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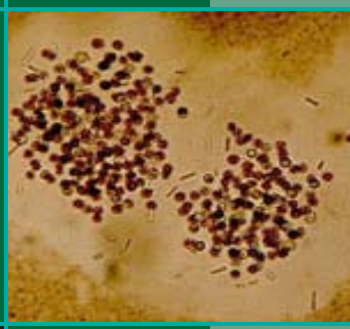

Research Report 74

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# Management Strategies for Cyanobacteria (Blue-Green Algae): A Guide for Water Utilities

**Gayle Newcombe, Jenny House, Lionel Ho, Peter Baker and Michael Burch**

## Research Report No 74




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Management Strategies for Cyanobacteria (Blue-Green Algae) and their Toxins: a Guide for Water Utilities

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# FOREWORD

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# EXECUTIVE SUMMARY

The *Management Strategies for Cyanobacteria (Blue-Green Algae): A Guide for Water Utilities* is a comprehensive manual, which consolidates current knowledge on the management of cyanobacteria (blue-green algae). The guide covers management strategies of source water and all stages of the treatment process.

The guide provides an introduction to cyanobacteria including an outline of the health effects of toxins and description of the tastes and odours associated with cyanobacteria. The current guidelines and standards that relate to toxic cyanobacteria are described.

Source water management is covered including a description of the life cycle of cyanobacteria and factors affecting growth to help the water supplier understand the effect of management strategies for the control of cyanobacteria. Sampling and monitoring programs and their rationale are also described. An overview of the best procedure for assessing the risk of toxic algal outbreaks in a water supply and a description of the monitoring aid known as the 'Alert Levels Framework' are covered. An evaluation of nutrient control, mixing strategies and algicides is included in the review of source water management practices.

Treatment strategies are described including advice on conventional treatment, oxidation by chlorine and ozone and adsorption by granular activated carbon (GAC) and powdered activated carbon (PAC), biological filtration, UV and membranes and multiple barrier options. Finally recommendations are made regarding the most cost-effective treatment strategies for given particular conditions.

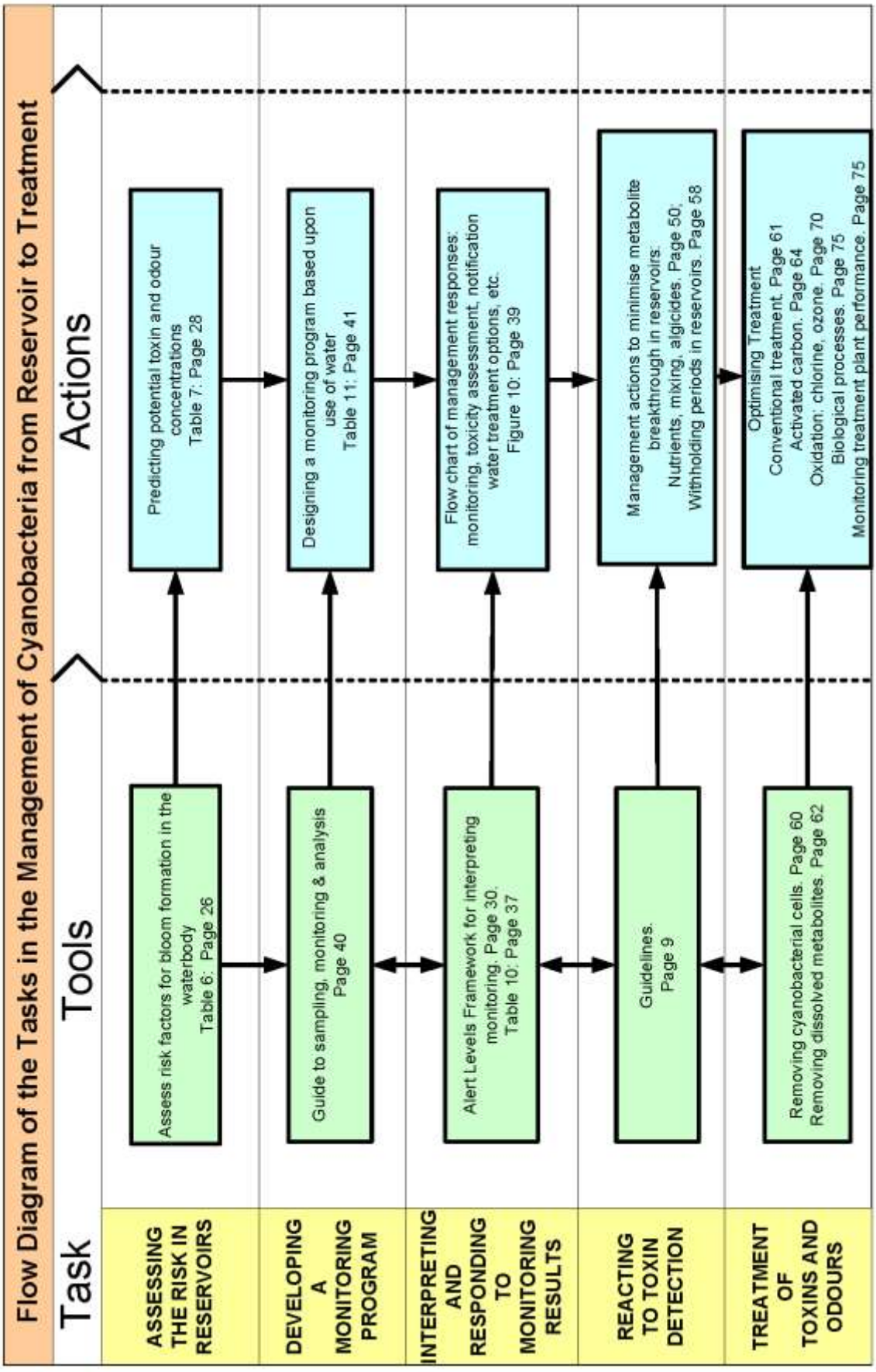
The following flow diagram (Figure 1) summarises the major management processes recommended when cyanobacteria are present in a water supply. The processes follow from the reservoir to the treatment plant and identify potential tools and actions to deal with each particular management task.

The flow diagram is designed so that a water manager can:

- identify when and where a problem exists
- identify a management tool or action that may be appropriate for their situation
- gain further information on that action from the relevant section in the guide.

A comprehensive summary of the treatment options available for the management of cyanobacteria and their metabolites is given in Tables 22 and 23.

Figure 1: Quick reference guide to tasks in management of cyanobacteria.



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# 1 INTRODUCTION

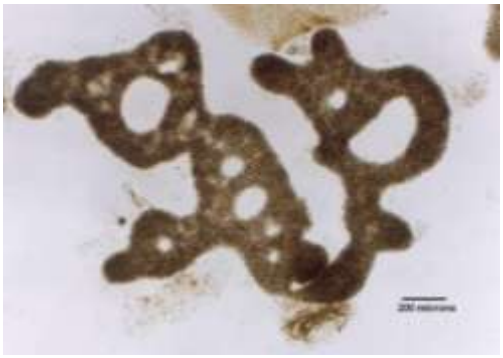
Cyanobacteria are widespread in the terrestrial and aquatic environment. The two main issues of concern for drinking water supply are the taste and odour compounds and toxins these organisms are known to produce. Toxic cyanobacteria have been reported in twenty-seven countries and are found on all continents, including Antarctica. However, for the consumer, the “earthy” taste and odour often associated with cyanobacteria is of significant concern, leading to the perception that the water may be unsafe to drink. Drinking water authorities world-wide are faced with the challenge of treating contaminated water, or with the possibility of a cyanobacterial bloom occurring sometime in the future. As a result, cyanobacteria have been identified as a major issue of concern in water supply reservoirs.

## 1.1 What are Cyanobacteria?

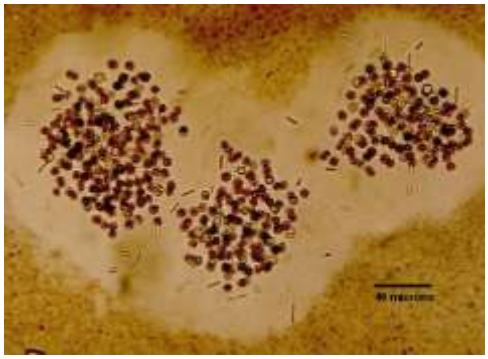
Cyanobacteria (blue-green algae) are microscopic photosynthetic organisms that form a common and naturally occurring component of most aquatic ecosystems [1]. Cyanobacteria belong to the group of organisms called prokaryotes, which also includes bacteria, and can be regarded as simple in terms of their cell structure. They are characterised by the lack of a true cell nucleus and other membrane-bound cell compartments such as mitochondria and chloroplasts. Eucaryotes by contrast, are all other organisms such as animals, plants, fungi, and protists, which includes the true algae. These all have their cells organised into compartmentalised structures called organelles, which in particular includes the nucleus.

Cyanobacteria come in a range of shapes and sizes and can occur as single cells, floating free within the water column, while others assemble into groups as colonies or filaments [2] and occur in many and varied environments. Filaments can be straight, coiled, or twisted, and have been reported up to several millimetres long. Colonial cyanobacteria, for example *Microcystis aeruginosa,* can be found as individual cells, and also in colonies of up to many millimetres. Filaments and colonies can also aggregate, to form much larger structures, of multiple filaments or colonies which are clearly visible to the naked eye.

The forms of some of the more common problem cyanobacteria found in Australia are shown in Figure 2. In low numbers they are an important contributor to the aquatic biology of waterways but can increase to such large numbers that they can dominate when conditions become favourable, causing unsightly colouring of the water, floating surface scums, and at times unpleasant smells [3].



*a) Microcystis aeruginosa*



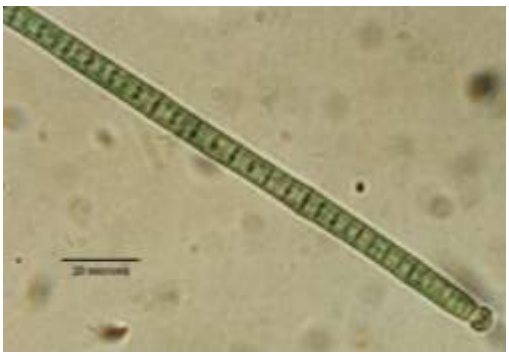
*b) Microcystis aeruginosa*

*c) Anabaena circinalis*

*d) Anabaena planktonica*

*e) Cylindrospermopsis raciborskii*

*f) Aphanizomenon ovalisporum*



g) *Phormidium amoenum*

h) *Nodularia spumigena*

Figure 2: Microscope photographs of some of the common toxic and odour producing cyanobacteria found in Australia.

### 1.1.1 Why are cyanobacteria a problem, particularly for drinking water supplies?

The water supply problems associated with cyanobacteria include offensive tastes and odours and the possible effects of toxins on public health. These have a deleterious effect upon drinking water quality. It is important to note that not all cyanobacteria produce toxins, including blooms containing known toxin-producing species.

## 1.2 Health Effects

Historically, there are well-documented and anecdotal reports of animal poisonings and deaths as well as human poisonings from drinking water contaminated with cyanobacterial blooms. Epidemiological evidence has also shown symptoms of poisoning or injury that have been attributed to the presence of cyanobacterial toxins in drinking water [4]. Toxicological studies carried out on animals have provided information on the role of the toxins in poisonings and on their comparative toxicity [4]. Little is known of the scale and nature of either long-term or short-term effects of these toxins [5]. Therefore the health significance of algal toxins in water supplies is an important issue.

### 1.2.1 What are the known health effects of cyanobacteria?

Cyanobacteria are known to produce toxins. There are two main groups of cyanotoxins, cyclic peptides and alkaloids. Another group, the lipopolysaccharides, are of similar structure to potent bacterial endotoxins, but current evidence suggests that the cyanobacterial versions are not as potent as their bacterial counterparts.

Table 1 lists the principal target organs for these toxins, and the cyanobacteria that produce them. Although the toxins listed are assumed to be the substances most significant for human health, it is unlikely that all cyanotoxins have been discovered.

The majority of cyanotoxins are associated with well-known planktonic and bloom forming cyanobacteria that are free floating in the water, such as *Microcystis*, *Anabaena* and *Cylindrospermopsis*. However it is important to note that some benthic or attached cyanobacteria, such as *Oscillatoria, Phormidium* and *Lyngbya* have also been shown to sometimes produce both neuro- and hepatotoxins the same as those found in planktonic species and therefore should also be considered as a possible toxicity hazard [6,7,8,9,10,11,12,13].

#### 1.2.1.1 Cyclic peptides

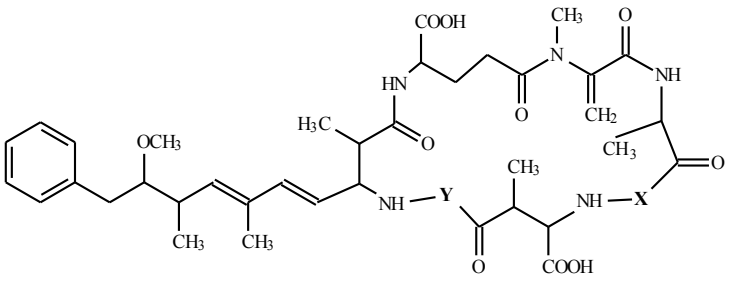
The microcystins and nodularin are known to cause liver damage (hepatotoxins). They block protein phosphatases 1 and 2a, which are “molecular switches” in all eukaryotic cells, with an irreversible covalent bond [14]. For vertebrates, a lethal dose of microcystin causes death by liver damage within hours to a few days.

There are two potential mechanisms for long-term microcystin damage to the liver, progressive active liver injury as described above [15], and promotion of tumour growth. Tumour-promoting activity of microcystins is well documented in animals, although microcystins alone have not been demonstrated to be cancer causing. The structures of the peptide hepatotoxins are shown in Figure 3.

Table 1: General features of the cyanotoxins [after 16,17,18,19,20].

| Toxin Group | Primary target organ in mammals | Cyanobacterial genera |
|---|---|---|
| **Cyclic peptides** | | |
| Microcystins | Liver, possible carcinogen in this and other tissues | *Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis, Aphanizomenon* |
| Nodularin | Liver, possible carcinogen | *Nodularia, Anabaena, Planktothrix (Oscillatoria), Aphanizomenon* |
| **Alkaloids** | | |
| Anatoxin-a | Nerve synapse | *Anabaena, Planktothrix (Oscillatoria), Aphanizomenon, Cylindrospermopsis* |
| Anatoxin-a(S) | Nerve synapse | *Anabaena* |
| Aplysiatoxins | Skin, possible tumour promoter | *Lyngbya, Schizothrix, Planktothrix (Oscillatoria)* |
| Cylindrospermopsins | Liver and possibly kidney. Possible genotoxic and carcinogenic | *Cylindrospermopsis, Aphanizomenon, Umezakia, Raphidiopsis, Anabaena* |
| Lyngbyatoxin-a | Skin, gastrointestinal tract, possible tumour promoter | *Lyngbya* |
| Saxitoxins | Nerve axons | *Anabaena*(1), *Aphanizomenon, Lyngbya, Cylindrospermopsis* |
| **Lipopolysaccharides (LPS)** | Potential irritant; affects any exposed tissue | All |
| **β-*N*-methylamino-L-alanine (BMAA)** | Brain - neurodegenerative disease | Many species |

(1) The common bloom forming cyanobacterium *Anabaena circinalis* often produces saxitoxins in Australia. The name of this genus has recently been revised internationally to *Dolichospermum.* The recognition and adoption of this new name is expected to come into common usage over time and this important bloom-forming species will therefore progressively become known as *Dolichospermum circinalis* [21]

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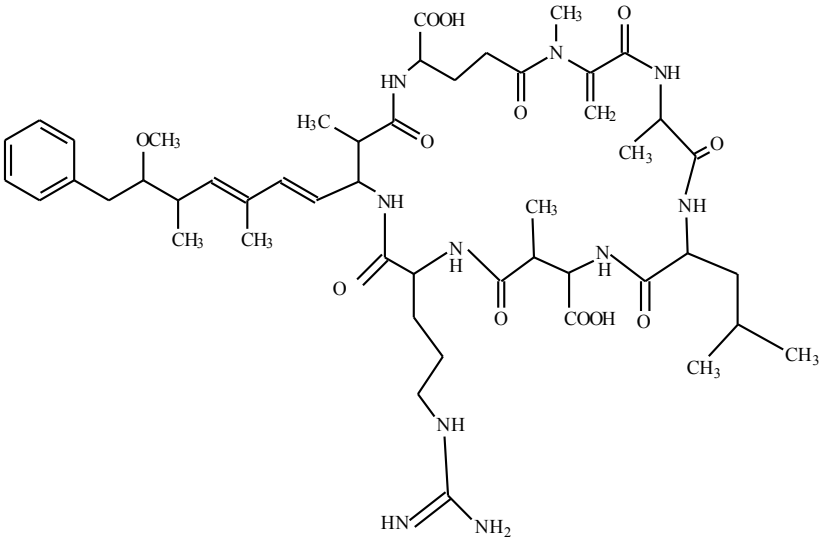


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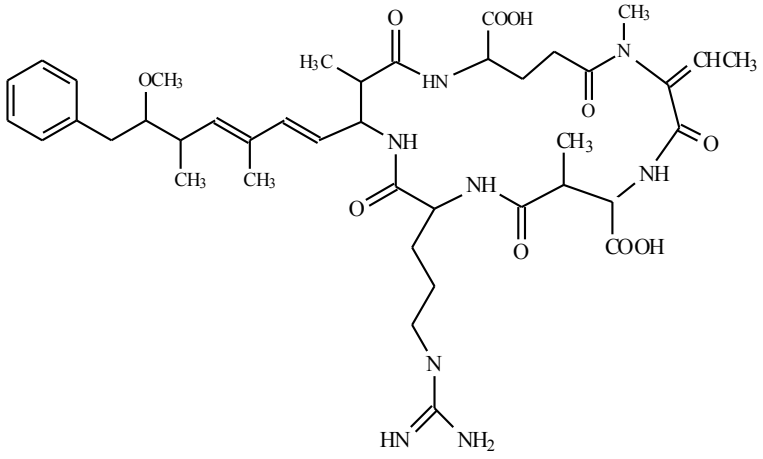



**(3)**

Figure 3: Structures of peptide hepatotoxins; (1) - General structure of microcystins with variant amino acids at positions X & Y, (2) - microcystin-LR, (3) – nodularin.

Microcystins initially appeared to contain 5 invariant and 2 variant amino acids. One of the invariant amino acids is a unique β-amino acid called Adda. A 2-letter suffix (XY) is ascribed to each individual toxin to denote the 2 variant amino acids [22]. At position 'X' the amino acid is commonly leucine, arginine or tyrosine, and at 'Y' is arginine, alanine or methionine. Variants of all the "invariant" amino acids have now been reported, e.g., desmethyl amino acids and/or replacement of the 9-methoxy group of Adda by an acetyl moiety. Currently there are in excess of 80 variants of microcystin which have been characterised [23,24]. Of these 80 compounds, microcystin-LR appears to be the microcystin most commonly found in cyanobacteria. It is also common for more than one microcystin to be found in a particular strain of cyanobacterium [25,26]. The microcystin variants also differ in toxicity [27]. The literature indicates that hepatotoxic blooms of *M. aeruginosa* containing microcystins occur commonly worldwide [24].

The cyclic pentapeptide nodularin contains amino acids similar or identical to those found in microcystins, namely arginine, glutamic acid, β-methylaspartic acid, N-methyl-dehydrobutyrine and also Adda [28].

### 1.2.1.2 Alkaloids

The alkaloid toxins produced by cyanobacteria include a range of compounds that interfere with nerve cell function (neurotoxins), including anatoxins and saxitoxins, as well as cylindrospermopsin, which is a recognised hepatotoxin, but which also causes general cell damage (cytotoxin).

While the neurotoxins have different modes of action, all have the potential to be lethal at high doses by inhibiting the ability to breathe - anatoxin-a and anatoxin-a (S) through cramps, and saxitoxins through paralysis. However, no human deaths from exposure to cyanobacterial neurotoxins are known.

The neurotoxic saxitoxins or paralytic shellfish poisons (PSPs) are one of a number of groups of toxins produced by dinoflagellates in the marine environment (Figure 4). Shellfish feeding on toxic dinoflagellates can themselves become toxic and hazardous if consumed, even causing human fatalities [29]. Poisoning incidents usually coincide with the sudden proliferation of these organisms to produce visible blooms, the so-called "red tides" [30,31].

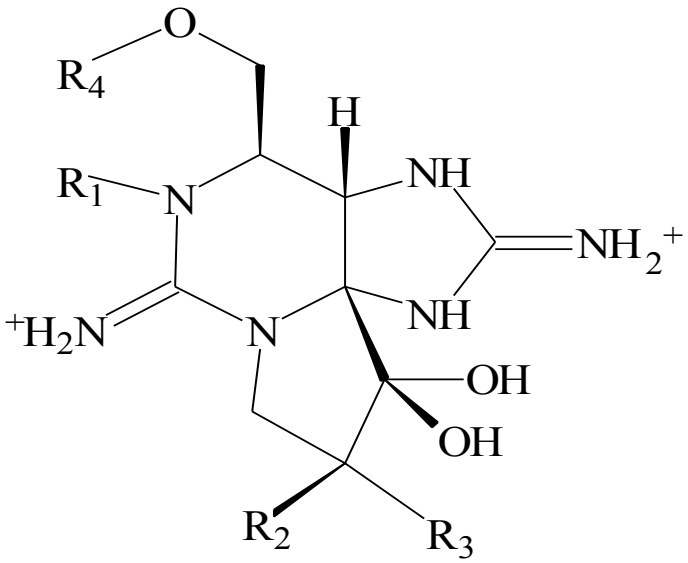



Figure 4: Structure of the saxitoxin class of cyanotoxins also known as paralytic shellfish poisons (PSPs). The groups $R_1$ to $R_4$ are variable on the saxitoxins, resulting in molecules of different charge, molecular weight, and toxicity.

Table 2: Relative toxicity of the range of chemical structural variants of the saxitoxin class of toxins (from [32]).

| | R1 | R2 | R3 | Net Charge | Relative Toxicity |
|---|---|---|---|---|---|
| **R4=$CONH_2$ (CARBAMATE TOXINS)** | | | | | |
| STX | H | H | H | +2 | 1 |
| neoSTX | OH | H | H | +2 | 0.924 |
| GTX1 | OH | H | $OSO_3^-$ | +1 | 0.994 |
| GTX2 | H | H | $OSO_3^-$ | +1 | 0.359 |
| GTX3 | H | $OSO_3^-$ | H | +1 | 0.638 |
| GTX4 | OH | $OSO_3^-$ | H | +1 | 0.726 |
| **R4 = $CONHSO_3^-$ (N-SULFOCARBAMOYL (SULFAMATE) TOXINS)** | | | | | |
| GTX5 (B1) | H | H | H | +1 | 0.064 |
| GTX6 (B2) | OH | H | H | +1 | - |
| C1 (epiGTX8) | H | H | $OSO_3^-$ | 0 | 0.006 |
| C2 (GTX8) | H | $OSO_3^-$ | H | 0 | 0.096 |
| C3 | OH | H | $OSO_3^-$ | 0 | 0.013 |
| C4 | OH | $OSO_3^-$ | H | 0 | 0.058 |
| **R4=H (DECARBAMOYL TOXINS)** | | | | | |
| dcSTX | H | H | H | +2 | 0.513 |
| dcneoSTX | OH | H | H | +2 | - |
| dcGTX1 | OH | H | $OSO_3^-$ | +1 | - |
| dcGTX2 | H | H | $OSO_3^-$ | +1 | 0.651 |
| dcGTX3 | H | $OSO_3^-$ | H | +1 | 0.754 |
| dcGTX4 | OH | $OSO_3^-$ | H | +1 | - |

Saxitoxins are also the neurotoxins present in *Anabaena circinalis*, the only cyanobacterium yet found to be neurotoxic in Australia [33,34,35,36,37]. As indicated previously the name of this genus has recently been revised to *Dolichospermum* [21]. The recognition and adoption of this new name is expected to come into common usage over time and this important bloom-forming species will therefore progressively become known as *Dolichospermum circinalis.* The widespread occurrence of saxitoxins, especially in Australian neurotoxic *A. circinalis*, makes them a very important class of cyanobacterial toxins, at least in this country. In relation to *A. circinalis* in Australia, toxin profiles appear to be relatively constant and dominated by the C toxins [36,37]. There is also some limited evidence that this cyanobacterium can produce both neurotoxins and hepatotoxins [38], a phenomenon which has been reported overseas with *Anabaena flos-aquae* [39,40].

The saxitoxins are a relatively complex class of 18 compounds with widely differing toxicities which can be divided into three groups as shown in Table 2. They can also be divided into three groups based on the net charge of the molecule under acidic conditions [41,42] (Table 2). This grouping comprises the saxitoxins (saxitoxin (STX), neosaxitoxin (neoSTX) and decarbamoyl derivatives) (charge +2), the gonyautoxins (GTXs) including decarbamoyl derivatives (charge +1) and C toxins (charge 0). These properties form the basis of analytical methods involving high performance liquid chromatography (HPLC).

Saxitoxins have now also been found to be responsible for neurotoxicity in three cyanobacterial species overseas; *Aphanizomenon flos-aquae* [43,44,45], *Lyngbya wollei* [46] and *Cylindrospermopsis raciborskii* [47].

Cylindrospermopsin is an hepatotoxic alkaloid toxin (Figure 5) that was first was isolated from *C. raciborskii* and was therefore named after it [5,48]. It is a general cytotoxin (cell toxin) with relatively slow onset of symptoms resulting in kidney and liver failure. Symptoms may become obvious only several days after exposure, so it will often be difficult to determine a cause–effect relationship. Results by Falconer and Humpage [49] suggest that cylindrospermopsin may also act directly as a tumour initiator, which has implications for long-term exposure.

Cylindrospermopsin has also subsequently been isolated from the cyanobacterium *Umezakia natans* in Japan [50] and *Aphanizomenon ovalisporum* in both Australia [51] and Israel [52]. Cylindrospermopsin can be classified as an hepatotoxic alkaloid but toxicological studies have shown

that, while the principal organ affected is the liver, other organs such as the kidney are also affected [53,54,55]. Recently a toxic minor component from a strain of *Aphanizomenon ovalisporum* from Israel, 7-epicylindrospermopsin, has been characterised [56] indicating that toxins other than cylindrospermopsin itself need to be considered when dealing with these cyanobacteria.

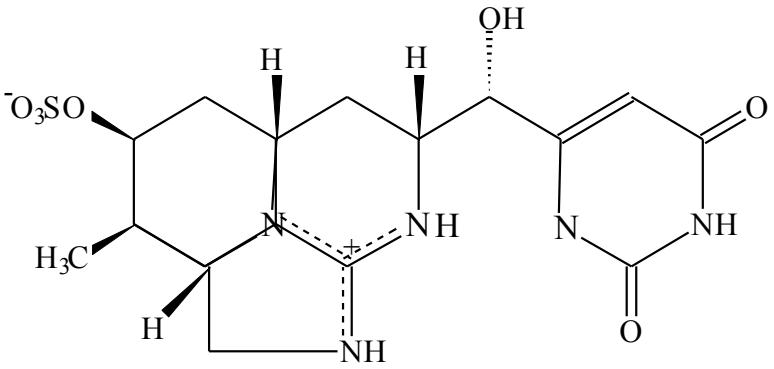



Figure 5: Structure of cylindrospermopsin

### 1.2.1.3 Lipopolysaccharides

Lipopolysaccharides (LPS) are an integral component of the cell wall of all cyanobacteria (as well as other types of bacteria), and help to determine and maintain the size and shape of the cell [57]. As LPS are always present in cyanobacteria it would appear to make LPS a potential issue of concern for exposure in recreational situations, relative to the other known toxins. These compounds have been shown to produce irritant and allergenic responses in human and animal tissues [14]. They are pyrogenic (fever-causing agents) and toxic [58]. An outbreak of gastro-enteritis is suspected to have been caused by cyanobacterial LPS [59]. Interestingly, however, cyanobacterial LPS are considerably less potent than LPS from some other types of bacteria such as *Salmonella* [60,61].

### 1.2.1.4 β-*N*-methylamino-L-alanine (BMAA)

Some cyanobacteria produce a neurotoxic amino acid, β-*N*-methylamino-L-alanine (BMAA), which has been associated with a fatal human neurodegenerative disease, with similarities to Alzheimer's and Parkinson's diseases [62]. Recent studies have suggested that BMAA is also produced widely by free-living cyanobacteria from freshwaters throughout the world.

Researchers have reported finding BMAA in brain tissue of people on the island of Guam who had died of Amyotrophic Lateral Sclerosis/Parkinson's Dementia complex (ALS/PDC) [63]. BMAA had previously been identified as a metabolite of a cyanobacterium living in a symbiotic relationship with cycads on the island [64]. It has been hypothesised that there is a possible route of exposure, by a process of biomagnification of this compound from the cyanobacteria in the cycads via flying foxes that eat them and then to humans who subsequently consumed the flying foxes. Although BMAA was suggested as a contributing factor in the illness, a subsequent study found no BMAA in the brains of affected individuals [65].

The detection of BMAA in a number of common cyanobacteria and the demonstrated capacity of BMAA to biomagnify raises some concern for the water industry. Research is needed to assess the level of risk of exposure from drinking waters. However, at this point in time, the association between BMAA and neuro-degenerative diseases must be considered tenuous.

### 1.2.1.5 Entry of cyanobacterial toxins into the human body

Exposure to cyanobacteria and their toxins can arise through three routes (either as whole cells and/or as dissolved toxins):

- Direct contact of exposed parts of the body (i.e. skin), including sensitive areas such as the ears, eyes, mouth and throat, and the areas covered by a bathing suit (which may collect cell material).
- Accidental swallowing of water containing cells or consumption of drinking water containing dissolved toxin.
- Aspiration (inhalation) of water containing cells (usually during recreational or occupational exposure to water sprays or mists).

### 1.2.2 Why are health effects important to water managers-what does it mean for operations?

We know that there are historically well-documented and anecdotal reports of animal poisonings and deaths and human poisonings from drinking water contaminated with cyanobacterial blooms. Epidemiological evidence has also shown symptoms of poisoning or injury that have been attributed to the presence of cyanobacterial toxins in drinking water [4].

The rapid onset and short-term acute health effects are the most obvious health risk associated with cyanobacterial toxins. In any population there are individuals who are at greater risk of suffering from health effects than others; for example, children who drink a higher volume of water in proportion to body weight than an adult, or individuals who are at risk of injury to organs such as dialysis patients, or patients with liver disease.

Experiments have also shown that long-term exposure to microcystin toxins can result in liver injury and the possibility of tumour growth promotion. Hence, it is the long-term health effects with low toxin levels as well as the short-term exposure to high toxin levels that may represent a health risk [4]. Consequently, it is important to know if drinking water contains cyanobacteria and associated toxins, as the health implications could be considerable. Managers always need to take primary guidance with regard to health considerations from the current drinking water guidelines for toxins as these are carefully developed to balance the current scientific information and risk to public health (Section 1.3).

Taste and odours (geosmin and methylisoborneol (MIB)) are also important water quality parameters and although they are a source of customer complaints, they are not known to be a health risk. They require early water treatment intervention as they are detectable at low cell numbers (from 500 cells $mL^{-1}$).

Water treatment is an important management tool to remove cyanobacterial toxins and tastes and odours from drinking water. Cyanobacterial cells should be removed whole if possible to reduce the amount of dissolved metabolites, which are more difficult to remove (see Chapter 3).

## 1.3 Guidelines and Standards

### 1.3.1 What are drinking water guidelines?

Drinking water guidelines are designed to protect public health and the safety of drinking water supplies by suggesting safe levels for constituents that are known to be hazardous to health. The World Health Organization (WHO) Guidelines for Drinking Water Quality [66,67] represent a scientific consensus on the health risks presented by microbes and chemicals in drinking water and are often used to derive guideline values for individual countries, states or regions. The guideline values are intended to be used in the development of risk management strategies. These values are associated with guidance on monitoring and management practices.

### 1.3.2 How should water managers use guideline values?

The guideline value is important for water supply authorities, as this value sets the concentration of toxin that is tolerable in drinking water, i.e. “at the tap”. The Australian Drinking Water Guidelines (ADWG) are not mandatory standards, however, they provide a basis for determining the quality of water to be supplied to consumers in all parts of Australia. In this circumstance the guideline level is effectively a recommendation from the health authorities, although this situation is changing with the introduction of more prescriptive drinking water standards in some jurisdictions. For some water

authorities in Australia the guidelines/standards become part of the *de facto* contractual standards. They are therefore required to comply with the guideline values as part of their standards of service.

For other countries the guideline level can be a standard that must be met and compliance monitoring may be required (see Table 3).

### 1.3.3 What are the drinking water guideline values for cyanobacterial toxins in Australia?

The WHO guideline values were used as a basis to develop the ADWG as part of the National Water Quality Management Strategy coordinated by the National Health and Medical Research Council (NHMRC) and the Natural Resource Management Ministerial Council (NRMMC).

The ADWG provide information and recommendations for four individual classes of toxins: microcystins, nodularin, saxitoxins, and cylindrospermopsin (NHMRC/ARMCANZ, 2004) [5], and can be found at:

http://www.nhmrc.gov.au/_files_nhmrc/file/publications/synopses/adwg_11_06_fact_sheets.pdf

A guideline value has been recommended only for total microcystins. The guideline recommends that the concentration of total microcystins in drinking water in Australia should not exceed 1.3 μg $L^{-1}$.

No guideline values could be set for concentrations of nodularin, saxitoxins or cylindrospermopsin due to the lack of adequate data. In relation to lipopolysaccharides (LPS) produced by cyanobacteria, there is currently insufficient information to carry out a critical assessment on occurrence and significance of LPS and so no fact sheet has been produced.

The most recent review of the ADWG has now led to the recommendation that, although the strength of data is insufficient to establish a guideline value for cylindrospermopsin in drinking water, a range of information can be used to develop a 'Health Alert' value for cylindrospermopsin of 1 μg $L^{-1}$.The data used to develop this health alert come from a range of Australian toxicological studies one of which provided sub-chronic oral doses of the toxin to mice and demonstrated responses to the toxins after an extended trial of 11 weeks. [68]. The recommendation of a health alert acknowledges that health authorities should be notified if blooms of *Cylindrospermopsis raciborskii* or other producers of this toxin are present in water supply.

