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10 Control and management of Harmful Algal Blooms

10.1 Introduction

Everyone would agree that the best cure is prevention. This is certainly applicable in the case of cyanobacterial blooms and their tremendous and widespread impact on human and environmental health, natural and man-made assets, as well as overall ecosystem services. There have even been suggestions and also some evidence that cyanobacteria blooms may have a negative impact on the housing markets in some parts of the world. Certainly, common sense would dictate that the level of appreciation and the livability of a neighborhood may be positively influenced by the presence of a nice lake (natural or artificial); however, the opposite is true when that lake is affected by water quality issues including odor and toxins from the development of algal blooms. Prevention of cyanobacterial blooms has been at the heart of the nutrient management strategies around the world and has resulted in many success stories. However, blooms still occur and they also occur in assets that the public are not necessarily exposed to, such as drinking water reservoirs, wastewater stabilization ponds and retention dams, in addition to natural systems such as lakes and rivers. When prevention fails or less than satisfactory results are achieved, we need to tackle the problem through direct mitigation technologies that aim at reducing or eliminating the risk generated by the presence of cyanobacteria and their toxins. In this chapter, we will discuss a broad range of mitigation approaches that have been applied with various degrees of success, and we also explore the opportunities for future development of innovative solutions for this important problem.

10.2 Global water crisis

Humans utilize water in agriculture, industry, the household and for recreation [1]. Water also provides incidental ecosystem services to humans and other organisms, such as habitats, climate control and media for nutrient cycling [2]. It is a natural resource essential to life on earth, and adequate management of water is required to maintain these vital services to the global population and the environment.

Water resources are generally not well managed, and as a result, water quality remains of significant concern. Waterways are continually polluted through the addition of nutrients and heavy metals, high-quality water is wasted on activities where it is not suited to the purpose, and water is not equally distributed between people of all nations and social status. These anthropogenic impacts on water quality and quantity have led to a crisis that is affecting the entire biosphere of the earth [1, 3, 4]. This crisis

is increasing with the concurrent rise in global population and climate change [4, 5]. In order to combat this, water resource management must be adaptive, flexible and engage with stakeholders at multiple levels, from the local to the global.

One of the most significant anthropogenic impacts on waterways is eutrophication. Eutrophication occurs when high nutrient loads enter a water body, often as a result of agricultural and industrial processes. This increase in nutrients, particularly nitrates and phosphates, can detrimentally affect ecosystems and reduce the quality of water for reuse purposes [6, 7]. Eutrophication is often a precursor to the occurrence of harmful algal blooms, which commonly contain toxin-producing cyanobacterial species and are a threat to human and environmental health [8].

10.3 Cyanobacteria and cyanotoxins

Cyanobacteria are prokaryotic phytoplanktons that occur in fresh, brackish and salt water systems throughout the world [9, 10]. Species of cyanobacteria differ in their morphology and may exist as single cells, colonies and filaments [11]. When cells aggregate, they form dense cyanobacterial blooms, a potential threat to human and environmental health.

Cyanobacteria generally dominate in reservoirs containing high nutrient loads and stagnant water, although cyanobacterial blooms do occasionally occur in oligotrophic systems and favor water temperatures between 15 and 30 °C [8]. Studies into the dynamics of cyanobacterial blooms predict that the expected increase in global temperature will result in increased surface water temperatures and thermal stratification, as well as changing meteorological patterns, possibly stimulating increased cyanobacterial growth rates [12–19]. It is likely that this will result in an increased frequency of cyanobacterial bloom events. Of particular concern to water utility managers are those cyanobacterial species that form blooms in freshwater reservoirs that are used for drinking, recreation and irrigation.

Cyanobacterial blooms have several detrimental environmental effects. Blooms often proliferate in the surface layer of stratified reservoirs, shading organisms below, which can result in the death of pelagic and benthic organisms [20–23]. When blooms collapse, the release of organic cell matter to the water column increases the system's oxygen demand. The concentration of dissolved oxygen is lowered due to its consumption in reactions to degrade organic and inorganic compounds; this results in mass deaths of fish and other aquatic organisms [10, 24]. Such deaths are often observed by the general public and receive considerable media attention.

Many species of cyanobacteria also produce toxins. Cyanobacterial toxins (cyanotoxins) vary in their toxicity to humans and animals, and include hepatotoxins, dermatotoxins, cytotoxins, neurotoxins and lipopolysaccharides. Cyanotoxins can induce both acute and chronic effects, and can pose a risk to both humans and ecological systems [25–32].

The most common routes of human contact with cyanotoxins are through the contamination of drinking water, the recreational use of lakes and rivers containing cyanobacteria and via the ingestion of blue-green algal supplements [33–37]. Organisms within the environment are often harmed by direct exposure to cyanotoxins or through bioaccumulation [38–48]. Bioaccumulation can lead to the magnification of cyanotoxins throughout food webs, potentially altering growth patterns, grazing behavior and development, and leading to significant health risks for organisms, including humans, that predate species which have bio-accumulated cyanotoxins [29, 41, 49, 50].

The shading of underlying organisms, reduction of dissolved oxygen and bioaccumulation of cyanotoxins can lead to shifts in ecological assemblages and potentially ecosystem collapse, as well as significant threats to human health. As such, it is imperative that the risks of cyanobacterial blooms in various freshwater bodies are assessed and mitigated so that they can be appropriately managed to avoid detrimental effects.

10.4 Cyanobacterial prevention and mitigation

Many techniques for cyanobacterial bloom prevention and mitigation have been investigated (Tab. 10.1 and Tab. 10.2). Some have been applied directly in reservoir management, while others have been trialed only under laboratory conditions. The success of preventative and mitigation techniques depends upon the underlying conditions present, and the characteristics of individual water bodies must be considered when determining the most appropriate management strategies to apply.

Prevention of cyanobacterial blooms has been achieved with varying success through techniques including nutrient reduction, artificial destratification, macrophyte establishment, predation, the addition of allelopathic chemicals, ultraviolet radiation (UVR) and ultrasonication (Tab. 10.1). Nutrient reduction and destratification have shown reasonable success in large reservoirs, though most success has been where nutrient inputs can be significantly reduced and reservoirs are relatively deep.

Despite preventative attempts, often cyanobacterial blooms still occur. It is therefore imperative that mitigation measures for controlling blooms are investigated. Many such methods have been trialed in both the laboratory and field, with varying success (Tab. 10.2).

It is common practice to remove cyanobacteria using copper sulfate, chlorine or coagulants and flocculants [109], although the dynamics of the removal of cyanobacteria from wastewater by such methods has not been thoroughly investigated. These cyanobacterial removal techniques currently practiced on a large scale may be environmentally damaging and ineffective for the removal of cyanotoxins [93, 109–112]. Several of the removal methods used in drinking water treatment are highly successful where cyanobacterial and cyanotoxin concentrations are low and the water will not

be released to the environment, but are often prohibitively expensive for use in highly eutrophic systems and generally less effective in reservoirs containing high concentrations of organic matter [112, 113] (Tab. 10.2).

Tab. 10.1: Commonly used prevention strategies for cyanobacterial blooms.

	Specific comments for use	References
Allelopathic chemicals	Chemicals generally secreted by decaying organic matter. Addition of organic matter increases WSP sludge production. May be unanticipated effects on non-target organisms.	[51–64]
Destratification	Can promote growth of non-buoyant phytoplankton over cyanobacteria. Often ineffective in shallow and highly eutrophic water bodies. Generally requires electrical connection on-site.	[65–71]
Macrophytes	Interfere with WSP processes. Provide breeding grounds for mosquitos and other disease vectors.	[72–81]
Nutrient reduction	50% of phosphorus in wastewater is from human waste and cannot be reduced. Likely that WSPs will be high in nutrients regardless of reduction measures. N:P ratio may be more important than actual phosphorus and nitrogen concentrations.	[8, 71, 82–85]
Predation	May alter WSP ecology, particularly if zooplankton are added and preferentially consume non-target phytoplankton. Consumption of cyanotoxins may result in the death of predators.	[86–91]
Ultrasonication	Only tested at reduced scales. May not be appropriate for full-scale WSPs.	[92–103]
Ultraviolet radiation	Cells in WSP are likely adapted to high UVR doses. May be practical in association with other treatment methods. Can only be used at pond inlets and outlets.	[104–108]

Tab. 10.2: Commonly used mitigation strategies for cyanobacterial blooms.

