FAECAL INDICATOR BACTERIA MONITORING IN BLUE-GREEN ALGAE CONTAMINATED WATER

by

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Abstract

Due to enhanced human and climatically-driven environmental changes, numerous water bodies including the Great Lakes area have seen increased harmful algal blooms (HABs) (including those caused by blue-green algae) in the past few decades. HABs affect the ecological integrity of the affected water by disrupting or altering the biological communities, bringing more challenges to water-quality monitoring and sustainable water management. Faecal indicator bacteria (FIB) monitoring for detection of possible microbial and pathogen contamination is a significant water-management practice, with implications for water resource sustainability.

Work in this thesis focuses on HAB impact on faecal indicator bacteria survival and thus the use of FIB for indicating microbial contamination. To serve this purpose, microcosms that contain the representative algae and indicator bacteria were established to explore the algae-bacteria dynamics at laboratory scale. Two species of *Microcystis* (*M. smithii* and *M. aeruginosa*) and four lab strains of *E. coli* (ATCC 25922, 8739, 51813, and 11775) were used. The sample water was collected regularly for *Microcystis* and *E. coli* screening. *Microcystis* was shown to dramatically reduce *E. coli* survival in the microcosms. Three possible factors contributing to the observed algae-*E. coli* dynamics, nutrient (nitrogen and phosphorus) availability, water pH, and microcystin production by *Microcystis*, were examined; microcystin production was the only factor found to be able to linked to *E. coli* death. Lastly, lake strains of *E. coli* and Total coliforms sampled from Lake Ontario were included and showed the same general behavior.

The results show that use of FIB (*E. coli* and Total coliforms) as indicators of faecal contamination in water with *Microcystis* spp. (and possibly other blue-green algae) present could be greatly compromised, especially if algal toxin is produced. On the other hand, FIB survival dynamics, if interpreted properly, might be used to detect the onset of toxin-producing harmful algal blooms.
Acknowledgements

I would like to express my deep gratitude to my supervisor, Dr. Stephen Brown, for his continuous support for my MES study and research, for his patience and motivation. His inspiring guidance and immense knowledge helped me in all the time of research and writing of this thesis, and would benefit me for life.

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<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>BLAST</td>
<td>basic local alignment search tool</td>
</tr>
<tr>
<td>BMAA</td>
<td>beta-methylamino-L-alanine</td>
</tr>
<tr>
<td>CCM</td>
<td>carbondioxide concentrating mechanism</td>
</tr>
<tr>
<td>CFU</td>
<td>colony forming unit</td>
</tr>
<tr>
<td>dH2O</td>
<td>distilled water</td>
</tr>
<tr>
<td>DIC</td>
<td>dissolved inorganic carbon</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DST</td>
<td>defined substrate technology</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>FIB</td>
<td>faecal indicator bacteria</td>
</tr>
<tr>
<td>FISH</td>
<td>fluorescence in situ hybridization</td>
</tr>
<tr>
<td>HAB</td>
<td>harmful agal bloom</td>
</tr>
<tr>
<td>L:D</td>
<td>light:dark</td>
</tr>
<tr>
<td>LC</td>
<td>liquid chromatography</td>
</tr>
<tr>
<td>MAA</td>
<td>mycospore-like amino acid</td>
</tr>
<tr>
<td>MF</td>
<td>membrane filtration</td>
</tr>
<tr>
<td>MPN</td>
<td>most probable number</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>MUG</td>
<td>4-methylumbelliferyl-β-D-glucuronide</td>
</tr>
<tr>
<td>NPS</td>
<td>non-point sources</td>
</tr>
<tr>
<td>ONPG</td>
<td>o-nitrophenyl-β-D-galactopyranoside</td>
</tr>
<tr>
<td>O. D.</td>
<td>optical density</td>
</tr>
<tr>
<td>PS</td>
<td>point sources</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain-reaction</td>
</tr>
<tr>
<td>P/A</td>
<td>present/absent</td>
</tr>
<tr>
<td>rpm</td>
<td>rounds per minute</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td><em>Taq</em></td>
<td><em>Thermus aquaticus</em></td>
</tr>
<tr>
<td>TMDL</td>
<td>total maximum daily loads</td>
</tr>
<tr>
<td>TTD</td>
<td>time to detection</td>
</tr>
<tr>
<td>U.S. EPA</td>
<td>United States Environmental Protection Agency</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

Water covers approximately 70% of Earth's surface, and is an essential resource for all life. Water pollution caused by human activities contaminates surface and ground water, and poses a considerable threat to human health and well-being as well as the preservation of the environment. The specific contaminants include a wide spectrum of pathogens and chemicals, from various sources such as sewage, fertilizer and agriculture runoff, and industrial discharges. Understanding and monitoring these contaminants is of great significance to inform the public, protects the water environment, and ensures access to safe water. This task, however, could be especially challenging when the detection matrix is complicated and more than one contaminant is of concern. Water contamination caused by faecal microbes and blue-green algae are the main focuses of the following sections, as the background prior to research objectives of this thesis.

1.1 Microbial contamination in water and the use of faecal indicator bacteria (FIB)

1.1.1 Microbial contamination

Transmission of microbial pathogens by water, especially drinking water, is a significant cause of waterborne illness worldwide. In developing countries, primarily in South Asia and Africa, water-related microbial diseases, such as cholera and typhoid, pose severe public health risks to local people. Those who live in areas with poor hygiene practices and health care, especially children under five, are the most affected (Fenwick, 2006). Industrialized areas such as North America and Europe eliminated many endemic enteric diseases through the implementation of filtration and chlorination for drinking water; however, waterborne outbreaks
associated with microbial pathogens still happen frequently. In the U.S.A., it has been estimated that each year 7.1 million cases of mild to moderate gastrointestinal infections are caused by the consumption of contaminated water; 560,000 people suffer from severe waterborne diseases which results in about 12,000 deaths (Medema et al., 2003). The drinking-water contamination event that occurred in Walkerton, Ontario, Canada in May 2000 caused seven deaths and over 2,300 illnesses (Hrudey, Payment, Huck, Gillham, & Hrudey, 2003).

It has been shown that the majority of pathogenic microbes are derived faecally, which include bacteria, viruses, and protozoa (Leclerc, Mossel, Edberg, & Struijk, 2001). The point sources (PS) of contamination mainly include untreated/undertreated sewage, hospitals, and industries (Cabral, 2010). Non-point sources (NPS) are largely from runoff water of agricultural and pasture land and urban areas (Cabral, 2010). The common waterborne pathogens along with the associated diseases/symptoms are listed in Table 1. In all cases, the infected people and animals shed large numbers of microbes in faeces, which again cause the contamination of surface and ground waters.

1.1.2 Faecal indicator bacteria

It is impossible and impractical to perform tests for all potential pathogenic microbes. Further, waterborne pathogens tend to travel through the environment and may be diluted below normal detection limits, even though still present at clinically important concentrations. These limitations led to the birth of the ‘indicator’ idea: To use easily detectable and representative microorganisms to indicate the presence of faecal contamination, and therefore an elevated risk of microbiological contamination in the water. Based on the generally accepted scientific consensus,
Table 1. Examples of waterborne pathogens and diseases (derived from (Aw & Rose, 2012))

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Disease/Symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td><em>Escherichia coli O157</em></td>
<td>Gastroenteritis, haemolytic, uraemic syndrome</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>Chronic gastritis</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em></td>
<td>Legionellosis</td>
</tr>
<tr>
<td><em>Mycobacterium avium</em></td>
<td>Lung infection</td>
</tr>
<tr>
<td><em>Salmonella enterica</em></td>
<td>Gastroenteritis, typhoid</td>
</tr>
<tr>
<td><em>Shigella spp.</em></td>
<td>Shigellosis</td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td></td>
</tr>
<tr>
<td><em>Adenoviruses</em></td>
<td>Gastroenteritis, respiratory illness, conjunctivitis</td>
</tr>
<tr>
<td><em>Caliciviruses (noroviruses)</em></td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td><em>Enteroviruses</em></td>
<td>Paralysis, meningitis, myocarditis, gastroenteritis</td>
</tr>
<tr>
<td><em>Hepatitis A virus</em></td>
<td>Infectious hepatitis</td>
</tr>
<tr>
<td><em>Hepatitis E virus</em></td>
<td>Infectious hepatitis</td>
</tr>
<tr>
<td><em>Polioviruses</em></td>
<td>Poliomyelitis</td>
</tr>
<tr>
<td><em>Rotaviruses</em></td>
<td>Diarrhoea, vomiting</td>
</tr>
<tr>
<td><em>Norwalk viruses</em></td>
<td>Diarrhoea, vomiting</td>
</tr>
<tr>
<td><strong>Parasitic protozoa</strong></td>
<td></td>
</tr>
<tr>
<td><em>Naegleria fowleri</em></td>
<td>Amoebic meningoencephalitis</td>
</tr>
<tr>
<td><em>Cryptosporidium parvum</em></td>
<td>Cryptosporidiosis</td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>Amoebic dysentery</td>
</tr>
<tr>
<td><em>Giardia lamblia cysts</em></td>
<td>Diarrhoea</td>
</tr>
</tbody>
</table>

Payment, Waite, and Dufour (2003) listed the criteria that an effective indicator microorganism should fulfill as many as possible:

- It should be present whenever the pathogens are present, and absent in unpolluted waters;
- It should not multiply in natural waters;
- It should be present in greater numbers than the pathogens to facilitate testing;
- It should react to the natural environment and treatment in a similar manner to the pathogens;
- It should be readily isolated, identified and enumerated;
- It should be analyzed at low cost to permit frequent testing;
- It should not be pathogenic itself to minimize the health risk associated with testing.

The earliest use of the indicator approach dates back to the late 19th century. In 1885, Escherich described a motile, rod-shaped microorganism in the faeces of new-born and sucking babies that could cause milk to clot. He named it Bacterium coli commune (now *Escherichia coli*, or *E. coli*) (Medema et al., 2003). It was subsequently found to exist ubiquitously in human faeces by other researchers (Ashbolt, Grabow, & Snozzi, 2001). In 1892, Schdinger first suggested the use of *E. coli* as an indicator ‘of the presence of faecal pollution and therefore of the potential presence of enteric pathogens’, giving the term faecal indicator bacteria (FIB) (Payment, Waite, & Dufour, 2003). The first edition of ‘Standard Methods for the Examination of Water and Wastewater’ released in 1905 chose *E. coli* as the indicator organism for drinking-water sources (Gilcreas, 1966).

Presently, Total coliforms, faecal coliforms, *E. coli*, faecal streptococci, and *Enterococci* are the most used FIB worldwide for microbiological water quality (as shown in Table 2, which
Table 2. International drinking water standards and guidelines (derived from (Mesquita & Noble, 2013))

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Canada</th>
<th>USA</th>
<th>United Kingdom</th>
<th>EU directive</th>
<th>WHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliforms</td>
<td>0/100mL in 90%</td>
<td>0/100mL in 95%</td>
<td>0/100mL</td>
<td>0/100mL</td>
<td>*</td>
</tr>
<tr>
<td>Thermotolerant coliforms (faecal coliforms) or E. coli</td>
<td>*</td>
<td>0/100mL</td>
<td>*</td>
<td>*</td>
<td>0/100mL</td>
</tr>
<tr>
<td>E. coli</td>
<td>0/100mL</td>
<td>0/100mL</td>
<td>0/100mL</td>
<td>0/100mL</td>
<td>0/100mL</td>
</tr>
<tr>
<td>Enterococci</td>
<td>*</td>
<td>*</td>
<td>0/100mL</td>
<td>0/100mL</td>
<td>*</td>
</tr>
<tr>
<td>Cryptosporidium parvum</td>
<td>*</td>
<td>99% removal or inactivation</td>
<td>&lt;1 oocyte /10L</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Clostridium perfringens (including spores)</td>
<td>*</td>
<td>*</td>
<td>0/100mL</td>
<td>*</td>
<td>a</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>0/250 mL</td>
<td>*</td>
</tr>
<tr>
<td>Colony count 22°C</td>
<td>*</td>
<td>*</td>
<td>No abnormal change</td>
<td>No abnormal change</td>
<td>*</td>
</tr>
<tr>
<td>Colony count 37°C</td>
<td>&lt;500 CFUs/mL&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;500 CFUs/mL&lt;sup&gt;b&lt;/sup&gt;</td>
<td>No abnormal change</td>
<td>20 CFUs/mL&lt;sup&gt;c&lt;/sup&gt;</td>
<td>*</td>
</tr>
</tbody>
</table>

* spaces left in blank indicate parameters not specified;
  a Necessary only if the water originates from or is influenced by surface water;
  b Heterotrophic plate count (35°C for 48h) or <200 background coliforms on a total coliform membrane filter;
  c Necessary only in the case of water offered for sale in bottles or container.
lists the drinking water criteria of some developed countries as well as the World Health Organization, WHO). They could be subsumed under two groups, coliforms and faecal streptococci as shown in Figure 1. Definitions of these bacteria are mostly based on the conventional cultivation methods, describing the common biochemical characteristics within each group as briefly discussed below.