Table 3: Guideline values or standards for cyanobacterial toxins in drinking water from various countries (Information derived from websites and [69] unless otherwise stated).

| Country | Guideline Value/Standard | Comments/Explanations |
|---|---|---|
| Argentina | Under revision | |
| Australia | 1.3 µg $L^{-1}$ total microcystins, expressed as toxicity equivalents of microcystin-LR | From the Australian Drinking Water Guidelines see section above for further explanations on the process of guideline derivation |
| Canada | 1.5 µg $L^{-1}$ cyanobacterial toxins as microcystin-LR | Canada uses guidelines as the standard of water quality. The guidelines are expressed with the unit of Maximum acceptable concentrations (MAC). These are derived from tolerable daily intake (TDI) which in turn is derived from a calculated no-observed adverse effect level (NOAEL) from data from human or animal studies. To derive a MAC from a TDI, adjustments are made for average body weight and drinking water consumption, as well as other considerations. In terms of health the guidelines ensure that the MACs are far below exposure levels at which adverse effects have been observed. The guideline is considered protective of human health against exposure to other microcystins (total microcystins) that may also be present |
| Czech Republic | 1 µg $L^{-1}$ microcystin-LR | Value as national legislation, follows WHO provisional guideline value. |
| China | 1 µg $L^{-1}$ microcystin-LR | WHO provisional guideline for microcystin-LR |
| France | 1 µg $L^{-1}$ microcystin-LR | Drinking water decree |
| Italy | 1 µg $L^{-1}$ microcystin-LR | WHO provisional guideline for microcystin-LR used as a reference by local authorities. |
| Japan | 1 µg $L^{-1}$ microcystin-LR | WHO provisional guideline for microcystin-LR |
| Korea | 1 µg $L^{-1}$ microcystin-LR | WHO provisional guideline for microcystin-LR. Algal alarming system – based on cell numbers and chlorophyll a. |
| New Zealand | For cyanobacteria: <1 potentially toxic cyanobacterium present in 10 mL of sample.<br>PMAV for cyanobacterial toxins:<br>Anatoxin (as STX-eq): 3.0 µg $L^{-1}$<br>Anatoxin-a (S): 1.0 µg $L^{-1}$<br>Cylindrospermopsin: 3.0 µg $L^{-1}$<br>LPS endotoxins: 3.0 µg $L^{-1}$<br>Microcystins: 1.0 µg $L^{-1}$<br>Nodularin: 1.0 µg $L^{-1}$<br>Saxitoxins:1.0 µg $L^{-1}$ | Maximum acceptable values (MAVs) for micro-organisms or organic determinands of health significance. Provisional MAV (PMAV) for cyanobacterial toxins. MAVs are based on the WHO 'Guidelines for Drinking Water Quality'. They are the concentration of a determinand, which is not considered to cause any significant risk to the consumer over a lifetime of consumption of water. The method of derivation varies according to NZ conditions and the way in that the determinand presents a risk. However they are derived with the use of a TDI. The MAVs are standards in NZ. The Standards provide compliance criteria and compliance is routinely monitored |

Table 3 contd.

| | | |
|---|---|---|
| Norway | 1 µg $L^{-1}$ microcystin-LR | Provisional WHO guideline for drinking water adopted |
| Oceania | None found | Clean drinking water supply to all people is the main current focus |
| Poland | 1 µg $L^{-1}$ microcystin-LR | National legislation in place for guideline value in drinking water |
| South Africa | 0-0.8µg $L^{-1}$ for microcystin-LR | Guideline levels for microcystins in potable water as a "Target Water Quality Range" |
| South America (Brazil) | 1.0 µg $L^{-1}$ for microcystins<br>3.0 µg $L^{-1}$ for saxitoxins (equivalents)<br>15 µg $L^{-1}$ for cylindrospermopsin | Guideline values for microcystins, saxitoxins and cylindrospermopsin, along with biomass monitoring programs. Guideline value for microcystins adopted as mandatory. Guideline values for equivalents of saxitoxins and for cylindrospermopsin included as recommendations. Use of algicides prohibited and toxicity testing/toxin analysis when cell counts exceed 10,000 cells $mL^{-1}$ or 1 $mm^3$ $L^{-1}$ biovolume. [i]. |
| Spain | 1 µg $L^{-1}$ microcystins | National legislation, maximum permissible amount in drinking water |
| Thailand | No guideline currently | Awareness for need for guidelines |
| United States of America | None currently known. | Maximum Contaminant Levels (MCLs) are the highest level of a contaminant that is allowed in drinking water. They are enforceable standards. Cyanobacteria and their toxins are listed as microbiological contaminants on the contaminant candidate list (CCL). This means that they are currently recognised as unregulated contaminants, but are known to occur in public water systems and may require regulation under the Safe Drinking Water Act. Contaminants on the CCL are a priority for the US Environmental Protection Agency with the aim to set MCLs |
| Uruguay | Under revision | |
| World Health Organization | 1.0 µg $L^{-1}$ for microcystin-LR | Refer to World Health Organisation Guidelines for Drinking-Water Quality, 1996 [67] |

[i] Azevedo, S.M.F.O., 2001. New Brazilian regulation for cyanobacteria and cyanotoxins in drinking water. Poster presentation at V. ICTC.

## 1.4 Taste and Odour

Unpleasant tastes and odours in drinking water are a constant concern for water managers. Although they do not produce known health problems, they are responsible for customer complaints when the flavour of the drinking water changes.

### 1.4.1 What causes tastes and odours in water supplies?

A range of organisms that grow naturally in source water are known to cause taste and odour problems in drinking water supplies. These include actinomycete bacteria, various types of cyanobacteria and algae, fungi and other aquatic micro-biota [70,71]. The organisms can be free-living in the water column (i.e. planktonic) or grow as benthic populations in sediments or attached to submerged rocks and walls. An issue for the water industry is the importance of the relative contribution and therefore risk to water quality of these various micro-organisms to the occurrence of tastes and odours, on a seasonal basis, in different reservoirs and source waters. Taste and odour production tends to be more of a problem during warmer weather when conditions are more favourable for algal growth (see section on algal growth). An important issue is that taste and odours can be detected at very low levels by some consumers and this may be associated with low cell numbers of the organisms responsible for producing them.

Table 4: Compounds causing nuisance tastes and odours in water supplies and their biological source [72,73]).

| Odour | Compound | Source |
|---|---|---|
| Earthy | Geosmin | Cyanobacteria (e.g. *Anabaena*(1), *Oscillatoria*) |
| | | Actinomycetes |
| Musty | 2-Methylisoborneol (MIB) | Cyanobacteria (e.g. *Phormidium*, *Planktothrix, Pseudanabaena*) |
| | | Actinomycetes |
| Grassy | β-Cyclocitral | Cyanobacteria |
| | | Green algae |
| | | Diatoms |
| Odorous sulphur | Mercaptans | Decomposed or living cyanobacteria |

Note: This table is by no means complete but concentrates on tastes and odours produced by cyanobacteria.

(1) Synonym: *Dolichospermum.*

The most common compounds which cause taste and odour problems are geosmin and 2-methylisoborneol (MIB). These two earthy-musty smelling metabolites are produced by a range of cyanobacteria and actinomycete bacteria.

Both geosmin and 2-methylisoborneol (MIB) are slightly polar, relatively low molecular weight aliphatic tertiary alcohols that are similar in structure and solubility. Their structures do not contain functional groups that are particularly susceptible to oxidation reactions, which are important in terms of water treatment and removal of these compounds, and this is discussed in detail later in Chapter 3. Figure 6 shows the molecular structures of the two compounds.


geosmin MIB

Figure 6: Molecular structures of geosmin and MIB.

Appendix 1 contains two tables: a list of the common algal species in South Australian waters together with the associated taste and odour compounds they produce based upon local experience, and a more comprehensive table compiled from international literature giving the range of species of planktonic and benthic cyanobacteria and actinomycetes that have been shown to produce either geosmin and/or MIB.

The problems associated with odours and toxins produced by planktonic cyanobacteria (e.g. *Anabaena, Planktothrix* – see Table 4) are well known in the Australian water industry. As a consequence planktonic cyanobacteria are usually the focus for management actions for odour and toxin control. Benthic cyanobacteria and actinobacteria are less well understood but have also been implicated as sources of unexplained taste and odour incidents in drinking water reservoirs.

Benthic cyanobacteria that have been linked to geosmin and MIB production in Australia include *Aphanizomenon*, *Oscillatoria*, *Phormidium* and *Pseudanabaena* [74]. These cyanobacteria grow in mats or films attached to sediments, rocks and aquatic plants on the banks of lakes and rivers. Light is the major factor determining their distribution with euphotic depth limiting growth in deeper waters and high irradiance levels suppressing growth in shallow water. Other controlling factors include temperature, nutrient availability and shear stress associated with wave action.

Both MIB and geosmin were originally isolated from Actinomycetes [75,76,77]. Actinomycetes are a group of gram-positive bacteria that share some features with fungi, primarily their growth in the form of mycelium, a branching network of filaments [78] and they are ubiquitous in aquatic habitats [79]. They have been identified in water samples, aquatic sediments [80] and attached to aquatic plants [81,82]. While many researchers have identified a link between the presence of aquatic actinomycetes and tastes and odours in water supplies [83,84,85] others suggest that this association is tenuous. It has been argued that actinomycetes may not actively grow in large bodies of water and that tastes and odours are produced by terrestrial species transported into water via run-off [86,87,88]. These terrestrial taste and odour producing organisms could also include fungi, bacteria and even amoebae [89]. Furthermore, not all actinomycetes produce MIB or geosmin, so the presence of this organism in a reservoir with a taste and odour problem doesn't necessarily imply it is the source.

## 1.5 Analysis for Cyanobacteria and their Metabolites

### 1.5.1 What should you monitor?

When considering cyanobacteria in reservoirs, you would usually want to know about any or all of the following: the species present, cell numbers, tastes, odours and toxicity. The results from these analyses provide a comprehensive assessment of the magnitude of the cyanobacterial problem in the reservoir. In drinking water the important parameters are dissolved toxins and taste and odour compounds. Depending upon the problem, management strategies including the Alert Levels Framework, drinking water guidelines, control measures or treatment options can be implemented (see following sections). It is important to note that there is no evidence of a link between the

production of taste/odour and toxin by cyanobacteria. Therefore tastes and odours cannot be used as a warning system for an impending toxic cyanobacterial bloom.

## 1.5.2 How do you estimate cyanobacterial abundance in a sample?

Estimation of phytoplankton abundance and species determination is achieved by microscopic examination and enumeration. Results are usually given as cell $mL^{-1}$ for a genus/species with an estimated confidence limit and a biovolume measurement may also be given. These results can then be used in the Alert Levels Framework and so will allow for the assessment of the water body in relation to its cyanobacterial load (see Chapter 2).

Enumeration protocols require a satisfactory level of precision in estimates of cell abundance for colonial and filamentous cyanobacteria and the detection of trends in cell abundance with reasonable confidence and the minimum counting effort.

For public health surveillance, it is important that potentially toxic cyanobacteria be identified to species level. This information is necessary to determine the analytical technique appropriate for determining toxin levels.

Many monitoring programs are established to detect cyanobacteria that are known to taint water supplies with disagreeable tastes and odours. Detection of odours is often possible at quite low cell concentrations (>500 cells $mL^{-1}$) and identification of nuisance types at an early stage can alert operators to the need for remedial measures in either raw or treated water. Appendix 1 contains a table of the common species known to produce tastes and/or odours.

## 1.5.3 What is the best way to test a sample for cyanobacterial toxin content?

There is an increasing range of analytical methods available for the detection and quantification of cyanotoxins, and they vary in their manner of detection, the information they provide and sophistication [90]. For a complete overview and review of methods please refer to the report "Evaluation of Analytical Methods for the Detection and Quantification of Cyanotoxins in Relation to Australian Drinking Water Guidelines" [91], together with a more recent international review [92].

The range of analyses available include immunological or biochemical screening techniques based on enzyme-linked immunosorbent assays (ELISA) and enzyme activity (protein phosphatase inhibition, PPIA) assays respectively, to quantitative chromatographic techniques based on high performance liquid chromatography (HPLC) and more sophisticated (and expensive) liquid chromatography-mass spectrometry (LC-MS). Animal bioassays (mouse tests), and in some cases assays based on isolated cell lines, are also available for screening the entire range of toxins. A comprehensive discussion of the range of cell-based screening assays used to detect cyanotoxins is given in CRC for Water Quality and Treatment Research Report 60 [93].

It is important that the appropriate analytical method is selected. For example, the technique which is most suitable to monitor microcystins in relation to the Australian Drinking Water Guidelines is high performance liquid chromatography with photo diode array detection or mass spectral detection (HPLC-PDA or HPLC-MS). The analytical methods available for saxitoxins are continuously evolving and are based upon either high performance liquid chromatography and fluorescence detection or mass spectral detection (LC/MS/MS). Internationally the only technique recognised by the Association of Official Analytical Chemists (AOAC) for analysing saxitoxins from shellfish (where they are commonly found) other than mouse bioassay is a technique based upon liquid chromatography with pre-column derivatisation [94]. This technique is not yet widely used for analysis of cyanobacterial material. The method recommended for cylindrospermopsin is liquid chromatography with tandem mass spectrometry (LC/MS/MS).

A summary of analytical techniques that are available for different classes of toxins, their detection limit and other issues to consider when using them are given in Table 5.

For the techniques described in the table the detection limits may vary depending upon the standards that are available and instrumentation used. A range of other methods used within various research laboratories for screening and analysis includes ELISA methods for microcystins, neuroblastoma cytotoxicity assay, saxiphilin and single-run HPLC methods for saxitoxins and protein synthesis inhibition assays for cylindrospermopsin. A promising area that is developing rapidly is the application of molecular techniques (quantitative PCR) for determination of genes for toxin production.

Table 5: Analytical methods commonly used commercially for cyanotoxin detection and analysis in Australia [38]

| TOXIN | ANALYTICAL METHOD | DETECTION LIMIT ($\mu g\ L^{-1}$) | DESCRIPTION |
|---|---|---|---|
| **Microcystins** | • Liquid Chromatography with Mass Spectrometry (LC-PDA/MS) | 0.5<br>< 1.0 for individual microcystins | • Detection of microcystins by HPLC/PDA provides a spectrum of the separated analytes and achieves a detection limit of considerably less than 1 $\mu g\ L^{-1}$ for individual microcystins when used with appropriate concentration and cleanup procedures. |
| | • Protein Phosphatase Inhibition Assay (PPIA) | around 0.1 $\mu g\ L^{-1}$ or less | • Useful as a screening tool, relatively simple to use and is highly sensitive, with low detection limits relative to guideline values. |
| | • Enzyme-Liked Immuno-sorbent Assay (ELISA) | 0.05 $\mu g\ L^{-1}$ | • Detection of microcystins by ELISA provides semi-quantitative results |
| | • Mouse Bioassay | N/A | • Qualitative, screening assay |
| **Nodularin** | • Liquid Chromatography with Mass Spectrometry (LC-PDA/MS)<br>• PPIA<br>• ELISA<br>• Mouse Bioassay | 0.5<br>< 1.0<br>0.1 $\mu g\ L^{-1}$<br>0.05 $\mu g\ L^{-1}$ | • Same as for microcystins (HPLC/PDA), see section on microcystins<br>• Both protein phosphatase and ELISA assays commercially available for detecting microcystins are also useful for screening for nodularin.<br>• Qualitative screening assay |
| **Cylindrospermopsin** | • Liquid Chromatography with Mass Spectrometry (LC-PDA/MS & LC MS-MS)<br>• ELISA<br>• Mouse Bioassay | Around 1.0<br>0.05 $\mu g\ L^{-1}$ | • Cylindrospermopsin can be detected using LC/MS/MS (without the sample requiring extraction/reconcentration step)<br>• Semi-quantitative screening assay capable of detecting low toxin concentrations<br>• Qualitative screening assay |
| **Saxitoxins (Paralytic Shellfish Poison – PSP's)** | Liquid Chromatography (HPLC) with post-column derivatisation<br>• ELISA<br>• Mouse Bioassay | Depends upon the variant C1:37, C2:14, GTX2: 25, GTX3: 6, GTX5:?, STX: 34, dcGTX2:?, dcGTX3:?<br>0.02 $\mu g\ L^{-1}$ | • Detection limits of saxitoxins (from Australian neurotoxic *A. circinalis*) have been determined using HPLC with post-column derivatisation and fluorescent detection and without sample concentration.<br>• Semi-quantitative screening assay. Has advantage of detection of low levels STX. Poor cross reactivity to some analogues.<br>• Qualitative screening assay |

## 1.5.4 How can tastes and odours be detected in water supplies?

The operator will be faced with a range of alternatives to detect and quantify odours in water and the following is a description of the types of techniques that may be offered by an analytical lab to track down and identify odour problems in drinking water.

Of course a combination of routine monitoring and customer complaints provides early warning of possible taste and odour problems in source water, however both sensory and chemical methods can be used to assess tastes and odours in water. For many years, the sensory methods (i.e., those using either the human nose or mouth as the "detector") were the only methods available. In recent decades, however, the development of chemical analysis methods such as gas chromatography and mass spectrometry has allowed identification and quantification of odour-causing organic substances in water. Sensory and chemical analysis methods are discussed separately in the following subsections.

### 1.5.4.1 Sensory analysis

*Flavour profile analysis*
The Flavour Profile Analysis (FPA) method was originally adapted by the Metropolitan Water District of Southern California from the procedure used in the food industry. Subsequently, the method has been used by water utilities and universities worldwide, and has been found to be very useful for the identification of problem odours in drinking water [95,96,97].

Flavour by definition refers to all taste and odour sensations experienced while eating food or drinking beverages [98]. The FPA method records intensities for each flavour contributing to the overall perception of a water sample. The sample is tested without dilution by a panel of at least four members, and the intensity of each descriptor is rated on a numerical scale. The procedure used for testing of water samples by the FPA method is described in detail in the Standard Methods for the Examination of Water and Wastewater 20th Edition [99].

*Other sensory techniques*
The threshold odour number (TON) test has been the method historically used for the quantitation of odours in drinking water. The procedure is described in detail in [99]. The basic principle of the test is that several aliquots of the sample to be evaluated are diluted using various amounts of odour-free water. These aliquots are then warmed and smelled by one or more persons. The highest dilution in which an odour is just detectable is reported as the threshold odour number. Further discussion of the test and details regarding the selection of panellists are provided by [97].

### 1.5.4.2 Chemical analysis

The chemical procedures used to analyse organic taste and odour compounds in water must be very sensitive, because many of these substances can be detected by sensory analysis (i.e., the human nose) at nanogram per litre levels. The most common method currently used for analysis is gas chromatography combined with mass spectrometry (GC/MS). However, as these compounds occur at very low concentrations, some method of pre-concentration is required. The most important methods used for the pre-concentration step are summarised below.

*Closed-loop stripping analysis (CLSA).*
This procedure has been widely used for the analysis of non-polar volatile organic compounds of intermediate molecular weight, at the nanogram to microgram per litre level. The compounds are stripped from the water by a recirculating stream of air and then adsorbed from the gas phase onto a few milligrams of activated carbon. They are then extracted from the carbon with a few microlitres of carbon disulphide for direct analysis. This method can be applied to both raw and treated waters. The main advantage of the method is that it does not require further concentration of the solvent. Prior to the widespread adoption of solid phase micro-extraction (see below) this method was considered the standard method for the isolation of MIB and geosmin [100]. The limit of detection (LOD) for this method is usually reported as 1-2 ng $L^{-1}$.

*Solid phase microextraction (SPME).*
As this technique is simpler and more cost-effective than CLSA it has gained popularity in recent years. The method uses a fused silica fibre, coated with a polymer, to adsorb the compound from the solution, or the headspace above the solution in a sealed vessel. In the latter case, salt is added and the sample is warmed slightly to obtain maximum recovery. The fibre is then placed directly in the hot GC injection chamber, where the compounds are volatilised and can be analysed by MS [101]. The LOD for this method is usually reported as 1-2 ng $L^{-1}$ for geosmin and slightly higher for MIB.

# 2 SOURCE WATER MANAGEMENT

## 2.1 Growth of Cyanobacteria

### 2.1.1 Why do cyanobacteria grow and form blooms?

It is broadly recognised that the growth of cyanobacteria in reservoirs is favoured by high nutrient concentrations, particularly phosphorus, in combination with the right physical conditions of elevated temperature, usually accompanied by thermal stratification and high light. However the dynamic seasonal and temporal combination of these factors is less well understood in individual circumstances.

To predict the occurrence of cyanobacteria and the risk of blooms it is important to consider both the role of the unique features of cyanobacteria and how they use these to exploit the environment and accumulate to high densities. It is also important to consider the important driving variables or triggers for excessive cyanobacterial growth. These characteristic features of cyanobacteria and their interaction with major environmental influences are reviewed below in this context.

### 2.1.2 Biological and environmental factors influencing the growth of cyanobacteria

#### 2.1.2.1 Cyanobacterial life cycle

The cyanobacterial life cycle involves the planktonic ("free-floating in the water") population and benthic ("bottom-dwelling") resting stages which can be either dormant colonies or akinetes [102]. Akinetes are thick-walled reproductive structures in some ways equivalent to spores or 'seed" in plants [103]. They are found in sediments and are very resistant to adverse environmental conditions, can survive many years and are thought to provide a resting stage that may enable the survival of a species. They germinate when environmental conditions are appropriate, thereby providing a source or inoculum for subsequent populations, particularly from one season to the next [102]. It is important to note that only one type of cyanobacteria produces akinetes, these are the filamentous, heterocystous cyanobacteria (Order Nostocales). Examples of genera in this group are *Anabaena* and *Aphanizomenon*. The cycle of akinete formation in the cyanobacterium *Anabaena* is illustrated in Figure 7.

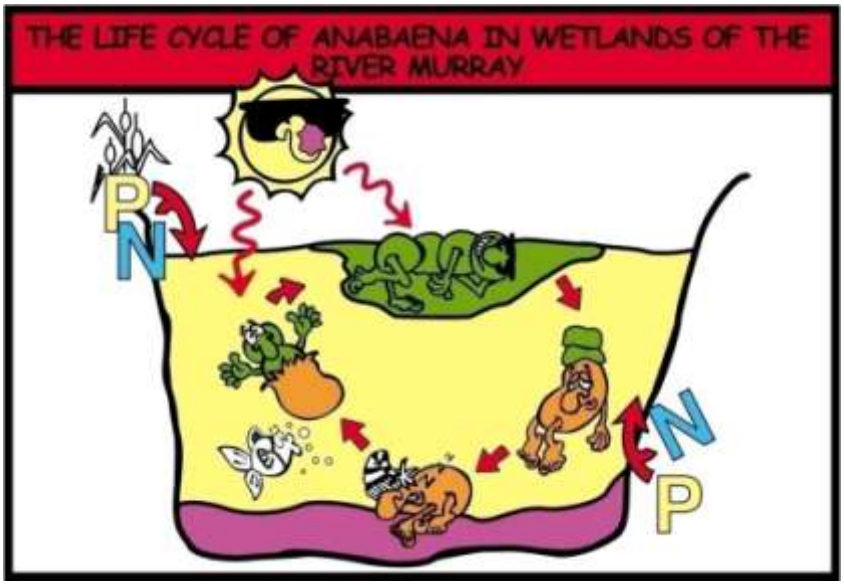



Figure 7: The typical life cycle of the cyanobacterium *Anabaena* showing akinete formation and germination.

The life cycle of akinete-producing cyanobacteria can be summarised in a number of steps. First, the filaments of cyanobacteria grow by cell division. Akinete production and release follows, usually for the population to survive over winter. Finally, growth from the akinetes occurs, which is triggered by environmental factors, including light and temperature, with new cyanobacteria maturing and growing by cell division for the new season's population [102,103].

Other filamentous or single cell/colonial cyanobacteria are not known to form akinetes or other resting-stage cellular structures. It has been suggested that some of the normal or regular growth cells called vegetative cells may rest over winter in a state of senescence in the sediment. For example *Microcystis* can 'overwinter' as vegetative colonies on the lake sediments, where they may survive for several years, apparently without light or oxygen [104]. The new population may then appear in spring from the normal growth of these colonies by cell division.

### 2.1.2.2 Buoyancy regulation

Bloom forming cyanobacteria possess gas vesicles that are structures within cells that collectively are called gas vacuoles. These structures are rigid hollow cylindrical chambers made of protein which contain atmospheric gas [105] and provide cells with buoyancy. Some cyanobacteria can combine this positive buoyancy with the accumulation and loss of carbohydrate which acts as ballast to regulate their buoyancy and this enables them to migrate up and down through the water column [105]. Colonies near the surface are exposed to high light and so have a high rate of photosynthesis and therefore build up carbohydrates within the cells. This makes them heavy and although they contain gas vacuoles the carbohydrate ballast makes them sink at a rate dependent upon their colony size and density of the cell. Large colonies sink faster than small ones. As the colonies sink down out of the euphotic depth they stop producing carbohydrate and start consuming it by respiration [106]. The colonies then become buoyant again and float back up to the surface euphotic zone. This is illustrated in Figure 8 in a stylised cartoon drawing of the daily migration cycle of *Anabaena.* Buoyancy regulation is a mechanism that positions the cyanobacteria at the best depth for capturing light for optimum growth and may also allow them to scavenge nutrients from the water column [107]. This may be a significant advantage over other phytoplankton algae particularly in stratified lakes where turbulence is low and heavy cells tend to sink. This mechanism only works well when the water body is not too turbulent and is also deep. One consequence of this buoyancy regulation mechanism is that cyanobacterial colonies may all become buoyant at night and rise to the surface and form the characteristic surface scums often seen in the morning when a lake is calm.

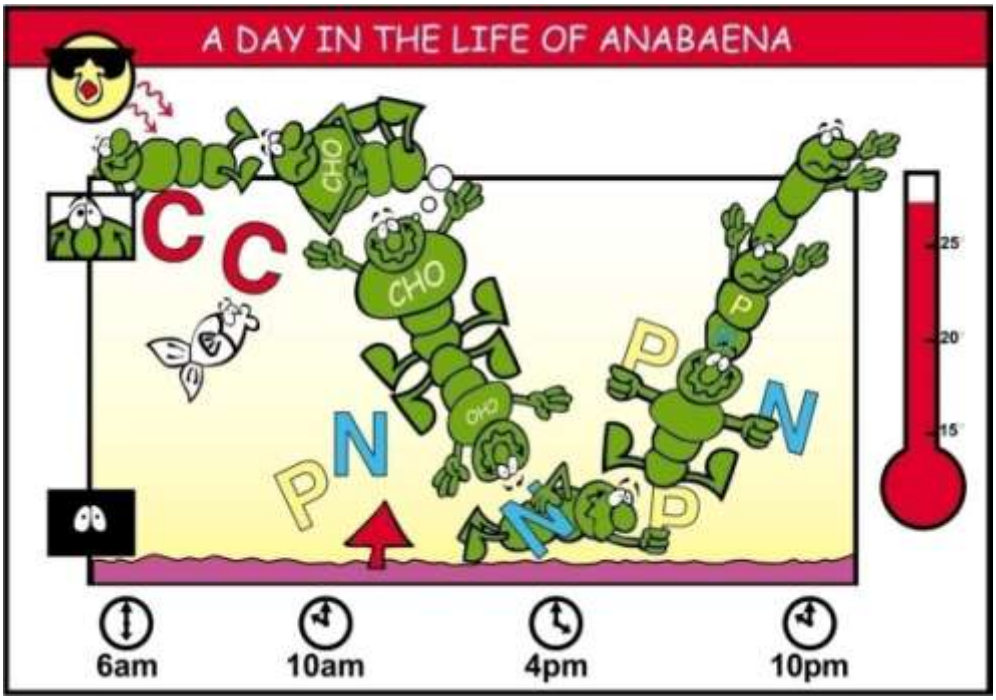



Figure 8: A stylised diagram of the daily cycle of buoyancy regulation and vertical migration of the cyanobacterium *Anabaena.*

### 2.1.2.3 Light

Cyanobacteria contain the photosynthetic pigment chlorophyll a, but unlike other phytoplankton they also contain phycobiliproteins. These are light harvesting pigments that are able to capture light in the green, yellow and orange part of the visible light spectrum (500-650 nm). This enables cyanobacteria to use light energy across the spectrum very efficiently. For example, in light limiting conditions cyanobacterial growth rates are higher than that of green algae, and this, in combination with buoyancy regulation, allows them to out-compete green algae in highly turbid waters.

Both turbidity and stratification can influence the amount of light received by cyanobacteria in a water body. The light conditions in a given water body determine the extent to which the physiological properties of cyanobacteria will be of advantage in their competition against other phytoplankton. Generally, the zone in which photosynthesis can occur is termed the euphotic zone. By definition, the euphotic zone extends from the surface to the depth at which 1 % of the surface light intensity is measured. The euphotic zone can be estimated by measuring the transmittance of the water with a 'Secchi' disk and multiplying the Secchi depth reading by a factor of approximately 2-3. Those cyanobacteria that display buoyancy regulation via gas vesicles overcome these problems when they float to water depths with optimal light conditions.

Light penetration into a water body is also important for growth of benthic cyanobacteria. The greater the light penetration the deeper benthic cyanobacteria can grow.

(Adapted from [108])

### 2.1.2.4 Temperature

Cyanobacterial and algal growth rates are temperature dependent. Growth can occur at low temperatures although there is significant potential for growth above about 15$^{o}$C and maximum growth rates are attained by most cyanobacteria at temperatures above 25$^{o}$C [109]. It has been suggested that these temperature optima are higher than for green algae and diatoms. However, there is an argument that the field studies on which these assumptions were based were undertaken on warm, thermally stratified water bodies, where the stratification may be the controlling factor in cyanobacterial growth, rather than the temperature [107]. Stratification is discussed further below.

(Adapted from [110])

### 2.1.2.5 Nutrients

Mass developments or blooms of cyanobacteria are generally associated with high nutrient concentrations, particularly phosphorus. Since cyanobacterial blooms often develop in eutrophic lakes, it has been assumed that they require high phosphorus and nitrogen concentrations. This contrasts to observations that cyanobacterial blooms often occur when concentrations of dissolved phosphate are lowest. Experimental data have shown that the affinity for nitrogen or phosphorus for many cyanobacteria is higher than for many other photosynthetic microalgae. If dissolved phosphate (soluble reactive phosphate determined from filtered samples) is detected at concentrations of only a few micrograms per litre, then cyanobacterial growth and biomass are not limited by phosphate availability. This means that they can out-compete other phytoplankton organisms under phosphorus or nitrogen limitation [108]. In addition to their high nutrient affinity, cyanobacteria also have a substantial storage capacity for phosphorus. They can store enough phosphorus to perform two to four cell divisions, which corresponds to a 4 - 32-fold increase in biomass [108].

In considering the potential for blooms of cyanobacteria there is a well-established relationship that relates nutrient concentrations to the occurrence of cyanobacteria. This is the concept that the maximum population size or 'carrying capacity' of a lake is controlled by the concentration of total phosphorus. This was developed from eutrophication programs pioneered in Europe in the 1960s and is discussed further below in relation to predicting the potential population of cyanobacteria in lakes [111].However, as a simple guide, a total phosphorus level of 10 – 25 µg $L^{-1}$ presents a moderate risk to support the growth of cyanobacteria. For levels of less than 10 µg $L^{-1}$ there is a low risk of cyanobacterial growth, and a level greater than 25 µg $L^{-1}$ provides high growth potential (see Table 6).

The 'Vollenweider' model relates the spring phosphorus loading, measured as total phosphorus, to the 'summer' or growing season biomass of all phytoplankton algae in the population, measured as chlorophyll [112,113,114]; see also extensive discussion in [115]. This extensive body of work established that a clear relationship existed between phosphorus loadings and algal biomass [113, 114]. This relationship has also been used for predictive purposes in a number of ways and is one of the best accepted paradigms of modern limnology. The conventional understanding is that eutrophication and the tendency for the occurrence of nuisance cyanobacterial blooms is initially driven by catchment processes that contribute excess nutrients and particularly phosphorus to the water body.

An additional and sometimes controversial paradigm is that there is a relationship between the total nitrogen: total phosphorus (TN:TP) ratio and the tendency for dominance in a lake by cyanobacteria over other phytoplankton groups [116]. A low ratio between nitrogen and phosphorus concentrations may favour the development of cyanobacterial blooms, and a range of empirical data based upon lake studies in North America and Europe indicated that at TN:TP ratios above 25-30, nitrogen-fixing cyanobacteria rarely form blooms [116]. However a review of the literature by Oliver and Ganf [117] suggested that there was little evidence to support the contention that TN:TP ratios are important in determining cyanobacterial dominance. They concluded that TN:TP ratios do not *per se* influence the occurrence of planktonic cyanobacteria, the more important issue is whether either nutrient could be considered limiting for cyanobacterial growth, or growth of other algae.

Potential sources of nutrients include both human related activities and natural inputs from catchments such as:

- sewage outfall
- on-site or other private sewage disposal systems
- intensive agricultural activities resulting in possible run-off from untreated animal effluent
- urban stormwater

The delivery of nutrients to reservoirs by storm or high rainfall events can also provide a trigger for cyanobacterial growth and blooms. The importance of big inflow events as triggers will vary between catchments and reservoirs and will depend upon the time in the season they occur relative to the cyanobacterial growing season. The nutrients in inflows may not immediately trigger an algal growth event but are often available within a lagged timeframe when other physical conditions (temperature, stratification, etc.) become conducive to support cyanobacterial growth in the growing season.

(Adapted from [109], [112])

### 2.1.2.6 Stratification of water bodies

Thermal stratification of a water body influences the depth at which cyanobacteria are likely to be found, the light levels they receive, and the concentrations of nutrients in the water body.

The shape and structure of lakes and reservoirs, the latitude, weather conditions and the physical nature of the water, all influence thermal stratification. Stratification can be determined by measuring vertical profiles of temperature within the water body. Where thermal stratification occurs, the water body usually develops two separate non-mixing layers (the epilimnion and the hypolimnion), with a transitional layer (thermocline) in between (Figure 9). The two layers may have different physicochemical characteristics. The upper, warmer, epilimnion can become wind-mixed and because of its exposure, can freely exchange dissolved gases (such as $O_2$ and $CO_2$) with the atmosphere. The hypolimnion, which is the colder bottom layer, is isolated from the epilimnion by the thermocline. The density change at the thermocline, caused by the temperature difference, acts as a physical barrier that prevents mixing of the upper and lower layers.

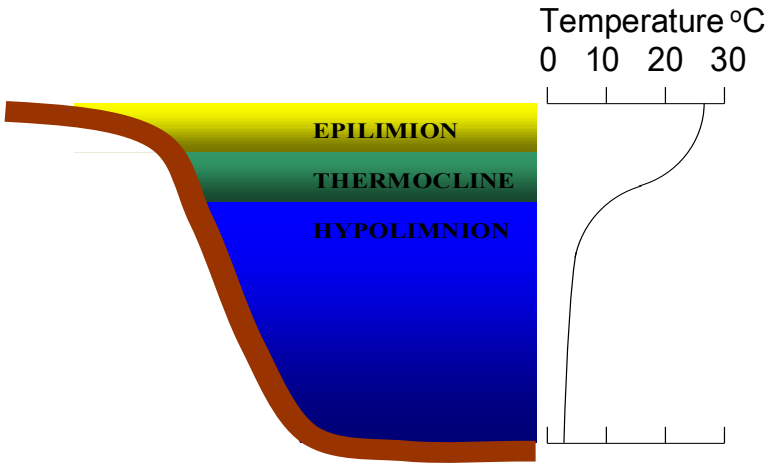



Figure 9: Cross section of a thermally stratified lake showing location of the epilimnion and hypolimnion and associated temperature changes.

In temperate climates, thermal stratification generally occurs seasonally (summer-autumn) in water bodies of appropriate depth, whereas in tropical climates it often follows daily or diurnal time patterns.

