CYANOBACTERIA BLOOMS: FROM IMPACTS ON THE ENVIRONMENT TO MANAGEMENT STRATEGIES

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Abstract

Bloom-forming cyanobacteria are harmful to both environment and public health because of the release of water soluble toxins. This report provides a broad overview of cyanobacteria and cyanotoxins and the current state of knowledge about the bloom control management. Cyanobacteria blooms usually flourish in warm, lentic, and eutrophic waters. Several environmental factors such as temperature, nutrients, light intensity, and turbulence can affect cyanobacterial growth and the formation of bloom. Cyanobacteria can synthesize multiple types of toxins, which cause human and animal toxications worldwide. Cyanobacterial blooms also cause detrimental effects on aquatic ecosystems, and the taste and odor problems in drinking water supplies. Due to the adverse effects, treatments that are used for removing both cyanobacterial cells and aqueous cyanotoxins should be carried out once cyanobacterial blooms occur in freshwaters. Strategies based on physical, chemical, and biological methods are carried out to remove the cyanobacteria and cyanotoxins. All of these strategies have both advantages and disadvantages: some physical treatment methods can remove cyanotoxins within the intact molecules, but the cost is usually high and further processing is needed; some chemical methods are cheap and can degrade the cyanotoxins, however, the toxicological characterization of the chemical and the by-products needs to be investigated; some biological treatments are more environmentally friendly, but the long reaction time and some other external factors also pose some problems that affect the efficiency of the treatments. The paper concludes that the key to success is to find a reasonable balance between those advantages and disadvantages, and the specific conditions of each unique aquatic ecosystem should be taken into consideration. As well, some suggestions are also proposed for the further development of more robust monitoring and management strategies.

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Chapter 1 - Introduction

Eutrophication is an increase in the rate of supply of artificial or natural nutrients, usually phosphorus and nitrogen, in an aquatic system (Nixon, 1995). Eutrophication has been considered as a pollution problem in many countries' lakes since the middle of the twentieth century (Rodhe, 1969), and has become more widespread now. Eutrophication can result in surface scums, increased turbidity, floating plant mats, changes in macrophyte species composition, and visible cyanobacterial blooms (Chorus & Bartram, 1999). Cyanobacterial harmful algal blooms (CHABs), which are rapid and massive expansions of cyanobacteria (blue-green algae) in aquatic ecosystems, have increased in recent decades, posing a serious threat to environment and public health. Cyanobacteria are believed to be the earliest known prokaryotic oxygenic photosynthetic organisms on Earth and have released oxygen into the atmosphere since over 2.5 billion years ago (Schopf, 2002). Cyanobacteria can be found in diverse terrestrial and aquatic environments worldwide, and they can exist in form of colonies, filaments, solitary, or free-living cells (Quiblier et al., 2013). In this paper, we will focus on the cyanobacteria in the freshwater ecosystem. Freshwater cyanobacteria blooms usually result from a combination of various environmental factors, including warm temperature, appropriate incidence of sunlight, excessive available nutrients, lack of disturbance, etc. (Perovich et al., 2008). Cyanobacteria blooms can be detrimental to aquatic ecosystems by degrading water quality, killing aquatic animals (Robarts et al., 2005), and altering the trophic structure and functionality of the ecosystems (Havens, 2008). They are also harmful to humans because of the various toxic compounds that produced by cyanobacteria (Dionysiou, 2010). Cyanobacteria produce a range of organic compounds, including those taste and odor compounds and cyanotoxins. Cyanotoxins are natural contaminants that occur worldwide, so far, cyanobacteria blooms have been reported

for more than 60 countries, and approximately half of these blooms are known to produce cyanotoxins (Codd, 1995; Svrcek & Smith, 2004). According to different acting sites, cyanotoxins can be classified as neurotoxins, hepatotoxins, cytotoxins, and skin irritants (Falconer, 2008). Humans usually ingest cyanotoxins from drinking water, by eating fish and shellfish from contaminated waters, or through recreational activities. Humans and animals which are exposed to cyanotoxins can be seriously ill, and even dead. All these conditions are undesirable and noxious, therefore, the efficient management and protection of freshwater ecosystem from the cyanobacterial blooms is of essential importance.

At present, various methods have been used for improving water quality and managing the cyanobacterial blooms, which can be classified as physical, chemical, and biological methods. The physical methods include activated carbon adsorption (Newcombe, 2003), ultrasound (Hao et al., 2004), clarification and flotation, filtration, sedimentation (Drabkova & Marsalek, 2007; Svrcek & Smith, 2004), etc. Most of them cannot provide permanent solutions and some of them are very expensive. The chemical agents used for cyanobacterial control, such as metal algaecides, photocatalysts, oxidants, herbicides, etc., have fast effects, but may result in secondary pollution (Wang et al., 2012). The biological methods consist of grazing by zooplankton and/or herbivorous fishes, planting macrophytes and periphyton on the windward lake shore, and biodegradation of cyanotoxins with the participation of cyanophages (viruses) and indigenous environmental bacteria (Drabkova & Marsalek, 2007). In this review, we will address the causes and environmental influences of cyanobacterial blooms, their ecological impacts on environment and human beings, and also the diversity of currently available treatments for cyanobacteria and cyanotoxins management. The effectiveness, advantages, and limitations of all the available methods are also discussed in this study.

Chapter 2 - Harmful Cyanobacteria

2.1 An overview of cyanobacteria

Cyanobacteria, also known as blue-green algae, belong to the Kingdom Prokaryota (Monera), Division Eubacteria, Class Cyanobacteria (Maynard et al., 2000). Cyanobacteria are the oldest oxygenic phototrophic organisms on the Earth, and are considered to be the earliest organisms to have released oxygen into the atmosphere (Schopf, 2002). The long evolutionary history has endowed them to adapt to climatic and geochemical changes and also to nutrient alterations and deficiencies (Carr & Whitton, 1982; Fogg, 2012). Cyanobacteria can be found all over the world. Their prominent habitats are limnic and marine environments, and occasionally in the soil and the fissures of rocks. Cyanobacteria flourish in waters with a great range of temperature and salinity. They are capable of living in brackish and fresh waters, Antarctic cold waters, volcanic hot springs, snow and ice in glaciers, and even some environments where no other organisms can exist (Chorus & Bartram, 1999; Svrcek & Smith, 2004; Takeuchi, 2001).

As a prokaryote, the cellular structure of cyanobacteria is most similar to bacteria: they don’t have defined nucleus or organelles, and their cell wall structure is similar to that of gram-negative bacteria (Gerba et al., 2000). Cyanobacteria have single circular chromosomes, and some of them also carry plasmids, which are small circular strands of DNA (Falconer, 2004; Kaneko et al., 1996; Schwabe et al., 1988). Many cyanobacteria have gas vacuoles that allow them to float in the surface waters, where they gain more light exposure. (Oliver, 1994). The ribosome, which is the protein-synthesizing organelles of cyanobacteria, is also same as bacterial. There are several kinds of pigments present in the cyanobacteria: chlorophyll-a and phycocyanin, which are contained in the photosynthetic membranes, provide the blue-green color of many species; pigment carotenoids and phycoerythrin, can provide a strong red color to some other

species (Whitton & Potts, 2000). Despite the common name blue-green algae, they are actually not eukaryotic, but prokaryotic cells. However, like the algae, but unlike bacteria, they can use water as the electron source and release oxygen gas through photosynthesis (Falconer, 2004). Depending on the species, the size of cyanobacteria cells vary from < 3 to 40 μm ("Cyanobacterial Toxins -- Microcystin-LR," 2000). In freshwater, the morphology of cyanobacteria varies with different environmental factors, and can be identified as three basic groups: unicells, undifferentiated-nonheterocystous filaments, and filaments containing differentiated cells (heterocysts) (Paerl et al., 2001) (Figure 2-1). Nitrogen fixation is an important function of some species of cyanobacteria, which is processed in heterocysts. Heterocysts are specialized cells, which usually occur within the filament of photosynthetic cells. They don't have photosynthetic membranes, and the cell wall is thicker than the normal cells. The nitrogenase within heterocysts can reduce nitrogen molecular to ammonia, which contributes to the incorporation of glutamine (Haselkorn, 1995).

Cyanobacteria include 2000 species in 150 genera, and based on morphology, they can be classified into five orders (Table 2-1). From the ecological aspect, freshwater cyanobacteria can be classified into several groups, including mat-forming species, bloom-formers, picocyanobacteria, colonial non-bloom-formers, and metaphytic species, etc (Vincent, 2009). In this review, we will focus on the bloom-forming cyanobacteria and their toxins.

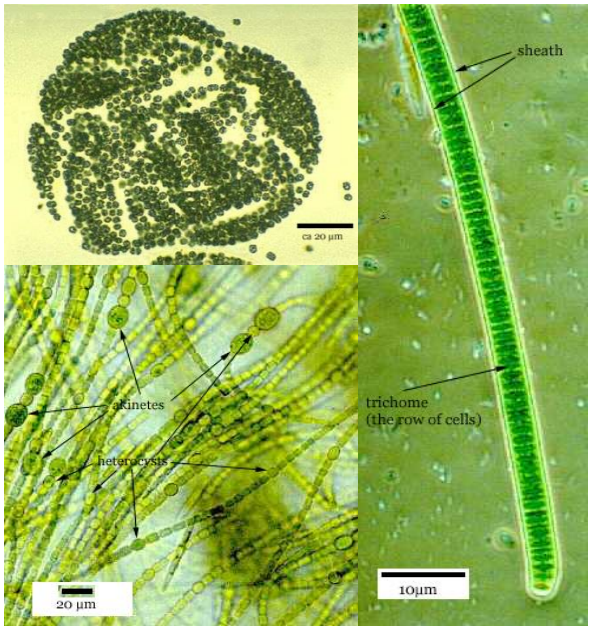

Figure 2-1 Morphology of cyanobacteria. Upper left, Chroococcales, colonies of unicells; lower left, Nostocales, coccoidal cells forming filaments with heterocysts; right, Oscillatoriales, single filamentous forms consisting of a trichome of cells surrounded by a sheath ("Precambrian Life I: Microfossils," 2001).

Table 2-1 Grouping of cyanobacteria based on morphology (Vincent, 2009)

Order	Characteristics	Representative genera
Chroococcales	Coccoid cells occur either solitary, in pairs or as colonies of unicells. Reproduced by binary fission or budding.	Aphanocapsa, Aphanothece, Gloeocapsa, Merismopedia, Microcystis, Synechococcus, Synechocystis
Pleurocapsales	Coccoid cells, aggregates or pseudo-filaments that reproduce by baeocytes.	Chroococcidiopsis, Pleurocapsa
Oscillatoriales	Single filamentous forms, without heterocysts or akinetes.	Lyngbya, Leptolyngbya, Microcoleus, Oscillatoria, Phormidium, Planktothrix
Nostocales	Filamentous cyanobacteria with heterocysts and akinetes.	Anabaena, Aphanizomenon, Calothrix, Cylindrospermopsis, Nostoc, Scytonema, Tolypothrix
Stigonematales	Division in more than one plane, have true branching and heterocysts	Mastigocladus (Fischerella), Stigonema

2.2 Cyanotoxins

Cyanobacteria can produce a wide range of secondary metabolites, which include peptides, glycosides, and macrolides (Namikoshi & Rinehart, 1996). Those compounds usually act as antibiotics, hormones, and toxins. Harmful cyanobacteria are notorious for their production of various types of cyanotoxins, which pose threats to both human and environmental health (Ettoumi et al., 2011; Hitzfeld et al., 2000). There are a variety of cyanobacteria genera that can produce toxins. Cyanotoxins involve more than 100 compounds with different structures and toxicological properties (Merel et al., 2013). Table 2-2 lists the cyanobacteria genera, associated toxins, and the toxicological characteristics of cyanotoxins.

Table 2-2 Characteristics of cyanotoxins (Lopez et al., 2008; Merel et al., 2013)

Toxin	Genera	Mode of action	Main effect
Microcystins	*Anabaena, Aphanocapsa, Hapalosphon, Microcystis, Nostoc, Planktothrix (Oscillatoria)*	Inhibit protein phosphatase	Liver failure and hepatic hemorrhage
Nodularins	*Nodularia spumigena*	Inhibit protein phosphatase	Liver failure and hepatic hemorrhage
Saxitoxins	*Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya*	Bind to sodium channels	Ataxia, convulsions and paralysis
Anatoxins	*Anabaena, Aphanizomenon, Planktothrix (Oscillatoria)*	Binds to nicotinic acetylcholine receptors	Muscular paralysis
Cylindrospermopsin	*Aphanizomenon, Cylindrospermopsin, Umezakia*	Inhibits protein synthesis	Liver and kidney failure
Lyngbyatoxins	*Lyngbya*	Activate protein kinase C	Tumour promotion and skin irritation
Beta-N-methylamino-L-alanine (BMAA)	*Anabaena, Cylindrospermopsin, Microcystis, Nostoc, Planktothrix (Oscillatoria)*	Binds to glutamate receptors	Neurodegenerative syndrome

As seen in the table, some cyanobacteria genera can synthesize multiple types of toxins, and the specific toxins can also be produced by different genera of cyanobacteria. Cyanotoxins can be classified into 3 groups according to their chemical structure: alkaloids, cyclic peptides, and lipopolysaccharides (Chorus & Bartram, 1999); and into 4 groups based on the target organ: hepatotoxins, neurotoxins, cytotoxins, and dermatotoxins (Corbel et al., 2014).

2.2.1 Hepatotoxins

From both fresh and brackish waters, cyclic peptide hepatotoxins are the most frequently found cyanotoxins in cyanobacterial blooms (Chorus & Bartram, 1999). Those hepatotoxins have molecular weights from 800 Da to 1100 Da. Some types of hepatotoxins, such as nodularins, contain five amino acids, and some types, such as microcystins, have seven amino acids (Figure 2-2).

