HARMFUL ALGAL BLOOMS AND BIOLOGICAL TOXINS PRODUCTIONS; FROM INTEGRATED MONITORING APPROACH TO ON-SITE SOLAR PHOTOCATALYTIC DECOMPOSITION

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Abstract

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Occurrence of Harmful Algal Blooms (HABs) worldwide has caused concern to environmental and health authorities because of their potential to generate and release biological toxins. In particular, microcystins (MCs) produced from cyanobacteria are of great concern. MCs are among the most powerful natural poisons. The presence of MCs in drinking water sources has raised major concern.

The overall goal of this research is to predict formation of biological toxins in water bodies and to develop a new sustainable approach to decompose them, if feasible, on-site and in real-time with minimal efforts, less chemicals, and low energy inputs.

In order to achieve the goal, the first objective was to predict MC-LR formation during harmful algal blooms utilizing easy-to-detect indirect parameters. There have been efforts to monitor HAB activities and toxin releases, including i) manual field sampling followed by in lab analysis to directly measure MCs (i.e., biological toxins), ii) remote sensing based on satellite image analysis to estimate cyanobacterial index (i.e., algal blooms), and iii) in situ sensing of easily measurable proxy parameters to algal blooms such as phycocyanin (an accessory pigment to chlorophyll associated with HABs). The

current observation systems for monitoring HABs discussed while pointing out their advantageous and disadvantages.

Second objective was to pioneer a high efficiency visible light-activated TiO_2 photocatalytic process to decompose biological toxins. For on-site applications, the TiO_2 firmly immobilized onto a glass substrate in form of nanoporous thin film. Effect of operation parameters such as type of surfactants, calcination temperature, number of coatings and pH was investigated. In order to evaluate potential field application of our system, we use lake water spiked with MC-LR under solar radiation and the result showed our film has high potential to decompose biological toxins under solar radiation within a reasonable time.

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Chapter 1

Introduction

1.1. Motivation

Cyanobacterial harmful algal blooms have recently become spatially and temporally more prevalent in the United States (US) and worldwide (Stewart and Falconer 2008). They have been found in eight of the fifteen largest continental lakes: Lake Victoria in Africa (Miles et al. 2013), Lakes Erie, Huron, Ontario, and Michigan in North America (Wilhelm 2008; Vanderploeg et al. 2001; Hotto et al. 2007; De Stasio and Richman 1998), Lake Winnipeg in North America (Schindler et al. 2012), and Lake Ladoga in Asia (Gromov et al. 1996). Other large bodies of water that have been impacted by cyanobacterial HABs include: Lake Taihu in China (Pearl et al. 2011), Kasumigaura in Japan (Islam et al. 2013), and Baltic Sea in Northern Europe (Funkey et al. 2014). These toxic blooms have impacted several important river systems such as Nile River in Africa (Zakaria et al. 2006), River Murray in Australia (Bormans et al. 1997), St. Johns River in North America (Burns 2008), and La Plata River in South America (Nagy et al. 2002). Regional surveys focusing on smaller rivers and lakes within urban settings and agricultural beltways suggest that cyanobacterial HABs occur in temporal, subtropical, and tropical climates. In 2010, twenty-three lakes with cyanobacterial blooms in Midwestern US were sampled and analyzed for thirteen cyanotoxins, and MCs occurred in all blooms (Graham et al. 2010).

Economic impacts of coastal HAB events in United States have been estimated over two billion dollars annually from recreation and angling cost, lake property values, biodiversity loss, and drinking water treatment cost (Dodds 2009).

In addition to cyanobacterial HABs' negative ecological, biochemical, and health impacts, they create the economic losses to local surrounding communities and water treatment facilities due to unpleasant odor and taste, de-oxygenation during decomposition of dead

fish, machinery clogging in filters and pumps, and increased costs of operating water treatment plants.

There have been federal efforts to monitor HAB activities and toxin release in United States particularly by National Oceanic and Atmospheric Administration (NOAA) (Hudnell, 2010). First, on site manual sampling followed by lab analysis is commonly used to identify algal species and to assay biological toxins species-by-species (Rivasseau, et al., 1999). In spite of its high accuracy and reliability, this approach is neither sustainable nor practical to meet the vast spatial and temporal measuring need. Second, monitoring relies on spectral images taken from satellites and aircrafts to provide the large spatial scale and high frequency of observations required to assess bloom locations and movements. The remote sensing approach is useful for monitoring general algal bloom activities by providing cyanobacterial index (CI) denoted with imaginary color (Stumpf, et al., 2012). Third, in situ sensing is a recent monitoring approach (Twardowski, et al., 2005). The in situ autonomous observing approach optically senses phycocyanin as an accessory pigment to chlorophyll often associated with HABs (as a surrogate chemical or proxy to HABs) (Marion, et al., 2012). The remote and in situ (real-time) monitoring approaches benefit immediate decision-making and timely response, which are crucial elements for developing an early warning system as a sustainable environmental infrastructure. However, such a color product is not specific to HABs. High level of chlorophyll may be or may not be associated with toxic blooms (Graham, et al., 2010). Even not all algal blooms are associated with the release of biological toxins (Anderson, 2009).

There is no research on comparison of different observing systems results. Each observing systems monitor different HAB parameters such as biological toxins (e.g., MCs), general algal blooms (e.g., CI), and proxy targets (e.g., phycocyanin). It is

hypothesized that they exhibit certain correlation because they are inherently designed to represent the same phenomenon, algal blooms. In addition, correlation between monitoring parameters might be site specific.

Comparison between different observing systems, which monitors different HAB parameters, would help us to understand advantages and disadvantageous of each system. Correlation among the monitoring parameters also helps us to evaluate the validity of the systems to assess HAB events. In this study, first, we introduce different monitoring strategies and then we compare different monitoring data from a sample location (Western part of Lake Erie, Great Lakes, United States) in 2013 to investigate any correlation between different harmful algal bloom observing systems.

During harmful algal bloom, some algae produce and release lethal biological toxins such as microcystins (MCs). Compared to particulate algae, which are easily removed by conventional processes, biological toxins dissolved in water are not easy to remove and thus they are not the focus of the treatment processes. Consequently, after detecting biological toxins using different monitoring approaches, next step would be removal of biological toxins in water resources is important, if feasible, on-site and in real-time with minimal efforts, less chemicals, and low energy inputs. It is hypothesized that TiO_2 photocatalysis could decompose biological toxins due to generation of hydroxyl radicals. In addition, visible light-activated TiO_2 could utilize solar radiation as a sole energy source for on-site decomposition of biological toxins in water.

Progress in chemical oxidation has resulted in advanced oxidation technologies (AOTs), one of the most powerful water treatment processes (Nfodzo, et al., 2013). AOTs are based on generation of hydroxyl radicals and sulfate radicals with high oxidation potentials. Particularly, TiO_2 photocatalysis has been highlighted as one of the most promising and green AOTs due to its effectiveness to generate hydroxyl radicals (Choi, et

al., 2010). For the generation of hydroxyl radicals from TiO_2, ultraviolet (UV) radiation which provides high photon energy above the band gap of TiO_2 is required. Use of UV irradiation is limit the application of TiO_2 photocatalysis process in real world and increase the cost of treatment process. Introduction of anionic dopants, especially nitrogen, to TiO_2 also makes it possible to reduce the TiO_2 band gap and thus to activate TiO_2 under visible light. Solar irradiation can use instead of UV lights, which can make the process much more applicable and cost effective.

In addition to the intrinsic photocatalytic activity of TiO_2, which is directly related with its crystal properties, the structural properties of porous TiO_2 catalysts, such as their surface area, porosity, and pore size and distribution are also important because of their potential role in enhancing the light absorbance of TiO_2 catalysts and the accessibility of reactants to the active catalytic. One of the methods to fabricate highly porous materials with desired pore size and structure for target-specific applications is to use amiphilic organic molecules such as surfactants and block copolymers as pore directing agents when TiO_2 is form from its molecular precursor in a sol-gel process. In particular, we used a nitrogen-containing surfactant in the sol-gel method, which is expected to produce N-doped porous TiO_2.

Moreover, sol stability and film homogeneity are also important. Conventional approaches immobilize already-synthesized (or commercially-available). Binding of the TiO_2 precursor to the glass surface at high temperatures, uniform and ultrathin nature, and nanocrystalline TiO_2 are believed to significantly enhance the mechanical stability of the TiO_2 film. Transparency is also another important factor, which is related with light penetration through the TiO_2/glass composite and thus overall photocatalytic reactivity. The second motivation of us in this study was to develop an innovative integrated material processing method to synthesize nanocrystalline N-doped TiO_2 with a controlled

porous structure and to fabricate a robust, transparent, and ultrathin TiO_2 film that can decompose MC-LR (i.e., biological toxins) under solar radiation.

1.2. Background

Harmful algal blooms (HABs) have been reported in various types of freshwater and saltwater bodies worldwide (Chorus and Bartram 1999). In particular, HABs associated with cyanobacteria (i.e., blue green algae) have been of great interest due to their significant environmental and health impact. The formation of cyanobacterial HABs is manipulated by anthropogenic, environmental, and climatic factors. Nutrient enrichment of water bodies has been a primary factor in the proliferation of cyanobacterial HABs. cyanobacterial HABs also cause negative impacts to other organisms by creating hypoxic zones, losing habitat, imposing mechanical damage, synthesizing toxic metabolites, and especially producing cyanotoxins. The subsequent risk of cyanobacterial HAB formation to environmental and human health is an increasingly relevant and timely topic.

From a worldwide freshwater concern, five classes of cyanotoxins have remained primary focus; microcystin, nodularin, saxitoxin, cylindrospermopsin, and anatoxin-a. Among them, microcystins (MCs) are the most widespread and have been found in North and South Americas, Africa, Europe, Asia, Australia, and Antarctica. Several strains from the cyanobacteria genera such as *Microcystis*, *Anabaena*, *Oscillatoria*, *Anabaenopsis*, *Planktothrix*, and *Nostoc* have been reported to produce MCs (Chorus and Bartram 1999).

1.2.1. Monitoring approaches and observing programs

Strategies and targets for monitoring algal blooms are various Algae species (e.g., living organisms) or biological toxins (e.g., chemical compounds) are monitored to determine

detrimental impacts of algal blooms. Usually, algae and toxins themselves are directly quantified while measuring easy-to-detect surrogate parameters for them (so-called proxies) is an alternative to indirectly estimate algal blooms. On site, in situ, or remote observing approach can be selected for the monitoring, depending on the size of concerning areas, frequency of observing needs, and technical difficulty level of measuring the parameters. Some approaches deliver general characteristics of algal blooms while others exactly qualify and quantify species by species. Current monitoring strategies include: i) manual on site sampling followed by in lab analysis (current norm), ii) remote sensing based on satellite image analysis. Iii) In situ sensing of proxy parameters such as phycocyanin, chlorophyll, or biomass will discuss below.

1.2.1.1. Manual on site sampling followed by in lab analysis

Manual sampling followed by lab analysis is the simplest method to monitor HAB activity and toxin release. The first step is visiting a site and taking water samples. Samples should preserve in standard condition and analyze within 36 hours after collection. Previous history of HAB events and satellite images can also be used to guide sampling locations. Some areas such as shoreline and downstream of reservoirs or rivers are expected to exhibit higher toxin concentrations. Highest concentrations of cyanobacterial toxins are usually observed in scums (just below dead materials at the surface of a water body) and within dense cyanobacterial blooms. In United State, Harmful algal blooms usually start in Jun and ends in October (Codd et al. 2005b). Sampling protocols are varies based on type of blooms, Surface-scum forming cyanobacteria like *Anabena flosaqua* for instance have gas-filled cavities that allow them to float to the surface of water so surface sampling is recommended, but some cyanobacteria such as *Planktothrix agardihii* is more uniformly distributed within a water column and thus depth

sampling is required (Westrick et al. 2010). Next step is to analyze samples in the lab qualitatively as well as quantitatively. There are several screening methods to measure algal blooms. Algae are generally microscopic organisms. Identifying algae species can be done manually based on microscopic observation of their size and shape. Measuring biological toxins in water can be an alternative to measuring algae species in order to monitor HAB activity. Cyanobacteria generate and release many cyanotoxins in water, including microcytins, anatoxin-a, cylindrospermopsin, and saxitoxins (Hawkins et al. 2005). Screening methods for detecting those toxins are divided into two general categories: biological assays and chromatographic methods. As one of the most powerful biological assays, neurochemical and enzyme-linked immunosorbent assay (so-called ELISA) utilizes antigen-antibody interaction to detect MCs. Color changes initiated by the interaction are detected by a screening kit. This method has high detection limit of up to 0.2 μg/L but it has limitation in specificity (Jianwu et al. 2007). Anatoxin-a and microcystin variants are found intracellularly during around 95% of algal bloom period. However, some chemical species such as cylindrospermopsin are released to water by living cyanobacterial cells (Codd et al. 2005b). Typically, biological assays cannot measure these extracellular toxins. Since antibodies used in ELISA have cross-reactivity with other type of MCs, total concentrations of MCs are measured.