Stratification can also result in substantial release of phosphorus from sediments, causing an increase in the internal nutrient loading to a water body. Under oxygenated conditions (i.e. well-mixed water body) phosphorus rich sediments are sealed by an oxidised surface layer of an iron-phosphorus complex. However, under stratified conditions (i.e. a non-mixed water body) the sediments become anoxic due to microbial activity using up the available oxygen, which is not replenished by normal gaseous diffusion from the water column above. Under these low redox conditions the complex breaks down resulting in phosphorus release from the sediments. During stratified conditions, sediment-bound phosphorus can become a major nutrient source for cyanobacteria if the nutrients are transferred to the surface layer by mixing and water movement. The amount of phosphorus released from the sediments is governed by water exchange rates, sediment chemistry, temperature, mixing conditions, and sediment disturbance.

Usually, shallow (e.g. 2-3 m), wind-exposed lakes are non-stratified. Lakes of intermediate depth (e.g. 5-7 m) may develop transient thermal stratification for a few calm and sunny days, which is then disrupted by the next rain or wind event. In temperate climates deeper lakes can exhibit a stable stratification from spring to autumn. The formation of stratified conditions can influence light intensities experienced by cyanobacteria, bloom formation and nutrient levels in the water.

### 2.1.3 Cyanobacterial blooms

“Blooms” tend to occur when a combination of favourable environmental conditions such as temperature, light, nutrient concentrations and thermal stratification coincide. Bloom development occurs through a series of phases, the seeding and initial development phase, followed by a rapid or exponential growth phase and a plateau phase and die-off phase. The plateau phase can last for some time if the appropriate environmental conditions persist. Under calm weather conditions excessively buoyant cells or colonies may accumulate at the surface. Light winds drive them to leeward shores and bays, where they form scums. In extreme cases, such agglomerations may become so dense that they acquire a gelatinous consistency. More frequently, they are seen as streaks or slimy scums that may even look like blue-green paint or jelly. This is characteristically termed a “bloom”. Blooms distributed evenly throughout the upper water layer may be dense enough to cause visible discolouration. Scum formation can lead to the concentration of cells in the water column by factors of thousands up to one-million and accumulations can display a pea-soup consistency.

It should be noted that the term “bloom” does not have one formal or recognised definition; however it is generally understood to mean an algal or cyanobacterial concentration that is significantly above

average for a lake or reservoir [108]. A bloom also usually has the connotation of impaired water quality, because of visible scums or the presence of algal metabolites such as tastes and odours or toxins. For water bodies used for drinking water supply or recreational activities a bloom can be defined by the concentration that causes a problem/ nuisance for these uses.

#### 2.1.3.1 Blooms of benthic cyanobacteria

The growth of benthic cyanobacteria follows a slightly different developmental process. It has been suggested that the growth of benthic cyanobacteria follows a three step process involving:

1) Initiation, when light and temperature conditions become favourable
2) Formation and growth of benthic mats that cover large areas of submerged sediments, and
3) Subsequent detachment and drift. [118]

In the attached form the benthic cyanobacteria may have high geosmin or MIB production rates but the potential for effect is localised to the area of growth of the benthic mats. However following detachment into the free-floating clumps the metabolite can be released and become more widely dispersed due to cell lysis and may be so smelly to be noticeable to people in the vicinity of the lake or reservoir. On occasions where large-scale detachment and drift of benthic cyanobacteria occurs, masses of floating clumps can take on the appearance of a more characteristic cyanobacterial bloom, but is usually considerably smaller.

## 2.2 Risk Assessment for the Growth of Cyanobacteria

Risk assessment is the process of using available information to predict how often identified hazards or events may occur and the magnitude of their consequences. Risk can be assessed at two levels: maximum risk in the absence of preventative measures and residual risk after consideration of existing preventative measures [119].

### 2.2.1 How can water managers assess the likelihood of toxic algae outbreaks in their water source?

Information on the importance and interrelationship of environmental variables has been used in a range of ways to determine the likelihood of the growth of cyanobacteria and the development of blooms in particular lakes. A range of approaches has been used for this risk assessment and these have been variously termed ‘susceptibility’ or ‘vulnerability’ assessments. A central underlying assumption of these assessments, which has been mentioned, is that for most freshwaters there is a relationship between phosphorus loading to a water body and algal productivity and biomass. The field of modelling the growth and prediction of algal blooms is a major research topic around the world and the discussion here is limited to the basic characteristics and assumptions of some of the simpler approaches that have been used.

The use of regression models, such as the classic chlorophyll:phosphorus relationships or ‘Vollenweider’ models, as management tools for individual lakes has been criticised [120] as these models integrate the behaviour of many lakes. A similar integrating empirical approach that assesses underlying lake water quality and capacity to sustain algal productivity is the risk assessment procedure of [121]. This approach was developed and seemed to work satisfactorily for assessment of a large number of European lakes and predicted ‘average’ lake water quality based upon a combination of a few physical parameters only – lake depth, residence time, lake surface area and catchment area.

In addition to these simple models based upon lake physical parameters [122], there are more complex deterministic 2D and 3D hydrodynamic models linked to water quality models which can be used to model the occurrence of different algal groups including cyanobacteria. These models are generally complex to run and calibrate and require a large amount of data for a wide range of physical and chemical variables for successful validation. There is a very good recent review of the potential use of some of these water quality models for the prediction of taste and odour events [122]. This concluded that although some of these models can simulate algal growth reasonably well, they are not a viable option to simulate geosmin and MIB production and release. This may be a realistic current assessment, although ongoing development and improvement of water quality and algal

growth simulation models to incorporate information on dynamics of taste and odour and toxin production and release may result in more robust models in the future.

A simple alternative risk assessment approach developed in Australia to assess water bodies for their susceptibility to cyanobacterial contamination is given in the NHMRC 'Guidelines for Managing Risks in Recreational Water' [123]. This approach assesses a range of major driving variables for cyanobacterial growth in a semi-quantitative way to determine the potential for cyanobacterial contamination. This technique was developed by the AWQC for the NHMRC document based upon empirical observations in Australian reservoirs and from the range of literature studies on the variables influencing cyanobacterial growth.

The variables used in the assessment are considered the four predominant drivers or indicators of the potential for cyanobacterial occurrence. These are: prior history of cyanobacterial occurrence, water temperature, total phosphorus concentration and a measure of thermal stratification. The combinations and the values of these parameters are assigned to categories and assessed in a matrix which defines the risk of the 'Potential for Cyanobacterial Growth' into five categories, ranging from 'Very Low' to 'Very High' (Table 6). It can be seen that this matrix is a linear continuum of the major variables from low to high that line up across the columns and is therefore rather simplistic. This is because it is possible to have a range of other combinations of variables that lead to intermediate risk. Nevertheless the approach is suitable for semi-quantitative application to reservoir data.

This risk assessment approach is to some extent also biased to determine the likelihood of conditions that favour occurrence of types such as *Microcystis* spp. (potentially toxic), and a range of *Anabaena* species including *Anabaena circinalis* (toxic and odour producing) i.e. 'buoyant bloom-formers', which are the major toxic and T&O nuisance cyanobacteria that occur in South-Eastern Australia. It may not apply as well to other important problem types such as *Cylindrospermopsis raciborskii* or *Aphanizomenon* spp.

Table 6: Assessment of the potential for cyanobacterial growth based on environmental parameters.

| | Environmental factor | | | |
|---|---|---|---|---|
| **Potential for Cyanobacterial Growth** | **History of Cyanobacteria** | **Water Temperature ($^oC$)** | **Nutrients Total Phosphorus ($\mu g\ L^{-1}$)** | **Thermal Stratification** |
| Very Low | No | <15 | <10 | Rare or Never |
| Low | Yes | <15-20 | <10 | Infrequent |
| Moderate | Yes | 20-25 | 10-25 | Occasional |
| High | Yes | >25 | 25-100 | Frequent and persistent |
| Very High | Yes | >25 | >100 | Frequent and persistent/strong |

## 2.3 Predicting Toxins and Odours

### 2.3.1 Is it possible to predict the concentrations of toxins and odours that could occur?

The risk assessment procedure above describes the susceptibility of a reservoir to cyanobacterial contamination, but does not provide a quantitative output for the potential maximum cyanobacterial population. Researchers at the CRC for Water Quality and Treatment developed a simplified empirical model, based only upon phosphorus concentration, to predict the potential levels of toxins and odours that could by generated in source water by cyanobacteria. This model is based upon empirical relationships between phosphorus and cyanobacterial cell numbers from the literature and actual cell content or 'cell quotas' of microcystin, saxitoxin and geosmin measured from natural cyanobacterial

populations in Australia. The resulting growth predictions can then be compared with actual cyanobacterial growth data from historical records to verify the calculations used in the model. This simple model can be used to estimate "worst case challenges", i.e. to answer the question "how bad could it get in my reservoir, and what scale of treatment might I need?" In reality, the magnitude of the risk over a season is determined by the period of time that favourable growth conditions persist, the carrying capacity of the reservoir (the total algal biomass that the physico-chemical conditions that the reservoir will support) and the types and effectiveness of management actions that can be implemented. This simple model does not consider those complex dynamic interactions that control growth but is a projection of likely maximum cell numbers and associated metabolites based simply upon phosphorus supply and availability as the yield-limiting variable for the final population size.

Within this model three different algal growth (and therefore toxins/odours) scenarios have been developed around the degree of availability of phosphorus as the yield-limiting variable. These are:

- ***Best* case**:* assumes that a low proportion of phosphorus is available for cyanobacterial growth (36%) and converted into phytoplankton, and a low fraction of this biomass is cyanobacteria, so problem cyanobacteria do not become dominant and toxin and odour production occur at the lowest potential rates.
- ***Most likely case**:* assumes median values for the availability of phosphorus (60%) and for conversion of phosphorus into cyanobacterial biomass; cyanobacteria do not dominate and there are median rates of toxin and odour production.
- ***Worst case**:* assumes that 80% of the phosphorus is bioavailable, that all of this phosphorus is translated into biomass of cyanobacteria, which become dominant, and toxins and odours are produced and released at the maximum reported rates.

**Best case* really means '*least worst case*' or '*most favourable'* in this circumstance for the occurrence of toxins and odours in a water body.

The outputs from this simple model are cyanobacterial numbers, and odour and toxin concentrations. An example of the output from this model is given in Table 7. This is for a reservoir with a current total phosphorus concentration of 80 µg $L^{-1}$. The projected outputs for cell numbers of the cyanobacteria *Microcystis* and associated microcystin, and *Anabaena*, and saxitoxin and total and dissolved geosmin indicate the range that could be encountered under current conditions, with a decrease or an increase in ambient nutrient levels. The model should be used with historical or current phosphorus concentrations where available. The scenarios can then be compared with drinking water guidelines and Alert Levels (see following sections) to assess the potential cyanobacterial hazard and be used for planning purposes to assess water treatment options for removal of contaminants.

Table 7: Scenarios for the growth of nuisance cyanobacteria and production of toxins and odours for different nutrient ambient concentrations in a reservoir. Model assumptions for the three cases are described in the text. These scenarios are developed as an example for a water supply reservoir in South Australia that has regular cyanobacterial blooms of *Anabaena circinalis*, which produce both geosmin and saxitoxins.

| | | | Predicted concentrations of cyanobacteria and their metabolites | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Reservoir nutrient status** | **Total Phosphorus** ($\mu g\ L^{-1}$) | **Scenario modelled*** | **Bioavailable Phosphorus** ($\mu g\ L^{-1}$) | ***Microcystis aeruginosa*** (cells $mL^{-1}$) | **Microcystin (Total)** ($\mu g\ L^{-1}$) | ***Anabaena circinalis*** (cells $mL^{-1}$) | **Geosmin (Total)** ($ng\ L^{-1}$) | **Geosmin (Dissolved)** ($ng\ L^{-1}$) | **Saxitoxin (Total)** ($\mu g\ L^{-1}$) |
| **Lower nutrient level** | 40 | **Best Case** | 14.4 | 2,000 | 0.03 | 1,000 | 36 | 5 | 0.07 |
| | | **Most Likely Case** | 24 | 27,000 | 1.15 | 13,000 | 960 | 240 | 0.9 |
| | | **Worst Case** | 32 | 89,000 | 12.8 | 44,000 | 4,800 | 1,920 | 2.9 |
| **Current nutrient level** | 80 | **Best Case** | 28.8 | 4,000 | 0.06 | 2,000 | 72 | 11 | 0.13 |
| | | **Most Likely Case** | 48 | 53,000 | 2.3 | 27,000 | 1,920 | 480 | 1.8 |
| | | **Worst Case** | 64 | 178,000 | 25.6 | 89,000 | 9,600 | 3,840 | 5.9 |
| **Higher nutrient level** | 160 | **Best Case** | 57.6 | 8,000 | 0.12 | 4,000 | 144 | 22 | 0.26 |
| | | **Most Likely Case** | 96 | 107,000 | 4.6 | 53,000 | 3,840 | 960 | 3.5 |
| | | **Worst Case** | 128 | 356,000 | 51.2 | 178,000 | 19,200 | 7,680 | 11.7 |

*** based upon assumptions for phosphorus availability and utilisation, cyanobacterial growth and different cell quotas of toxins and odours (refer Table 8)**

The assumptions and the range of values for the variables used to derive the scenarios in Table 7 are listed in Table 8.

Quite simply the model calculates chlorophyll yield from available phosphorus concentration, which can be modified depending upon the scenario selected. Chlorophyll *a* is then translated to cell numbers of *Microcystis* or *Anabaena* using published cell chlorophyll quotas. Cellular content or 'cell quota' ranges for geosmin, saxitoxin and microcystin are applied to estimate the likely yield of the cyanobacterial metabolites under the chosen scenarios.

Table 8: Assumptions and variables used in the simple cyanobacterial risk assessment model to derive growth, toxin and odour metabolite production by nuisance cyanobacteria based upon phosphorus supply in reservoirs. Chlorophyll a (chl *a*) concentration is a standard measure of algal or cyanobacterial biomass.

| Variable inputs and assumptions | Best Case | Most Likely Case | Worst Case |
|---|---|---|---|
| Proportion of total phosphorus (TP) pool in the reservoir that is bioavailable | 0.36 | 0.6 | 0.8 |
| Proportion of the bioavailable P that is converted to chl *a* | 0.5 | 0.8 | 1.0 |
| Proportion of chl *a* that is either *Anabaena* or *Microcystis* | 0.1 | 0.5 | 1.0 |
| The chl *a* content of *Anabaena circinalis* (pg cell$^{-1}$) [124] | 0.72 | 0.72 | 0.72 |
| The chl *a* content of *Microcystis aeruginosa* (pg cell$^{-1}$) [124] | 0.36 | 0.36 | 0.36 |
| The ratio of geosmin to *Anabaena* chl *a* (ng µg$^{-1}$) | 50 | 100 | 150 |
| The proportion of geosmin that is dissolved: i.e. extracellular and free in solution | 0.15 | 0.25 | 0.4 |
| The production of saxitoxins by *Anabaena* | 0.33 | 0.33 | 0.33 |
| The ratio of microcystin to *Microcystis* chl *a* | 0.04 | 0.12 | 0.4 |

The assumptions and calculations used with the simple cyanobacterial risk assessment model and their justification are as follows:

1) Two general starting assumptions apply for this model:
   - that the climatic conditions are favourable for cyanobacterial growth and therefore the eventual population size is determined by the carrying capacity of the reservoir.
   - that all other conditions for optimum growth are met and the phosphorus concentration is the limiting factor that will determine the eventual algal and cyanobacterial biomass.

2) Phosphorus concentrations: The level of total phosphorus (TP) in the example here (i.e. 80 µg L$^{-1}$ TP) was derived from the average spring/summer concentrations in an actual drinking water reservoir. For the scenario purposes the projected lower and upper levels were selected arbitrarily as half and double this concentration. If historical data are available for your reservoir then it is possible to select equivalent values for the model calculations.

3) Phosphorus availability: The proportion of TP that is bioavailable for uptake and utilisation by organisms will vary between water bodies and an empirical range is used here. The values selected here are: 0.36 for best case; 0.6 for most likely case; 0.8 for the worst case.

4) Incorporation of bioavailable P into algal biomass: The proportion of bioavailable P that is converted to chlorophyll *a* is assumed to be in the range of 0.5 - 1 (i.e. 50-100%). The assumption is that some bioavailable P will be taken up by other organisms, but most bioavailable P is taken up by phytoplankton and directly translated into chlorophyll *a*.

5) The proportion of chlorophyll *a* that is attributable to either *Anabaena* or *Microcystis* depends upon the degree of dominance achieved by the cyanobacteria and a range of 0.1 – 1 (10%-100%) is used here. Major blooms of cyanobacteria can form practically monospecific populations and the 'worst case' scenario assumes that 100% of the chlorophyll *a* is *Anabaena* or *Microcystis* accordingly. The 'most likely case' assumes a value of 50%. Reflecting the fact that minor blooms of cyanobacteria may account for less than half of the chlorophyll *a* in the reservoir, the 'best case' assumes that 10% of chlorophyll *a* is *Anabaena* or *Microcystis.*

6) The assumed chlorophyll *a* content of *Anabaena circinalis* is 0.72 pg cell$^{-1}$ and 0.36 pg cell$^{-1}$ for *Microcystis aeruginosa.* These values are based on values published by Reynolds [124]. This is used to determine the number of cells mL$^{-1}$ from the Chl *a* concentration.

7) The ratio of geosmin to *Anabaena* Chl *a*: The model selects a geosmin: chlorophyll *a* ratio in the range 50-150 ng $\mu g^{-1}$ for the three scenarios. This spans the range of 59-360 ng $\mu g^{-1}$ for *Anabaena circinalis* found by [125].

8) The proportion of extracellular or dissolved geosmin is derived from a range of actual field bloom data for a population of *Anabaena circinalis* in South Australia. This may be expected to vary with the strain, the stage of the bloom and environmental conditions. The 'worst case' scenario assumes 40% of geosmin is dissolved. This is reduced to 25% for the 'most likely scenario' and to 15% extra-cellular for the 'best case' scenario.

9) The ratio of microcystin to *Microcystis* chlorophyll *a* is derived from the published data and depends upon the strain and environmental conditions. The 'worst case' scenario assumes a ratio of microcystin to *Microcystis* chlorophyll *a* of 0.4, which is the maximum of the range published by [126]. This is reduced to 0.12 for the 'most likely case' and 0.04 for the 'best case' scenario (the mean of the range published by Chorus and Bartram, 1999) [126].

10) The production of saxitoxins by *Anabaena* can then be determined from the number of cells $mL^{-1}$ using the estimated saxitoxin yield of 0.33 $\mu g\ L^{-1}$ for *Anabaena* cell density of 5,000 cells $mL^{-1}$ (Humpage & Falconer, unpublished). Cell quotas for toxin production will be variable within and between natural populations and over time and other cell quotas can be used where they are available.

The output from this simple model should be considered in the light of a number of factors that will modify and reduce the risk from odours and toxins. For example:

- The cyanobacteria present may not necessarily produce toxins or odour metabolites, even if they are known toxigenic species or taste and odour producers.
- Management strategies are available in the reservoirs to reduce the growth or impact of the cyanobacterial population (e.g. variable off take height, algicide use, destratification).
- A range of variables associated with local conditions including water chemistry and weather patterns may make the conditions unsuitable for cyanobacterial growth.

Some factors such as weather patterns and the type of cyanobacteria can obviously not be controlled, however in some cases management strategies can be implemented to reduce the risks associated with cyanobacterial growth and contamination by toxins and odours and these are discussed below in the section on control techniques.

## 2.4 Alert Levels for Action

### 2.4.1 What is an Alert Levels Framework?

An 'Alert Levels Framework' (ALF) is a monitoring and action sequence that operators and regulators can use for a graduated response to the onset and progress of a potentially toxic cyanobacterial bloom in a drinking water source. Although the ALF is intended to assist in the management of potentially toxic cyanobacterial blooms, the approach of systematic monitoring and assessment is applicable to the occurrence and growth of all cyanobacteria in drinking water supplies. This is because all cyanobacteria should be treated with caution, until the absence of toxicity is confirmed by testing; or advice based upon past local knowledge indicates the absence of hazard.

The ALF described here is a generic model for drinking water, however it is possible to translate the format for monitoring and management of cyanobacteria in waters used for other purposes such as recreation and agriculture. The level thresholds, indicators and actions for these uses would be different from those applied for drinking water management and would need to be developed based upon appropriate guidelines and risk assessment procedures.

The intention of the ALF is that it is a situation assessment tool based around data from cyanobacterial cell counts and equivalent cell biovolume, which are to be used in conjunction with the relevant drinking water guidelines for toxins to assess the potential hazard from a cyanobacterial bloom. The rationale for the use of cell counts to prompt management actions is that, for most

practical purposes, cell counting is still used primarily by most water authorities to detect algal-related water quality problems. This is because cell counting is widely available and provides relatively rapid and cost-effective information. By contrast, toxin testing is still generally not widely available and can have slow turn-around time for results. The cell counts (and biovolume) are regarded as an indicator or "surrogate" for a potential toxin hazard. It is important to note that cell counts do not replace toxin analyses, which are required for health risk assessment, but rather are used as relatively conservative triggers in the management plan. The counts can be used to prompt toxin monitoring, which can then be assessed in relation to the relevant guideline to determine the hazard and risk.

The framework is developed from the perspective of the water supply operator or the manager of the raw water supply. The circumstances and operational alternatives for use with the ALF will vary depending upon the source of supply and water treatment facilities available. The associated monitoring program for cyanobacteria will also be site and season specific. Further, the monitoring program will depend upon the level of expertise of the operators, and on the degree of access to toxicity testing and analytical capacity for toxins. The progress through this sequence, particularly in relation to consultation and warnings, will vary depending upon whether the water source is a river or a reservoir, and whether management options are available either in the source or in a drinking water treatment plant.

## 2.4.2 History of ALFs

The concept of the ALF was first developed for algal management in South Australia in 1991, and modified and adopted nationally in 1992. It was subsequently adopted and used internationally by the World Health Organization (WHO) as a model system for response to cyanobacterial blooms [127] and has been adapted by other users to incorporate recreational and agricultural waters. The ALF given here is an updated version of the earlier Australian model which now references the Australian Drinking Water Guidelines (NHMRC/NRMMC) for microcystin toxins in particular [128].

## 2.4.3 How to use the ALF for drinking water

The framework follows the development of a potentially toxic cyanobacterial bloom through a monitoring program with associated actions in four stages called Alert Levels. The actions accompanying each level include additional sampling and testing, operational interventions, consultation with health authorities and other agencies, and customer and media releases. The sequence of alert levels is based upon initial detection of cyanobacteria at the Detection Level, progressing to moderate cyanobacterial numbers at Level 1, where notification, additional sampling and assessment of toxicity may occur. For the next stage at Level 2, the higher cell numbers can indicate the potential for the occurrence of toxins above guideline concentrations. Alert Level 2 represents the point where the operators and health authorities may decide to issue a health warning or notice in relation to suitability of the water for drinking water supply consumption. This would follow a full health assessment and depend upon circumstances such as availability and performance of water treatment, consumption patterns, etc. It is possible of course that an operator may decide to issue advice or a notice at cell numbers lower than that equivalent to the guideline. The sequence can also continue to escalate to Alert Level 3 for very high cyanobacterial biomass in raw water. This level represents the situation where the potential risk of adverse health effects is significantly increased if treatment is unavailable or ineffective. Alert Level 1 and 2 ideally require an assessment of toxicity and toxins in raw water and assessment of both the drinking water and the performance of the treatment system for toxin removal.

## 2.4.4 Levels of the Framework

### 2.4.4.1 Derivation and definition of the Levels

The generic Alert Levels Framework described here and presented in Table 10 was originally developed for tracking populations of potentially toxic *Microcystis aeruginosa* using cell counts as a surrogate for the toxin hazard. The ALF is also presented in the form of flow chart in Figure 10. It assumes a worst case where the *Microcystis* population is potentially highly toxic (toxin cell quota of 0.2 pg total microcystins per cell; mean cell volume of 87 $\mu m^3$). For this toxic population of *M. aeruginosa* a cell density of approximately 6,500 cells $mL^{-1}$ (biovolume of 0.6 $mm^3$ $L^{-1}$) would be

equivalent to the guideline of 1.3 µg $L^{-1}$ microcystin-LR if the toxin were fully released into the water. This is the definition of Alert Level 2 given above and is the recommended "Alert" for *M. aeruginosa* in the ADWG.

The ALF then assumes that other unknown or uncharacterised cyanobacterial populations can be assessed for potential toxin hazard by comparison of their biovolume with the equivalent biovolume for the population of highly toxic *M. aeruginosa*. The underlying assumption is that, based upon the same cell quota per biovolume ratios, other unknown cyanobacteria may contain a toxin hazard equivalent to a highly toxic *M. aeruginosa.*

In reality cell toxin quotas in natural populations will be highly variable and the relationship between toxin concentrations and biovolume will not necessarily be valid for *M. aeruginosa* or other species or populations. However, the assumption is regarded as reasonable for the purpose of preliminary hazard assessment in the absence of toxin testing.

As information about toxicity of different cyanobacteria became available definitions of Alert Levels for other species of toxic cyanobacteria were developed. Specific notification and alert levels have now been recommended for populations of *Anabaena circinalis*, *Cylindrospermopsis raciborskii* and *Nodularia spumigena* containing saxitoxins, cylindrospermopsin and nodularin respectively and these are described in detail below. For other species and uncharacterised cyanobacterial populations managers can still use the generic biovolume levels and definitions given in Table 10 and Figure 10 to apply the ALF for toxin hazard assessment.

The threshold definitions for the generic ALF and the recommended actions are summarised in Table 10, and more detailed description and rationale follows.

***Detection Level***

This level encompasses the early stages of bloom development, where cyanobacteria are first detected at low levels in raw water samples. The cell numbers for this level are somewhat arbitrary, and are $\geq$ 500 & < 2,000 cells $mL^{-1}$ for *Microcystis aeruginosa* or a total biovolume of $\geq$ 0.05 & < 0.2 $mm^3$ $L^{-1}$ for other cyanobacteria (individual species or combined total).

Taste and odours may become detectable in the supply, although this does not necessarily indicate the presence of toxic cyanobacteria. If a routine monitoring program is not in place, this is the appropriate time to collect and deliver samples to a laboratory for confirmation of the presence of cyanobacteria. If there is no routine program the recommendation for monitoring is to commence weekly sampling and cell counts at representative locations in the water body. The presence of low population densities of cyanobacteria could still mean there is the potential for the formation of localised surface scums and operators should regularly inspect raw water offtakes for scums or discoloured water.

***Alert Level 1***

Alert Level 1 represents the level at which the cyanobacterial population has become established, and localised high numbers may occur.

The threshold for this level is a cell number ≥ 2,000 cells $mL^{-1}$ and < 6,500 cells $mL^{-1}$ of *Microcystis aeruginosa* for a sample taken at the source water intake for the drinking water supply or a total biovolume of all cyanobacteria of ≥ 0.2 & < 0.6 $mm^3$ $L^{-1}$ (Table 10).

The variability around a cell count result of 2,000 cells $mL^{-1}$ is likely to be in the range of 1,000-3,000 cells $mL^{-1}$. This is based upon a likely precision of ±50% for counting colonial cyanobacteria such as *Microcystis aeruginosa* at such low cell densities. For counting filamentous cyanobacteria such as *Anabaena circinalis* the precision is likely to be much better at these cell densities (~±20%), giving an actual likely cell density in the range of 1,600-2,400 cells $mL^{-1}$ for a reported result of 2,000 cells $mL^{-1}$.

The definition for Level 1 is relatively conservative and has been chosen to indicate a point that represents a cell density providing a buffer, or time margin, of 4-6 days before the guideline for toxin concentration in raw water could be exceeded (i.e. Level 2 conditions) if the population is toxic and is

actively growing. This is based upon a population doubling rate of 4 days which is equivalent to a growth rate of $\mu$=0.17 $d^{-1}$.

Alert Level 1 may require notification and consultation with health authorities and other agencies for ongoing assessment of the status of the bloom. Although contact with health authorities is recommended when this level is reached, it may not be required on a weekly frequency if local conditions deem this unnecessary. For instance, if the dominant cyanobacterium present is not known to be a problem based on prior testing and experience (e.g. small pico-cyanobacteria such as *Aphanocapsa* sp.), this alert level can be adjusted to suit the local situation.

The requirement for toxicity assessment at this level will depend upon advice and discussion with health authorities. It will also depend upon circumstances such as: whether the cyanobacteria are known toxigenic species, past history of toxicity, nature of the supply and associated water treatment, local sensitivity in relation to this supply, etc. This consultation should be initiated as early as possible and continue after the results of toxicity testing and/or toxin analysis become available.

The bloom population should be sampled to establish the extent of its spread and variability. Special samples (concentrated scums and/or grab samples representative for the raw water intake) should be collected and dispatched for toxicity testing or toxin analysis.

This level may warrant operational actions such as the deployment of booms adjacent to offtakes, or changing the depth of drinking water abstraction. Mixing or destratification may be useful in some circumstances to reduce cyanobacterial growth. Treatment with algicides may be an emergency measure in some situations and should be restricted to reservoirs only; its use and application also depends upon local environmental regulations.

***Alert Level 2***

Alert Level 2 is the next stage at slightly higher cell numbers of potentially toxic cyanobacteria. The threshold for Level 2 (in the absence of toxin information) is cell numbers and/or biovolume that could indicate the potential for a toxin hazard at or above the guideline level if:

1. the population was highly toxic, and
2. all toxins were released and water treatment is ineffective for their removal

This level is characterised in general terms by an established bloom with moderately high numbers showing a trend upwards over several successive samples at sampling frequencies of at least twice per week. The cyanobacterial population is likely to have developed to the extent that localised surface scums may form where scum-forming species are prevalent.

Two thresholds definitions for Level 2 (Table 10) are:

1. Cell numbers $\geq$ 6,500 cells $mL^{-1}$ for *Microcystis aeruginosa* or
2. Total biovolume of other cyanobacteria of $\geq$ 0.6 $mm^3$ $L^{-1}$
   (Note that this is given at 1 significant figure)

The cell numbers for Level 2 ($\geq$ 6,500 cells $mL^{-1}$) is the "Alert" given in the Australian Drinking Water Guidelines for toxic *Microcystis aeruginosa* equivalent to the microcystin guideline of 1.3 µg $L^{-1}$. The approximate biovolume of 0.6 $mm^3$ $L^{-1}$ for other cyanobacteria (toxigenic or of unknown toxicity status) is equivalent to the biovolume for 6,500 cells $mL^{-1}$ of *M. aeruginosa*. This biovolume of cyanobacterial cells could be equivalent to the ADWG guideline for microcystins if the cyanobacteria were found to be toxic and to produce microcystins. Furthermore, it is assumed that for blooms and populations of uncharacterised cyanobacteria, the hazard from toxicity is unlikely to exceed the worst case for an equivalent biovolume of highly toxic *M. aeruginosa* containing microcystin. Therefore using this biovolume as indicator of potential toxin hazard in the first instance should allow protection from significant risk while further assessments are made.

Alert Level 2 represents the point where the operators and health authorities may decide to issue a health warning or notice in relation to suitability of the water for consumption. This would follow a health assessment and depend upon circumstances such as availability and performance of water

treatment, consumption patterns, etc. It is also possible that an operator may decide to issue advice or a notice at cell numbers lower than these thresholds (Table 10).

It may be acceptable to continue to supply drinking water from the source water even with a positive toxicity result, dependent upon a risk assessment by the health authorities that may recommend specific action to protect more susceptible population groups. The operational interventions at this level are the same as those for Alert Level 1.

***Alert Level 3***

The threshold definition for Alert Level 3 is cell numbers of $\geq$ 65,000 cells $mL^{-1}$ of the toxic species *M. aeruginosa* (i.e. toxins confirmed by analytical or bioassay techniques) in the raw water adjacent to the offtake. Alert Level 3 is alternatively defined by the total biovolume of other toxic cyanobacteria $\geq$ 6 $mm^3$ $L^{-1}$ (see Table 10). The cell number for Level 3 represents ten times the Australian Drinking Water Guidelines for toxic *Microcystis aeruginosa* of 6,500 cells $mL^{-1}$, and is also equivalent to approximately 13 µg $L^{-1}$ microcystin-LR. This describes an established toxic bloom with high cell numbers and possibly localised scums. The sampling program will have indicated that the bloom is widespread with no indication of a cyanobacterial population in decline in the short term. Conditions in Level 3 are indicative of a significant increase in the risk of adverse human health effects from the water if it were untreated, or treated by an ineffective system, even for short-term exposure.

The cell count in Level 3 can be a trigger for the immediate notification to health authorities, but this would only be in a situation where this has not occurred earlier (at Level 1 or 2). This would occur where there was no prior information from an ongoing monitoring program, and treatment is limited or its performance for toxin removal is untested. This could be a scenario where a one-off sample or result is the initial discovery of a major bloom in the source water. By definition the circumstances for Level 3 are that there is some potential for adverse public health outcomes if these high numbers are present in the source water or supply combined with the nature of the water treatment, the population sensitivity, and their consumption patterns. High cell numbers also mean there is potential for much higher localised concentrations, i.e. surface scums and, depending upon the position of the offtake, this could then mean that very high cell numbers could be entering the supply for short periods and this may not be captured by the monitoring program.

If activated carbon (powdered or granular) or advanced oxidation processes such as ozone are available in the treatment process, it is likely it will be needed at this level. The treated water should be monitored for the specific cyanotoxins to confirm their removal.

The application of algicides in this phase can potentially enhance problems for treatment by releasing high concentrations of dissolved toxins as a result of cell disruption. Where coagulation and filtration systems generally remove cell-bound toxins, dissolved toxin is more likely to break through the treatment system (see Chapter 3).

If water treatment is unsatisfactory for toxin removal, and toxins are present in supply at concentrations significantly above the guideline then Level 3 may result in the activation of a contingency water supply plan that is appropriate for the operator and the system. This may involve switching to an alternative supply for human consumption, or in some circumstances the delivery of safe drinking water to consumers by tanker or in bottles. More extensive media releases and even direct contact, with appropriate advice to customers may be necessary. Where advice is provided to the public because of a cyanobacterial hazard to human health it may be appropriate to indicate that the water would be suitable for purposes such as washing, laundry, toilet flushing etc. Closure of a public drinking water supply because of a cyanobacterial hazard in source water is not likely to be justified since potential hazards from disruption of supply (public hygiene and fire-fighting, etc.) are likely to be worse than the cyanobacterial hazard.

Monitoring of the bloom should continue, to determine when it is in decline, so that normal supply can be resumed. Monitoring is usually only warranted at 3-7 day intervals. Experience suggests that the toxicity of a cyanobacterial population can change, but it is unlikely to become completely non-toxic or to decline in a period of a few days.

The sequence of actions at Level 3 should follow through to deactivation of an emergency with advice and media releases to confirm this. It is possible that the collapse of a bloom, or management action such as flushing and control of scum, could lead to a rapid decline from Level 3 back to Level 1 or beyond. Likewise the sequence might escalate rapidly, bypassing Level 1 & 2, if adequate monitoring and early warning information is not available. The collapse of a bloom may be associated with the release of dissolved toxin into the water and the length of time for toxins to degrade is discussed in Section 2.6.4.3. Generally withholding times to avoid toxin contamination can vary from a minimum of several days to weeks depending upon the toxin and the waterbody.