**Figure 1. Bacterial indicator relationship schematic** (underlined bacteria are indicator bacteria; size of ovals not necessarily reflects proportion)

**Total coliforms** refer to bacteria that are rod-shaped, facultative anaerobic, Gram-negative, and non-spore forming, which can ferment lactose with the production of carbon dioxide gas and acid (such as lactic acid) when incubated in a medium with bile salts and detergents at 35–37 °C (APHA, 1998; Payment et al., 2003). A more genotypic definition is that Total coliforms are members of genus or species within the family *Enterobacteriaceae* capable of growth at 37 °C and possessing β-galactosidase (Payment et al., 2003). Regardless of the definitions assumed, the total coliform bacteria are a heterogeneous group. It traditionally comprises the genera of *Escherichia, Citrobacter, Enterobacter*, and *Klebsiella*, and sometimes includes members of genera such as *Budvicia, Serratia* and *Rahnella*. 
All members of the total coliform group can occur in human faeces, and are also spread widely in animal faeces and the natural environment. They can as well survive in drinking-water distribution systems by taking advantage of indigenous bio-films (Camper, McFeters, Characklis, & Jones, 1991; LeChevallier, Welch, & Smith, 1996). In many countries, they are no longer considered as a reliable index of faecal contamination for either recreational water or drinking water. However, coliform levels could be of great use as an indicator of water-quality changes, water treatment failures, and possible infiltration in distribution systems (Payment et al., 2003).

**Thermotolerant coliforms** (also known as faecal coliforms), a subset of total coliform bacteria, are defined as coliforms that are able to ferment lactose at temperature of 44-45 °C in a medium with bile salts (APHA, 1998; Payment et al., 2003). They comprise largely species of Escherichia and to a lesser extent, members of the KESC group (*Klebsiella, Enterobacter, Serratia* and *Citrobacter*).

Thermotolerant coliforms are more faecal-specific in origin compared to Total coliforms. Hence, the term ‘faecal coliforms’ was historically employed and is still in use. Incubation under elevated temperature was believed to be able to distinguish the coliforms of purely faecal origins from those who are not, which is eventually proved not entirely true. For example, it is reported that up to 84% of the fecal coliforms were *Klebsiella*, while some species of *Klebsiella* were found to have non-faecal sources such as pulp and paper mill waste (Caplenas & Kanarek, 1984). Therefore, the use of faecal coliforms as an index of faecal contamination is compromised, although many areas are still utilizing them as faecal indicators (Tallon, Magajna, Lofranco, & Leung, 2005).
**E. coli** is a single species in the faecal coliform and *Escherichia* groups. It is taxonomically defined as a member of the family *Enterobacteriaceae* that possesses the enzymes β-galactosidase and β-glucuronidase (Payment et al., 2003). It generally can ferment lactose and mannitol at 37 °C and 44-45 °C with the production of acid and gas (APHA, 1998; Payment et al., 2003), although some strains cannot grow at 44-45°C, some do not possess β-glucuronidase enzyme, and some do not produce gas (Payment et al., 2003).

*E. coli* is an indigenous commensal bacterium of the gastrointestinal tract of humans and warm-blooded animals, and comprises about 1% of the total bacterial biomass in these organisms (Leclerc et al., 2001). It was found to represent over 94% of thermotolerant coliforms in human faeces, while the KESC members made up only 3.2-7.4% (Dufour, 1977). Moreover, the majority of *E. coli* strains are not disease-causing. Therefore, *E. coli* has been traditionally considered and still widely acknowledged as the most suitable faecal indicator organism for drinking water quality (Gilcreas, 1966; Payment et al., 2003). However, it also has limitations. As stated in ‘Guidelines for Drinking-water Quality’ of WHO (WHO, 2008):

*Enteric viruses and protozoa are more resistant to disinfection; consequently, the absence of *E. coli* will not necessarily indicate freedom from these organisms. Under certain circumstances, it may be desirable to include more resistant micro-organisms, such as bacteriophages and/or bacterial spores.*

**Faecal streptococci** are members of the genus *Streptococcus*, defined as facultative anaerobic, Gram-positive, non-sporeforming, and chain forming cocci that grow at 35 °C in a medium containing bile salts and sodium azide (APHA, 1998; Payment et al., 2003).

Faecal streptococci generally occur in the digestive systems of humans and other warm-blooded animals. In the past, faecal streptococci were monitored together with faecal coliforms. A ratio of faecal coliforms to faecal streptococci was calculated to determine whether the
contamination was of human or non-human origin. However, this is no longer recommended as a reliable test (Payment et al., 2003; Sinton, Finlay, & Hannah, 1998).

**Enterococci** are a subset of the faecal streptococci. They were separated from *Streptococcus* in the 1980s and grouped under the genus *Enterococcus* since then. Compared with most faecal streptococci, enterococci are generally more tolerant of high NaCl (up to 6.5%) and alkaline pH (up to 9.6) (Payment et al., 2003). The important Enterococci with respect to human faecal contamination are *E. faecalis*, *E. faecium* and *E. durans* (Payment et al., 2003).

Enterococci are typically more human-specific than the larger fecal streptococci group. Concentrations of enterococci in swimming waters were reported to be correlated with risks of gastrointestinal illness in swimmers in 1982 (Cabelli, Dufour, McCabe, & Levin, 1982). Similar findings led agencies such as U. S. EPA and WHO (Payment et al., 2003) to recommend Enterococci as the quality indicator for recreational salt water as well as fresh water.

While the above-mentioned FIB are widely recognized and employed, other microbial variables for water quality are also developed in some countries. For example, in the United Kingdom, spores of *Clostridium perfringens* are tested as an index of more robust virus and protozoa; measurement for *Cryptosporidium* oocysts is adopted to assess water treatment efficiency for the removal of aerobic spore pathogens that are resistant to disinfection (see Table 2). However, these organisms may not be good faecal indicators because they are highly resistant to some disinfection methods, and therefore may not correlate well with the presence of some viruses/protozoa. Also, *Clostridium perfringens* are anaerobic and require complicated methodologies for isolation and detection by culture methods.
Overall there is not a single indicator or even a set of indicators that are ideal for all waters and pathogens. The indicator assumptions could be invalid in many circumstances. However, it is generally accepted that the indicator approach is the most practical scheme for water monitoring. It has provided a high level of public health protection from microbial contamination since its establishment in the 1950s. It is important to choose the ‘best’ indicator and detection method according to the circumstances and to conduct monitoring as frequently as possible (Payment et al., 2003).

1.1.3 Faecal indicator bacteria detection

1.1.3.1 Cultivation methods

The traditional indicator detection methods are largely developed from microbiological cultivation techniques. They can provide presence/absence (P/A) results by visible changes of presumptive reactions after incubation in a proper medium for a certain period of time (24-48 hours). The variables measured include evolution of gas (detected by bubbles, pressure change or specific gas sensors for oxygen, O₂, or carbon dioxide, CO₂), change in pH (using electronic or coloured pH indicators), colour/fluorescence changes (from enzyme-substrate reactions), and visible presence of growth. Detection of \textit{E. coli}, Total coliforms or fecal coliforms is traditionally based on lactose fermentation, which produce gas and acid (decrease in pH) within 48 hours at 35 °C or 44-45 °C (APHA, 1998).

Cultivation methods provide quantitative information in two means. The classic plate technique counts the colony forming units (CFUs) after proper inoculation on an agar plate. It can process a maximum sample volume of around 1 ml directly. For higher sample volumes,
membrane filtration (MF) is employed with filters of standard diameter and pore size below 1 µm. Broth-based cultivation with multiple-tube fermentation offers quantitative enumeration. A series of tubes with appropriate decimal dilutions of the water sample are employed for incubation. The P/A result of each tube is used to statistically estimate the target density reported as the Most Probable Number (MPN).

With the advent of enzymatic reaction based procedures, cultivation methods became more specific, time-saving, and user-friendly (Köster et al., 2003; Rompré, Servais, Baudart, de-Roubin, & Laurent, 2002). Two highly specific enzymes, β-D-glucuronidase and β-D-galactosidase, are utilized for detection of E. coli and Total coliforms, respectively (Rompré et al., 2002). Specific chromogenic/fluorogenic substrates are added to the selective medium to produce color/fluorescence upon cleavage by the corresponding enzyme. Defined Substrate Technology (DST) boosts the target bacteria growth by highlighting the vital nutrient input for them (Edberg, Allen, Smith, & Kriz, 1990). Qualitative and quantitative measurements can be achieved by visible color/fluorescence change and multi tube MPN techniques.

Table 3 shows some regularly used chromogenic/fluorogenic substrates for detection of indicator bacteria. The commercially available media have gained increasing popularity in the past decade due to their ease of use. The Colilert® medium includes a bacterial cultivation broth and two enzyme substrates, o-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG). The chromogenic substrate, ONPG, can react with β-D-galactosidase enzyme found in Total coliforms, while the fluorogen, MUG, can detect the β-D-glucuronidase enzyme that indicates E. coli. After 18 or 24 hours of incubation at 35°C, the medium turning yellow shows a total-coliform-positive result; the fluoresce under a long-wave ultraviolet light (366 nm) indicates the presence of E. coli. Colilert allows for quantitative
detection by use of a specially designed disposable incubation tray called the Quanti-Tray®, which conducts MPN by separating a 100 ml samples into a series of test wells and provides quantification based on statistical distributions. Colilert-18/Quanti-Tray has become the new International Organization for Standardization (ISO) standard 9308-2:2012, by replacing a previous MPN method in ISO 9308-2:1990 (Lange, Strathmann, & Oßmer, 2013). Colilert-18 is also U.S. EPA-approved and included in ‘Standard Methods for Examination of Water and Wastewater’ (EPA, 2008).

Another U.S. EPA-approved coliform testing system, the TECTA™ B16 detection instrument (ENDETEC, Kingston, ON) (as seen in Table 3), is based on enzyme-substrate assay as well (EPA, 2012). Development of the TECTA started in 2003 by a group of university and industry researchers led by Dr. Stephen Brown of Queen’s University. It features a polymer-based optical sensor to enable the automatic detection of the fluorescent indicators of target bacteria (ENDETEC, 2011). Every TECTA instrument has 16 chambers that read TECTA™ CCA test cartridges. Each cartridge contains bacterial growth media, enzyme-specific chemical substrates, and an optical polymer sensor. Figure 2 shows the TECTA instrument, test cartridge, and detection principle. After adding a water sample into the cartridge, target bacteria such as *E. coli* begin to multiply. They produce a specific enzyme (e.g. glucuronidase for *E. coli*) that interacts with the substrate, releasing fluorescent molecules from the substrate. These fluorescent molecules then diffuse into the polymer sensor due to hydrophobic forces, enabling UV excitation and fluorescence detection. The TECTA possesses several key advantages over conventional
### Table 3 Examples of chromogenic substrates for the detection of indicator bacteria (derived from (Köster et al., 2003))

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Chromogenic substance</th>
<th>Enzyme tested</th>
<th>Commercial kits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliform bacteria</td>
<td>o-nitrophenyl-(\beta)-Dgalactopyranoside (ONPG)</td>
<td>(\beta)-D-galactosidase ((\beta)-GAD)</td>
<td>Colilert® (IDEXX)</td>
</tr>
<tr>
<td></td>
<td>6-bromo-2-naphtyl-(\beta)-Dgalactopyranoside</td>
<td></td>
<td>m-ColiBlue® (Hach)</td>
</tr>
<tr>
<td></td>
<td>5-bromo-4-chloro-3-indolyl-(\beta)-Dgalactopyranoside (XGAL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>5-bromo-4-chloro-3-indolyl-(\beta)-glucuronide (XGLUC)</td>
<td>(\beta)-D-glucuronidase ((\beta)-GUD)</td>
<td>ColiComplete® (BioControl)</td>
</tr>
<tr>
<td></td>
<td>4-methylumbelliferyl-(\beta)-Dglucuronide (MUG)</td>
<td></td>
<td>Chromocult® (Merck)</td>
</tr>
<tr>
<td></td>
<td>p-nitrophenol-(\beta)-D-glucuronide (PNPG)</td>
<td></td>
<td>TECTA™ B16 (Endetec)</td>
</tr>
<tr>
<td>Enterococci</td>
<td>4-methylumbelliferyl-(\beta)-D-glucoside (MUD)</td>
<td>(\beta)-D-glucosidase ((\beta)-GLU)</td>
<td>Enterolert® (IDEXX)</td>
</tr>
<tr>
<td></td>
<td>indoxyl-(\beta)-D-glucoside</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
coliform tests (ENDETEC, 2011). It provides the continuous information throughout the incubation process, allowing for early alerts of bacteria detection. It has high dynamic range from 1 to over $10^6$ CFUs in a 100 ml water sample without need for multiple dilutions. The optical polymer sensor can extract and concentrate the fluorescent indicators, minimizing the interferences from color and turbidity in samples that can obscure conventional detection. These advantages make the TECTA system suitable for *E. coli* and total coliform tests in colored and turbid samples such as water with algae present.

