CHAPTER 2
LITERATURE REVIEW

Blue green algae have a cosmopolitan distribution, they are common in all kinds of habitats, ranging from the cold deserts of the Antarctic continent to the stones and sand of the world's hot desert. However, the major location is in moist or aquatic environments (Falconer, 2005). The names "cyanobacteria" and "blue-green algae" are valid and compatible systematic terms. This group of microorganism comprises unicellular to multicellular prokaryotes that possess chlorophyll *a* and perform oxygenic photosynthesis (Castenholz and Waterbury, 1989; Mur *et al.*, 1999). They have also been characterized by their ability to form the phycobilin and phycocyanin pigment. They are one of most diverse groups of gram-negative photosynthetic prokaryotes in terms of their morphology, physiology and metabolism (Codd, 1995). They are very important photosynthetic organisms, especially in the freshwater ecosystem together with the eukaryotic phytoplankton and form the basis of the aquatic food web. However they may also be a source of considerable nuisance in many situations. Their mass population led to undesirable properties of water such as odor and color, depletion of dissolved oxygen and high pH. Moreover, they can produce a wide range of potent toxins which pose a health hazard for humans, domestic animals and wildlife (Kuiper-Goodman *et al*., 1999)

Blue green algae can form dense blooms that hamper recreation by diminished water clarity or generated bad odour and taste. The blooms are caused by an estimated 40 genera but the main genera are *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Microcystis* and *Planktothrix* (*Oscillatoria*) (Hoeger *et al*., 2005). Moreover, some blue green algae can produce toxins (cyanotoxins) and cause incidental illness or death of cattle, dogs and humans, or may even affect entire ecosystems with disastrous impact on fish, birds and eukaryotic algae (Carmichael, 1992; Codd, 1995; Codd, 1998; Jochimsen *et al*., 1998; Codd *et al*., 2005). Toxins can occur within the cells or be released from cells to water under certain conditions of growth and/or external stress factors responsible for cell lyses (Codd, 1995). The most studied and

widely distributed are the hepatotoxic microcystins. They accumulate in aquatic organisms and are transferred to higher trophic levels. This is an issue of major concern in aquatic toxicology, as it involves the risk for human exposure through the consumption of contaminated fish and other aquatic organisms. The persistence and detoxification of microcystins in aquatic organisms are important issues for public health and fishery economics (Martins and Vasconcelos, 2009).

2.1 Nile tilapia and giant freshwater prawn in Thailand

The Nile tilapia (*Oreochromis niloticus*) was first introduced into Thailand in 1965 when the Emperor of Japan gave a few fish to H.M. King of Thailand. The fish were bred at Chitralada Palace and hence the name Chitralada strain was born. Since then, many other strains of Nile tilapia have been introduced, most notably the GIFT strain in the mid nineties. Nile tilapia are mostly raised using manures and other recyclable wastes in earthen ponds, as low cost rather than quality is the main priority. Tilapia can be reared in tanks, cages or earthen ponds both in fresh and brackish water up to 25 ppt salinity. Unlike most other fish species, tilapia have the ability to consume minute phytoplankton that they filter out of the water. For this reason, commercial pelleted feeds are not necessary for growing tilapia and nutrient-enriched water ("green water"), produced by the addition of animal manure or fertilizer, is sufficient to achieve a marketable fish of 300 to 500 grams in six months (Belton and Little, 2008). Thai tilapia production has increased, almost exponentially, from an officially recorded 22,800 tons in 1990 to 203,700 tons in 2005 (DOF, 2007). Thailand is the fourth ranking tilapia producer in the world since 2000. It was consumed and exported to other countries (Whangchai *et al*., 2008).

The giant freshwater prawn, *Macrobrachium rosenbergii*, was one of the first species to become scientifically known. In Thailand, giant freshwater prawns are commercially important because they are widely used for human consumption. Domestic consumption was 70 % of total production (DOF, 2007). The prawns can be cultured in all freshwater bodies some areas in the northern part are suitable for raising freshwater prawns such as Chiang Rai and Pijit provinces, production is not enough to support the market demand. However, there is a high potential to increase

production to cater for high consumption rates in the tourist cities of the northern region (Whangchai *et al*, 2007).

Green water systems that predominated in Thailand can cause eutrophication of surface water, resulting in increased occurrences of toxic *Microcystis* blooms. Microcystins have become a global health threat to humans, wild animals, or domestic livestock. They accumulate in aquatic organisms and are transferred to higher trophic levels. It involves the risk for human exposure through the consumption of contaminated aquatic organisms (Martins and Vasconcelos, 2009). Therefore the situation, accommulation and eradication of toxic blue green algae including their toxins in fish and prawn ponds have been carried out. It was useful for management and protection of public health from the damaging effect of blue green algae and their toxins.

2.2 Occurrence and distribution of toxic *Microcystis*

The occurrence of toxic blue green algal blooms, scum or mats had been reported in about 60 countries (Metcalf and Codd, 2004). Light microscopy has traditionally been used in water monitoring for blue green algal taxa and their abundance. Bloom-forming genera include *Microcystis, Anabaena, Planktothrix, Aphanizomenon, Cylindrospermopsis, Raphidiopsis* and *Noduralia*. Scum production is particularly common with *Microcystis, Anabaena, Anabaenopsis, Planktothrix* and *Aphanizomenon*. Mat and biofilm-forming genera with toxigenic members include *Phormidium, Oscillatoria* and *Lyngbya*. Beside the typical scum and bloom-forming blue green algae, there are also some species of very small cell size (typically 0.2-2 μm.), which usually do not form scum and called pico-blue green algae (Maeda *et al.,* 1992). They are known to produce several toxic substances (Bláha and Mařálek, 1999).

Microcystis is one of the most common species of freshwater cyanobacteria, and often dominates the phytoplankton of eutrophic lakes all over the world (Reynolds *et al*., 1981; Zohary and Robarts, 1990; Xie *et al*. 2003; Visser *et al*. 2005).