***Notification and Alert Levels for common toxic cyanobacteria in Australia***

The recent revision of the Australian Drinking Water Guidelines for cyanobacteria and toxins has recommended trigger levels for initial notification to health authorities for a range of known toxin producing cyanobacteria. These levels are based upon cell numbers and biovolumes and are derived in a manner to be equivalent to Alert Levels 1 & 2 for *Microcystis aeruginosa* given in Table 10.

These levels are derived such that the 'Notification' (*Alert Level 1*) is equivalent to cell number or biovolume that is approximately 30% of the value equal to the health alert (or guideline value in the case of microcystin) and the 'Alert' (*Alert Level 2*) being the cell number or biovolume equivalent to the cell concentration of the particular species at the health alert or guideline value.

This is illustrated by the example given for cylindrospermopsin:

"Initial notification to health authorities could be provided when numbers of *C raciborskii* reach 30% of the density equivalent to 1 µg $L^{-1}$ cylindrospermopsin (4,500 cells $mL^{-1}$; biovolume 0.18 $mm^3$ $L^{-1}$), while an alert could be provided when cell numbers are equivalent to 1 µg $L^{-1}$ cylindrospermopsin (15,000 cells $mL^{-1}$; biovolume 0.6 $mm^3$ $L^{-1}$). For cylindrospermopsin producing species other than *C raciborskii* notifications and alerts should be based on biovolumes." [5].

The Notification and Alerts for the known toxin producers *Microcystis aeruginosa, Anabaena circinalis*, *Cylindrospermopsis raciborskii* and *Nodularia spumigena* which are recommended in the ADWG as triggers in the context of a tiered framework such as the Alert Levels Framework are summarised in Table 9.

Table 9 Threshold definitions – lower threshold or trigger level for 'Notification' (Alert Level 1) & 'Alert' (Alert Level 2) for range of known toxic cyanobacteria given in the ADWG: (NHMRC/NRMMC 2004) [5].

| Species or Type | Notification (Alert Level 1) | | Alert (Alert Level 2) | |
|---|---|---|---|---|
| | Cell Numbers (cells $mL^{-1}$) | Biovolume ($mm^3$ $L^{-1}$) | Cell Numbers (cells $mL^{-1}$) | Biovolume ($mm^3$ $L^{-1}$) |
| ***Microcystis aeruginosa*** | 2,000 | 0.2 | 6,500 | 0.6 |
| ***Anabaena circinalis*** | 6,000 | 1.5 | 20,000 | 5 |
| ***Cylindrospermopsis raciborskii*** | 4,500 | 0.18 | 15,000 | 0.6 |
| ***Nodularia spumigena*** | 12,000 | 2.7 | 40,000 | 9.1 |

## 2.4.5 Customer and media information

Providing information to consumers and media liaison are important aspects of managing water quality problems associated with cyanobacterial blooms. Information should be prompt and concise with detail about reasons for changes to supply and explanation for any differences in water quality. It is important for all of the agencies involved to provide coordinated and consistent advice.

The Alert Levels Framework suggests a number of points where media releases could be issued. These are in situations where consumers may experience changes in water quality, e.g. due to changes in source water quality, switching to another source water, changes in treatment, implementation of a contingency plan, or warning notices for recreational use of the source water.

The approach to releasing information will depend on the nature of the supply and the problem. For example, in major urban water supplies with sophisticated treatment infrastructure, it may not be necessary to advise consumers, as water quality changes will not be evident. In circumstances with limited treatment, as is often the case in rural or remote areas, or if the bloom occurs in a multiple use water resource (for instance those also used for recreation) it is important to inform consumers of the extent of the problem as part of the management strategy.

Table 10: Alert level definitions and actions for a general Alert Levels Framework for management of toxic cyanobacteria in drinking water.

| Level | Derivation and Background intention | Threshold Definition<br>These apply to a sample location in source water immediately adjacent to the water supply intake [1] | Recommended Actions |
|---|---|---|---|
| **Detection Level** | *LOW ALERT*<br><br>Detection | ≥ 500 & < 2,000 cells/mL<br><br>*Microcystis aeruginosa*<br><br>**-or-** a total biovolume of all cyanobacteria ≥ 0.05 & < 0.2 $mm^3/L$ (individual species or combined total)<br><br>***Cyanobacteria detected at low levels*** | *Have another look*<br>➢ Regular monitoring<br>➢ Weekly sampling and cell counts<br>➢ Regular visual inspection of water surface for scums adjacent to offtakes |
| **Alert Level 1** | *MEDIUM ALERT*<br><br>Potential for these cell numbers or equivalent biovolume to give rise to a toxin concentration that is 1/3 to 1/2 of the potential drinking water guideline concentration for microcystin. | ≥ 2,000 [2] & < 6,500 cells/mL<br><br>*Microcystis aeruginosa*<br><br>**-or-** the total biovolume of all cyanobacteria ≥ 0.2 $mm^3/L$ and < 0.6 $mm^3/L$ [3]<br><br>*Trigger value for this level can be adjusted for local conditions (see text)*<br><br>***Cyanobacteria detected at levels that indicate that the population is established, and high to very high numbers may occur in localised patches due to wind action.*** | *Talk to the health regulators*<br>➢ Notify health authorities<br>➢ Increase sampling frequency to 2x weekly at offtake and at representative locations in reservoir to establish population growth and spatial variability in source water<br>➢ Establish the representativeness (i.e. variability) of the offtake sample over time<br>➢ Decide on requirement for toxicity assessment or toxin monitoring |
| **Alert Level 2** | *HIGH ALERT*<br><br>Potential for these cell numbers or equivalent biovolume to give rise to a toxin concentration that is around or greater than the drinking water guideline concentration for microcystin. | ≥ 6,500 cells/mL<br><br>*Microcystis aeruginosa*<br><br>**-or-** the total biovolume of all cyanobacteria ≥ 0.6 $mm^3/L$ [4]<br><br>***Established bloom of cyanobacteria with the potential for toxin concentration to exceed guideline if the*** | *Assess the significance of the hazard in relation to the guidelines*<br>➢ Advice from health authorities on risk to public health, i.e. health risk assessment considering toxin monitoring data, sample type and variability, effectiveness of available treatment<br>➢ Consider requirement for advice to consumers if supply is unfiltered<br>➢ Continue monitoring as per Level 1 |

Table 10 continued

| | | | |
|---|---|---|---|
| | Assumes microcystin toxicity is the worst case for potential toxicity in any unknown sample or population of cyanobacteria. This applies whether or not the cyanobacteria present are known toxin-producers. | ***population is toxic and if the available treatment is ineffective.*** | ➢ Toxin monitoring of water supply (finished water) may be required, dependent upon advice from the relevant health authority |
| **Alert Level 3** | *VERY HIGH ALERT*<br><br>Potential for these cell numbers or equivalent biovolume to give rise to a toxin concentration that is greater than 10x the drinking water guideline concentration for microcystin. | ≥ 65,000 cells $mL^{-1}$<br>*Microcystis aeruginosa*<br><br>*-or-* the total biovolume of all cyanobacteria ≥ 6 $mm^3 L^{-1}$ (5)<br><br>***In circumstances without water treatment, or ineffective treatment, there may be an elevated risk of adverse human health outcomes if alternative water supplies or contingency advanced water treatment is not implemented.*** | *Assess potential risk immediately if you have not already done so*<br>➢ Immediate notification of health authorities if this has not already occurred at Level 1 or 2<br>➢ Requires advice to consumers if the supply is unfiltered<br>➢ Toxicity assessment or toxin measurement in source water and drinking water supply if not already carried out<br>➢ Continue monitoring of cyanobacterial population in source water as per Level 1<br>➢ In absence of treatment and subject to health risk assessment this level may require alternative contingency water supply<br>➢ Continue toxin monitoring after cell numbers significantly decline (e.g. for 3 successive zero results) |

1) The cell numbers for the Alert Levels are from samples that are taken from the source water location adjacent to, or as near as possible to, the water supply offtake (i.e. intake point). It must be noted that if this location is at depth, there is potential for higher cell numbers at the surface at this or other sites in the source water.
2) The variability around a cell count result of 2,000 cells $mL^{-1}$ is likely to be in the range 1,000 - 3,000 cells $mL^{-1}$. This is based upon a likely precision of +/-50% for counting colonial cyanobacteria such as *Microcystis aeruginosa* at such low cell densities.
3) These biovolume values are rounded up to express the value to one significant figure, e.g. 0.17 to 0.2 $mm^3 L^{-1}$; 0.57 to 0.6 $mm^3 L^{-1}$
4) This biovolume (≥ 0.6 $mm^3 L^{-1}$) (rounded up from 0.57) is approximately equivalent to those numbers of *M. aeruginosa* for Level 2
5) This biovolume (≥ 6 $mm^3 L^{-1}$) (rounded up from 5.7) is approximately equivalent to those numbers of *M. aeruginosa* for Level 3

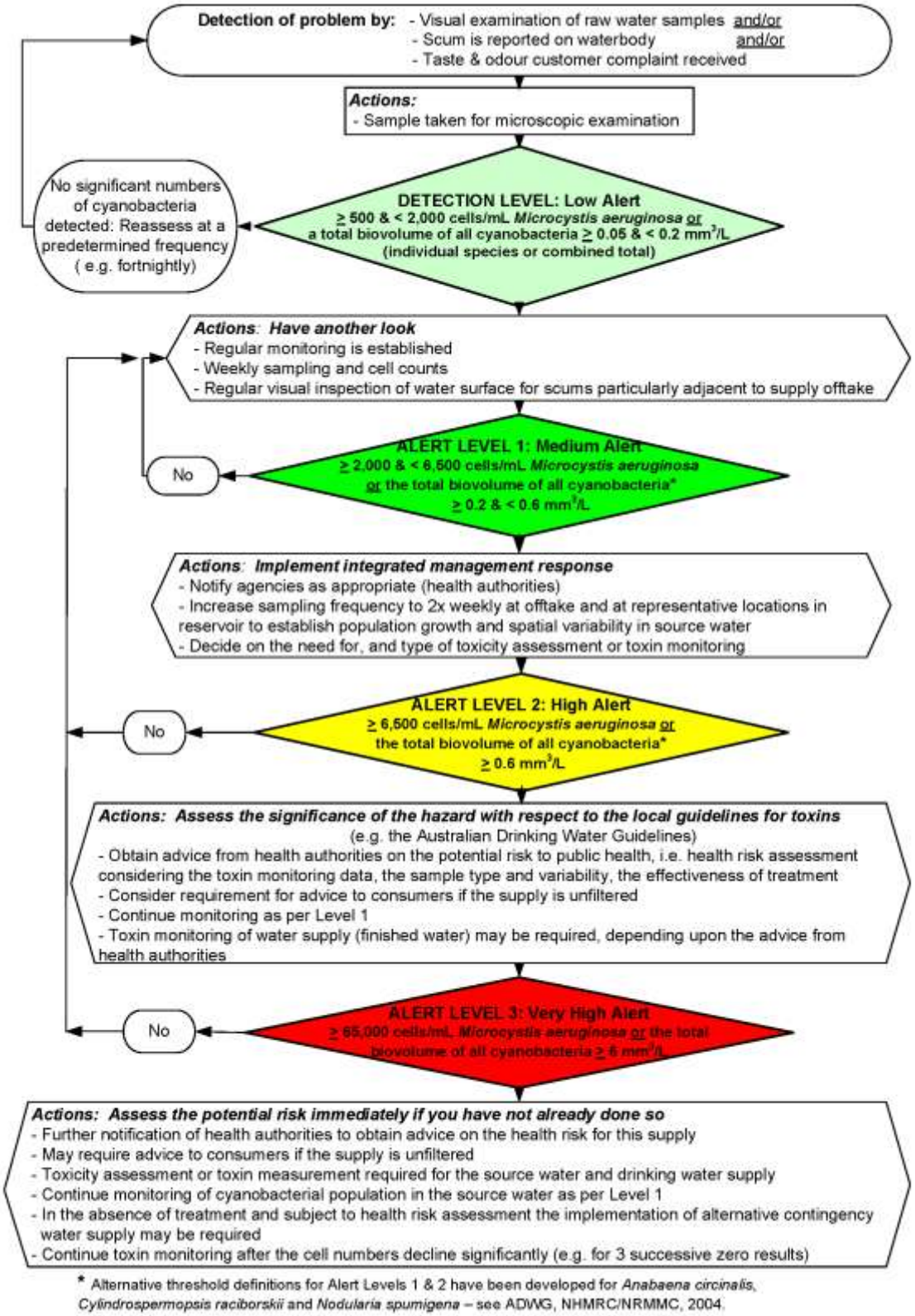



Figure 10: Flow chart of the Alert Levels Framework for management of cyanobacteria in drinking water.

## 2.5 Sampling and Monitoring

Monitoring can be defined as including two components - sampling of the water body and analysis of the samples in the laboratory. Together they provide the information for the early warning system and for tracking the development of cyanobacterial blooms [129]. A short overview of some of the key components of monitoring is given below.

When choosing an organisation to sample and analyse cyanobacterial samples it is recommended that the testing laboratory is accredited by a national laboratory accreditation authority. For example in Australia the National Association of Testing Authorities (NATA) accredits and recognises facilities that are competent in specific types of testing, measurement, inspection and calibration. It is important to note that not all accredited laboratories use the same methods for testing and this is not particularly important provided the individual methods are accredited. It may however, make it difficult to compare results when samples are analysed by more than one laboratory.

### 2.5.1 Sampling program design

#### 2.5.1.1 How do you design a sampling program?

Developing an appropriate sampling strategy will depend most importantly upon the primary objective of the monitoring program. The objective will be determined by the immediate use of the water, which in turn determines the level of confidence required in the monitoring results. For example if the water is being used directly to supply consumers, i.e. is in service, then you will want a very high degree of confidence in the monitoring result for any potential hazards from the occurrence of cyanobacteria. However if the reservoir is not directly in service at this time or is a bulk water storage, then you may have less need for a high degree of confidence in the results. This objective-based approach can be used to design a program based upon the level of sampling effort which translates to resource needs and cost for the program and this is outlined in Table 11.

For most purposes, the clear aim should be to obtain samples that are representative of the water body as a whole, or the part of a water body that is in use (e.g. recreational bathing along a shoreline). A distinction can also be made between different water body types which include storage reservoirs and lakes, direct service reservoirs, rivers and river weir pools.

Once the aim of the monitoring program is established the required level of sampling effort is described as high, moderate or low, depending on combinations of the following components:

- Type of access required for sample collection
- Sample type or the method used to collect a sample
- Number of samples collected at any one time
- Frequency of sampling

These components, which are given in Table 11, are outlined in more detail below.

#### 2.5.1.2 Access for sample collection

Cyanobacteria tend to be extremely patchy in distribution, both vertically and horizontally. Vertical patchiness results from the development of a stratified water column in warm calm weather, allowing those cyanobacteria which are buoyant to maintain their position at the surface for extended periods. Horizontal patchiness is common for most phytoplankton, but can be particularly pronounced in cyanobacteria due to the effect of prevailing winds, which cause accumulation downwind along shorelines of reservoirs or bends in river reaches. Benthic cyanobacteria are also known to cause problems associated with water use and therefore may need to be sampled.

Depth integrated sampling in open water provides, in general, a better representation of the ‘true’ or average cyanobacterial population in a water body and is therefore the preferred option. Open water, mid-stream or benthic sampling is normally achieved by working from a boat, but can also be achieved in some circumstances from a bridge over a river, or from an open water structure such as a reservoir offtake platform. For drinking water supplies, sampling the appropriate depth next to, or from, the water offtake tower is recommended. Due to the resources required for open water sampling (i.e. boat and two people), it is often reserved for high priority public health surveillance (i.e. drinking water

supplies). Open water sampling may also be necessary for public health surveillance of deeper bathing waters in a lake or for ecological studies and monitoring.

If open water sampling is not possible due to the non-availability of a boat or offtake structure, the second option for monitoring drinking water supplies is to sample from reservoir/lake shorelines or riverbanks. Such samples may not be representative of the 'true' cyanobacterial population due to the bias in spatial distribution discussed above and the limited choice of suitable locations. In choosing a location for sampling the likely effects of the prevailing winds and water currents should be taken into account.

In certain circumstances, open water sampling may not be the preferred option. This is often the case for monitoring of recreational waters, where the risk of exposure to cyanobacteria from bathing and water contact sports is higher adjacent to shorelines where scum forming species predominate.

Table 11: Recommendations for design of a monitoring and sampling program for cyanobacteria based upon the purpose of the monitoring and type of water body. The scale of sampling effort and procedures for monitoring are determined by the purpose of the monitoring.

| Purpose of Monitoring | Confidence required from Results | Water Body Type | Sampling Effort required | Access required for Sampling | Sample Type (method)[1] | Number of Samples[2] | Frequency of Sampling[3] |
|---|---|---|---|---|---|---|---|
| **Public health surveillance of drinking supplies**: ***in direct service*** | **Very High** | Reservoirs & lakes | High | Supply offtake<br>*and*<br>Open water by boat | Discrete sample at offtake depth<br>*and*<br>Integrated depth | Both offtake location and multiple open water sites | Weekly or 2x-weekly |
| | | Rivers and weir pools | | Mid-stream by boat; from bridge or weir | Integrated depth | | |
| **Public health surveillance of drinking supplies: *bulk water storage / not in service*** | **High** | Reservoirs & lakes | Moderate | Supply offtake location<br>*and/or*<br>Open water by boat | Discrete sample at offtake depth<br>*and/or*<br>integrated depth | Multiple sites | Weekly or 2x-weekly |
| | | Rivers and weir pools | | Mid-stream by boat; from bridge or weir | Integrated depth | | |
| **Public health surveillance of recreational water bodies & non-potable domestic supplies** | **Moderate** | Reservoirs & lakes | Low | Shoreline | Surface Sample | Limited number of sites | Weekly or fortnightly |
| | | Rivers and weir pools | | River bank | Surface Sample | | |

1. Integrated depth samples are collected with a flexible or rigid hosepipe, depth (2-5m) depending on mixing depth; surface or depth samples are collected with a closing bottle sampler (van Dorn or Niskin sampler); shoreline or bank samples collected with a 2m sampling rod which holds a bottle at the end.
2. Multiple sites should be a minimum of 100m apart (except in smaller water bodies such as farm dams), including one near the offtake. Multiple samples can also be pooled and one composite sample obtained. River monitoring should include upstream sites for early warning. Samples from recreational waters should be collected adjacent to the water contact area.
3. Frequency of sampling is determined by a number of factors including the category of use, the current alert level status, the cost of monitoring, the season and the growth rate of the cyanobacteria being tracked. Sampling should be programmed at the same time of day for each location. Visual inspection for surface scums should be done in calm conditions, early in the morning.

### 2.5.1.3 What sample collection methods should be used?

The choice of methods for sample collection will vary depending upon whether the sites require access by boat, shore or platform and will include integrated water column (hosepipe) sampling, discrete depth (grab) sampling, grab sampling from an extension pole, sediment sampling by grab or corer for benthic cyanobacteria and sampling from a pipeline. Different methods are used to collect samples for cyanobacterial identification, for toxin analysis or for assessing benthic cyanobacteria. In addition different techniques may be used to collect these samples from a boat, from depth, from the shoreline or a pipeline. These methods are summarised below.

#### 2.5.1.3.1 Water samples for cyanobacterial identification and counting

*Reservoir/river sampling by boat*

Reservoir sampling is ideally done using a boat. The sampling stations in a reservoir should preferably be chosen in a stratified random pattern; that is, randomly within several defined sectors, representing the entire water body. For boat sampling the use of permanent moorings with marker buoys placed in each of the sectors is the most practical approach and makes open water sampling easier, especially in windy weather. Having permanent sampling sites also gives consistency which enables the comparison of results at each site over a given time frame. If permanent marker buoys are unable to be placed in a water body, then a GPS should be used to ensure the consistency of sampling points over time. One way to introduce randomness when boat sampling is to move sampling station moorings within sectors on a yearly basis. For monitoring rivers, randomness of sampling sites is less critical due to instream flow.

*Integrated water column samples*

Integrated water column samples are also called ‘hosepipe’ samples and are recommended for open water sampling, where a representative sample of the water column over depth is desirable. The samples should be collected using a flexible hose pipe or rigid plastic pipe (Figure 11). A rigid pipe can be fitted with a one way valve, which tends to simplify the operation of withdrawing the pipe and sample from the water. The depth that the sample pipe is dipped should reflect the approximate depth to which cyanobacterial cells are likely to be mixed. In Australian water bodies, this can vary from approximately 2-10 metres, depending on the degree of stratification and exposure of the reservoir to winds. When the stratification status is uncertain, a temperature probe, if available, may be used to determine the depth of any thermocline present. If this equipment is not available, a 5 metre long flexible pipe is recommended, but a 2 metre long pipe may be more appropriate in shallower water bodies (those that are less than 3 metres deep). The inner diameter of the pipe should be at least 2.5 cm and flexible pipes are probably more practical than rigid pipes for pipe lengths greater than two metres. The recommended method of obtaining a ‘hosepipe’ sample is shown in Figure 11.

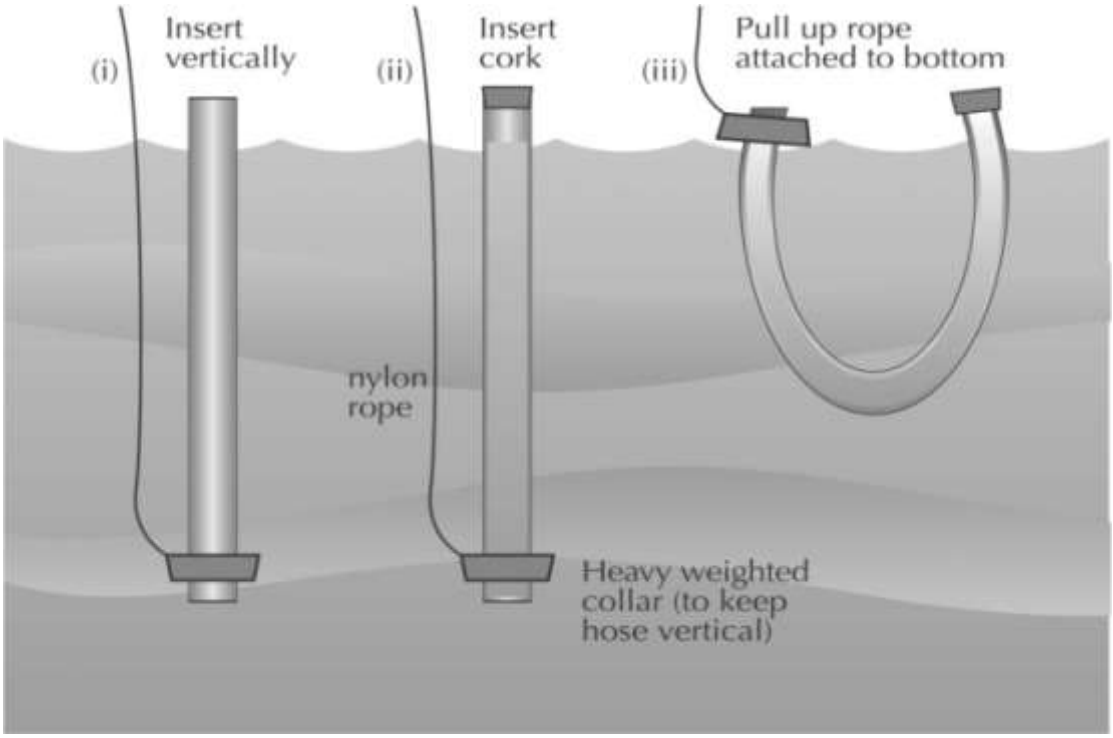



Figure 11: Using a hosepipe sampler to collect an integrated water column sample.

*Discrete depth samples*

Water sampling for public health surveillance is often required at the raw water abstraction or intake point for reticulation to a drinking water treatment plant. For this purpose discrete depth samples or 'grab' samples are often collected with a sampling bottle apparatus (e.g. 'Van Dorn' or 'Niskin' samplers), that can be triggered to be filled at a specific depth below the surface corresponding to the offtake depth (Figure 12). The rationale for this is to determine the total load of cyanobacteria (and their toxins) to the water treatment plant. In addition, the degree of cell lysis and toxin release through the reticulation system can be measured from an accurate assessment of intact cells at the offtake point. This is important information for determining the appropriate strategy for cell and toxin removal in the treatment plant. When choosing a sampling site near the water abstraction point in a reservoir the size of the offtake and the abstraction pumping rate should be considered. If pumping rates are high, vortices may occur around the offtake or abstraction valves which indicate that surface water is being drawn down into the offtake. If this situation is present in the reservoir, a number of samples at depths ranging from the surface to the offtake depth should be taken to determine the total load of cyanobacteria cells and toxins entering the water treatment plant. The method for collecting a water sample at depth is depicted in Figure 12.

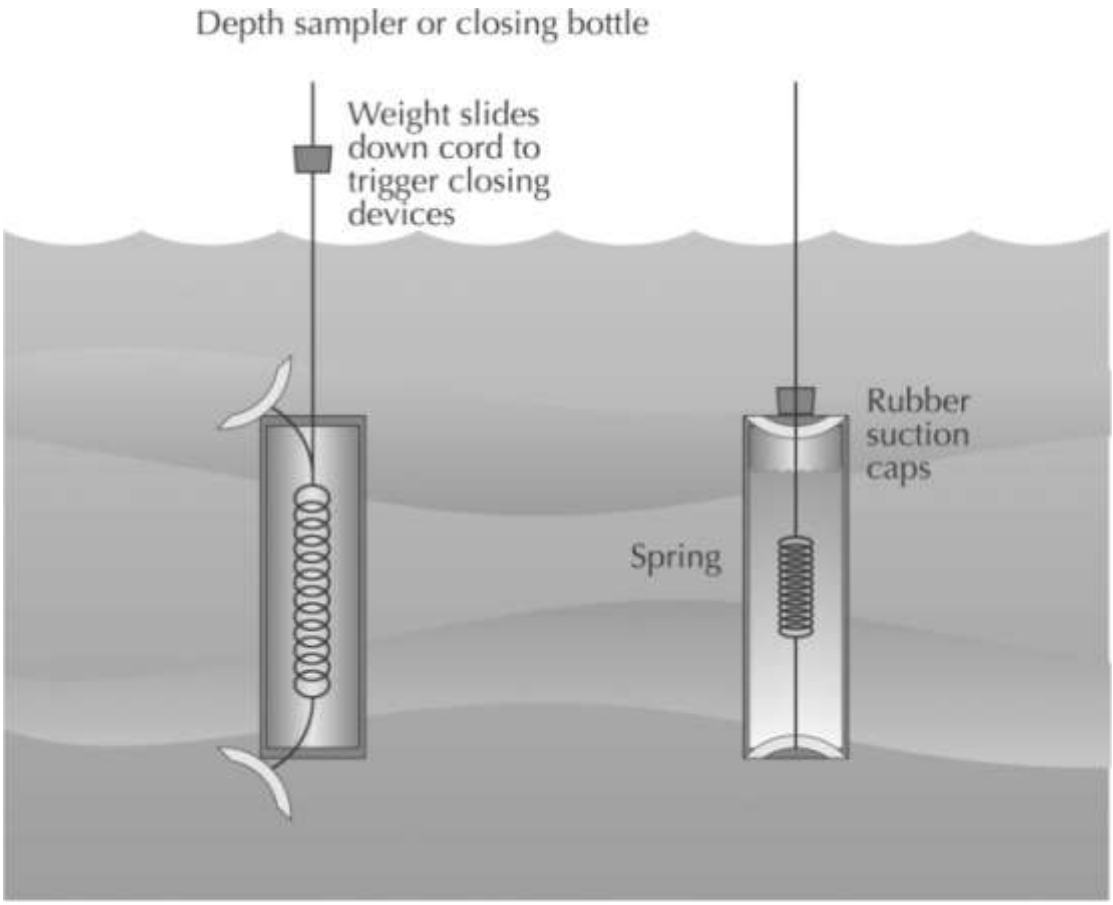



Figure 12: Using a depth sampler or closing bottle to collect a grab sample at a discrete depth.

*Surface grab samples from shoreline*

Sampling from a bank or shoreline is comparatively simple, but introduces a risk of excessive bias of samples from patchy shoreline accumulations. A 'pole-type' sampler can be used, where the bottle is placed in a cradle at the end of an extendable pole of 1.5-2 metres length. This procedure is depicted in Figure 13. Alternatively, a spear sampler as described in [130] is a useful sampling device for collecting an integrated depth water sample when standing on the bank or shoreline. It is also important to note that in using either the pole or spear sampler, scum accumulations near to the shoreline will not be sampled. A separate dip sample of any accumulations may be needed for further analysis such as toxin analysis. It is recommended to take surface grab samples from immediately below the surface to avoid dense floating surface patches of scum. This is usually at a standard depth of 25 or 30 cm and it is important to be consistent with this depth between sites.

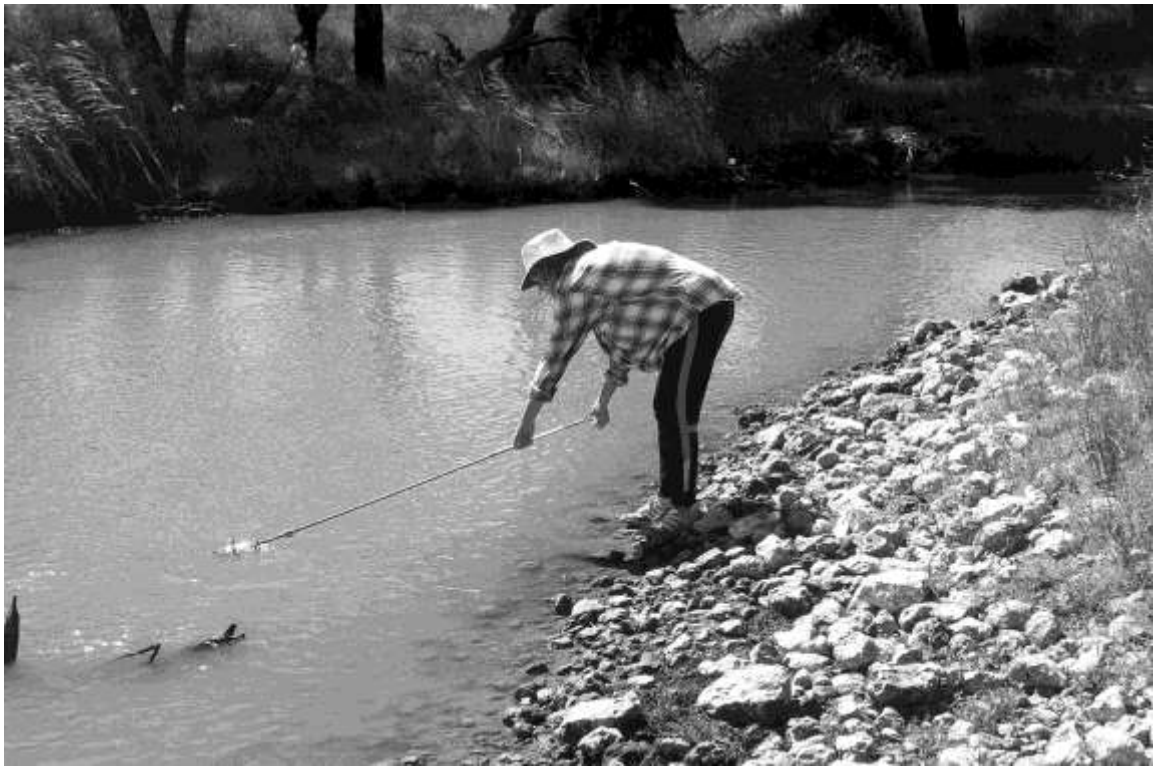


Figure 13: Taking grab samples from the shoreline with an extension pole.

#### 2.5.1.3.2 Samples for toxin analysis

*Qualitative*

Qualitative toxin analysis is done by mouse bioassay and is usually carried out either when more sophisticated techniques are unavailable, or the identity of the toxin is initially unknown. The samples are generally collected from dense accumulations of scum along shorelines and riverbanks if these are present. Alternatively, cells may be concentrated by either trailing a phytoplankton net (25-50 µm nylon mesh) from a boat or from the shoreline, or by collecting a large volume of water that can be concentrated in the laboratory. The volume of sample required depends upon the concentration of scum or cyanobacteria collected. Up to 2 litres of sample may be required if cyanobacterial concentrations are low, or if species present are small enough to pass through a phytoplankton net and samples therefore require concentration by other means such as filtration or centrifugation.

It is important to note that animal bioassay tests are usually only recommended or permitted in cases where there is a requirement to assess toxicity for the protection of public health. This is particularly the case if there is no suitable alternative chemical or biochemical analytical test available. This is due to ethical considerations regulating the use of animal testing. This will vary between state jurisdictions and should be established with analytical service providers before planning to undertake this testing.

*Quantitative*

Quantitative toxin analysis is performed using a variety of methods suited to the type of sample and toxin present. Samples are collected in the same manner as those taken for phytoplankton identification and enumeration and the volume of sample required is dependent upon the type of analysis to be used. In general, at least 500 mL of water should be collected.

#### 2.5.1.3.3 Samples for benthic cyanobacterial surveys

In some instances it may be necessary to collect benthic samples for identification of cyanobacteria. In most cases benthic samples are not collected routinely and are generally for qualitative analysis only. This may be the case if high levels of taste and odour compounds are detected in water bodies or supplies, but little or no cyanobacteria is detected in water samples. Samples can be collected using a benthic sampler such as an ‘Eckman’ grab or a rigid plastic corer (e.g. PVC or polycarbonate pipe).

A transect in a shallow, protected bay, should be chosen to sample. Duplicate samples at varying depths down to approximately 5 metres are collected either by grab or corer and emptied into a container with a fitted lid. If large quantities of sediment/sample are collected, a subsample can be taken and stored in a smaller specimen jar.

An alternative cause of taste and/or odour problems may be due to cyanobacteria growing attached on dam walls or offtake structures. Cyanobacteria attached to these structures can be scraped off, most easily when water levels drop.

If a quantitative assessment of benthic cyanobacteria growth is required, removable artificial substrates can be used. Artificial substrates of known surface area are placed in appropriate locations and depth within the water body and left deployed for periods of 1-2 months to allow benthic cyanobacteria to colonise the substrate surface. Once removed, the benthic cyanobacteria and algal biofilm is scraped off and measured quantitatively. Substrates can then be returned to the water body for the next assessment period. This technique for assessment of benthic algal growth would usually be part of a special investigation or ecological study and would require planning and some method development for each particular water body being studied.

#### 2.5.1.3.4 Sampling from a pipeline

Water samples are generally collected from a tap situated along, or at the end of a pipeline. In this case, a tap should be opened and allowed to run for a sufficient period of time to allow a fresh water sample to be collected. It is recommended that water samples collected from a pipeline are used for qualitative (i.e. identification) purposes only as the water in the pipeline may be treated with chemicals such as chlorine or copper sulphate.