Such a screening test is positive, samples are sent to a laboratory for further analysis even to qualify and quantify specific toxin species by using more accurate chromatographic techniques such as high performance liquid chromatography (HPLC). More than 80 different microcystin species can be identified by HPLC (Pyo et al. 2005). Traditionally, MCs have been analyzed by HPLC with an ultraviolet detector. However, analytical methods are shifting towards HPLC with more sophisticated mass

spectrometry (MS/MS) with high accuracy and responsiveness in spite of its high cost (Tomoyasu and Keiji 1996).

As an alternative to the measurement of algae or toxins, the total toxicity of water samples can also be measured by mouse bioassay and protein phosphate inhibition assay (Jianwu et al. 2007; Hawkins et al. 2005).

Many local health and water authorities follow this simple monitoring approach to analyze water samples in their areas periodically and thus to release HAB information to the public. There are also federal level efforts. A HAB monitoring project by National Oceanic and Atmospheric Administration (NOAA) has regularly sampled Bear Lake, Muskegon Lake, Western Lake Erie, and Saginaw Bay around Great Lakes.

Table 1.1 shows the variation of microcystin concentration in water taken from one of Western Lake Erie sampling stations. In addition to MCs, other water quality parameters and cell counts are monitored weekly. Samples are taken at the surface because they best represent the portion of the water column that most likely comes into contact with many users. Total intracellular concentrations of MCs are quantified by ELISA technique. Considering most MCs are retained in the cells until cell death, the reported microcystin concentration is very close to total water microcystin concentration for new and peak blooms but it is not exactly the same. Before 2015, NOAA just measured particulate (intercellular) microcystin concentration using ELISA method but after that time, they also started measuring dissolved microcytin concentration using HPLC method. Since July 2015 samples are taken from surface scum (at top), surface (0.75 m deep) and bottom (1 m of bottom of the lake) instead of just surface of water as it was before. Chlorophyll a and phycocyanin concentrations are also measured since 2015 (NOAA, 2015).

Table 1.1 Microsystin concentration in Western Lake Erie (location: N 41º42.454, E 83º23.000) during August and September of 2013 (Data source: NOAA-Center of Excellence for Great Lakes and Human Health; NOAA, 2013)

Sampling date	Microcystin concentration (µg/L)
August 19, 2013	56.4
August 26, 2013	43.2
September 3, 2013	20.8
September 10, 2013	8.78

1.2.1.2. Remote sensing based on satellite image analysis

Monitoring relies on spectral images taken from satellites and aircrafts to provide the large spatial scale and high frequency of observations required to assess bloom locations and movements.

The demonstration of remote sensing of cyanobacterial blooms goes back four decades. Wrigley and Horne (1974) showed the potential of remote sensing with aerial infrared photography of a bloom of *Aphanizomenon flos-aquae* in Clear Lake, California.

Several factors have to be considered in using remote sensing to monitor cyanobacterial (or other harmful) algal blooms. The most obvious is the spatial resolution. Satellite data is obtained as “pixels” that cover an area on the ground. The pixel at the shoreline typically contains both land and water retrievals so that any water body must be more than three pixels wide to obtain even one pixel that might be useful. Ocean color sensors like Moderate Resolution Imaging Spectroradiometer (MODIS) have pixels that cover about 1 km and thus a water body must be at least 3 km wide to provide a useful pixel. Landsat with 30 m pixels can resolve much smaller water bodies.

Spectral bands at different wavelength irradiate to water surface and reflectance will detect using appropriate sensors. Interpretation of surface reflections by implying suitable algorithms helps to quantifying algal bloom intensity.

water bodies with little turbidity other than that caused by algae may be reliably monitored by relatively simple measures of water brightness (Kahru et al. 2007). This type of approach has been successfully applied to several routine monitoring programs of the Baltic Sea (Stumpf et al. 2010). However, more spectral bands are needed to identify pigments. Total pigment concentration may be estimated using two spectral bands. While this is more informative than water brightness, interpreting the data still requires caution. Ratios of visible bands (blue, green, and red) are effective in water containing only algae. These algorithms do not adequately discriminate between chlorophyll, dissolved pigments, and other pigmented compounds like iron.

Red (600−700 nm) and near-infrared (NIR) bands (700−800 nm) are far more effective for bloom discrimination in inland and coastal turbid waters. Chlorophyll-a absorbs strongly around 680 nm while other water pigments have slight and spectrally uniform absorption in the red and NIR. Algorithms based on band ratios of water reflectance between 680 nm and 709 nm have been demonstrated to estimate chlorophyll in eutrophic water (Gilerson et al. 2010) and cyanobacterial blooms (Simis et al. 2005)

Spectral curvature algorithms, which use three or more bands, can detect and quantify blooms and circumvent the problem of atmospheric correction. The spectral curvature, which is essentially the second derivative, is essentially insensitive to atmospheric correction. Two frequently used algorithms are the maximum chlorophyll index (MCI) (Gower et al. 2005) and the cyanobacterial index (CI) (Wynne et al. 2008). Both indices correspond to the amount of biomass (Wynne et al. 2010; Matthews et al. 2012; Binding

et al. 2013) and can be applied routinely for monitoring (e.g., Wynne et al. 2013). These algorithms have been reliably applied to data that has not been atmospherically corrected (Gower et al. 2005; Wynne et al. 2008).

$$CI = -R(681nm) + R(665nm) + \{R(709nm) - R(665nm)\}\frac{(681 - 665)}{(709 - 665)}$$

CI= Cyanobacteria Index (dimensionless number from 0 to 0.031)

R= reflectance at different wavelengths.

NOAA has monitored Lake Erie every summer for five years, creating bulletins and forecasts that are consistent from image to image (Wynne et al. 2013), as shown in Figure 1.1 as an example.

Although these algorithms are not sensitive to chlorophyll below about 10−15 μg/L (Matthews et al. 2012), their sensitivity is enough to detect concentrations of concern in most practical cases (WHO 2000). Between the two algorithms, the MCI shows sensitivity to sediment turbidity at the low end of its chlorophyll concentration (Binding et al. 2013) while the CI has not shown such sensitivity. With the MERIS 620 nm band, a curvature algorithm can also show the presence of phycocyanin (Matthews et al. 2012).

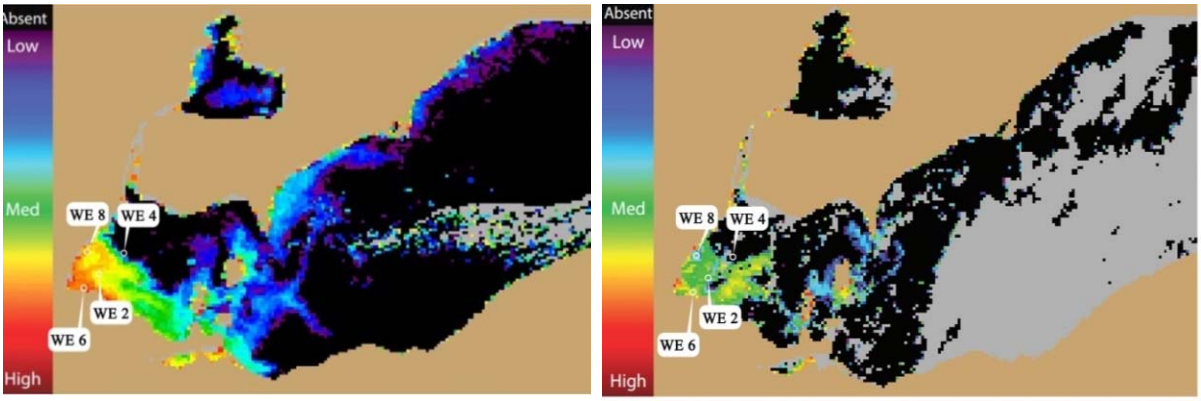


Figure 1.1 Remote sensing images of Western Lake Erie in September 4th 2013 (left) and October 16th 2013 (right). Cyanobacterial index based on image analysis are labeled with from warm colors (high potential of algal blooms) to cold colors (low potential of algal blooms) (Data source: NOAA-Center of Excellence for Great Lakes and Human Health; NOAA, 2013)

While MERIS failed in 2012, the European Space Agency is planning the launch of its replacement, the OLCI, on Sentinel-3 in 2015. The OLCI has the MERIS bands, assuring continuity of monitoring into the future. The existing 10-years of MERIS data (comprehensive at 1 km, and somewhat more irregular at 300 m before 2009) allows for evaluation of recent trends in blooms. For example, Stumpf et al. (2012) used the data set to determine the nutrient loading factors driving inter-annual variations in the Lake Erie cyanobacterial blooms. For higher resolution, Sentinel-2 will have not only Landsat bands, but will also have an additional NIR band that should improve separation of algal blooms from other pigments in water. Between these and future hyperspectral satellite sensors, comprehensive routine monitoring will be possible into the future.

1.2.1.3. In situ autonomous observing systems

Local governments and water quality managers are dependent on effective monitoring programs. However, most established programs are labor intensive and consequently have limited spatial-temporal coverage of their sampling (e.g. no more than weekly sampling at a few sites). These sampling limitations can significantly limit the timely detection of HABs and ultimately our understanding of the environmental factors that control HAB dynamics.

A number of autonomous buoy platforms are now available that particularly address the temporal measurement needs. One of the most famous systems belong to Land Ocean Biogeochemical Observatory (LOBO) that provide integrated and automated water quality measurements with real-time telemetry of results. The LOBO system is composed of a floating platform and instrument frame, power and wireless telemetry system, integrated sensor suite, automated processing, and web-based data visualization software. Autonomous buoy measurements can be continuously conducted on short time scales (e.g. every 30 min to 1 hour) over long deployment times (several months) to provide excellent temporal sampling of HABs and associated critical ecological parameters.

Since the buoy systems described above are generally deployed as surface floats and make their measurements in the upper meter of the water column, this deployment scheme may not be adequate in deeper and continuously stratified ecosystems where HABs can occur with little or no surface manifestation (Twardowski et al. 2005; McManus et al. 2008; Sullivan et al. 2010).

Photo-spectrometric detection part has number of compact, energy efficient sensors that can measure phycocyanin. Phycocyanin is a pigment-protein complex from the light-

harvesting phycobiliprotein family, along with allophycocyanin and phycoerythrin. It is an accessory pigment to chlorophyll.

Although these instruments do not directly quantify HAB species or abundance, their measurements can use in developing optical proxies for HAB detection and monitoring.

LOBO data from a recent deployment in Western Lake Erie demonstrates the power of these high temporal scale measurements when compared to typical weekly monitoring programs.

1.2.2. Microcystins

Microcystins (MCs) are water soluble and stable molecules, which allow them to persist in the environment even after a bloom dissipates (Chorus and Bartram 1999). Synthesis of MCs is influenced by environmental conditions and genetic composition. The gene cluster, mcyA-J, was identified as the origin of biosynthesis (Kaebernick and Neilan 2001). The non-ribosomal assemblage of MCs is accomplished using a multienzyme complex including peptide synthetase and polyketide synthase (Dittmann and Wiegand 2006; Kaebernick and Neilan 2001). MCs are cyclic hepatotoxins with the principal amino acid sequence, cyclo-(D-Ala^{1}-L-X^{2}-D-MeAsp^{3}-L-Z^{4}-Adda^{5}-D-Glu^{6}-Mdha^{7}). The D-MeAsp is D-erythro-b-methylaspartic acid and the Mdha is N-methyldehydroalanine. The X and Z represent a variation of L-amino acids. For example, one of the commonly reported MCs is microcystin-LR, where X is leucine (L) and Z is arginine I (Figure 1.2).