Figure 2. TECTA microbiology system and the detection principle diagram (ENDETEC, 2011)
1.1.3.2 Nucleic acid methods

Nucleic acid based methods target specific segments of the genome (DNA or RNA) of the analyte organisms after being labelled and/or amplified. The core reaction is the complementary sequence recognition between a nucleic acid primer/probe and a nucleic acid target (e.g. by DNA-DNA hybridization or DNA-RNA hybridization). The polymerase chain-reaction (PCR) and fluorescence in situ hybridization (FISH) are the two most prominent examples (Payment et al., 2003).

**PCR** is the most common DNA amplification method in molecular biology. A classic PCR process includes three steps: denaturation, annealing and extension. Six elements are essential to complete the replicative cycles for amplification: the thermostable DNA polymerase (usually *Thermus aquaticus* (*Taq*) DNA polymerase), template DNA, two oligonucleotide primers (for both strand of DNA), nucleotide triphosphates (to build the copied DNA), buffer (with salts and additives) and thermocycler (to accurately control the temperature). The double-stranded DNA is denatured at 94-96°C into single strands by disrupting the hydrogen bonds between the bases. Then lower temperature is needed for primers to bind to the template DNA, which varies from 45 to 65°C depending on specific primers. After successful annealing, primer extension starts by flanking to their target sequences, with the DNA polymerase catalysis under the optimal temperature (typically between 72 to 74°C). Thus, the target DNA strands are doubled after every three-step cycle and become the templates of the next cycle. After 30 to 50 cycles of the exponential amplification within a few hours, adequate quantity of DNA are synthesized for further analysis. Due to its specificity and rapidity, PCR has, since its publication in 1985, found rapidly expanding clinical and biological applications, including the detection of waterborne bacterial, viral and protozoan pathogens (Wose Kinge, Mbewe, & Sithebe, 2012).
Primer development is crucial to a successful PCR experiment. Bej, Steffan, DiCesare, Haff, and Atlas (1990) chose primer pairs based on lacZ, a gene encoding β-galactosidase, and achieved a detection level as low as 1 cell/100ml for the coliform group. However, Fricker and Fricker (1994) later reported that the same primers failed to distinguish some Hafnia alvei and Serratia odorifera strains in their specificity research on 324 cultured coliform strains. Compared to the heterogenetic coliform group, primers targeting E. coli have more options. Genes associated with proteins and enzymes that are specific to E. coli, such as malB and uidA, have been reported to be successfully applied to E. coli detection (Iqbal et al., 1997; Spierings, Ockhuijsen, Hofstra, & Tommassen, 1993).

PCR techniques have been subjected to continuous modifications in the past three decades for better performance. Nested PCR includes two consecutive runs of amplification, and can reduce unintended primer binding in the second round by using primers from the first amplification (that is, the primers are nested). It has been shown to accurately detect low concentrations of E. coli and pathogens in drinking water thanks to the enhanced specificity (Juck, Ingram, Prévost, Coallier, & Greer, 1996; Tallon et al., 2005). Multiplex PCR incorporates several pairs of primers to different binding sites at the same assay. Bej, McCarty and Atlas (1991) utilized it for simultaneous detection of the total coliform group, E. coli, Salmonella spp. and Shigella spp. and found it only worked well with certain compositions and lengths of primers. Development of bioinformatics tools such as BLAST (Basic Local Alignment Search Tool) facilitates primer design and enables the broader applications of Multiplex PCR for its high throughout and cost-effective features (Kong, Lee, Law, & Law, 2002). Quantitative real-time PCR (qPCR) combines PCR with fluorescence detection to record the accumulation of amplicons in ‘real time’ during the exponential phase of the PCR amplification. It enables more accurate and sensitive detection than traditional end-point detection such as electrophoresis in an agarose gel.
with two commonly used fluorescent reporters, SYBR Green and TaqMan probe. This approach has been applied to the quantitative detection of *E. coli* stains in drinking water (Livak, Flood, Marmaro, Giusti, & Deetz, 1995; Wittwer, Herrmann, Moss, & Rasmussen, 1997).

PCR techniques utilizing DNA as templates are limited in that they cannot distinguish between viable and non-viable organisms, since DNA can survive long after cell death. Reverse transcription PCR (RT-PCR) (including RT-qPCR) overcomes this weakness by detecting RNA through coupling the PCR procedures to a preceding reverse transcription, which synthesizes complementary DNA (cDNA) from RNA templates. Messenger RNA (mRNA) has a much shorter half-life than DNA and therefore is less likely to be detected in the environment outside a living cell. It has been successfully used for enteropathogen detection in natural waters (Fuhrman, Liang, & Noble, 2005). However, the difficulties of extracting intact RNA, especially intact mRNA, impede its wider application in water or other environmental samples (C. J. Smith & Osborn, 2009).

**Fluorescence in situ hybridization (FISH)** is based on the fluorescent labeling realized through in situ DNA probe-target hybridization. A typical FISH protocol for water tests includes filtration of a water sample, fixation and permeation of bacterial cells, hybridisation with a fluorescent probe, washing to eliminate unbound or non-specifically bound material, and microscopic examination (e.g. epifluorescence microscopy or laser scanning electron microscopy). Possible hybridization targets are mRNA, and ribosomal RNA (rRNA, related to the ribosome content and hence the physiological state of cells), allowing for the detection of viable but non-culturable organisms. Fluorescent probes can be acquired commercially or designed according to specific applications with necessary tests against reference microorganisms. Hybridization conditions such as temperature, buffer, and probe concentration are curial to the
desirable specificity and stringency. Different fluorescent labels can be used for multiple-target monitoring.

Due to the lack of taxonomic definition, no specific 16S RNA probe is available for the coliform group. Instead, probes for the family Enterobacteriaceae were developed, such as ENTERO and ENT1 (Loge, Emerick, Thompson, Nelson, & Darby, 1999; Mittelman, Habash, Lacroix, Khoury, & Krajden, 1997). Regnault, Martin-Delautre, Lejay-Collin, Lefèvre, and Grimont (2000) developed a probe for *E. coli* detection. It was shown to be successful in drinking water samples (Delabre et al., 2001). A species-specific Peptide Nucleic Acid (PNA) oligonucleotide probe was reported for the detection of *E. coli* in tap water (Prescott & Fricker, 1999). A combination of Streptavidin-Horseradish Peroxidase and a tyramide signal amplification system was used to for detection, obtaining comparable results to plate count methods under 3 hours. However, PNA probes are less specific than regular oligonucleotide probes and require validation testing beforehand.

1.1.3.3 Immunological methods

Immunological methods are based on the specific recognition between an antibody and the antigen to which it is directed. Due to the high specificity and affinity towards antigens, antibodies are ideal tools for the detection of the target microorganisms present in the environment. The methods mainly include enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assay (IFA). Two types of antibodies are usually employed: polyclonal and monoclonal antibodies. Obst, Hubner, Wecker and Bitter-Suermann (1989) developed a monoclonal antibody against the enterobacterial common antigen for ELISA of *Enterobacteriaceae* in drinking water, necessitating a pre-cultivation to reach a detection limit of
100,000 cells/ml. Research done by Hubner, Steinmetz, Obst, Giebel, and Bitter-Suermann (1992) and Levasseur et al. (1992) with the same antibodies showed improved sensitivity, but also high rates of false positives due to cross-reactivity. An innovative antibody probe of IFA aiming for the stage-specific growth proteins (Dnak, Dps, and Fis) was reported for viability assessment of coliforms in municipal effluent (Rockabrand, Austin, Kaiser, & Blum, 1999). Compared with nucleic acid methods (e.g. FISH), the more limited application of immunological methods in drinking water tests might be due to the fact that monoclonal antibodies have approximately the same specificity as the 16S rRNA oligonucleotide probes (Mittelman et al., 1997), while are much more laborious to produce.

In summary, although cultivation methods are traditionally considered time-consuming and labour-intensive, their low cost, together with the acceptance and standardization of the test kits, maintain them as a strong tool of routine water tests. Molecular methods provide enhanced specificity and rapidity, but suffer from false positives, quantitation difficulties, and high expense. They are more likely to be applied for epidemiology and outbreak investigations than for routine water testing (Ashbolt et al., 2001). Immunological methods are comparatively less prevalent to detect faecal pollution due to false positives and/or unsatisfactory sensitivity and detection limits (Fayer, Morgan, & Upton, 2000).

1.2 Harmful algal blooms (HABs) and blue-green algae

1.2.1 Harmful algal blooms

Algae are plant-like organisms that exist ubiquitously in fresh, estuarine, and marine waters. They encompass a variety of taxonomic groups with both eukaryotic and prokaryotic
members. They range in size from microscopic unicellular phytoplankton, microalgae, with sizes of typically a few micrometers, to the large seaweeds, macroalgae, as long as 60 to 100 meters (Graham & Wilcox, 2000). Most of the algae can undergo photosynthesis like plants. Different pigments (e.g., chlorophylls, carotenoids, and phycobilins) in algae capture different wavelengths of sunlight and lead to the distinctive coloration of algae, such as green and red (Graham & Wilcox, 2000). By utilizing light, CO$_2$ as well as organic and inorganic nutrients in the habitats, algae are able to harvest energy and material in the form of organic carbon compounds. This primary production provides the fundamental organic matter upon which the indigenous aquatic organisms of higher trophic levels feed, either directly or via food web interactions. When the consumption cannot keep pace with the production, and when the hydrographic physical and chemical conditions are in favor, rapid and massive algal proliferation and accumulation with/without visible discoloration, known as blooms, take place. The enhanced development of algae is normally beneficial and important for the sustainability of aquatic ecosystems (Pozdnyakov, Kondratyev, & Pettersson, 2001). However, if the biomass abundance is overwhelming and/or toxin-producing species are involved, the algal bloom could be harmful from organismal, environmental, and human health perspectives, and is termed as a ‘harmful algal bloom’ (HAB). HABs are mostly caused by microalgae or phytoplankton, with fewer cases of macroalgae. There are approximately three hundred species of microalgae that are reported at times to form blooms, which include the eukaryotic green algae or chlorophytes, dinoflagellates, cryptophytes, chrysophytes (including diatoms), and prokaryotic blue-green algae (Hudnell, 2008).