### 2.5.1.4 How many samples should be collected?

For monitoring trends in cyanobacterial abundance, an indication is required of the 'true' cyanobacterial population, representing the entire water body. This can be achieved by collecting a suite of discrete samples from different sampling sites, which are counted separately and then may be averaged. As an alternative to undertaking separate counts on samples collected at several sites, samples may be pooled or composited. These samples are collected at three or more individual sites and then pooled into one container. The sub-sample for counting is then taken from the container after its contents have been thoroughly mixed. If composite samples are made, then the individual samples being pooled must be of equal volume to prevent bias. An alternative to pooling samples in the field is to send discrete samples to a laboratory, where they can be sub-sampled, pooled and analysed. Using this process, a portion of the original discrete sample can be retained for further analyses if required. The trade off from compositing is that there is a decrease in statistical power for subsequent data analysis against the three-fold or greater reduction in counting costs.

The number of sampling sites in a water body is chosen to determine the spatial variability of the cyanobacterial population and will also be influenced by time and cost considerations. It is recommended that a minimum of three sites be used when cyanobacterial counts exceed Alert Level 1 conditions (> 2,000 cells $mL^{-1}$) for both open water sampling and shoreline sampling. For lakes and reservoirs, the sampling stations should be at least 100 m apart (where possible), while for rivers, replicate samples should represent different 'parcels' of water. When sampling from a boat, replicate samples should preferably be taken at the downstream end first to avoid re-sampling the same 'parcel' of water.

A case study that provides an example of an actual sampling program for a drinking water service reservoir that has regular populations and occasional blooms of the cyanobacterium *Anabaena circinalis* is given in Box 1.

At lower cyanobacterial abundances, a single site may be sufficient, although the inherent variability in cyanobacterial numbers and a lack of multiple sites makes it difficult to be confident about detecting population growth, let alone rates of change.

**Box 1: A case study of sampling program design for cyanobacteria for Myponga Reservoir, South Australia.**

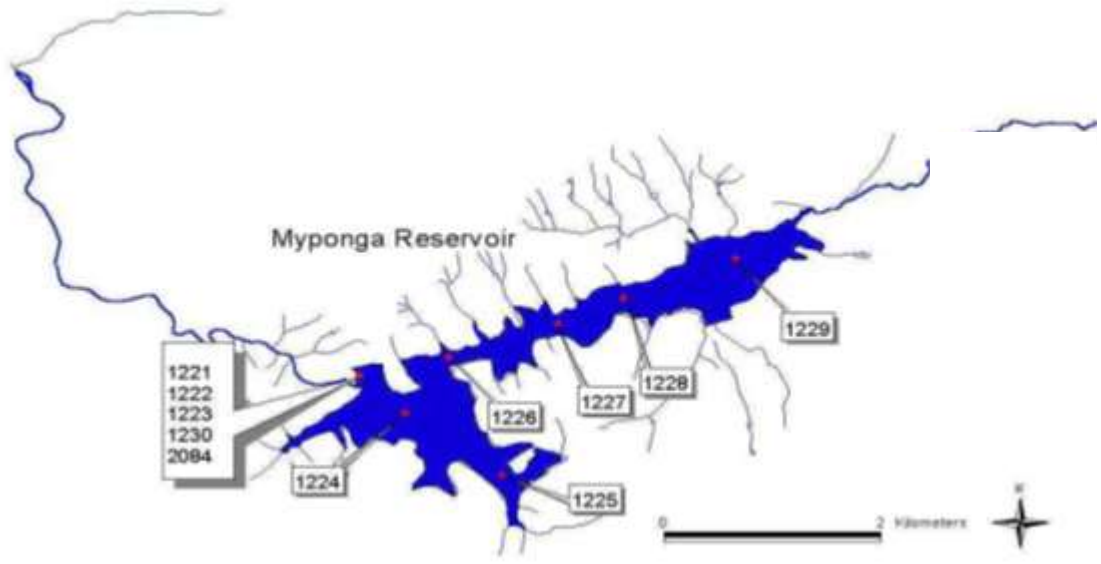
Myponga Reservoir is a moderate-sized drinking water reservoir that has regular growth of the nuisance cyanobacterium *Anabaena circinalis* each summer. The reservoir is used directly for drinking water supply after alum coagulation, dissolved air flotation (DAF) and dual media filtration. Chlorine is used for disinfection and the plant has the capacity to dose with powdered activated carbon (PAC) for taste, odour and toxin control.

**Site description**

Myponga Reservoir (S 35° 21' 14", E 138° 25' 49") is located 70 km south of Adelaide in South Australia. The reservoir has a capacity of 26,800 ML at a full supply level of 211.0 m AHD (Australian Height Datum), an average depth of 15 m, a maximum depth of 36 m and a surface area of 2.8 $km^2$. The mean retention time based upon abstraction is approximately 3 years. Water is removed from the reservoir via a series of offtake valves located on the dam wall. These offtakes are located at depths of EL 201.25, 184.54 and 177.75 which equate to 9.75, 26.5 & 33.25 m below the surface respectively at full supply level. The reservoir is operated such that the variable offtake is set to the lowest level during a cyanobacterial bloom in order to minimise algal cells and associated metabolites such as geosmin from entering the water treatment plant.

**Routine sampling program**

Samples are collected once per week in winter and twice-weekly in the summer growth season for identification and counting of phytoplankton from up to 10 separate locations. Sampling is concentrated at the offtake site where 4 separate samples are collected: a 0-5m integrated surface sample (Location 1221) and three discrete depth samples at 10, 20 & 30m (Locations 1222, 1223 & 1230). Spatial variability is assessed by collecting integrated column samples (0-5m) at 6 locations (Locations 1224-1229) spaced throughout the reservoir. The winter sampling frequency is weekly for 6 months from April - September which then increases to twice-weekly from October - March inclusive. The sampling program in winter has recently incorporated a process of collecting and 'pooling' (compositing) samples from the 6 reservoir locations which are then processed for a single cell count. If cyanobacteria are recorded in this pooled sample above a certain threshold (200 cells $mL^{-1}$) the individual sites will be re-assessed individually. Note that this pooling is only used in winter and all locations are sampled and counted individually in summer.




**Sampling Locations in Myponga Reservoir**

## 2.5.1.5 Frequency and timing of sampling

### 2.5.1.5.1 How often to sample?

The appropriate frequency of sampling will be dictated by a number of factors including the category of use, the current alert level status, the cost of monitoring, the season and the growth rate of the cyanobacteria being tracked. Apart from cost, the underlying consideration in operations monitoring is the possible health consequences of missing an early diagnosis of a problem. Growth rates are generally related to seasonal conditions and previous studies have shown that cyanobacteria in the field can exhibit growth rates from 0.1 - 0.4 $d^{-1}$ (equivalent to population doubling times of nearly a week (6.93 days) to less than two days (1.73 days), respectively). These estimated growth rates can be used to construct a set of theoretical 'growth curves' for a population of cyanobacteria starting from an initial count of either 100 or 1,000 cells/mL (Table 12). Historical data should be used as an indicator of likely rates of the growth and increase in cyanobacterial numbers for a particular water body.

Table 12: Cyanobacterial concentrations that can be achieved from an actively growing population by applying two different growth rates and initial starting concentrations.

| **Population Doubling Time (days) (Growth Rate)** | **Initial Concentration (Cells $mL^{-1}$)** | **Cyanobacterial Concentration** | | | |
|---|---|---|---|---|---|
| | | after 3 days | after 7 days | after 14 days | after 28 days |
| 6.93 (μ=0.1) - *slow* | **100** | ***130*** | ***200*** | ***400*** | ***1,600*** |
| 1.73 (μ=0.4) - *fast* | **100** | ***330*** | ***1600*** | ***27,000*** | ***7,300,000*** |
| 6.93 - *slow* | **1,000** | ***1,300*** | ***2,000*** | ***4,000*** | ***16,500*** |
| 1.73 - *fast* | **1,000** | ***3,300*** | ***16,500*** | ***270,000*** | ***73,000,000*** |

Notes

1. Cell numbers in the table are rounded. Concentrations shown illustrate the maximum potential numbers and would not necessarily be reached by natural populations. The calculation assumes exponential growth without accounting for loss due to factors such as sedimentation and grazing.
2. Shading used in the table indicates Alert Level status (See Figure 10) as follows:
   **Detection Level**
   **1. Alert Level One**
   **2.-Alert Level Two**
   **3.-Alert Level Three**
3. Growth rates applied in the table span the range for natural populations of cyanobacteria. The Relative Growth Rate is denoted as μ (sometimes also signified as R or k) and has units of $day^{-1}$

Table 12 illustrates the cyanobacterial cell concentrations that could be reached over a one to four week period when two different growth rates are considered. For a high (fast) growth rate at a moderate starting cell concentration of 1,000 cells $mL^{-1}$ Alert Level 2 status will be exceeded in approximately 5 days, and Alert Level 3 status can be reached within as little as 10 days. However at the slower growth rate and for the same starting numbers (1,000 cells $mL^{-1}$) Alert Level 2 would not be reached for around 19 days.

Correspondingly when starting from quite low numbers (100 cells $mL^{-1}$), which could be considered a background population, the Alert Level 2 is reached sometime between 1 and 2 weeks (approximately 10 days) at high growth rates and is still not reached after 4 weeks when growth rate is low.

Based upon this assessment, it is recommended that sampling for high risk/high security supplies (i.e. drinking supplies) should occur at least weekly and probably twice-weekly when Alert Level 1 status (cyanobacterial counts > 2,000 cells $mL^{-1}$) is reached. For supplies where the public health risk is deemed to be low (i.e. low cell counts in non-supply reservoirs), fortnightly sampling may be adequate, but caution is advised given the rate at which the cyanobacterial population may increase.

### 2.5.1.5.2 When should you sample?

The timing of sampling for buoyant cyanobacteria can be important during calm, stratified periods especially if depth integrated samples are not collected. Buoyant cyanobacteria tend to accumulate near or at the water surface overnight, which can result in an over-estimation of cell concentration in

surface samples collected early in the morning or an under-estimate in those collected at depth at the same time. Temporary surface scums may be observed early in the morning, but they tend to disperse as winds increase and may even be mixed back into the water column during the day. Thus, a sample that is less biased by scum formation is, on average, more likely to be obtained later in the day. If the option exists, it is preferable to delay sampling to later in the day, but whatever time is chosen it is best to adhere to the same sampling times for each location on each sampling occasion if possible.

### 2.5.1.6 Visual inspection

Visual inspection for water discolouration or surface scums of cyanobacteria is an important part of any monitoring program in all water bodies, irrespective of their use. This can be a secondary form of surveillance for higher classes of monitoring, or if few other resources are available, the principal form of surveillance used for remote or non-specialised field personnel. Be warned however, that the visual inspection method may not detect high numbers of all cyanobacteria, for example *Cylindrospermopsis,* which does not form a scum. Cyanobacteria such as *Cylindrospermopsis* may be present in dangerously high concentrations and the only indication of a bloom may be a slight green discolouration of the water. In situations where non-bloom forming cyanobacteria are present it is essential that samples are collected for analysis to determine the abundance of cyanobacteria in the water body.

In cases where bloom-forming cyanobacteria are present, a qualitative assessment of cyanobacterial presence can be a useful indicator of water quality and the relative hazard of a water body. The frequency of visual inspections may vary depending on seasonal and weather conditions. Daily monitoring of recreational areas in summer may provide a suitable alternative to the closing of bathing areas in response to cell counts from samples collected several days before. If visual inspection is the only monitoring being carried out, the position and extent of scum formation should be recorded on a special report sheet.

The first ‘on site’ indication of cyanobacteria may be the presence of small green particles in the water that may be more obvious by holding a jar of contaminated water up to the light. Scum formation will not normally be observed until open water concentrations of bloom forming cyanobacteria exceed 5,000-10,000 cells $mL^{-1}$, but exceptions are possible. They are usually most apparent early in the morning following calm days or nights, but as cell concentrations increase, or during prolonged periods of calm weather, scums may persist at the surface for days or weeks. Scum accumulations will normally be observed at the downwind end of a reservoir, lake or river reach and also in sheltered back waters, embayments and river bends.

In general, a healthy cyanobacterial scum will appear like bright green or olive green paint on the surface of the water. Scums only look blue in colour when some or all of the cells are dying. As the cells die, they release their contents, including all their pigments, into the surrounding water. Cyanobacteria have three main pigment types: chlorophyll, phycobiliproteins, and carotenoids. In healthy cells, the green chlorophyll colour normally masks the other pigments, although these other pigments may give blooms a more yellow-green or olive-green colour in some cases. When the cells die, the chlorophyll is rapidly bleached by sunlight, while the blue phycobiliprotein pigment (called phycocyanin) persists.

Cyanobacterial scums should not be confused with scums or mats of filamentous green algae, which appear like hair or spider web material when a gloved hand is passed through the water. There are blooms of other phytoplankton that look very similar to cyanobacterial scums, but these cannot be readily distinguished without a microscope. Scums or mats of filamentous green algae are more common in slow flowing, shallow streams and irrigation channels and drains.

Another tell-tale sign of cyanobacterial blooms is their odour. Some cyanobacteria produce a distinctive earthy/musty odour that can often be smelt at some distance before the bloom/scum can be seen. Therefore it is useful to conduct ‘odour surveillance’ in conjunction with any visual inspection program.

### 2.5.1.7 Transport and storage of samples

#### 2.5.1.7.1 Samples for cyanobacterial identification and enumeration

Samples should be preserved as soon as possible after collection by the addition of 1% acid Lugol's iodine preservative. There are manuals that give the recipe and instructions for the preparation of this iodine solution [131]. It is sometimes useful to retain a portion of sample in a live (unpreserved) state as cyanobacteria are often easier to identify in this way. This may be the case when a new water body is being sampled or a new problem occurs in an existing site. To ensure reasonably rapid turn-around time for reporting results of monitoring, samples should be received at the analytical laboratory used for cyanobacterial counting within 24 hours of collection. If received on the same day as collection, the receiving laboratory may assume responsibility for preservation of samples. In remote rural areas, it is sometimes advantageous to avoid sampling on Thursdays and Fridays so that samples do not remain in a courier or mail sorting depot over the weekend.

The preserved cyanobacterial samples are reasonably stable as long as they are stored in the dark. If samples are unlikely to be examined microscopically for some time, they should be stored in amber glass bottles with an airtight seal or PET plastic (soft drink) bottles. Polyethylene (fruit juice) bottles tend to absorb iodine very quickly into the plastic and should not be used for long term storage. Live samples will begin to degrade quickly especially if there are high concentrations of cyanobacteria present. These samples should be refrigerated and examined as soon as possible after collection.

#### 2.5.1.7.2 Samples for toxin analysis

Careful handling of samples is extremely important to ensure an accurate determination of toxin concentration. Microcystin and cylindrospermopsin toxins are readily degraded both photochemically (i.e. in light) and microbially. Samples should be transported in dark cold conditions and kept refrigerated and in the dark prior to analysis. Samples should be analysed as soon as possible or preserved in an appropriate manner [131].

Another important aspect of the analysis of cyanotoxins is the percentage of the toxin that is found within the cell. Cyanotoxins can be in the dissolved state, after release from the cyanobacteria, or within the cell, or intracellular. The percentage of the toxin in each state will depend on the species, the state of health, and the period in the growth cycle of the cyanobacteria. For example, a healthy *Microcystis aeruginosa* cell during the exponential growth phase will probably contain around 98-100% of the toxin in the intracellular form while during bloom collapse most of the toxin might be released into the dissolved state. In contrast cylindrospermopsin can be up to 100% extracellular even in a healthy cell. This has important implications for risk mitigation through water treatment processes (Chapter 3) and should be an integral part of the monitoring program if high concentrations of toxic cyanobacteria are likely to enter the treatment plant.

## 2.6 Control Techniques

## 2.6.1 What can you do to minimise the risks of having a bloom?

There are several ways to control or minimise the growth and impact of cyanobacteria in reservoirs. They are summarised simply into physical and chemical control techniques and are described in detail below. The options within each category include:

- Physical controls
  - Nutrient management
  - Artificial destratification
  - Offtake selection
- Chemical controls
  - Algicides

## 2.6.2 Nutrient management – What can be done to control nutrient inputs?

Cyanobacterial growth can be limited by reducing nutrient concentrations in the reservoir. Phosphorus should be the main target as it is usually the critical nutrient promoting growth and bloom formation. Nutrients can be limiting to cyanobacteria in two ways; they can limit the rate of growth and they can limit the maximum biomass or size of a bloom. There is a common perception that cyanobacterial blooms are the direct result of eutrophication, however, the reality is that cyanobacteria can exceed problem levels at concentrations barely above the detection limit. Concentrations of phosphorus of less than 10 $\mu g\ L^{-1}$ as filterable reactive phosphorus (FRP) are considered to be growth limiting [115] and 100 $\mu g\ L^{-1}$ soluble inorganic nitrogen is considered the minimum concentration to maintain growth during the growing season [132]. Higher concentrations support rapid growth and higher biomass.

The major nutrient sources are usually from the catchment, although there can be an internal load derived from sediment and a contribution from atmospheric deposition. Managing catchments to reduce the external load is a highly desirable long-term goal but it is complex and costly and often not sufficient by itself to eliminate cyanobacterial blooms. Strategies to reduce nutrient export and retain phosphorus in the catchment include removal of point source contributions such as sewage effluent and intensive agriculture, control of diffuse sources by stormwater management, regulating animal stocking practices and protecting streams with buffer strips. Other techniques include soil treatment with amendments such as gypsum to bind phosphorus and optimising fertiliser application, i.e. making sure not to over-apply fertiliser. These all have a role and will reduce the sustainable algal biomass in lakes and reservoirs but the results and benefits are likely to be long-term. In some instances catchments are naturally high in phosphorus and consequently attempts to reduce phosphorus to limiting levels would be difficult. In these cases alternative strategies to control algae should be sought.

The nutrient source that reservoir managers have the ability to control is the internal nutrient load. The internal load is the release of phosphorus and nitrogen from the sediments in reservoirs that become stratified, particularly if the bottom waters adjacent to the sediments become anoxic as a result of reduced mixing and intense biological activity.

There are many options for nutrient control within lakes and a very comprehensive discussion including a cost-benefit analysis is given by [133]. Invariably most of the techniques have the aim of reducing the internal nutrient load supplied to the lake from anoxic sediments. Techniques include sediment removal (dredging), destratification and mixing, hypolimnetic aeration and sediment treatment for phosphorus inactivation. A detailed discussion of all of these techniques is beyond the scope of this guide however some brief comments can be made on each option.

Sediment removal is an expensive option and is often regarded as a last resort for lake restoration. It is usually used in shallow productive lakes and can have major disruptive consequences for water quality while it is undertaken, and should only be done in conjunction with significant complementary effort to reduce the external load. Destratification and mixing can be very effective in promoting circulation and oxygen diffusion to deep water to reduce sediment phosphorus release and techniques for destratification are discussed below.

Hypolimnetic aeration is a sophisticated technique that involves injecting either air or pure oxygen into the deep and usually isolated and anoxic hypolimnion layer. The aim is to increase the dissolved oxygen concentration adjacent to the sediments to reduce the release of phosphorus, ammonia, iron and manganese from these anoxic sediments. When properly designed, hypolimnetic aeration and oxygenation systems can replenish dissolved oxygen in water bodies while preserving stratification [134]. The intention is generally not to completely mix the reservoir but preserve stratification while adding oxygen to deal with the deficit at depth. The technique has been used in a few cases in Australia for dealing with anoxia in shallow rivers with the goal of achieving nutrient reduction and another case of achieving Fe and Mn reduction in a small reservoir (B Sherman, pers. comm.). None of these applications approach the scale of large oxygen delivery systems for reservoirs used in the US and Europe [134]. Factors that would need to be considered critically before application of hypolimnetic aeration would include the suitability for the particular reservoir, selection of appropriate engineering design and the likely cost - both capital and recurrent expenditure relative to other conventional aeration systems [133].

Sediment and water treatment for phosphorus inactivation by chemical means has traditionally been done with alum (aluminium sulphate) which has been used to both strip phosphorus from the water column and bind it into the sediment [133]. Alum is not a favoured lake treatment in Australia as it involves addition of a heavy metal which may potentially be toxic to biota depending upon pH and alkalinity of the receiving water, and the effects of treatment can be relatively short term. Recently, products have been specifically developed for phosphorus flocculation and for sediment capping as a means of algal control in lakes. The best known product is a lanthanum modified bentonite clay which was specifically designed to bind phosphorus and maintain it in a complex under most conditions encountered in aquatic systems [135]. Other products include lime ($CaCO_3$ & $Ca(OH)_2$) and minerals based upon zeolite. Limited published results seem to indicate that the modified clay is effective under a range of environmental conditions for phosphorus removal from water and binding into sediment even under reducing conditions. Issues to consider are dose rates and longevity of treatment depending upon local water chemistry conditions.

It is important to consider the merits of different management practices for controlling nutrient supply and this will depend upon the relative contributions of external and internal sources to the nutrient budget of the particular reservoir. Comprehensive studies to accurately determine the relative sources of nutrients in each reservoir are therefore important to allow informed cost-benefit decisions regarding the direction of management effort.

## 2.6.3 Artificial destratification

Perhaps the most environmentally sound method to control cyanobacterial growth in reservoirs is to manipulate the environment to favour other phytoplankton over cyanobacteria. The reduced mixing and turbulence in reservoirs is the central factor in promoting cyanobacterial growth in reservoirs, and it is for this reason that artificial mixing and destratification has received the most attention as a potential management technique to reduce their growth in Australia. Artificial mixing not only discourages cyanobacteria but can also address the release of iron, manganese and nutrients from the sediments, which occurs when reservoirs become stratified.

### 2.6.3.1 How does artificial destratification work?

A major problem in reservoirs experiencing periods of warm stable conditions is that the water becomes stratified. These conditions provide cyanobacteria with optimum conditions for growth and the potential for cell numbers to increase. During stratification the deeper layer or hypolimnion is effectively separated from the atmosphere and becomes depleted of oxygen. Under these conditions the sediments become chemically reduced and contaminants such as ammonia, phosphorus, iron and manganese are re-solubilised from the sediment.

There are basically two types of artificial destratification systems available; bubble plume aerators and mechanical mixers. Both systems generate turbulence which weakens stratification and allows the influence of the prevailing wind (wind-forcing) to then more readily mix the reservoir.

Bubble plume aerators operate by pumping air through a diffuser hose near the bottom of the reservoir. As the small bubbles rise to the surface they entrain water and this rising plume develops unique temperature and density characteristics. The plume will rise to the surface and then plunge back to the level of equivalent density in the reservoir and an intrusion will then propagate horizontally away from the aerator plume at that depth. As the intrusion moves through the reservoir there is return flow above and below the intrusion and these circulation cells facilitate exchange between the surface layer and the deeper water or hypolimnion (Figure 14).

The role of bubble plume aerators is to weaken stratification and work synergistically with wind to mix the reservoir and to oxygenate the hypolimnion. To control contaminant resolubilisation the hypolimnion must receive sufficient oxygen to satisfy the sediment oxygen demand.

Mechanical mixers, in the form of impellers directed vertically, have been used as an alternative method of destratifying reservoirs. Mechanical surface mixers operate by means of a large impeller mounted on a raft in the reservoir (Figure 14). Rotation of the impeller draws water from the surface layer and transports it through a large column (draft tube) to the desired depth. Water movement due

to the impeller increases kinetic energy within the system and creates thermal instability. Mixers have been used in the same way as aerators to promote circulation and gas exchange from the surface to depth. An additional motivation behind using this type of destratification technique is to decrease the residence time of water in the illuminated surface layer. The aim is to circulate the cyanobacteria, from the surface layer, through the water column into deeper and darker water thereby inducing light limitation.

Surface-mounted mechanical mixers do not work effectively when the reservoir has significant surface heating as the lower density of the jet, relative to the colder hypolimnion, means that the water floats rapidly back to the surface without generating a lot of mixing and forming an intrusion. Both types of destratifiers have been shown to mix the surface layers very well close to the mixing device but not as effectively outside the immediate influence of the plume. As a consequence there are still often stable zones or habitats in sheltered parts of the reservoir for buoyant cyanobacteria to exploit. One mixing approach to consider is to use aerators to generate the large basin-wide circulation cells and use mixers to target the surface stratification outside the direct influence of the aerator plume.

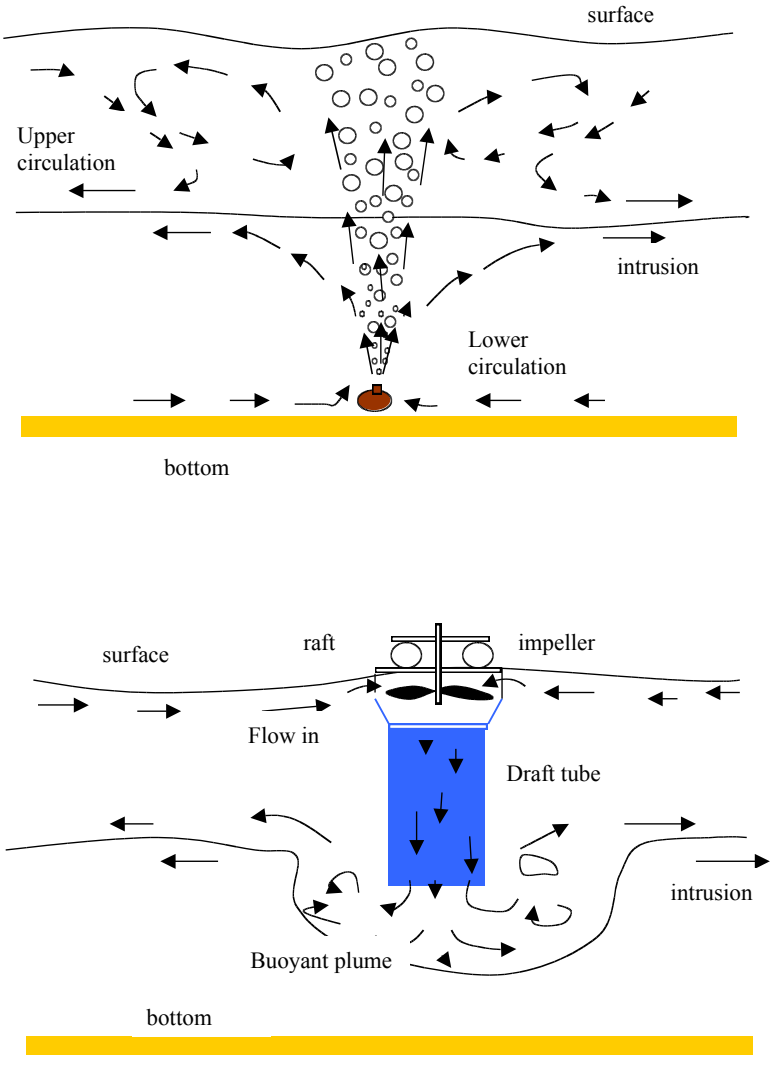



Figure 14: Flow and circulation fields created by a bubble plume aerator and a surface-mounted mechanical mixer in reservoirs.

### 2.6.3.2 Designing and operating destratification systems

The design or sizing of a bubble plume aerator should be done before installation to ensure it has the capacity to adequately mix the reservoir. The aerator configuration will depend upon the reservoir size, depth and the maximum temperature stratification. Detailed information on the design of an aeration system, including conceptual design, hydrodynamic modelling of the conceptual design and pneumatic and practical design, is given in CRC Research Report 67: A Practical Guide to Reservoir Management [136].

Destratification is normally employed during late spring, summer and autumn depending upon the latitude and altitude which affect the amount of surface water heating. Historical records of temperature would give a guide to when destratifiers should be used. Regular temperature profiles will provide information on how well mixed the reservoir is. Ideally temperature profile information should be collected using a thermistor chain, and systems are available commercially to operate these so that the information can be logged and accessed by telemetry. This level of information provides a great degree of flexibility for the operation of a destratification system.

### 2.6.3.3 Does destratification work for algal control?

Artificial destratification has achieved good results in reducing iron and manganese problems for water treatment plants [137,138,139], however the results in relation to the control of nuisance algae and cyanobacteria have been more variable [140]. This is most likely due to the complex interaction of the effects of destratification upon the availability of nutrients and light which are both required for the growth of photosynthetic organisms such as algae and cyanobacteria.

Destratification systems operating in deep reservoirs (mean depth >15m) have generally been more successful in changing the composition of the phytoplankton community (e.g.141, 142), while studies in shallower water bodies show less impact [143, 144]. Even in deep reservoirs destratifiers may not be able to prevent the development of a stratified surface layer, outside of the immediate influence of the plume or mixer, which means that there is still a habitat for buoyant cyanobacteria to exploit [142].

It is likely that in situations where artificial destratification has failed to reduce cyanobacterial growth, neither nutrients nor light were limited sufficiently to impact on growth. Either there was a large enough external load to continue to supply adequate nutrients, and therefore limiting the internal load was inconsequential, or the artificial mixing was not adequate to light-limit the cyanobacteria.

The theoretical requirements for mixing and destratification to reduce cyanobacterial growth and biomass can be explained as follows. The reduction of cyanobacterial biomass is dependent upon the relationship between the depth to which the water column is mixed ($Z_{mix}$) and the depth of the penetration of light or photosynthetically active radiation (PAR, 400-700nm) into the water column. Light penetration is often described as the euphotic depth ($Z_{eu}$) which is the depth to which 1% of the subsurface irradiance penetrates [145]. The ratio between these depths can be used to evaluate the potential for light availability to limit the growth of phytoplankton which are circulating within the surface mixed layer. For example $Z_{mix}$:$Z_{eu}$ ratios of 2.5 [143] or 3 [145] are regarded as ratios that will not support cyanobacterial growth. This means that the surface layer must mix to much deeper than light penetrates. Therefore both the mixing and the clarity of the water column determine the $Z_{mix}$:$Z_{eu}$ ratio. It follows that if a water body is inherently turbid or coloured it is theoretically more suitable to use mixing as a control technique than in clear water, because the euphotic depth is shallower in turbid water.

A detailed description and comparison of the use of aerators and mechanical mixers to control cyanobacteria is provided in the CRC Report 59 [146].

### 2.6.3.4 Can cyanobacterial growth in rivers be controlled?

Low flow conditions in rivers can lead to thermal stratification, which favours the growth of cyanobacteria. There is a well known correlation between low flow and blooms of a range of *Anabaena* species, particularly *Anabaena circinalis,* in the Darling and Murray Rivers during drought conditions in South-Eastern Australia. This suggests that the manipulation of flow could be used to control cyanobacteria by disrupting the stratification that allows them to flourish. The degree of stratification in a flowing water column is determined by the relative supply rates of stratifying thermal

energy from heating by the sun and of destratifying turbulent kinetic energy (TKE) that comes from flow and wind. In regulated rivers the magnitude and timing of discharge can theoretically be manipulated to disrupt stratification every few days thereby controlling cyanobacterial growth. Practically however, the availability of sufficient water upstream and the capacity to deliver it may be another matter.

The determination of the flows required has been determined by a CSIRO study [147], that developed a mixing criterion for turbid rivers which allows you to calculate the flow rate required to disrupt stratification and control cyanobacteria. The proviso for this management strategy is that, in addition to the availability of water, managers must consider the implications of this flow regime on aquatic macrophytes and aquatic organisms that are adapted to particular water regimes.

## 2.6.4 Algicides

### 2.6.4.1 How do algicides work and how do you use them?

Algicides have long had a role in management strategies to control toxic cyanobacteria. When used correctly they have the attraction of terminating the problem at the source in the reservoir, and this is a "once-off" treatment if it is successful. The algicide of choice has traditionally been copper sulphate. Copper sulphate has been used widely to control algal blooms in water supply storages and lakes for nearly 100 years. Increasingly, copper is regarded less favourably as a preferred option due to awareness of its adverse environmental impacts on the aquatic ecosystem [148].

There are several important issues to be aware of when treating cyanobacteria with algicides. Firstly, they should be applied at the early stages of bloom development when cell numbers are low. This will reduce the potential for the release of high concentrations of intracellular toxins and odour metabolites associated with dense blooms. These dissolved metabolites will then disperse and be diluted throughout the water body. Secondly, the rupture of cells by algicides compromises the effectiveness of metabolite removal by conventional filtration. Metabolites contained in intact cells are relatively well removed by coagulation and flocculation in the treatment process. Release of metabolites into the dissolved state increases dependence upon additional treatments such as adsorption by activated carbon or advanced oxidation to achieve effective removal. Thirdly, it is good practice to have a withholding period after algicide treatment to allow metabolites, particularly toxins, in the reservoir to dilute, disperse and degrade (see Section 2.6.4.3 below on withholding periods).

The compounds that have been used as algicides are listed in Table 13 along with key references which describe their properties and effectiveness. They include the popular copper-based compounds and a range of other agents that have been used rarely and for which there is scant reliable scientific data on their effectiveness. Many of these formulations are also not available or registered for use in Australia.

Table 13: Algicides, their formulations and key references (after [149])

| Compound | Formulation | References |
|---|---|---|
| Copper sulphate | $CuSO_4.5H_2O$ | 149, 150,151,152 |
| *Copper Chelates* | | |
| Copper II alkanolamine | Cu alkanolamine.$3H_2O^{++}$ | 153 |
| Copper-ethylenediamine complex | $[Cu(H_2NCH_2CH_2NH_2)_2(H_2O)_2]^{++}SO_4$ | 154 |
| Copper-triethanolamine complex | Cu $N(CH_2CH_2OH)_3.H_2O$ | 154 |
| Copper citrate | $Cu_3[(COOCH_2)_2C(OH)COO]_2$ | 150,153, 154,155 |
| Hydrogen peroxide | Sodium Carbonate Peroxyhydrate | |
| Potassium permanganate | $KMnO_4$ | 151, 156 |
| Chlorine | $Cl_2$ | 151 |
| Lime | $Ca(OH)_2$ | 157 |
| Barley straw | Unknown | 158, 159 |

The effectiveness and use of some of the more commonly used algicides is discussed below.

#### 2.6.4.1.1 Copper sulphate

As indicated previously copper sulphate has been regarded as the algicide of choice because it is economical, effective, relatively safe and easy to apply, has no significant human health implications, and has been considered not to cause extensive environmental damage [150, 160]. The last point has been a controversial issue for some time [161] because copper tends to accumulate in lake sediments [162, 163]. In some cases it appears not to be remobilised and is bound permanently to the bottom sediments [161,163]. In contrast a study of 10 small drinking water reservoirs in Canada found that copper in the sediments, previously accumulated from copper sulphate treatments, was released back into the open water under low dissolved oxygen conditions in the hypolimnion in summer [164].

The effectiveness of copper-algicide treatment is determined by a combination of chemical, physical and biological factors. Chemical factors relate to the characteristics of the receiving water. The toxicity is a function of water chemistry, particularly pH, water hardness and organic content (DOC), all of which influence the complexation of the metal which 'de-toxifies' the added copper. Physical factors, particularly thermal stratification in the reservoir, affect the distribution of copper after application, which in turn may determine contact with the target organism. The important biological factor is the sensitivity of the target organism to copper. Cyanobacteria are generally regarded as being relatively sensitive to copper toxicity. The relative toxicity of copper sulphate to a range of common nuisance cyanobacteria is given in Table 14.