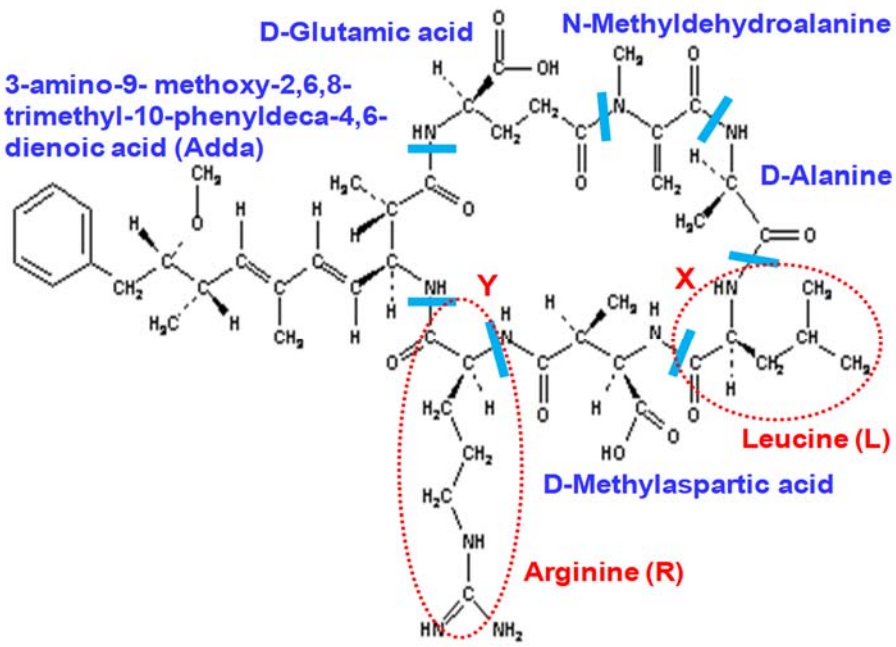


Figure 1.2 Molecular structure of microcystin-LR (Kondo, et al., 1992)

The conversed Adda (2S, 3S, 8S, 9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) is the primary region that causes inhibition of protein phosphatase 1 and 2A. This inhibition results in severe tissue and organ damage, especially liver damage where the toxin is concentrated (Chorus and Bartram 1999). MCs have been reported to promote liver tumors and have genotoxic potentials.

MCs have been found to be the causative agent in numerous wildlife and domestic animal and human poisonings (Codd et al. 2005a; Drobac et al. 2013; Stewart et al. 2008). The primary human exposure routes to MCs are drinking water, fish consumption, and recreational waters. Although it would be rare to be exposed to a natural toxin like MCs intravenously, such an exposure through hemodialysis was reported in Brazil (Jochimsen et al. 1998). Symptoms included vomiting, visual disturbance, gastroenteritis

liver damage, tinnitus, and nausea. A 1996 incident in Caruaru in north-east Brazil caused the death of 60 patients (Pouria et al. 1998). A study by Chen (2009) analyzed serum samples from Lake Chaohu fishermen. These fishermen's primary water and food supplies were derived from Lake Chaohu that has seasonal cyanobacterial blooms. Their statistical analysis supported a positive relationship between serum MCs and liver function enzymes (alanine aminotransferase and aspartate aminotransferase). These data suggest a risk of health from chronic exposure to MCs. Potentially, the 1999 World Health Organization's recommended no observable adverse health effect level of MC-LR at 40 μg/kg/d and drinking water equivalent concentration of MC-LR at 1 μg/L may need further investigation (Chorus and Bartram 1999).

1.2.3. TiO_2 photocatalysis

Progress in chemical oxidation results in advanced oxidation technologies (AOTs), one of the most powerful water treatment processes. AOTs are based on generation of hydroxyl radicals with high oxidation potentials. Particularly, TiO_2 photocatalysis has been highlighted as one of the most promising and green AOTs due to its effectiveness to decompose wide variety of pollutant.

TiO_2 is a semiconductor that has a gap between its valence and conduction band. When TiO_2 catalysis face to external source of energy (usually by UV radiation with above the band gap energy of TiO_2, 3.2 Ev), an electron would excite and move from valence band to conduction band which result in generation of whole in valence band (h^+) and unpaired electron in conduction band (e^-). The electron would react with O_2 in redox reaction and produce superoxide radical anion ($O_2^{-\bullet}$) and hole would interact with Hydroxyl ion in water and produce Hydroxyl radical. ($OH^\bullet$) Hydroxyl radicals non-selectively and readily attack and decompose organic contaminants and convert them to

carbon dioxide, water and other inorganic species. Figure 1.3 shows generation of Hydroxyl radical on surface of TiO_2 under UV radiation:

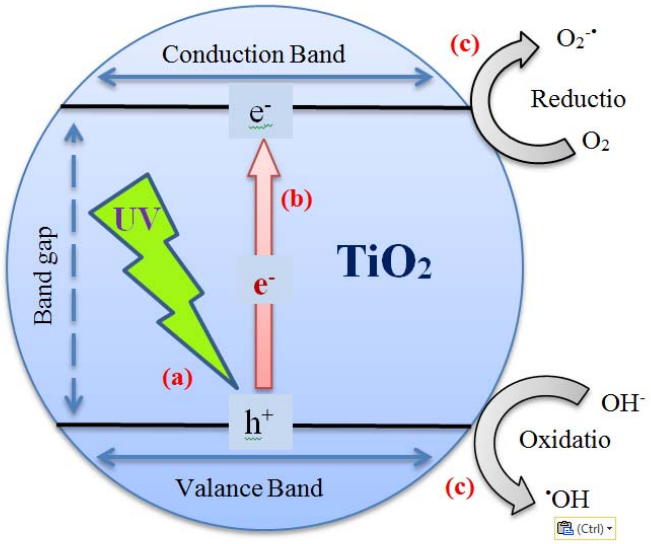


Figure 1.3 Generation of Hydroxyl radicals at TiO_2 surface on UV radiation

Most of lab-scale photocatalytic reactors are in slurry mode that TiO_2 particles are suspended in reactor. These systems benefit more catalytic active surface for reaction and less mass transfer limitation but they have an important drawback as well. TiO_2 particles have to separate and remove from the reactor after treatment process and this separation process could be time consuming and cost a lot. Another type of photocatalytic reactors are fixed bed reactors, which TiO_2 particles would immobilize on surface of a supporting substance like glass and make thin films that can use several times in continuous form without need have separation process at the end of treatment reaction.

1.2.4. Nitrogen doping of TiO_2

As mentioned before, photocatalytic reactions need external source of irradiation with high photon energy more than high band gap between valence and conduction band which is about 3.2 Ev in case of conventional TiO_2. Ultraviolet light irradiation usually uses to provide this energy due to it is energy content and wavelength. UV light irradiation increase the cost of treatment process and make the real world application of photocatalytic reaction difficult. Use of solar irradiation has been founded a suitable and sustainable substitute for UV light. The problem is only 5% of solar radiation is in UV range. To utilize visible light in solar radiation for TiO_2 activation, dye-sensitized or metal ion-doped TiO_2 has been developed and the TiO_2 has shown promising results for the degradation of chlorinated compounds and nitrogen oxides (Bae and Choi, 2003). Introduction of anionic dopants, especially nitrogen, to TiO_2 also makes it possible to reduce the TiO_2 band gap and thus to activate TiO_2 under visible light. Synthesize process of nitrogen doped TiO_2 usually has two steps. First, synthesize TiO_2 and then dope of nitrogen from nitrogen containing materials such as ethylamine, urea or nitrogen gas at high temperature.

1.2.5. Sol-gel Method

Sol-gel method is one of the most applicable methods to form solid inorganic materials from liquid molecular precursor. The first step is sol preparation. During hydrolysis and condensation reactions of alkoxide inorganic precursors. Polymeric or particulate sol containing inorganic materials will produce. The substrate should deposit into sol solution and solvent and other volatile component should evaporate for solidification of the gel. Drying process would occur at low temperature to form more condense network. The last

step is heat treatment at high temperature to remove organic substances and crystallization of solid materials.

Modified sol-gel method using pore templating agent is one the newest methods to make porous TiO_2 structure. Surfactant molecules are self-organized in water so by adding Titanium alkoxide precursor to surfactant solution it will hydrolyze and condense to form TiO_2 inorganic network around the self-assembled surfactant molecules. After removal of organic template by thermal treatment, porous inorganic TiO_2 network will form.

Using nitrogen contain surfactant as pore templating agents has two benefits. It can produce pore structure, which increase active surface area as well as selectivity and reduce the band gap of TiO_2 so that solar radiation can use as source of energy instead of UV light.

As liquid molecular precursor of titanium (TTIP) adds to surfactant solution, TTIP is hydrolyzing and accumulating around the self-assembled surfactants to form a surfactant organic core/TiO_2 inorganic shell composite. During calcination process the surfactant template will remove and leaving porous structure. Moreover, Nitrogen atoms in surfactant will defused and incorporate into the crustal TiO_2 in a form of Ti-N-Ti or Ti-O-N-Ti (Choi, et al., 2007). Figure 1.4 illustrates the schematic of reaction.

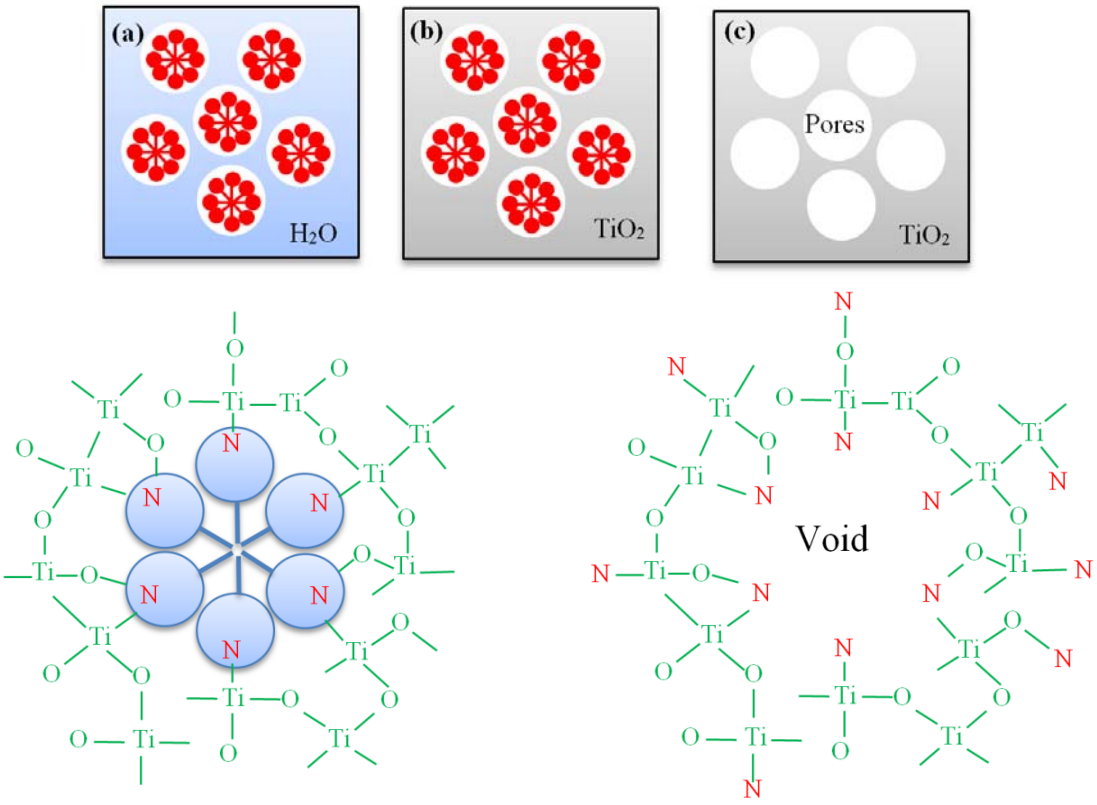
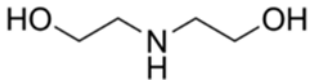
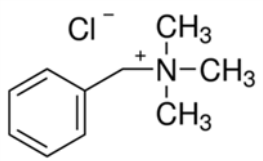
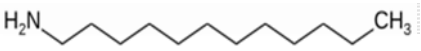
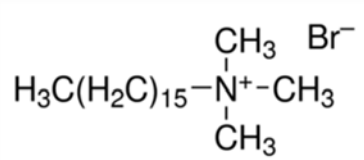
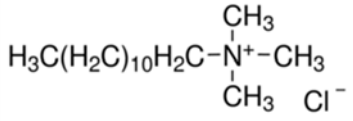


Figure 1.4 Incorporation of TiO_2 Network onto self-organized surfactant micelles followed by the removal of the organic templates to form N-TiO_2 with mesoporous structure (Choi, et al., 2007)

Different nitrogen containing surfactants selected for which are relatively biodegradable, inexpensive and non-toxic compare to other commonly used ionic tinplating agents. Molecular structure as well as physical and chemical properties of all surfactant summarized in Table 1.2

Table 1.2 physical and chemical properties of different surfactant (The source of the chemical structures is Sigma-Aldrich Co.)