The genus *Microcystis* is characterized by colonies with irregulary agglomerated spherical cells in common, not stratified, colourless mucilage. Colonies are micro- up to macroscopic, they live in freshwater plankton and form

morphologically different stages during the vegetation cycle. The cells of all the species are able to produce gas vesicle gathered in aerotopes which are reversible and they control buoyancy of colonies in the water column (Reynolds *et al.*, 1981; Komárek and Komáková-Legnerová, 2002b).

Among genus *Microcystis*, *Microcystis aeruginosa* is the most frequently blooms worldwide. It ranges from cold, temperate climates to tropical environments and it forms highly toxic scums that have killed a thousand of farm livestock (Falconer, 2005). Nevertheless, *M. aeruginosa* does not occur as a dominant species in the polar regions, where filamentous forms predominate (Vincent, 2000). *M. aeruginosa* is often dominant under nutrient-rich conditions, especially where there is a significant supply of ammonia, although it also forms blooms in less polluted water (Sivonen and Jones, 1999).

Extensive water blooms have been reported from many countries. For example, mass populations of *M. aeruginosa* cells form in calm weather in Harbeessport Dam, South Africa with cell densities up to 1.76×10^9 cells.ml^{-1} at 10 cm. depth. The cells within the scum remain viable for 11 weeks, indicating survival in the absence of light or dissolved oxygen (Zohary, 1985; Scott, 1983).

In Brazil, *M. aeruginosa* is commonly formed in natural and artificial lakes, forming a permanently high population in eutrophic lakes, which are often used to provide drinking water. It has similarly been recorded in Argentina (Amé, 2003), China, Japan and Australia (Falconer, 2005).

The eutrophication of waterbodies, with resulting blue green algal bloom development, is common in eastern Asia. Eutrophication is closely associated with fish culture. Blue green algal blooms are not thought to be toxic in many traditional communities but are regarded as useful food sources for zooplankton and fish.

In Japan, *Microcystis* blooms in lakes mainly consist of *M. aeruginosa* and *M. wesenbergii* (Watanabe *et al*., 1986). Their spatial and temporal dynamics in natural habitats have been studied by some authors (Watanabe *et al*., 1986; Amemiya *et al*., 1990; Tsujimura, 2003: Ozawa *et al*., 2005).

Eutrophication in Chinese lakes has progressed rapidly, resulting in frequent outbreak of toxic blue green algal blooms in many large lakes such as Lake Chaohu

and Lake Taihu where production of freshwater shrimps are an important industry (Chen and Xie, 2005).

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2.3 Cyanotoxin (microcystins)

Cyanotoxin are secondary metabolites synthesized within the cells of some species of blue green algae. The natural function of these toxins is unclear, although some obtain effect on other biota. Several hypotheses have been put forward to explain blue green algal toxin production (Turner and Tester, 1997). Research has focused on compounds that affect humans and livestock either as toxins or pharmaceutically useful substances. Up until now, many toxic metabolites with various biological activities have been identified and isolated from blue green algae (Dow and Swoboda, 2000).

Among cyanotoxin, the hepatotoxic microcystins (MCs) are considered to be one of the most dangerous groups (Carmichael, 1997; Chorus and Bartram, 1999), because they are highly toxic to mammals with the LD_{50} of MC-LR i.p. or i.v. in mice and rats ranged between 36 and 122 $\mu g.kg^{-1}$, comparable to the toxicity of the chemical organophosphate nerve reagents (Dawson, 1998). The exposure to MCs has been implicated in acute death of terrestrial animals and through haemodialysis also caused death of humans (Carmichael, 2001). The long-term exposure to MCs is related to chronic human intoxication such as primary liver cancer (Yu, 1989; 1995). Now, MCs are of great concern to public due to their potential risk to human health.

MCs were named after the first organism found to produce them, *Microcystis aeruginosa*, but later studies also showed their occurrence in other blue green algal genera (Chorus and Bartram, 1999). They have been characterised from planktonic *Anabaena*, *Oscillatoria (Planktothrix)*, *Nostoc*, and *Anabaenopsis* species, and from terrestrial *Hapalosiphon* genera (Sivonen and Jones, 1999). *Microcystis* produces not only MCs but also a variety of other cyclic or linear bioactive oligopeptides (Reschef and Carmeli, 2006). Some are known peptides, but other remains unidentified.

All MCs have a common cyclic heptapeptide structure (Figure 2.1) consisting of (–D-Ala–L–X–D-MeAsp–L– Z-Adda–D-Glu–Mdha), where MeAsp stands for erythro-b-methylaspartic acid, Mdha for N- methyldehydroalanine, Adda for 3-amino-9-methox- y-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, and X and Z for the

variable amino acids that give its name to the molecule (Carmichael, 1992). Over 70 variants of MCs were recorded over the world (Codd *et al*, 2005). Most of structural variants of microcystins are highly toxic within a comparatively narrow range, i.p. mouse toxicities largely in the range about 50-300 $\mu g.kg^{-1}$ b.w. Microcystin-L.R. is highest toxic (Kuiper-Goodman *et al*., 1999)