Table 14: Relative toxicity of copper sulphate to different cyanobacteria. Modified after Palmer [152].

| Very Susceptible | Susceptible | Resistant |
|---|---|---|
| *Anabaena*<br>*Microcystis*<br>*Aphanizomenon*<br>*Gomphosphaeria*<br>*Rivularia* | *Cylindrospermum*<br>*Planktothrix (Oscillatoria)*<br>*Plectonema* | *Nostoc*<br>*Phormidium* |

The important point to remember about copper is that it is a broad-spectrum aquatic biocide, which can be toxic to non-target species such as zooplankton and fish, and this can have significant adverse environmental effects. The environmental fate and persistence of copper is dependent upon how it is distributed in the environment. Copper in aquatic ecosystems can be present in soluble form or it may be associated with particles in mechanisms such as sorption, chelation, co-precipitation and biological accumulation by plankton. Removal from an aqueous phase occurs primarily through the adsorption of copper to sediments, where it will remain indefinitely if it is not physically removed [149].

In Australia the use of algicides to control algal blooms is generally not recommended by government environment agencies and will usually only be permitted in emergency cases [165]. This is because the policy position is that the use of algicides is not regarded as an effective long-term solution to algal problems. Water authorities can use copper under conditions related to their permits or licences from the local EPA, but most would prefer to move away from copper algicide use and find a more environmentally sound long-term management strategy for nuisance algal and cyanobacterial blooms.

#### 2.6.4.1.2 Chelated copper algicides

The problem of the reduced effectiveness of copper sulfate treatment in hard alkaline water has long been recognised [166,167]. Chelated copper algicides were developed and have been widely used over the last 40 years to overcome the problems of the complexation and precipitation loss of toxic copper under these circumstances. These are a variety of liquid formulations of individual or combined mixtures of complexes of copper which is chelated to mono- di- and tri-ethanolamine, citrate, gluconate, and ethylene diamine (Table 13). These liquid chelates and complexes are claimed to "increase contact time of the soluble copper, reduce toxicity to non-target organisms, improve treatment accuracy and efficiency, and increase efficacy." These compounds have not been widely used in drinking water reservoirs in Australia; however they are a registered product that can have application under certain water chemistry conditions.

#### 2.6.4.1.3 Hydrogen peroxide (stabilised)

Recently a range of stabilised hydrogen peroxide compounds have been developed in the US specifically to provide an alternative to overcome the environmental issues associated with copper algicides (Table 13). Several manufacturers have now had these formulations added to the list of USEPA registered pesticides as algicides for use in drinking water reservoirs. The formulations have solid granules of sodium carbonate peroxyhydrate which are directly applied to a water body releasing sodium carbonate and hydrogen peroxide. The hydrogen peroxide then degrades further into hydroxyl free radicals which are claimed to cause oxidative damage to cell membranes and to intercellular physiological processes. The compounds are not registered for use in Australia, however they are undergoing evaluation for effectiveness compared to copper compounds in a laboratory based research study supported by Water Quality Research Australia.

#### 2.6.4.1.4 Barley straw

The use of decomposing barley straw for the control of algae and cyanobacteria has been the subject of considerable interest and investigation over the last 20 years [168, 169, 170, 171, 172, 173, 174, 175]. The effect of rotting barley straw in reducing the growth of filamentous green algae was first demonstrated in an irrigation canal [169]. Subsequently algistatic effects were shown in laboratory cultures with the cyanobacterium *Microcystis aeruginosa* [170]. The reasons for the inhibitory effects were suggested as being due to either the production of antibiotics by the fungal flora or the release of phenolic compounds from the decomposition of straw cell walls [169].

Inhibitory effects to cyanobacteria have also been demonstrated in reservoir trials with barley straw [172, 173, 174]. The activity of barley straw is usually described as being algistatic (prevents new growth of algae) rather than algicidal (kills already existing algae).

The evidence on the efficacy of barley straw from Australia conflicts with overseas studies. Of the two published studies a lab investigation failed to find any inhibitory effects from extracts derived from rotting straw on isolates of *M. aeruginosa* [170], and a comprehensive field-based trial also found no algicidal or algistatic effects from barley straw over a 6-month period.

These contradictory findings and the unknown identity of the phytotoxic compounds in rotting barley straw would indicate that this technique is still too poorly understood to recommend for widespread use as an algal control measure, particularly in drinking water supply situations.

### 2.6.4.2 Copper sulphate dosing techniques: dose rates and application

A range of methods is available for copper sulphate dosing. The commonly used method involves applying dry granular copper sulphate alongside or behind powerboats. Copper sulphate can also be dosed by conventional aerial application similar to other agricultural chemicals. The method of application of copper sulphate may have important effects on copper dispersal and ultimately the toxicity and success of treatment. It is important to try to achieve the best possible coverage of the reservoir surface and avoid missing shallow inaccessible zones where cyanobacteria can accumulate.

When determining the dose rate it is recommended to obtain the current pH, alkalinity and dissolved organic carbon (DOC) of the water as these parameters will influence the impact of the copper sulphate. The water chemistry conditions that are challenging for successful copper sulphate treatment and will significantly reduce its toxicity are alkaline pH i.e. >7.5-8.0; high alkalinity i.e. > 40 mg $L^{-1}$ as $CaCO_3$; and moderate to high DOC i.e. > 4 mg/L. Guidelines for copper sulphate treatment are given by [176].

To accurately determine the required dose rate it is useful to do a range-finding bioassay test with the target organism in the reservoir water you want to treat. This is like a water treatment 'jar' test where cyanobacterial cells are treated with a range of concentrations of copper sulphate ($CuSO_4.5H_2O$) - for example 6-8 concentrations in the range from 0.01 to 0.5 mg Cu $L^{-1}$, and maintained at room temperature for either 24 or 48 hours. Subsamples are removed and either stained with cell activity stains and assessed by fluorescence microscopy and/or counted by conventional cell counts. This allows the calculation of the $MLD_{100}$ or "Minimum Lethal Dose to 100% of cells" at the time end point you require – either 24 or 48 hours. From this data the amount of copper required for the dosing can be calculated for the volume to be treated. In some cases for treating buoyant cyanobacteria it may only be necessary to dose a zone of the top 5m, which is approximately equivalent to the surface

mixed layer in the reservoir. The majority of cells will be located in this layer if conditions are calm and stable and especially if the reservoir is stratified. It follows that if treatment is done under these conditions there is a greater of chance of achieving the maximum contact of toxic copper with the target cyanobacterium as the copper dissolves and disperses at a high concentration throughout the surface layer. Also when stratification is present, it is recommended to dose early in the day, as buoyant cyanobacteria are more likely to be at the surface of the water column.

If treatment is done on a regular basis it is recommended to develop a procedure to track and guide the boat using GPS to move in a systematic pattern to achieve optimum coverage of the entire reservoir surface with chemical.

Once a water body has been dosed with copper sulphate it is important to monitor the water for copper residuals, to ensure that guidelines for drinking water are not likely to be exceeded. For species of cyanobacteria known to be toxic or taste and odour producers it may also be necessary to monitor for toxins, tastes and odours.

A flow diagram giving recommendations for copper sulphate dosing is given in Figure 15.

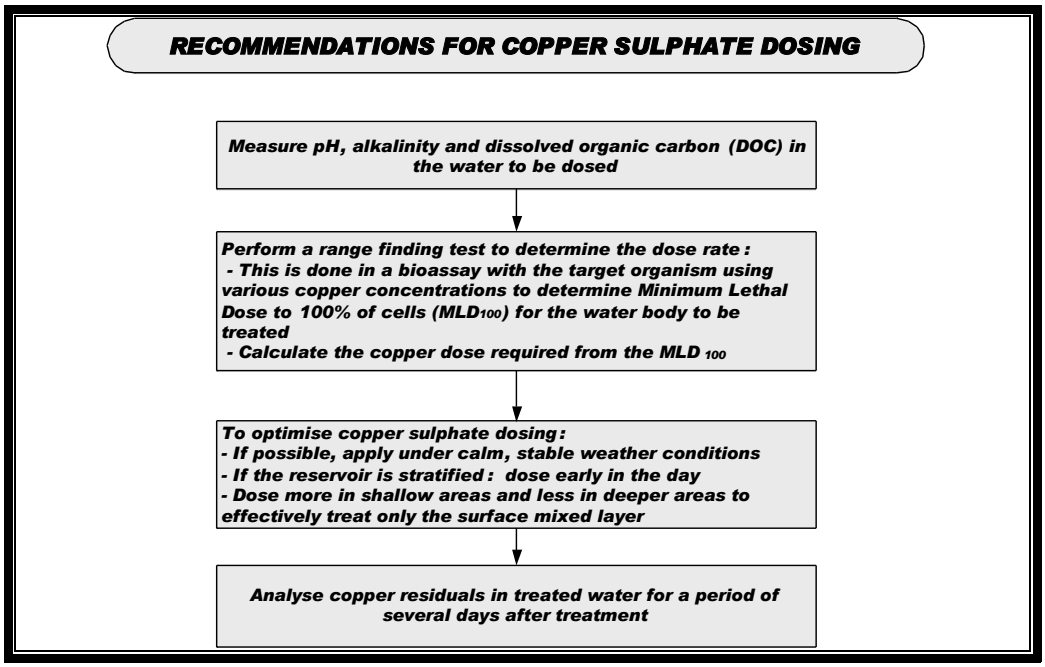



Figure 15: Flow diagram for copper sulphate dosing: determining dose rates, application guidelines and follow-up monitoring.

### 2.6.4.3 After using algicides and toxins are released - how long will they persist in the water?

A range of microorganisms have been shown to very effectively degrade several of the major cyanotoxins, including microcystins and cylindrospermopsin [177, 178]. However the time taken for total toxin degradation varies widely from 3-4 days to weeks or months depending upon the circumstances. Therefore it is recommended that monitoring be undertaken to determine the amount of toxin remaining in the water body after treatment with an algicide.

Generally, microcystins are known to degrade readily in a few days to several weeks [179, 180, 181, 178]. Cylindrospermopsin has been shown to persist in the water body for extended periods and its degradation is dependent upon the presence in the reservoir of the right microorganisms with the necessary enzymes for cylindrospermopsin degradation [178]. However, in water bodies where the cylindrospermopsin is found regularly then degradation has been shown to occur relatively rapidly [182].

Saxitoxins have not been shown to be degraded by bacteria so, if a toxic bloom of *Anabaena circinalis* is dosed, it may be necessary to have water treatment strategies for dissolved toxin removal [183]. In addition, although saxitoxin appears not to biodegrade it can undergo biotransformations involving conversion from less toxic forms to more toxic variants [184].

# 3 TREATMENT STRATEGIES

## 3.1 Cyanobacterial Cell Removal

### 3.1.1 Why is it important to remove cyanobacteria cells intact?

As mentioned previously, a healthy cyanobacteria cell can have very high levels of toxin and/or taste and odour compounds confined within its walls. Therefore, high cell numbers can result in very high total algal metabolite concentration. For example, for *Microcystis aeruginosa* more than 95% of the toxin could be contained within healthy cells, and a similar situation is often seen for *Anabaena circinalis* containing geosmin. In contrast, the number would be around 50% or less for *Cylindrospermopsis raciborskii* [185]. Often the most effective initial barrier for high total algal metabolite concentrations entering the treatment plant is to remove the cells, intact and without damage. Any damage may lead to cell leakage, and an increase in the algal metabolite concentration entering the distribution system. Dissolved algal metabolites are not removed by conventional treatment technologies, and the aim should be to minimise the levels entering the treatment plant.

### 3.1.2 Physical and chemical variations in cyanobacteria and their potential effects on removal

***Or, Why there is no easy answer to the question "How much alum will I need during a blue-green algal bloom?"***

As indicated in Section 1.1 cyanobacteria come in a wide range of shapes and sizes (Figure 2). This variation in particle size and shape will lead to differences in the response of the cyanobacteria to physical removal processes such as coagulation, settling, sand filtration, and membrane treatment. For example the cell wall composition differs between species and this can lead to variations in cell surface chemical properties, cell wall strength and surface charge between species, and even within the same species growing under different water quality conditions. Some cyanobacteria forming colonies or filaments have a sheath to hold the cells in place, providing a different external surface from the cell wall itself [186]. The robustness and composition of the cell wall will also depend on the stage of the growth phase. Stressed cells are often less robust and possibly have weaker cell walls. All of these variables will affect the physical removal methods, mentioned above, as well as chemical treatments such as charge neutralisation by coagulants/polymers, and oxidation. Most of the conflicting advice given in the literature can be attributed to these factors. The guidance given below can be applied regardless of the type of cyanobacteria present in the source water.

### 3.1.3 What is the best treatment procedure when our source water contains algal cells?

#### 3.1.3.1 Pre-oxidation

When pre-oxidation is applied in the presence of cyanobacteria the advice is always "STOP!" Chemical oxidation can have a range of effects on cyanobacteria cells, from minor damage to cell walls to cell death and lysis [187]. Although it has been reported in the literature that oxidation at the inlet of the treatment plant can improve the coagulation of algal cells through a number of mechanisms [188,189], the risk of damaging the cells and releasing algal metabolites into the dissolved state is high. If pre-oxidation must be applied in the presence of cyanobacteria cells the levels of oxidant should be sufficient to meet the demand of the water including cells, and result in a residual required for destruction of dissolved toxins (see following sections on removal of dissolved toxins). If insufficient oxidant is applied there is a risk of high levels of dissolved toxin and organic carbon entering the treatment plant and adversely influencing subsequent removal processes. Taste and odour compounds may also be released due to damage of cells by pre-oxidation. As geosmin and MIB are not readily oxidised, this can result in high levels of dissolved tastes and odours (T&O) entering the treatment plant, and subsequent higher treatment costs due to PAC dosing in many cases. Copper sulphate dosing in reservoirs can also result in damage to cells, lysis, and release of algal metabolites into the water. Table 15 shows the results of sampling for total and dissolved geosmin prior to, and four and six hours after, copper sulphate dosing of Happy Valley Reservoir.

Table 15: Variation in total and dissolved geosmin at the inlet to Happy Valley Treatment Plant after copper sulphate dosing of the reservoir.

| **Time** | **06:15** | | **12:00** | | **14:00** | |
|---|---|---|---|---|---|---|
| **Geosmin (ng $L^{-1}$)** | Total | Dissolved | Total | Dissolved | Total | Dissolved |
| **Raw** | 86 | 5 | 52 | 48 | 58 | 60 |

### 3.1.3.2 Conventional treatment

As mentioned above, the response of cyanobacteria to coagulants and other chemicals used during the coagulation/flocculation process depends strongly on the type of organism and its form (i.e. individual cells, filamentous etc). As a result, specific guidelines for coagulation are not possible. However, general tips for optimum removal of cyanobacteria will be helpful as a first treatment step.

*Optimise the coagulation process for the conditions of increased cyanobacteria numbers.* If optimisation of coagulation is maintained for the normal parameters (turbidity, UV removal etc) under the conditions of high numbers of cyanobacteria, significant removal of cells, and therefore intracellular algal metabolites, should be achieved [190]. Evidence in the literature is conflicting regarding the most effective coagulant, polyelectrolyte, etc., so optimising the existing processes should be the first response. Evidence is also conflicting in terms of damage to the cells during the coagulation process [191]. Whether there is some damage during the process appears to be dependent on the health of the cells, and the stage of growth of the bloom. In a natural bloom there will probably be cells in all stages of growth. However, an optimised coagulation process will provide a very effective first barrier to algal metabolites in the treatment plant.

Dissolved air flotation (DAF) is very effective for the removal of cyanobacterial cells, particularly for those species with gas vacuoles that may render them more difficult to settle. The same advice for the optimisation of the process applies for the DAF process.

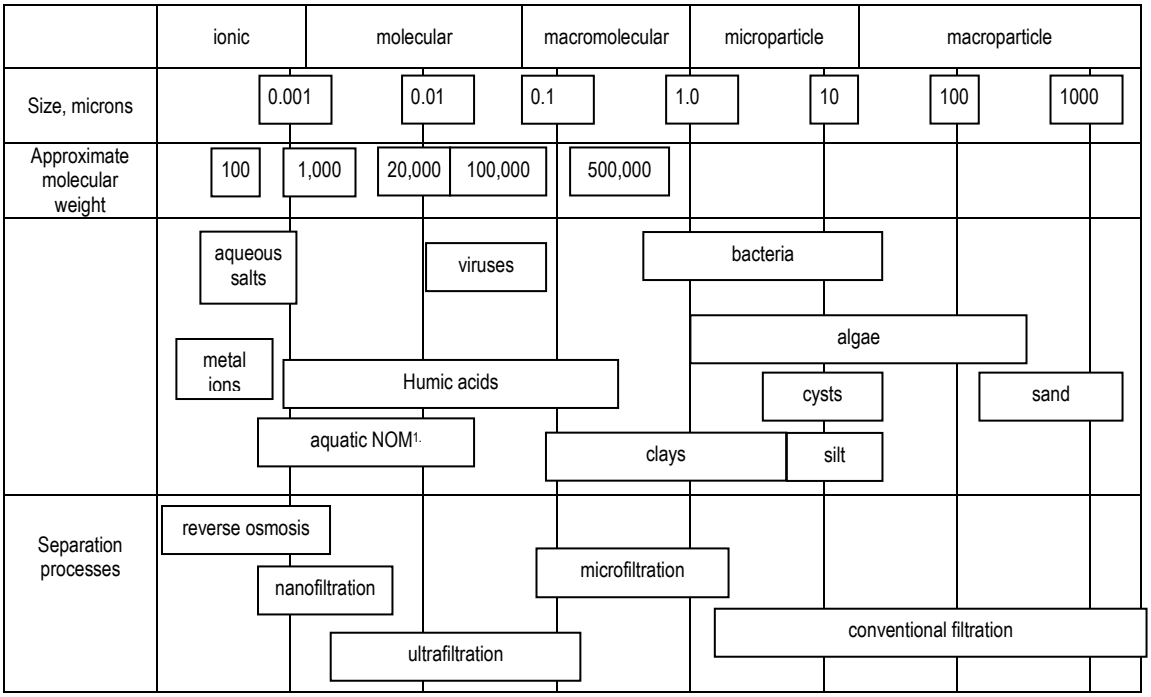
*Sludge and backwash disposal.* Once confined in sludge of any type, cyanobacteria may lose viability, die, and release dissolved algal metabolites into the surrounding water [192]. This can occur within one day of treatment for some cyanobacteria, and could potentially result in very high dissolved concentrations of algal metabolites. Similarly, algal cells carried onto sand filters, in flocs or individually, could rapidly lose viability. As a result, where cyanobacteria are potentially toxic, all sludge and sludge supernatant should be isolated from the plant until the toxins have degraded sufficiently, wherever this is possible. Microcystins are readily biodegradable [193] so this process should take 1-4 weeks. Cylindrospermopsin appears to be slower to degrade [194] and the biological degradation of saxitoxins has not yet been studied. However, the latter are known to be stable for prolonged periods in source water, so caution is recommended. Intracellular geosmin and MIB may also be released in sedimentation tanks and sludge treatment facilities. This could result in increased taste and odour levels through the plant, or in the sludge supernatant which, if it is returned to the head of the plant, could contribute significantly to the levels entering the treatment plant. The possibility of this occurring in individual treatment plants should be the focus of regular in-plant sampling (see Section 3.3, Monitoring Treatment Plant Performance).

During a bloom situation where some cells are carried through to the filters, backwash frequency will probably increase. This is desirable to reduce the risk of dissolved algal metabolites released into the filtered water. Operators should be aware of the possibility of algae in the backwash water, and consequent risk of elevated dissolved metabolite levels.

### 3.1.3.3 Membrane filtration

Experimental and full-scale studies for the removal of cyanobacteria using membranes are scarce. Generally cyanobacteria cells and/or filaments or colonies can be expected to be 1 micron in size or larger. Therefore membranes with a pore size smaller than this will remove cyanobacteria cells. Figure 16 is a representation of the removal efficiency of various filtration processes. As the figure shows, in general, micro- and ultra-filtration membranes could be expected to remove cyanobacteria cells effectively. In reality, actual pore size distributions will vary between manufacturers, and membrane materials, so specific information should be sought regarding pore sizes. Clearly the

efficiency of removal will also depend on the integrity of the membranes. Processes such as nanofiltration (NF) and reverse osmosis (RO) membrane filtration will have a pre-treatment step designed to remove particulates and dissolved organic carbon to minimise fouling of the membranes. Therefore, if these pre-treatment processes are working effectively only dissolved algal metabolites could be expected to challenge these membranes. In the case of micro- and ultra- filtration, healthy cyanobacteria cells may be concentrated at the membrane surface. The extent of damage to the cells will depend on the flux through the membranes, pressure and the time period between backwashes [195]. As with coagulation, optimisation of the processes is recommended, with frequent backwashing, and isolation of the backwash water from the plant due to the risk of the cells releasing dissolved toxin. Ultra- and micro- filtration membranes cannot be expected to remove dissolved algal metabolites released from damaged cells on the membrane surface (see following section). In practice, some removal has been noted. As this is most likely due to the adsorption of the algal metabolites onto the membrane surface, it would be expected to vary between different membrane materials, and to decrease significantly with time as the adsorption sites are occupied by the algal metabolite molecules.




1. NOM: Natural organic matter

Figure 16: Efficiency of various filtration processes.

## 3.2 Dissolved Metabolite Removal

### 3.2.1 The cells have been removed, how do we know if we have dissolved toxin?

Assume the worst until chemical analysis tells you otherwise. If there is the possibility of toxins entering the treatment plant it is always wise to send samples for chemical analysis for the toxin most likely to be present. This knowledge will come from a history of observation and monitoring as described in Section 2.5 of this guide. It is likely that the analysis will take at least 24 hours, possibly more, so it is prudent to take treatment measures to remove the maximum level of the toxin most likely to be present. This can be estimated as a first step using the cell numbers and biovolumes of the potentially toxic species, discussed in the section on the Alert Level Framework (ALF) (Section 2.4).

## 3.2.2 Types of metabolites, physical properties and effects on treatment

***Or, why there is no easy answer to the question "How do I remove toxins and tastes and odours in our drinking water treatment plant?"***

Processes to remove dissolved microcontaminants from drinking water are strongly influenced by the structure (i.e. size, shape, charge and solubility) of the target compound [196,197]. A brief description of the most important algal metabolites in Australia is given in the following sections. More information regarding metabolite production, characteristics and structures are given in Chapter1.

### 3.2.2.1 Taste and odour compounds

#### 3.2.2.1.1 Geosmin and 2-methylisoborneol

Geosmin and 2-methylisoborneol (MIB) are slightly polar, relatively low molecular weight aliphatic tertiary alcohols that are similar in structure and solubility. Their structures do not contain functional groups that are particularly susceptible to oxidation reactions. Figure 6 shows the molecular structures of the two compounds.

### 3.2.2.2 Toxins

#### 3.2.2.2.1 Microcystins

Figure 3 shows the chemical structures of common microcystins. As indicated in Section 1.2.1.1 these are cyclic heptapeptides, with the structural differences indicated in the figure. These differences determine the letters following "microcystin" in the name of the toxin. The different amino acids groups in different microcystins are responsible for differences in the overall charge of the molecule (0, -1 or -2) and their solubility. Such structural differences can be expected to influence response to different treatment processes.

#### 3.2.2.2.2 Saxitoxins

The saxitoxins (see 1.2.1.2) are a group of carbamate alkaloids ranging in molecular weight from 176 to 369 g $mol^{-1}$. They can have a net charge of +2, +1 or 0, and display a wide range of toxicities. Structures and toxicities of the saxitoxins found in Australian *Anabaena circinalis* are shown in Figure 4 and Table 2 respectively. As with the microcystins, the wide variations in structures within this group of toxins results in different responses to treatment processes.

#### 3.2.2.2.3 Cylindrospermopsin

Cylindrospermopsin is a hepatotoxic alkaloid compound of molecular weight 415 g $mol^{-1}$ (Figure 5). The cylindrospermopsin molecule is reasonably soluble, with two charged groups imparting a net zero charge to the molecule.

## 3.2.3 How do we remove dissolved toxins and odour compounds in our conventional treatment plant?

As mentioned earlier, conventional treatments such as coagulation etc, are not effective for the removal of dissolved metabolites. Three categories of water treatment processes that can be applied for the effective removal of dissolved algal metabolites are:

***Physical processes***, removal using activated carbon, membranes
***Chemical processes***, oxidation with chlorine or ozone, chloramines and potassium permanganate
***Biological processes***, filtration through sand or granular activated carbon (GAC) supporting a healthy biolfilm

These processes are discussed in detail in the following sections.

## 3.2.3.1 Physical processes

### 3.2.3.1.1 What is activated carbon?

Activated carbon is a porous carbonaceous material with a very high surface area. For example, a level teaspoon of a good quality powdered carbon has an internal surface area larger than a standard soccer pitch.

Activated carbon can be manufactured from a range of natural or synthetic organic materials providing they contain carbon and oxygen as the major components. The raw material is heated in the presence of steam or chemicals to a temperature high enough to volatilise contaminants and other components and convert the remaining carbon and oxygen into a hydrophobic, porous structure. The most common raw materials used for the production of activated carbon for water treatment are:

- wood
- coconut
- coal
- anthracite
- lignite
- peat

The adsorption sites for algal metabolites are in the internal porous structure of the activated carbon particle.

Activated carbon can be used in powdered form (powdered activated carbon, PAC), or granular form (granular activated carbon, GAC). When used in the powdered form, activated carbon is usually dosed at the head of the plant and removed during the subsequent particle separation process, coagulation, or membrane filtration. It is then disposed to waste. The granular form is used either in separate filters after the treatment process as a final polishing step, or incorporated into existing filters as a replacement for all, or some of the filtration media.

An important aspect of the application of activated carbon is the adverse effect that natural organic material (NOM) has on the adsorption of other contaminants. In practical terms this leads to increased PAC dose requirements and shorter lifetime of GAC filters. As all NOM is different, it is strongly recommended that any testing of activated carbon for the removal of algal metabolites be carried out in the water that will be treated by the carbon.

A detailed description of the production, properties and standard testing methods for activated carbon is given in Appendix 2.

### 3.2.3.1.2 How do we use it to remove algal metabolites?

*Geosmin and MIB*

Geosmin and MIB are relatively low molecular weight, small compounds which adsorb into a fairly narrow range of very small pores < 8 nanometres in width (primary micropores). Although the concentration of NOM in the same size range that might compete effectively with these compounds for adsorption sites is a relatively low proportion of the total NOM, it is still present in high concentrations compared with typical geosmin and MIB levels. Therefore NOM impacts substantially on the adsorption of these algal metabolites.

Typically, the ideal activated carbon for removal of geosmin and MIB is a good quality steam activated coal, wood or coconut based carbon with a high volume of primary micropores, and a sufficient volume of larger, transport pores to allow rapid diffusion to the adsorption site. Unfortunately it is not possible to use surrogate compounds or tests to evaluate activated carbons for the removal of these compounds. Adsorbents must be tested specifically for the removal of MIB and geosmin to determine the most effective carbon, and the appropriate doses (see following sections and appendices).

*Microcystins*

Microcystins are relatively large molecules compared with the other toxins. From molecular modelling the size can be approximated to around 1-2 nm, although it is very difficult to estimate the hydrodynamic size of a charged molecule in solution. The charged groups, carboxylic acid groups and

arginine amino acids, are hydrophilic ("water loving") groups, whereas the microcystins also have sections that are hydrophobic ("water fearing"). In addition the microcystins are in the size range of a large proportion of the NOM competing for adsorption sites on the carbon. The influences on the removal of microcystins by activated carbon are therefore quite complex.

The best activated carbon for the microcystin toxins is a good quality carbon with a high volume of pores in the size range > 1 nm. This type of carbon will also display good rates of removal. Most wood-based chemically activated carbons have the desired properties. However, these carbons can be quite expensive, and some coal-based carbons also have a reasonably high proportion of larger pores. In the case of microcystins, it is desirable to test several carbons, along with a good quality wood-based carbon, to determine the best one for a particular water quality. If it is not possible to compare carbons for the adsorption of microcystins, the tannin number test, or even the adsorption of dissolved organic carbon (DOC), would serve as a good surrogate testing procedure. Once the tests have been completed, it is advisable to do a cost analysis of the carbons to determine which is the best value for money (see Appendix 2). For example, a more expensive carbon may be the most cost effective if much lower doses are required.

*Saxitoxins*
Saxitoxins are smaller molecules than microcystins, and can be expected to adsorb in smaller pores. As a result, carbons with a large volume of pores < 1 nm are more effective for these toxins. Good quality, steam-activated wood-, coconut- or coal-based carbons are usually the best. The comparison of activated carbons specifically for the removal of saxitoxins is probably not an option for most water authorities due to the high cost of the analysis. However, as a general rule, carbons that are effective for the removal of tastes and odour compounds MIB and geosmin are also effective for saxitoxins. As the presence of saxitoxins in Australian water sources will probably be accompanied by high levels of geosmin, treatment for the odour compound will also result in at least some removal of the saxitoxins (see following section for the dose recommendations). When no other test is available, carbons with a high iodine number or surface area of around 1000 $m^2 g^{-1}$ may be suitable (see Appendix 2).

*Cylindrospermopsin*
There are very limited data available describing the removal of cylindrospermopsin by activated carbon. The molecular weight of the molecule (415 g $mol^{-1}$) indicates that it would be removed by carbons similar to those recommended for saxitoxins. However, laboratory results have shown that carbons possessing higher volumes of larger pores are the most effective, suggesting the molecule has a larger hydrodynamic diameter than indicated by its molecular weight [198]. Thus it appears that the carbons that are effective for microcystins are also effective for cylindrospermopsin.

#### 3.2.3.1.3 General recommendations for types of activated carbon

***Geosmin and MIB:*** Coal or coconut carbons with high volume of primary micropores, also some larger pores for better rates of adsorption. Some steam-activated wood-based carbons with these features have also been shown to be effective. Test for adsorption of MIB and geosmin in the water where the carbon is to be used.

***Microcystins and cylindrospermopsin:*** wood-based, chemically-activated carbons, or coal/lignite-based carbons with similar properties. Test for adsorption of specific toxin, if this is not feasible, compare tannin numbers, or adsorption of dissolved organic carbon.

***Saxitoxins:*** Steam-activated wood, coal or coconut carbons with high volume of micropores, also some larger pores for better rates of adsorption. As above, test for adsorption of saxitoxins in the water where the carbon is to be used. If this is not feasible, the activated carbon that is most effective for MIB and geosmin should also be effective for these toxins.

Where possible, compare a number of carbons with the desired properties, in the water of interest, and undertake a cost analysis. A description of a useful comparative test for PAC is given in Appendix 3.

#### 3.2.3.1.4 Do these recommendations also apply to GAC?

Broadly speaking yes they do. Both overall capacity and adsorption rates are also important for GAC filtration. As for PAC, a comparative test is recommended. Details of a useful comparative test for GAC are given in Appendix 4. It should be noted that this test will give an indication of relative

performance of new GACs under the specific evaluation conditions, it will not be indicative of long-term effectiveness of the carbons.

#### 3.2.3.1.5 Where should we apply PAC for optimum performance?

One disadvantage with PAC is that the contact time is usually too low to utilise the total adsorption capacity of the carbon. To obtain optimum value for the cost outlay, contact time should be maximised where possible. This could involve applying the PAC upstream of coagulation in a separate PAC contact basin, or in a pipeline where there is some distance between the source water off-take and the treatment plant. The PAC can also be applied after coagulation. The advantage of this placement is that a significant proportion of the competing compounds, the NOM, has been removed during the coagulation process. The disadvantage is that the contact time, where the PAC is mixed efficiently through the water, is greatly reduced. There is some evidence that a layer of PAC on top of the conventional filters may provide some additional removal. This has not been shown conclusively for the removal of algal metabolites, so could not be recommended as an effective barrier.

#### 3.2.3.1.6 How much PAC should we dose?

As mentioned above, NOM plays a large role in controlling the removal of microcontaminants using activated carbon. The NOM, which is present in all water sources, has a wide range of molecular sizes and is always present in much higher concentrations than the target compound. For example, a concentration of 5 µg $L^{-1}$ of toxin entering a treatment plant would be considered quite high, whereas a concentration of 5 mg $L^{-1}$ of DOC in surface water would be relatively common. In this situation the concentration of NOM (approximately 2 x DOC) is 2000 times that of the target compound, the toxin. Clearly it offers very high competition for adsorption sites on the activated carbon. The case with MIB and geosmin is more extreme, with the total NOM concentration several million times that of the odour compounds. The difficulty in providing guidelines for the dosing of PAC for the removal of any compound is the overriding influence of the competing NOM. Every water source will have NOM of different concentration and character, and these factors are controlled by site-specific conditions such as vegetation, soil type, climactic conditions etc. As a result, only broad guidelines can be given and, as with the choice of activated carbon, it is suggested doses are determined on a site-specific basis (see Appendix 5).

The dose recommendations given in the following sections are reliant on operator knowledge of the incoming algal metabolite concentration. In practice metabolite analysis undertaken in a qualified laboratory may have a turnaround time of several days. An effective monitoring program as recommended in Section 2.5 should allow water quality managers to estimate the maximum toxin or T&O concentration that could be expected to enter the plant. In this case it would be wise to dose assuming the highest probable concentration, then adjust the PAC downwards when actual concentrations are known. For MIB and geosmin it is less straightforward, as the range of possible concentrations entering a plant is much larger. However, a systematic monitoring program, such as those described in earlier sections, will allow operators to estimate inlet concentrations until actual values are known. Estimates of algal metabolite concentration can often be made using the algae type and numbers given in Section 2.3 of this manual.

***Determination of required PAC doses***

*Homogenous surface diffusion model*

The homogenous surface diffusion model (HSDM) is a theoretical model which can be used to predict the adsorption of microcontaminants onto activated carbon for a range of initial contaminant concentrations and carbon doses [199,200]. The HSDM can also be used to predict required PAC doses for a range of initial algal metabolite concentrations. Laboratory data for equilibrium capacities and time dependent removal are required for the application of this method. The model can then be used to predict time dependent removal under different conditions of inlet concentration and contact time, and from this information a PAC dose table similar to the example below (Table 16) can be constructed. This information applies to a particular water quality and activated carbon only. At present the Australian Water Quality Centre is the only laboratory in Australia capable of undertaking such predictions for algal toxins.

Table 16: Predicted PAC doses required to obtain a concentration of 1 µg $L^{-1}$ after 60 minutes contact

| Inlet concentration (µg $L^{-1}$) | m-LR PAC dose (mg $L^{-1}$) | m-LA PAC dose (mg $L^{-1}$) |
|---|---|---|
| 10 | 38 | >>100 |
| 5 | 29 | 95 |
| 2 | 15 | 50 |

*PAC dose curves*

It has been confirmed by a number of research groups that the percentage removal of microcontaminants (contaminants that are present at concentrations orders of magnitude lower than the NOM concentration) by PAC is dependent on the dose and contact time, and independent of the initial concentration of the contaminant [201,202]. Therefore, using the appropriate carbon, and the water from the plant at the position where the carbon will be used, a graph of percent removal of algal metabolite vs activated carbon dose can be constructed from experimental data, and this can be converted to a figure which can be used to estimate doses required for a particular inlet concentration. An example of the process and the use of the graph is given below (Figure 17).