1.2.2 Blue-green algae

1.2.2.1 Blue-green algae basics
Blue-green algae, or cyanobacteria, are the oldest known oxygenic phototrophs, which have been living on Earth for over 3.5 billion years (Graham & Wilcox, 2000; Schopf, 2000). They have experienced numerous environmental alternations, including extreme temperature and irradiance fluctuations, the biosphere changing from anoxic to aerobic, the abundance and scarcity of nutrients (N, P, and minor elements), the particularly wet and dry weathers (Schopf, 2000). They now have a wide geographic distribution, ranging from tropical to polar zones, from hypereutrophic lakes to subsurface soils. The long evolutionary history and tremendous spatial scales enable the blue-green algae to develop diverse adaptive strategies among the various genera to cope with environmental perturbation (Huisman & Hulot, 2005). Their typical ecophysiological characteristics include (Briand, Leboulanger, Humbert, Bernard, & Dufour, 2004; Paerl, Fulton III, Moisander, & Dyble, 2001; Wiedner, Rücker, Brüggemann, & Nixdorf, 2007):

- forming heat and desiccation-tolerant resting cells, or akinetes, and cysts to survive hostile environments;
- using photoprotective sheaths, capsules and pigments to protect them from UV damage when conducting photosynthesis;
- mediating buoyancy with gas vesicles for better light and nutrient availability;
- converting atmospheric nitrogen (N$_2$) into biologically-available ammonia;
- sequestering iron (Fe) and storing P for nutrient needs;
- producing secondary metabolites, including toxins, to counter potentially adverse conditions

The bloom-forming blue-green algae can be morphologically classified into three groups. As shown in Table 4, the three types are coccoid, filamentous non-heterocystous and filamentous heterocystous genera. Coccoid genera are mainly non-N$_2$ fixing; filamentous heterocystous
### Table 4. Representative blue-green algae and their morphology and toxicology facts (sources from (Gaulke, Wetz, & Paerl, 2010; Kuosa, 1991; Paerl, 2014))

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Genera</th>
<th>Toxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coccoid</td>
<td><em>Synechococcus</em></td>
<td>Microcystin; beta-Methylamino-L-alanine (BMAA)</td>
</tr>
<tr>
<td></td>
<td><em>Microcystis</em></td>
<td>Microcystin; Aeruginosin; beta-Methylamino-L-alanine (BMAA); Cyanopeptolin</td>
</tr>
<tr>
<td>Filamentous non-heterocystous</td>
<td><em>Oscillatoria</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Planktothrix</em></td>
<td>Microcystin; Aeruginosin; Anatoxin-a /Homoanatoxin-a; beta-Methylamino-L-alanine (BMAA); Cyanopeptolin</td>
</tr>
<tr>
<td></td>
<td><em>Lyngbya</em></td>
<td>Lyngbyatoxin; Aplysiatoxins; Jamaicamides</td>
</tr>
<tr>
<td></td>
<td><em>Trichodesmium</em></td>
<td>beta-Methylamino-L-alanine (BMAA)</td>
</tr>
<tr>
<td>Filamentous heterocystous</td>
<td><em>Anabaena</em></td>
<td>Microcystin; Anatoxin-a /Homoanatoxin-a; Anatoxin-a(S); beta-Methylamino-L-alanine (BMAA); Cyanopeptolin</td>
</tr>
<tr>
<td></td>
<td><em>Aphanizomenon</em></td>
<td>Microcystin; Anatoxin-a /Homoanatoxin-a; beta-Methylamino-L-alanine (BMAA)</td>
</tr>
<tr>
<td></td>
<td><em>Cylindrospermopsis</em></td>
<td>Anatoxin-a /Homoanatoxin-a; beta-Methylamino-L-alanine (BMAA); Microcystin</td>
</tr>
<tr>
<td></td>
<td><em>Nodularia</em></td>
<td>Nodularin; beta-Methylamino-L-alanine (BMAA)</td>
</tr>
<tr>
<td></td>
<td><em>Calothrix</em></td>
<td>beta-Methylamino-L-alanine (BMAA)</td>
</tr>
<tr>
<td></td>
<td><em>Scytonema</em></td>
<td>beta-Methylamino-L-alanine (BMAA)</td>
</tr>
<tr>
<td></td>
<td><em>Lyngbya</em></td>
<td>Anatoxin-a /Homoanatoxin-a; Aplysiatoxins; beta-Methylamino-L-alanine (BMAA)</td>
</tr>
<tr>
<td></td>
<td><em>Oscillatoria</em></td>
<td>Microcystin; Anatoxin-a /Homoanatoxin-a; Aplysiatoxins</td>
</tr>
</tbody>
</table>
genera are generally N\textsubscript{2} fixers; filamentous non-heterocystous genera include both types. Many genera can produce potent toxins that affect the hepatopancreatic, digestive, endocrine, dermal, and nervous systems of mammals (including humans), fish, birds, etc. (Aráoz, Molgó, & De Marsac, 2010; Brand, Pablo, Compton, Hammerschlag, & Mash, 2010; Carmichael, 2001). Hepatotoxins are the most commonly occurring blue-green algae toxins, the largest group being cyclic heptapeptides, known as microcystins. To date, more than 80 congeners of microcystins have been identified, with potency varying with the specific chemical structure (Pearson, Mihali, Moffitt, Kellmann, & Neilan, 2010). Many blue-green genera are accountable for microcystin production, including \textit{Synechococcus}, \textit{Microcystis}, \textit{Planktothrix}, and \textit{Anabaena} (see Table 4).

1.2.2.2 Impacts of blue-green algae

Over the past few decades there has been extensive proliferation and increased incidence of HABs caused by blue-green algae, which are often referred to as cyanobacteria HABs, or CyanoHABs (Paerl et al., 2001). The affected geographic spectrum covers coastal regions of numerous resourceful water bodies, including Lake Victoria (Africa), Lake Erie and Lake Michigan (USA–Canada), Lake Okeechobee (Florida, USA), Lake Ponchartrain (Louisiana, USA), Lake Taihu (China), the Baltic Sea, Caspian Sea, tributaries of Chesapeake Bay, North Carolina’s Albemarle-Pamlico Sound, Florida Bay, the Swan River Estuary in Australia (Paerl, Hall, & Calandrino, 2011). During the completion of this thesis, Lake Erie and in Lake St. Clair were reported to have a severe toxic algal bloom in July, which could probably rival the bloom of 2011, the most severe bloom in decades (Matheny, 2015).

The notorious syndromes of CyanoHABs start with water clarity loss, which then leads to visible biomass accumulation and bloom development, sometimes accompanied with repulsive
odours. The gradual dominance of blue-green algae in the aquatic system prevents other microphytes/macrophytes from light acquisition for photosynthesis, which inevitably affects invertebrate and fish habitats by altering food web dynamics (Glibert, Anderson, Gentien, Graneli, & Sellner, 2005). Respiration of massive algae and bacterial decomposition of dying blooms cause oxygen depletion (hypoxia and anoxia), and result in the loss of benthic fauna and flora and fish deaths (Paerl et al., 2001). Dense algae biomass itself also causes fish deaths by damaging or clogging fish gills (Hudnell, 2008).

In addition, a large amount of blue-green algae genera (e.g. *Nodularia*, *Microcystis*) can release cyto-, hepato-, and neurotoxic secondary metabolites to the aquatic ecosystems. They have been reported to be responsible for mass mortality and morbidity events involving wild and farmed fish and shellfish, mammals, seabirds, etc. (Carmichael, 2013; Stewart, Seawright, & Shaw, 2008). The affected waters that are used for drinking water, irrigation, fishing and recreational purposes pose severe hazards to human health. Ingestion (including through seafood), dermal contact, and aspiration or inhalation of the noxious water has been linked to liver, digestive and skin diseases, neurological impairment and even death (Carmichael, 2001; Humpage, 2008).

In addition to their negative ecological, biogeochemical and health impacts, CyanoHABs also result in enormous economic losses on tourism, fisheries, and increased water management costs. It is estimated that CyanoHABs cause overall US$2.2-4.6 billion of economic costs annually in the USA (Dodds et al., 2009) and AU$180-240 million in Australia (Atech, 2000).
1.2.2.3 Causes of blue-green algae

The two main driving forces behind the massive and extensive development of blue-green algae worldwide are nutrient over-enrichment (leading to eutrophication) and climatically-induced environmental change (e.g. global warming, increased frequencies and intensities of storm events, more severe and persistent droughts) (Elliott, 2010; Paerl et al., 2001; Paerl & Huisman, 2008). The inherent superiorities of blue-green algae enable them to maximize growth under the beneficial conditions, outcompeting the indigenous/beneficial phytoplankton, and hence maintaining their dominance.

1.2.2.3.1 Anthropogenic eutrophication

Of all the factors that contribute to bloom formation, anthropogenic eutrophication is the one that has received the most attention. Eutrophication, by definition of Nixon (Nixon, 1995), refers to ‘an increase in the rate of supply of organic matter to an ecosystem’. Natural eutrophication in water bodies is the aging process resulting from natural nutrient enrichment, typically from an oligotrophic lake, to a nutrient-rich pond, then to a marsh, over hundreds to thousands of years (Wetzel, 1993). Human activities accelerate the natural process by promoting nutrient inputs, especially P and N, from the primary production of indigenous organisms, as well as adjacent waters and land plagued by PS and NPS nutrient discharges. PS include sewage treatment plants and other identifiable industrial and municipal discharges that are close to watersheds. NPS are mostly diffuse inputs from fertilizer usage, livestock and pet waste, atmospheric deposition, etc.; they are accountable for the major part of anthropogenic nutrient loading, and are harder to locate and control than PS. Numerous bloom outbreaks have been reported to have strong links to increased nutrient loading. For example, red tides in Tolo Harbor,
Hong Kong, showed a remarkable increase from 1976 to 1986 that strongly paralleled the rise of N and P discharges of 25- and 6-fold, respectively (Lam & Ho, 1988). Elevated nutrient loading into an embayment in Brazil has resulted in the enrichment of toxic blue-green algae species, *Microcystis aeruginosa* (Ferrao, Domingos, & Azevedo, 2002).

P and N are the key nutrients for primary productivity due to their relative scarcity in aquatic ecosystems; one or both of them could become the limiting factor of algal growth depending on the availability and ratio of the two. In marine and estuarine waters (salinity>5 practical salinity units, or psu), N is usually considered as the principle nutrient that limits algal growth because it is less abundant (relative to demand) than P; freshwaters and oligohaline estuarine water with lower salinity (<5 psu) and thus less P are more P-limited in terms of algal development (Paerl & Otten, 2013). Excess P (relative to N) loading has been identified as contributing to the CyanoHABs, especially for the N\textsubscript{2}-fixing genera (e.g., *Aphanocapsa*, *Raphidiopsis*, *Woronochinia*) since they can partially supply their own N needs (Downing, Watson, & McCauley, 2001; V. H. Smith, 1983). Traditionally, watershed nutrient restriction efforts toward bloom controls have focused on P reduction, especially in the case of freshwater CyanoHABs. Measures such as the phosphate detergent bans since the late 1970s, the restricted application of P-fertilizers, and the monitoring of total maximum daily loads (TMDL) for P have worked jointly to decrease bloom activities in large European and Asian lakes (e.g., Lakes Constance and Lucerne, Germany-Switzerland; Lake Trummen, Sweden; Lago Maggiore, Italy; Lake Biwa, Japan) as well as the Great Lakes of North America (Paerl et al., 2001).

With the shift to the use of synthetic N fertilizers, continuing growth of human population and the accompanied domestic waste, and atmospheric deposition with more nitrogen compounds (e.g., nitric oxide, or NO; nitrogen dioxide, or NO\textsubscript{2}), the nutrient loading dynamics
have changed substantially. Currently the N loads are frequently eclipsing P inputs in the receiving waters (Boyer et al., 2006; Paerl, 1997). Excessive N, together with P, has increasingly become the trigger of CyanoHABs in downstream freshwater and marine ecosystems (Paerl, 2009). Microcystis and other non-N$_2$ fixing genera are now the dominant component of the populations of new or resurgent CyanoHAB events, partly because of the input of organic and inorganic forms of N. For example, Lake Eire, USA-Canada, which had been bloom-free for almost twenty years due to effective nutrient (mostly P) management, began once again to experience algal blooms since the 2000s, with non-N$_2$ fixing taxa such as Microcystis and Anabaena as the key culprits (Rinta-Kanto, Konopko, et al., 2009; Rinta-Kanto, Saxton, et al., 2009).

1.2.2.3.2 Climate change and other factors

In addition to N and P enrichment, climate change and the associated hydrological, physical and chemical disturbances also play an important role in the expansion and proliferation of blue-green algae (Briand et al., 2004; Elliott, 2010; Paerl & Huisman, 2008; Paul, 2008).

