1.0 Introduction

1.1 Background

Malpas Dam is the main provincial water storage servicing the city of Armidale on the New England Tablelands of NSW (Figure 1.1). Since its construction in 1968 it has been the site of regular blooms of cyanobacteria.

Figure 1.1 North-east NSW.

These blooms, which have consisted mainly of *microcystis* and *anabaena spp.* are the source of many concerns for the managerial authority, Armidale Dumaresq Shire Council (ADSC). Specifically, in Malpas Dam cyanobacteria are believed to impact on:

- Public health,
- Taste and odours,
- Increased water treatment costs,
- Treatment difficulties,
- General water quality.

In recent history cyanobacteria were directly responsible for poor water quality during the summer of 1994/95. During this period there were on average 60 formal complaints from residents received by ADSC per day (Leys 1995).

In response to these problems ADSC incorporated a Powdered Activated Carbon stage (PAC) during water treatment (1986[1]) as well as an artificial destratification unit within the dam and in close proximity to the offtake point from the dam (1988). PAC has been shown to be effective at removing both taste and odours associated with algal blooms but less so against the odours associated with manganese, iron, and sulphur (Blackburn et al. 1997).

Artificial destratification is used to remove the risk of cyanobacteria blooms by reducing their competitive advantage over other algae and disrupting their buoyancy regulation through artificial mixing. The 'bubble plume' is the most widely adopted mixing method in Australia, however it has been of limited success. McAuliffe and Rosich (1989) found in a study of 52 lakes this method of control was ineffective in over 60% of cases. Anecdotal evidence suggests that this may also be the case in Malpas Dam. Historically some blooms have been partially impaired through the management use of destratification (M. Porter 1998, pers. comm., 3 October) however it has not been uncommon for algal blooms to continue to flourish in Malpas during destratification operations (Hawkins & Tansitt 1995).

The continued persistence of cyanobacteria during the isothermal conditions induced by destratification may in part be due to the presence of other factors that aid their growth and development. It is generally accepted that the optimum conditions for growth of cyanobacteria in surface waters include (Pressedee & Hart 1991, Qld Water Quality Task Force 1992, Sas 1998, Seip et al 1992, Reynolds 1992 and Cooke et al. 1993 cited in Whitton & Potts 2000:179):

- A readily available source of organic carbon,

[1] The PAC was installed in 1986 however due to problems with dosing and operation it was not used consistently and reliably until around 1992 (ADSC 2004, pers. comm., 2 July 2004).

- Nitrogen (in order of preference in the form of ammonia, nitrate, nitrite, or free nitrogen >100μg/L),
- Phosphorus (preferably as Soluble Reactive Phosphorus >10μg/L),
- Water temperature of 15-25 °C,
- Thermal stratification,
- Calm water conditions,
- Low sunlight attenuation (i.e. low turbidity),
- Total Nitrogen/Total Phosphorus Ratio <16.

From previous research and monitoring programs some of these conditions are being met within Malpas Dam (Boulton et al. 1995, Hawkins & Tansitt 1995). However the exact combination of factors which initiate excessive algal growth are as yet unclear. Nutrient availability appears not to be a limiting factor as year round levels of bio-available nitrogen and phosphorus are consistently and significantly above that considered limiting for algal growth (Hawkins & Tansitt 1995).

Although nutrients may not be limiting cyanobacteria growth in Malpas Dam they are necessary and as such need to be controlled for long term management of eutrophication and subsequent algal blooms in the dam (Hawkins & Tansitt 1995, Boulton et al. 1995, Banens 1988). The main nutrient sources have been shown to include both insitu release from dam sediments and loads associated with inflow from the catchment following significant rainfall events (Boulton & Faulkner 1995, Leys D. Unpublished 1996, Wilson 2001). Inflow also influences other water quality parameters in the dam such as turbidity, temperature, dissolved oxygen and stability, all of which are important in the life cycle of cyanobacteria.

Given that these aspects of water quality may exert some influence on cyanobacteria growth in Malpas Dam it is apparent that these parameters should be monitored. In conjunction with the monitoring of the presence of cyanobacteria in the dam it would be possible to clarify the specific conditions which lead to excessive growth in the dam. Once understanding of the

conditions under which cyanobacteria flourish in Malpas Dam is achieved the next step is to be able to predict or model when these conditions will occur.

Prediction or modelling of all aspects of the hydrological cycle is possible to varying degrees of accuracy depending on the method applied and the utility and serviceability of the model to the problem at hand. In Malpas Dam what is required is a simple rainfall-runoff model, a method by which to estimate loads of nutrients and sediments being delivered to the dam from inflow and the resultant impact on these same variables in the dam. From the study on the causal conditions of cyanobacteria in Malpas Dam the impact that these inflows have on cyanobacteria growth and general water treatment procedures can then be assessed and appropriate management steps undertaken.

1.2 Aim

This research aims to provide information to the ADSC on possible causes of the recurrent blooms of cyanobacteria and general water quality issues in Malpas Dam. To this end, two broad fields of investigation were undertaken to:

1. Determine the significance of the various parameters outlined in section 1.1 thought to be conducive for blooms in Malpas Dam.
2. Evaluate the impact that catchment inputs of nutrients and sediments have on water quality in Malpas Dam and possible implications for algal blooms.

2.0 Malpas Locality

2.1 Malpas Catchment

Malpas catchment is elongated in a north-south direction and incorporates Ben Lomond[2] (alt. 1500m), two small reservoirs, half the township of Guyra and about 70 rural landholders (Figure 2.1). It covers an area of approximately 20,000 hectares and is part of the larger Macleay River catchment in northern NSW.