As presented in Figure 2-2, a microcystin molecular has cyclic structure with seven amino acids. Within these amino acids, the Adda amino acid ((2S, 3S, 4E, 6E, 8S, 9S)-3-Amino-9-methoxy-2, 6, 8-trimethyl-10-phenyldeca-4, 6-dienoic acid) is associated with the toxicity of the molecule through the conjugated diene (Dawson, 1998). The X and Z are variable amino acids that identify and name different variants. The X amino acid is usually arginine (R), tyrosine (Y), or leucine (L), and the Z amino acid is commonly arginine (R), methionine (M), or alanine (A) (Svrcek & Smith, 2004). Therefore, the microcystin which has leucine and arginine can be identified as microcystin-LR. The nodularin structure is similar to microcystin, also contains a carbon ring with an Adda amino acid, which causes toxicity. However, it only has five variable amino acids. Comparing to microcystins, nodularins contain less variants and mainly exist in brackish water environment (Fitzgerald et al., 1999).

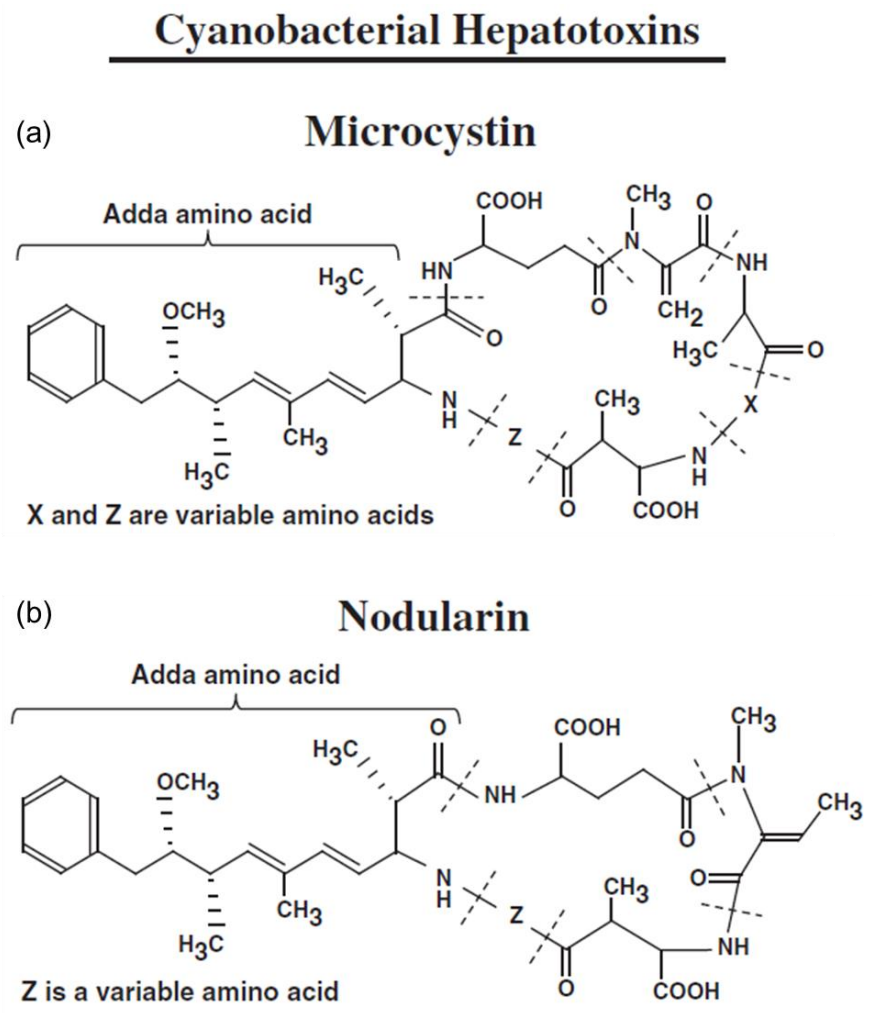

Figure 2-2 Chemical structure of (a) Microcystin, and (b) Nodularin (Merel et al., 2013)

Microcystins (MCs) are the most famous cyanotoxins because they are most widespread and most frequently studied. MCs are water soluble and very stable (Chorus & Bartram, 1999). They can damage the liver resulting from hypovolemic shock and accumulating excessive blood in the liver, and also by binding to the protein phosphatase (Zanchett & Oliveira, 2013). Lots of human and animal intoxications by drinking or touching water with MCs have been reported (Hilborn et al., 2007; Soares et al., 2006; Stewart et al., 2008). And the World Health Organization (WHO) gave a guideline of 1 μg/L for MC-LR in the drinking water (WHO, 1998).

2.2.2 Neurotoxins

Neurotoxic cyanobacteria have been reported in Europe, North America, and Australia where they caused human and animal intoxications. Although they are not as widespread as hepatotoxins in waters (Svrcek & Smith, 2004), they are the most toxic toxins that produced by cyanobacteria (Zanchett & Oliveira, 2013). Anatoxin-a is a 165 Da alkaloid. It is highly soluble, and unstable when pH>10 and exposed to sunlight. Under the sunlight, it will transfer to a non-toxin form (Merel et al., 2013).

Anatoxin-a can bind muscarinic acetylcholine receptors like the action of acetylcholine, but it cannot be degraded by the acetylcholinesterase. Therefore, the cells which are blocked with anatoxin-a would be over stimulated and thereby resulting to muscle paralysis (Corbel et al., 2014). Anatoxin-a can result in animals convulsion, vomiting, and respiratory arrest. Luckily, there were no human intoxications reported as so far. There is no official guideline for anatoxin-a in drinking water. However, 3 μg/L is suggested as a maximum acceptable level in drinking water (Merel et al., 2013).

2.2.3 Cytotoxins and dermatotoxins

The alkaloid cylindrospermopsin is a cytotoxin with molecular weight of 415 Da (Falconer, 1999). It was first found in the tropical waters in Australia (Hawkins et al., 1985), and since then has shown up in Japan, Israel, Hungary, and USA (Chorus & Bartram, 1999). Cylindrospermopsin can be found in waters with large concentrations even when the cyanobacteria cells are intact and healthy, which is different with other cyanotoxins (Carmichael, 2001). This type of toxin is relatively stable and water-soluble, it is not sensitive to sunlight, and can be decomposed slowly with temperature ranging from 4°C to 50°C, in the neutral pH environment. Boiling cannot degrade it significantly (Chiswell et al., 1999). Cylindrospermopsin

can block protein synthesis in both vegetable and mammal cells (Merel et al., 2013). In mammals, it will induce pathological symptoms in various organs, including spleen, thymus, kidney, intestine, eye, and heart (Svrcek & Smith, 2004).

The common cyanobacterial dermatotoxins include debromoaplysiatoxins, lyngbyatoxins, and aplysiatoxins, mainly produced by *Lyngbya*, *Schizothrix* and *Oscillatoria* in marine water. They can induce dermatitis and oral and gastrointestinal inflammation among humans who are exposed to the coastal waters. These dermatotoxins are also potent cancer promoters by activating the protein kinase C (Chorus & Bartram, 1999; Merel et al., 2013).

2.3 Preferred bloom conditions and life cycle of blooms-forming cyanobacteria

Since cyanobacteria are phototrophic organisms, the blooms primarily happen in surface waters, but not ground waters. Although some species prefer to grow in flowing waters, most cyanobacteria do not adapt to such conditions. In general, cyanobacterial blooms usually flourish under their optimal growth conditions as: lentic waters with little or no wind, which leads to the water stratification; pH of water is neutral to weakly alkaline (pH 6~pH 9); warmer water temperature (25 ^{0}C or above); and eutrophic water conditions (Svrcek & Smith, 2004). Under the above bloom-forming conditions, cyanobacteria can rapidly dominate a fresh water body. The relationships between different environmental factors and cyanobacterial growth will be discussed later in detail.

Previous studies have shown that the life cycle of cyanobacteria plays an important role in controlling the time and duration of blooms, and also the species which will dominate the blooms (Anderson & Rengefors, 2006; Kremp et al., 2008). So far, a number of models have been developed for cyanobacteria life cycle, most of them focus on the growth phase and the

nitrogen fixation stage of cyanobacteria. The model introducing here is the CLC (cyanobacteria life cycle) model developed by Hense and Beckmann in 2006 (Hense & Beckmann, 2006). The CLC model divides the life cycle into four phases: vegetative cells (VEGs), vegetative cells with heterocysts (HETs), akinetes (AKIs), and recruiting cells (RECs) (Figure 2-3).

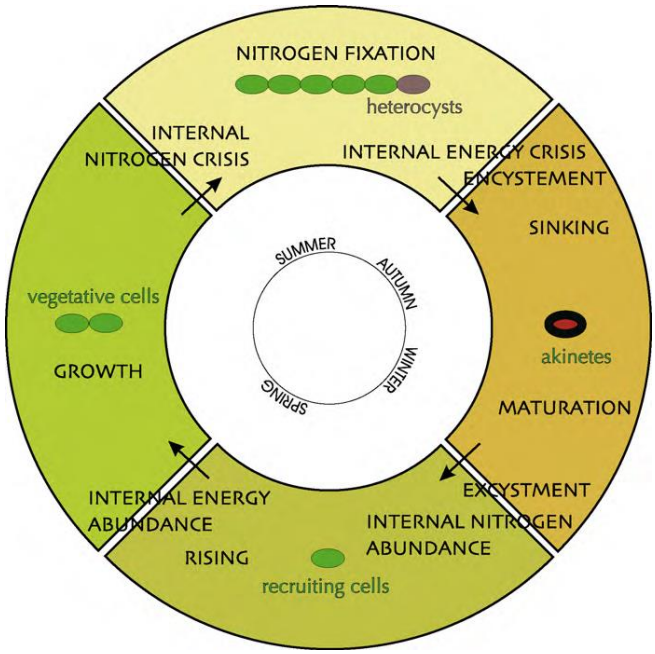

Figure 2-3 Schematic summary of the CLC model, with four life cycle phases (Hense & Beckmann, 2010)

As shown in Figure 2-3, the life cycle shows a seasonal style, and includes benthic and pelagic phases. In early spring, as the weather gets warmer, the overwintering cyanobacteria are recovering from dormancy and start to reproduce. After the RECs phase, cyanobacteria move up by their own buoyancy or by external forces, such as current and wind. The VEGs have high energy and high nitrogen quotas. They can grow as long as nitrogen is available. And they are growing in forms of filaments. After spring blooms, the nitrogen in the water is exhausted, which strongly limits the growth of VEGs. Under this condition, a cell differentiation of cyanobacteria is carried out and the heterocysts are formed. These specialized cells have the ability to fix

nitrogen. They have additional envelopes and do not have photosystem II, which protects them from being destroyed by oxygen. By the end of summer, nitrogen fixation and nutrient uptake are reduced because of the decrease of temperature. In addition, since the solar radiation is also decreased, the internal energy of cyanobacteria cell is depleted and parts of the biomass transfer to AKIs, which are kind of resting spores. These AKIs sink to the bottom of waters and permanently disappear from the column. They maintain a low level of metabolic activities and gradually mature by refilling nitrogen. When they have abundance internal nitrogen, they become RECs and rise to the water surface again, where they can take up enough energy which is necessary for the growth. That is the entire life cycle of cyanobacteria by CLC model (Hense & Beckmann, 2010).

2.4 Health and ecological effects

Cyanotoxins have caused human toxications worldwide (Table 2-3). Among the reported cases, most of them are because of the ingestion of contaminated water and food (aquatic animals and agricultural products) and dermal exposure to contaminated recreational water.

Table 2-3 A list of documented cases of human illness associated with cyanotoxins. (Cheung et al., 2013; Lopez et al., 2008; WHO, 2003)

Exposure source	Year	Country	Case description
Drinking Water	1931	USA	Illness of around 8,000 people whose drinking water came from tributaries of Ohio River, where a large cyanobacteria bloom had occurred
	1968	USA	GI Illnesses were documented to occur in association with massive blooms of cyanobacteria
	1979	Australia	Serious illness & hospitalization of 141 people associated with toxic bloom in drinking water reservoir, which had been treated with copper sulfate
	1988	Brazil	Death of 88 and illness of around 2,000 people associated with toxic cyanobacteria in drinking water reservoir after flood
	1993	China	Liver cancer incidence found higher for populations

			using surface waters where cyanobacteria occurred in drinking water rather than groundwater
	1994	Sweden	Illness (gastrointestinal and muscle cramps) of 121 (out of 304) inhabitants of a village whose drinking water supply was accidentally cross-connected with cyanobacterial contaminated untreated river water
Recreational Water	1959	Canada	Illness (headache, muscular pains, gastrointestinal) of 13 people after recreational exposure to cyanobacterial bloom
	1989	England	Illness in soldiers training in water with cyanobacterial bloom; 2 developed serious pneumonia
	1995	Australia	Human illness (gastrointestinal) associated with recreational water contact in waters with cyanobacteria
	2004	USA	GI illness & dermal irritation associated with recreational exposure to a CHAB event in Nebraska
Water used for Hemodialysis	1974	USA	Chills, fever, hypotension in 23 dialysis patients in Washington, D.C. associated with cyanobacteria in local water source
	1996	Brazil	Death of 52 dialysis patients & illness of 64 others associated with microcystin toxins in water used for dialysis

Cyanotoxins in drinking water can cause serious poisoning in humans, even leading to death (Falconer & Humpage, 2005). The liver is most affected by cyanotoxins in humans. The hepatotoxins can rupture the liver structure and accumulate excess blood in the liver and eventually cause death to animals or humans by liver failure (Carmichael et al., 1997). The symptoms induced by hepatotoxins include vomiting, diarrhea, headache, muscle cramps, weakness, labored breathing, and anorexia. Some subjects who ingest huge amount of hepatotoxins would be dead after having symptoms such as coma and muscle tremors for several hours to several days. Studies show that hepatotoxins can induce tumors in the liver in experimental animals and some indirect evidence also show that humans who drink the contaminated water have a high risk of developing tumors (Svrcek & Smith, 2004). Besides the

liver, some other organs, such as the colon and kidneys, are also affected by exposure to cyanotoxins. Human poisoning by cyanotoxins is not only through drinking water, but also through the food chain. There are some fishes, like carp and tilapia, can graze cyanobacteria in water. When these fishes are consumed by humans, the toxins transfer to human bodies through the food chain and are accumulated in different organs (Ferrão Filho, 2009).