	Cyanobacterial removal	Toxin removal	Specific comments for use	References
Adsorption (naturally occurring particles)		✓	Occurs naturally, but may be insufficient for complete cyanotoxin removal. Cyanotoxin variants adsorb differently. Adsorption decreases as pH increases. Often biodegradation is greater than adsorption when in contact with particles. If cyanotoxins are filtered through natural soil and adsorption is insufficient, this can endanger aquifers.	[114–123]
Adsorption (activated carbon)		✓	Other organic compounds compete for adsorption-sites. May increase sludge loading.	[124–130]
Biodegradation		✓	Detailed in Tab. 10.3.	Tab. 10.3
Chlorine and chlorinated compounds	✓	✓	Ineffective at removing microcystins at pH > 8. Phytoplankton cells, rather than cyanotoxins, may preferentially react with chlorinated compounds. Cyanotoxins may be released from cells more quickly than they can be degraded by chlorinated compounds in solution. Produces by-products dangerous to humans and the environment (e.g. trihalomethanes).	[93, 106, 131–142]
Coagulation and flocculation	✓		Increases sludge loading. Generally does not affect membrane integrity, so cyanotoxins are not released. If flocs are not removed, cyanotoxins accumulate in sludge. Must consider flow environment of WSP. Increases concentration of aluminum in the environment.	[111, 143–150]

Tab. 10.2 (continued)

	Cyanobacterial removal	Toxin removal	Specific comments for use	References
Copper sulfate	✓		Traditional method of cyanobacterial removal in WSPs. Releases cyanotoxins to the dissolved state, but does not subsequently degrade them. Increases copper concentration in the environment.	[110, 151–156]
Filtration and reverse osmosis	✓	✓	Impractical for water containing high suspended sediment loads. Most degradation in successful studies appears to be biological, except where nanofiltration is used.	[157–163]
Hydrogen peroxide	✓	✓	Effectiveness may be increased by presence of UVR and/or iron. Cyanotoxin degradation decreases with increasing pH. May release cyanotoxins to the dissolved state over hours/days. Often found ineffective on scales of minutes when not coupled with other methods. May affect microcystin synthesis within cells.	[129, 140, 141, 152, 164–179]
Ozone	✓	✓	Cost prohibitive – may preferentially react with other organic compounds. Cyanotoxin degradation decreases with increasing pH. Release of cellular organic compounds increases the ozone dose required. Requires electricity on-site for the production of ozone.	[93, 106, 111–113, 129, 132, 140, 180–182]

Tab. 10.2 (continued)

	Cyanobacterial removal	Toxin removal	Specific comments for use	References
Permanganate		✓	Does not seem to be significantly affected by pH. Likely produces harmful by-products.	[93, 106, 132, 136, 140, 141, 183, 184]
Predation and biomanipulation	✓	✓	May alter WSP ecology, particularly if zooplankton are added and preferentially consume non-target phytoplankton. Addition of organic matter increases sludge loading.	[51–54, 56, 58–60, 86–90, 185]
Titanium dioxide			Large amounts of catalyst are required – up to mg per l – so impractical in wastewater.	[167, 186–188]
Ultrasonication	✓	✓	Only tested at reduced scales. May not be appropriate for full-scale WSPs. Requires electricity on-site.	[92–102]
Ultraviolet radiation	✓	✓	Occurs naturally, but can be enhanced by coupling with other removal methods. Can reduce cyanotoxins through photosensitized reaction with compounds including phycocyanin. Currently used to ensure the removal of coliforms at some WWTP outlets. Impractical alone where cell concentrations are high. Requires electricity on-site.	[93, 105, 106, 165, 170, 174, 181, 189–194]

Effective techniques for the mitigation of both cyanobacteria and cyanotoxins must be determined by considering the underlying properties of the water system in question, including depth, pH values, concentrations of suspended solids, and dissolved organic and inorganic compounds [195, 196]. To be successful, any mitigation approach must reduce both cyanobacteria and cyanotoxins and pose no or negligible threat to ecosystems.

10.5 Cyanobacterial management

The management of cyanobacteria in any freshwater resource must consider the entire cycle of water through catchments, water reservoirs, treatment plants and distribution systems [71]. Appropriate management of cyanobacterial blooms is imperative to reducing their negative impacts on human and ecological health, water treatment processes and income-generating activities, including tourism and property development. This is not simply about implementing prevention and mitigation strategies. It is also important to assess the potential risks associated with such blooms, so that they can be treated effectively and efficiently at the site of interest. Incorporating risk assessment into management will allow plans to be developed which minimize the costs of bloom mitigation and the potentially undesirable environmental effects of many cyanobacterial removal methods. Such plans should be developed for all freshwater resources that suffer from potentially toxic blooms. This will reduce or eliminate the undesirable consequences of cyanobacteria and cyanotoxins on both humans and ecosystems. A management plan should consist of the following actions required during four distinct time periods (Fig. 10.1):

1. Prior to bloom;
2. Hazardous bloom suspected;
3. Hazardous bloom identified;
4. Mitigation ineffective.

Barrington et al. [197] offered a detailed approach for the appropriate monitoring regime to minimize the risk of undetected or undetectable incidents. Currently the authorities rely on some form of either public reporting of an incident or some form of monitoring which is usually a response to a visual inspection of the water system (Fig. 10.2).

This form of risk assessment assumes that hazardous events will always be correctly identified. Although useful, such an approach may lead to overly cautious and cost-intensive behavior by following the precautionary principle, whereby potentially hazardous events are assumed to be dangerous regardless of their actual characteristics [198, 199]. If a monitoring methodology is overly precautionary, costs may be incurred by implementing unnecessary control measures to treat “false positive” results. This traditional method of assessment also fails to consider the associated risk should

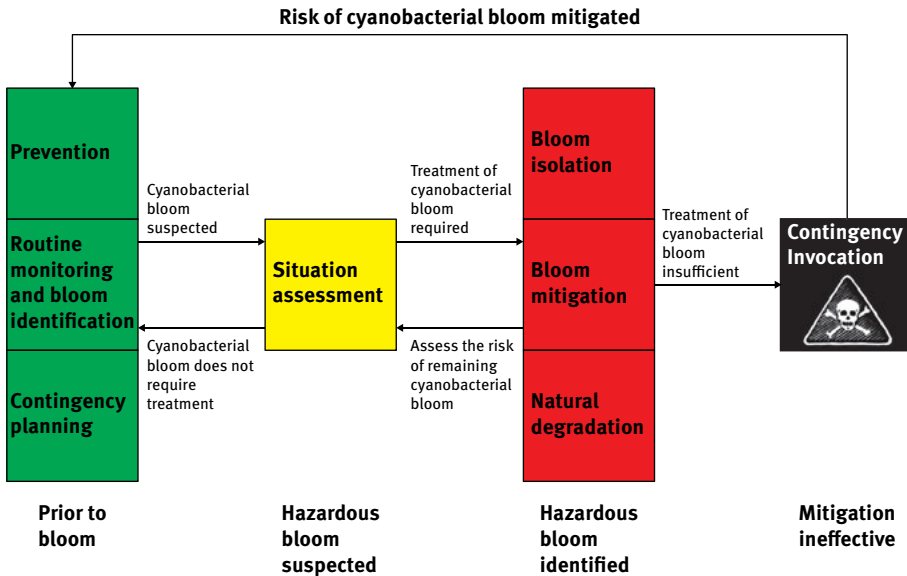


Fig. 10.1: Management framework for the removal of toxic cyanobacteria from water bodies.