Over the past few decades, the world has been experiencing more intense rain and snow storms as well as more severe and protracted droughts (ICCP, 2007). In the short term, the storm events may prevent bloom forming by enhanced flushing rates which reduces nutrient levels. However, given sufficient time, the resultant larger total nutrient discharges will inevitably lead to enrichment and promote algal outbreaks (Paerl & Otten, 2013). Moreover, the enhanced nutrient loading and the summer droughts, combined with increased water withdrawal by humans, have caused rising salinity in fresh water (Nielsen & Brock, 2009; Schallenberg, Hall, & Burns, 2003). Many blue-green algae, including Anabaena, Microcystis and Nodularia, are salt-
tolerant, even though they are mostly found in freshwater (Moisander, McClinton, & Paerl, 2002). They can thrive in freshwater of appreciable salinity like they do in other brackish waters, such as the Baltic Sea and the San Francisco Bay Delta (Paerl & Fulton III, 2006).

As mean temperatures rise within the global warming context, growth of blue-green algae is generally favored over eukaryotic phytoplankton (Butterwick, Heaney, & Talling, 2005; Watkinson, O’Neil, & Dennison, 2005). Research shows that Microcystis, representative taxa of blue-green algae, achieves and maintains optimal growth when the temperature exceeds 25 °C; the growth rates of other eukaryotic representatives either decline or level off before 25 °C (Grzebyk & Berland, 1996; Reynolds, 2006; Yamamoto & Nakahara, 2005). The high temperature endurance of blue-green algae is mostly due to their prokaryotic nature, which endows them with vitality in harsh conditions such as high temperature and high salinity. Also, their possession of photoprotective pigments (e.g. carotenoids) and UV-absorbing compounds (mycosporine-like amino acids (MAAs), scytonemin) helps to reduce UV stress (e.g. irradiance, production of antioxidant enzymes) while maintaining a surface position to shade out and suppress competitors (Carreto & Carignan, 2011; Paul, 2008). Further, the increased temperature lowers the nutrient thresholds required for blue-green algae blooms, with CyanoHABs proceeding at lower nutrient levels at elevated temperatures than would be required at cooler temperatures (Paerl & Paul, 2012). This gives blue-green another advantage when competing for nutrient budgets. The increased temperature also benefits bloom formation (including the blue-green algal species) by increasing the annual growth period (early melting of the ice covers and longer growing windows) (Peeters, Straile, Lorke, & Livingstone, 2007; Wiedner et al., 2007). This impact is especially obvious for the polar species of blue-green algae; extended ice-free period and warmer water temperatures allow for enhanced algal reviving, nutrient cycling and photosynthetic production, facilitating bloom formation and dominance (Vincent, 2002). Lastly,
with the massive growth of blue-green algae under desirable temperature (and other) conditions, the photosynthetic activity is intensified and absorbs enormous amounts of energy and heat. This in turn increases the ambient temperature and forms a positive feedback loop, further perpetuating the competitive advantages of blue-green algae (Ibelings, Vonk, Los, van der Molen, & Mooij, 2003).

Another consequence resulting from the climatic disturbances is enhanced vertical stratification of lakes, which also encourages blue-green algae growth. Stratification stems from the physical or chemical differences of the water masses that affect its density. The increased salinity and temperature gradients between the surface and the deeper water as discussed above promote and intensify stratification (Paerl & Fulton III, 2006). Furthermore, the stratification period is lengthened by the earlier start in spring and persistence until later in fall due to the increased temperature (Elliott, 2010; Peeters et al., 2007). The stratified water hinders nutrient cycling and the mobility of phytoplankton; some blue-green algae, however, are less confined by these barriers. By altering their buoyancy, mediated by gas vesicles, they are capable of conveniently migrating up to the surface area for optimal photosynthesis conditions (Huisman & Hulot, 2005), as well as down to deeper water or other euphotic zones for better nutrient acquisition (Ibelings, Mur, & Walsby, 1991; Reynolds, 2006). The stratification of the water column can also cause bottom water hypoxia or anoxia, which often promotes internal transferring of P from P loading, from the sediments in the redox sensitive Fe-bound and organic P pools, and fuels bloom formation (Paerl, 1988).

With better access to surface water, buoyant blue-green algae enjoy richer CO₂ resources than sub-surface phytoplankton, in addition to the more desirable higher temperature and more intense light. With the increasing concentration of atmospheric CO₂, the high CO₂ demands of
photosynthesis of blue-green algae in blooms are greatly facilitated. Utilizing the effective single-cell CO₂ concentrating mechanism (CCM) (Van, Wang, Nakamura, & Spalding, 2001; Vance & Spalding, 2005), blue-green algae can intercept atmospheric CO₂ and reduce further diffusion into the bulk water as dissolved inorganic carbon (DIC) (Paerl & Ustach, 1982). The limited DIC, coupled with poor light penetration, further disadvantages the photosynthetic growth of eukaryotic algal populations below the surface scums (Huisman et al., 2004).

1.3 Links between blue-green algae and faecal indicator bacteria

Algal-bacterial interactions play an important role in aquatic ecosystems. Algae generally represent the primary sources of organic nutrients for heterotrophic bacteria; bacterial remineralization of organic nutrients produces biogenous elements algae need. The interactions can be categorized as one of three types: (1) symbiotic, where both algae and bacteria benefit from presence of each other for nutrient acquisition or other growth factors; (2) antagonistic, where algae can inhibit bacterial growth with antibiotics or other bioactive substances, and bacteria can lyse algae by penetrating into the periplasmic space of algal cells; (3) commensalistic, where algae and bacteria can live together with no positive or negative impacts on each other (Doucette, 1995; Grossart, 1999; Grossart & Simon, 2007; Pernthaler & Rudolf, 2005; Sapp et al., 2007). These interactions could exist simultaneously and be highly variable depending on the specific environments and physiological status of participants (Grossart, 1999; Grossart & Simon, 2007).

In the symbiotic category, Wang, Shen, Shi, Chen, Ni, and Xie (2015) recently reported that five heterotrophic bacteria (*Aeromonas veronii, Enterobacter aerogenes, Exiguobacterium acetylicum, Bacillus cereus* and *Shewanella putrefaciens*) were found to be able to facilitate
Microcystis aggregation by inducing exopolymer production, while feeding on extracellular organic matter released from Microcystis cells. During the bloom formation of Anabaena oscillarioides, the attachment of Pseudomonas aeruginosa on A. oscillarioides heterocysts was observed to be related to the amount of algal biomass production and N\textsubscript{2} fixation (Paerl & Gallucci, 1985).

In the antagonistic category, some bacteria possess algicidal activity (e.g. lytic effect) towards blue-green algae (e.g., Microcystis aeruginosa) and can be employed as an environmentally-friendly means to control CyanoHABs (Kim et al., 2007; Shao et al., 2014). On the other hand, blue-green algae were studied for antibacterial characteristics for pharmaceutical purposes; some of them produced biomolecules that showed inhibitory activity against E. coli (Madhumathi, Deepa, Jeyachandran, Manoharan, & Vijayakumar, 2011; Volk & Ferkert, 2006; Yadav, Sinha, & Tyagi, 2012).

Commensalistic co-occurrence of E. coli and algae was mostly seen with macroalgae such as Cladophora spp and Ulva (Beckinghausen, Martinez, Blersch, & Haznedaroglu, 2014; Byappanahalli, Shively, Nevers, Sadowsky, & Whitman, 2003; Quero, Fasolato, Vignaroli, & Luna, 2015). Cladophora algal mats were reported to be able to harbor high amounts of E. coli and other FIB, with some exceeding $10^5$ CFUs/cm\textsuperscript{2} (Beckinghausen et al., 2014; Ksoll, Ishii, Sadowsky, & Hicks, 2007).

To the best of the author’s knowledge, there are no available reports addressing directly the dynamics of blue-green algae and E. coli or other faecal indicators in the same sample medium. Previous reports of antagonistic effects of compounds from blue-green algae on E. coli might lead us to predict that E. coli will not grow readily in a culture with this algae, but
experiments to directly monitor the growth and survival of the bacteria are needed. This is especially important from the point of view of the effectiveness of FIB to indicate water quality when there is a risk of HABs being present at the same time.

1.4 Research objectives

The prevalent anthropogenic nutrient enrichment, the alternation of the water environment induced by global climate change, together with the diverse suite of adaptive strategies of prokaryotic phytoplankton, have made blue-green algae increasingly common fixtures in various water systems. The influence that their presence exerts on the aquatic community composition and dynamics remains unclear and is subjected to extensive investigation. This thesis will concentrate on the possible influence of blue-green algae on the monitoring of FIB, sentinels for faecal contamination events. As discussed in the previous section, the effectiveness of indicator systems for tracking faecal microbial contamination is built on the assumption that the presence/abundance of FIB can reflect the status of some other pathogens of concern. Therefore, it is of great significance to investigate the factors that could potentially change the growth pattern of FIB, either supportively or oppressively, which could undermine the use of FIB.

Research of this thesis intends to investigate the growth pattern of selected FIB in the presence of the blue green algae *Microcystis*, and to explore the factors that may affect the algae-FIB dynamics. To this end, laboratory microcosms to co-grow faecal indicator bacteria (*E. coli* consortium) and blue-green algae (*Mycrocystis aeruginosa; Myrcocystis smithii*) were established; Chapter 2 will be dedicated to explaining the design of the experiments, including the main apparatus, methodology and the preliminary tests. The complete description of the
experiments, results and discussion will be presented in Chapter 3, with emphasis on the lab strains of *E. coli* and a slight extension to lake water strains of FIB. Lastly, Chapter 4 will conclude with main findings of the experiment, the implication for water monitoring and water-quality problems associated with faecal contamination and harmful algal blooms, and suggest directions for future work.
Chapter 2
Experimental Design and Methodology

Chapter 1 reviewed microbial contamination from faecal sources and the use of FIB such as *E. coli*, as well as the increasingly concerning HAB events caused by blue-green algae. Research questions arising from the review are: Would the presence of blue-green algae affect the growth pattern of FIB, and thus interfere with their use to indicate potential faecal contamination in water? If yes, what could be the possible affecting factors? What is the implication for water monitoring and water-quality problems associated with both faecal contamination and harmful algal blooms? Work in this thesis attempts to tackle these questions at the lab scale, as an initial step to explore the algae-FIB dynamic interactions. Laboratory microcosms to co-grow blue-green algae (*Microcystis* spp.) and faecal indicator bacteria (*E. coli* consortium) were established for *E. coli* monitoring. This chapter describes the experimental design using *Microcystis* spp. cultures, *E. coli* determination with a TECTA microbiology system, and investigations of the possible dynamic regulators.

2.1 Materials and apparatus

Isolates of two *Microcystis* species, *M. smithii* and *M. aeruginosa*, were provided courtesy of Dr. Yuxiang Wang’s group (Department. of Biology, Queen’s University, Kingston, ON). Lab strain *E. coli*, ATCC® 25922, 8739, 51813, and 11775, were purchased from ATCC (Thermo Fisher Scientific Oxoid Ltd, Basingstoke, UK). Lake water was collected in Lake Ontario near Kingston, ON, in September, 2014 as a source of naturally occurring strains of *E. coli* and Total coliforms. The main apparatus utilized for *E. coli* and algae growth monitoring and...
variable measurement were TECTA™ B16 microbiology system and TECTA™ CCA test cartridge (ENDETEC, Kingston, ON), Colilert®-18/Quanti-Tray® (IDEXX Laboratories, Inc., Westbrook, ME), hemacytometer (Qiujing, Shanghai, China), Labophot microscope (Nikon, Japan) with camera attachment, SpectraMax® Plus 384 spectrophotometer (Molecular Devices, Sunnyvale, CA) with 1 cm pathlength glass and quartz cuvettes, Orbitrap Velos Pro™ Hybrid Ion Trap-Orbitrap Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA) and QuantiPlate™ Kit for Microcystins (Envirologix, Portland, OR). 47-mm Whatman® GF/F glass microfiber filters (Whatman, UK) and vacuum filtration assembly flasks were used for sample filtration. 4 L Erlenmeyer flasks were used for algae culturing; other vessels included 25 mL and 50 mL volumetric flasks and 15 mL centrifuge tubes with Teflon lined caps for N and P determination. Chemicals used in the experiments were of analytical grade or of the highest purity available from Thermo Fisher Scientific (Ottawa, ON), including sodium hydroxide (NaOH), hydrochloric acid (HCl), potassium nitrate (KNO₃), potassium dihydrogen phosphate orthophosphate (KH₂PO₄), potassium persulfate (K₂S₂O₈), ammonium molybdate ((NH₄)₆Mo₇O₂⁴·4H₂O) and acetone.