In addition to ingestion of cyanotoxins, human have been reported to take in cyanotoxins through hemodialysis, which used the cyanobacteria contaminated water in dialysate. The toxins entered the patients' blood and then made them ill (Funari & Testai, 2008).

In recreational waters with blooms, humans can be exposure to cyanotoxins by dermal contact or by accidentally ingesting water (Funari & Testai, 2008). Fever and gastrointestinal illness are the most common symptoms reported that are associated with exposure to contaminated recreational waters. Pneumonia, headache, and myalgia have also been reported (Giannuzzi et al., 2011). It is difficult to assess the recreational exposure to cyanobacteria because there are not many exposed people, and the sensitivity to toxins, the time of exposure, and the toxin types and concentrations are all different (Koreiviene et al., 2014). The WHO established a guideline value of 20-100 μg/L MCs as the acceptable recreational exposure level (WHO, 2003).

Cyanobacterial blooms may also cause detrimental effects on aquatic ecosystems. The dense cyanobacterial blooms can accumulate as thick scums and mats, which results in deoxygenation of the bottom waters. The lack of oxygen would lead to an increased mortality rate in fish, shellfish, aquatic invertebrate, and plant populations. The blooms may also affect benthic fauna and flora since the light penetration is decreased. Toxic blooms may inhibit the growth of other phytoplankton by competing for the sunlight and nutrients. They can also

suppress the zooplankton grazing. All of these effects induce the changes in community structure and composition of the aquatic ecosystems (Quiblier et al., 2013; Zanchett & Oliveira, 2013).

In addition to the production of toxins, cyanobacteria blooms are also associated with taste and odor problems in drinking waters. In shallow bays, algal scums can assemble together for a relative long time. During that time, cells may disintegrate and die. The dead cells release their contents, not only toxins, but also a variety of odor and taste compounds, into the water. Those compounds produced by blooms include geosmin and 2–methylisoborneol (MIB), which are not toxic but are a nuisance to the public. Odor and taste compounds cause drinking water and fish taste malodorous and unpalatable. And also cause the loss of recreational and aquacultural revenue and the increase of cost for bloom treatment (EPA, 2013).

2.5 Detection methods of blooms and toxins

Monitoring chlorophyll a concentrations and the total number of cyanobacteria cells are recommended by the World Health Organization (WHO) as the main methods to detect the cyanobacterial blooms (Chorus & Bartram, 1999). In addition, the timely identification and quantification of cyanotoxins are the essential preconditions for the successful treatment of cyanobacterial blooms. They can also provide an early warning of serious health and ecological problems due to the toxins in the water (Svrcek & Smith, 2004). There are a wide range of methods for detecting cyanotoxins: from in vivo bioassays (mouse bioassay), immunological assays (ELISA), biochemical assays (PPIA), to quantitative chromatographic techniques (HPLC) (Merel et al., 2013; Svrcek & Smith, 2004).

Before analysis, samples usually need specific preparation according to the expected results. After sampling, samples need to be stored at low temperature (4 ^{0}C). Timely analysis is very important since the distribution of intracellular and extracellular toxins will change with

time. Direct filtration of samples is effective for the detection of extracellular toxins. However, in order to determine the intracellular or total levels of toxins, cell lysis is needed. The cells are lysed usually by a freezing-thawing process, which can damage the cell membranes and liberate the intracellular toxins (Nicholson & Burch, 2001). Some other studies suggest that sonication is more effective at cell lysis and can be used as a pre-treatment method for the toxins analysis (Rapala et al., 2002). In addition, toxins after filtration need further purification and concentration processes to be measured by analytical techniques.

Mouse bioassay is used for assessing the cyanotoxins biological effects and the entire range of toxins, but not for the exact identification of toxins. The samples are injected in at least three mice. After 24 hours, anatomize these mice. From the symptoms of different organs, researchers can identify the presence of hepatotoxins or neurotoxins in experimental subjects (Falconer, 1993). Due to its low sensitivity and the development of new methods, the mouse bioassay is rarely used in toxicological research.

Enzyme linked immunosorbent assay (ELISA) is a fast and highly sensitive technique for the cyanotoxins analysis. Using ELISA assay, cyanotoxins are detected by recognizing and binding to specific antibodies, and the result is the total toxin concentration. There are various commercial ELISA kits used for the detection of microcystins in water (Hilborn et al., 2005; Rapala et al., 2002). They can obtain a lower detection limit of 4 ng/L and an upper limit of 2 μg/L for MC-LR (Lindner et al., 2004). ELISA has good reproducibility, repeatability, and low variability, which are comparable to HPLC test. However, it also has some limitations. This assay cannot identify different microcystin variants, and the cross reactivity of the antibodies with other compounds will induce the overestimate of the toxin concentration (Svrcek & Smith, 2004).

The protein phosphatase inhibition assay (PPIA) can be used for detecting cyanotoxins such as MCs and NODs because those toxins can inhibit protein phosphatase (Almeida et al., 2006; Bouaicha et al., 2002). The enzyme used in the assay is first exposed to the sample with toxins, and then incubated with the relevant substrate. The absorbance of the mixture is measured at a specific wavelength to detect the substrate and thereby measure the enzyme activity. Therefore, the concentration of toxin can be assessed since it is usually inversely proportional to the enzyme activity (Merel et al., 2013). PPIA is a fast method with the detection limit as 0.01 μg/L (Almeida et al., 2006). However, it can only detect MCs and NODs, and cannot distinguish these two toxins. Therefore, if it is needed to detect the other cyanotoxins in the sample, some further analysis should be taken (Merel et al., 2013).

Cyanotoxins can also be detected by chromatographic techniques coupled to different quantification detectors. Chromatography can separate different compounds present in the sample. The most common separation method for cyanotoxins is using liquid chromatography (LC), which usually uses a reversed phase C18 column and methanol/water as a mobile phase. This technique is rapid and flexible, and adapts to a wide range of detectors. Gas chromatography (GC) can also be used for separation of cyanotoxins, but it is not commonly used, because most cyanotoxins are large molecules and cannot volatilize easily (Kaushik & Balasubramanian, 2013). After separation, variants of sample can be identified and quantified by different detectors depends on the retention time and the comparison with suitable standards. Detection by fluorescence, UV absorbance, or mass spectrometry are most commonly used after LC separation. The chromatographic techniques can provide information about toxicity of different variants in the sample, but it requires longer time and more expensive equipment, and

sometimes the lack of suitable standards would hamper the analyses (Merel et al., 2013; Svrcek & Smith, 2004).

2.6 Factors affecting cyanobacterial growth and bloom formation

There are several environmental factors, such as temperature, nutrients, light intensity, turbulence and mixing, and water residence time, can mediate cyanobacterial bloom expansions. Most of them related to human activities.

2.6.1 Temperature/climate change

Due to the increasing carbon dioxide (CO_2) produced by the burning of fossil fuels present in the atmosphere, the global temperature increased by approximately 1°C in the past century (IPCC, 2001), and is expected to increase an additional 2-5°C in this century (Houghton et al., 2001). Warmer temperature favors the bloom-forming cyanobacterial genera since their growth has a preference for warmer conditions. Harmful cyanobacteria such as *Microcystis* have their maximal growth rates at the water temperature over 25^0C. During the warmest periods of the year, cyanobacteria always dominate phytoplanktons, especially in the eutrophic environment (Figure 2-4) (Paerl, 2014). In addition, the toxin content of some genera of cyanobacteria also increases with the rising temperature, and reach their optima at above 25^0C (Rapala et al., 1997). Warmer surface waters are also good for vertical stratification. The waters stratify because warm surface water and the cold water beneath have different densities (Paerl & Otten, 2013). Vertical stratification is another preferred bloom-forming condition, and as the temperature rising, the period of stratification becomes longer which induces the blooms persist longer in the water system.

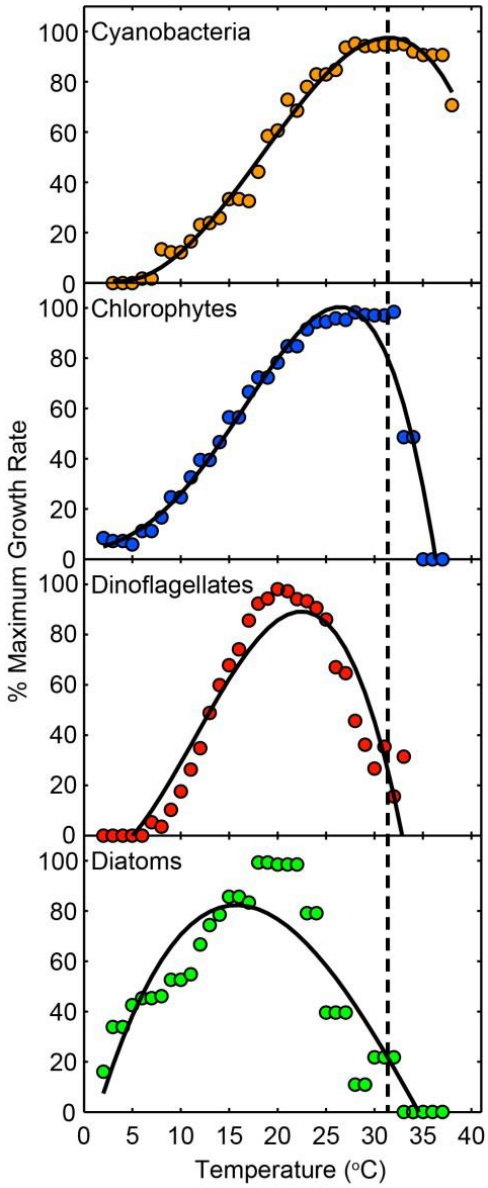

Figure 2-4 Temperature-specific growth rate relationships for four different phytoplankton classes. It can be seen that when the growth rates of other eukaryotic classes decline due to warmer temperature, the growth rate of cyanobacteria obtains the maximum (Paerl, 2014).

2.6.2 Nutrients

Eutrophication is cited as another key factor which promotes harmful cyanobacterial blooms. The increase of nutrients, especially nitrogen (N) and phosphorus (P) in their diverse forms, is considered as the main cause of eutrophication (Vasconcelos, 2006). The increase of nutrients input can be caused by natural processes, such as leaf decay and natural forest fires. It

is also caused by anthropogenic activities, including agricultural activities, domestic and industrial effluents, and the improper watershed management. The anthropogenic activities are pointed out as the major causes of eutrophication (Vasconcelos, 2006).

Phosphorus is usually considered as the "limiting" nutrient for growth of cyanobacteria. Its enrichment, especially relative to nitrogen enrichment, favors the development of harmful cyanobacterial blooms (Paerl & Otten, 2013). Previous studies show that some cyanobacteria genera have a higher affinity for phosphorus and nitrogen than other photosynthetic organisms. That means that when the phosphorus or nitrogen is limited, cyanobacteria are more competitive than the other phytoplankton organisms. In addition, cyanobacteria have stronger capacity for phosphorus, which means that they can store enough phosphorus to produce substantial biomass (Chorus & Bartram, 1999). The N_2 fixing cyanobacterial genera are more likely to bloom with phosphorus enrichment. These genera can supply their own nitrogen needs through the N_2 fixing process (Paerl & Otten, 2013). A low ratio between N and P concentrations may favor the development of N_2 fixing cyanobacteria. And a previous study considered a mass ratio of 22:1 (TN: TP) as the boundary between lakes dominated by N_2 fixing cyanobacteria and lakes have low levels of those algae (Smith et al., 1995).

In the past several decades, the increasing application of N-fertilizers, storm water runoff, and human and agricultural wastes all lead to the enrichment in N relative to P. The N-rich aquatic ecosystems also favor the development of cyanobacterial blooms, especially those non-N_2 fixing cyanobacteria (Paerl & Fulton, 2006). Therefore, the eutrophic systems, especially those with sufficient P, are more prone to bloom with additional N inputs. Hence, the reduction of both N and P inputs are needed to prevent eutrophication and cyanobacterial blooms expansion.

2.6.3 Light intensity

Since cyanobacteria contain chlorophyll a and can conduct photosynthesis, light is the primary energy source for their growth, which enables them to carry out all the metabolic processes (Markou & Georgakakis, 2011). Both light quality and quantity can affect photosynthesis. Cyanobacteria harvest light mainly in the 400-500 nm and 600-700 nm wavelength range, which can hardly be used by other phytoplanktons. The chlorophyll a, together with phycobiliproteins, enables them to absorb light energy efficiently and to live with only green light (Chorus & Bartram, 1999).

In the whole photosynthetic system, photosystem II (PSII) is more sensitive to light than the other parts. With high light densities, the photosynthetic capacity of cyanobacteria decreases due to the light-induced damage to PSII. And the cyanobacterial growth is inhibited accordingly. Exposure to high light intensity (320 µE m^{-2} s^{-1}) for a long time is lethal for many genera (Van Liere & Mur, 1980). However, when the light density is below the saturation point, higher light density usually induces higher cyanobacterial growth rates (Figure 2-5). Cyanobacteria which form surface blooms have a higher tolerance for high light intensities (Chorus & Bartram, 1999).

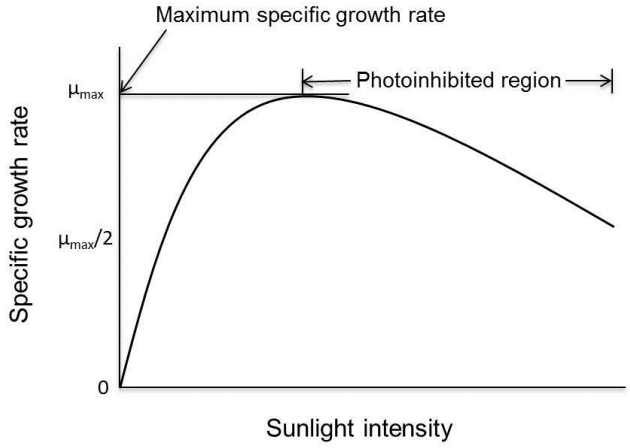

Figure 2-5 Light saturation and photoinhibition. Above the point of light saturation, a further increase in light intensity reduces the biomass growth rate (Kin, 2010).