Surfactant	**Molar mass** ($g \cdot mol^{-1}$)	**Toxicity** (LD_{50} Rat, oral mg/kg)	**Chemical formula**	**Molecular structure**
Diethanolamine (DEA)	105.14	710	$C_4H_{11}NO_2$	
Benzyltrimethylammonium chloride (BTAC)	185.69	43	$C_6H_5CH_2N(Cl)(CH_3)_3$	
Dodecylamin (DDAD)	185.35	1020	$CH_3(CH_2)_{11}NH_2$	
Hexadecyltrimethylammonium bromide (CTAB)	364.45	410	$CH_3(CH_2)_{15}N(Br)(CH_3)_3$	
Dodecyltrimethylammonium chloride (DDAC)	263.89	5045	$CH_3(CH_2)_{11}N(CH_3)_3Cl$	

1.3. Objectives and Challenges

1.3.1. Comparison of various observation systems for monitoring harmful algal blooms

The first objective of this study was to predict MC-LR formation during harmful algal blooms utilizing easy-to-detect indirect parameters. The first challenge to achieve this objective was to find a location that has two characteristics; 1) the frequency of Harmful Algal bloom should be high in the location and 2) accessibility to different observing system data at that specific location. Western part of Lake Erie was selected as it has both conditions (Figure 1.5). Maumee Bay originated from Maumee River flowing through Northern Ohio followed by the City of Toledo was a particular focus.

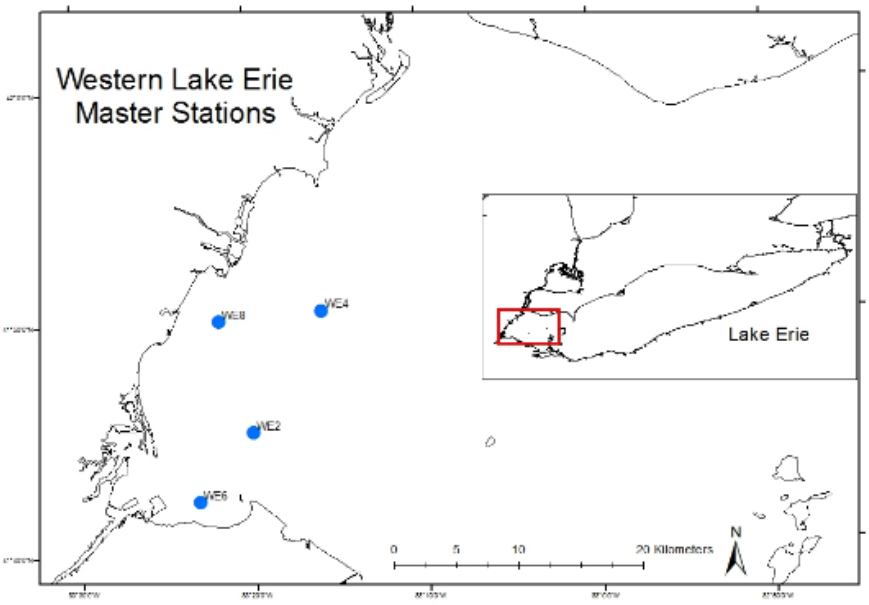


Figure 1.5 western part of Lake Erie that selected to study different observing system for monitoring harmful algal blooms (Data source: NOAA-Center of Excellence for Great Lakes and Human Health; NOAA, 2013)

After selecting a case study location, we had to collect data from different sources. As mentioned before, there are three different observing methods for monitoring HABs. i) manual field sampling followed by in lab analysis to directly measure MCs (i.e., biological toxins), ii) remote sensing based on satellite image analysis to estimate cyanobacterial index, CI (i.e., algal blooms), and iii) in situ sensing of proxy parameters to algal blooms such as phycocyanin (an accessory pigment to chlorophyll associated with HABs). Microcystin concentration has measured by cooperation with The Center of Excellence for Great Lakes and Human Health at NOAA. They are monitoring the distribution of MCs in many areas around Western Lake Erie frequently from 2009.

Satellite images from the moderate resolution imaging spectroradiometer (MODIS) were processed to propose cyanobacterial index (CI). MODIS is a key instrument equipped in Terra (EOS AM) and Aqua (EOS PM) satellites. Terra MODIS and Aqua MODIS have viewed the entire Earth's surface every 1 to 2 days, acquiring data in 36 spectral bands or groups of wavelengths (NASA 2014). Spectral curvature algorithm called CI should used to quantify blooms and circumvent the problem of atmospheric correction (Stumpf, et al., 2012). CI corresponds to the amount of biomass. Data collected by cooperation with The National Centers for Coastal Ocean Science at NOAA. Lake Erie Land and Ocean Biochemical Observatory (LOBO) has an autonomous observing buoy for monitoring and collecting water quality and environmental data, which are used in statistical ecological niche models to develop predictive capabilities for harmful HABs. Their monitoring device is located next to the Toledo Harbor Light on Maumee Bay (N 41º 49.533 and W 83º 11.617) and can measure phycocyanin concentration every hour. Data can collect by cooperation with them

After collecting data from different sources at same time and same location, the last challenge was to find the correlation between them using regression analysis methods.

Since MCs, CI, and phycocyanin have not any functional dependency, correlation rather than simple linear regression can apply to investigate the linear relationship between parameters (Puth, 2014). In addition, the monitoring parameters were measured with different time schedules. As a result, the Pearson Product-Moment (PPM) correlation equation selected to use (Harring and Wasko, 2011).

1.3.2. Photocatalytic decomposition of microsystin-LR using nanostructure porous nitrogen-doped TiO_2 thin film under solar irradiation

Second objective of this study was to pioneer a high efficiency visible light-activated TiO_2 photocatalytic process to decompose biological toxins.

The first challenge was to synthesize N-doped TiO_2 thin films using sol-gel methods modified with surfactant. Transparent films are favorable as light radiation can easily penetrate through films if we have to put numbers of film together. Therefore, we had to find out a suitable surfactant that we could prepare transparent films using them. The surfactant has to have low toxicity and price as well.

After preparation of different thin films, In order to check whether the film works under visible light, first we tested decomposition of methylene blue (MB) as a model organic dye. After that, we switched target chemical to decompose from MB to MC-LR.

The next challenge was to study the effect of operation parameters such as calcination temperature, pH and number of coated layers. The final step was to evaluate the potential real field application of our system so we tested our TiO_2 thin films using lake water under solar radiation.

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Chapter 2

Comparison of various observation systems for monitoring harmful algal blooms: a case study on western Lake Erie

Occurrence of Harmful Algal Blooms (HABs) worldwide has caused concern to environmental and health authorities because of their potential to generate and release toxic biological metabolites. In particular, microcystins (MCs) produced from cyanobacteria are of great concern. There have been significant efforts to monitor harmful bloom events and cyanotoxin levels, including: i) manual field sampling followed by lab analysis to directly measure MCs, ii) remote sensing based on satellite image analysis to estimate cyanobacterial index (CI), and iii) in-situ sensing of proxy parameters to cyanobacterial blooms such as phycocyanin. This present study compared the observation systems highlighting western Lake Erie to find any correlation of the monitoring parameters based on the Pearson Product-Moment equation. A close relation between measured MC concentration and estimated CI was revealed. Phycocyanin concentration was highly correlated with CI while it was weakly correlated with MCs. Relationship between MCs and CI was found to be site-specific. Discussion on geographical, ecological, meteorological, and analytical factors specific to the locations was made to explain the observed correlations and variations. The results imply that combining the data by integrating the current HAB observation systems, which monitor different parameters independently, would be helpful to reliably monitor algal bloom activities.

2.1. Introduction

Several environmental factors, such as nutrients, light, wind, temperature, pH, and hydrology, develop frequent massive and prolonged blooms of cyanobacteria and algae, forming harmful algal blooms (HABs). Cyanobacteria (blue-green algae) are of particular concern in freshwater bodies. More than 50 species of cyanobacteria are known to produce cyanotoxins such as microcystins (MCs), anatoxin-a, and cylindrospermopsins

(Codd *et al.* 2005). In particular, MCs are among the most powerful natural poisons and up to 50% of the recorded blooms can be expected to contain MCs (Carmichael 1992). Cyanobacteria and their toxins are currently in the U.S. Environmental Protection Agency's Drinking Water Contaminant Candidate List (EPA 2016a).

Direct economic impacts of coastal harmful blooms events in U.S. have increased significantly each year (Hoagland & Scatasta 2006). The majority of the impacts are associated with public health and commercial fishery sectors. In 2014, HABs and MCs outbreak in drinking water resources in the city of Toledo, OH triggered close attention of general public all around the nation (Elizabeth 2014). As a result, monitoring and publicizing HAB activity can provide a major mechanism for reducing or preventing exposures to toxins during HABs (Seltenrich 2014).

Cyanobacteria and toxins can be directly identified while measuring easy-to-detect surrogate parameters to them (i.e., proxy) can be an alternative to the direct measurement in order to simplify the monitoring process (Menetrez 2012). On-site, in-situ, or remote observing approach is selected for the monitoring (Seltenrich 2014). Some approaches indicate general idea on algal bloom activity while others exactly qualify and quantify algae and toxins in water to determine the actual risk and toxicity level of HABs. There have been huge efforts to monitor HAB activities and toxin releases in U.S. particularly by National Oceanic and Atmospheric Administration (NOAA) (Hudnell 2010). Table 2.1 summarizes current observing systems for monitoring HABs and their characteristics.

Table 2.1 Comparison of current observation systems for monitoring harmful algal blooms

	Measuring target	Measurement directness	Data scale [a]	Final Information
On site sampling followed by lab analysis	Biological toxins (microcystins) and algal species	Direct	Microscale	Exact algae community and toxin release
Remote sensing based on satellite image analysis	Cyanobacterial index (color image)	Indirect	Macroscale	General algal blooms
In situ sensing for monitoring a proxy parameter	Phycocyanin, an accessory pigment to chlorophyll	Indirect	Macroscale	General algal blooms

[a] Microscale data: detailed information for a defined area and macroscale data: general information for a vast area.

First, on-site manual sampling followed by lab analysis is commonly used to identify cyanobacterial species and to assay biological toxins species by species (Rivasseau *et al.* 1999). In spite of its high accuracy and reliability, this approach is neither sustainable nor practical to meet the vast spatial and temporal measuring need. Second, monitoring relies on spectral images taken from satellites and aircrafts to provide the large spatial scale and high frequency of observations required to assess bloom locations and movements. The remote sensing approach is useful for monitoring general cyanobacterial bloom activities by providing cyanobacterial index (CI) denoted with imaginary color (Stumpf *et al*. 2012). Third, in-situ sensing is a recent monitoring approach (Twardowski *et al.* 2005). For example, in-situ autonomous observing approach optically senses phycocyanin as an accessory pigment to chlorophyll often associated with HABs (i.e., as a surrogate chemical or proxy to HABs) (Marion *et al.* 2012). The remote and in-situ (real-time) monitoring approaches benefit immediate decision-making and timely response. However, such a color product is not specific to HABs. High level of

chlorophyll may be or may not be associated with toxic blooms (Graham *et al.* 2010). Even not all cyanobacterial blooms are associated with release of biological toxins (Anderson 2009).

In spite of its significance, no one has attempted to collect and compare such HAB observing parameters to see any correlation among the parameters and thus to better assess, interpret, and even forecast HABs. As a result, the objective of this present study is to compare and correlate the current observation systems and thus to evaluate the effectiveness of each system to monitor and assess HAB events. The observation systems monitor different HAB parameters such as biological toxins (e.g., MCs), general cyanobacterial blooms (e.g., CI), and proxy targets (e.g., phycocyanin). It is hypothesized that they exhibit certain correlation because they are inherently designed to represent the same phenomenon, algal blooms. HAB-associated data, meteorological conditions, and geographical information were collected for western Lake Erie in 2013, only in which all of the three monitoring parameters were available. Correlation of the HAB data was interpreted to prove the hypothesis above and to better understand relation between HAB outbreak and toxin release. This is the first study to compare and correlate the different observation parameters that exclusively target at monitoring and interpreting the same phenomenon, HABs.

2.2. Methodology

2.2.1. Selection of a HAB study site

Western Lake Erie was selected as a HAB study site due to frequent observation of algal blooms and toxin releases (Wynne *et al.* 2010). Maumee Bay originated from Maumee River flowing through Northern Ohio followed by the city of Toledo was focused (Elizabeth 2014). The site was labeled as WE2, WE4, WE6, and WE8. Figure 2.1 shows

western Lake Erie and the location of the four study sites. There have been significant monitoring activities to obtain HAB-associated data around the locations (Wynne *et al.* 2010).

Figure 2.1 Study locations in western Lake Erie: WE2 (N41º45.825; W83º19.701), WE4 (N41º49.663; W83º11.649), WE6 (N41º 42.454; W83º23.000), and WE8 (N41º49.998; W83º21.895). Inset shows the whole Lake Erie and the rectangle in the inset shows the western Lake Erie (adapted and modified from Google map at https://www.google.com/maps).