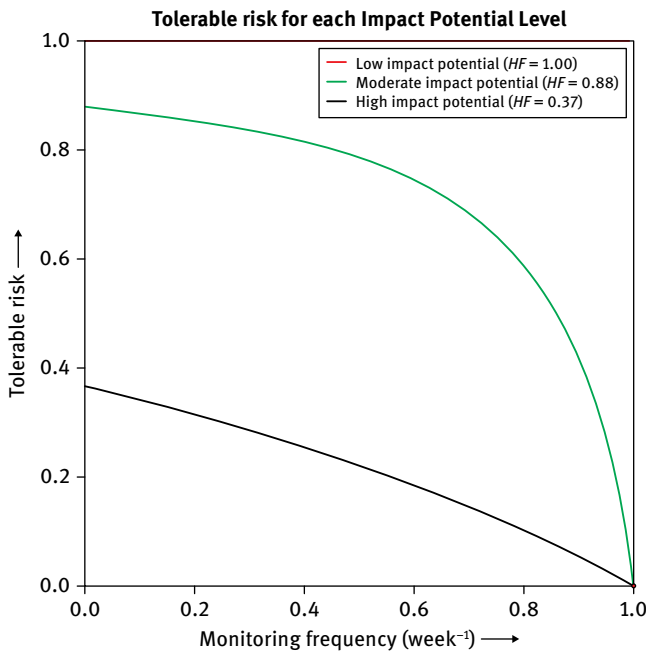


Fig. 10.2: Tolerated/tolerable risk according to monitoring frequency for each impact potential level, for Swan Coastal Plain Lakes. The tolerated risk indicates the probability that a cyanobacterial bloom at or above each impact potential level will not be detected given it is occurring. HF = Hazard Frequency. For further details on the methodology and definitions, please refer to [203].

a hazardous event not be identified by the monitoring regime (a “false negative” result). Since most monitoring methods are not of significant accuracy to identify every occurrence of a hazardous event, it is imperative that water managers are aware of the possibility that a dangerous event may occur which is not identified by the current monitoring methodology (Fig. 10.3). Water utilities and managers must optimize their monitoring regimes to reduce the occurrence of both “false positive” and “false negative” results, which will in turn reduce the risks and costs associated with hazardous events such as toxic cyanobacterial blooms.

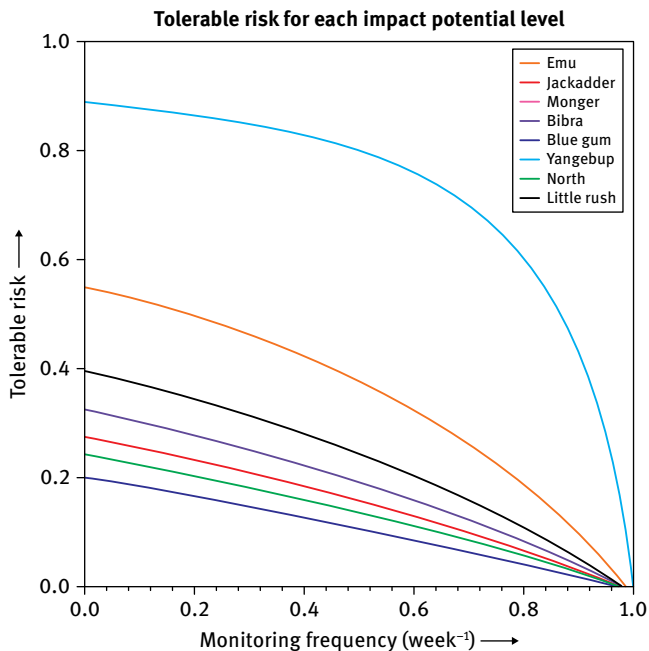


Fig. 10.3: Tolerated risk according to monitoring frequency for each impact potential level, at each of the investigated lakes of the Swan Coastal Plain. The tolerated risk indicated the probability that a cyanobacterial bloom at or above each impact potential level will not be detected given it is occurring. For further details on the methodology and definitions, please refer to [203].

A form of risk assessment that is used in medical diagnosis can be applied to environmental conditions and determines the probability that monitoring results will correctly identify hazardous situations (so long as the approximate frequency of the hazardous event is known). The development and application of this risk assessment methodology is outlined in Barrington et al. [197]. This assessment considers the relative probability of diagnostic tools returning “true positive”, “true negative”, “false positive” and “false negative” results [200]. This can assist in the development of monitoring programs and environmental decision-making [201, 202].

10.6 Case study: The management of cyanobacteria in waste stabilization ponds

Waste stabilization ponds (WSPs) are one form of freshwater body that have received minimal research with regards to the management of toxic cyanobacteria. Within such systems, the health and environmental risks associated with cyanobacterial blooms are coupled with the negative effects of cyanobacteria upon wastewater treatment processes, which may result in further indirect health, ecological and economic concerns. Cyanobacterial blooms are a serious problem in these systems, where they result in substantial increases to operational and maintenance costs of these assets. Hence the timely management of blooms in such reservoirs is essential.

Water containing human excreta has been treated since the link between sewage and human health was first recognized. There are many methods of wastewater treatment currently utilized, but the most commonly used process throughout both the developed and developing worlds consists of systems of WSPs [196, 204]. Such wastewater treatment plants (WWTPs) are generally utilized in rural and remote areas (Fig. 10.4), but plants servicing upwards of one million people have shown success where the land is available and reasonably priced [204].



Fig. 10.4: Examples of waste stabilization ponds (WSPs) in Australia.

WSPs are a simple, highly efficient, low-cost, low-maintenance and robust process for treating wastewater [204–206]. In WSPs, wastewater constituents are removed by sedimentation or transformed by biological and chemical processes, and a sludge layer forms due to the sedimentation of influent suspended solids, algae, and bacteria [205, 207]. In addition, WSPs are more efficient at removing pathogens than the electrochemical methods utilized in most urban WWTPs [195, 208].

After coarse screening to remove large objects, wastewater enters an initial deep WSP where sedimentation removes settleable particles including helminth eggs and protozoan cysts. These ponds are likely anaerobic due to the high biological oxygen demand (BOD) loading, and such oxygen-depleted conditions result in the significant reduction of BOD. Following sedimentation and anaerobic processing, wastewater enters facultative WSPs, which primarily remove pollutants through algal-bacterial mutualism. The photosynthetic algae present in these WSPs produce oxygen, which is then consumed by the heterotrophic bacteria that degrade any remaining organic and

inorganic compounds (Fig. 10.5) [195]. This further reduces the BOD of the water, such that it will not consume large amounts of dissolved oxygen when discharged. In the final WSP of the WWTP, referred to as the maturation or polishing pond, the majority of suspended sediments and pollutants have been reduced to acceptable levels for release. The primary function of the maturation pond is to kill dangerous wastewater organisms, including coliform bacteria and viruses, by the presence of natural radiation, high pH values and adsorption to settleable solids (Fig. 10.6) [195, 209]. In an ideal WWTP system, passage through these multiple WSPs will have decreased suspended sediment, organic and inorganic compounds, and dangerous wastewater organisms

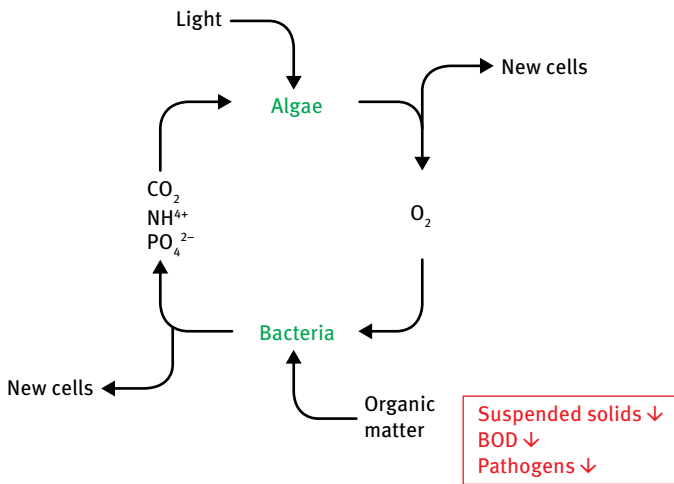


Fig. 10.5: Algal-bacterial mutualism in waste stabilization ponds (adapted from [195]).

