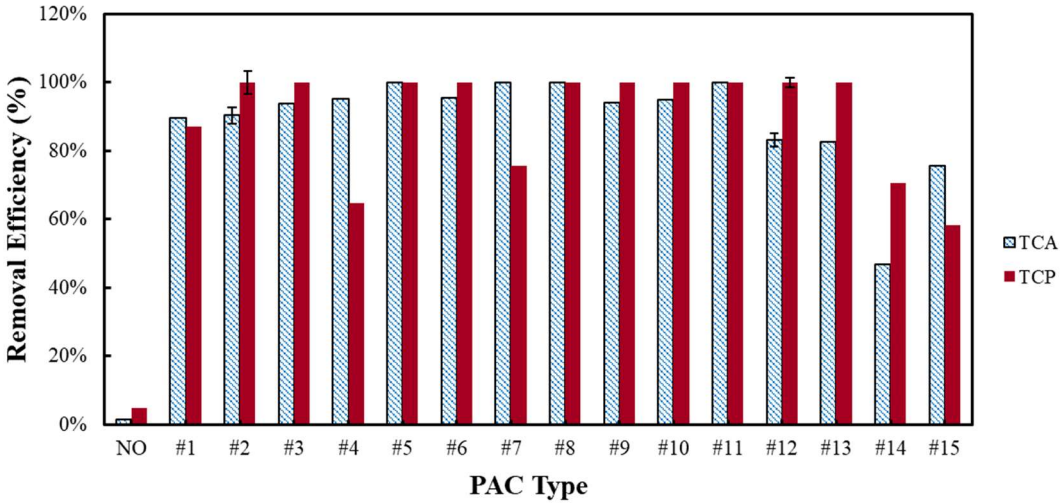

Figure 2. The removal efficiencies of TCA and TCP with different PACs (dosage amount: 5 mg/L, contact time: 4 hours).

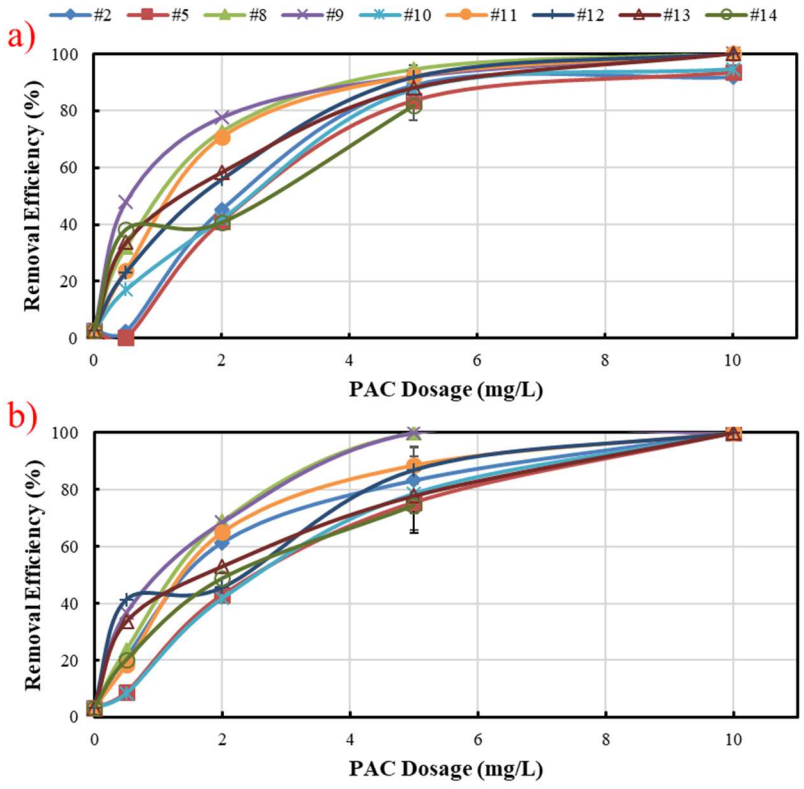

Figure 3. The effect of dosage amount on a) TCA and b) TCP removal efficiencies with nine types of selected PACs (contact time: 4 hours).

Dosage and contact time study: The removal efficiencies of 2,4,6-TCA and 2,4,6-TCP with different dosage and contact times of nine selected PACs were shown in Figures 3 and 4. The nine kinds of PACs with different material and parameters had the same removal trend on T&O compounds absorption. From PAC concentrations 0.5 mg/L to 5 mg/L, the removal efficiencies increased sharply, but the PACs dosage increase from 5 mg/L to 10 mg/L, the removal efficiencies were relatively stable. With 5 mg/L dosage amount, #8 and #9 were shown to be more suitable than others, which had 94% and 92% removal for 2,4,6-TCA, 100% and 100% removal for 2,4,6-TCP, respectively. However, there are no obvious difference among the nine selected PACs for 2,4,6-TCA removal, especially when the dosage amount reached to 5 and 10 mg/L. With the increase of contact time from 5 minutes to 4 hours, the removal efficiency of all PACs for both 2,4,6-TCA and 2,4,6-TCP increased significantly. However, from 4 hours to 24 hours, the removal effeciencies either no significant change or increase very slowly. Therefore, to removal the T&O compounds (i.e., 2,4,6-TCA and 2,4,6-TCP) in DWTP, 5 mg/L of PACs with a basin that could provide 4 hours contact time should be enough.