2.2.2 On-site monitoring of MC concentration

NOAA Great Lakes Environmental Research Laboratory in collaboration with the Cooperative Institute for Limnology and Ecosystems Research at the University of Michigan has operated a sampling program for Lake Erie and publicized the distribution of MCs in many locations around the western Lake Erie (NOAA 2016a). Water samples were collected from the four different locations denoted as WE2, WE4, WE6, and WE8 during typically May-October when algal bloom was high. The samples were taken at the surface, which is believed to be most representative of the portion of water column that

recreational users contact. The surface portion also corresponds to the focus of satellite images. As one of the most powerful biological assays, neurochemical and enzyme-linked immunosorbent assay (ELISA) was introduced to quantify the intracellular concentration of MCs in the water samples (Rivasseau *et al.* 1999).

2.2.3 Satellite-based remote sensing for CI

NOAA National Centers for Coastal Ocean Science and Great Lakes Environmental Research Laboratory have analyzed satellite images around Great Lakes and published the Lake Erie Harmful Algal Bloom Bulletins (NOAA 2016b). A spectral curvature algorithm called CI was used to quantify blooms and circumvent the problem of atmospheric correction (Stumpf *et al.* 2012). CI indirectly corresponds to the amount of biomass. The estimated threshold for cyanobacteria detection is at 35,000 cells/Ml. The satellite images published in the Lake Erie Harmful Algal Bloom Bulletins were analyzed to extract CI. A number between 1 and 250 was assigned to each pixel of a satellite image based on its color. CI was calculated based on Eq. 1, where DN is pixel number based on color from 1 (coolest color) to 250 (warmest color) and CI ranges from 0.0001 to 0.031.

$$CI = 10^{\frac{DN}{100}-4} \qquad (1)$$

2.2.4 In-situ sensing of phycocyanin

Erie Land and Ocean Biochemical Observatory (LOBO) has monitored and publicized phycocyanin concentration in western Lake Erie as a surrogate parameter to HABs (SEA-BIRD COASTAL 2016). The Erie LOBO is an autonomous observing buoy for monitoring and collecting water quality and environmental data, which are used in

statistical ecological niche models to develop predictive capabilities for HABs. The Erie LOBO location was next to the Toledo Harbor Light on Maumee Bay (N41º49.533; W83º11.617), which is very close to WE4. The LOBO was equipped with phycocyanin fluorescence that was calibrated based on regular field sampling.

2.2.5 Correlation of HAB parameters

The observing systems monitored different HAB parameters. Since these parameters do not have any functional dependency, correlation rather than simple linear regression was applied to investigate the linear relationship between parameters. Both regression and correlation coefficient approaches offer similar behavior in terms of testing the null hypothesis of no association. However, the computation of a correlation coefficient is useful to measure the relationship, or association, between variables whether or not a regression is appropriate (Path *et al*. 2014). In addition, the monitoring parameters were measured at different time schedules. As a result, the Pearson Product-Moment (PPM) correlation equation was used (Eq. 2), where X and Y are all independent variables and r is the PPM correlation coefficient ranging $-1 \leq r \leq +1$ (Harring & Wasko 2011). The Pearson correlation coefficient of two variables *X* and *Y* is formally defined as the covariance of the two variables divided by the product of their standard deviations (which acts as a normalization factor). The equation is widely used as a measure of the degree of linear dependence between two independent variables. Relationship of two parameters was quantified with the correlation coefficient. If r is greater than zero, the two parameters show positive relationship. Very strong, strong, moderate, weak, and negligible (or no) relationships are indicated by r values, 1.0−0.7, 0.7−0.4, 0.4−0.3, 0.3−0.2, and 0.2−0.01, respectively. Correlation refers to quantitative relationship between two variables that are measured on either ordinal or continuous scales.

Correlation implies an association between two variables rather than causation (Wernet *et al.* 2008).

$$r = \frac{n(\sum xy)(\sum x)(\sum y)}{\sqrt{[n(\sum x^2)-(\sum x)^2][n(\sum y^2)-(\sum y)^2]}} \qquad (2)$$

2.3 Results and discussion

Concentrations of MCs have been directly measured by weekly water sampling at WE2, WE4, WE6, and WE8 locations during May-October since 2009 (NOAA 2016a). A time series of CI-embedded satellite images for western Lake Erie have been developed weekly since 2009 (NOAA 2016b) and CI values for WE2, WE4, WE6, and WE8 locations were extracted from the images. Concentrations of phycocyanin have been detected every hour at the Toledo Harbor Light since 2013, which is close to WE4 location (< 0.2 mile) (SEA-BIRD COASTAL 2016). Considering availability of the spatial (measuring location should be close enough) and temporal (measuring time should be close enough) monitoring data, comparison between MCs and CI was valid for WE2, WE4, WE6, and WE8 in 2013 while comparison between phycocyanin and CI and comparison between phycocyanin and MCs were valid only for WE4 in 2013.

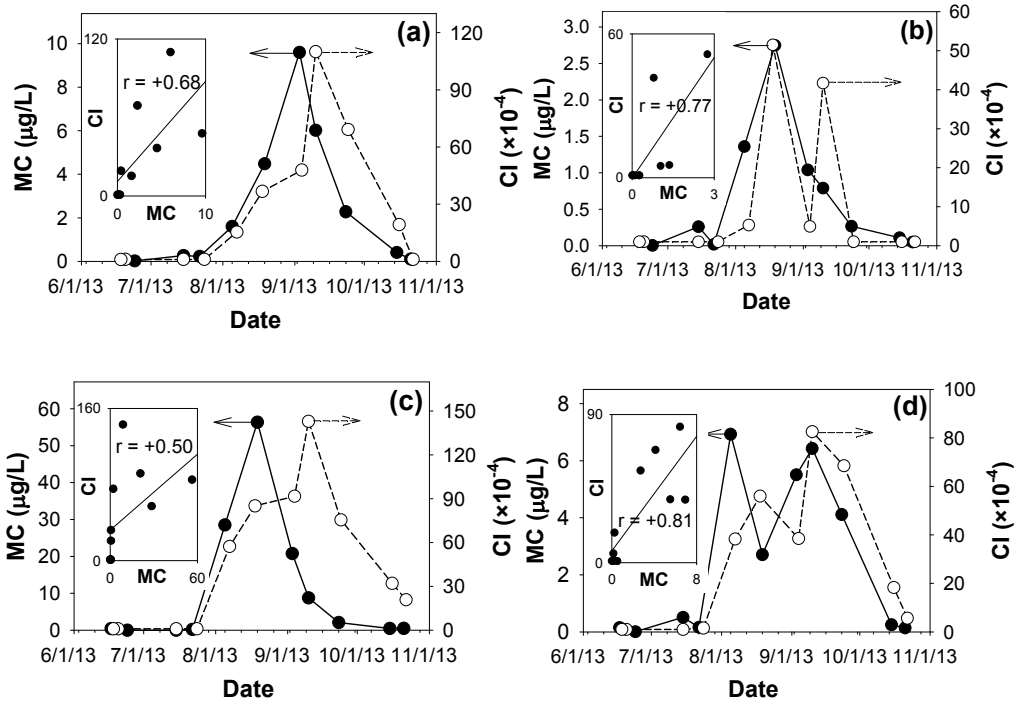

Figure 2.2 Comparison of microcystin concentration (biological toxin) and cyanobacterial index (cyanobacterial bloom) in 2013 for (a) WE2, (b) WE4, (c) WE6, and (d) WE8 in western Lake Erie. It should be noted that different X and Y axis scales were used for each location to align the maximum concentrations of the two parameters to the same level so that correlation of the two parameters can be easily visualized in each location. Inset shows correlation between MC and CI paired and measured within 24-hour time difference. The correlation coefficient I of the Pearson Product-Moment equation has shown (data source: NOAA 2016a and 2016b).

2.3.1 Correlation of MCs with CI

MCs (biological toxins) were compared with CI (algal blooms) for WE2, WE4, WE6, and WE8 based on data collected in 2013, as shown in Figure 2. It should be noted that the two parameters were not measured simultaneously because the two observing systems

were operated independently. Observing dates showing high algal bloom tendency labeled with CI also showed high MC concentrations in water. For some dates and locations, MC concentrations were very low or negligible in spite of high CI (e.g., WE4 and WE6 in middle September). Based on the observation of MCs and CI for the locations, in general, the production of biological toxins was found to be highly associated with HAB activity. WE8 seems to have the highest correlation of MCs and CI. In order to investigate the degree of correlation for a set of two parameters, variables measured within 48-hour difference were paired and plotted, and then the PPM equation was applied to calculate a PPM correlation coefficient I, as shown in the inset of Figure 2. Although there were some outliers, in general MCs were linearly correlated with CI (i.e., r is greater than 0). WE2, WE4, and WE8 locations showed very strong correlation at r of +0.68, +0.77, and +0.81, respectively, while WE6 showed strong correlation at +0.50. Relationship between MCs and CI was also found to be site-specific. For example, MC level for WE4 changed within a very narrow range of only 0.0−2.8 μg/L while its CI changed greatly from 0 to 50×10^{-4}. Meanwhile, MC level for WE6 changed within a wide range of 0.0−57 μg/L while its CI changed from 0 to 142×10^{-4}. This means MC concentration of WE4 is very low compared to that of WE6, with given CI's expressing cyanobacterial bloom tendency.

Many geographical, ecological, meteorological, and analytical factors specific to the locations might have been involved in the observed variations. In cloudy weather, satellites cannot properly capture high resolution images for the areas of concern, which impacts calculation of CI. The accuracy of MCs measurement also significantly decreases at low concentrations due to the nature of the ELISA method (Jianwu *et al.* 2007). The average water temperature increased up to 2.5 Cº between the middle August and the early September. Since higher temperature is favorable for the growth of

cyanobacteria, both CI and MCs were high at that period. As cyanobacteria concentrate on water surface, blue green scum generates and can be clearly identified on satellite images.

Looking at MCs and CI carefully for all the locations (particularly WE2 and WE6), CI peaks slightly followed MC peaks in 1-2 weeks. This is particularly true to the end of algal bloom season. During new and peak bloom periods, the intercellular MCs measured by the ELISA is very close to total MCs in cells and water because most of MCs are retained in the cells until cell death (i.e., negligible MCs in water) (Otten *et al.* 2012). However, ageing cells during a dying bloom release MCs into water, which are not counted by the MC measurement, while satellite images still keep capturing algal blooms. This resulted in low MCs but still high CI in each observing time during September.

In fact, the situation is more complicated when vertical movement of cyanobacteria over time is considered. Some cyanobacteria, such as *Anabaena flos-aquae*, have gas-filled cavities that allow them to float and rise from bottom level to water surface. Some cyanobacteria, such as *Planktothrix agardhi*, can be found in bottom sediment and may float to surface when mobilized by severe storm events and other sediment disturbance (Falconer & Humpage 2006). Such a cyanobacterial movement also depends on light condition, nutrient level, water temperature, and wind speed, and it typically takes several days. For example, unusually significant decrease in air temperature (from 24.5 to 23.7 ºC) and rapid increase in wind speed (from 2.6 to 10.3 m/sec which is above 7.7 m/sec, a threshold wind speed strong enough to mix blooms through water column) were reported for WE4 location in September 4, 2013(NOAA 2016a).

2.3.2 Correlation of phycocyanin with CI and MCs

Phycocyanin (as a proxy to cyanobacterial blooms) was compared with CI (algal blooms) and MCs (biological toxins) monitored around WE4 location, as shown in Figures 2.3 and

4, respectively. Peak points for phycocyanin in accordance with CI and MCs occurred in the middle August and the early September, most probably due to the rapid increase in water temperature (from 22.5 to 25.5 ºC) which is favorable for algal blooms. Dates showing high CI and MCs generally exhibited high phycocyanin concentration, implying the production of phycocyanin is highly associated with HAB activity.

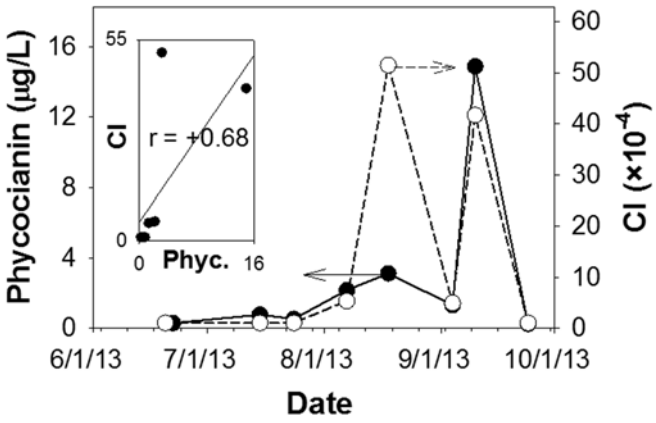

Figure 2.3 Comparison of phycocyanin concentration (proxy to cyanobacterial bloom) with cyanobacterial index (CI) in western Lake Erie in 2013. Phycocyanin has been monitored only for the location (N41°49.533 and W83°11.617) very close to WE4 since 2013. Inset shows correlation between two comparing parameters paired and measured almost at the same time. The correlation coefficient I of the Pearson Product-Moment equation is shown (data source: NOAA 2016b and SEA-BIRD COASTAL 2016).