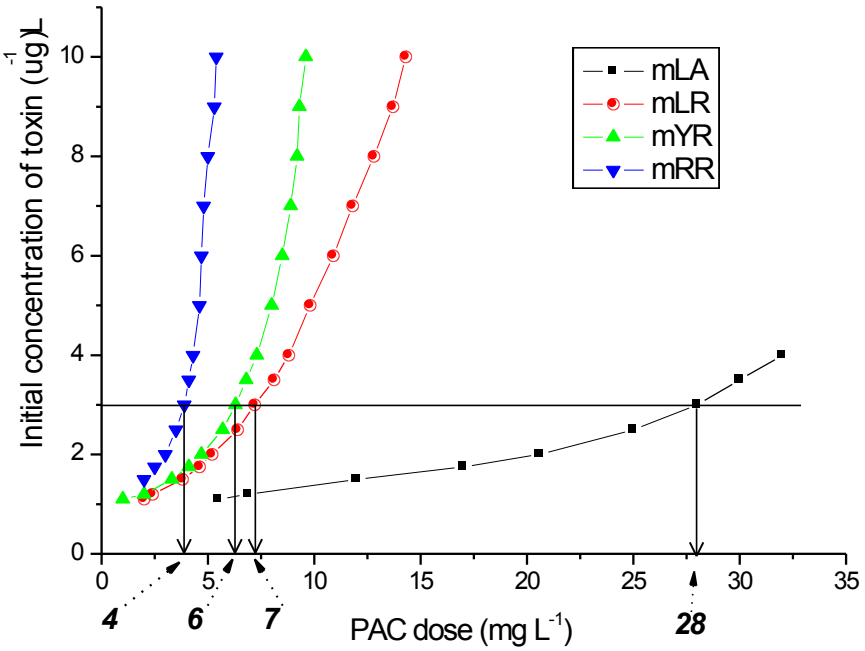



Figure 17: Hypothetical PAC dose curves for removal of microcystins to a concentration of 1 µg $L^{-1}$.

Note this graph should not be used to estimate PAC doses for toxin removal, it is for illustrative purposes only.

Figure 17 shows toxin concentration vs PAC dose required to reduce the initial concentrations of four microcystins to 1 µg $L^{-1}$. If the inlet concentration of the toxin is known to be approximately 3 µg $L^{-1}$ the required PAC dose would be 4 mg $L^{-1}$ for microcystin RR, 6 for microcystin YR, 7 for microcystin LR and around 28 for microcystin LA. The graph would apply for a particular carbon and contact time. If variations in contact time are expected due to variations in flow, separate graphs should be constructed, perhaps for a maximum flow (shortest contact time), and minimum flow (longest contact time). It should also be kept in mind that a change in water quality could change dose requirement, in particular, an increase in DOC may result in an increased PAC requirement. Details for the construction of a PAC dose requirement curve are given in Appendix 5.

### 3.2.3.1.7 General recommendations for PAC dose

It is always preferable to test activated carbons in your own water before deciding on dose requirements. The suggestions given below should be taken as a general guide to approximate dose requirements only.

*Geosmin and MIB*

Table 17 gives some typical values of PAC doses that may be required to reduce odour levels to below the values that would be detected by most consumers (around 10 ng $L^{-1}$). As geosmin is more readily removed than MIB by activated carbon, lower doses are required for the same percentage removal. These values are based on a contact time of 60 minutes of efficient mixing prior to the addition of any other chemicals. The actual removal seen in the treatment plant will depend on DOC concentration and character, point of addition of PAC, mixing regime and effective contact times.

Table 17: Typical doses required to remove MIB and geosmin to levels below 10 ng $L^{-1}$ *

| Algal Metabolite | Inlet concentration (ng $L^{-1}$) | PAC dose (mg $L^{-1}$) | Type of PAC |
|---|---|---|---|
| Geosmin | 10-30 | 4-15 | Good quality wood, coal or coconut, steam activated |
| | 30-100 | 15-35 | |
| MIB | 10-30 | 6-30 | |
| | 30-100 | 30-55 | |

*These doses were estimated from many laboratory experiments but the actual doses required will depend strongly upon water quality. Site specific testing is recommended.

*Microcystins*

As can be seen from Figure 17, the extent of removal by PAC, and therefore the required PAC doses, varies significantly for the microcystins. If microcystins are present in source water, and activated carbon is to be a major process for their removal, it is necessary to get a scan of the microcystins present. Although microcystin LR (MCLR) is the most common microcystin worldwide, it seldom occurs without other variants also present in the water. It is not uncommon in Australia to find a bloom producing a mix of 50:50 MCLR and microcystin LA (MCLA). Microcystin LA is as toxic as MCLR, but is considerably more difficult to remove using PAC. In contrast, microcystin RR (MCRR) is readily removed by PAC, but is considerably less toxic. There are many other microcystins that may be present in source water, but there is no information on the removal of these compounds by PAC.

Table 18: General recommendations for PAC doses for microcystin toxins in source water with a DOC of 5 mg $L^{-1}$ or less, and contact time 60 minutes *

| Microcystin | Inlet concentration 1-2 µg $L^{-1}$ | Inlet concentration 3-4 µg $L^{-1}$ |
|---|---|---|
| MCLA | 30-50 | Not recommended |
| MCLR | 12-15 | 15-25 |
| MCYR | 10 | 10-15 |
| MCRR | 8 | 10 |

*These doses were estimated from many laboratory experiments but the actual doses required will depend strongly on water quality. Site specific testing is recommended

The presence of a mixture of toxins does not appear to affect the doses, therefore, for a mixture of MCLR and MCLA at 1 µg $L^{-1}$ each, add the doses for each toxin individually, i.e. approximately 40 mg $L^{-1}$. It should also be noted that it is very unlikely that dissolved microcystin concentrations > 4 µg $L^{-1}$ would be encountered in practice. If this occurs on a regular basis advanced treatments such as ozone/GAC would be recommended.

*Saxitoxins*

Similar to microcystins, the different variants of the saxitoxins adsorb to different extents on PAC. Fortunately in this case, the most toxic variants are generally present in the lowest concentrations in most natural blooms of *Anabaena circinalis* and are removed more readily. In general a dose of 20 to 30 mg $L^{-1}$ and a contact time of at least 60 minutes would be recommended for an inlet concentration of 10 µg $L^{-1}$ STX equivalents, and a finished water goal concentration of <3 µg $L^{-1}$.

*Cylindrospermopsin*

From the limited information available, PAC doses recommended to achieve a target of 1 µg $L^{-1}$ for cylindrospermopsin would be 10-20 mg $L^{-1}$ for an inlet concentration 1-2 µg $L^{-1}$ and 20-30 for an inlet concentration of 3-4 µg $L^{-1}$.

Table 19 gives a summary of the general recommendations for PAC application

Table 19: General recommendations for PAC application in source water with a DOC of 5 mg $L^{-1}$ or less, and contact time 60 minutes *

| Algal Metabolite | | Inlet concentration (µg $L^{-1}$) | PAC dose (mg $L^{-1}$) | Type of PAC |
|---|---|---|---|---|
| geosmin | | 10-30 | 4-15 | Good quality wood, coal or coconut, steam activated |
| | | 30-100 | 15-35 | |
| MIB | | 10-30 | 6-30 | |
| | | 30-100 | 30-55 | |
| Microcystins | MCLR | 1-2 | 12-15 | Wood-based, chemically activated, or high mesopore coal, steam activated |
| | | 2-4 | 15-25 | |
| | MCLA | 1-2 | 30-50 | |
| | | 2-4 | NR** | |
| | MCYR | 1-2 | 10-15 | |
| | | 2-4 | 15-20 | |
| | MCRR | 1-2 | 8-10 | |
| | | 2-4 | 10-15 | |
| Cylindrospermopsin | | 1-2 | 10-20 | As above |
| | | 2-4 | 20-30 | |
| Saxitoxin | | 5-10 STX eq | 30-35 | Coal wood or coconut, steam activated |

*These doses were estimated from laboratory experiments using the most effective PAC. The actual doses required will depend strongly on water quality and effectiveness of activated carbon. Site and PAC specific testing is recommended
**NR-not recommended

#### 3.2.3.1.8 We have GAC, how long will it last for algal metabolite removal?

This is a very important question for water authorities contemplating installing GAC filters. Currently there are no facilities in Australia for the regeneration of used GAC, so when the filter no longer produces water of acceptable quality the GAC must be removed, and replaced with new. There are a number of tests designed to predict breakthrough of microcontaminants on GAC, and some of these have been reasonably successful overseas when used for microcontaminants that are present in the water constantly. However, there are two main reasons why these tests cannot be reliably applied for the prediction of algal metabolite breakthrough:

*Transient nature of the problem*
Algal metabolites are rarely present in source water constantly; the problem is of a transient nature, often appearing regularly in a particular season each year. In most cases the life of the GAC is controlled by the adsorption of the wide range of organic compounds in NOM, which is present year-round. A short-term laboratory test to determine the removal capacity for algal metabolites will not give an estimate of the length of time GAC can be expected to remove occasional episodes of algal metabolites.

*Biological degradation*
MIB, geosmin, microcystins and cylindrospermopsin are all readily biodegradable under certain conditions (see following section on biological filtration). If a GAC filter is consistently degrading the algal metabolites, the lifetime could be indefinite. Or, more likely, the GAC filter may initially allow some breakthrough of the compounds, and then the biological function of the filter could commence resulting in no algal metabolites detected in the outlet water. This is described in more detail in Section 3.2.5.

Although it is very difficult to predict the "*lifetime"* of GAC for the removal of algal metabolites, it is recommended that a filter be tested, or monitored, for removal, if this is to be a major barrier to algal metabolites entering the distribution system. This type of testing can give an estimate of the ability of the GAC ***at the time*** to remove the algal metabolites, but cannot predict ***how much longer*** it will effectively remove the compounds. Details of an appropriate test are given in Appendix 5.

Although the use of GAC for metabolite removal is very complex, some general suggestions can be given based on pilot- and laboratory-scale studies for the odour compounds and microcystins and saxitoxins. No data exists for the long term removal of cylindrospermopsin by GAC. Recommendations for microcystins could also be applied for cylindrospermopsin until more information is available.

*MIB and geosmin*
Pilot studies have indicated that, with an empty bed contact time of 15 minutes, GAC can last for longer than 12 months without breakthrough of MIB or geosmin. It has also been shown that if the water is pre-chlorinated the efficiency of the GAC for MIB and geosmin removal is greatly reduced.

*Microcystins*
Reports of length of time until breakthrough vary for microcystins, but would be expected to be between 3 and 12 months from commissioning if the filter is challenged with the toxins on an intermittent basis.

*Saxitoxins*
Saxitoxins appear to be well removed by GAC, and good removals (up to 75% removal of toxicity) have been reported after 12 months of running laboratory scale GAC columns.

#### 3.2.3.1.9 Will membranes remove algal metabolites?

Membranes are physical filtration barriers, and the main factor influencing removal of microcontaminants is the size, or hydrodynamic diameter, of the compound compared with the pore size distribution of the membrane. Other factors, such as electrostatic interactions and a build up of NOM and particles on the membrane can also alter the permeability of the membranes to particular compounds. However these factors are very difficult to predict, and cannot be taken into account for metabolite removal. Figure 16 shows the approximate ranges of pore size of common membranes, and molecular weight and size of the compounds and particles they can reject. According to this figure, microcystins should be rejected by RO membranes and nanofiltration membranes with a pore size distribution in the lower range. Saxitoxins and cylindrospermopsin could also be expected to be removed by RO. However, according to this figure, even RO membranes may allow the smaller toxin molecules or taste and odour compounds to permeate the membrane. The crucial issues are the pore size distribution of the particular membrane, which should be available from the manufacturer, and the integrity of the membrane. As mentioned earlier, membranes contain a range of pores, and larger pores could allow a percentage of the molecules to permeate.

### 3.2.3.2 Chemical processes

The most common chemicals used for the removal of microcontaminants are chlorine and ozone. Both are oxidants which react with some portion of the molecular structure of the target compound. As with activated carbon, water quality is a major influence on the effectiveness of the oxidation process, and therefore a major influence on the optimum conditions such as oxidant dose and contact time. The first oxidant to be discussed, chlorine, does not have a significant effect on the concentrations of MIB and geosmin.

#### 3.2.3.2.1 What doses are required for reduction of toxin concentration using chlorination?

Chlorine is an oxidant which will react with many organic compounds, including algal toxins and NOM. The most reactive form of chlorine is hypochlorous acid (HOCl), which is in equilibrium with the hypochlorite ion ($OCl^-$) in solution. The chemical equation is given below.

$HOCl \Leftrightarrow H^+ + OCl^-$

The concentration of hypochlorous acid is dependent on the pH of the water. An example of the relative concentrations of the two major forms of chlorine over a moderate range of pH is given in Table 20. From the table it can be seen that a small change in pH can result in a large change in the concentration of the most reactive form, therefore the reaction of chlorine with any compound will be dependent on pH.

Table 20: Ratio of HOCl to $OCl^-$ and concentrations of the species at different pH. Initial concentration 5.4 mg $L^{-1}$ as $Cl_2$

| **pH** | **6.0** | **6.5** | **7.0** | **7.5** | **8.0** | **8.5** | **9.0** |
|---|---|---|---|---|---|---|---|
| **HOCl:$OCl^-$** | 32:1 | 10:1 | 3.2:1 | 1:1 | 0.32:1 | 0.1:1 | 0.03:1 |
| **HOCl (mg $L^{-1}$)** | 3.9 | 3.6 | 2.9 | 2.0 | 1.1 | 0.4 | 0.1 |
| **$OCl^-$ (mg $L^{-1}$)** | 0.1 | 0.4 | 1.1 | 2.0 | 2.9 | 3.6 | 3.9 |

Chlorine reacts rapidly with a range of molecules, depending on their molecular structure and susceptibility to oxidation. Once applied, the concentration of chlorine decreases rapidly as a result of reaction with the complex mixture of organic molecules comprising NOM. When we use chlorine for the removal of algal toxins we should be aware that a competitive effect is produced between the different types of NOM and the toxins. Some molecules, or structures within molecules are more reactive than others and the rates of reaction between chlorine and organic compounds will depend on their structure. The result of these effects is a large variation in rate and extent of chlorine decay in different waters. An example of the effect of different concentration of DOC on chlorine consumption is shown in Figure 18. After dosing with 0.5 mg $L^{-1}$ of chlorine, a chlorine residual could still be detected in Morgan water after 30 minutes, whereas no chlorine would be detected in Myponga water after 10 minutes. As NOM is a mixture of unknown character it is very difficult to predict the reaction of chlorine with NOM and the toxins. To take into account this variation of chlorine reaction with NOM the concept of chlorine exposure, or CT (concentration x time) is introduced to help describe the reaction of the available chlorine with microcontaminants such as toxins. The CT value is the area under a plot of chlorine residual vs time, and describes the amount of free chlorine to which the solution has been exposed.

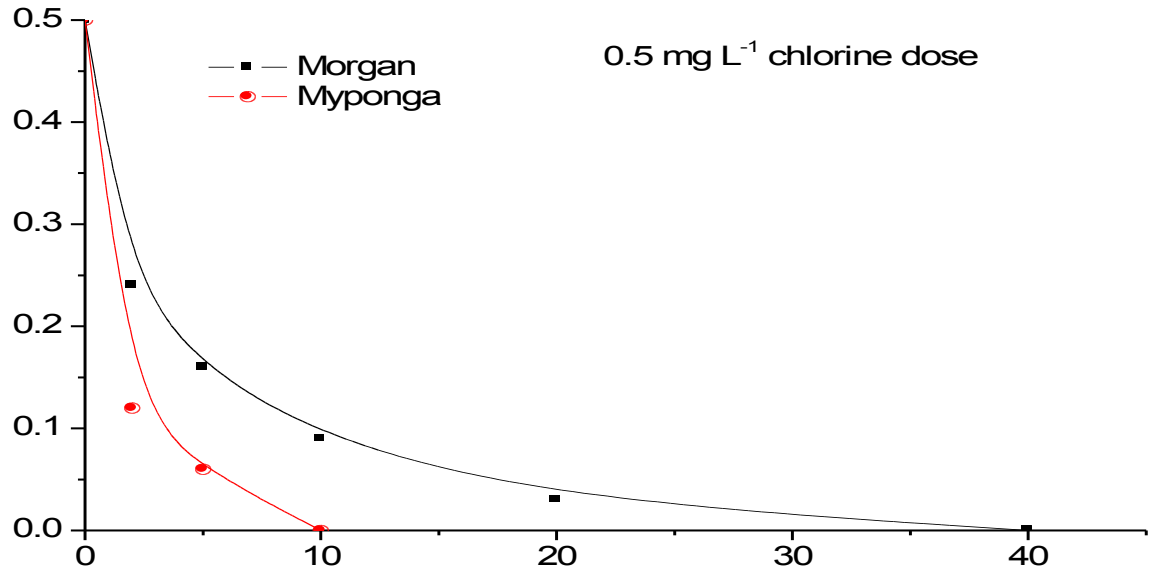



Figure 18: Chlorine residual as a function of time in two waters. Myponga water DOC = 5.0 mg $L^{-1}$, Morgan DOC = 2.9 mg $L^{-1}$

The rate of reaction of chlorine with many organic compounds is dependent on the concentrations of the reactants. Therefore, the rate of reaction will be higher for a higher concentration of chlorine. This brings into question advice often given regarding chlorination of algal toxins, i.e. that we should aim

for a set residual after a particular contact time. This may be appropriate advice for waters with a moderate chlorine demand, but for waters requiring a low dose of chlorine to maintain a residual, this may not be sufficient. In addition, as with all chemical reactions, temperature is also an important factor, with reaction rates with chlorine usually increasing with increasing temperature.

Given all of the variables discussed above, the results of chlorination under a range of conditions reported in the literature can be used as a guide for recommendations for chlorine doses [203, 204, 205].

*Microcystins*
Microcystins are reactive with chlorine. They have a conjugated double bond in their structure which is susceptible to chlorine, as well as reactive amino acid groups. As these amino acid groups vary with the type of microcystins, the toxins themselves vary in their reactivity [206]. Of the four most common microcystins, the ease of oxidation by chlorine is given by:
MCYR>MCRR>MCLR>MCLA.

As a general rule for the oxidation of all microcystins to below the guideline value the following conditions should be achieved:

pH <8
Residual >0.5 mg $L^{-1}$ after 30 minutes contact
Chlorine dose > 3 mg $L^{-1}$
CT values in the order of 20 mg min $L^{-1}$ have been shown to be effective

Laboratory work has shown little effect of temperature on the chlorination of microcystins.

*Saxitoxins*
Saxitoxins are not as reactive with chlorine as microcystins, as their structures do not contain very reactive sites. However, laboratory results have shown that the reactivity with chlorine increases with increasing pH. This suggests that the effect of pH on the saxitoxin structure renders it more reactive, even though chlorine is in its less reactive form at higher pH [207]. More recent work undertaken at the AWQC has shown moderate reactivity between chlorine and saxitoxins at neutral pH, so that chlorine is now considered an effective process in the multi-barrier approach to saxitoxin removal.

*Cylindrospermopsin*
The limited data available on the chlorination of cylindrospermopsin suggests it is susceptible to chlorination, and may even be more reactive than microcystins [208]. The conditions outlined above for the chlorination of microcystins could be expected to also be applicable for cylindrospermopsin.

#### 3.2.3.2.2 General recommendations for chlorine

*Microcystins, saxitoxins, and cylindrospermopsin:*

pH <8
Residual >0.5 mg $L^{-1}$ after 30 minutes contact
Chlorine dose > 3 mg $L^{-1}$
CT values in the order of 20 mg min $L^{-1}$

Destruction of the toxins could be expected to range between almost 100% for cylindrospermopsin and the more susceptible microcystins to approximately 70% for saxitoxins.

#### 3.2.3.2.3 What doses are required for reduction of algal metabolite concentration using ozonation?

Ozone, like chlorine, is an oxidant. It is extremely reactive and, also like chlorine, is present in water in more than one form. The ozone molecule (structure of three oxygen atoms $O_3$) reacts with organic molecules present in the water. It also breaks down spontaneously – auto-decomposes – to produce hydroxyl radicals. This is a very reactive chemical species, and it is not discriminating in the structures it attacks. The formation of hydroxyl radicals is dependent upon pH, and predominates at pH > 8. The decomposition of ozone, formation of hydroxyl radicals, and the reactions of both species with NOM can be described as a chain reaction where NOM plays a part as both an initiator and inhibitor in the creation of hydroxyl radicals [209,210].The alkalinity of the water is also important for ozonation, as the carbonate ion plays a strong role in inhibiting the formation of the hydroxyl radicals. Therefore,

while high alkalinity water may maintain an ozone residual for longer, this is at the expense of the formation of hydroxyl radicals, the most reactive species.

*MIB and geosmin*
MIB and geosmin are relatively recalcitrant to oxidation. However, studies have shown that the hydroxyl radical can oxidise these compounds rapidly [211]. The removal of MIB and geosmin by ozone is therefore very dependent on the water quality parameters such as pH, DOC concentration and character and alkalinity. The conditions that favour hydroxyl radical production will favour MIB and geosmin removal. Although it seems counter intuitive, for these compounds a stable ozone residual is not favourable and more removal will be obtained in conditions where the molecular ozone decomposes to hydroxyl radicals.

Full scale, pilot plant and laboratory results indicate that approximately 50% removal of MIB and geosmin can be expected when an ozone residual of 0.3 mg $L^{-1}$ is maintained for 10 minutes.

*Microcystins*
As mentioned above, microcystins have structures present in the molecule that are susceptible to oxidation, therefore the ozone molecule will react with them. In addition, the hydroxyl radical would be expected to react strongly with the microcystins [212]. There is a competitive effect with NOM, always at higher concentration than the toxins, as it can be expected that there will be some sites present in NOM that are as reactive as those on the microcystin molecule.

As with chlorine, the reduction in the concentration of microcystins will also depend on the initial dose, but it appears from laboratory and pilot scale work that the maintenance of a residual of 0.3 mg $L^{-1}$ for at least 5 minutes will result in the reduction of microcystins to below detection (by HPLC) in most waters. Water with DOC higher than 5 mg $L^{-1}$ may require higher doses.

*Saxitoxins*
As mentioned above, saxitoxins are not as susceptible to oxidation as the microcystins, and are not readily removed by ozonation [213,214] An increase in pH, with a consequent increase in hydroxyl radical formation may result in higher levels of removal, but this has not been proven in the laboratory or pilot plant. Conditions suggested for microcystin, above, could be expected to reduce the concentration of saxitoxins by no more than 20%, according to laboratory scale experiments.

*Cylindrospermopsin*
The limited data existing on the ozonation of cylindrospermopsin suggests that the conditions recommended for microcystin will also apply for the removal of cylindrospermopsin [215].

#### 3.2.3.2.4 General recommendations for ozone

*MIB and geosmin*

pH>7
Under optimal conditions, 50% removal of MIB and geosmin can be expected when an ozone residual of approximately 0.3 mg $L^{-1}$ is maintained for 10 minutes.

*Microcystins and cylindrospermopsin*

pH > 7
Residual >0.3 mg $L^{-1}$ for at least 5 minutes contact
CT values in the order of 1.0 mg min $L^{-1}$ have been shown to be effective

*Saxitoxins*
Ozonation not recommended as a major treatment barrier, at this stage, more research is required.

Table 21: General recommendations for toxins for ozone application

| Toxin | pH | Residual after 5 minutes (mg $L^{-1}$) | CT (mg min $L^{-1}$) |
|---|---|---|---|
| Microcystins | >7 | >0.3 | 1 |
| Cylindrospermopsin | >7 | >0.3 | 1 |
| Saxitoxins | | Not recommended | |

#### 3.2.3.2.5 Will chloramine be effective?

Unfortunately the answer to this is simple – probably not. Monochloramine is a much weaker oxidant than either chlorine or ozone, so could not be expected to have an effect on geosmin or MIB concentration and only very high doses and long contact times have been shown to have any effect on microcystin concentration [216]. No data is available for the other toxins.

#### 3.2.3.2.6 What about potassium permanganate?

Potassium permanganate is not effective for the removal of MIB or geosmin, although it has been shown to reduce the concentration of microcystins considerably [217,218] and may also be effective for the reduction of cylindrospermopsin. If potassium permanganate application is practised to control manganese it should be maintained in the presence of these toxins. Unfortunately the data currently available is not sufficient to allow recommendations for dose requirements or to allow us to consider potassium permanganate as an effective barrier.

#### 3.2.3.2.7 Other oxidants

*Chlorine dioxide:* Not effective with doses used in drinking water treatment
*Hydrogen peroxide*: Not effective on its own. In combination with ozone or UV it produces hydroxyl radicals that are very strong oxidising agents. Insufficient information exists to recommend doses
*UV radiation:* Capable of degrading microcystin-LR and cylindrospermopsin, but only at impractically high doses or in the presence of a catalyst.

#### 3.2.3.2.8 Will any toxic by-products be produced during oxidation?

Laboratory tests using mouse bioassay and other toxicity tests have shown that the oxidation of algal toxins using chlorine or ozone does not result in the production of by-products that display the same mode of toxicity as the original toxins [219]. There are no data on the by-products of oxidation of MIB and geosmin. In practice, many other organic compounds will be oxidised simultaneously, and many different types of by-products will be formed. Some of these may be potentially harmful at high concentrations. Identifying the individual by-products of metabolite oxidation separately from those produced from oxidising of NOM is a difficult exercise. However, with the current knowledge it is certain that the oxidation of water containing algal toxins results in a consequent reduction of acute toxicity related to consumption of that water. Whether the oxidation of any or all of the organic components in water poses any real long term threat to health is still under debate. In addition, in most cases the presence of toxins will be of a short term nature, and therefore less likely to cause chronic effects.

#### 3.2.3.2.9 How can we optimise ozone/GAC for algal metabolite removal?

*MIB and geosmin*
The combination of ozone and GAC has been shown to be very effective for long term removal of MIB and geosmin when the ozone contactors sustain an ozone residual of 0.3 mg $L^{-1}$ for at least 10 minutes and the GAC has an empty bed contact time of approximately 15 minutes.

*Microcystins and cylindrospermopsin*
Maintenance of an ozone residual as recommended in the previous section will result in no toxins reaching the GAC filters. Therefore the GAC filters can be maintained/replaced as required for the other purposes (DOC removal, taste and odour removal, etc.). It should be noted that, in the absence of an ozone residual the filters will function as normal GAC filters, and if the adsorption capacity for the toxins has been utilised by NOM, toxin breakthrough could result. If this is a possibility, the aim should be to maintain maximum DOC removal, as this will give some indication of the potential to remove these toxins.

*Saxitoxins*
Ozone/GAC is an excellent combined barrier for saxitoxin removal. The ozone reduces the concentration of saxitoxin entering the GAC filter, and, as mentioned above, GAC is an effective treatment for saxitoxins [220,221]. As with GAC alone, if tastes and odour compounds are effectively removed by adsorption onto the GAC, it is likely that saxitoxins would be removed. If there is doubt regarding the ability of an existing GAC filter to remove saxitoxins, it is wise to perform a laboratory test to determine the likely removals at full scale. Details of a GAC test are given in Appendix 5.

### 3.2.3.3 Biological processes

Biological filtration is an attractive treatment process for water authorities for a number of reasons. Biological processes generally:

- are of low technology, requiring little maintenance
- require relatively low infrastructure and running costs
- do not require additional treatments and are therefore processes involving the *removal* of contaminants without the *addition* of chemicals

Factors that have a major impact on the removal of organic compounds through biological filtration processes include:

- variety and numbers of microorganisms present, biomass
- temperature
- water quality (pH, dissolved organic carbon character and concentration, alkalinity)
- filter contact time, hydraulic loading
- filter medium

#### 3.2.3.3.1 Will our sand filters remove any algal metabolites?

Once again there is no simple answer to this question. MIB, geosmin, microcystin variants and cylindrospermopsin show great potential for significant biological removal, even at flow rates approaching those encountered in rapid sand filters [222,223]. Full scale data at Morgan water filtration plant in Adelaide showed clearly that biological removal of both MIB and geosmin was taking place in rapid sand filters until a chloramine residual was introduced into the backwash water [223].

Only particular strains of certain microorganisms are capable of degrading algal metabolites, and sufficient numbers must be present on the biological filters to result in biological removal. In addition, both microcystins and cylindrospermopsin display a “lag phase” between the time the toxin enters the filter, and when the biofilm begins to remove the toxins. That is, the biofilm is said to require time for “acclimation” to the compounds. Knowledge of the origin of the lag phase, and the ability to eliminate it is essential before biological removal can be confidently relied upon as an effective barrier against these toxins. If the presence of toxins in sand filters is a common occurrence, it is possible that some biological removal will take place. However, if pre-filter chlorination is practised as a means of reducing particle counts, it is very unlikely that sufficient biological activity will be maintained for toxin removal.

Slow sand filtration and bank infiltration, practiced in some European countries, are processes where very long contact times and high biological activity result in excellent removal of taste and odour compounds and microcystins [224]. There is also good preliminary evidence that these processes will be effective for cylindrospermopsin removal [223].

#### 3.2.3.3.2 Will our BAC remove algal metabolites?

Biological activated carbon (BAC) is a term often used to describe a GAC filter preceded by ozone. Algal metabolite removal by BAC is covered in the section on optimisation of ozone/GAC, or if it is functioning only as a biological filter, see previous section.

## 3.3 Monitoring of Treatment Plant Performance

Many water treatment plants have a treated water monitoring regime that does not give any indication of treatment plant performance. Inlet water and finished water metabolite data are not sufficient to judge the value of each treatment step for the removal of the compounds. Each water treatment plant

is different and variations, such as mixing regimes and point of addition for PAC for example, can have a dramatic effect on removal efficiencies. It is also possible that the PAC is working effectively at the beginning of the treatment process, but algal metabolites released from sludge in the sedimentation basins are increasing the concentration of the contaminant *after* the major barrier. Another mistake can be made if PAC doses are determined by the total inlet algal metabolite concentration. If 98% of the algal metabolites are taken out through coagulation, most of the PAC will be serving no real purpose. A knowledge of the performance of each of the major treatment steps for algal metabolite removal will allow the optimisation of treatment, and result in lower treatment costs overall. Several full scale investigative sampling efforts are recommended during each algal metabolite challenge to develop a database of information of process effectiveness as a tool for optimisation of algal metabolite removal. To obtain the full value from investigative sampling several important steps should be followed:

- Measurement of total and dissolved algal metabolite should be undertaken at the inlet to the plant, so removal of each form can be monitored
- When PAC is used samples should be taken to determine the effectiveness of the PAC for the removal of dissolved algal metabolite. The point at which these samples are taken will vary, depending on the point of addition. It should be assumed that the PAC is removing algal metabolites only while in suspension, and samples should be taken prior to the sedimentation step.
- Samples to determine the removal of cell-bound algal metabolites during the flocculation stage should be taken at the very end of the flocculation bay at the ***appropriate time lapse*** after a floc bay inlet sample. Total and dissolved algal metabolite should be measured. This allows sampling of approximately the same plug of water through the plant and will indicate the removals through PAC vs coagulation/flocculation. It is very important to estimate the approximate time lapse through the plant as there can be significant changes in the inlet water concentrations, and we are looking for relatively small changes in concentration through the plant.
- Similarly, to determine if any dissolved algal metabolite is released by the sludge during sedimentation, the settled water outlet should be sampled after the appropriate time lapse. Occasional sampling for cyanobacteria cells in the raw water and settled water would indicate the removal efficiency of live cells through the plant.
- If the product water algal metabolite level is to be directly compared with the raw water level, it should also be sampled after the appropriate time lapse.
- The time lapse information is also important for monitoring inlet and outlet to ozone and GAC contactors
- Periodic analysis of sludge thickener supernatant for total and dissolved algal metabolite will show any additional release of the compounds from the cells during sludge treatment.

## 3.4 Water Treatment Summary

The advice given in the previous sections on water treatment is summarised in Table 22 for MIB and geosmin, and Table 23 for algal toxins.

Table 22: Summary of water treatment options for removal of MIB and geosmin

| Treatment process | Intact cells |
|---|---|
| Coagulation sedimentation | Very effective for the removal of intracellular T&O provided cells accumulated in sludge are isolated from the plant |
| Rapid filtration | Very effective for the removal of intracellular T&O provided cells are not allowed to accumulate on filter for prolonged periods |
| Slow sand filtration | As for rapid sand filtration, with the additional possibility of biological degradation of dissolved T&O |
| Combined coagulation/ sedimentation/filtration | Extremely effective for the removal of intracellular T&O provided cells accumulated in sludge are isolated from the plant and any free cells are not allowed to accumulate on filter for prolonged periods |
| Membrane processes | Very effective for the removal of intracellular T&O provided cells are not allowed to accumulate on membrane for prolonged periods |
| Dissolved Air Flotation (DAF) | As for coagulation/sedimentation |
| Oxidation processes | Not recommended as a treatment for cyanobacteria cells as this process can lead to cell damage and lysis and consequent increase in dissolved T&O levels |
| | Dissolved metabolites |
| Adsorption -powdered activated carbon (PAC)<br>(doses required vary with water quality) | A microporous carbon (coconut or coal based, steam activated wood) 60 minutes contact time recommended<br>High doses may be required for high concentrations of T&O |
| Adsorption -granular activated carbon (GAC) | GAC adsorption is an effective treatment for T&O. The time required for breakthrough will depend on the contact time and water quality. Removal is not reliable in the presence of free chlorine |
| Biological filtration | When functioning at the optimum this process can be very effective for the removal of T&O. However, factors affecting the removal such as biofilm mass and composition, acclimation periods, temperature and water quality cannot be easily controlled |
| Ozonation | A residual of at least 0.3 mg $L^{-1}$ for 10 minutes should result in up to 50% removal of the T&O. Doses will depend on water quality |
| Chlorination | Ineffective |
| Chloramination | Ineffective. |
| Chlorine dioxide | Not effective with doses used in drinking water treatment |
| Potassium permanganate | Not effective with doses used in drinking water treatment |
| Hydrogen peroxide | Not effective on its own |
| UV Radiation | Ineffective |
| Membrane Processes | Depends on membrane pore size distribution. Only the tightest RO or NF membrane could be expected to remove these T&O compounds |

Table 23: Summary of water treatment options for removal of toxins.

| Treatment process | | Treatment efficiency |
|---|---|---|
| | Intact cells | |
| Coagulation sedimentation | | Very effective for the removal of intracellular toxins provided cells accumulated in sludge are isolated from the plant |
| Rapid filtration | | Very effective for the removal of intracellular toxins provided cells are not allowed to accumulate on filter for prolonged periods |
| Slow sand filtration | | As for rapid sand filtration, with the additional possibility of biological degradation of dissolved toxins |
| Combined coagulation/ sedimentation/filtration | | Extremely effective for the removal of intracellular toxins provided cells accumulated in sludge are isolated from the plant cells and any free cells are not allowed to accumulate on filter for prolonged periods |
| Membrane processes | | Very effective for the removal of intracellular toxins provided cells are not allowed to accumulate on membrane for prolonged periods |
| Dissolved Air Flotation (DAF) | | As for coagulation/sedimentation |
| Oxidation processes | | Not recommended as a treatment for cyanobacteria cells as this process can lead to cell damage and lysis and consequent increase in dissolved toxin levels |
| | Dissolved metabolites | |
| Adsorption -powdered activated carbon (PAC) (doses required vary with water quality) | Microcystins (except m-LA) | Wood-based, chemically activated carbon is the most effective, or similar, 60 minutes contact time recommended |
| | Microcystin LA | High doses required |
| | Cylindrospermopsin | Wood-based, chemically activated carbon is the most effective, or similar, 60 minutes contact time recommended |
| | Saxitoxins | A microporous carbon (coconut or coal based, steam activated wood) 60 minutes contact time recommended effective for the most toxic of the variants |
| Adsorption -granular activated carbon (GAC) | All dissolved toxins | GAC adsorption displays a limited lifetime for all toxins. This can vary between 2 months to more than one year depending on the type of toxin and the water quality |
| Biological filtration | All dissolved toxins | When functioning at the optimum this process can be very effective for the removal of most toxins. However, factors affecting the removal such as biofilm mass and composition, acclimation periods, temperature and water quality cannot be easily controlled |
| Ozonation | All dissolved toxins | Ozonation is effective for all dissolved toxins except the saxitoxins. A residual of at least 0.3 mg $L^{-1}$ for 5 minutes will be sufficient. Doses will depend on water quality |
| Chlorination | All dissolved toxins | If a residual of 0.5 mg $L^{-1}$ is maintained for at least 30 minutes, and a dose of at least 3 mg $L^{-1}$ is applied, most microcystins and cylindrospemopsin should be destroyed. Microcystin LA and saxitoxins may require a higher residual |
| Chloramination | All dissolved toxins | Ineffective. |
| Chlorine dioxide | All dissolved toxins | Not effective with doses used in drinking water treatment |
| Potassium permanganate | All dissolved toxins | Effective for microcystin, limited or no data for other toxins |
| Hydrogen peroxide | All dissolved toxins | Not effective on its own |
| UV radiation | All dissolved toxins | Capable of degrading microcystin-LR and cylindrospermopsin, but only at impractically high doses or in the presence of a catalyst |
| Membrane processes | All dissolved toxins | Depends on membrane pore size distribution |

# APPENDIX 1: KNOWN TASTE AND ODOUR PRODUCERS

All information relating to cyanobacteria is based on knowledge obtained at the Australian Water Quality Centre (M Burch & P Baker).