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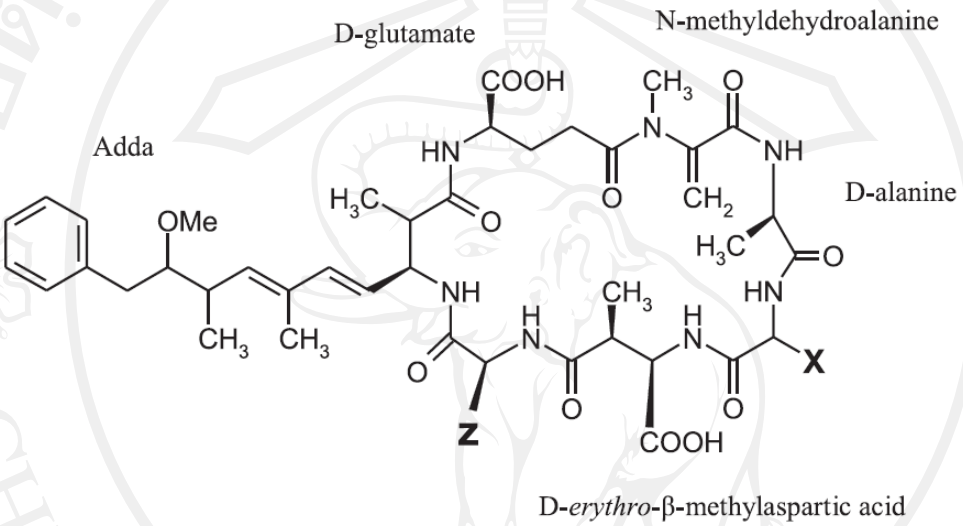

Figure 2.1 Structure of microcystins in which X and Z are variable L amino acids (Sivonen and Jones, 1999)

As a result of an increasing concern with their health implications, the World Health Organisation has set a drinking water guideline value of 1.0 $\mu g.L^{-1}$ for microcystin-LR (WHO, 2006). The level of MC accumulation sufficient to pose risk to humans is uncertain, and will depend on levels of consumption and the severity of toxic blooms in the area where fish or shellfish are caught or collected (Magalhaes *et al.*, 2001). In order to set safe levels of toxicants or contaminants in food or drinking water, it is first necessary to determine the dose level in humans that is considered to be without adverse effects when taken daily over a lifetime; this is known as the Tolerable Daily Intake (TDI) (Kuiper-Goodman *et al.*, 1999). TDI 0.04 $\mu g.kg^{-1}$ b.w. for microcystin-LR is recommended by WHO (2006).

2.4 Factor affecting the bloom formation by *Microcystis*

Although blue green algae are important producer in the aquatic food web, they are occasionally regarded as harmful organisms because of their ability to form bloom and produce a wide range of potent toxins especially, *Microcystis*. They become one of the most severe problems related to freshwater ecosystems. Indeed, all of the common bloom- and scum-forming blue green algal genera are capable of producing toxins (Gorham and Carmichael, 1988; Codd *et al*., 1989). The growth of *Microcystis* and the formation of blooms are influenced by a variety of physical, chemical and biological factors (Mur *et al*., 1999).

2.4.1 Temperature

Maximum growth rates of blue green algae are at the temperatures above 25 ^{o}C. Blue green algal blooms usually occur during warm periods, at the temperature above 20 ^{o}C (Robarts and Zohary, 1987). These optimum temperatures are higher for green algae and diatoms. *Microcystis* has been found to be more temperature sensitive in comparison to *Anabaena*, *Aphanizomenon* and *Planktothrix* (Oliver and Ganf, 2000) and its growth was found to decline sharply at temperatures below 15 ^{o}C (Robarts and Zohary, 1987).

2.4.2 Light and Buoyancy

Light availability has a strong influence on the dominant species of blue green algae and the depth at which they occur. Like algae, blue green algae contain chlorophyll *a* as a major pigment for harvesting light and conducting photosynthesis. They also contain other pigments such as phycobiliproteins which include allophycocyanin, phycocyanin and phycoerythrine. These pigments harvest light in the green, yellow and orange part of the spectrum (500-600 nm.), which are hardly used by other phytoplankton species (Mur *et al*., 1999).

Microcystis photosynthesizes at optimal rates and resists photoinhibition in high surface irradiances. Some blue green algae are also capable of changing the content of light harvesting pigments depending on available wavelengths (Tandeau de Marsac and Houmard, 1993). Blue green algae which form surface blooms seem to have a higher tolerance to high light intensities. Furthermore, they can maintain a

relative higher growth rate than other phytoplankton when light intensities are low. Therefore *Microcystis* will have a competitive advantage in lakes which are turbid due to dense growths of other phytoplankton (Mur *et al*., 1999)

Microcystis contain aerotopes (former = gas vacuoles; Komárek and Anagnostidis, 2005) same as many planktonic blue green algae. These structures are filled with an array of gas vesicles and have a very low density compared with cytoplasm. Thus the aerotope has a density approximately one-fourth that of water and thus gas vesicles can give blue green algal cells a lower density than water (Oliver and Ganf, 2000). It provides a competitive advantage to the organism, which cannot be found in diatoms or green algae. They can move up and down photosynthesizing at the surface layer and nutrient up taking in the deep layer (Ganf and Oliver, 1982). According to Stoke's Law, the sinking rate is dependent on the difference in density between the water and the cells and on the square of the colony size (Mur *et al*., 1999). *Microcystis aeruginosa* has effective buoyancy control due to the presence of aerotopes and large colony size so the presence of *Microcystis* cannot be related strictly to the level of eutrophication. This genus is found in mesotrophic, eutrophic and hypertrophic waters.

2.4.3 Nutrients

Generally, either nitrogen or phosphorus is the limiting nutrient in aquatic systems. Enrichment of waters with one or both of these nutrients stimulates algal growth. (Oliver and Ganf, 2000). Because blue green algal blooms often develop in eutrophic lakes, it is assumed that they require high phosphorus and nitrogen concentrations. This assumption is maintained even though blue green algal blooms often occur when concentration of dissolved phosphate is lowest (Mur *et al*., 1999).