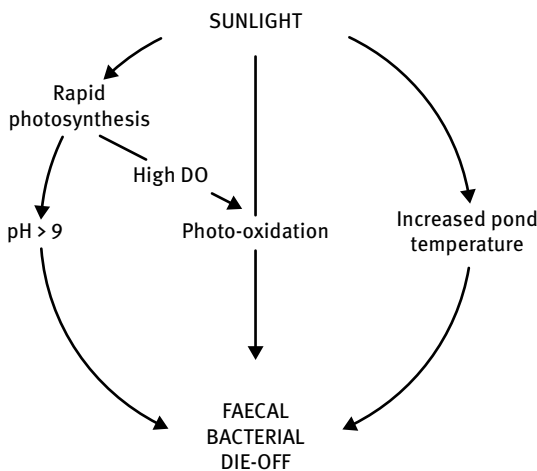


Fig. 10.6: Conceptual mechanisms for faecal-bacterial die-off in facultative and maturation waste stabilization ponds (reproduced from [195]).

to levels which will not harm humans or animals upon their release [195, 196]. Treated water is generally discharged to on-site evaporation, the environmental flow or human reuse [210].

Cyanobacteria have been recorded in WSPs throughout the world (e.g. [211–216], Fig. 10.7). Cyanobacterial blooms increase the sludge and suspended solids loadings of WSPs and change their ecology. By altering WSP ecology, cyanobacteria inhibit the natural processes of water purification anticipated by design engineers, particularly algal-bacterial mutualism, and this can result in the discharge of inadequately treated wastewater effluent.

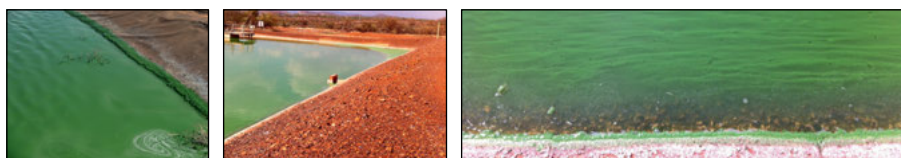


Fig. 10.7: Cyanobacterial bloom at in WSP in central Western Australia (Photos: [217, 218]).

Cyanobacteria and cyanotoxins impact upon WSP ecology through physical, chemical and biological mechanisms [219]. The ability of cyanobacteria to regulate their buoyancy gives them a competitive advantage over other phytoplankton, forming dense blooms and surface scums that shade the organisms below [21–23]. This shading inhibits the growth of other autotrophic organisms required for wastewater treatment and the removal of coliform bacteria and viruses by natural radiation. Cyanobacterial blooms may also alter the BOD, either by inhibiting natural wastewater treatment or by increasing the BOD when cells decay. This in turn decreases dissolved oxygen concentrations, which can have dire effects on aquatic species when effluent is discharged to the natural environment [10, 24]. Cyanotoxins are harmful to the aquatic biota involved in WSP treatment, including other phytoplankton, zooplankton and protozoa (reviewed in [220]), which can negatively impact treatment processes.

These changes in pond ecology caused by cyanobacteria have the potential to cause a shift away from beneficial wastewater treatment organisms, thus inhibiting treatment. This decreases the removal of wastewater pollutants such as coliform bacteria, nutrients and BOD, increasing the risk that insufficiently treated wastewater effluent will be discharged to reuse or the environment.

In highly eutrophic, shallow WSPs, prevention of blooms through these measures may not be practical or possible. Other preventative measures may also be of limited use in the WSP environment. The establishment of macrophyte communities has been shown to lower cyanobacterial concentrations, but is impractical in WSPs as macrophyte communities may impact upon wastewater treatment processes and provide breeding habitats for insects that carry vector-borne disease. Predation by the addi-

tion of zooplankton and fish, and biomanipulation by the addition of decaying organic matter and allelopathic chemicals, have been successful in some reservoirs, but may alter WSP ecology or significantly increase sludge production, further decreasing WWTP efficiency. Ultrasonication to prevent the formation of large cyanobacterial blooms has shown some promise in reduced-scale experiments, but is yet to be trialed on scales large enough to infer its preventative efficiency at the full-scale. Ultraviolet radiation may prevent cyanobacteria in some situations, but where cyanobacteria are already exposed to UVR they have likely developed defense mechanisms, reducing the suitability of UVR as a preventative technique.

Waste stabilization ponds are eutrophic, shallow systems which experience high levels of natural irradiance, so it is unlikely that cyanobacterial blooms can always be prevented.

Consideration of the cyanobacterial and cyanotoxin removal methods studied in the literature (Tab. 10.3) suggests that hydrogen peroxide (H_2O_2) may be suitable for reducing cyanobacterial and cyanotoxin concentrations, and may be more successful when coupled with other mitigation techniques. Hydrogen peroxide degrades within hours of addition [221–224], is not considered to be carcinogenic [225] and is unlikely to impact significantly upon aquatic biota at the concentrations required for cyanobacterial removal [177, 226, 227]. Hydrogen peroxide should not pose a risk to ecosystems or humans if treated effluent is discharged to the environment or for reuse.

Hydrogen peroxide has been used occasionally for cyanobacterial management in WSPs, although there has been minimal scientific investigation into the removal dynamics of cyanobacteria and cyanotoxins by this method. The addition of H_2O_2 alone has often been considered inadequate for cyanotoxin treatment [140, 165, 170, 174, 228]. However, there are many physical, chemical and biological properties of WSPs that differ from laboratory studies, and the presence of such factors may improve the potential for cyanobacterial and cyanotoxin treatment by H_2O_2 under WSP conditions.

10.7 Treatment of cyanobacteria and cyanotoxins with hydrogen peroxide

Cyanobacterial and cyanotoxin removal by H_2O_2 proceeds via the generation of hydroxyl ($\bullet\text{OH}$) and hydroperoxyl ($\bullet\text{OOH}$) radicals and is illustrated in Fig. 10.8. Hydroxyl and hydroperoxyl radicals are produced naturally from H_2O_2 through interaction with chemical catalysts (e.g. iron) and UVR. These radicals damage cells via multiple pathways, including membrane disruption, mutagenesis, bleaching of pigments, oxidation of photosystem II, reduction of carbon dioxide fixation and the division of peptides [164, 166, 179, 229–233]. These mechanisms lead to oxidative stress within cells, similar to the effects caused by photo-inhibition under high natural radiation doses [234]. Most phytoplankton are able to repair systems damaged by photo-

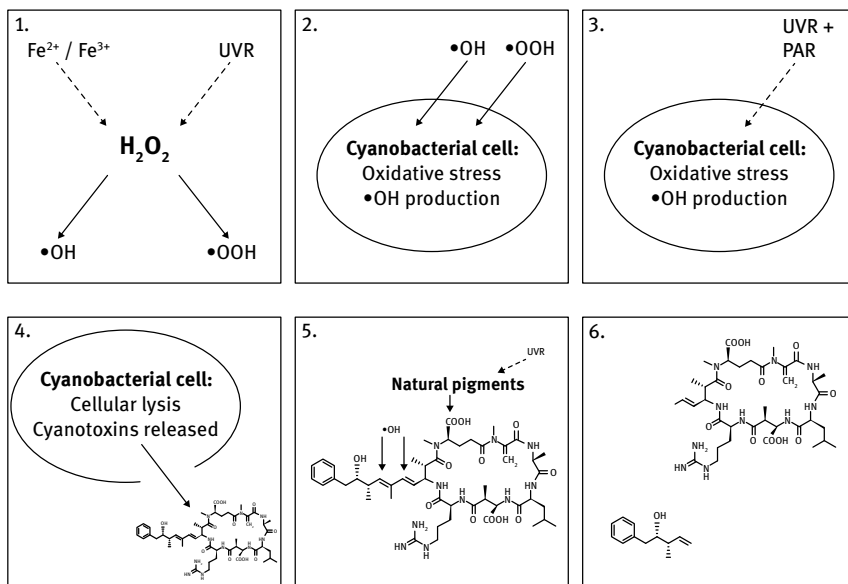


Fig. 10.8: Method of action of hydrogen peroxide (H_2O_2) for cyanobacterial and cyanotoxin removal.