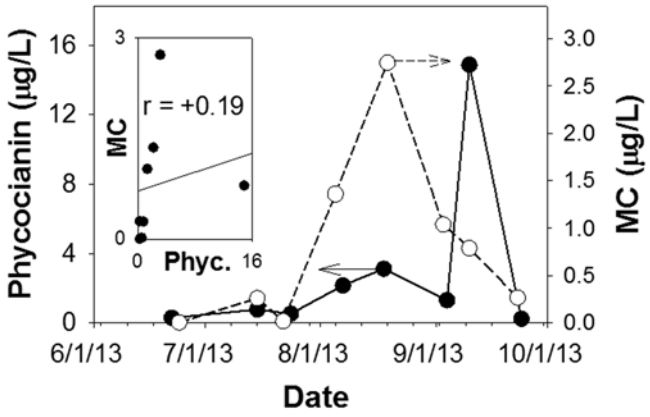

Figure 2.4 Comparison of phycocyanin concentration (proxy to cyanobacterial bloom) with microcystins concentration (MCs, biological toxins) in western Lake Erie in 2013. Phycocyanin has been monitored only for the location (N41°49.533 and W83°11.617) very close to WE4 since 2013. Inset shows correlation between two comparing parameters paired and measured almost at the same time. The correlation coefficient I of the Pearson Product-Moment equation is shown (data source: NOAA 2016a and SEA-BIRD COASTAL 2016).

Correlation of phycocyanin with CI and MCs is shown in the inset. Unlike paired data of MCs and CI measured within 48-hour difference, a pair of phycocyanin and CI (or MCs) was measured almost simultaneously. Correlation coefficient of phycocyanin with CI was at r = +0.68, indicative of strong relationship. In fact, phycocyanin is an accessory pigment to chlorophyll generally associated with cyanobacterial blooms. Meanwhile, correlation coefficient of phycocyanin with MCs was at only +0.19, indicative of weak relationship (but still positive relation). It is known that not all of cyanobacterial blooms produce MCs (Anderson 2009). Up to 50% of recorded blooms are expected to contain such toxins (Carmichael 1992).

2.4. Conclusions

It was proven that the HAB observing parameters (MCs, CI, and phycocyanin) are generally well correlated because they inherently represent the same phenomenon, HABs. In particular, measured biological toxin concentration (MCs) was strongly aligned with algal bloom activity (CI) estimated by satellite image analysis. Relationship between MCs and CI seemed to be site-specific. Phycocyanin had strong correlation with CI, implying that measuring an easy-to-detect proxy parameter in-situ and in real-time is effective for monitoring algal blooms. Combining data by integrating the current HAB monitoring systems and observing programs would be helpful to reliably assess HAB activities with high accuracy. This study comparing only three major HAB parameters can be extended to include many other observing targets associated with HABs, including chlorophyll and phycoerythrin. More observing locations and longer monitoring periods, once established in future, enable to propose more comprehensive correlation of the current monitoring systems and to understand the behavior and functioning of HABs. When such a site-specific correlation is found through this kind of study, one will be able to better forecast biological toxin release from other HABs-associated data such as CI and to better understand relationship between HAB activity and toxin release. As a result, this study can significantly contribute to the areas of water supply, water quality, and algal bloom monitoring.

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Chapter 3

Solar-driven photocatalytic decomposition of microcystin-LR using N-TiO_2 film: from lab development to on-site demonstration

Harmful algal blooms (HABs) found in various water bodies worldwide have been a huge concern due to their adverse impacts on human health and ecosystem. In particular, HABs associated with cyanobacteria have been of great interest because of their potential to generate and release biological toxins, in particular, lethal microcystins (MCs). The overall goal of this study was to develop a new sustainable approach to decompose MCs, if feasible, on-site and in real-time with minimal efforts, less chemicals, and low energy inputs. To achieve the goal, a high efficiency nitrogen-doped TiO_2 photocatalytic film immobilized onto a glass substrate was fabricated via integrated materials synthesis processing. The film was characterized with visible light activated, nanoporous, and transparent nature. Effects of surfactant type, calcination temperature, coating layers, and reaction pH on the photocatalytic decomposition of MC-LR under visible light were investigated. Eventually, the TiO_2 film was able to successfully decompose MC-LR on site in a lake under solar radiation in real-time. This study implied the high potential of the TiO_2 film for on-site and real-time decomposition of many organic contaminants in water by using sustainable solar energy.

3.1. Introduction

Contamination of water resources with natural and anthropogenic chemicals has been a huge concern worldwide. Particularly, increasing occurrence of harmful algal blooms (HABs) alarms water and health authorities and general public (Nfodzo *et al.* 2013). Most seriously, HABs associated with cyanobacteria produce and release lethal biological toxins such as microcystins (MCs) (Chorus & Bartram 1999). MCs are a group of natural toxins known to promote liver cancer (Antoniou *et al.* 2005). Animal poisoning and fish kills have been reported in conjunction with MCs and have resulted in significant economic losses (Hitzfeld *et al.* 2000; Andersen *et al.* 1993). Among around 60 MC

congeners, microcystin-LR (MC-LR) is the most famous due to its high toxicity and frequent occurrence (Antoniou *et al.* 2005). World Health Organization (WHO) established a provisional concentration limit of 1 μg/L for MC-LR in drinking water (WHO 1998) and United States Environmental Protection Agency (USEPA) has placed MCs on the Drinking Water Contaminant Candidate List (USEPA 2005) for further investigation and assessment.

Unlike particulate algae that can be easily removed by conventional water treatment processes, biological toxins dissolved in water such as MC-LR are hard to remove and are recalcitrant (Lawton & Robertson 1999). Many technologies, including activated carbon adsorption, coagulation/sedimentation, and membrane separation, have been tested for treatment of MC-LR (Feitz *et al.* 1999; Lui *et al.* 2003; Lawton *et al.* 2003; Song *et al.* 2006; Lee *et al.* 2006; Yuan *et al.* 2006). However, these ex-situ treatment approaches benefit only those who directly use treated water. They do not provide a systematic tool to protect living creatures and ecosystem in HAB sites.

As a result, the overall goal of this study is to develop an on-site (or in-situ) treatment approach for removal of biological toxins. Considering many limitations for on-site applications, treatment approach should be characterized with minimal efforts, less chemicals, and low energy inputs. Once developed, such an approach is also important for source water management. In order to achieve the goal and to fulfill the requirements, this study proposes to use a high efficiency nitrogen-doped TiO_2 photocatalytic film immobilized onto a glass substrate.

TiO_2 photocatalysis is one of the most effective water treatment processes (Choi *et al.* 2010). Strong hydroxyl radicals generated from TiO_2 non-selectively and readily attack and decompose organic contaminants in water including MCs (Song *et al.* 2006). The catalytic process does not either require addition of other chemicals or consume

TiO_2 materials. However, only requirement is to irradiate TiO_2 surface with UV with high photon energy above the band gap of TiO_2. This greatly inhibits the utilization of solar radiation as a sustainable energy source for the TiO_2 activation because only 5% of the incoming solar energy onto the earth's surface is in UV (Anpo and Takeuchi 2003). Consequently, activation of TiO_2 under visible light can facilitate the development of promising processes for on-site remediation of contaminated water under solar radiation without introduction of complicated facilities for generating and introducing UV. For the activation of TiO_2 under visible light, anion dopants, especially nitrogen, are popular, which are known to narrow the band gap of TiO_2 (Lin *et al.* 2006).

Meanwhile, for on-site applications of TiO_2 photocatalysis, TiO_2 should be firmly immobilized onto a transparent substrate such as glass and the TiO_2 film itself should be transparent to improve light utilization (Vaiano *et al.* 2015; Singh *et al.* 2015). To exhibit high reactivity and thus to decompose MCs in real-time under solar radiation, the structural properties of TiO_2 film should also be controlled. In particular, porous structure is beneficial to enhancing light absorbance of TiO_2 and accessibility of reactants to TiO_2 (Zakersalehi *et al.* 2013). Surfactants and block copolymers as pore templates during sol-gel synthesis of TiO_2 have been widely used to control the porous structure (Bosc *et al.* 2004; Yang *et al.* 1999). In particular, use of nitrogen-containing surfactants is interesting. They can play dual roles as pore template to make porous TiO_2 and as nitrogen source to dope the porous TiO_2 with nitrogen (Choi *et al.* 2007a).

In this study, such a high efficiency nitrogen-doped porous transparent TiO_2 photocatalytic film immobilized onto a glass substrate was fabricated via integrated materials synthesis processing employing surfactant template-based sol-gel, dip coating, and calcination. Effects of surfactant type, calcination temperature, multiple coating layers, and reaction pH on the photocatalytic decomposition of MC-LR under visible light

were investigated. Eventually, the TiO_2 film was able to successfully decompose MC-LR on site in a lake under solar radiation. This study, for the first time, demonstrated the high potential of the TiO_2 film for on-site and real-time decomposition of organic contaminants in water by using sustainable solar energy.

3.2. Materials and methods

3.2.1 Fabrication of TiO_2 film

Three nonionic long chain nitrogen-containing surfactants, diethanolamine (DEA), benzyltrimethylammonium chloride (BTAC) and dodecylamin (DDAD) purchased from Aldrich, were used as pore directing agent and nitrogen doping source. Briefly, each surfactant was dissolved in isopropanol (i-PrOH, Fisher) and then acidic acid (Fisher) was added to the solution for the esterification reaction with i-PrOH, as demonstrated elsewhere (Choi *et al.* 2006). Finally, titanium tetraisopropoxide (TTIP, Aldrich) was added under vigorous stirring. The molar ratio of surfactant/i-PrOH/acetic acid/TTIP was 1:45:6:1. Transparent TiO_2 sol was prepared.

A borosilicate glass with an effective surface area of 10 cm^2 (20 cm^2 for both sides) was dip-coated with the TiO_2 sol using a PTL-MM01 (MTI Corporation) dip-coating device at a coating rate of 15 cm/min. After coating, TiO_2 film was dried at room temperature for 1 hr and calcined in a programmable furnace (Paragon HT-22-D, Thermcraft). The heat-treatment temperature was increased at ramp rate of 60 ºC/hr to 100 ºC and maintained at 100 ºC for 1 hr. The film was further calcined at different temperatures at 350, 400, 450, and 500 ºC for 2 hr and cooled down naturally, forming nitrogen-doped TiO_2 (N-TiO_2). To increase the number of coating layers up to 7, the dip-coating and calcination process was repeated. Control TiO_2 prepared without the

surfactants was calcined at 500 ºC which generally results in high crystallinity and thus high reactivity (Aphairaj *et al.* 2011).

3.2.2 Reactivity evaluation for TiO_2 film

To quickly check whether TiO_2 can be activated under visible light, decomposition of methylene blue (MB) was first tested because its decomposition can be visually monitored and easily detected. Two 15 W fluorescent lamps (Philips) were used at the intensity of 3.52 mW/cm^2 measured by photonics power meter (Ophir version 15.01). Figure 3.1 shows the spectrum of fluorescent lamp measured by factory. The lamps were mounted with a UV block filter (UV420, Bower) to cut the spectral range below 420nm. Figure 3.2 shows the picture of photocatalytic reactors and photonic power meter. Concentration of MB was 5 mg/l and volume of the solution was 10 ml where one TiO_2 film was emerged. The solution was mixed by using a programmable shaker. Temperature was maintained at around 25 ºC and initial pH at 6.5 was not adjusted. The solution was initially kept in dark condition for 1 hr and then irradiated with visible light for 4 hr.