Phosphorus

The main factor influencing the growth rate of blue green algae is phosphorus. If soluble phosphorus concentration in water drop below 10 $\mu g.L^{-1}$, blue green algal population growth is likely to be nutrient limited (Cooke *et al*., 1993). Increased total phosphorus has been shown to increase the blue green algal and phytoplankton biomass (Schindler, 1977; Trimbee and Prepas, 1987; Watson *et al*.,

1997; Peerapornpisal *et al.*, 1999). In general, blue green algae do not have as high demand for phosphorus as do other phytoplankton. In addition, they can store polyphosphate sufficient for two to four cell divisions in phosphate deficient water (Mur *et al*., 1999) and can migrate vertically to a depth where phosphate availability is higher. *Microcystis* can utilize these advantages, with a large capacity for phosphorus storage and high variable buoyancy (Falconer, 2005).

Nitrogen

Blomqvist *et al*. (1994) and Hyenstrand *et al*. (1998) proposed that the favoring source of nitrogen for nitrogen fixing blue green algae were dissolved inorganic nitrogen, non-nitrogen fixing blue green algae prefer ammonium and eukaryotic algae prefer nitrate. Although nitrogen limitation in lakes is not as common as phosphorus limitation, it does occur occasionally (Wetzel, 2001). Buoyant blue green algae like *Microcystis* can benefit from the migration to ammonium sources in deeper water. Nitrogen can be stored by blue green algae in the form of two proteins, cyanophycin and phycocyanin.

Numerous studies indicate the importance of nutrient concentrations as regulators of blue green algal biomass and community compositions (Rapala and Sivonen 1998, Kotak *et al*. 2000, Chorus *et al*. 2001, Lepistö *et al.* 2005, Willame *et al.* 2005). In general, these studies indicate that blue green algal will dominate when a low ratio between nitrogen and phosphorus concentrations occurs (Oliver and Ganf, 2000). A statistical analysis using the environmental data gathered during mass occurrences in the 1980’es in Finland indicated that the most distinguishing factors among hepatotoxic, neurotoxic, and nontoxic mass occurrences were the dissolved nutrient concentrations (Rapala and Sivonen 1998).

2.4.4 Biological factor

The role of blue green algae in aquatic food webs is very complex. In general, phytoplankton are grazed upon by zooplankton, which in turn are consumed by fish.

Grazing can be a major factor for modifying the biomass and community composition of the phytoplankton. Blue green algae are not easily digested by zooplankton (Mur *et al.*, 1999). Colonial blue green algae are deemed unsuitable food

for zooplankton. Ghadouani *et al.* (1993) concluded that zooplankton communities are negatively affected by *Microcystis* blooms similar to Boing *et al*. (1998) suggested that *M. aeruginosa* was the main grazing-resistant phytoplankton species in the reservoir. Macrophytes compete with blue green algae and other phytoplankton for nutrients and light and may also suppress phytoplankton by releasing inhibitory compounds. Other aquatic bacteria can also compete with blue green algae for nutrients. Blue green algae are attacked by viruses, bacteria and actinomycetes, but the importance of natural enemies for the breakdown of populations is not well understood (Mur *et al*., 1999). Recently, viral infection has been implicated as an important factor in algal mortality (Wommack and Colwell, 2000; Muhling *et al*., 2005) and in termination of algal blooms (Bratbak *et al*., 1993; Tomaru *et al*., 2004). Moreover, several different types of virus have been reported as algicidal agents for specific host species in marine and freshwater environments (Wilson *et al*., 1993; Suttle, 2000). Nevertheless, *Microcystis* have few natural enemies, and their capacity for buoyancy regulation prevents sedimentation so the loss rate of blue green algal population is generally low (Mur *et al*., 1999).

2.5 Factors affecting microcystin production

In the field, several factors may vary at the same time. This makes it difficult to assess causal relationships between environmental factors and MC production (Edwin *et al*., 2005). Laboratory studies with pure strains of blue green algae indicated that environmental factors can induce changes in toxicity or toxin concentration (Sivonen and Jones, 1999). Environmental parameters such as light intensity, temperature, nutrients and trace metals have been mimicked under laboratory conditions and investigated to their effect on cyanotoxin production (Kaebernick and Neilan, 2001) in batch and continuous culture experiments.

The effect of temperature on toxin levels is comparable in most blue green algae. MC content of batch cultures grown between 10 and 35 ^{o}C are high between 18 and 25 ^{o}C (Sivonen and Jones, 1999). The relationship between light intensity and the toxin content of cells was not clear (Falconer, 2005). However, lowest toxin concentrations have been documented at low light intensity, with highest levels between 20 and 142 μmol photons $m^{-2}s^{-1}$ depending on the study (Kaebernic and

Neilan, 2001). Most toxic *Microcystis* strain showed no major effects of light intensity (Falconer, 2005). Different blue green algal species have different light requirements: *Planktothrix* prefers low light intensities for growth, *Anabaena* prefers moderate and *Aphanizomenon* prefers high light intensities. All strains produce most toxins when grown under their optimum light conditions (Sivonen and Jones, 1999).

Nutrients, such as nitrogen and phosphorus are essential for blue green algal growth. Decreased amounts of MC have been reported under the lowest phosphorus concentrations tested (Sivonen and Jones, 1999). An exception to this being increased MC of *Microcystis aeruginosa* recorded under phosphorus limiting condition (Oh *et al*., 2000)

Nitrogen affects the production of cyanotoxins differently in nitrogen-fixing and non-nitrogen-fixing blue green algae. Non-nitrogen-fixing species produce more toxins under nitrogen-rich condition. Whereas nitrogen fixing species are not dependent on the nitrogen in the media for their toxin production. They show highest levels of MC, anatoxin-a or nodularin when present in a nitrogen free medium (Rapala *et al*., 1993; Rapala *et al*., 1997; Lehtimaeki *et al*., 1997; Saker *et al*., 1999).

Blue green algae require a variety of trace metals such as iron, molybdenum, and copper for key enzymes, growth, photosynthesis, and nitrogen metabolism (Pirjo, 2005). Utkilen and Gjølme (1995) proposed that iron-limited conditions influenced toxin production by *M. aeruginosa*, and iron uptake was light dependent. The toxin-producing *M. aeruginosa* strain has more efficient iron uptake system than the strain that does not produce toxin.