Cyanobacteria require little energy to maintain cell structure and functions, which means that they can achieve a higher growth rate than other phytoplanktons with lower level light intensities. Cyanobacteria have capacity of growing under low light intensities, which enables them to grow in the shadow of other phytoplankons. In addition, in the lakes which are highly turbid due to dense growth of other phytoplankton, cyanobacteria have a competitive advantage and could finally out-compete other phytoplankton organisms (Chorus & Bartram, 1999).

2.6.4 Turbulence and mixing/Water residence time

Some physical factors, such as turbulence, mixing, and water residence time, also play essential roles of harmful cyanobacterial blooms in the aquatic ecosystem (Paerl, 2014). Cyanobacteria are very sensitive to the stability of the water column, especially to the vertical stratification (thermal or salinity stratification) (Reynolds, 1984). Therefore, disruption of the stratification by altering turbulence or vertical mixing may modulate the bloom dynamics. Studies show that increasing turbulence can damage the cells and filaments, cause disaggregation and rapid death of cells, and thus inhibit the cell activities and growth (Paerl, 1990). Strong wind and mixing by tide also affect the distribution of cyanobacterial blooms. In those waters, their ability to maintain optical position by regulating gas vesicles could be overcome by mixing, and thus presenting a potential obstacle to their growth and expansion (Kononen et al., 1996; Reynolds, 1987). Long water residence (replacement or flushing) times favor cyanobacteria growth over other phytoplankton, mainly because comparing to those eukaryotic phytoplankton, cyanobacteria have relatively slow growth rates (Paerl, 2014). Hence decreasing the water residence time, usually by increasing the flushing rates, can effectively control the blooms expansion. More physical methods that used to control the blooms are introduced in next chapter.

Chapter 3 - Harmful bloom management

The management of a cyanobacterial bloom is a complex and long-lasting task since the blooms are driven by a complex set of physical, chemical, and biotic factors. The objective of harmful bloom management is to prevent, monitor, and mitigate this phenomenon in an environmentally friendly way. There are various strategies that are used for bloom management. However, for different potentially contaminated sites, different strategies should be selected because of the distinct situations for each site.

3.1 Nutrient input controls- the prevention of bloom occurrence

Nutrient input reductions should be the first and most important step for improving the water quality of lakes or reservoirs. The nutrient input could be classified into point and non-point source loading. The point sources are some well-defined discharge sites, like municipal wastewaters, industrial effluent, and other distinct discharge sources. They are relatively easy to control by building wastewater treatment (WWT) plants. The non-point sources of nutrient input usually includes agriculture and urban runoff, and erosion from the deforested areas, which is more difficult to target and control. Therefore, the non-point source input control is likely to play a key role in the blooms remediation (Drabkova & Marsalek, 2007; Paerl & Otten, 2013).

For a long time, the phosphorus input reduction is considered as an effective method of reducing cyanobacterial dominance in freshwater ecosystems (Smith & Schindler, 2009). However, increasing studies show that nitrogen input reduction is also of great importance (Grantz et al., 2014; Paerl, 2013; Paerl & Scott, 2010). To control non-point source pollution, establishing N and P input thresholds is very important. The Total Maximum Daily Load Program (TMDL) is such a program implemented by the US Environmental Protection Agency

(EPA) (EPA, 2012). The TMDL program tries to identify the quality limited waters, establish priority waters, and develop TMDLs for listed waters that can achieve the water quality standards. The TMDL program is a key tool for controlling and reducing non-point source pollution delivered to the surface waters (Merel et al., 2013).

Since the runoff of nutrients is tightly associated with the increasing of runoff from landscape, in many cases, the preventative measures are similar to those anti-flooding measures. These measures include straightening of rivers and streams, restoration of wetlands, rehabilitation of riparian zones, and construction of retention ponds. The higher the diversity of the landscape is, the higher its buffering and nutrient fixation capacities are (Cooke et al., 2005). The prevention of nutrients input can also be achieved by managements of post-discharge nutrients removal, which include: dredging sediments, precipitating, binding, or immobilizing nutrients in the sediments, harvesting the macrophytes which can assimilate nutrients, and removing higher level consumers, such as shellfish and finfish, to remove and stop the nutrients passing to the next level in the food chain (Robb et al., 2003).

Although the restriction of nutrients input can have a positive long-term effect, in fact, the chance of sufficiently removing nutrients from watershed is usually limited. Because of the internal recycling of nutrients in the waters, the measures we talked above may be insufficient. In addition, nowadays, for most areas across the world, the significantly decrease of nutrients input is almost impossible and unavailable because of the economical limitations: it is usually a very expensive remedial measure. Therefore, some other methods are developed for the already occurring blooms management.

3.2 Physical methods

3.2.1 Sediment removal and capping

Sediment removal is a very effective method for bloom control. The upper layer of the lake bottom sediments, which has a higher capacity to bind phosphorus, is usually removed. At the same time, most of the cyanobacteria inoculum is also removed within the sediments (Drabkova & Marsalek, 2007). There are various methods of sediments removal. The most common one for small ponds is pumping the water out to expose the bottom sediment, and then remove the sediment. But this method is not suitable for bigger lakes, or the lakes that require aquatic life conservation. Another environmentally-friendly technique is the use of the suction dredgers. This technique can remove the sediment without re-suspension of the undesirable sediments into the water. However, this technique is very expensive because the big volume of sediment and water mixture (90% of it is water) that need to be transferred. In addition, the sediment dredging also intervenes in the lake ecosystem. The most obvious effect is to the benthic organisms. Once the lake basin is dredged completely, the ecosystem needs a couple years to re-establish the benthic fauna. However, in many cases, considering the long term benefits derived, the effect on the benthic fauna can be acceptable (Cooke et al., 2005; Drabkova & Marsalek, 2007).

Sediment capping is an alternative technique and usually cheaper than sediment removal. The capping process involves the placement of a cover over the top of the sediment. Thus, the sediment is sealed and the release of contaminants and nutrients to the water column is prevented. The cover material is acting as a physical barrier, which can be 30-40 cm thick sediment, sand, or gravel, but should be coarser than the original sediment. Various calcite materials are used for sediment capping (Hart et al., 2003). The mixture of aluminum salts and ballast materials is also

reported to reduce phosphorus release from sediments. As for now, no negative aspects of using this material were reported (Drabkova & Marsalek, 2007).

3.2.2 Activated carbon adsorption

Activated carbon adsorption is usually used in the water treatment industry for the removal of cyanotoxins (Jones et al., 1993). Two forms of activated carbon are used in the drinking water treatment: powdered activated carbon (PAC) and granulated activated carbon (GAC). PAC is used for adsorption and usually for transient contaminants. GAC is generally used in continuous flow through column reactors, and to reduce natural organic matters and taste and odor compounds (Westrick et al., 2010). The activated carbon is naturally microporous with pore size smaller than 2 nm diameter. The mesopores (2 to 50 nm) are less common (Pontius, 1990). Although activated carbon has no impact on cyanobacteria and the intracellular toxins, it can effectively remove the extracellular cyanotoxins, such as MCs, STXs, ANTX-a, and CYL (Merel et al., 2013).

Numbers of studies in the past two decades have investigated the use and the doses of activated carbon for cyanobacterial toxin removal. In the earliest study in 1976, Hoffman used activated carbon to successfully remove two unknown peptide toxins from an *M. aeruginosa* bloom. After being treated with PAC (in 80 mg/L and 800 mg/L), those toxins were no longer toxic to mice (Hoffmann, 1976). Some other early studies also concentrated on the doses of PAC needed to remove cyanotoxins in the conventional water treatment processes. In a lab scale experiment, simultaneously applied PAC (5 mg/L) and coagulant could remove up to 34% of MCs and more than 50% of the neurotoxins. In another pilot scale experiment, the application of 20 mg/L of PAC after the conventional treatment processes yielded a 90% removal of cyanotoxins from a cyanobacterial bloom. The GAC has been reported to remove the

cyanotoxins as well. A pilot scale experiment showed that the MCs were reduced by more than 90% after treated with GAC filter (Bruchet et al., 1998; Hart et al., 1998; Mouchet & Bonnelye, 1998). The choosing of adsorbent should depend on the type of toxins: for those toxins with smaller size molecules, activated carbons with more micropores could be more efficient, and vice versa, the activated carbons with more mesopores should be used to remove the toxins with bigger size molecules.

Although the activated carbon can quickly and effectively remove cyanotoxins from water, due to its short lifetime, it has to be changed frequently, which significantly increases the costs. In addition, the adsorption efficiency of activated carbon will decrease with time (Lambert et al., 1996). Therefore, a study showed that activated carbon filters with regeneration and replacement could be more effective for the removal of MCs (Alvarez et al., 2010).

3.2.3 Filtration

Filtration is a unit process used to remove suspended particulates from water. These particulates include coagulated floc, clay and silt, and also microorganisms. The slow sand filtration is used to remove both cyanobacteria and cyanotoxins during water treatment process (Grutzmacher et al., 2002). Slow sand filtration is usually operated at a low speed and could develop a biofilm over the top of the filter. The biofilm is formed by the growth of bacteria from the surface water, and it allows the biodegradation of MCs, with a latency period. The biodegradation of MCs is through some enzymatic pathways. The efficiency will decrease at the lower water temperature. Although plugging the filters is a potential problem, slow sand filtration has been considered as an efficient method and expected to remove up to 99% of algal cells (Svrcek & Smith, 2004).

Membrane filtration is a physical separation process that uses a membrane to divide a water stream into two different fractions: the permeate that can go through the membrane and the retentate that is stopped by the membrane. The membrane filtration can be classified into several processes according to the pore size of the associate membrane. Microfiltration (0.1-10 μm) and ultrafiltration (1-100 nm) are efficiently used for the removal of cyanobacteria and the intracellular toxins (Merel et al., 2013). However, the extracellular toxins cannon be removed by microfiltration because of the pore size of the membrane. Ultrafiltration is expected to remove extracellular MCs, but not for the smaller toxins (Lee & Walker, 2008). Although the filtration technique has some concerns such as clogging and cell lysis, there is no damage of cell membranes during the processes, which prevents the increase of extracellular cyanotoxins in the permeate. By both membrane filtrations, one of the cyanotoxins, *M. aeruginosa*, can be removed by 98% from the drinking water resources (Chow et al., 1997). Cyanobacteria should be theoretically removed by nanofiltration (~1 nm) and reverse osmosis (0.1 nm), but clogging would happen immediately for these membranes. However, these two processes are very efficient for the retention of the extracellular cyanotoxins. Previous studies show that more than 90% of extracellular toxins can be removed by nanofiltration or reverse osmosis (Merel et al., 2013).

Although the membrane filtration appears to be suitable technologies for the removal of both cyanobacteria and cyanotoxins, the methods are very complex, and the high costs associated with the energy requirement makes them unaffordable and unavailable for most drinking water treatment units.

3.2.4 Ultrasound

Ultrasound, which has sound waves of a frequency higher than 20 kHz, can induce the disruption of both structure and function of cyanobacterial cells, and is a potential treatment for cyanobacterial bloom control (Phull et al., 1997). The effect of ultrasound on cyanobacteria depends on frequency, intensity, and the processing time (Wu et al., 2011). Ultrasound would rupture the gas vacuoles, break the cell wall and membrane, inhibit the photosynthetic activity, and interrupt the cell division and cell cycle, thereby inhibit the growth of cyanobacteria (Rajasekhar et al., 2012b).

When applying ultrasound to water, it could cause acoustic cavitation. Frequency is a very important parameter that could affect the cavitation. At lower frequencies, the physical and mechanical effects play a major role because of the high energies released from the rupture of bubbles. However, at higher frequencies, there is not enough time for cavitation bubble to grow, thus the big bubbles cannot be produced. Under this condition, the chemical effects from the radicals produced predominate (Rajasekhar et al., 2012b; Wu et al., 2011; Zhang et al., 2006). In addition, as the frequency increases, greater ultrasound intensities are needed to attain cavitation. A study shows that for 400 kHz frequency ultrasound, 10 times more power is needed than that for 10 kHz to attain cavitation (Khanal et al., 2007). And at the same frequency, higher sound intensity will lead to greater cavitation effects and eventually cell lysis (Figure 3-1). Besides frequency and intensity, the duration of exposure is also an important parameter that affects the cavitation. Figure 3-1 shows that a longer duration time will lead to stronger cavitation effects, and thus lead to stronger sonochemical effects (Suslick, 1990).