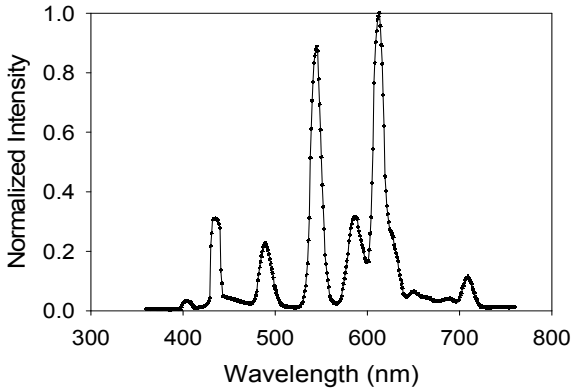

Figure 3.1 Fluorescent lamp spectrums
(http://www.lighting.philips.com/main/prof/lamps/fluorescent-lamps-and-starters)

After the preliminary test with MB, decomposition of MC-LR (Cayman Chemicals) was targeted under the same condition except for initial concentration of MC-LR at 1 mg/l. The effects of various parameters including calcination temperature, multiple coating layer, and reaction pH on the decomposition of MC-LR were investigated. Standard conditions (calcination temperature of 500 ºC, coating layers of 3, and natural pH of 6.5) were fixed while one parameter varied. Reaction pH towards acidic conditions was adjusted by using H_2SO_4 (Fluka) and sodium phosphate buffer (Fluka).

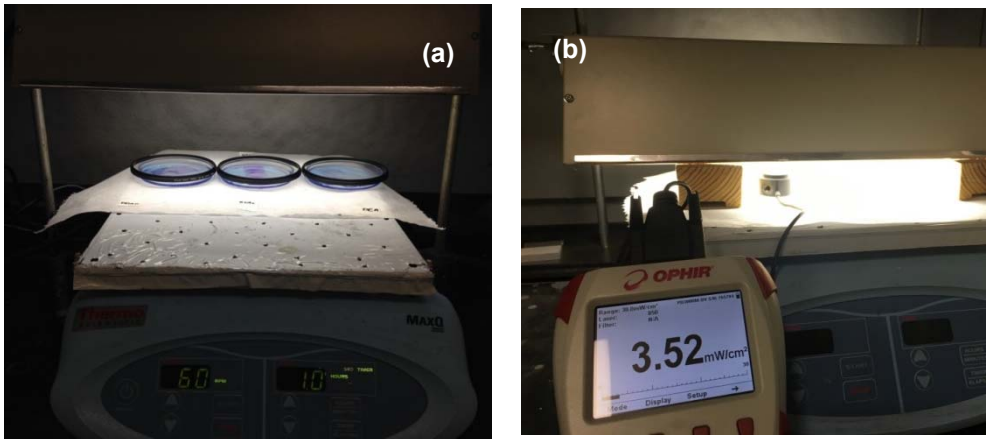

Figure 3.2 Picture of (a) photocatalytic reactors and (b) photonic power meter

3.2.3 On-site decomposition of MC-LR

To evaluate the field application potential of the TiO_2 film, on-site test was conducted in Lake Arlington (Arlington, TX), which has shown HABs and toxin releases. MC-LR concentration was periodically monitored from July to October in 2016. However, the level of MC-LR during the period was too low to implement such a field test and thus lake water was taken, put into a confined area, and spiked with MC-LR at 0.1 mg/L. After installing TiO_2 film, the whole system was exposed to solar radiation for 4 hr. Solar

intensity at the top of the sector was 1.55 mW/cm^2 measured with photonics power meter (Ophir version 15.01).

3.2.4 TiO_2 characterization and chemical analysis

A Tristar II 3020 (Micromeritics) porosimetry analyzer was used to determine the structural properties of TiO_2 including surface area, pore volume, and pore size and distribution. Point of zero charge (PZC) was measured using a Zeta potential analyzer (SZ-100, Horiba, Japan). A Kristalloflex D500 diffractometer (Siemens) was employed to examine the crystal phase of TiO_2. Nitrogen content in TiO_2 was measured with an X-ray photoelectron spectroscope (XPS, Kratos axis) with at takeoff angle of 90° and vacuum pressure of 2.3-7.6 × 10^{-9} Torr. Concentration of MB was measured using a UV-Vis spectrophotometer (UV-2550, Shimadzu). MC-LR was monitored with a reversed-phase high performance liquid chromatography (1200 series, Agilent) and UV detector at 238 nm as described elsewhere (Shamsollahi *et al*. 2015). Concentration of natural organic matter (NOM) in Lake Arlington was measured by using total organic carbon (TOC) analyzer (Shimadzu TOC-Vcsn).

3.3 Results and discussion

3.3.1 Photocatalytic decomposition of MB under visible Light

To quickly check whether TiO_2 films prepared with different surfactants are reactive under visible light, decomposition of MB was investigated, as shown in Fig. 3.3 Although MB absorbs visible light mainly at 609 nm and 668 nm, there was no photolysis most probably due to the low light intensity at 3.52 mW/cm^2 (Vaiano *et al.* 2015). Around 4-8% of MB was absorbed under dark condition onto N-TiO_2 and control TiO_2. Control TiO_2 showed negligible reactivity under visible light above 420 nm while all N-TiO_2 films

demonstrated significant decomposition of MB. The result implies that the surfactants worked effectively as a nitrogen doping source for visible light activation of TiO_2. In particular N-TiO_2 made with DEA showed fastest MB decomposition kinetics, i.e., 67% decomposition for 4 hr.

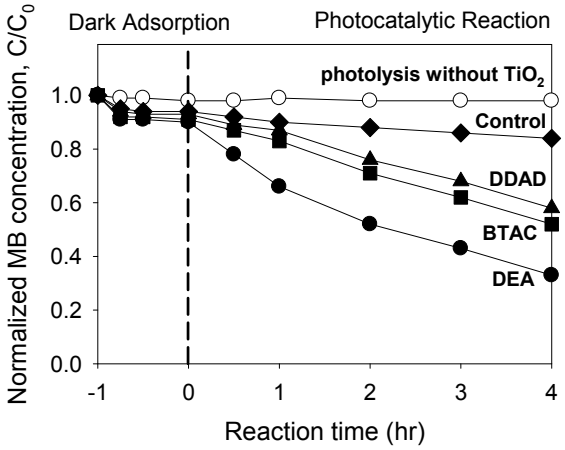

Figure 3.3 Photocatalytic decomposition of MB under visible light (> 420 nm) by TiO_2 thin films prepared with different surfactants (N-TiO_2) (MB concentration: 5.0 mg/L, calcination temperature: 500 ºC, number of coatings: 3, and pH: natural at around 6.5). Control TiO_2 was also prepared without surfactants.

3.3.2 Photocatalytic decomposition of MC-LR under visible Light

N-TiO_2 film made with DEA was further tested with MC-LR, as shown in Fig. 3.4 There was no photolysis of MC-LR since MC-LR does not absorb visible light. Adsorption of MC-LR to TiO_2 films was negligible. Under visible light, control TiO_2 showed negligible reactivity with MC-LR while N-TiO_2 exhibited significant reactivity, i.e., 47% decomposition of MC-LR for 4 hr. The result confirms that DEA worked effectively as a nitrogen doping source. Considering different concentrations for MC-LR (1 mg/L) and MB

(5 mg/L) were used, decomposition of MC-LR was much slower than MB due to its recalcitrant cyclic structure (Kenefick et al. 1993).

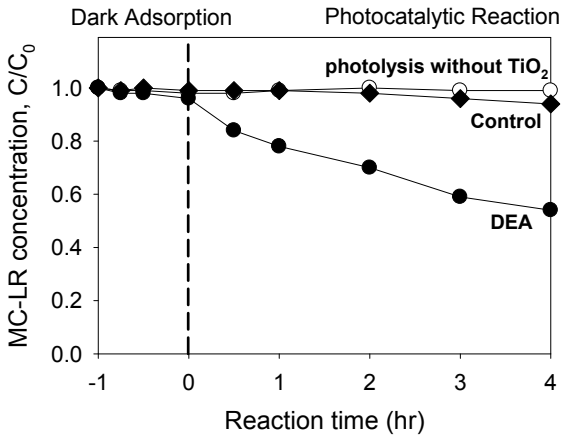

Figure 3.4 Photocatalytic decomposition of MC-LR under visible light (> 420 nm) by TiO_2 thin films prepared with DEA (N-TiO_2) and without DEA (control TiO_2) (MC-LR concentration: 1.0 mg/L, calcination temperature: 500 ºC, number of coatings: 3, and pH: natural at around 6.5).

3.3.3. Physiochemical properties of N-TiO_2 and control TiO_2

To explain the high reactivity under visible light, the physicochemical properties of N-TiO_2 made with DEA and control TiO_2 were investigated, as summarized in Table 3.1. In addition, a series of N-TiO_2 films were synthesized at different calcination temperatures. Synthesis temperature is known to greatly influence the properties of TiO_2, in particular, crystal phase, structural properties, and nitrogen content, and thus its reactivity (Sathish et al. 2005). Surface area decreased from 151 to 61.1 m^2/g and porosity also decreased from 41% to 22% when calcination temperature increased from 350 to 500 ºC. Calcination is required to remove surfactant templates to create porous structure while prolonged calcination at high temperatures also collapse the formed porous structure

(Asahi et al. 2001). All N-TiO_2 films were found to have anatase crystal phase and to be transparent.

Table 3.1. Physicochemical properties of TiO_2 films prepared at different calcination temperatures

TiO_2 films	Calcination temperature (ºC)	Surface Area (m^2/g)	Pore Volume (cm^3/g)	Pore Size (nm)	Porosity (%)	Crystal phase	Nitrogen content (%)	Band gap energy (eV)	PZC
N-TiO_2	350	151	0.181	3.7	41	Anatase	6.3	2.39	6.2
N-TiO_2	400	136	0.142	4.5	35	Anatase	5.2	2.55	6.4
N-TiO_2	450	88.8	0.109	4.8	30	Anatase	3.8	2.64	6.5
N-TiO_2	500	61.1	0.074	4.7	22	Anatase	3.1	2.73	6.7
Control TiO_2	500	50.1	0.012	5.7	4.0	Anatase	0.5	2.95	5.4

Nitrogen content decreased from 6.3 to 3.1% over temperature from 350 to 500 ºC because nitrogen was subject to thermal decomposition during calcination process. Optical band energy of N-TiO_2 (made with DEA) at different calcination temperature and control TiO_2 were determined using UV-Vis absorption spectra. As shown in Figure 3.5, the absorption spectrum shoulder of N-TiO_2 calcined at 350 °C was extended toward the visible light range, indicating a red-shift effect of nitrogen doping. In order to measure the effective optical band gap, the linearly extrapolated wavelength was used as the effective wavelength λ_g^{eff} (Sun et al. 2005). The λ_g^{eff} of N-TiO_2 was approximately 520 nm (E_g^{eff} = 2.39 eV) while that of control TiO_2 was 420 nm (E_g^{eff} = 2.95 eV). The same procedure repeated for calculating band gap energy of N-TiO_2 at different calcination temperature. As shown in Table 3.1, by increasing calcination temperature from 350 to 500 ºC band gap energy will also increase due to loss of nitrogen atoms incorporated in the TiO_2 lattice as nitrogen atoms tend to be replaced with oxygen atoms in the air at high temperature (Sathish et al. 2005) N-TiO_2 showed lower band gap energy (2.73 eV) than

control TiO_2 (2.95 eV) at 500 ºC. Results demonstrate the effect of nitrogen doping in reducing band gap energy of TiO_2.

PZC of N-TiO_2 was at around 6.2-6.7. In comparison between N-TiO_2 (made with DEA) and control TiO_2 prepared at 500 ºC, which were used for the experiments in Figs. 3.2 and 3.3, N-TiO_2 showed higher surface area (61.1 m^2/g) than control TiO_2 (50.1 m^2/g) and superior porosity (22%) to control TiO_2 (4.0%). In addition, N-TiO_2 contained 3.1% nitrogen while control TiO_2 showed negligible nitrogen (0.5% most probably from the impurities of the ingredients used). The properties of N-TiO_2 could explain its decomposition capability for MB and MC-LR under visible light. All the results proved the dual role of DEA as nitrogen doping source and pore directing agent.

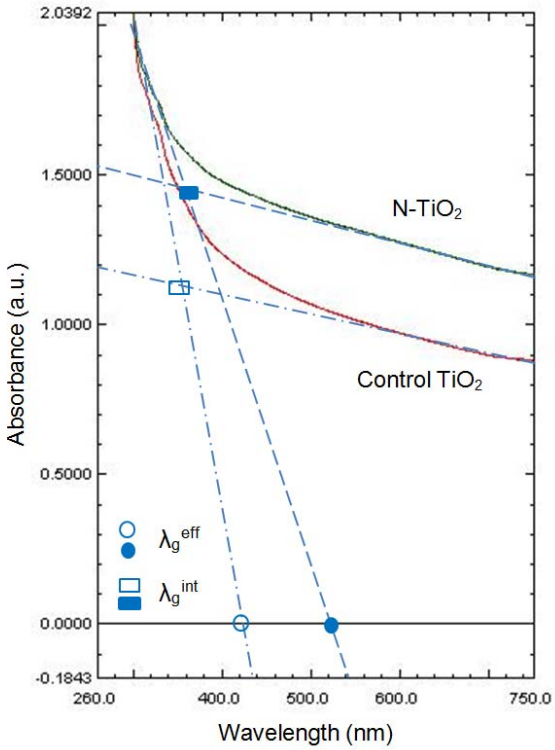

Figure 3.5 Optical UV-visible absorption spectra of control TiO_2 and N-TiO_2 calcined at 350 °C.