Colony size appears to be affective on toxin production also. A study on the relationship between colony size and MC content in Wannsee Lq wake, Germany, in summer showed that the largest colonies had the highest MC content per cell (Kurmayer *et al*., 2003)

2.6 Effect and accumulation and of microcystins in aquaculture organism

Summarizes ecological effects of blue green algal blooms and their potential adverse impacts was shown in figure 2.2. During intense blooms photosynthetic activity depletes free CO_2 from water and pH is driven up. Some have argued that this favors dominance of blue green algae, which for the most part are superior

competitors when CO_2 is rare. Low CO_2 also may stimulate formation of surface scums and extreme dominance by blue green algae taxa that can move to the air–water interface where CO_2 is most available, shading other algae in the process (Paerl and Ustach 1982). There is evidence that high pH during intense blue green algal blooms may be toxic to certain species of fish (Kann and Smith 1999), although this presumably might occur with blooms of any kind of phytoplankton (bacterial or algal) or in dense beds of plants. Oxygen depletion that occurs in the water during blooming also can have biological impacts, the most visible being fish kills. There also are observations of adverse impacts of high levels of ammonia during blooming.

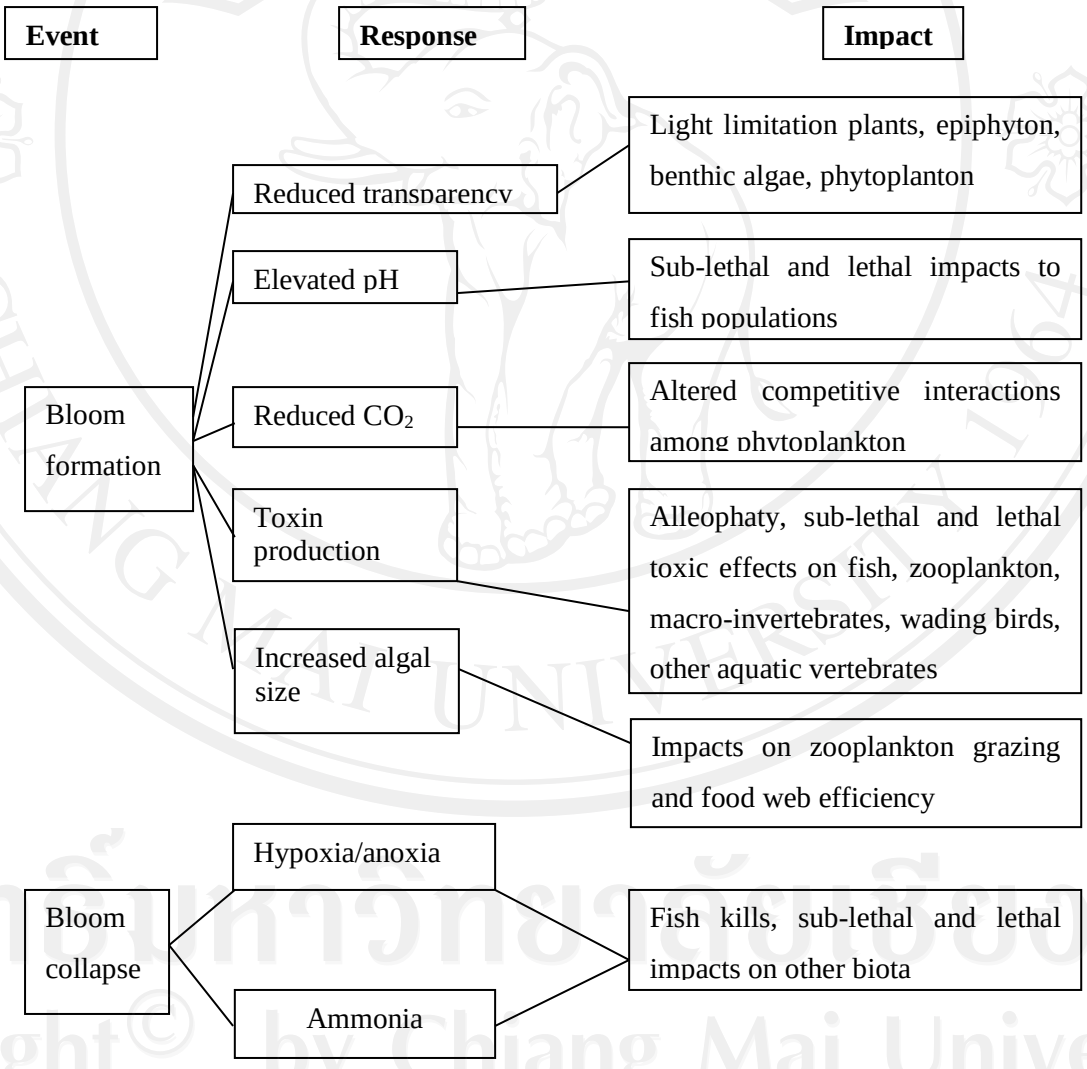

Figure 2.2 A summary of ecological responses and impacts associated with blooms of blue green algae in lakes, rivers, and estuaries.
(Havens, 2008)

Microcystis interacts with a range of organisms in its environment: it competes for nutrients and light with other phytoplankton and is consumed by zooplankton, freshwater bivalves and planktivorous fish. The toxic compounds produced by *Microcystis* often play a role in these interactions.

Many evidences indicate wide ranging effects of different blue green algal toxins on various aquatic organisms (see Ibelings and Havens, 2005). Effects vary from mortality to subtle changes in behavior.