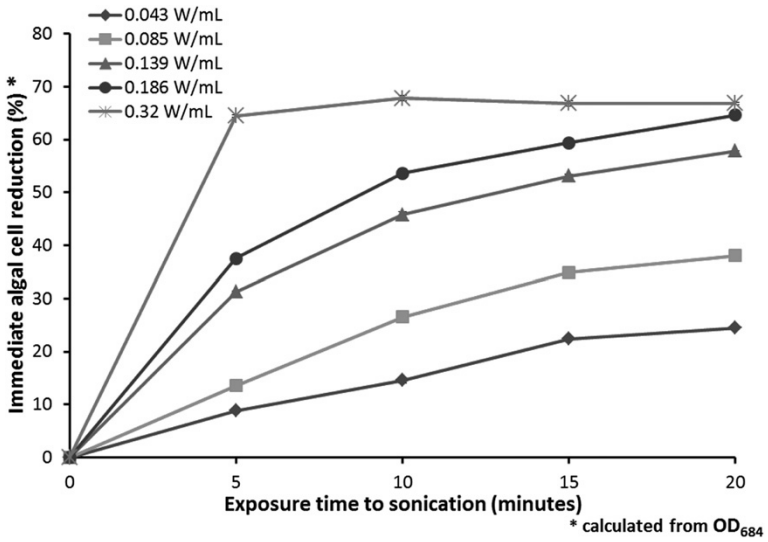

Figure 3-1 Sound intensity and duration effects on *M. aeruginosa,* at 20 kHz (Rajasekhar et al., 2012b)

The gas vacuoles within cyanobacterial cells provide buoyancy to cells and thus the cells can regulate their vertical position in the water column. The vertical position determines the extent of the light exposure and thus their growth (Reynolds, 1972). The inhibition of cyanobacterial growth by ultrasound is mainly due to the rupture of gas vacuoles, which results in cell lysis. However, studies have been reported that gas vacuoles could regenerate within 24 hours after sonication (Lee et al., 2000). Research also shows that the application of ultrasound could damage the photosynthetic components and inhibit the photosynthesis, which reduce the cell growth rate (Zhang et al., 2006). Comparing to some unicellular algae, sonication has a greater inhibition effect on filamentous cyanobacterial species such as *Aphanizomenon flos-aquae* and *Anabaena circinalis*. It indicates that the ultrasound may affect these algae by disrupting their filaments and the cell walls (Rajasekhar et al., 2012a).

There are also some drawbacks of sonication. In addition to the short time effect, ultrasound is also known to lyse cells and release the intracellular toxins into water, which are highly undesirable. Therefore, developing correct parameters for sonication is of great importance for bloom control.

3.3 Chemical methods

3.3.1 Metals

3.3.1.1 Coagulation agents

Coagulation is a process that uses chemicals to make smaller particles aggregate into larger ones for further removal. Aluminum (Al) salts and polymers are the most common coagulants used for nutrients removal during the water treatment process. Actually, aluminum can control the cyanobacterial bloom through different ways, besides removing phosphorus from the water column, it can also prevent the release of phosphorus from the sediment, and can remove cyanobacterial cells by the formation of floccules. The effect of nutrient/cyanobacteria removal can be affected by several factors such as pH, alkalinity, mixing, coagulant concentration, and cell size (Witters et al., 1996). The use of Al is cheap and relatively safe, and it will not induce serious damage to the environment because it does not cause serious cell damage, which release the toxins into the water. However, the application of Al may cause a long term decrease in pH in the water (Jancula & Marsalek, 2011).

Iron (Fe) is another metal that is used for phosphorus removal in the waters. Several compounds of iron can be used as coagulants. But unlike Al, once they settle at the bottom, the phosphorus can migrate into the water column again when short of oxygen, which is common in stratified reservoirs and lakes (Hupfer & Lewandowski, 2008).

Calcium (Ca) is usually applied in the form of calcite or lime. It can reduce both chlorophyll and phosphorus for a long time and does not cause any toxin release. However, if it is used in water bodies with a short residence time, it cannot have long-term positive effects (Jancula & Marsalek, 2011).

3.3.1.2 Algaecide

Copper (Cu) is the most common algaecide for algae control. It is usually used as a copper sulfate, or as some commercial products, such as Cobre Sandoz BR[@], Cuprogarb 500[@], and Clearigate[@] (Murray-Gulde et al., 2002). Copper has been used for the control of phytoplankton in waters for over one hundred years because of its low price and its toxicity against algae. Once Cu enters cells, it will substitute for magnesium in the chlorophyll. It can also affect other biochemical processes in the cells. The toxicity of Cu can be affected by several factors, such as pH, alkalinity, DOC, Cu dose, and exposure time (Jancula & Marsalek, 2011). Silver has been reported to be used together with Cu, which can inhibit both microbial and cyanobacterial growth (Choi et al., 2008).

Copper is usually used in low concentrations and needs to be re-applied because of its rapid dynamics in waters. The use of Cu is harmful to some non-target aquatic species. And Cu will cause damage in cyanobacterial cells and release the intracellular toxins into the water (Jancula & Marsalek, 2011).

3.3.2 Photocatalysts

Photolysis is a natural process that can destruct and remove toxic organic compounds from water. Photolysis degradation happens when the compounds absorb the same wavelength radiation as the emitted light, or when the contaminant is present with other compounds (photocatalysts) that can be photo-excited by the emitted light and will form active radical species to degrade that contaminant (Lawton & Robertson, 1999). Microcystins are the most popular and predominant cyanotoxins cross the world. Since the sunlight has higher wavelength than the MCs absorption range, the toxin cannot be degraded by sunlight alone. However, the

MCs degrade rapidly in the presence of photocatalysts, such as TiO_2 and ZnO (De La Cruz et al., 2011).

Titanium dioxide (TiO_2) is one of the most promising photocatalysts for the complete destruction of some organic contaminants, especially for cyanotoxins. TiO_2 is abundant in nature and hence is inexpensive. The common form of TiO_2 is nanoparticles. TiO_2-mediated photocatalytic process could be direct under UV and visible light, and will not produce hazardous compounds (Pantelic et al., 2013). TiO_2 is activated by UV irradiation and forms reactive oxygen species such as $HO^{\bullet}$, $O^{\bullet}{}_2^-$, and $HO^{\bullet}{}_2^-$. These species will react with contaminants and mineralize the contaminants completely (Hoffmann et al., 1995). Studies show that with UV radiation, the degradation of MCs is not significant, but along with TiO_2 results in the rapid and complete degradation and removal of MCs from the freshwaters (Liu et al., 2009; Pelaez et al., 2009).

There are only a few literatures studied the zinc oxide (ZnO) photocatalysis. But all of them show that ZnO absorbs a larger fraction of the sunlight spectrum than TiO_2, and hence has greater ability of photocatalytic degradation of MCs in presence of sunlight (Elmolla & Chaudhuri, 2010; Jacobs et al., 2013).

Photocatalysis has many advantages as a water decontamination process such as the rapid and efficient removal of MCs, the toxic by-products, and microbes. However, it is only suitable for lower level cyanotoxins concentrations (Pantelic et al., 2013).

3.3.3 Oxidant

The common chemical oxidants used in the oxidation methods include chlorine (Cl_2, ClO_2, HOCl), permanganate ($KMnO_4$), ozone (O_3), hydrogen peroxide (H_2O_2), and Fenton reagent. Chlorine is still the most frequently used reagent for the water treatment in the world. It

is very effective, easy to apply, and inexpensive. Chlorine is a strong oxidant, and widely used in the water treatment process for both disinfection at the beginning of the treatment and controlling the growth of microbes in the post-treatment process (Deborde & Von Gunten, 2008). Chlorine is generally applied in different forms, such as chlorine gas, sodium hypochlorite solution, chlorine dioxide, and dry calcium hypochlorite. HOCl is the primary reactive species, and the hydroxylation of the Adda moiety is the major oxidation site (Sharma et al., 2012; Westrick et al., 2010). Some more recent studies have investigated that chlorine could effectively destroy toxins such as MCs and nodularin, and the efficiency depends on several factors: chlorine forms, concentration, exposure time, and pH of solution. The concentration of MCs can be decreased by over 95% by calcium hypochlorite and aqueous chlorine, and only by 40% by sodium hypochlorite (Nicholson et al., 1994). At pH<8.0, the cyanotoxins can be inactivated to the most extent by the chlorination process (Westrick et al., 2010). However, the chlorination process also has drawbacks: chlorine reacts with some natural organic compounds in the water and forms some toxic by-products, its effectiveness will decrease with time due to its volatility, and drinking water after chlorination will change the taste and odor to some consumers (Sharma et al., 2012).

Permanganate is another strong oxidant, but it is a poor disinfectant (EPA, 1999). Permanganate can oxidize organic compounds by different pathways: react with double bonds through oxygen donation, hydrogen atoms abstraction, and electron exchange (Campbell, 1964). Potassium permanganate was found to remove up to 95% of MCs from drinking water (Xagoraraki, 2007). Under the experimental conditions, 1.1 mg/L of permanganate was shown to completely remove MCs within one hour, with the final concentrations lower than the WHO guideline (1 μg/L). The efficiency of MCs oxidation by potassium permanganate is not pH

dependent, at least in the pH range that has been studied (from pH 6.2-8.2) (Sharma et al., 2012). Compared to other oxidants, permanganate is easy to apply, effective over a wide pH range, cheap, and relative stable in the water. However, the process needs longer contact time, the permanganate may be toxic and irritating when touching skin, and care has to be taken about the permanganate dose because high levels of it will cause cell lysis (Pantelic et al., 2013).

Ozone is one of the most frequently used oxidants in the water treatment industry. Ozone can react with all types of the common cyanotoxins but is less efficient with STXs (Merel et al., 2013). It is very effective and rapid in degrading MCs, even at relatively high concentrations. For ozone, the oxidation is achieved by reacting with alkene groups, neutral amine groups, and activated aromatic groups (Von Gunten & Hoigne, 1994). For a pH lower than 7.0, the oxidation is very efficient for MCs, but for pH 7.0 or greater, the oxidation efficiency is decreased and cannot remove the MCs completely. For anatoxin-a, the most efficient pH range is from 7.0 to 10.0, and for cylindrospermopsin is from 4.0 to 10.0 (Pantelic et al., 2013). There are also disadvantages of using ozone for disinfection and oxidation purposes. First, the ozonation equipment is very expensive and also needs higher level of maintenance and operation skill. Second, ozone is very toxic and corrosive, thus the operation of ozone should be highly careful (EPA, 1999).

Hydrogen peroxide (H_2O_2) is an effective oxidant. Several studies indicated that comparing to other eukaryotic phototrophs, cyanobacteria are more sensitive to H_2O_2 (Barroin & Feuillade, 1986). Barrington and Ghadouani showed that cyanobacteria decreased twice faster than the green algae and diatoms after application of the H_2O_2 into the wastewater samples (Barrington & Ghadouani, 2008). Later work found that 50% of the cyanobacteria biomass can be removed within 48 hours with the application of H_2O_2 to the wastewater treatment ponds

(Barrington et al., 2011). These studies also showed that in the presence of UV radiation, the dose of H_2O_2 required can be reduced by an order of magnitude (Barrington & Ghadouani, 2008; Barrington et al., 2011). H_2O_2 is existent in all surface waters in low concentrations, and some organisms can even produce it. H_2O_2 decays into water and oxygen rapidly, thus it will not accumulate in the environment. However, since H_2O_2 degrades too fast, it has very short effect on cyanobacteria. So the application of H_2O_2 to the real ecosystem should be repeated in short time periods. In addition, because the eukaryotic phytoplankton is less susceptible to H_2O_2, the appropriate dose needs to be determined in order to ensure other aquatic organisms are largely unharmed (Barrington et al., 2013; Jancula & Marsalek, 2011).

Fenton reagent is a mixture of hydrogen peroxide (H_2O_2) and ferrous ion (Fe(II)). The ferrous ions can catalyze the hydrolysis of H_2O_2 to form hydroxyl radicals (Al Momani et al., 2008). Therefore, the Fenton reagent is a powerful oxidant that is usually used to degrade the organic contaminants. Fenton reagent is very efficient for the MC-LR removal, and the efficiency depends on the initial concentrations of both Fe(II) and H_2O_2. A study showed that MC-LR can be completely degraded within 80 s, with initial Fe(II) concentration at 0.05 mg/L and initial H_2O_2 concentration at 0.02 mg/L (Al Momani et al., 2008). Fenton reagent is a promising method because the ferrous is naturally abundant and non-toxic, and also because the H_2O_2 is easy to handle and relatively environmentally friendly. Furthermore, the photo-Fenton process was found to be more efficient than using Fenton alone for the degradation of MC-LR (Pantelic et al., 2013).

3.3.4 Herbicides

There are several herbicides that used as algaecides such as diuron and endothall. They are toxic to cyanobacteria due to their ability of inhibit photosynthesis. At the level of

photosystem II, those herbicides can prevent oxygen production and block the electron transfer, and hence inhibit the growth of algae. The disadvantages of using herbicides include the adverse effect on other organisms, their high persistence in water and sediment, and the resistance to those herbicides built up by algae (Jancula & Marsalek, 2011).

3.4 Biological methods

Biological controls include increasing grazing pressure on cyanobacteria, planting macrophytes and periphyton to inhibit cyanobacterial growth, and introduction of bacteria and cyanophages that can lyse cyanobacterial cells.

3.4.1 Grazing effect

Cyanobacteria can be controlled by the top-down control of zooplankton by piscivore, which is a biomanipulation process based on the food web management. This process is implemented by harvesting the non-predatory fish and introducing the predatory fish, hence the feeding pressure of fish on zooplankton will decrease. Under this circumstance, the zooplankton will be dominant, which can effectively control the developing of phytoplanktons (Shapiro et al., 1975). However, the effectiveness of this method is limited. First, most filaments or colonies cyanobacteria are too large for zooplankton to ingest. Second, the feeding of zooplankton can be inhibited by the toxic effects of cyanobacteria, which are sometimes enhanced by exposure to zooplankton. However, some zooplankton species, such as daphnia, may be already resistant to cyanotoxins because they are living in the water with commonly present cyanobacteria (Drabkova & Marsalek, 2007).

3.4.2 Macrophytes and periphyton

As a rooted plant, macrophyte can reduce the resuspension of sediments generated by wind or boat, can provide a refuge for daphnia that graze algae, and provide a shade to keep the

water cool in the littoral zones. Macrophytes can also remove part of nutrients and release some allelopathic chemicals that can inhibit cyanobacteria (Drabkova & Marsalek, 2007). In addition, macrophytes can act as careers for periogyton, which can further remove the dissolved phosphorus (McComas, 2004).

However, there are some factors that hinder this effect. First, lakes have a resistance to the decreasing of nutrient load, thus the water quality may not get better even if the nutrient levels are substantially reduced (Cooke et al., 2005). Second, the action of waves, limitation of light, animals eating the plants, and nonactive seeds all may prevent the growth of macrophytes (Drabkova & Marsalek, 2007). Last, the macrophyte can only dominate the shallow lakes where the colonized macrophyte may potentially occupy 100% of the area. For those deep lakes that have small littoral zones, the macrophyte effect is limited (Cooke et al., 2005).