2.2 Blue-green algae, *Microcystis* spp., culturing system

Two *Microcystis* species, *M. smithii* and *M. aeruginosa*, were cultured with MA medium (Ichimura, 1979). This medium was specifically developed for *Microcystis* strains, characterized by a high pH and high levels of elements such as boron (B), manganese (Mn), zinc (Zn). The recipe for 1 L of MA medium is shown in Table 2.1. The preparation procedure was as follows: into 900 mL distilled water (dH₂O), the listed ingredients were dissolved, then the final volume brought to 1 L with distilled water and the pH adjusted to 8.6 with 1 mol/L sodium hydroxide.
(NaOH). The medium was autoclaved at 121 °C for 30 minutes to ensure sterility before being used for algal culturing.

**Table 5. MA medium recipe for 1 L of culture** (Ichimura, 1979)

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock Solution (g/L dH₂O)</th>
<th>Quantity Used</th>
<th>Concentration in Final Medium (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>50.0</td>
<td>1 mL</td>
<td>5.88 x 10⁻⁴</td>
</tr>
<tr>
<td>KNO₃</td>
<td>100.0</td>
<td>1 mL</td>
<td>9.89 x 10⁻⁴</td>
</tr>
<tr>
<td>Ca(NO₃)₂ · 4H₂O</td>
<td>50.0</td>
<td>1 mL</td>
<td>2.12 x 10⁻⁴</td>
</tr>
<tr>
<td>Na₂ β-glycerophosphate · 5H₂O</td>
<td>50.0</td>
<td>1 mL</td>
<td>1.63 x 10⁻⁴</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>40.0</td>
<td>1 mL</td>
<td>2.82 x 10⁻⁴</td>
</tr>
<tr>
<td>MgCl₂ · 6H₂O</td>
<td>50.0</td>
<td>1 mL</td>
<td>2.46 x 10⁻⁴</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>20.0</td>
<td>1 mL</td>
<td>3.23 x 10⁻⁴</td>
</tr>
<tr>
<td>Na₂EDTA · 2H₂O</td>
<td>5.0</td>
<td>1 mL</td>
<td>1.34 x 10⁻⁵</td>
</tr>
<tr>
<td>FeCl₃ · 6H₂O</td>
<td>0.5</td>
<td>1 mL</td>
<td>1.85 x 10⁻⁶</td>
</tr>
<tr>
<td>MnCl₂ · 4H₂O</td>
<td>5.0</td>
<td>1 mL</td>
<td>2.53 x 10⁻⁵</td>
</tr>
<tr>
<td>CoCl₂ · 6H₂O</td>
<td>5.0</td>
<td>1 mL</td>
<td>2.10 x 10⁻⁵</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>0.5</td>
<td>1 mL</td>
<td>3.67 x 10⁻⁶</td>
</tr>
<tr>
<td>Na₂MoO₄ · 2H₂O</td>
<td>0.8</td>
<td>1 mL</td>
<td>3.31 x 10⁻⁶</td>
</tr>
</tbody>
</table>

A photograph of the culture system is showed in Figure 3. Five 4 L flasks were thoroughly cleaned, filled with 3 L of MA medium and labelled from left to right as #1 to #5. After medium being autoclaved, axenic *M. smithii* inoculum was transferred from the stock culture to flasks #1 and #2; axenic *M. aeruginosa* was added to flasks #3 and #4. Flask #5 served as the control with only axenic *E. coli* addition. All the cultures were incubated with aeration at
room temperature of 22±1 °C, illuminated by four 32-watt T8 48-inch cool white fluorescent tubes on 16:8 light:dark (L:D) cycle. Normally after 7 to 14 days, the algal culture reached maximum growth, depending on the species and conditions of initial algal inoculum.

![Figure 3. Blue-green algae culture system assembly: flasks containing 3 L MA medium were incubated with “cool white” fluorescent lamps on a 16:8 light:dark cycle at room temperature (22±1 °C). Flasks were aerated continuously using a standard aquarium aerator.](image)

**2.3 TECTA microbiology system for *E. coli* and total coliform detection**

**2.3.1 Operation of the TECTA microbiology system**

Due to its advantages for coloured and turbid sample tests (see Section 1.1.3.1, Chapter 1), the TECTA B 16 microbiology detection instrument was used for *E. coli* and total coliform monitoring in this thesis. Specifically, 100 mL of water sample (or diluted sample) was transferred aseptically to the test cartridge and sealed tightly. Dilution ratio of 1:9 was adopted for most of the experiments to reduce consumption of medium with regular testing but still sensitive
enough for tracking bacteria populations. The cartridge was thoroughly shaken until the ingredients were fully dissolved, which usually took several minutes with water of around 21 °C. After setting the test variables, such as test mode and test type, the cartridges were loaded into the chambers of the TECTA and the lid was closed to start incubation and testing. The TECTA provides P/A results and the time to detect the growth of bacteria, called time to detect, or TTD. When the calibration curve is determined for a given matrix (see Section 2.3.2 for details), the TECTA also gives an estimated bacteria count of *E. coli* and Total coliforms.

### 2.3.2 Calibration of the TECTA response

The TTD values determined by the TECTA are inversely related to the number of bacteria initially present in a sample. To calibrate the TECTA response, samples spiked with known amounts of *E. coli* were prepared, ranging from 492 to 4917 CFUs/100mL. These samples were tested in the TECTA system, providing the corresponding TTD values. A graph of TTD (as the y-axis) against the base-10 logarithm of the *E. coli* cell levels was plotted, as shown in Figure 4. The linear regression equation (R² = 0.97) derived from the graph was used for *E. coli* quantitation in the following experiments.
Figure 4. Calibration curve for *E. coli* determination with TECTA system: Time to detection (TTD) was plotted against the base-10 log concentration of *E. coli* organisms, where concentration was determined using a laboratory reference test for the same inoculation stock culture.

2.3.3 Algal matrix interferences

Some matrix components present in algae culture samples could affect the performance of TECTA system by interfering with the target enzyme activity or the uptake of fluorescent products into the optical detection path. These interferences, if present, would lead to inaccurate quantitation results. Therefore, TECTA performance with the algal matrix was investigated as part of the method development and validation. The algal matrix interferences were examined with *M. aeruginosa* samples. After 10 days of culturing, sample water from the culture vessel was taken for TECTA analysis. When the results were confirmed to be *E. coli* negative, 0, 5 and 20 mL of algae water were diluted to 100 mL in a TECTA cartridge, then spiked with the same amount of *E. coli* 25922 (10-100 CFUs). Each sample was run in duplicates. TTD of the samples with distinctive algae biomass didn’t present significant differences (relative standard deviation, or RSD, 0.30-3.3%), as illustrated on the primary y-axis in Figure 5. The CFU counts were
derived from the calibration equation showed in Figure 4 and mapped out on the secondary y-axis of Figure 5. The mean recoveries of *E. coli* were 113% and 101% for samples with 5 mL and 20 mL algae culture, respectively, suggesting negligible algal matrix interference in the TECTA tests in the proposed experimental method.

![Graph showing TTD and E. coli concentration results](image)

**Figure 5.** TTD and *E. coli* concentration results provided by the TECTA instrument for analysis of samples with different dilution volumes of algal cultures. All samples were inoculated with the same number of *E. coli* bacteria after dilution.
2.4 Chlorophyll $a$ measurement and cell counts for algal growth monitoring

To determine the possible interferences from *E. coli* in chlorophyll $a$ measurements, the algae growth with and without *E. coli* spiking were monitored and compared. Chlorophyll $a$ is a major algal pigment and was used as an indicator of algal biomass. The measurement procedure was a standard method using visible wavelength spectrometry (Glover, Campbell, & Prezelin, 1986), as follows:

**Sample preservation and preparation**

i. Chlorophyll $a$ (algae) samples were handled in subdued light from time of collection to prevent photochemical breakdown of the chlorophyll.

ii. Samples were kept in a dark cooler and stored in the refrigerator at 4 °C until further processing within 48 hours of collection.

iii. A 10 to 50 mL volume of sample was filtered, depending on the expected chlorophyll $a$ content, through a 47-mm glass fiber filter, applying vacuum until the sample was dry.

iv. The filter was folded into quarters, wrapped in aluminum foil and stored frozen (-20 °C) in ziplock bags for at least 24 hours. Frozen samples were held for up to 28 days from the time of filtration until extraction.

**Chlorophyll $a$ extraction**

i. Samples were frozen at -20 °C and left for 20 minutes; then thawed immediately at room temperature for 5 minutes. This freeze/thaw cycle was repeated three more times to break the cell membranes, facilitating the later extraction.

ii. Sample filters were removed from the foil wrap, placed in a 15 mL centrifuge tube, and steeped in 10 mL 90% acetone to extract chlorophyll from the algal cells. The centrifuge tubes were vortexed vigorously to mix well and then stored at 4 °C for 8-10 hours, to allow for complete extraction.
Spectrophotometric reading and calculation

i. The spectrophotometer was turned on at least 5 minutes before use.

ii. A 1 cm pathlength cuvette was filled with 1 mL 90% acetone to zero the spectrophotometer.

iii. The extract was clarified by centrifuging for 15 min at 3500 rpm.

iv. 1 mL of the clear supernatant was carefully transferred to a cuvette and the absorbance at 750, 664, 647, and 630 nm were measured using the multi wavelength mode of the spectrophotometer.

v. The absorbance at 750 nm is affected by background contributions to absorbance, such as turbidity, and was subtracted from the 664, 647, and 630 nm values for background correction.

vi. The chlorophyll $a$ concentration was calculated using Jeffrey and Humphrey’s Trichromatic Equation (Glover et al., 1986):

$$\text{Chlorophyll } a \ (\frac{\mu g}{L}) = \frac{[11.85(A_{664} - A_{750}) - 1.54(A_{647} - A_{750}) - 0.08(A_{630} - A_{750})]V_1}{V_2L}$$  \hspace{1cm} (1)

Where

- $A_{664}$ = absorbance at 664 nm
- $A_{647}$ = absorbance at 647 nm
- $A_{630}$ = absorbance at 630 nm
- $A_{750}$ = absorbance at 750 nm
- $V_1$ = the volume of 90% acetone used for extraction (mL)
- $V_2$ = the volume of water filtered (L)
- $L$ = the cell path length (cm)

Another growth variable examined was the algae cell count. It was determined with a 20 $\mu$L sample on a haemocytometer slide (Qiuqing) under a Nikon Labophot microscope (x400 magnification). The cells in the designated boxes were counted and recorded.
2.5 Nitrogen and phosphorus measurement

Nitrogen (N) and phosphorus (P) are the key nutrients to the growth of both algae and E. coli. The continuous monitoring of the nutrient change are crucial to interpreter the algae-bacteria dynamics (see Section 3.1.2, Chapter 3 for details). The total nitrogen (TN) and total phosphorus (TP) determination in this work took the associated international standards, ISO 11905-1 and ISO 6878 (ISO, 1997, 2004), as references. For TN measurement, alkaline K$_2$S$_2$O$_8$ was used to oxidize the organic nitrogen and ammonia to nitrate, which, together with the inorganic nitrate present, represents TN. Absorbance of nitrate, which is proportional to the TN concentration, was measured using a spectrometer at 220 nm, wavelength with maximum absorbance of nitrates. Since some dissolved organics also have 220 nm absorbance, a second absorption at 275 nm was measured for correction. At this wavelength, nitrates do not absorb, but dissolved organics do. The final nitrate absorption was calculated by the following equation:

$$A_{TN} = A_{220} - 2A_{275}$$

Where

- $A_{TN}$ = absorbance of nitrate
- $A_{220}$ = absorbance at 220 nm
- $A_{275}$ = absorbance at 275 nm

In TP determination, the orthophosphate in the presence of ascorbic acid reacts with (NH$_4$)$_6$Mo$_7$O$_{24}·4H$_2$O to form a strongly colored molybdenum blue complex. The maximum absorbance of this complex at 700 nm was measured to determine the concentration of orthophosphate present. Organophosphorus compounds were mineralized to orthophosphate by K$_2$S$_2$O$_8$ prior to the color development to evaluate the TP level. The TN and TP measuring procedures are described as follows.
TN Detection and spectrometric reading

i. A 10 mL of sample or diluted sample, depending on estimated TN level, was added into a 25 mL volumetric flask.

ii. 1 mL of 1.88 M NaOH was added to the flask to create an alkaline environment that the following oxidation needs.

iii. 5 mL of 0.15 M recrystallized K$_2$S$_2$O$_8$ was added to the flask as the oxidizing agent.

iv. The flask was autoclaved at 121 °C for 30 minutes to complete the oxidation.

v. After the liquid had cooled to room temperature, 1 mL of 1M HCl was added to eliminate potential interferences on ultraviolet absorbance measurement of nitrate.

vi. Ultrapure water was added to the flask to bring the final solution volume to 25 mL.

vii. 1 mL of solution in the above flask was added to a 1 cm pathlength quartz cuvette.

viii. Spectrometric reading was performed at 220 nm and 275 nm wavelengths for the nitrate absorbance.

ix. The total nitrate concentration was calculated using the nitrate absorbance and calibration curve (see Figure 6 for details).