For phytoplankton, they generally compete for nutrients (nitrogen and phosphorous) and light. *Microcystis* is a superior competitor for light because of its buoyancy. Buoyancy gives *Microcystis* better access to light compared to non-buoyant species (Ibelings *et al*., 1991a,b; Huisman *et al.*, 2004), and buoyancy prevents sedimentation losses (Visser, *et al*., 1995). Non-buoyant species of phytoplankton depend on water mixing to get access to light and to remain in suspension. In addition, the toxic compounds produced by *Microcystis* can have allelopathic effects on other groups of phytoplankton like green algae (Vincent and Silvester, 1979, Kearns and Hunter, 2001), dinoflagellates (Sukenik, *et al*. 2002) and other cyanobacteria (Singh, *et al*. 2001)

Effects in fish include changes in liver enzymology, liver damage and ionic imbalance. Effects of microcystins on the embryonic development of fishes have been studied in two species: zebra fish and loach. Whereas immersion of zebra fish embryos in a solution of purified microcsytins did not result in morphological changes except at the very highest concentration (Oberemm *et al*., 1999), embryonic development of loach was affected by exposure to MCs (Liu *et al*., 2002).

Effect studies are especially rich in the zooplankton literature. It can be seen that effects on zooplankton vary from feeding inhibition to reduced reproduction, growth and mortality. Feeding inhibition may actually serve as a protective mechanism, and there is some evidence that especially species that are highly susceptible to MCs may protect themselves by strong inhibition of the intake of blue green algae (Demott, 1999). Blue green algae are generally believed to be food of low quality to zooplankton, especially Daphnia, so that direct toxic effects cannot always be separated from the effect of insufficient food of good quality (LaurenMaatta *et al*. 1997).

Many studies have suggested that blue green algal toxins bioaccumulate in aquatic biota and that this may increase the risk of exposure of biota higher up in the food web. There are many more studies on fish where mass mortalities have been attributed to blooms of toxic blue green algae. Toxins in fish have been analyzed after exposure via different routes, but the majority of work actually involves studies of fish caught in lake or sea, i.e. fish that has been exposed to toxins via natural routes (exposure through the food web or to dissolved toxins after lysis of blooms). In the ecosystem the feeding guild of a fish species appears to be a primary determinant of exposure to toxins. Phytoplanktivorous fish like silver carp (e.g. Jang *et al*. 2004) directly consume blue green algae. Zooplanktivorous fish like sticklebacks and smelt (Ibelings *et al*. 2005) feed on zooplankton that directly consume blue green algae. Piscivorous fish that prey on zooplanktivorous fish are one step further removed from the toxin producing blue green algae. It may be expected that in the absence of biomagnifications MC concentrations decrease in the order phytoplanktivorous > zooplanktivorous > piscivorous fish. Xie *et al.* (2005) found that MC in various tissues and organs varied as carnivorous > omnvivorous > phytoplanktivorous fish. Fischer and Dietrich (2000) explain that there are several differences in GI tract between a carnivorous fish like rainbow trout and planktivorous and herbivorous cyprinids like carp. Cyprinids possess a much longer ileum with larger surface area and higher resorption capacities, so that carnivorous fish would accumulate less MCs (Ibelings and Havens, 2005)

The occurrence of blue green algal waterblooms is a worldwide concern in environmental health. Not only do these blooms degrade the ecosystem and interfere with quality targets and lake restoration (Ibelings and Chorus, 2007), the toxins produced by blue green algae may be harmful to humans. MCs in human diet were shown in Table 2.1

Table 2.1 Microcystins in human diet, examples taken from literature

Organism	Organ(s)/tissue	Toxin conc. ($mg.g^{-1}$) (gram of wet (fresh) weight.)
Fish		
Odontesthes bonariensis	Muscle	0.05/0.34
Hypophthalmichthys molitrix	Muscle	0.00025-0.097
Cyprinus carpio	Muscle	0.038
Tilapia rendalli	Muscle	0.002-0.337
Unidentified fish spp.	Muscle	0.04
Oreochromis niloticus	Muscle	0.102
Hypophthalmichthys molitrix	Muscle	0.0016
Platichthys flesus	Muscle	0.0005-0.1
Rutilus rutilus	Muscle	0.0004-0.2
Unidentified fish spp.	Muscle	0.075-1.5
Oncorhynchus mykiss	Muscle	0.035
Mussels		
Anodonta woodiania	Foot/muscle	0.009/0.026
Hyriopsis cumingii	Foot/muscle	0.022/0.039
Cristaria plicata	Foot/muscle	0.01/0.023
Lamprotula leai	Foot/muscle	0.021/0.058
Anodonta woodiania	Whole	0.064
Hyriopsis cumingii	Whole	0.188
Cristaria plicata	Whole	0.096
Lamprotula leai	Whole	0.131
Unidentified mussels	Whole	2.5
Crayfish		
Unidentified crab spp.	Muscle	0.103
Procambarus clarkia	Muscle	0.005/0.010
Shrimps		
Palaemon modestus	Muscle	0.006/0.026
Macrobrachium nipponensis	Muscle	0.004/0.012
Palaemon modestus	Whole	0.114
Macrobrachium nipponensis	Whole	0.051
Unidentified prawns	Flesh	0.005-0.022

(Modified from Ibelings and Chorus, 2007).

2.7 Eradication of *Microcystis*

Despite studies of the effects of various environmental factors on the growth of *Microcystis* species, the mechanisms that determine bloom dynamics and termination have not been studied sufficiently (Oliver and Ganf, 2000). Recent observations have shown that in addition to physical factors such as temperature and irradiation, chemical factors such as nutrients, and biological factors (predators), mortality induced by bacteria and virus may be one of the important factors that control these algal blooms (Brussaard, 2004; Choi *et al*., 2005; Nagasaki *et al*., 2004; Tomaru *et al*., 2004). The effective measures for reduction of blue green algal concentrations in reservoirs are best undertaken when the limiting factors for blue green algal growth have been identified (Falconer, 2005).