3.3.4 Decomposition of MC-LR under various conditions

The series of N-TiO_2 films prepared at different calcination temperatures were examined for the decomposition of MC-LR under visible light, as shown in Fig. 3.6 All N-TiO_2 films showed high reactivity. When increasing calcination temperature from 350, 400, and 450 ºC, the reactivity significantly increased although nitrogen content decreased and porous structure collapsed. This can be explained with TiO_2 crystallization during calcination (Aphairaj *et al.* 2011). Initial amorphous TiO_2 is transformed to the most active anatase phase at calcination temperatures above 300 ºC and to less active rutile phase at calcination temperatures above 550 ºC (Yu and Wand 2010). It has been well reported TiO_2 prepared at around 450-550 ºC shows best reactivity due to the formation of anatase phase with high crystallinity (Kang and Chen 2010; Lin *et al.* 2015; Vaiano *et al.* 2015). Further increase in calcination temperature to 500 ºC rather resulted in slight decrease in the reactivity probably due to too low nitrogen content (Choi *et al.* 2007a).

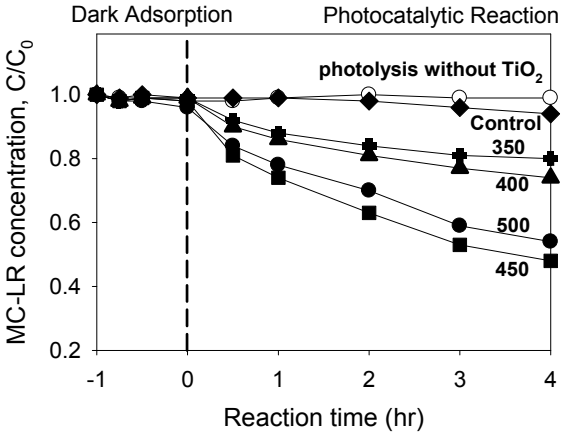

Figure 3.6 Photocatalytic decomposition of MC-LR under visible light (> 420 nm) by TiO_2 thin films prepared with DEA (N-TiO_2) at different calcination temperatures (MC-LR concentration: 1.0 mg/L, number of coatings: 3, and pH: natural at around 6.5). Control TiO_2 was also prepared without surfactants at 500 ºC.

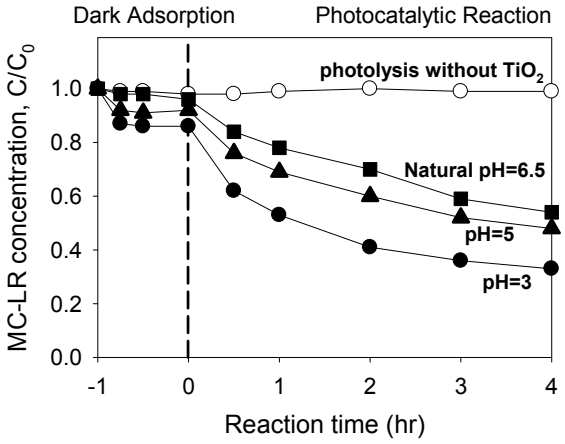

Figure 3.7. Photocatalytic decomposition of MC-LR under visible light (> 420) nm at different reaction pHs by TiO_2 thin films prepared with DEA (N-TiO_2) (MC-LR concentration: 1.0 mg/L, calcination temperature: 500 ºC, and number of coatings: 3).

Figure 3.7 shows the effect of reaction pH on the photocatalytic decomposition of MC-LR under visible light. Significant decomposition of MC-LR was observed under natural pH of 6.5 without pH adjustment. When pH was adjusted to acidic conditions, MC-LR was decomposed much faster. Acidic pH was reported to be favorable for the photocatalytic degradation of MC-LR (Song et al. 2006). Under low pH, the surface of MC-LR is negatively charged due to dissociation of its free carboxylic group while N-TiO_2 with PZC of 6.7 is positively charged.

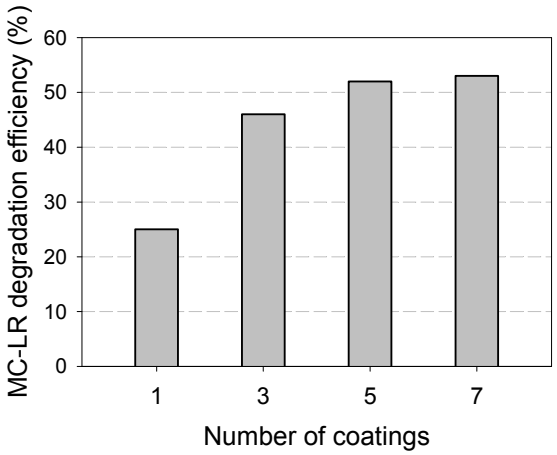

Figure 3.8 Photocatalytic decomposition of MC-LR under visible light (> 420 nm) for 4 hr by TiO_2 films prepared with DEA (N-TiO_2) with different number of coating layers (MC-LR concentration: 1.0 mg/L, calcination temperature: 500 ºC, and pH: natural at around 6.5).

The effect of the number of coating layers up to 7 was also examined, as shown in Fig. 3.8 When the coating layer increased from 1 to 3, MC-LR decomposition almost doubled. Then, the number of coating layers did not affect the reactivity significantly (Choi *et al.* 2006). In general, multiple coating brings more TiO_2 loading to films, resulting in high reactivity. The disproportional increase in the reactivity could be explained by the porous structure of inner TiO_2 layers becomes more collapsed during the repeated calcination process and thus less available for chemical reaction. In addition, a reaction rate-limiting factor might be the low intensity of visible light, rather than TiO_2 loading.

3.3.5. On-site decomposition of MC-LR under solar radiation

The previous experiments were conducted under laboratory conditions employing MC-LR in deionized water and artificial visible light. As a result, a field test was conducted to evaluate whether the TiO_2 film can be activated under solar radiation and MC-LR in a real

water matrix can be decomposed, as shown in Fig. 3.9 TOC of the lake water representing NOM, as competing component with MC-LR for TiO_2, was very high at 15.5 mg/l and solar intensity was at 1.55 mW/cm^2. The lake water was spiked with MC-LR to achieve 0.1 mg/L, which is much greater than 0.087 mg/L, average of maximum detection of MCs in eight major lakes in US (USEPA 2015). MC-LR was not decomposed under solar radiation in the absence of TiO_2 and control TiO_2 was not able to decompose MC-LR under solar radiation. Only N-TiO_2 showed significant reactivity under solar radiation to decompose MC-LR. Around 47% of MC-LR was decomposed after 4hr exposure of N-TiO_2 to solar radiation. The result is significant considering the low solar intensity, the high initial MC-LR concentration, and even the presence of NOM at 15.5 mg/L.

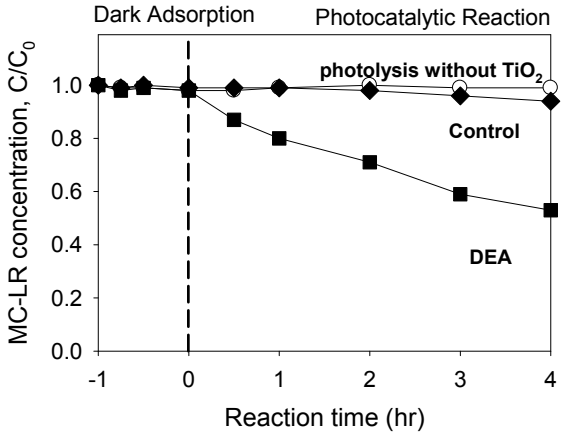

Figure 3.9 *On-site* photocatalytic decomposition of MC-LR in lake Arlington (Arlington, Texas) under solar radiation by TiO_2 thin film prepared with DEA (N-TiO_2) (spiked MC-LR concentration: 0.1 mg/L, light intensity: 1.55 mW/cm^2, calcination temperature: 450 ºC, and number of coatings: 5).

3.4. Conclusions

The overall goal of this study was to develop a new sustainable approach to decompose MCs, if feasible, on-site and in real-time with minimal efforts, less chemicals, and low energy inputs. A high efficiency N-TiO_2 photocatalytic film immobilized onto a glass substrate was fabricated via integrated materials synthesis processing. N-TiO_2 film was characterized with highly porous, anatase crystal, transparent, and nitrogen-doped nature. All the results proved the dual role of DEA surfactant as nitrogen doping source and pore directing agent. N-TiO_2 prepared with DEA at 450-500ºC exhibited fast decomposition of MC-LR under visible light due to its compromised properties with respect to surface area, crystal phase, and nitrogen content. N-TiO_2 film was able to successfully decompose MC-LR in a lake containing high NOM under solar radiation. This study implies the high potential of the N-TiO_2 film for on-site and real-time decomposition of organic contaminants in water by using sustainable solar energy.

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Chapter 4

Recommendations and potential applications

4.1. Recommendations

In this study, first we introduce different observing systems for monitoring harmful algal blooms. Considering more sites and longer periods can helps better judgment about correlation between different observation systems to monitoring harmful algal blooms systems. geographical, ecological and meteorological of sampling location can affect the observing system results so having more data from other location and more time periods benefits better comparison between data from different observing systems.

In case of synthesis of nanostructure porous nitrogen-doped TiO_2 thin film, more analytical characterization could help to better understand physiochemical and structural properties of N-doped TiO_2. X-ray photoelectron spectroscope (XPS,) can use to determine the fine elemental and electronic structure of TiO_2 (Choi, et al., 2007) and high-resolution transmission electron microscope (HR-TEM) could shows morphology and pore structure of thin films (Choi, et al., 2006).

4.2. Potential applications

Comparison between different monitoring systems revealed advantages and limitation of each method. The remote sensing and in situ sensing of phycocyanin are real time monitoring approaches which benefit immediate decision-making and timely response, but, those observing system just monitoring general algal bloom indirectly. There is no observing system that can directly detect biological toxins in situ and in real time at trace level. One potential substitutive to existing monitoring approaches could be an in situ sensing system, which directly identifies and quantifies biological toxins. In comparison with on-site sampling followed by in lab analysis, the proposed In situ sensing benefits the advantage of direct measurement of biological toxins and overcome its limitations as

it is real time and can cover vast area. We are currently investigating on developing this innovative in situ sensing system to monitor harmful algal blooms.

As discussed before, TiO_2 photocatalysis has been highlighted as one of the most promising and green advanced oxidation technologies (AOTs) in water treatment processes (Choi, et al., 2010). In spite of its high potential power to decompose vast variety of contaminants in water, it has two main drawbacks that can limits it is application in real world. First, ultraviolet (UV) radiation is needed and TiO_2 particles separation if used in slurry reactors (Vaianoi, et al., 2015). Our N-doped TiO_2 film overcome those limitations by using solar radiation instead of UV lamps and immobilized TiO_2 instead of suspended particles. This research showed the potential field on-site application of nitrogen-doped TiO_2 film to decompose biological toxins. By modification of this system and scale up the number and dimension of films, it can be used in real world for on-site decomposition of biological toxins under solar irradiation in real time. More films in bigger size will be needed and operation parameters can modified based on-site location characteristics such as MC-LR concentration at algal bloom season, solar radiation intensity and water parameters such as NOM concentration and pH.

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Biographical Information

Hesam Zamankhan Malayeri earned his bachelor degree in civil engineering at Iran University Science and Technology in 2008 and his master degree in environmental engineering at Tarbiat Modares University in 2011. She started his PhD degree in environmental engineering program at the University of Texas at Arlington in 2012. During his PhD academic study, he worked on two main projects. First, In situ sensing system for the selective and sensitive detection of biological toxins in HABs funded by NIH and NSF) and Second, in-situ decomposition of biological toxins on nitrogen-doped nanostructure TiO_2 films under solar radiation funded by texas higher education coordinating board through the Norman Hackerman advanced research program as a graduate research assistant in Dr. Hyeok Choi's lab in civil and environmental engineering department at University of Texas at Arlington.

He is interested in advanced oxidation technologies, environmental nanotechnologies, physical and chemical processes in water and wastewater treatment, and sensing system for monitoring biological toxins in different water bodies. He did his Ph.D. research on "harmful algal blooms and biological toxin productions, from integrated monitoring approach to on-site solar photocatalytic decomposition".