TP Detection and spectrometric reading

i. A 25 mL of sample or diluted sample, depending on estimated TP level, was added into a 50 mL volumetric flask.

ii. 4 mL of 0.18 M K$_2$S$_2$O$_8$ was added to the flask for oxidation.

iii. The flask was autoclaved at 121 °C for 30 minutes.

iv. After the liquid had cooled to room temperature, 1 mL of ascorbic acid and 2 mL of ammonium molybdate were added to the flask to form blue phosphomolybdenum complex for spectrometric measurement.

v. Ultrapure water was added to the flask to bring the final volume to 50 mL.
vi. 1 mL of solution in the above flask was added to a 1 cm pathlength cuvette.

vii. Spectrometric reading was performed at 700 nm within 15 minutes.

viii. The total phosphorus concentration was calculated using the orthophosphate absorbance and calibration curve (see Figure 6 for details).

Calibration curves

Standard solutions were prepared with KNO₃ and KH₂PO₄ solutions for TN and TP determination, respectively. Specifically, 0.20, 0.50, 1.00, 3.00 and 7.00 ml of 10 mg/L KNO₃ solutions were transferred, by means of a volumetric pipette, to 25 mL volumetric flasks and brought to 10 ml with ultrapure water. These solutions represented KNO₃ amounts of 2.00 µg to 70.0 µg. 0.50, 1.00, 3.00, 5.00 10.0 and 15.0 ml of 2 mg/L KH₂PO₄ solutions were transferred to 50 mL volumetric flasks and brought to 25 ml with ultrapure water, giving KH₂PO₄ amounts ranging from 1.00 µg to 30.0 µg. These standard solutions were processed with the TN/TP detection procedures. The absorbance values from the spectrophotometric measuring were recorded to plot ‘TN/TP mass per sample (concentration) versus absorbance’ graph, as shown in Figure 6. The relationship between concentration and absorbance was linear for both TP and TN calibration. Linear regression equation was derived from the graph for concentration determination of tested samples in the following experiments.
Figure 6. Calibration curves for TN and TP measurement (mass of TN/TP vs. absorbance)

Interferences

Major interferences to TN/TP detection can come from contamination of glassware or reagents. To prevent this, glassware were washed in 1 M HCl, rinsed thoroughly with distilled water and stored in an ammonia-free environment if it was for TN detection. Detergents containing phosphate were avoided if it was for TP detection. In TN determination, high-purity sodium hydroxide (≥ 99.9%) and K$_2$S$_2$O$_8$ were needed. Recrystallization of crude K$_2$S$_2$O$_8$ from water was performed weekly since it can easily get contaminated. Blank tests using the same amount of pure water sample (10 ml for TN detection and 25 ml for TP detection) and the same detection procedure described above were carried out in parallel with every determination, and absorbance of the blank sample was subtracted from the value measured for the corresponding sample (including the standard solutions). If absorbance of the blank test was abnormal (e.g., ≥ 0.030 in TN determination), water, chemicals, glassware and utensils were carefully checked for possible contamination sources, and the experiments were repeated with the contamination removed.
2.6 Microcystin determination in algal cultures

Microcystin is another variable that was examined for the algae-bacteria dynamic investigation (see Section 3.2.1.2, Chapter 3 for details). Microcystins are the most common toxins that are produced by blue-green algae. They are cyclic peptides with seven amino acids, and named for the various amino acids on their structures. Microcystin-LR is the most common and also the most toxic one. The WHO drinking water quality guideline of 1 µg/L microcystin is based on this congener. The detection methods for microcystins can be classified into two categories: physic-chemical methods such as liquid chromatography (LC) with mass spectrometric (MS) detection (Bogialli et al., 2006; Wang, Pang, Ge, & Ma, 2007) and bioassay methods such as immunoassays (Weller, 2013). The *M. aeruginosa* culture was used for preliminary tests with both LC-MS and immunoassay. A Thermo Fisher Orbitrap LC-MS with C18 column (100Å, 5 µm, 4.6 mm×250 mm) at 40 °C provided results within 35 min without sample pre-treatment. Microcystin-LR and –RR were detected in the sample as shown in the mass chromatogram (Figure 7).

Due to the cost and availability issue, the toxin measurement was conducted with an immunoassay test kit, Envirologix QuantiPlate Kit for Microcystins. The kit is a competitive enzyme-linked immunosorbent assay (ELISA). In the test, microcystin toxin in the sample competed with enzyme (horseradish peroxidase)-labeled microcystin for a limited number of antibody binding sites on the inside surface of the test wells. After washing off the non-binding material, the outcome of the competition was visualized with a color development step; the sample concentration was inversely proportional to color development. For quantitative analysis purpose, the calibration curve was produced with the standard calibrators, according to manufacturer's instructions. As shown in Figure 8, the microcystin levels show a good linear relationship with the
Figure 7. Mass chromatogram from analysis of *M. aeruginosa* culture samples with microcystin-LR and -RR detected. Chromatographic and mass spectrometric details are in the text. Chemical structures of two microcystins shown in the right-upper corner.

relative optical density of the samples, with a linear range of 0.16-2.5ppb. The results for these three standard calibrators are in agreement with the expected results according to the manufacturer. The calibration equation shown in the figure was used for microcystin calculation in the following experiments.
Figure 8. Standard curve for microcystin measurement with QuantiPlate Kit for Microcystins (relative optical density vs. log concentration of microcystins)

\[ y = -0.269 \ln(x) + 0.3642 \]

\[ R^2 > 0.99 \]
Chapter 3

Determination of *E. coli* and Total coliforms in algal cultures

Based on the preliminary tests on the algal culturing practice and the biological and chemical variable detection, the algae-*E. coli* dynamic experiments were carried out as described in this chapter. Four lab stains of *E. coli* were examined for their survival and growth pattern in algal cultures. Two species of *Microcystis*, *M. smithii* and *M. aeruginosa*, were selected to determine their effect on *E. coli* survival and growth. At the same time, variables such as chlorophyll *a* and algae cell counts in cultures with and without *E. coli* were recorded to determine if the *E. coli* bacteria would influence algal growth. To explore the potential factors affecting the algae-*E. coli* culture dynamics, P and N nutrient availability, culture pH, and microcystin levels were also measured. Lastly, lake water samples containing natural strains of *E. coli* and Total coliforms were included to verify the behavior of naturally occurring FIB, paving the way for future research.

3.1 Growth of lab strain *E. coli* in *Microcystis* cultures

3.1.1 Growth record of *E. coli* consortium in *Microcystis* cultures

After incubating *M. smithii* in flask #1 and #2 and *M. aeruginosa* in flask #3 and #4 for three days, *E. coli* consortium comprised of four lab stains, ATCC 25922, 8739, 51813, and 11775, were spiked into flask #1, #3, and #5 at the initial concentration of approximately 40,000 CFUs (10,000 CFUs for each strain) per 100 mL. Flask #5 was a control with only *E. coli* in the same culture media. These three flasks were sampled and monitored for *E. coli* survival and
growth from day one after \textit{E. coli} addition. The sample interval varied from one to three days depending on the previous results. The monitoring results versus the sampling days were plotted in Figure 9. The control sample results show that \textit{E. coli} organisms are normally stable in the MA medium. After initial inoculation, the apparent number increased and then slowly decreased but remained above the Day 1 level for at least 28 days. When inoculated into a culture with \textit{M. smithii}, the \textit{E. coli} counts dropped rapidly with an average half-life of 0.58 day (the half-life was derived from the kinetics model for first order exponential decay), with no \textit{E. coli} being detected after 9 days. When inoculated into \textit{M. aeruginosa}, the \textit{E. coli} counts dropped even more rapidly (the average half-life was 0.23 day), with no \textit{E. coli} being detected after 3 days. Two parallel experiments verified the similar observation, as illustrated as (a) and (b) in Figure 9. These results indicate that some variables associated with the \textit{Microcystis} culture, especially \textit{M. aeruginosa}, appear to be detrimental to the bacteria survival.

While the short lifetime of \textit{E. coli} in the two algae cultures was repeatedly observed, the growth of \textit{algae} was also monitored for the possible interferences from \textit{E. coli} present. Chlorophyll \textit{a} concentrations and algae cell counts of two \textit{Microcystis} cultures with and without \textit{E. coli} spiking were measured and plotted in Figure 10. To better examine the link between the examined variables and \textit{E. coli} survival, \textit{E. coli} counts are also remained in Figure 10, as well as in the figures of other factorial experiments. The figure shows that chlorophyll \textit{a} concentration in \textit{M. smithii} and \textit{M. aeruginosa} both increased slowly in the first six days, and then rose almost exponentially through day 14. Cell counts of algae in two species also presented a significant increase, except that they started to increase greatly after three days. The overlapping (within error) light and dark lines, which represent the chlorophyll \textit{a} and cell count results for cultures with and without \textit{E. coli}, respectively, indicate that the cultures with \textit{E. coli} added did not show
Figure 9. *E. coli* levels in two *Microcystis* cultures and control medium recorded at various times after inoculation of the culture (data from two parallel experiments, a and b)

significant differences from the pure *Microcystis* cultures, suggesting that the presence of *E. coli* had little impact on *Microcystis* growth.

The above algae-*E. coli* dynamic investigation shows that *E. coli* survival could be severely affected (shortened) by the presence of *Microcystis*, especially *M. aeruginosa*. This is particularly significant to the use of *E. coli* as FIB for assessing the quality of surface water,
including for recreational use. One of the characteristics of an ideal faecal indicator is it should react to the natural environment in a similar manner to the pathogens, as discussed in Section 1.1.2, Chapter 1. According to the observed dynamics, this will not be true if the presence of Microcystis in a sample can prevent detection of the E. coli while not affecting the survival of other pathogens like viruses and protozoa. Persistence of the other pathogens is likely as viruses do not depend on key enzymes that are the normal target for microcystins (Hernández, Lopez-Rodas, & Costas, 2009) and many protozoa are known to grow in the presence of HABs (Davis & Gobler, 2011; Wilken, Wiezer, Huisman, & Van Donk, 2010). Lack of E. coli in samples may give a false sense of water safety regarding microbial contamination at the same time risk of
HABs is increasing, even if the presence of HABs will eventually be noticed and trigger a warning about quality of the water.

3.1.2 Investigation on influencing factors of *E. coli* survival in *Microcystis*

Previous discussion (Section 1.3, Chapter 1) has described that the algae-bacteria dynamics are extremely diverse and intricate. They are usually regulated by a combination of numerous environmental factors, which include physical (e.g. weather and water temperature), chemical (e.g. pH variation and nutrient flux) and biological (e.g. bioactive substance production) forces (Doucette, 1995; Grossart & Simon, 2007). Laboratory settings provide controllable and measurable conditions and are therefore desirable for factorial experiments to identify the major dynamic regulator. The focuses of laboratory investigation in this thesis are the effects of nutrient (N and P), pH and microcystins produced by *Microcystis*.