2.7.1 Nutrient reduction

Microcystis biomass can be influenced by the combination of light availability, phosphorus, nitrogen, and the hydrophysical characteristics of the water body. Although, the component that has received most attention for the prevention and decreasing of blue green algal bloom is phosphorus (Chorus and Mur, 1999) but nitrogen is significant to the gas vacuolated blue green algae, as it is an essential component in the systhesis of their gas vesicle. *Microcystis* require inorganic nitrogen for growth. Nitrogen limitation will be particularly damaging to the non-nitrogen fixing bloom-forming blue green algae and may be a critical factor in their replacement by other phytoplankton species (Oliver and Ganf, 2000). Phosphorus inputs to aquatic environments are often easier to control than nitrogen inputs. Methods for elimination of phosphorus from domestic sewage are well developed and currently more cost-effective than nitrification and denitrification (Bartram *et al*., 1999).

2.7.2 Removal by pressure devices

Microcystis not only lack an advantage over other phytoplankton, they are at a considerable disadvantage if without gas vesicles. This incident offers a method of controlling these organisms. In engineering a device for collapsing gas vesicles, the cost will rise with the pressure required. The devices i.e. ultrasonic transducer

(Nakano *et al.*, 2001), explosive (Menday and Buck, 1972) and deep concentric pipes (Clarke and Walsby, 1988) have been tested for the lake-scale collapse of gas vesicles of *Microcystis*.

2.7.3 Aquatic bacteria

It is generally accepted that bacteria can affect algal dynamics, either negatively or positively. In the laboratory, a number of experiments have supported the antialgal ability of bacteria (Daft *et al*., 1975; Kim *et al*., 2003; Kodani *et al*., 2002; Lee *et al*., 2000; Manage *et al*., 2000; Sigee *et al*., 1999; Yamamoto *et al*., 1998). Interest in harmful algal blooms has revealed that bacteria are capable of stimulating or inhibiting algal growth or killing algae (Manage *et al*., 2000). Salomon *et al*. (2003) found that *Alcaligenes denitrificans* had algicidal effect on *Microcystis* spp. Choi *et al*. (2005) reported an aquatic bacterium capable of eliminating the cyanobacterium, *Streptomyces neyagawaensis*. These results suggested that indigenous bacteria isolated from sediments may have potential application in controlling harmful blue green algal blooms in freshwaters.

2.7.4 Cyanophage

The ecological impact of phages on *Microcystis* populations is not clear; however, reports have suggested that phage may play an important role in regulating bloom dynamics. Manage *et al*. (1999) observed that an increase in cyanophage titers (the numbers of particles forming plaques on an *M. aeruginosa* lawn) was accompanied by a large decrease in the abundance of *M. aeruginosa* in a natural freshwater environment. Tucker and Pollard (2005) recently identified two types of podovirus-like particles that inhibited growth of *M. aeruginosa* in natural lake samples collected during an *M. aeruginosa* bloom.

2.8 Eradication of microcystins

MCs are chemically stable in water (Jones and Orr, 1994; Tsuji *et al.*, 1994) and have been documented to be recalcitrant to conventional water treatment processes (Hoffman, 1976; Keijola *et al.*, 1988; Himberg *et al.*, 1989; Lahti and Hiisvirta, 1989). Chlorination is generally an effective treatment option for removing

MCs, with CT values (product of the disinfectant concentration C in mg.L^{-1} and the contact time T in min). The effectiveness of chlorine on MCs greatly decreases at higher pH (>8.0) often associated with blue green algal blooms. Activated carbon adsorption and ozone oxidation have been shown to be successful in their removal from drinking water (Jones *et al*., 1993; Rositano *et al*., 2001; Newcombe *et al*., 2003; Ho, 2004). However, the presence of natural organic material (NOM) reduces the effectiveness of both of these treatment processes for the removal of MC. For activated carbon, NOM decreases the adsorption capacity for these metabolites through competitive adsorption and/or pore blockage mechanisms (Lambert *et al*., 1996; Newcombe *et al*., 2003), while NOM can consume ozone thereby reducing its concentration in solution (Rositano *et al*., 2001; Ho, 2004).

Although, several chemical treatments of water are proposed, it is possible that the chemical treatments sometimes may produce carcinogenic substances and other mutagens (Ishii *et al*., 2004). Biodegradation is one of the safe and mild treatments for removing blue green algal toxins from water. Many studies have reported biological degradation of MC in natural lakes and reservoirs (Ho *et al*., 2006).

Microcystin degrading bacteria

A number of studies have reported biological degradation of MC in samples from lakes and sediments (Christoffersen *et al*., 2002; Cousins *et al*., 1996; Jones and Orr, 1994; Rapala *et al*., 1994). It is now known that MC can be degraded by aquatic bacteria but only a few bacterial strains with the ability to degrade MCs have been isolated and characterized (Edwards and Lawton, 2009). *Microcystis* spp. has numerous bacteria associated with its extracellular mucus zone (Hoppe, 1981). Maruyama *et al*. (2003) reported that MC-degrading bacteria are present in the mucilage of *Microcystis*.

Jones *et al.* (1994) isolated an MC-degrading bacteria, *Sphingomonas* sp. strain ACM-3962 (MJ-PV) and *Pseudomonas* sp., from Australian river water. Bourne *et al*. (1996, 2001) reported its degradation pathway and genes coding the MC-degrading enzymes of this bacterium. Recently, they reported the application of MJ-PV for the biodegradation of MCLR in natural water and a pilot scale of slow sand

filtration water treatment systems (Bourne *et al*., 2006). Previously identified bacteria belonged to the Proteobacteria, and with the exception of one isolate (*Sphingomonas* sp.), they were all shown to degrade MC-LR via the same degradation pathway: formation of linear MC-LR following cleavage at the 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-deca-4,6-dienoic acid (Adda)–Arg bond and hydrolysis at Ala-Leu to yield a tetrapeptide with Adda as the final product detected (Bourne *et al*., 1996; Imanishi *et al*., 2005; Ishii *et al*., 2004). It has been shown that the presence of a geen mlrA, encoding the most important enzyme for MC degradation, it unique to MC degraders but not only genus *Sphingomonas* (Saito *et al.*, 2003). Therefore, other MC-degrading bacteria were found such as *Methylobacillus* (Hu *et al*., 2009), *Burkholderia* (Lemes *et al.*, 2008), Rapala *et al*. (2005) described a novel bacterium, *Paucibacter toxinivorans* capable of degrading MCs and nodularin (NOD).