Simulation test: A six-gang stirring was used to simulated the DWTP treatment processes. The amount of PAC added during T&O events was normally 10 mg/L in the selected DWTP. However, based on the dosage study, 5 mg/L PAC should be able to control the issues. Therefore two dosage concentrations were tested: 5 mg/L and 10 mg/L. The results of removal efficiencies for 2,4,6-TCA and 2,4,6-TCP after each treatment process are shown in Figure 5. With 5 mg/L PAC added, the T&O compound removal efficiencies were about 40-60% for 2,4,6-TCA and 30-60% for 2,4,6-TCP after Step 1.The removal efficiencies with 10 mg/L PAC were all higher than 80%. there was about 20-30%

2,4,6-TCA and 2,4,6-TCP lost for the control without adding any PAC,, which means the strong mixing and other chemicals added could lead to some removal of T&O. After Step 2, no matter the dosage amount of PACs, both 2,4,6-TCA and 2,4,6-TCP concentrations were below the method detection limits. Therefore, to control the T&O event in normal DWTP, if the event is caused by 2,4,6-TCA, 5 mg/L of PAC will be able to control the issue, at least for the water matrix tested in this study.

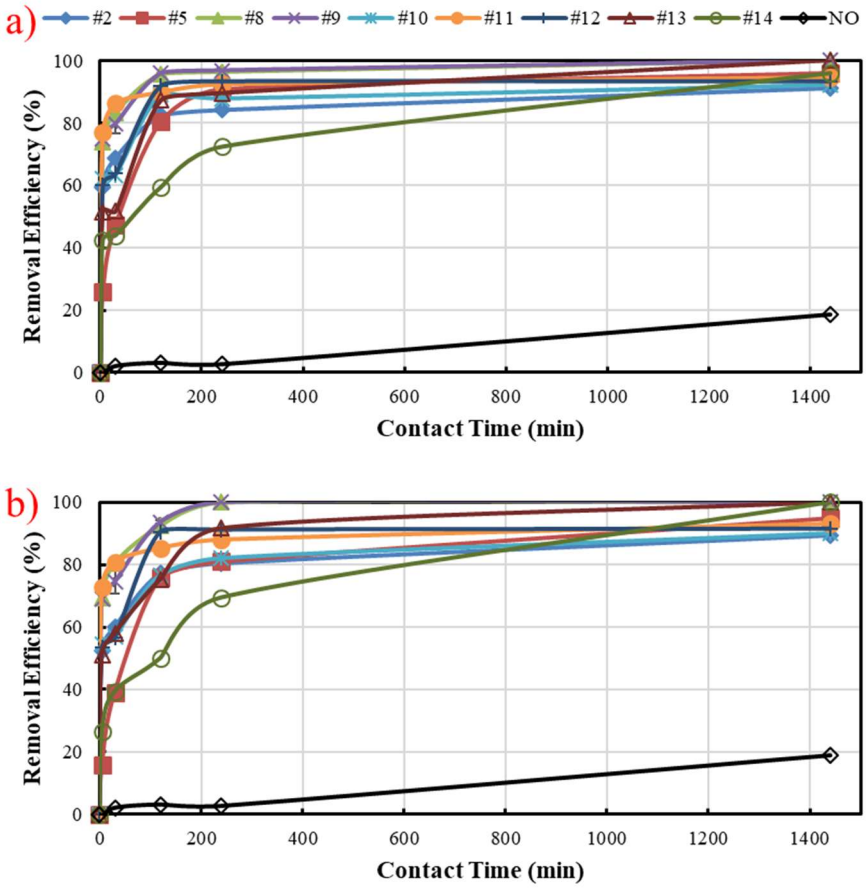

Figure 4. The effect of contact time on a) TCA and b) TCP removal efficiencies with nine types of selected PACs (dosage amount: 5 mg/L).