- H_2O_2 is added to the water column. Chemical catalysts (e.g. iron) and ultraviolet radiation (UVR) react with H_2O_2 to produce hydroxyl ($\bullet\text{OH}$) and hydroperoxyl ($\bullet\text{OOH}$) radicals.
- $\bullet\text{OH}$ and $\bullet\text{OOH}$ radicals attack cyanobacterial cells, inducing oxidative stress and an increased cellular production of $\bullet\text{OH}$.
- Oxidative stress may lead to death if cellular processes are unable to repair the damage to core systems. Damaged cells become more susceptible to damage by UVR and photosynthetically active radiation (PAR).
- Cell death and lysis occurs. This releases cyanotoxins (here microcystin-LR) into the water column.
- $\bullet\text{OH}$ and $\bullet\text{OOH}$ radicals attack the conjugated bonds of cyanotoxins, which can lead to oxidation and ultimately cleavage. UVR interacts with natural pigments in the water column, which can lead to isomerization of the cyanotoxin molecule.
- The toxicity of the cyanotoxin is destroyed.

inhibition and oxidative stress within hours [229, 232–235]. Where these mechanisms are insufficient, permanent damage occurs, and it is likely that core photosynthetic activities have been lost, resulting in cyanobacterial death. The ensuing cell lysis releases cyanotoxins to the water column. Hydroxyl and hydroperoxyl radicals destroy the toxicity of cyanotoxins by targeting their conjugated diene structure, forming dihydroxylated products and inducing cleavage of the molecule [236]. Other natural processes such as thermal decomposition, isomerization, adsorption and biodegradation also destroy the toxicity of dissolved cyanotoxins, and studies have determined that oxidation of cyanotoxins results in non-toxic by-products [180, 188, 237]. Hydrogen peroxide may thus be effective for treating both cyanobacteria and cyanotoxins in WSPs.

Most studies into the use of H_2O_2 have been conducted within the laboratory, often on batch cultures of single cyanobacterial species [e.g. 168, 140, 164, 171, 166, 237] or purified cyanotoxin compounds in distilled water [e.g. 172, 173, 165, 170, 227, 174, 175]. The results of such investigations are not necessarily indicative of the dynamics of cyanobacteria and cyanotoxins following H_2O_2 addition to WSPs.

The effects of H_2O_2 on mixed phytoplankton assemblages under environmental conditions may be altered by a number of physical, chemical and biological variables. Natural irradiance increases the concentration of $\bullet OH$ and $\bullet OOH$ in solution [168] and further damages cells already inhibited by oxidation [239]. Mixing and stratification alter the position of phytoplankton and algicides within the water column, directly affecting the treatment of cells [240, 241]. The presence of other biota and compounds that may react preferentially with $\bullet OH$ and $\bullet OOH$ may reduce the removal of cyanobacteria and cyanotoxins by H_2O_2 , and the dynamics of H_2O_2 as an algicide may differ between prokaryotic cyanobacteria and eukaryotic phytoplankton species present in the assemblage [166, 168, 177, 242]. Although H_2O_2 does induce cell death in various phytoplankton, the decay of cyanobacterial cells appears to occur more rapidly than eukaryotic phytoplankton, suggesting that H_2O_2 may be a selective algicide when applied under environmental conditions [177]. Such environmental variables must be investigated to determine the applicability of H_2O_2 as an algicide.

Many studies into the effectiveness of H_2O_2 for removing cyanobacteria and cyanotoxins have been conducted on short timescales and at low temporal resolution (e.g. [140, 165, 167, 168, 170, 172–175, 228, 243]). In these investigations, particularly where H_2O_2 has not been coupled with other physical or chemical mitigation techniques, the removal of cyanotoxins by H_2O_2 has been considered negligible [140, 165, 170, 174, 228]. Short measurement periods have been considered sufficient given the rapid decay of H_2O_2 and the requirement for fast removal of cyanobacteria and cyanotoxins in rapid flow-through systems such as drinking water treatment. However, in systems such as WSPs, immediate cyanobacterial cell death and cyanotoxin removal is not required. Where H_2O_2 induces oxidative stress, cells may die within a timescale longer than that of H_2O_2 decay, releasing cyanotoxins to the dissolved state. Natural mechanisms may then degrade dissolved cyanotoxins given sufficient retention time, and it is thus important to monitor the effectiveness of H_2O_2 addition on timescales that allow for the induction of cellular stress followed by death, as well as natural degradation of cyanotoxins. In WSPs, water can be retained for several days following algicidal treatment, and the degradation of cyanobacteria and cyanotoxins over a longer timeframe than those traditionally investigated may be suitable.

Cyanobacterial treatment methods that result in the release of cyanotoxins from cells (e.g. [110, 244–246]) have generally been considered unfavorable for water management. Most past studies into the use of H_2O_2 have been conducted within the laboratory, so the degradation of cyanotoxins by natural mechanisms following H_2O_2 addition has not been thoroughly investigated. Cyanotoxins may be degraded more

rapidly from the dissolved state than whilst cell bound [247], suggesting that cyanobacterial mitigation techniques that induce cell lysis may be suitable where dissolved cyanotoxins can be degraded naturally. The methods of detoxication vary between water bodies, but studies indicate that thermal, photolytic, adsorptive and biodegradation of cyanotoxins occur within the environment.

Thermal decomposition of cyanotoxins is generally most effective at acidic pH values [248], which are rarely encountered in WSPs. Photolytic degradation can occur rapidly in systems containing high concentrations of pigments, but it is often not a significant removal mechanism in the short-term [191, 249, 250]. Adsorption onto natural matter may be unreliable for ensuring cyanotoxin degradation, particularly where cyanotoxins are suspended and not undergoing filtration [114–117, 119, 122].

Biodegradation has shown considerable success in degrading cyanotoxins (reviewed in [251]) (Tab. 10.3). Complete cyanotoxin removal has been observed to occur within two to three weeks under most environmental conditions. Cyanotoxins may act as substrates for certain bacteria, so the increase in bacterial populations may increase the rate of cyanotoxin removal. This suggests that higher concentrations of cyanotoxins may be biodegraded more rapidly than lower concentrations [252]. If natural biodegradation processes can be relied upon for the removal of cyanotoxins from WSPs, the expensive toxin removal techniques often utilized in drinking water treatment will be unnecessary.

The use of H_2O_2 for the removal of cyanobacteria and cyanotoxins from reservoirs introduces a larger scale than that investigated through laboratory experiments. Although controlled microcosm experiments are important when initially investigating the use of cyanobacterial and cyanotoxin removal methods, it is difficult to replicate environmental phenomena and heterogeneity at smaller scales. Such characteristics may significantly impact the use of H_2O_2 as a mitigation technique. The position of cyanobacterial cells and cyanotoxins within the water column is an example of a larger-scale phenomenon that may impact H_2O_2 use. Cells and cyanotoxins may be influenced by buoyancy regulation or stratification [253, 254], which themselves depend upon temperature, radiation and wind conditions [255], phenomena not often included in laboratory-scale studies. In order to test the true management potential of H_2O_2 as an algicide, scaled field trials are required to infer the differences in cyanobacteria and cyanotoxin dynamics following full-scale application [256–258].

Hydrogen peroxide has shown promise in reducing cyanobacteria and cyanotoxin concentrations in multiple studies. However, there has been limited investigation into the use of H_2O_2 for removing cyanobacteria and cyanotoxins from natural phytoplankton assemblages, particularly under field conditions and at the reservoir-scale, and no previous work has investigated the dynamics of cyanobacteria and cyanotoxin removal using H_2O_2 in WSPs. Should H_2O_2 be determined to be a suitable method for the removal of cyanobacteria and cyanotoxins from WSPs, a framework for the management of cyanobacterial blooms within WWTPs using H_2O_2 may be developed for use by water utilities.

Tab. 10.3: Studies into the biodegradation of cyanotoxins.