Maruyama *et al*. (2006) reported the discovery of three strains of bacteria that degrade the blue green algal hepatotoxin microcystin, Y2T, MDB2 and MDB3, were isolated from a eutrophic lake, Lake Suwa, and the Tenryu River, Japan, and characterized. They were identified as a new genus and species of the family Sphingomonadaceae with the name *Sphingosinicella microcystinivorans* (Maruyama *et al*., 2006*)*. Recently, degradation capability of MC-LR by novel bacteria isolated from Loch and river waters in Scotland, UK were recorded by Manage *et al*. (2009). Phylogenetic analysis (16S rRNA) identified them as *Arthrobacter* spp., *Brevibacterium* sp., and *Rhodococcus* sp. that belong to the Actinobcateria. Until recently, only members of genus *Sphingomonas* was reported to be able to degrade microcystin and the gene cluster responsible for microcystin degradation (*mlr*) has been reported for all Proteobacteria (Rapala *et al*., 2005; Lemes *et al*., 2008) where Manage *et al*. (2009) recorded isolates belonged to the Actinobacteria and no PCR products specific for proteobacteria detected, whereas all target genes (*mlrA*, *mlrB*, *mlrC* and *mlrD*) produced PCR products in the positive control. Thus, the recent work possibly recorded new gene for MC degradation pathways (Manage *et al*., 2010).

2.9 Effective Microorganisms (EM)

The technology of Effective Microorganisms (EM) was developed during the 1970's at the University of Ryukyus, Okinawa, Japan (Higa *et al.*, 2002). Studies have

suggested that EM may have a number of applications, including agriculture, livestock, gardening and landscaping, composting, bioremediation, cleaning septic tanks, algal control and household uses.

Effective Microorganisms is a mixture of groups of organisms that has a reviving action on humans, animals, and the natural environment (Higa, 1995) and has also been described as a multi-culture of coexisting anaerobic and aerobic beneficial microorganisms. The main species involved in EM include:

- Lactic acid bacteria i.e. *Lactobacillus plantarum, L. casei, Streptoccus lactis*
- Photosynthetic bacteria i.e. *Rhodopseudomonas palustrus, Rhodobacter spaeroides*
- Yeasts i.e. *Saccharomyces cerevisiae, Candida utilis*
- Actinomycetes i.e. *Streptomyces albus, S. griseus*
- Fermenting fungi i.e. *Aspergillus oryzae, Mucor hiemalis* (Diver, 2001).

The basis for using these EM species of microorganisms is that they contain various organic acids due to the presence of lactic acid bacteria, which secrete organic acids, enzymes, antioxidants, and metallic chelates. The creation of an antioxidant environment by EM assists in the enhancement of the solid-liquid separation, which is the foundation for cleaning water (Higa and Chinen 1998).

The use of effective microorganisms (EM) for reducing volumes of sewage sludge has often been suggested as feasible in wastewater treatment systems. They contain various microorganisms which may be microcystin-degrading bacteria. If they are, they will be applied to water quality management of small water resources especially; aquatic farms which blue green algal bloom occur.

2.10 Studies on toxic blue green algae in Thai aquaculture

In Thailand, research on diversity of algae including blue green algae in various freshwater resources are abundant. The occurrence of blue green algal genera with known toxin-producing taxa were present in many reservoirs in all region of Thailand i.e. *Anabaena*, *Cylindrospermopsis*, *Microcystis* and *Oscillatoria* (Lewmanomont *et al*, 1995; Peerapornpisal, 2005). Those focusing on toxin-producing genera and their toxins are rarely documented. Most research was on their

diversity and occurrence in the water resources for water supply. They occurred widespread throughout the country (Peerapornpisal *et al*., 1999; Mahakhant *et al*., 2001; Yongmanitchai *et al*., 2001; Peerapornpisal *et al*., 2002, Pekkoh, 2008). Although the evidence of acute effects of cyanotoxins on aquatic, wildlife and domestic animals including people have not been recorded in Thailand. Cyanotoxins are unlikely to be ingested by humans in amounts high enough for a lethal acute dose, but the damage produced by chronic effect is more probable if there is long-term frequent exposure (Magalhães *et al.*, 2003). In addition, human illness and chronic effect due to blue green algal toxins are never or rarely considered in contrast with alternative causes such as bacterial, viral or protozoal infection. It cannot be concluded that the incident never happens in this country. Furthermore, cyanotoxins may be accumulated in the trophic web and produce diverse intoxication symptoms and chronic effects that are difficult to diagnose and prevent (Smith and Haney, 2006).

There are many agricultural ponds throughout Thailand. Traditional cultivation of tilapia and prawn, farmers usually used feeding method and green water system. It is a habitat that typically experience blue green algal bloom. A research which recorded the occurrence of *Microcystis aeruginosa* and microcystins in prawn ponds was studied by Prommana *et al.* (2006). They found high amount of *M. aeruginosa* and 3.0-11.5 $\mu g.l^{-1}$ of microcystins**.** However, the accumulation of MCs in prawn was not studied, therefore, it is urgently needed to clarify whether MCs are able to accumulate in these prawns including other aquatic organism or not. Furthermore, using EM for improvement of water quality and elimination of microcystins in ponds will be conducted. The data will be used for evaluate quantitatively consumptive risk and useful for humans for the awareness, prevention and management of agricultural ponds including public health concern with consumption of aquatic organisms in Thailand.