3.4.3 Bacteria

MCs are usually resistant to enzymatic hydrolysis by normal proteases, but can be degraded by some bacteria species (Dziga et al., 2013). A number of *Sphingomonas* strains have been isolated from lakes and reservoirs and reported that have ability of degrading MCs. Some other bacterial species were reported to be capable of degrading MCs, such as *Rhodococcus sp., Brevibacterium sp., and Arthrobacter spp* (Manage et al., 2009). Furthermore, some probiotic bacteria have been shown to be capable of degrading cyanotoxins, but not very efficiently (Meriluoto et al., 2005). Studies show that the ability of MCs degradation is related to the presence of *mlrA* gene, and the cell wall associated proteinases may be involved in the degradation of MCs (Saito et al., 2003). Comparing to MCs, nodularin is more resistant to biodegradation. Most bacteria species talked above have only the ability of degrading MCs but not nodularin (Pantelic et al., 2013).

The use of bacteria to remove cyanotoxins from waters is a reliable and cost-efficient method, in which the harmful chemicals are not involved. However, the long reaction time it requires makes it not viable all the time (Pantelic et al., 2013).

3.4.4 Viruses/Algae/Fungi

Many other aquatic organisms have been studied to inhibit the cyanobacterial growth. Cyanophages are viruses of cyanobcateria presented in marine and freshwaters. They have been reported to be used for cyanobacterial bloom control (Safterman & Morris, 1964). Gons et al. (2002) showed that cyanobacterial biomass declined suddenly with the occurrence of cyanophages. However, the effect of inhibition is temporary because the cyanobacteria will become resistant to cyanophages with time. And the cyanophages are strain specific that cannot affect cyanobacteria in other species (Drabkova & Marsalek, 2007).

Some filamentous algae and planktonic algae can produce allelopathic chemicals which can inhibit the growth of cyanobacteria. A study shows that the waters dominated by filamentous algae usually are free of cyanobacteria (Wu et al., 1998). Using chytridiaceous fungus *Rhizophidium planktonicum* as parasite of cyanobacteria has been reported, but the effect was later indicated to be limited because the fungi is difficult to be cultured in large scale (Canter, 1954; Daft et al., 1985).

Chapter 4 - Conclusion, challenges and prospects

Harmful cyanobacterial bloom is a worldwide phenomenon, which poses a threat to both public water supplies and aquatic ecosystems across the world. This paper introduces some basic knowledge about cyanobacteria and cyanotoxins, health and ecological effects, toxin detection methods, important environmental factors that affect cyanobacterial growth, and emphasizes the management of the cyanobacterial bloom.

Cyanobacterial blooms are very prevalent, and can be found in surface water, which is used for drinking water supply, fishing industry, recreation, and irrigation. The prevention and mitigation of cyanobacterial blooms are difficult and sometimes impossible to implement. The only long-term solution is to restrict nutrient inputs to the water bodies, using measures such as agricultural and urban runoff control, the using of phosphate-free detergents, the regulation of local drainage and septic systems, and the control of spills of industrial waste. However, these measures seem to be unavailable in most of the areas due to the large financial costs. Some other conventional and advanced water treatment processes are carried out, but all of them have some disadvantages. For example, using activated carbon can effectively remove the cyanotoxins, but the carbon will be exhausted within a short time thus needed to be re-applied frequently. Ultrasound can inhibit the growth of cyanobacteria, but it has a short time effect and will lyse cells and release the intracellular toxins into water. Artificial mixing of the waters is very costly. Using algicides usually induce the release of toxins by lysing cells, and may generate toxic by-products. As every measurement has both advantages as well as disadvantages, the key to success is to find a reasonable balance between them.

In addition, because the water systems and the quality of the areas' source water are unique, some measurements that work in one area may not work effectively in others. The local

water treatment agencies should investigate more carefully and determine the best strategy to protect the local aquatic ecosystem and the public health. The strategy should usually involve a combination of different measurements. The public education is also of key importance, because the public awareness of this issue is essential for reducing the nutrients input into waters and minimizing the exposure of human beings to cyanobacterial blooms.

There are also some challenges for the analysis of cyanotoxins. First, since the current techniques for cyanotoxins analysis all have limitations and cannot detect all the toxins in the same water sample, the development of new and more sophisticated techniques to identify and quantify more types of toxins along with their different variants simultaneously is needed. Second, the robustness and detection limit of current detection techniques should be improved, which will reduce the possibility of underestimation. Furthermore, the analytical methods currently being used are too complicated for operators with limited knowledge and skill, so relatively simple techniques are required. Finally, the safe levels of some cyanotoxins other than MCs, such as nodularins and alkaloid, also need to be determined to give a guideline for the establishment of the bloom control strategies.

Lastly, if we cannot find a comprehensive and effective strategy to control the greenhouse gas emissions, the further climatic warming and its impact on the aquatic ecosystems will definitely induce further expansion of cyanobacterial blooms and deterioration of the ecosystems.

References

Al Momani, F., Smith, D. W., & El-Din, M. G. (2008). Degradation of cyanobacteria toxin by advanced oxidation processes. *Journal of Hazardous Materials, 150*(2), 238-249. Doi 10.1016/j.jhazmat.2007.04.087

Almeida, V. P. S., Cogo, K., Tsai, S. M., & Moon, D. H. (2006). Colorimetric test for the monitoring of microcystins in cyanobacterial culture and environmental samples from southeast - Brazil. *Brazilian Journal of Microbiology, 37*(2), 192-198. Doi 10.1590/S1517-83822006000200017

Alvarez, M., Rose, J., Bellamy, B. (2010). Treating Algal Toxins Using Oxidation, Adsorption, and Membrane Technologies. Orlando, FL: Water Research Foundation. Retrieved January, 27th, 2015, from http://www.sjrwmd.com/surfacewaterwithdrawals/pdfs/algal_toxins_study.pdf

Anderson, D. M., & Rengefors, K. (2006). Community assembly and seasonal succession of marine dinoflagellates in a temperate estuary: The importance of life cycle events. *Limnology and Oceanography, 51*(2), 860-873.

Barrington, D. J., & Ghadouani, A. (2008). Application of Hydrogen Peroxide for the Removal of Toxic Cyanobacteria and Other Phytoplankton from Wastewater. *Environ Sci Technol, 42*(23), 8916-8921. Doi 10.1021/Es801717y

Barrington, D. J., Ghadouani, A., & Ivey, G. N. (2011). Environmental Factors and the Application of Hydrogen Peroxide for the Removal of Toxic Cyanobacteria from Waste Stabilization Ponds. *Journal of Environmental Engineering-Asce, 137*(10), 952-960. Doi 10.1061/(Asce)Ee.1943-7870.0000401

Barrington, D. J., Reichwaldt, E. S., & Ghadouani, A. (2013). The use of hydrogen peroxide to remove cyanobacteria and microcystins from waste stabilization ponds and hypereutrophic systems. *Ecological Engineering, 50*, 86-94. Doi 10.1016/j.ecoleng.2012.04.024

Barroin, G., & Feuillade, M. (1986). Hydrogen-Peroxide as a Potential Algicide for Oscillatoria-Rubescens Dc. *Water Research, 20*(5), 619-623. Doi 10.1016/0043-1354(86)90026-6

Bouaicha, N., Maatouk, I., Vincent, G., & Levi, Y. (2002). A colorimetric and fluorometric microplate assay for the detection of microcystin-LR in drinking water without preconcentration. *Food and Chemical Toxicology, 40*(11), 1677-1683. Doi 10.1016/S0278-6915(02)00103-5

Bruchet, A., Bernazeau, F., Baudin, I., & Pieronne, P. (1998). Algal toxins in surface waters: analysis and treatment. *Water Supply, 16*(1), 619-623.

Campbell, T. W. (1964). Oxidation Mechanisms. Applications to Organic Chemistry. Journal of the American Chemical Society, 86(16), 3404-3404.

Canter, H. M. (1954). Fungal parasites of the phytoplankton. III. Transactions of the British *Mycological Society, 37*(2), 111-IN114.

Carmichael, W. W. (2001). Health effects of toxin-producing cyanobacteria: "The CyanoHABs". *Human and Ecological Risk Assessment, 7*(5), 1393-1407. Doi 10.1080/20018091095087

Carmichael, W. W., Evans, W. R., Yin, Q. Q., Bell, P., & Moczydlowski, E. (1997). Evidence for paralytic shellfish poisons in the freshwater cyanobacterium Lyngbya wollei (Farlow ex Gomont) comb. nov. *Appl Environ Microbiol, 63*(8), 3104-3110.

Carr, N. G., & Whitton, B. A. (1982). *The biology of cyanobacteria*. Berkeley and Los Angeles, CA. University of California Press.

Cheung, M. Y., Liang, S., & Lee, J. (2013). Toxin-producing cyanobacteria in freshwater: A review of the problems, impact on drinking water safety, and efforts for protecting public health. *Journal of Microbiology, 51*(1), 1-10. Doi 10.1007/s12275-013-2549-3

Chiswell, R. K., Shaw, G. R., Eaglesham, G., Smith, M. J., Norris, R. L., Seawright, A. A., & Moore, M. R. (1999). Stability of cylindrospermopsin, the toxin from the cyanobacterium, Cylindrospermopsis raciborskii: Effect of pH, temperature, and sunlight on decomposition. *Environmental Toxicology, 14*(1), 155-161. Doi 10.1002/(Sici)1522-7278(199902)14:1<155::Aid-Tox20>3.0.Co;2-Z

Choi, O., Deng, K. K., Kim, N. J., Ross, L., Jr., Surampalli, R. Y., & Hu, Z. (2008). The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water Research, 42*(12), 3066-3074. Doi: 10.1016/j.watres.2008.02.021

Chorus, I., & Bartram, J. (1999). *Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management*. Routledge, London. E & FN Spon.

Chow, C. W. K., Panglisch, S., House, J., Drikas, M., Burch, M. D., & Gimbel, R. (1997). A study of membrane filtration for the removal of cyanobacterial cells. *Journal of Water Services Research and Technology-Aqua, 46*(6), 324-334.

Codd, G. A. (1995). Cyanobacterial toxins: Occurrence, properties and biological significance. *Water Science and Technology, 32*(4), 149-156. Doi 10.1016/0273-1223(95)00692-3

Cooke, G. D., Welch, E. B., Peterson, S. A., Nichols, S. A. . (2005). *Restoration and Management of Lakes and Reservoirs* (G. D. Cooke, Taylor an Francis, Boca Raton Ed. 3rd ed.). Florida.

Corbel, S., Mougin, C., & Bouaicha, N. (2014). Cyanobacterial toxins: modes of actions, fate in aquatic and soil ecosystems, phytotoxicity and bioaccumulation in agricultural crops. *Chemosphere, 96*, 1-15. Doi: 10.1016/j.chemosphere.2013.07.056

Cyanobacterial Toxins -- Microcystin-LR. (2000). Retrieved September, 1st, 2014, from http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/cyanobacterial_toxins/index-eng.php

Daft, M. J., Burnham, J. C., & Yamamoto, Y. (1985). Lysis of Phormidium-Luridum by Myxococcus-Fulvus in Continuous-Flow Cultures. *Journal of Applied Bacteriology, 59*(1), 73-80. Doi 10.1111/j.1365-2672.1985.tb01778.x

Dawson, R. M. (1998). The toxicology of microcystins. *Toxicon, 36*(7), 953-962.

De La Cruz, A. A., Antoniou, M. G., Hiskia, A., Pelaez, M., Song, W. H., O'Shea, K. E., He, X., Dionysiou, D. D. (2011). Can We Effectively Degrade Microcystins? - Implications on Human Health. *Anti-Cancer Agents in Medicinal Chemistry, 11*(1), 19-37.

Deborde, M., & Von Gunten, U. (2008). Reactions of chlorine with inorganic and organic compounds during water treatment - Kinetics and mechanisms: A critical review. *Water Research, 42*(1-2), 13-51. Doi 10.1016/j.watres.2007.07.025

Dionysiou, D. (2010). Overview: Harmful algal blooms and natural toxins in fresh and marine waters - Exposure, occurrence, detection, toxicity, control, management and policy. *Toxicon, 55*(5), 907-908. Doi: 10.1016/j.toxicon.2009.12.024

Drabkova, M., & Marsalek, B. (2007). A review of in-lake methods of cyanobacterial blooms control and management. *The Glogal Database of Methods for Cyanobacterial Blooms Management.* Retrieved September, 1st, 2014, from http://www.cyanodata.net/revie-w.php

Dziga, D., Wasylewski, M., Wladyka, B., Nybom, S., & Meriluoto, J. (2013). Microbial Degradation of Microcystins. *Chemical Research in Toxicology, 26*(6), 841-852. Doi 10.1021/Tx4000045

Elmolla, E. S., & Chaudhuri, M. (2010). Comparison of different advanced oxidation processes for treatment of antibiotic aqueous solution. *Desalination, 256*(1-3), 43-47. Doi 10.1016/j.desal.2010.02.019

EPA. (1999). Alternative disinfectants and oxidants guidance manual. Retrieved February, 1st, 2015, from http://www.epa.gov/ogwdw/mdbp/alternative_disinfectants_guidance.pdf

EPA. (2012). Impaired waters and total maximum daily loads. Retrieved September, 1st, 2014, from http://water.epa.gov/lawsregs/lawsguidance/cwa/tmdl/index.cfm

EPA. (2013). Health and Ecological Effects. Retrieved December, 2nd, 2014, from http://www2.epa.gov/nutrient-policy-data/health-and-ecological-effects

Ettoumi, A., El Khalloufi, F., El Ghazali, I., Oudra, B., Amrani, A., Nasri, H., & Bouaïcha, N. (2011). Bioaccumulation of cyanobacterial toxins in aquatic organisms and its consequences for public health. *Zooplankton and Phytoplankton: types, characteristics and ecology.* New York: Nova Science Publishers.