Study	Bacterial source	Toxin source	Experimental environment	Light regime	Initial cyanotoxin concentration	Temp. (°C)	Results
[259]	River	Extract from natural bloom	river water with biofilm (L)	AD (0/12 $\mu\text{E m}^{-2} \text{s}^{-1}$)	160 $\mu\text{g MC-LR/-YR l}^{-1}$	22	Half-life of 20–23 hours
[259]	River	Extract from natural bloom	tap water with biofilm (L)	AD (0/12 $\mu\text{E m}^{-2} \text{s}^{-1}$)	160 $\mu\text{g MC-LR/-YR l}^{-1}$	22	Half-life of 17–84 hours
[260]	River	Extract from natural bloom	river water (L)	—	50 $\mu\text{g MC-LR l}^{-1}$	—	90 % reduction in 2 days, 100 % reduction in 12 days
[261]	Lake	Extract from natural bloom	culture medium (L)	—	0.7 $\mu\text{g MC-LR l}^{-1}$ / 1.7 $\mu\text{g MC-RR l}^{-1}$	30	100 % reduction in 24 hours at pH 7, less degradation at other pH values
[262]	Lake	Lysed natural algal material	lake water (L)	D	10–136 $\mu\text{g MC-LR eq. l}^{-1}$	20	Decreased to < 1 $\mu\text{g/l}$ after 7 days, 100 % reduction in 21 days
[262]	Lake	Lysed natural algal material	Lake water (F)	ND	2–54 $\mu\text{g MC-LR eq. l}^{-1}$	15.5–21.5	Decreased to < 1 $\mu\text{g/l}$ after 1–4 days, reduced to detection levels after 8 days
[263]	Reservoir water and bed sediment	Stock solution	Reservoir water and bed sediment (L)	AD	10 $\mu\text{g MC-LR l}^{-1}$	17–21	100 % reduction in 6–7 days
[264]	River and loch	Extract from culture	River and loch water (L)	—	1000–5000 $\mu\text{g MC-LR/-RR/-LW/-LF/NOD l}^{-1}$	29	100 % reduction in 7–19 days

Tab. 10.3 (continued)

Study	Bacterial source	Toxin source	Experimental environment	Light regime	Initial cyanotoxin concentration	Temp. (°C)	Results
[265]	Lake (during cyanobacterial bloom)	—	Lake water (L)	AD	20 µg NOD l ⁻¹	8–25	100 % reduction in 2–15 days
[265]	Lake (no cyanobacterial bloom present)	—	Lake water (L)	AD	20 µg NOD l ⁻¹	20–25	100 % reduction in 7 days
[163]	Biological sand filter	Extract from natural bloom	Reservoir water (L)	—	3–25 µg MC-LR/-LA l ⁻¹	22–30	100 % reduction in 2–15 days
[252]	Tertiary treated effluent	Stock solution	Tertiary treated effluent (L)	—	6–20 µg MC-LR l ⁻¹	22	100 % reduction in 3–4 days
[252]	Activated sludge treated effluent	Stock solution	Tertiary treated effluent (L)	—	20 µg MC-LR l ⁻¹	10–22	100 % reduction in 7–22 days
[266]	Lake sludge	Extract from culture	Culture medium (L)	—	≈ 2–4 µg MC-LR l ⁻¹ / ≈ 4 µg MC-RR l ⁻¹	30	100 % reduction in 4–17 hours
[267]	Lake	Extract from natural bloom	Culture medium (L)	D	6000 µg MC-RR/NOD l ⁻¹	30	100 % reduction in 4 days
[268]	Lake	Lake (naturally occurring)	Mesocosms within lake (F)	ND	0.14–8.93 µg MC-LR/-RR l ⁻¹	Variable	Toxin removal varied over several months
[244]	Lake bloom treated with copper sulfate	Lake bloom treated with copper sulfate	Lake bloom treated with copper sulfate (F)	ND	1300–1800 µg MC-LR eq. l ⁻¹	Variable	9 day lag phase before degradation began, 94 % degradation after 12 days

Tab. 10.3 (continued)

Study	Bacterial source	Toxin source	Experimental environment	Light regime	Initial cyanotoxin concentration	Temp. (°C)	Results
[269]	Culture	Extract from natural bloom	Drain and dam water (L)	—	1000 µg MC-LR l ⁻¹	20	3–6 day lag phase before degradation began, >95 % reduction in 10 days
[269]	Culture	Extract from natural bloom	Lake water (L)	—	1000 µg MC-LR l ⁻¹	20	No significant degradation in 12 days
[269]	Culture	Extract from natural bloom	River water (L)	—	1000–16 000 µg MC-LR l ⁻¹	20	Degradation rates increased with initial MC concentration > 95 % reduction in 23 days
[110]	Lake	Lake	Lake water treated with copper sulfate (L)	AD	≈ 1 µg MC-LR l ⁻¹	20	MC released to dissolved phase over 4 days Half-life of 3 days following maximum release
[270]	Lake	Lake	Mesocosms within lake (F)	D/ND	0.06–3.2 µg MC-LR l ⁻¹	Variable	90 % reduction in 15–30 days
[271]	Final effluent from activated sludge WWTP	Extract from culture	Sewage effluent (L)	AD	210–1620 µg MC-LR l ⁻¹	25	Undetected by day 13–27
[272]	Lake	Stock solution	Filtered and sterilised lake water (L)	D	10 000 µg MC-LR/-LF/-LW/-LY/-RR/NOD l ⁻¹	25	Half-life of 5–> 10 days
[273]	Estuarine water	Stock solution or lysed culture material	Estuarine water (L)	—	≈ 1000 µg MC-LR/[D-Leu ¹]MC-LR l ⁻¹	14–15	100 % reduction in 10–20 days

Tab. 10.3 (continued)

Study	Bacterial source	Toxin source	Experimental environment	Light regime	Initial cyanotoxin concentration	Temp. (°C)	Results
[274]	Culture	Extract from culture	Culture medium (L)	—	1/10/100 µg MC-LR l ⁻¹	4/22/37	Varied significantly between bacterial strains. Greatest reduction 60.1 % in 24 hours
[275]	Culture	Extract from culture	Culture medium (L)	—	Various concentrations of CYN and MC-LR/-RR/-LW/-LY/-LF	37	Varied. Greatest reductions 60.3 % MC-LR, 62.8 % MC-RR, 77.4 % MC-LF, 31.6 % CYN
[276]	Culture	Extract from culture	Culture medium (L)	—	100–4000 µg MC-LR/-RR l ⁻¹	—	99–100 % reduction in 5–9 days
[277]	Lake	Extract from natural bloom	Culture medium (L)	—	20 µg MC-LR/-RR/-YR l ⁻¹	27	100 % reduction in 6 days
[277]	Lake	Extract from natural bloom	Culture medium (L)	D	3–37 µg MC-LR/-RR/-YR l ⁻¹	5–30	10–30 °C, 100 % reduction in 6 days 5 °C, 60 % reduction in 7 days
[278]	Lake	—	Culture medium (L)	D	1000 µg MC-LR/-RR/-YR l ⁻¹	30	95–98 % reduction in 2.5 hours
[279]	Cultures	—	Culture medium (L)	—	100 µg MC-LR l ⁻¹	22/37	Varied between bacteria, greatest reduction 80 % in 25 hours
[280]	Lake	Stock solution	Culture medium (L)	—	50 000 µg MC-LR l ⁻¹	25	95.5 % reduction in 21 days

Tab. 10.3 (continued)

Study	Bacterial source	Toxin source	Experimental environment	Light regime	Initial cyanotoxin concentration	Temp. (°C)	Results
[281]	Lake water and sediment	Stock solution and extract from natural bloom	Culture medium (L)	D	200 µg MC-LR/-RR l ⁻¹	27	< 1 day for total degradation
[282]	Reservoir	—	Culture medium (L)	—	200 µg MC-LR/-RR l ⁻¹	—	100 % degradation in 36 hours
[283]	Lake sediment	Stock solution	Culture medium (L)	—	42 300 µg MC-RR l ⁻¹	30	100 % degradation in 10–36 hours
[284]	Culture	Culture	Culture medium (L)	—	114 µg MC-LR l ⁻¹	—	Up to 82.7 % removal in 40 hours

Experimental environment:

L: laboratory, F: field.