3.1.2.1 TN and TP concentration correlation with bacteria counts

N and P are the key nutrients to the growth of both algae and *E. coli*. Competition for N or P has been reported to be one of the decisive causes of the other algae-bacteria dynamics (Elser, Chrzanowski, Sterner, Schampel, & Foster, 1995; Mindl et al., 2005). Therefore, the TN and TP levels in the four algae cultures were measured, as illustrated in Figure 11, for the possible nutrient competition between algae and *E. coli*. In the figure, no significant fluctuation of TN or TP was observed corresponding to the end of bacteria survival in the four cultures. A slight decrease of TN and TP appeared in the end of the experiment, when algae entering thrive stage (see the chlorophyll *a* and cell count increase after day nine in Figure 10. The TN and TP profiles of *M. smithii* and *M. aeruginosa* showed similar pattern, suggesting little difference between the two species as to the nutrient impacts on *E. coli* survival. Since the observed *E. coli* survival is
very different between the *M. smithii* and *M. aeruginosa* cultures, it is unlikely that consumption of N and P are responsible for the loss of *E. coli* counts.

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**Figure 11.** Panels indicate *E. coli* counts in microcosms of *E. coli* lab strains with a) *M. smithii* indicating TN levels; b) *M. smithii* indicating TP levels; c) *M. aeruginosa* with TN d) *M. aeruginosa* with TP recorded on various days after inoculation of the culture (columns indicate *E. coli* concentration; light and dark lines indicate TN (a and c) or TP (b and d) in cultures with and without *E. coli*, respectively; blue columns and lines represent results in *M. smithii* cultures; red columns and lines represent results in *M. aeruginosa* cultures)

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3.1.2.2 pH correlation with bacteria counts

In addition to nutrients, pH is another important variable that regulates the water environment and habitat dynamics (Chen & Celia, 1994; Grossart & Simon, 2007). To investigate the pH change and its possible influence, pH values of all the cultures were screened
throughout the algae and *E. coli* growth. The culture with only *E. coli* spiking remained close to the initial pH of 8.2. *M. aeruginosa* and *M. smithii* cultures both experienced the gradual increasing pH from 8.2 to 9.6 and 9.9, respectively, as demonstrated in Figure 12. The elevated pH was due mostly to CO$_2$ uptake during photosynthetic activity, which is also a key feature of blue-green algae plagued systems. At high pH, blue-green algae gain advantages over other organisms and predators by directly utilizing HCO$_3^-$, providing more effective CO$_2$ concentration ability and buoyancy (for better access to atmospheric CO$_2$) (Huisman, Matthijs, & Visser, 2006; Miller & Colman, 1980; Shapiro, 1990; Whitton, 2012). In this experiment, high pH is expected to prevent *E. coli* growth, since the optimal pH range for *E. coli* growth is 6-8. However, survival may persist as they are reported to have base resistance up to pH 10.2 (Small, Blankenhorn, Welty, Zinser, & Slonczewski, 1994). By comparing the pH curves with and without the presence of *E. coli* (Figure 12), it is concluded that *E. coli* had no effect on the pH in either the *M. aeruginosa* or *M. smithii* cultures. However, high pH was probably not the key cause of *E. coli* death. Figure 12 shows that *E. coli* was still alive in *M. smithii* when pH rose up to 8.82 on Day 9, but already dead in *M. aeruginosa* before pH hit 8.41 on Day 6. Elevated pH may have been one of the adverse conditions that worked jointly with other factors to cause the frailty of *E. coli* in the two *Microcystis* cultures.
Figure 12. pH value of two *Microcystis* cultures with and without *E. coli* spiking recorded on various days after inoculation of the culture (columns stand for *E. coli* concentration; light and dark lines indicate pH in cultures with and without *E. coli*, respectively; blue columns and lines represent results in *M. smithii* cultures; red columns and lines represent results in *M. aeruginosa* cultures)

3.1.2.3 Microcystin correlation with bacteria counts

Microcystins are the most common and concerning toxins produced by many blue-green algae genera, including *Microcystis*. Water monitoring results of Lake Sagami and Tsukui during 1992-1995 shows that the microcystin concentrations ranged from 0.02 to 378 µg/L (Tsuji et al., 1996). Microcystin levels of the 187 Florida lakes were reported to range from <0.1 to 12 µg/L (Bigham, Hoyer, & Canfield Jr, 2009). Given the high potency in humans and tested mammals, microcystin and other cyanotoxins have long been assumed to serve a defensive function against predators or competitors (Anderson, Cembella, & Hallegraeff, 2012). Some research also suggest that they play more complicated role in the bloom ecology (Cembella, 2003). To determine the role microcystin played in the setting in this experiment, microcystin detection was conducted throughout the *E. coli* and algal growth. The results were revealed in Figure 13. Combined with growth variables in Figure 10, it is obvious that microcystin concentrations in both species increase with the thriving of algae. Meanwhile, *M. aeruginosa* constantly produced more
microcystin than *M. smithii* at comparable chlorophyll *a* cell count levels. Microcystin concentration increased from 0.60 to 15.00 ppb in *M. smithii* with *E. coli* spiking, and 1.12 to 26.71 ppb in *M. aeruginosa* with *E. coli*. This can probably be linked to the shorter lifespan of *E. coli* in *M. aeruginosa* than in *M. smithii*.

**Figure 13.** Microcystin concentration in two *Microcystis* cultures with and without *E. coli* spiking recorded on various days after inoculation of the culture (columns stand for *E. coli* concentration; light and dark lines indicate microcysts in cultures with and without *E. coli*, respectively; blue columns and lines represent results in *M. smithii* cultures; red columns and lines represent results in *M. aeruginosa* cultures)

To further explore the toxin factor, toxin-spiking experiments were carried out. Samples with different concentrations of microcysts were prepared by adding different volumes of filtered algae sample. Same amount of *E. coli* was then spiked in, and monitored for survival time. The results show that the survival time decreased from 21 to 6 days as microcystin concentration increased from 0.5 to 5 ppb, as shown in Table 6. Plot of *E. coli* counts versus survival time is showed in Figure 14. The *E. coli* loss rate and half-life derived from the plot are listed in Table 6. Compared to the system with algae growing at the same time, *E. coli* generally has longer lifespan (shorter half-life) in the filtered samples at similar microcystin levels. For
example, at microcystin level of approximately 1 ppb, *E. coli* lived for 19 days (half-life, 0.69 day) at system without algae present and 3 days (half-life, 0.23 day) with algae (*M. aeruginosa*) present. These findings point to the conclusion that microcystin is one but not the only one main cause of *E. coli* death; other factors that associated with algal presence might also contribute.

### Table 6. *E. coli* survival record, loss rate and half-life in different levels of microcystin

<table>
<thead>
<tr>
<th>Microcystin (ppb)</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival time (day)</td>
<td>&gt;25</td>
<td>21</td>
<td>19</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Loss rate (day(^{-1}))</td>
<td>0.01</td>
<td>0.76</td>
<td>1.00</td>
<td>2.03</td>
<td>2.18</td>
</tr>
<tr>
<td>(t_{1/2}) (day)</td>
<td>63.46</td>
<td>0.91</td>
<td>0.69</td>
<td>0.34</td>
<td>0.32</td>
</tr>
<tr>
<td>Rsq</td>
<td>0.05</td>
<td>0.92</td>
<td>0.94</td>
<td>0.97</td>
<td>0.99</td>
</tr>
</tbody>
</table>

**Figure 14. *E. coli* counts in different levels of microcystin**
3.2 Growth record of lake strain *E. coli* and Total coliforms in *Microcystis* cultures

Lake strain *E. coli* and Total coliforms were sampled from Lake Ontario, with cell counts of 6,450 and 43,520 CFUs/100mL, respectively. Lake water was spiked into the *M. smithii*, *M. aeruginosa* and MA media to explore the survival and growth of natural lake bacteria strains. As seen in Figure 15, the lake strains showed similar survival patterns to lab strains of *E. coli*; they lasted longer in *M. aeruginosa* than in *M. smithii*, while survival was unaffected after 14 days in control medium. The half-life values for *E. coli* in *M. aeruginosa* and *M. smithii* were 0.32 and 0.78 day, respectively, which are both slightly longer than for the lab strains of *E. coli*. This might be due to the fact that lake strains of *E.coli* have adapted more to the environment and hence are more resistant to stresses like algal toxins. Total coliforms also have a longer half-life in *M. smithii* (0.94 day) than in *M. aeruginosa* (0.28 day). Meanwhile, a green algae, *Chlorella* (identified by microscopy), was observed to grow in the control sample without any effect on the survival of *E. coli* and Total coliforms (Figure 15, control data). These results confirm that survival of *E. coli* and Total coliforms was affected by toxin-producing blue-green algae, and these FIB may not reliably indicate water quality in the presence of HABs. The *Chlorella* results suggest that non-toxin producing algae have no effect on the bacteria survival. Future research is needed to further confirm this behavior (e.g., by comparison of toxin-producing blue-green algae and green algae) in natural waters.

On the other hand, these results could indicate a new opportunity for the use of *E. coli* and coliform monitoring. If a water system normally has *E. coli* or coliform bacteria present, the sudden reduction or loss of these bacteria populations could indicate the onset of *Microcystis* growth as in an HAB. Therefore, the relationship between the disappearance of *E. coli* and the
level of algal toxins could be of great use for water quality management that pertains to FIB screening and HAB prediction and control.

Figure 15. Survival records of lake stain *E. coli* (a) and Total coliforms (b) in two *Microcystis* cultures and control medium recorded on various days after inoculation of the culture
4.1 Conclusions

Based on the experimental results, major conclusions could be reached as follows:

- **Viability of the tested *E. coli* was highly affected by presence of two *Microcystis* species, especially *M. aeruginosa***.
  Both the lab strain *E. coli* and lake strain *E. coli* and Total coliforms presented the similar pattern when comparing their growth in the designed algae and no-algae culturing environments: the same amount of *E. coli* and Total coliforms lasted the shortest in *M. aeruginosa*, slightly longer in *M. smithii* and the longest in the pure growth medium without algae presence.

- **Microcystin level was inversely related to the survival time of *E. coli*. Other factors might work in concert with microcystin to cause *E. coli* death.**
  To investigate the observed algae-*E. coli* dynamics, three possible affecting factors, nutrient (N and P) availability, pH influence and microcystin releasing, were examined for pure algae cultures and algae cultures with *E. coli* spiking. Neither N nor P level showed severe fluctuations during the growth of two *Microcystis* in all cultures that with or without *E. coli* addition. This result indicates that the competition for N or P nutrient (scarcity of N or P nutrient) was not the key cause to *E. coli* death. During the pH monitoring throughout algae and *E. coli* growth, it was observed that pH in both algae cultures increased, which mostly was due to photosynthetic activities of algae, with
decline of *E. coli* counts. Although the increase exceeded the optimal pH range for *E. coli* growth, it was still in their reported bearable areas. Therefore, the high pH might serve as an auxiliary element to facilitate work of other main contributors. Microcystin releasing, among the three factors, was the only one that could be linked to the different *E. coli* lifespans in different culture systems. It was observed that the same amount of *E. coli* last the shortest in *M. aeruginosa*, which produced more microcystins than *M. smithii* at similar biomass levels. In the following microcystin-spiking experiments, samples of different microcystin levels were prepared by filtering algae samples. The same phenomenon was confirmed: the higher the microcystin level, the shorter *E. coli* lasted.

At the comparable microcystin concentrations (e.g., 1 ppb), *E. coli* survival in the system without algal biomass was generally shorter than in the system with algae presence (not just microcystin), which suggests that microcystin was one but not the only one major factor that regulated the dynamics. Other factors that are associated with algal biomass existence must have worked jointly.

- **Use of *E. coli* as indicators of faecal contamination in blue-green algae contaminated water could be greatly compromised, especially if toxin is produced.**

The research findings of this thesis point toward the possibility that *E. coli* absence/presence/abundance might not be able to reflect microbial contamination situation properly if blue-green algae (especially the toxin-producing taxa) exist in the same or nearby water bodies. The interpretation of *E. coli* test results should take into account the possible influence from co-existing blue-green algae. Especially when the test shows *E. colli* negative, supplementary tests are needed to confirm the cause of *E. coli* absence and possibility of microbial contamination.
4.2 Recommendation for future work

Work in this thesis is an initial step to explore the algae-FIB dynamic complexity for better interpretation of FIB tests to indicate potential microbial contaminations. The future work could be carried out in a number of ways: to extend the research objectives to other blue-green algae taxa and FIB; to explore impact of other dynamic regulators; to validate the laboratory findings in natural waters with a wider variety of bacteria in more complicated community structures, and a wider variety of nutrients than in the laboratory medium; to determine if bacteria survival dynamics can be used to determine the presence of toxin-producing harmful algal blooms.
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