Falconer, I. R. (1993). Measurement of toxins from blue-green algae in water and foodstuffs. In I. R. Falconer (Ed.), *Algal toxins in seafood and drinking water* (pp. 165-175). London, UK: Academic Press.

Falconer, I. R. (1999). An overview of problems caused by toxic blue–green algae (cyanobacteria) in drinking and recreational water. *Environmental Toxicology, 14*(1), 5-12.

Falconer, I. R. (2004). *Cyanobacterial Toxins of Drinking Water Supplies*. CRC Press.

Falconer, I. R. (2008). Health effects associated with controlled exposures to cyanobacterial toxins. *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs, 619*, 607-612.

Falconer, I. R., & Humpage, A. R. (2005). Health risk assessment of cyanobacterial (blue-green algal) toxins in drinking water. *Int J Environ Res Public Health, 2*(1), 43-50.

Ferrão Filho, A. D. S. (2009). Bioacumulação de cianotoxinas e seus efeitos em organismos aquáticos. *Oecol. Bras., 13*, 272-312.

Fitzgerald, D. J., Cunliffe, D. A., & Burch, M. D. (1999). Development of health alerts for cyanobacteria and related toxins in drinking water in South Australia. *Environmental Toxicology, 14*(1), 203-209. Doi 10.1002/(Sici)1522-7278(199902)14:1<203::Aid-Tox26>3.0.Co;2-X

Fogg, G. (2012). *The blue-green algae*. New york. Academic Press. Doi 10.1126/science.184.4141.1066-a

Funari, E., & Testai, E. (2008). Human health risk assessment related to cyanotoxins exposure. *Critical Reviews in Toxicology, 38*(2), 97-125. Doi 10.1080/10408440701749454

Gerba, C. P., Maier, Raina M., Pepper, Ian L. (2000). *Environmental Microbiology*. 126-127. Elsevier.

Giannuzzi, L., Sedan, D., Echenique, R., & Andrinolo, D. (2011). An Acute Case of Intoxication with Cyanobacteria and Cyanotoxins in Recreational Water in Salto Grande Dam, Argentina. *Marine Drugs, 9*(11), 2164-2175. Doi 10.3390/Md9112164

Gons, H. J., Ebert, J., Hoogveld, H. L., van den Hove, L., Pel, R., Takkenberg, W., & Woldringh, C. J. (2002). Observations on cyanobacterial population collapse in eutrophic lake water. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 81*(1-4), 319-326. Doi 10.1023/A:1020595408169

Grantz, E. M., Haggard, B. E., & Scott, J. T. (2014). Stoichiometric imbalance in rates of nitrogen and phosphorus retention, storage, and recycling can perpetuate nitrogen deficiency in highly-productive reservoirs. *Limnology and Oceanography, 59*(6), 2203-2216. Doi 10.4319/lo.2014.59.6.2203

Grutzmacher, G., Bottcher, G., Chorus, I., & Bartel, H. (2002). Removal of microcystins by slow sand filtration. *Environmental Toxicology, 17*(4), 386-394. Doi 10.1002/Tox.10062

Hao, H., Wu, M., Chen, Y., Tang, J., & Wu, Q. (2004). Cyanobacterial bloom control by ultrasonic irradiation at 20 kHz and 1.7 MHz. *Journal of Environmental Science and Health, Part A, 39*(6), 1435-1446. Doi: 10.1081

Hart, J., Fawell, J. K., & Croll, B. (1998). The fate of both intra- and extracellular toxins during drinking water treatment. *Water Supply, 16*(1), 611-616.

Hart, J., Roberts, S., James, R., Taylor, J., Donnert, D., & Furrer, R. (2003). Use of active barriers to reduce eutrophication problems in urban lakes. *Water Science and Technology, 47*(7-8), 157-163.

Haselkorn, R. (1995). The Molecular-Biology of Cyanobacteria - Bryant,Da. *Science, 269*(5227), 1121-1121.

Havens, K. E. (2008). Cyanobacteria blooms: effects on aquatic ecosystems. *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs, 619*, 733-747. Doi: 10.1007/978-0-387-75865-7_33

Hawkins, P. R., Runnegar, M. T. C., Jackson, A. R. B., & Falconer, I. R. (1985). Severe Hepatotoxicity Caused by the Tropical Cyanobacterium (Blue-Green-Alga) Cylindrospermopsis-Raciborskii (Woloszynska) Seenaya and Subba Raju Isolated from a Domestic Water-Supply Reservoir. *Applied and Environmental Microbiology, 50*(5), 1292-1295.

Hense, I., & Beckmann, A. (2006). Towards a model of cyanobacteria life cycle - effects of growing and resting stages on bloom formation of N-2-fixing species. *Ecological Modelling, 195*(3-4), 205-218. Doi 10.1016/j.ecolmodel.2005.11.018

Hense, I., & Beckmann, A. (2010). The representation of cyanobacteria life cycle processes in aquatic ecosystem models. *Ecological Modelling, 221*(19), 2330-2338. Doi 10.1016/j.ecolmodel.2010.06.014

Hilborn, E. D., Carmichael, W. W., Soares, R. M., Yuan, M., Servaites, J. C., Barton, H. A., & Azevedo, S. M. F. O. (2007). Serologic evaluation of human microcystin exposure. *Environmental Toxicology, 22*(5), 459-463. Doi 10.1002/Tox.20281

Hilborn, E. D., Carmichael, W. W., Yuan, M., & Azevedo, S. M. F. O. (2005). A simple colorimetric method to detect biological evidence of human exposure to microcystins. *Toxicon, 46*(2), 218-221. Doi 10.1016/j.toxicon.2005.04.009

Hitzfeld, B. C., Hoger, S. J., & Dietrich, D. R. (2000). Cyanobacterial toxins: Removal during drinking water treatment, and human risk assessment. *Environmental Health Perspectives, 108*, 113-122. Doi 10.2307/3454636

Hoffmann, J. R. (1976). Removal of Microcystis toxins in water purification processes. *Water SA, 2*(2), 58-60.

Hoffmann, M. R., Martin, S. T., Choi, W. Y., & Bahnemann, D. W. (1995). Environmental Applications of Semiconductor Photocatalysis. *Chemical Reviews, 95*(1), 69-96. Doi 10.1021/Cr00033a004

Houghton, J. T., Ding, Y., Griggs, D. J., Noguer, M., van der Linden, P. J., Dai, X., Maskell, K., Johnson, C. A. (2001). Climate change 2001: the scientific basis., 881. Cambridge University Press.

Hupfer, M., & Lewandowski, J. (2008). Oxygen Controls the Phosphorus Release from Lake Sediments - a Long-Lasting Paradigm in Limnology. *International Review of Hydrobiology, 93*(4-5), 415-432. Doi 10.1002/iroh.200711054

IPCC. (2001). A report of working group I of the Intergovernmental Panel on Climate Change. Summary for Policymakers and Technical Summary. Retrieved January, 28th, 2015, from https://www.ipcc.ch/pdf/assessment-report/ar4/wg1/ar4-wg1-spm.pdf

Jacobs, L. C. V., Peralta-Zamora, P., Campos, F. R., & Pontarolo, R. (2013). Photocatalytic degradation of microcystin-LR in aqueous solutions. *Chemosphere, 90*(4), 1552-1557. Doi 10.1016/j.chemosphere.2012.09.004

Jancula, D., & Marsalek, B. (2011). Critical review of actually available chemical compounds for prevention and management of cyanobacterial blooms. *Chemosphere, 85*(9), 1415-1422. Doi 10.1016/j.chemosphere.2011.08.036

Jones, G., Minatol, W., Craig, K., & Naylor, R. (1993). *Removal of low level cyanobacterial peptide toxins from drinking water using powdered and granular activated carbon and chlorination-Results of laboratory and pilot plant studies.* Paper presented at the Proceedings of the 15th Federal AWWA Convention, Gold Coast, Queensland, Australia.

Kaneko, T., Sato, S., Kotani, H., Tanaka, A., Asamizu, E., Nakamura, Y., Miyajima, N., & Tabata, S. (1996). Sequence analysis of the genome of the unicellular cyanobacterium Synechocystis sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. *DNA Res, 3*(3), 109-136.

Kaushik, R., & Balasubramanian, R. (2013). Methods and Approaches Used for Detection of Cyanotoxins in Environmental Samples: A Review. *Critical Reviews in Environmental Science and Technology, 43*(13), 1349-1383. Doi 10.1080/10643389.2011.644224

Khanal, S. K., Grewell, D., Sung, S., & Van Leeuwen, J. (2007). Ultrasound applications in wastewater sludge pretreatment: A review. *Critical Reviews in Environmental Science and Technology, 37*(4), 277-313. Doi 10.1080/10643380600860249

Kin, S. (2010). Light saturation and photoinhibition. *Biofuels Project 2010.* Retrieved January, 27th, 2015, from http://biofuels2010.blogspot.com/2010/11/light-saturation-and-photoinhibition.html

Kononen, K., Kuparinen, J., Mäkelä, K., Laanemets, J., Pavelson, J., & Nommann, S. (1996). Initiation of cyanobacterial blooms in a frontal region at the entrance to the Gulf of Finland, Baltic Sea. *Limnology and Oceanography, 41*(1), 98-112.

Koreiviene, J., Anne, O., Kasperoviciene, J., & Burskyte, V. (2014). Cyanotoxin management and human health risk mitigation in recreational waters. *Environmental Monitoring and Assessment, 186*(7), 4443-4459. Doi 10.1007/s10661-014-3710-0

Kremp, A., Tamminen, T., & Spilling, K. (2008). Dinoflagellate bloom formation in natural assemblages with diatoms: nutrient competition and growth strategies in Baltic spring phytoplankton. *Aquatic Microbial Ecology, 50*(2), 181-196. Doi 10.3354/Ame01163

Lambert, T. W., Holmes, C. F. B., & Hrudey, S. E. (1996). Adsorption of microcystin-LR by activated carbon and removal in full scale water treatment. *Water Research, 30*(6), 1411-1422. Doi 10.1016/0043-1354(96)00026-7

Lawton, L. A., & Robertson, P. K. J. (1999). Physico-chemical treatment methods for the removal of microcystins (cyanobacterial hepatotoxins) from potable waters. *Chemical Society Reviews, 28*(4), 217-224. Doi 10.1039/A805416i

Lee, T. J., Nakano, K., & Matsumura, M. (2000). A new method for the rapid evaluation of gas vacuoles regeneration and viability of cyanobacteria by flow cytometry. *Biotechnology Letters, 22*(23), 1833-1838.

Lee, J., & Walker, H. W. (2008). Mechanisms and factors influencing the removal of microcystin-LR by ultrafiltration membranes. *Journal of Membrane Science, 320*(1-2), 240-247. Doi 10.1016/j.memsci.2008.04.007

Lindner, P., Molz, R., Yacoub-George, E., Durkop, A., & Wolf, H. (2004). Development of a highly sensitive inhibition immunoassay for microcystin-LR. *Analytica Chimica Acta, 521*(1), 37-44. Doi 10.1016/j.aca.2004.05.059

Liu, I., Lawton, L. A., Bahnemann, D. W., Liu, L., Proft, B., & Robertson, P. K. J. (2009). The photocatalytic decomposition of microcystin-LR using selected titanium dioxide materials. *Chemosphere, 76*(4), 549-553. Doi 10.1016/j.chemosphere.2009.02.067

Lopez, C. B., Jewett, E. B., Dortch, Q., Walton, B. T., & Hudnell, H. K. (2008). Scientific assessment of freshwater harmful algal blooms. *Interagency Working Group on Harmful Algal Blooms, Hypoxia, and Human Health of the Joint Subcommittee on Ocean Science and Technology*, 65. Retrieved January, 25th, 2015, from http://www.cop.noaa.gov/stressors/extremeevents/hab/habhrca/FreshwaterReport_final_2008.pdf

Manage, P. M., Edwards, C., Singh, B. K., & Lawton, L. A. (2009). Isolation and Identification of Novel Microcystin-Degrading Bacteria. *Applied and Environmental Microbiology, 75*(21), 6924-6928. Doi 10.1128/Aem.01928-09

Markou, G., & Georgakakis, D. (2011). Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review. *Applied Energy, 88*(10), 3389-3401. Doi 10.1016/j.apenergy.2010.12.042

Maynard, T., Roder, D., El Saadi, O., Turczynowicz, L., Fitzgerald, J., San Soong, F., & Ressom, R. (2000). Health effects of toxic cyanobacteria (blue-green algae). National Health and Medical Research Council. Retrieved January, 25th, 2015, from http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/eh14.pdf

McComas, S. (2004). *Lake and pond management guidebook*. Lewis publishers.

Merel, S., Walker, D., Chicana, R., Snyder, S., Baures, E., & Thomas, O. (2013). State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. *Environment International, 59*, 303-327. Doi 10.1016/j.envint.2013.06.013

Meriluoto, J., Gueimonde, M., Haskard, C. A., Spoof, L., Sjovall, O., & Salminen, S. (2005). Removal of the cyanobacterial toxin microcystin-LR by human probiotics. *Toxicon, 46*(1), 111-114. Doi 10.1016/j.toxicon.2005.03.013

Mouchet, P., & Bonnelye, V. (1998). Solving algae problems: French expertise and world-wide applications. *Journal of Water Services Research and Technology-Aqua, 47*(3), 125-141.

Murray-Gulde, C. L., Heatley, J. E., Schwartzman, A. L., & Rodgers, J. H. (2002). Algicidal effectiveness of clearigate, cutrine-plus, and copper sulfate and margins of safety associated with their use. *Archives of Environmental Contamination and Toxicology, 43*(1), 19-27. Doi 10.1007/s00244-002-1135-1

Namikoshi, M., & Rinehart, K. L. (1996). Bioactive compounds produced by cyanobacteria. *Journal of Industrial Microbiology & Biotechnology, 17*(5-6), 373-384. Doi 10.1007/Bf01574768

Newcombe, G. (2003). Removal of Algal Toxins From Drinking Water Using Ozone and GAC. *Sci-Tech News, 57*(1), 49.