Light regime:

D: dark, AD: artificial diurnal, ND: natural diurnal, AC: artificial constant.

Concentration:

MC-LR: microcystin-LR, MC-YR: microcystin YR, MC-RR: microcystin-RR, MC-LW: microcystin-LW, MC-LF: microcystin-LF, MC-LA: microcystin-LA, [D-Leu¹]MC-LR: [D-Leu¹] microcystin-LR, NOD: nodularin, CYN: cylindrospermopsin, MC-LR eq.: microcystin-LR equivalents.

—: indicates that details of the property were not stated.

10.8 New techniques for the control and characterization of cyanobacterial blooms

10.8.1 Allelopathic control of cyanobacteria

As discussed previously, there are many control methods for blooms; however, in some situations only a few of them are applicable due to other factors, including secondary pollution, high cost or no-target ecosystem effects. Consequently, there is a need for anti-algal agents that are more specific, environmentally-friendly and cost-effective.

Allelopathy is the direct or indirect effect of plants (including microorganisms) on others through the production of chemicals. This technique could be utilized for the development of an anti-algal agent for the control of harmful algal blooms; the allelopathic activity of barley straw to many kinds of algae including *Microcystis* has already been documented in the field and laboratory [58, 285–287].

Barley, *Hordeum vulgare L.*, is one of the earliest cultivated crops in the world and can be divided into two distinct groups: occidental and oriental. Most studies on cyanobacteria control using barley were undertaken in Europe and America with the cultivated occidental barley as an anti-algal agent. Oriental barley, i.e. Tibetan hulless barley (*Hordeum vulgare L. var. nudum*) originated from the Qinghai–Tibetan Plateau and is regarded as the progenitor of cultivated barley [288].

One of the key interests in allelopathy in China is to assess the acute, mid and long-term effects of Tibetan hulless barley straw extract on the growth, physiology and morphology of *Microcystis aeruginosa* at a single cell level [54, 63]. Recent data shows that a dosage of 2.0 g (dry weight) l⁻¹ of Tibetan hulless barley straw reduces the *in vivo* chlorophyll-*a* (chl-*a*) fluorescence of *M. aeruginosa* cells, resulting in a significant decline of the cell density (Fig. 10.9). These studies show promise for future use of Tibetan hulless barley straw algicidal agent for *M. aeruginosa* [54, 63, 64].

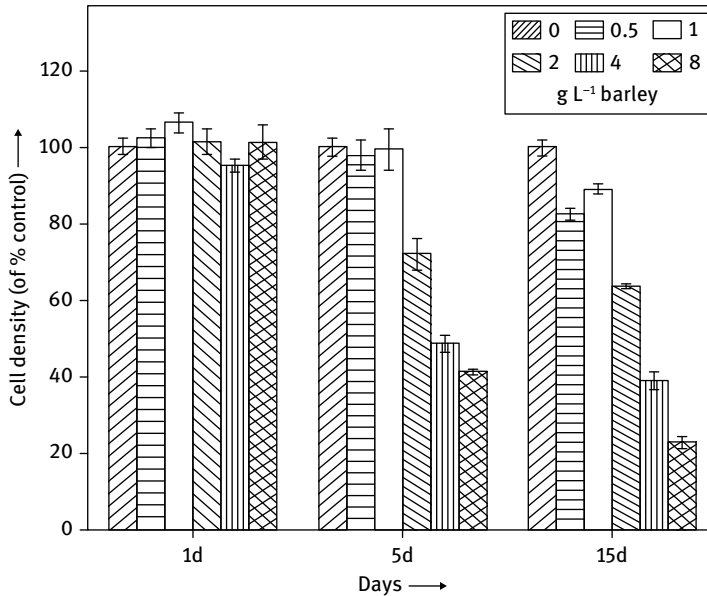


Fig. 10.9: Inhibition effect on growth of *Microcystis aeruginosa* cultures after a 1-, 5-, and 15-d Ti-betan hulless barley straw exposure. All error bars correspond to the standard deviation. Reproduced from [54].

10.8.2 Optimization of the FDA-PI method using flow cytometry to measure metabolic activity of cyanobacteria

The control of *M. aeruginosa* blooms has been the focus of many studies because of the widespread occurrence and the spectacular nature of the bloom events (Fig. 10.10), where this particular species can completely dominate the phytoplankton community [289, 290].



Fig. 10.10: Spectacular *M. aeruginosa* algal bloom in Lake Taihu, China. (Photos: Hohai University, Nanjing, China).

Advanced methods for the detection and assessment of the physiological status of this particular species have been developed to ensure a reliable diagnosis of the algal blooms. One such method is the exploration of the inhibition of enzyme activity, which is now a widely accepted method to determine acute and sub-lethal endpoints for bioassays, and to assess the integrity of the cell membrane during the blooms [291]. The release of large amounts of endotoxins can constitute a major environmental and human health hazard. Therefore, it is important to develop reliable diagnostic tools to monitor the blooms events, for a better risk assessment [292, 293].

Many kinds of fluoresceins had been used to detect the enzyme activities and cell membrane integrity. Among them, fluorescein diacetate (FDA) and propidium iodide (PI) were most frequently used. FDA is a non-polar, hydrophobic, non-fluorescent esterified compound; it readily permeates the cell membrane, and is hydrolyzed by non-specific esterases producing a fluorescein [294]. The mean fluorescence intensity per cell (MFI) of FDA-converted fluorescein was used to estimate the the enzyme activity (i.e. hydrolysis rate of esterase) in algae. PI is a fully cell-membrane impermeable fluorescent dye which has been used to indicate dead cells for a wide range of microorganisms [295, 296]; it can only combine with DNA in dead cells or cells with damaged membranes [295, 297]. In contrast to PI, the use of FDA was first reported for detecting the viability of marine phytoplankton after the exposure of environmental contaminants using a fluorescence microscope [298].

Cells with an intact cell membrane are stained bright green by FDA; in contrast, cells with a broken cell membrane are stained bright orange with PI [299, 300]. Furthermore, FDA also indicates the presence of active esterase [294, 300]. Cells with an intact cell membrane and inactive esterase do not stain with FDA or PI. Afterwards, the efficiency of PDA/PI detection was greatly improved by the detection of the fluorescence of individual cells using flow cytometry [301]. More recently, Franklin et al. [291] developed a rapid enzyme inhibition bioassay based on PDA/PI for marine and fresh-water microalgae with the use of flow cytometry, but no evidence of *M. aeruginosa* was detected in this study. Moreover, FDA has been used to evaluate the esterase activity of *M. aeruginosa*, but dosages used ranged from 1.6 to 16 mg l⁻¹ and the incubation time differed from 8 min to 2 h [302, 303].