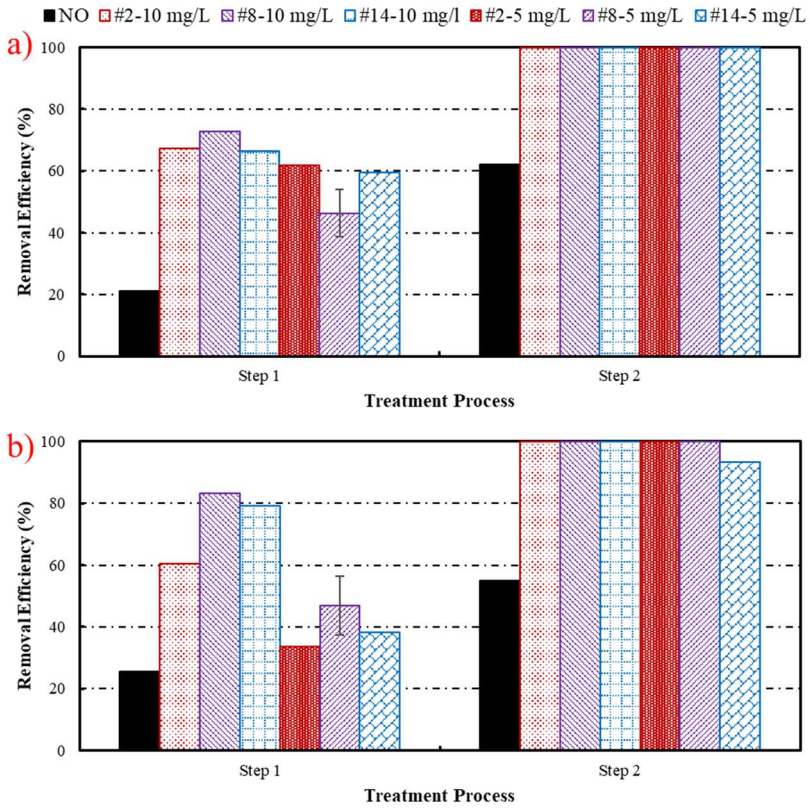

Figure 5. The removal efficiencies of a) TCA and b) TCP after two steps simulating DWTP treatment processes.

3.4. ENVIRONMENTAL IMPLICATIONS

It has been shown in this study that 2,4,6-TCA is a compound causing several T&O events in Missouri, USA recently. The formation of 2,4,6-TCA in the drinking water systems is not only due to chlorination but also to some microorganisms that would appear under specific circumstance like high P, snow smelting etc. Several fungal strains have been isolated in wine and DWTPs that could produce 2,4,6-TCA [22, 23, 47, 48]. Methyltransferase and gene coding have been purified from mycelia of *Trichoderma longibrachiatum*, which is specifically induced by several chlorophenols, especially the

compounds containing three or more chlorine atoms [49, 50]. The presence of T&O compounds in one of the events is caused by the poor maintenance of the sedimentation basin in the drinking water treatment system. Periodically checking the status of all the basins and treatment tools is very necessary to avoid this kind of situation. In the surface water, a large rain or snow-melt causing some bacteria in the soil being releasing to the surface water could lead to the presence of 2,4,6-TCA in investigated DWTPs. PAC, which is widely used in DWTPs, would be an effective way to remove the T&O compounds (2,4,6-TCA and TCP). However, PAC still may not decrease the concentration to its threshold since the threshold of taste is so low. Therefore, finding the precursors of 2,4,6-TCA and using suitable ways to control them would be beneficial. In our study, chlorination would promote the formation of 2,4,6-TCA so other kinds of disinfectants may be used during T&O events those caused by 2,4,6-TCA. A model should be set up to predict the occurrence of 2,4,6-TCA based on the water parameters, treatment processes, and other conditions, and then related drinking water treatment methods could be applied suitably.

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SECTION

2. CONCLUSIONS

This work investigated the chlorine demand for releasing and removal rates of cyanotoxin (i.e., MC-LR and CYN) with the related cyanobacteria. Low dosages of chlorine with no measurable free chlorine exposure can cause the majority of intracellular MC-LR to be released from the *M. aeruginosa* cells with little destruction of the total toxin present. The role of even very low levels of ammonia in the raw water is important due to its effect on the rapid conversion of the reactive free chlorine to non-reactive species and the releasing rates of intracellular toxins with ammonia adding were much lower than without ammonia. The chlorine dosage necessary to oxidize and remove *C. raciborskii* cells and CYN in natural water was highly dependent on the cell and organic matters concentrations in the water. Increasing oxidant exposure was shown to cause increased rates for both MC-LR and CYN release from cells. The effects of chlorination on the release of investigated cyanotoxins from cyanobacteria cells were greater than permanganate and other oxidants.

The occurrence, formation and control of T&O compounds 2,4,6-TCA has been studied. The formation of 2,4,6-TCA in the drinking water systems is due to not only chlorination but also to some microorganisms. Fifteen species of PACs with different brands and materials were investigated for 2,4,6-TCA and its precursor 2,4,6-TCP removal. Results shown that, to control the T&O event in normal DWTP, if the event is caused by 2,4,6-TCA, 5 mg/L of PAC will be able to control the issue.

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