Table 24: Common cyanobacteria in southern Australia, potential toxicity and odours.

**Common Cyanobacteria in southern Australia Potential Toxicity and Odours.**

| Genus/species | Toxicity status | Odour when algae are - Moderate | Abundant | Metabolite produced |
|---|---|---|---|---|
| *Anabaena bergii* | **Potentially toxic (cylindrospermopsin)** | | | |
| *Anabaena circinalis* | **Potentially Toxic (saxitoxins-PSP)** | Earthy, musty | Earthy, musty | Geosmin |
| *Anabaena crassa* | Not known to be toxic in Australia | Earthy, musty | Earthy, musty | Geosmin |
| *Anabaena* (other coiled spp) | Not known to be toxic in Australia | Some spp produce earthy, musty odours | Some spp produce earthy, musty odours | Geosmin |
| *Anabaena* (straight) spp | Not known to be toxic in Australia | Some spp produce earthy, musty odours | Some spp produce earthy, musty odours | Geosmin |
| *Anabaena solitaria* | Not known to be toxic in Australia | Earthy, musty | Earthy, musty | Geosmin |
| *Anabaenopsis* | Not known to be toxic in Australia | | | |
| *Aphanizomenon gracile* | Not known to be toxic in Australia | | Earthy, musty | Geosmin, MIB |
| *Aphanizomenon ovalisporum* | **Potentially toxic (cylindrospermopsin)** | | | |
| *Arthrospira* | Not known to be toxic in Australia | | | |
| *Cylindrospermopsis raciborskii* | **Potentially toxic (cylindrospermopsin)** | | | |
| *Microcystis aeruginosa* | **Potentially toxic (microcystins)** | | Sulphurous | Beta cyclo-citral, iso-propyl mercaptan, disulphides |
| *Microcystis flos-aquae* | **Potentially toxic (microcystins)** | | Sulphurous, earthy | Disulphides, geosmin |
| *Microcystis incerta* | Not known to be toxic in Australia | | | |
| *Microcystis wesenbergii* | **Potentially toxic (microcystins)** | | Sulphurous | Disulphides, iso-propyl mercaptan |
| *Nodularia spumigena* | **Potentially toxic (nodularin)** | | | |
| *Oscillatoria spp* | Not known to be toxic in Australia | Earthy, musty | Earthy, musty | MIB/geosmin |
| *Phormidium spp* | **Potentially toxic** Toxin unknown | Earthy, musty | Earthy, musty | MIB/geosmin |
| *Planktolyngbya* | Not known to be toxic in Australia | | | |
| *Planktothrix spp* | Not known to be toxic in Australia | Some spp produce earthy, musty odours | Some spp produce earthy, musty odours | MIB |
| *Pseudanabaena spp* | Not known to be toxic in Australia | | Some spp produce earthy, musty odours | MIB/geosmin |

Table 25: Cyanobacteria and actinomycetes identified as geosmin and/or 2-methylisoborneol (MIB) producers

| Benthic Cyanobacteria | Odour compound | | Planktonic Cyanobacteria | Odour compound | | Actinomycete | Odour compound | |
|---|---|---|---|---|---|---|---|---|
| | Geo | MIB | | Geo | MIB | | Geo | MIB |
| *Geitlerinema* | + | | *Anabaena circinalis* | + | | *Penicillium* spp. | + | + |
| *Jaaginema geminatum* | | + | *A. crassa* | + | | *P. expansum* | + | |
| *Leibleinia subtilis* | + | | *A. lemmermannii* | + | | *Aspergillus* spp. | + | + |
| *Lyngbya aestuarii* | | + | *A. macrospora* | + | | *Streptomyces albidoflavus* | + | |
| *Oscillatoria curviceps* | | + | *A. solitaria* | + | | *S. avermitilis* | + | |
| *O. tenuis* var. *levis* | | + | *A. viguieri* | + | | *S. citreus* | + | |
| *O. variabilis* | | + | *Aphanizomenon flos-aquae* | + | | *S. griseus* | + | |
| *Phormidium allorgei* | + | | *Aphanizomenon gracile* | + | | *S. griseofuscus* | + | + |
| *P. amoenum* | + | | *Oscillatoria limosa* | | + | *S. halstedii* | + | |
| *P. breve* | + | + | *Planktothrix agardhii* | + | + | *S. psammoticus* | + | |
| *P. chalybeum* | | + | *P. cryptovaginata* | | + | *S. tendae* | + | |
| *P. cortianum* | + | | *P. peromata* | | + | *S. violaceusniger* | + | + |
| *P. favosum* | | + | *P. peromata* var. *attenuata* | | + | *Streptomyces* spp. | + | + |
| *P. formosum* | + | | *Pseudanabaena catenata* | + | + | | | |
| *P.* strain LM689 | | + | *Pseudanabaena limnetica* | | + | | | |
| *P. simplissimum* | + | | *Symploca muscorum* | + | | | | |
| *P.* sp. strain NIVA 51 | + | + | | | | | | |
| *P. tenue* | | + | | | | | | |
| *P. uncinatum* | + | | | | | | | |
| *P. viscosum* | + | | | | | | | |
| *Planktothrix prolifica* | + | | | | | | | |
| *Porphyrosiphon* | | + | | | | | | |
| *Symplocastrum müllieri* | + | | | | | | | |
| *Tychonema bornetii* | + | | | | | | | |
| *Tychonema granulatum* | + | + | | | | | | |
| *Hyella* sp. | | + | | | | | | |
| *Microcoleus* sp. | + | | | | | | | |

Geo – Geosmin. Table modified from Jüttner and Watson (2007) [70]

# APPENDIX 2 MANUFACTURE, PROPERTIES AND STANDARD TESTING PROCEDURES FOR ACTIVATED CARBON

Activated carbon is formed by the conversion of carbon from primary materials such as coal, wood, peat or coconut shells. The material is converted into a highly porous structure by heating in the presence of steam, air, or sometimes chemicals to temperatures in the range of 600-1000 $^{o}$C. During this process the raw material is converted to layers of six membered carbon rings which are bound by physical forces into groups called microcrystallites. The spaces between these microcrystallites, the pores, provide the very large surface area for adsorption. Due to the nature of the starting materials there is always some inorganic material remaining on the surface (N, Fe, S, P, Na, Cl, Si), however, by far the most abundant elements present on the surface of activated carbon are carbon (approximately 80 to 98%) and oxygen (approximately 2 to 20%). The oxygen is present mainly as carbon-oxygen surface groups such as phenolic and carboxyl groups [225]. The internal structure of activated carbon, ie the sizes and numbers of the pores, as well of the chemistry of the surface, will depend on the starting material and the activation processes, and will affect the adsorption of target compounds such as algal metabolites [226].

Activated carbon is available in two forms, granular activated carbon (GAC) and powdered activated carbon (PAC). Powdered activated carbon can be added before coagulation, during chemical addition, or during the settling stage, prior to sand filtration. It is removed from the water during the coagulation process, in the former cases, and through filtration, in the latter. As the name implies, PAC is in particulate form, with a particle size typically between 10 and 100 μm in diameter. One of the advantages of PAC is that it can be applied for short periods, when problems arise, then discontinued when it is no longer required. With problems that may arise only periodically such as algal metabolites, this can be a great cost advantage. A disadvantage with PAC is that it cannot be reused and is disposed to waste with the treatment sludge or backwash water. Granular activated carbon is used extensively in Europe and the United States for the removal of micropollutants such as pesticides, industrial chemicals and tastes and odours, and is becoming more widely used in Australia, particularly for taste and odour removal and insurance against the possibility of a toxic algae bloom in the water source. The particle size is larger than that of PAC, usually between 0.4 and 2.5 mm. Granular activated carbon is generally used as a final polishing step, after conventional treatment and before disinfection. The advantages of GAC are that it provides a constant barrier against unexpected episodes of tastes and odours or toxins, and the mass of carbon provides a very large surface area. The disadvantage is that it has a limited lifetime, and must be replaced or regenerated when its performance is no longer sufficient to provide high quality drinking water. Filtration through GAC is often used in conjunction with ozone. When used in conjunction with ozone it is sometimes called BAC, or biological activated carbon; however, this description can be misleading as all GAC filters function as biological filters to some extent within a few weeks to months of commissioning.

## How does activated carbon work?

Physical adsorption is the primary means by which activated carbon works to remove contaminants from water. The highly porous structure provides a large surface area for contaminants (adsorbates) to collect. Physical adsorption occurs because all molecules exert attractive forces, especially molecules at the surface of a solid. The large internal surface area of carbon has many attractive forces which work to attract other molecules. One of the main forces is the attraction between the hydrophobic (“water fearing”) carbon surface and a hydrophobic molecule, or one with hydrophobic parts. The oxygen functional groups impart polarity and, if they dissociate, a charge to the surface, thus they allow adsorption through hydrogen bonding or electrostatic attraction [227].

Removal of contaminants by activated carbon is a complex process. Figure 19 is a schematic representation of the major processes occurring during adsorption, these are largely diffusion related. In order to be removed by activated carbon a molecule must diffuse:

-to the particle surface from the bulk liquid (1)
-through the liquid surface layer (2)
-through the pore structure of the carbon (3),

finally being removed from solution at the adsorption site (4) (see Figure 19)

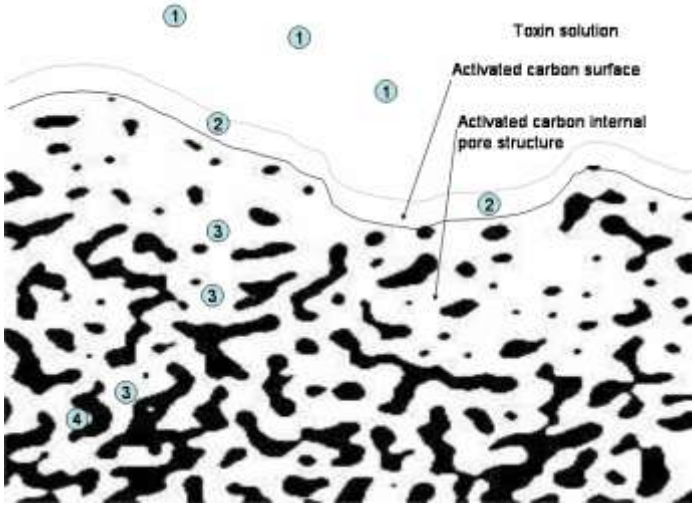



Figure 19: Representation of diffusion into the activated carbon structure.

Processes 1 and 2 depend on the physical parameters of the system, for example mixing conditions for PAC, flow rates for GAC. Processes 3 and 4 are dependent on the activated carbon pore size distribution and surface chemistry/hydrophobicity. In general, the most favourable energy for adsorption is provided by pores slightly larger than the adsorbing molecule, as there are more contact points for the compound to adhere, and it fits "snugly" into the pore. In water treatment another very important factor is how quickly the contaminant can reach a suitable adsorption site. This is strongly influenced by the access to the internal structure through the pores on the external surface, as well as the structure and size of the "transport pores", those the contaminant must travel prior to reaching the adsorption site (i.e. step 3, Figure 19).

Due to its very effective porous nature activated carbon adsorbs most compounds present in water to some extent. Although carbon has a very high surface area, invariably there are limited suitable adsorption sites available. A competition is set up between the different species for those adsorption sites, and adsorption of the compound of interest will usually be reduced [228,229]. The main competing species in surface water are those compounds formed by the breakdown of vegetable and animal matter in the environment, dissolved natural organic material (NOM). This mixture of compounds is collectively measured by dissolved organic carbon (DOC) analysis, or ultraviolet (UV) absorbance measurements.

## How do we choose the best activated carbon for the removal of algal metabolites?

As mentioned earlier, the factors that influence the adsorption of contaminants, such as pore size distribution and surface characteristics, are dependent on the starting material and method of activation. Even small variations in the chemical composition of the raw material and activation conditions can result in large differences in the finished product. Figure 20 shows some scanning electron micrographs of two types of activated carbon. The very different external structure of the carbons is also reflected in the internal porous structure and surface chemistry.

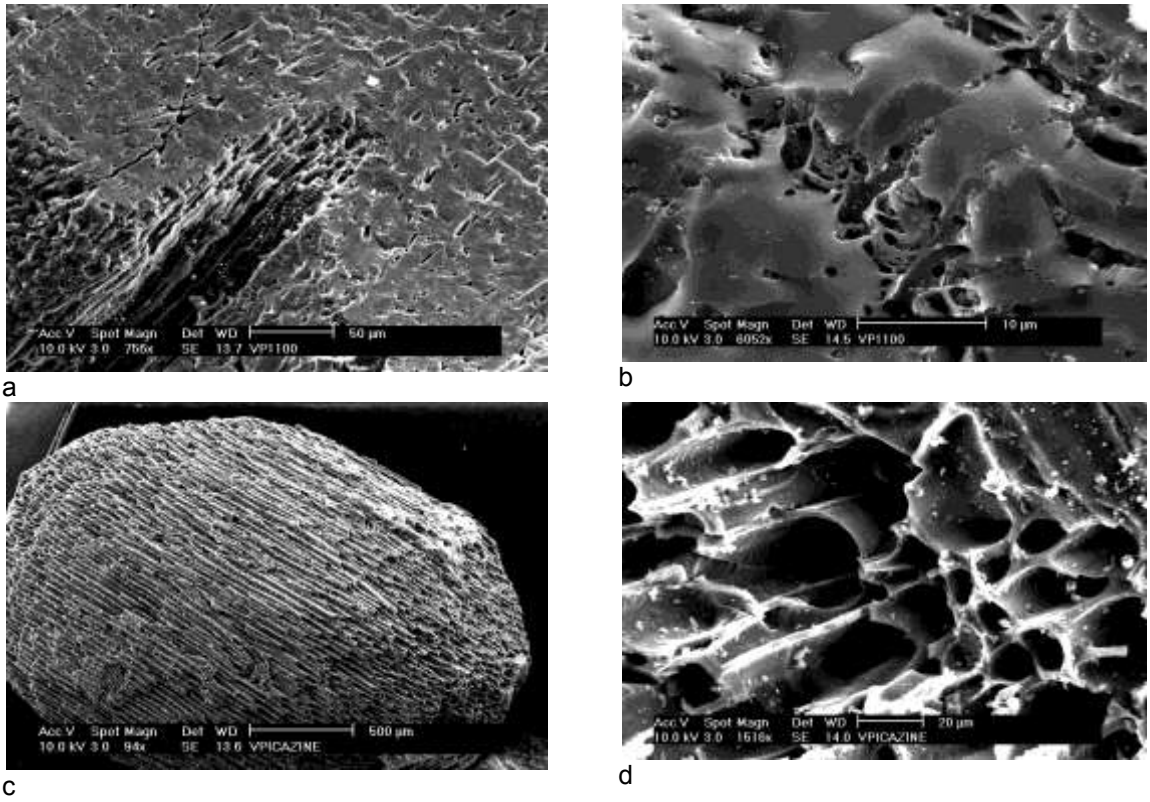



Figure 20: Scanning electron micrographs of external activated carbon structure, a) and b) coconut-based activated carbon c) and d) wood-based, chemically activated carbon

A range of tests is available to characterise activated carbons with the aim of determining the most appropriate adsorbent for a particular contaminant, these include:

**Surface area determination and pore size distributions:**
These parameters are usually determined using gas adsorption, most commonly nitrogen. The amount of nitrogen adsorbed is measured as a function of the relative pressure, and, based on the size of the $N_2$ molecule, and using one of a number of theoretical models, surface area and pore size can be calculated. The surface area can be a useful general guide for determining the overall area available for adsorption. For example, a carbon with a surface area of about 500 $m^2$ $g^{-1}$ would probably not be suitable for the removal of tastes and odours. However, a surface area of 1200 $m^2$ $g^{-1}$ (relatively high for an activated carbon) would not guarantee a high level of removal of these compounds, as the effectiveness of the adsorbent depends on the range of factors, mentioned above. The pore size distribution (PSD) will give a more reliable hint of whether the carbon will be suitable for a particular purpose, as the aim would be to have a carbon with a high volume of pores in the size range of the target molecule, as well as larger pores that will act as transport pores for the contaminant. The disadvantage of using PSDs is that the analysis is difficult, very low relative pressures of nitrogen are required, and the reproducibility between laboratories is not high.

The pores on activated carbon are categorised according to their size as follows-

| | |
|---|---|
| Primary micro pores | < 0.8 nanometre (nm) |
| Secondary micro pores | 0.8 - nm - 2 nm |
| Mesopores | 2 nm -50 nm |
| Macropores | > 50 nm |

[230]
One nanometre is one millionth of a millimetre.

**Iodine number**
The iodine number is obtained from a series of adsorption experiments measuring the amount of iodine removed from solution by activated carbon. As iodine is a relatively small molecule it is assumed that the iodine number is an indication of the number of micropores, or the surface area. A value of 800 or higher suggests a high surface area, high "activity" carbon [231,232].

**Molasses number**
For this test a solution of backstrap molasses is prepared, and the activated carbon is added. The removal of colour in the solution is measured using UV spectroscopy. Molasses is the syrup remaining after processing sugar cane or sugar beet to obtain sugar. Backstrap molasses is the darkest of the by-products, and contains an unknown mixture of large organic molecules, some of which are highly coloured. It is assumed that the more colour that is adsorbed by the carbon, the more effective it will be for the adsorption of large organic compounds from water. In reality the number may reflect the volume of large pores, perhaps mesopores, in the carbon structure. A reasonable value for activated carbon is around 250.

**Tannin number**
The tannin number is defined as the concentration of carbon, in mg $L^{-1}$ required to reduce a standard tannin solution from a concentration of 20 to 2 mg $L^{-1}$. The standard Merck tannic acid recommended for use in this test has a molecular weight of approximately 1700 g $mol^{-1}$. The tannin number can give an indication of the adsorption capacity of the carbon for DOC, and the lower the tannin number the better the adsorption of tannin.

Essentially the four methods above give good general information, but give specific removal information only about the compound used in the test (e.g. iodine, tannin). Details of tests that can be used to characterise activated carbon are given in [231].

**Density**
This parameter is often quoted by manufacturers. In general, a carbon with low density has a large volume of larger pores, such as macropores and mesopores, and relatively fewer micropores. It is also more likely to float, or be abraded during backwashing, which may be an issue for GAC.

**Abrasion resistance**
This number gives an indication of the "robustness" of an activated carbon particle. Of particular importance with GAC, where losses can be high through abrasion of particles during frequent backwashing

**Particle size**
For GAC filtration, the particle size required will be determined by the physical requirements for effective filtration at the flow rates experienced in the plant, as well as the mode of backwashing utilised. The particle size of PAC is a major influence on the rate of removal of target compounds; the smaller the particle, the higher the rate of removal. As a result, shorter contact times and lower doses are required for smaller PAC particles. However, the advantages are somewhat overcome by the difficulties of removing and handling very small particles of black powder. A diameter of approximately 11 micron has been found to result in high rates of adsorption without major difficulties in removal and handling.

Details of most of the tests described above are given in the AWWA standard methods [232]. The interpretation of the data obtained using these tests is not trivial, and any perceived relationship between the iodine number and, for example, the amount of cylindrospermopsin adsorbed in 30 mins, is tenuous at the best. Although this information is useful, and many of these parameters can be supplied by the activated carbon manufacturer, it is very difficult to use them to help decide on a brand, or raw material for the removal of a particular compound (except, of course, if the target compound is iodine, tannin, or molasses).

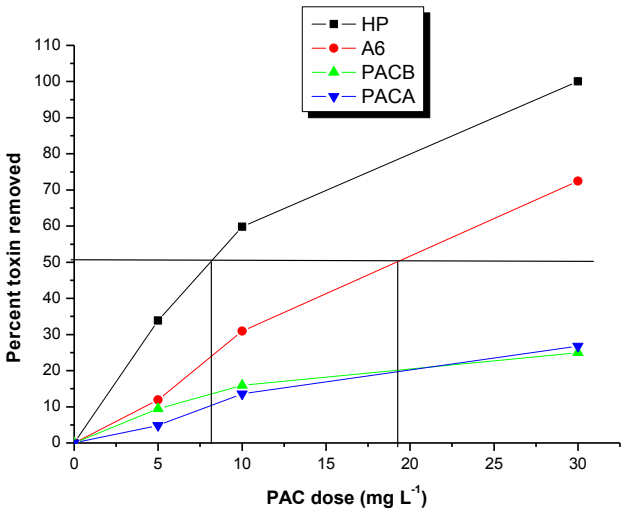
# APPENDIX 3 COMPARATIVE TEST FOR PAC

This test can be applied to determine the most cost-effective PAC for application in a water treatment plant.

1 Choose 3-6 good quality activated carbons with the general attributes required for the AM of interest (see main text). The manufacturer will give general guidance regarding raw materials and average pore sizes.
2 Sample water from the position in the plant where the PAC will be applied. Spike the water with the concentration of AM that might be expected at the application point. If this is unknown, 5 µg $L^{-1}$ of toxin (for saxitoxins STX equivalents), or 50 ng $L^{-1}$ geosmin and/or MIB is a value that will give representative results if converted to percent removals. Take a sample for analysis
3 Place 500 mL of spiked water into each of three jar testing vessels
4 Add 5, 10, and 30 mg $L^{-1}$ of PAC* into the separate jar test vessels, with stirring.
5 Continue stirring for the average contact time expected after the point of application in the plant. This could be the middle of the range expected over the period of possible contamination. Assume the effective contact time is only while the particles are in suspension in the plant. Disregard time during settling when determining contact time.
6 After the appropriate contact time, filter sample through membrane filter (0.45 µm), analyse samples for algal metabolite concentration, or send to appropriate laboratory.
7 Undertake this test for each PAC
8 Estimate the PAC dose required for 50% removal of the AM. This can be determined approximately by interpolating a graph of percent removal vs carbon dose (see Figure 21).
9 Multiply the cost per kilogram of the carbon by the dose required, and a simple cost analysis of the carbons can be achieved.

* Prepare a slurry for each carbon by adding 50 mg to 50 $cm^3$ of milli-Q water or 1:1, one day before running the test

An example of this procedure for microcystin LR for 4 carbons is shown in Figure 21 below.




| PAC | Dose required for 50% removal | Cost per kilogram | Cost per ML of water treated |
|---|---|---|---|
| 1 | 8 | 4.20 | **$34** |
| 2 | 19 | 1.80 | **$35** |
| 3 | >>30 | 3.80 | **>>$ 114** |
| 4 | >>30 | 2.30 | **>> $69** |

Figure 21: Microcystin removal by four PACs, including cost analysis.

In this example the most expensive carbon is the most cost effective for the removal of this contaminant.

# APPENDIX 4 COMPARATIVE TEST FOR GAC

Testing to determine the most effective GAC in the laboratory is not as straightforward as PAC. Under normal conditions (i.e. 12-20 minute contact time) most virgin GAC will adsorb contaminants to below detection, perhaps for a prolonged period. It is therefore very difficult to compare several GACs. Long term pilot plant studies are recommended to determine the most effective GAC and the approximate time until breakthrough of the contaminant. However, these tests are difficult, expensive and time consuming. A simple alternative to determine the most effective GAC is the short-bed adsorber test in combination with an equilibrium isotherm test. Equilibrium isotherms can be used to compare the capacities of the GACs for the contaminant, and short bed adsorber tests give an indication of the rates of adsorption. Two sets of experiments are required.

***Equilibrium isotherms:***

1 Sample water at the point in the treatment plant where the GAC will be situated, spike in toxin at a concentration of approximately 5 µg $L^{-1}$, or 50 ng $L^{-1}$ geosmin and/or MIB.
2 Place equal volumes of spiked water in each of 5 glass vessels. Volumes of 250-500 mL are preferred
3 Add GAC, ground to < 45 µm, to 4 of the vessels at doses of 2, 6, 10 15 mg $L^{-1}$. The 5$^{th}$ vessel will act as the control.
4 Mix vessels consistently to maintain activated carbon in suspension for 3 days.
5 Filter all samples and analyse
6 Undertake his test for each carbon and plot percent removed vs carbon dose for each carbon (see Figure 22 a)

***Short bed column tests:***

These tests are designed to force breakthrough of the contaminant for the comparison of different carbons

1 Pack GAC into small diameter column (1 cm) to a bed depth of 4 cm.
2 Pump spiked test water (see section above) through column at a flow rate equivalent to the filtration rate expected on the filters
3 Collect column outlet samples at regular intervals for a period of 2 hours
4 Analyse samples and plot percent breakthrough vs time (Figure 22 b)

A GAC that shows superior equilibrium capacity and removal in the short bed adsorber test could be expected to perform best at the full scale. In Figure 22 below, GACs 1 and 3 appear equivalent and the decision would depend on relative costs

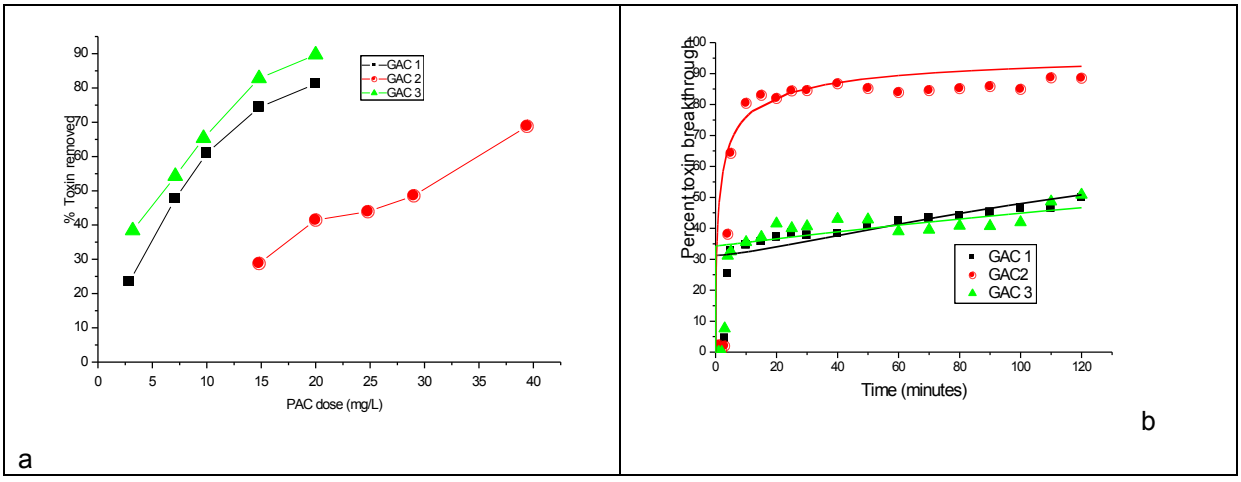



Figure 22: Comparative test for GAC a) equilibrium removal of microcystin; b) mini column breakthrough of microcystin.

# APPENDIX 5 CONSTRUCTION OF PAC DOSE REQUIREMENT CURVES

This method is simply an extension of the comparative test described in Appendix 2. Once the most cost effective activated carbon has been chosen a series of jar tests should be carried out over a larger range of doses, to obtain percent removals from 20 to 90%. These results can be applied to any concentration of AM as the percent removal is independent of the initial concentration. At least 5 carbon doses should be used to obtain an accurate removal vs dose curve. This should be undertaken at two contact times if the plant could experience a variation in flow affecting the contact time for the PAC. An example is given in Figure 23 a), below. To improve the ease of use of this graph, percent removal could be converted to initial concentration (see Figure 23 b) as example). If we assume a target concentration of 1 µg $L^{-1}$ of toxin, the y axis data can be converted to initial concentration using the equation:

Initial concentration =100/(100-percent removal)

For example, 50% removal on the graph would apply to 100/(100-50)=100/50=2 µg $L^{-1}$

In other words, if the aim is to reduce the concentration of toxin to 1 µg $L^{-1}$ from 2 µg $L^{-1}$ the removal we need is 50%.

Figure 23 b) shows the same data as Figure 23 a), with the percent removal axis converted to initial concentration. Both graphs are equally valid, although b) might be preferred for simplicity.

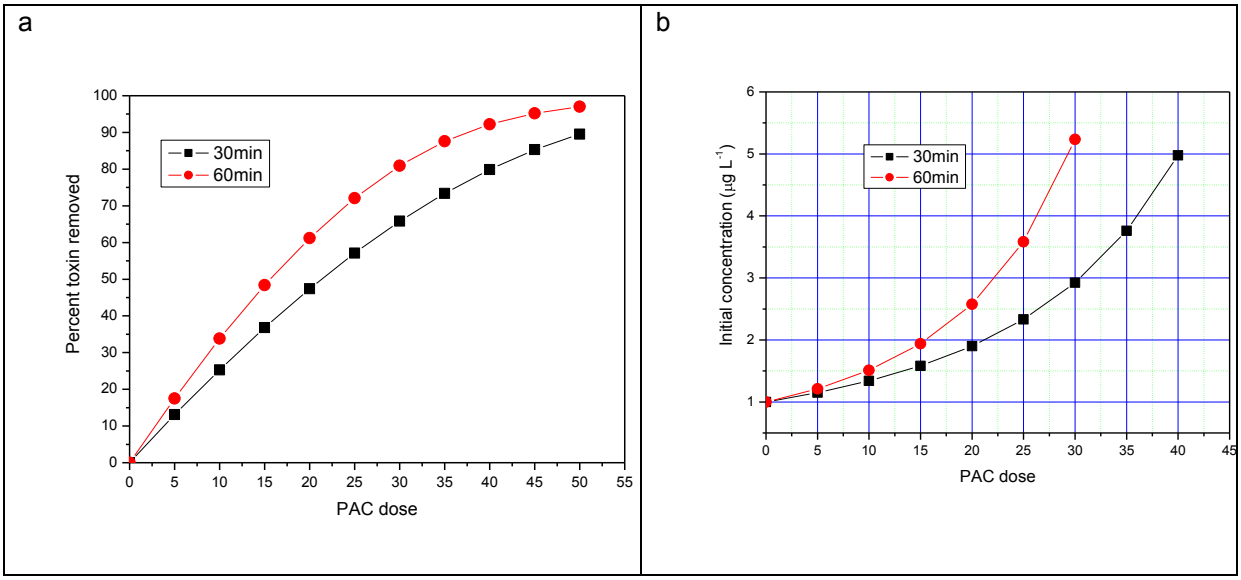



Figure 23: PAC dose calculators a) Percent toxin removal vs dose; b) initial toxin concentration vs dose to achieve 1 µg $L^{-1}$.

It is relatively easy to determine from Figure 23 b that an inlet concentration of 2 µg $L^{-1}$ will require a PAC dose of 15 mg $L^{-1}$ with a contact time of 60 minutes, and 20 mg $L^{-1}$ for a contact time of 30 minutes.

# APPENDIX 6 GAC MONITORING TEST

When GAC has been in use for 6 months or more it is worthwhile to begin to monitor for removal efficiency. For example, if a bloom of *Microcystis* were possible as the warmer months approach, a simple test for microcystin removal will give an indication of the GAC filter's ability to remove the toxin effectively.

Laboratory scale filter columns can be used for this test. A column diameter of 2.5 cm and a bed depth of 7-8 cm has been shown to be optimum. Larger pilot columns can also be used; in this case large volumes of water containing the AM to be tested will be required. This may prove an expensive exercise if the test is undertaken using commercial toxin standards.

The test can be conducted as follows

1 Take duplicate samples of 100 mL from the top of each GAC filter after backwash.
2 Place in glass column, 2.5 cm diameter, to a bed depth of 7-8 cm.
3 Pump water, sampled from the plant prior to the GAC filters and spiked with the AM of interest, at a flow rate to achieve the same empty bed contact time as the full scale GAC filters.
4 After several hours take samples from the inlet to the column, and the outlet.
5 Repeat for other GAC samples
6 Analyse samples and calculate average percent removal.

Clearly this is not a definitive test to determine full scale removals as the samples will not necessarily be representative of the whole filter. However, it can be used to give an *indication* of how the GAC filters would perform. For example, if the small scale column showed an average of 50% removal of microcystin, and this is the level of removal that would be necessary in the plant, it would be wise to consider replacement of the GAC.

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The Cooperative Research Centre (CRC) for Water Quality and Treatment operated for 13 years as Australia's national drinking water research centre. It was established and supported under the Australian Government's Cooperative Research Centres Program.

The CRC for Water Quality and Treatment officially ended in October 2008, and has been succeeded by Water Quality Research Australia Limited (WQRA), a company funded by the Australian water industry. WQRA will undertake collaborative research of national application on drinking water quality, recycled water and relevant areas of wastewater management.

The research in this document was conducted during the term of the CRC for Water Quality and Treatment and the final report completed under the auspices of WQRA.

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