Nicholson, B. C., & Burch, M. D. (2001). Evaluation of analytical methods for detection and quantification of cyanotoxins in relation to Australian drinking water guidelines. National Health and Medical Research Council of Australia. Retrieved February, 25th, 2015, from https://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/eh22_evaluation_analytical_detection_quantification_cyanotoxins_aust_drinking_water_guidelines_131223.pdf

Nicholson, B. C., Rositano, J., & Burch, M. D. (1994). Destruction of Cyanobacterial Peptide Hepatotoxins by Chlorine and Chloramine. *Water Research, 28*(6), 1297-1303. Doi 10.1016/0043-1354(94)90294-1

Nixon, S. W. (1995). Coastal Marine Eutrophication - a Definition, Social Causes, and Future Concerns. *Ophelia, 41*, 199-219.

Oliver, R. L. (1994). Floating and Sinking in Gas-Vacuolate Cyanobacteria. *Journal of Phycology, 30*(2), 161-173. Doi 10.1111/j.0022-3646.1994.00161.x

Paerl, H. W. (1990). Physiological Ecology and Regulation of N2 Fixation in Natural-Waters. *Advances in Microbial Ecology, 11*, 305-344.

Paerl, H. W. (2013). Combating the global proliferation of harmful cyanobacterial blooms by integrating conceptual and technological advances in an accessible water management toolbox. *Environ. Microbiol, Rep 5*, 12-14.

Paerl, H. W. (2014). Mitigating harmful cyanobacterial blooms in a human- and climatically-impacted world. *Life (Basel), 4*(4), 988-1012. Doi: 10.3390/life4040988

Paerl, H. W., & Fulton, R. S. (2006). Ecology of harmful cyanobacteriaEcology of harmful algae (pp. 95-109): Springer Berlin Heidelberg.

Paerl, H. W., Fulton, R. S., Moisander, P. H., & Dyble, J. (2001). Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *ScientificWorldJournal, 1*, 76-113. Doi: 10.1100/tsw.2001.16

Paerl, H. W., & Otten, T. G. (2013). Harmful cyanobacterial blooms: causes, consequences, and controls. *Microb Ecol, 65*(4), 995-1010. Doi: 10.1007/s00248-012-0159-y

Paerl, H. W., & Scott, J. T. (2010). Throwing fuel on the fire: synergistic effects of excessive nitrogen inputs and global warming on harmful algal blooms. *Environ Sci Technol, 44*(20), 7756-7758. Doi: 10.1021/es102665e

Pantelic, D., Svircev, Z., Simeunovic, J., Vidovic, M., & Trajkovic, I. (2013). Cyanotoxins: Characteristics, production and degradation routes in drinking water treatment with reference to the situation in Serbia. *Chemosphere, 91*(4), 421-441. Doi 10.1016/j.chemosphere.2013.01.003

Pelaez, M., de la Cruz, A. A., Stathatos, E., Falaras, P., & Dionysiou, D. D. (2009). Visible light-activated N-F-codoped TiO2 nanoparticles for the photocatalytic degradation of microcystin-LR in water. *Catalysis Today, 144*(1-2), 19-25. Doi 10.1016/j.cattod.2008.12.022

Perovich, G., Dortch, Q., Goodrich, J., Berger, P. S., Brooks, J., Evens, T. J., Gobler, C. J., Graham, J., Hyde, J., Karner, D., Paul, V., Paerl, H., Piehler, M., Rosen, B. H., Santelmann, M., Tester, P., & Westrick, J. (2008). Chapter 9: Causes, prevention, and mitigation workgroup report. *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs, 619*, 185-215.

Phull, S. S., Newman, A. P., Lorimer, J. P., Pollet, B., & Mason, T. J. (1997). The development and evaluation of ultrasound in the biocidal treatment of water. *Ultrasonics Sonochemistry, 4*(2), 157-164. Doi 10.1016/S1350-4177(97)00029-1

Pontius, F. W. (1990). *Water quality and treatment: a handbook of community water supplies*. American Water Works Association. Retrieved January, 6th, 2015, from http://www.globalspec.com/reference/80698/203279/water-quality-and-treatment-a-handbook-of-community-water-supplies-fifth-edition

Precambrian Life I: Microfossils. (2001). Retrieved January, 6th, 2015, from https://www2.bc.edu/~strother/GE_146/labs/lab7/

Quiblier, C., Wood, S., Echenique-Subiabre, I., Heath, M., Villeneuve, A., & Humbert, J. F. (2013). A review of current knowledge on toxic benthic freshwater cyanobacteria - Ecology, toxin production and risk management. *Water Research, 47*(15), 5464-5479. Doi 10.1016/j.watres.2013.06.042

Rajasekhar, P., Fan, L. H., Nguyen, T., & Roddick, F. A. (2012a). Impact of sonication at 20 kHz on Microcystis aeruginosa, Anabaena circinalis and Chlorella sp. *Water Research, 46*(5), 1473-1481. Doi 10.1016/j.watres.2011.11.017

Rajasekhar, P., Fan, L. H., Nguyen, T., & Roddick, F. A. (2012b). A review of the use of sonication to control cyanobacterial blooms. *Water Research, 46*(14), 4319-4329. Doi 10.1016/j.watres.2012.05.054

Rapala, J., Erkomaa, K., Kukkonen, J., Sivonen, K., & Lahti, K. (2002). Detection of microcystins with protein phosphatase inhibition assay, high-performance liquid chromatography-UV detection and enzyme-linked immunosorbent assay - Comparison of methods. *Analytica Chimica Acta, 466*(2), 213-231. Doi 10.1016/S0003-2670(02)00588-3

Rapala, J., Sivonen, K., Lyra, C., & Niemela, S. I. (1997). Variation of microcystin, cyanobacterial hepatotoxins, in Anabaena spp as a function of growth stimulation. *App. Environ. Microbiol., 63*, 2206-2212.

Reynolds, C. S. (1972). Growth, gas vacuolation and buoyancy in a natural population of a planktonic blue‐green alga. *Freshwater Biology*, *2*(2), 87-106.

Reynolds, C. S. (1984). *The ecology of freshwater phytoplankton*. Cambridge, England. Cambridge University Press.

Reynolds, C. S. (1987). Cyanobacterial Water-Blooms. *Advances in Botanical Research Incorporating Advances in Plant Pathology, 13*, 67-143. Doi 10.1016/S0065-2296(08)60341-9

Robarts, R. D., Waiser, M. J., Arts, M. T., & Evans, M. S. (2005). Seasonal and diel changes of dissolved oxygen in a hypertrophic prairie lake. *Lakes & Reservoirs: Research & Management, 10*(3), 167-177.

Robb, M., Greenop, B., Goss, Z., Douglas, G., & Adeney, J. (2003). Application of PhoslockTM, an innovative phosphorus binding clay, to two Western Australian waterways:

preliminary findings *The Interactions between Sediments and Water* (pp. 237-243): Springer Netherlands.

Rodhe, W. (1969). Crystallization of eutrophication concepts in North Europe *Eutrophication, Causes, Consequences, Correctives*. Washington D.C.: National Academy of Sciences.

Safterman, R. S., & Morris, M. E. (1964). Control of algae with viruses. *Journal (American Water Works Association)*, 1217-1224.

Saito, T., Okano, K., Park, H. D., Itayama, T., Inamori, Y., Neilan, B. A., Burns, B. P., & Sugiura, N. (2003). Detection and sequencing of the microcystin LR-degrading gene, mlrA, from new bacteria isolated from Japanese. *Fems Microbiology Letters, 229*(2), 271-276. Doi 10.1016/S0378-1097(03)00847-4

Schopf, J. W. (2002). The fossil record: tracing the roots of the cyanobacterial lineage. In: *The ecology of cyanobacteria* (pp. 13-35): Springer Netherlands.

Schwabe, W., Weihe, A., Borner, T., Henning, M., & Kohl, J. G. (1988). Plasmids in Toxic and Nontoxic Strains of the Cyanobacterium Microcystis-Aeruginosa. *Current Microbiology, 17*(3), 133-137. Doi 10.1007/Bf01573468

Shapiro, J., Lamarra, V. A., & Lynch, M. (1975). Biomanipulation: an ecosystem approach to lake restoration. Retrieved January, 6th, 2015, from http://www.indiana.edu /~lynchlab/PDF/Lynch2.pdf

Sharma, V. K., Triantis, T. M., Antoniou, M. G., He, X. X., Pelaez, M., Han, C. S., . . . Dionysiou, D. D. (2012). Destruction of microcystins by conventional and advanced oxidation processes: A review. *Separation and Purification Technology, 91*, 3-17. Doi 10.1016/j.seppur.2012.02.018

Smith, V. H., Bierman, V. J., Jones, B. L., & Havens, K. E. (1995). Historical trends in the Lake Okeechobee ecosystem IV. Nitrogen:phosphorus ratios, cyanobacterial dominance, and nitrogen fixation potential. *Archiv fu¨r Hydrobiologie, 107*, 71-88.

Smith, V. H., & Schindler, D. W. (2009). Eutrophication science: where do we go from here? *Trends in Ecology & Evolution, 24*(4), 201-207. Doi 10.1016/j.tree.2008.11.009

Soares, R. M., Yuan, M., Servaites, J. C., Delgado, A., Maglhaes, V. F., Hilborn, E. D., Carmichael, W. W., & Azevedol, S. M. (2006). Sublethal exposure from microcystins to renal insufficiency patients in Rio de Janeiro, Brazil. *Environmental Toxicology, 21*(2), 95-103. Doi 10.1002/Tox.20160

Stewart, I., Seawright, A. A., & Shaw, G. R. (2008). Cyanobacterial poisoning in livestock, wild mammals and birds - an overview. *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs, 619*, 613-637.

Suslick, K. S. (1990). Sonochemistry. *Science, 247*(4949), 1439-1445. Doi 10.1126/science.247.4949.1439

Svrcek, C., & Smith, D. W. (2004). Cyanobacteria toxins and the current state of knowledge on water treatment options: a review. *Journal of Environmental Engineering and Science, 3*(3), 155-185. Doi 10.1139/S04-010

Takeuchi, N. (2001). The altitudinal distribution of snow algae on an Alaska glacier (Gulkana Glacier in the Alaska Range). *Hydrological Processes, 15*(18), 3447-3459. Doi 10.1002/Hyp.1040

Van Liere, L., & Mur, L. R. (1980). Occurrence of Oscillatoria agardhii and some related species, a survey. *Hypertrophic ecosystems* (pp. 67-77): Springer Netherlands. Retrieved January, 15th, 2015, from http://link.springer.com/chapter/10.1007%2F978-94-009-9203-0_8

Vasconcelos, V. M. (2006). Eutrophication, toxic cyanobacteria and cyanotoxins: when ecosystems cry for help. *Limnetica, 25*(1), 425-432.

Vincent, W. F. (2009). Cyanobacteria. *Protists, Bacteria and Fungi: Planktonic and Attached*, 226-232.

Von Gunten, U., & Hoigne, J. (1994). Bromate formation during ozonization of bromide-containing waters: interaction of ozone and hydroxyl radical reactions. *Environ Sci Technol, 28*(7), 1234-1242.

Wang, Z., Li, D., Qin, H., & Li, Y. (2012). An integrated method for removal of harmful cyanobacterial blooms in eutrophic lakes. *Environ Pollut, 160*(1), 34-41. Doi: 10.1016/j.envpol.2011.09.003

Westrick, J. A., Szlag, D. C., Southwell, B. J., & Sinclair, J. (2010). A review of cyanobacteria and cyanotoxins removal/inactivation in drinking water treatment. *Anal Bioanal Chem, 397*(5), 1705-1714. Doi 10.1007/s00216-010-3709-5

Whitton, B. A., & Potts, M. (2000). *The ecology of cyanobacteria: their diversity in time and space*.Springer Science & Business Media.

WHO. (1998). Guidelines for drinking-water quality (Vol. 2). Retrieved January, 19th, 2015, from http://www.who.int/water_sanitation_health/dwq/2edaddvol2a.pdf

WHO. (2003). Guidelines for safe recreational water environments *Coastal and fresh waters* (Vol. 1). Geneva, Switzerland. Retrieved January, 25th, 2015, from http://www.who.int/water_sanitation_health/bathing/srwg1.pdf

Witters, H. E., VanPuymbroeck, S., Stouthart, A. J. H. X., & Bonga, S. E. W. (1996). Physicochemical changes of aluminium in mixing zones: Mortality and physiological disturbances in brown trout (Salmo trutta L). *Environmental Toxicology and Chemistry, 15*(6), 986-996. Doi 10.1897/1551-5028(1996)015<0986:Pcoaim>2.3.Co;2

Wu, X. G., Joyce, E. M., & Mason, T. J. (2011). The effects of ultrasound on cyanobacteria. *Harmful Algae, 10*(6), 738-743. Doi 10.1016/j.hal.2011.06.005

Wu, J. T., Kuo-Huang, L. L., & Lee, J. (1998). Algicidal effect of Peridinium bipes on Microcystis aeruginosa. *Current Microbiology, 37*(4), 257-261.

Xagoraraki, I. (2007). *Fate of pharmaceuticals during water chlorination*. Paper presented at the Water Quality Technology Conference, Charlotte, NC.

Zanchett, G., & Oliveira, E. C. (2013). Cyanobacteria and Cyanotoxins: From Impacts on Aquatic Ecosystems and Human Health to Anticarcinogenic Effects. *Toxins, 5*(10), 1896-1917. Doi 10.3390/toxins5101896

Zhang, G. M., Zhang, P. Y., Liu, H., & Wang, B. (2006). Ultrasonic damages on cyanobacterial photosynthesis. *Ultrasonics Sonochemistry, 13*(6), 501-505. Doi 10.1016/j.ultsonch.2005.11.001

Zhang, G. M., Zhang, P. Y., Wang, B., & Liu, H. (2006). Ultrasonic frequency effects on the removal of Microcystis aeruginosa. *Ultrasonics Sonochemistry, 13*(5), 446-450. Doi 10.1016/j.ultsonch.2005.09.012