Recently, a new procedure based on an optimized FDA/PI condition has been developed for short-term bioassays [54, 304]. This new procedure takes working conditions such as pH and impure cultures into consideration, could avoid algal cell damages in sample preparation and separate algal cells from non-algal particles by fluorescence triggering. This procedure has been used to assess the toxicity of copper on *M. aeruginosa* in a short-term exposure (36 h). As copper concentrations increased, the esterase activity was found to decrease in a concentration-dependent manner and the membrane fragments increased (Fig. 10.11). Moreover, esterase activity was a good indicator of copper toxicity in *M. aeruginosa*. The EC₅₀ value based on MFI was 101.5–146.2 µg/l (95 % confidence intervals) [304]. Therefore, this new procedure has the potential to be sub-lethal endpoint detection, and could be used for the selec-

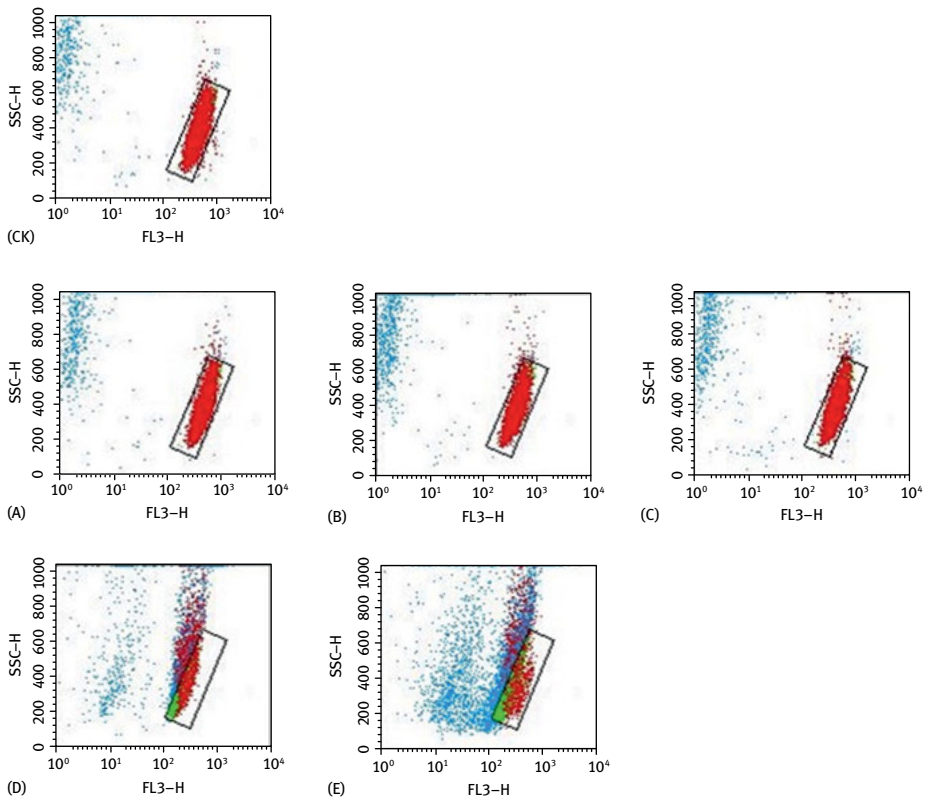


Fig. 10.11: Flow cytometry images of *M. aeruginosa* cells after 36 h copper exposure by Side Scatter (SSC) and FL3 detectors. (CK: blank control; A: 25 μg (Cu)/l; B: 40 μg (Cu)/l; C: 63 μg (Cu)/l; D: 100 μg (Cu)/l; E: 158 μg (Cu)/l).

tion of *M. aeruginosa* control methods or investigation of the *M. aeruginosa* activity inhibition mechanism as a rapid and cost-effective bioassay.

10.9 New perspectives and future directions

In recent years, the study of waste stabilization ponds (WSPs) as ecological systems has revealed new considerations that are likely to influence how we see a wide range of aquatic systems with cyanobacteria including a lakes, rivers and reservoirs. In particular, it was determined that the interplay between the hydrodynamics and ecology within these systems can explain the occurrence, magnitude and frequency of cyanobacterial blooms (Fig. 10.12).

Sludge accumulation can impact performance by reducing pond effective volume and changing the shape of the bottom surface, thus altering pond hydraulics [205,

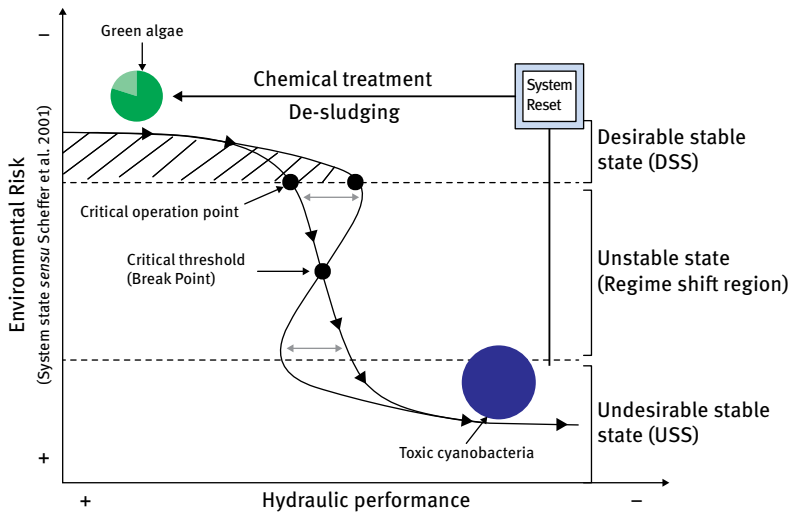


Fig. 10.12: Conceptual framework inspired by Scheffer's theory of catastrophic shift in ecosystems applied to a situation of intense eutrophication of WSP systems. The shaded area (lines) represents the desirable operational state of the system. Below the critical operation point small changes in hydraulic performance can lead to catastrophic shifts, driving the system into an undesirable stable state. A similar situation could be expected in hypereutrophic systems where intense cyanobacterial blooms occur. Reproduced from: Ghadouani and Coggins [218].

305, 306]. While periodic sludge removal is required, it is rarely considered integral to pond design [205], and the long-term sustainability of WSP systems is dependent on the safe management of sludge [206]. Previous studies have shown that distribution of sludge in ponds can be very uneven [205, 206, 217] (Fig. 10.13) and that different climatic regions have an effect on sludge accumulation rates [206]. Despite the number of WSPs worldwide (e.g. in regional Western Australia there are 84 wastewater treatment plants using 302 WSPs for treatment), there is still little information available on sludge distribution, sludge characteristics, accumulation rates, and their effect on wastewater treatment efficiency.

Besides sludge accumulation, factors that influence pond hydraulic performance are mainly related to shape, flow, inlet/outlet configuration, wind and temperature [307–310]. Optimal flow within treatment systems is described as flow with a uniform velocity profile, and it is recommended that ponds be designed to adhere to plug flow [307]; this flow regime provides mean maximum residence time. However, in reality, water in ponds does not move homogeneously, but with eddies and recirculation [307], and the actual mean residence time is always less than the nominal residence time [308]

Microbial and phytoplankton communities are essential for the functionality of WSPs, and community health is important for overall treatment efficiency. Microbial processes in ponds, such as algal growth, aerobic and anaerobic heterotrophic

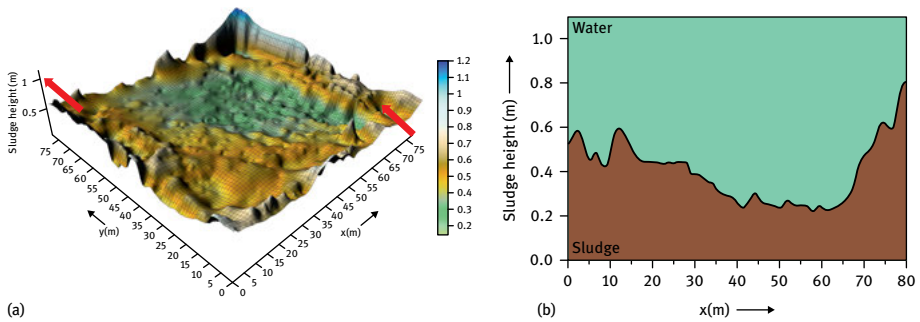


Fig. 10.13: (a) Sludge profile of a Western Australian WSP. z-axis shows sludge height from pond bottom in meters. Inlet and outlet configuration shown (red arrows). (b) Cross section of pond at $y = 40$ m. Note the formation of a channel between the inlet at outlet (a), and the variable horizontal distribution (b). Depth of pond: 1.1 m.

metabolism, nitrification and denitrification, work in conjunction with physical processes and exposure to sunlight to remove pathogens, nitrogen and organic contaminants [207]. The highly complex microbial communities present in wastewater treatment systems are not well understood, despite their importance in the treatment process [311]. Recent studies have indicated that there is a link between microbial diversity, community structure and treatment efficiency [312, 313], and that total bacterial cell counts [314] and phytoplankton presence [315] can be used as descriptive parameters for treatment processes and performance.

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