The township of Guyra is located 40 km north of Armidale and has an approximate population of 4300 people comprising 810 properties, half within the Malpas Dam catchment. The New England Highway runs directly through the town effectively marking the western boundary of the catchment (USC 2002).

Within the township a variety of industries have and continue to potentially influence the water quality of runoff from the immediate area. These include the Guyra abattoir which closed in 1975, a pet food manufacturing plant and activity at the sale yards in the north of Guyra. Both of which are still operating. In 1997-98 period 5,737 cattle and 82,097 sheep and lambs passed through the saleyards (L. Kelly 1998, pers. comm., 5 November 1999). Other possible influential sites within the area include the town cemetery and a retired landfill site, now the 'modern transfer and recycling centre'. All rubbish is now directed to a sanitary landfill site at Llangothlin which is located in the neighbouring Murray Darling catchment.

[2] A major basaltic eruptive centre in the north.

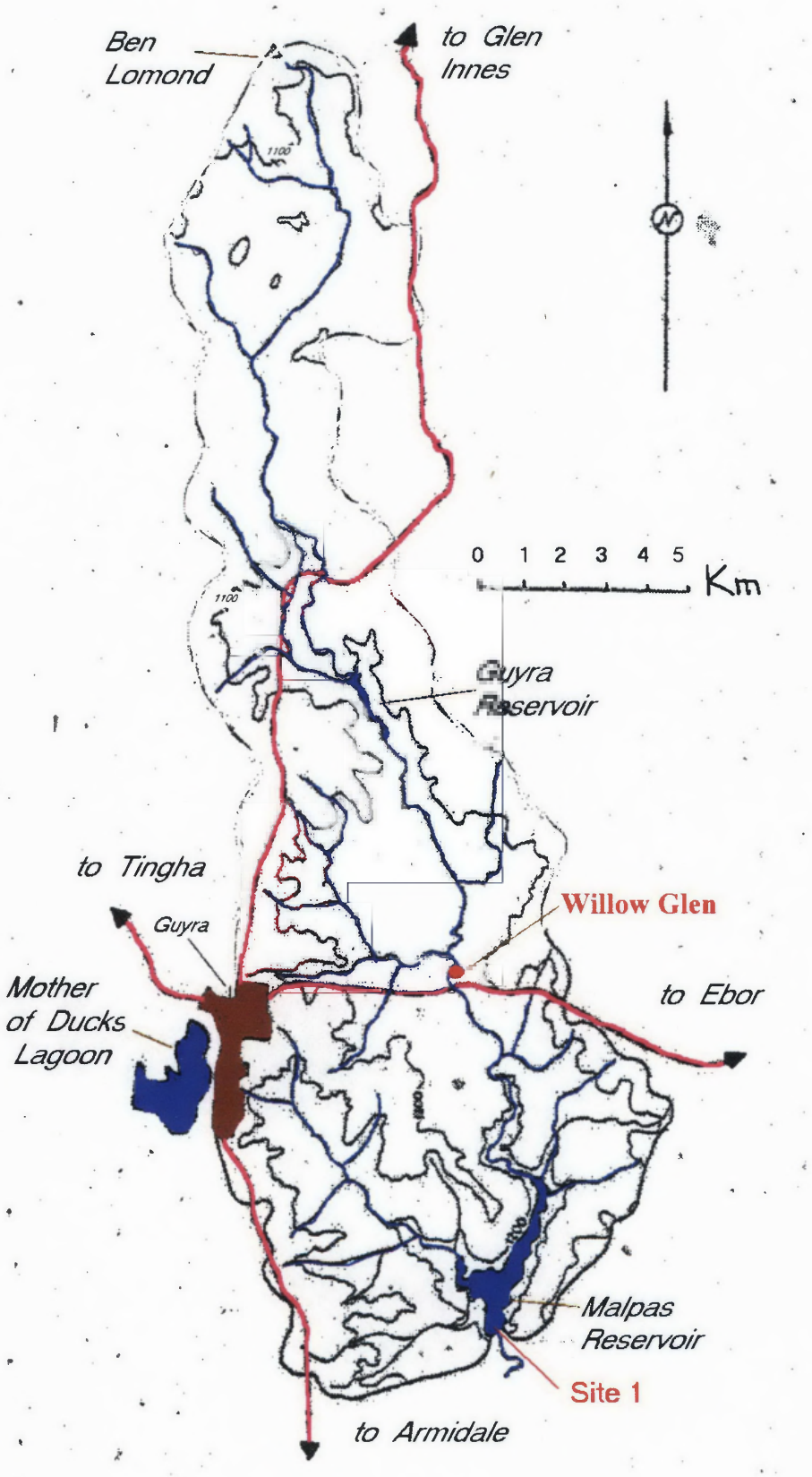


Figure 2.1 Malpas Dam Catchment

2.1.1 Land Uses

There are a variety of land uses within Malpas Catchment. The most prevalent being beef cattle and cross-bred sheep production on improved pastures. Most pastures have been improved through the addition of phosphate and nitrate based fertilizers which has been a constant landuse practice for many years. Methods of fertiliser application have included both aerial as well as direct injection and slow release pellet application. There are also areas of cereal crop production and potato production in the northern, steeper part of the catchment.

2.1.2 Topography/Geology

The Malpas Catchment is located within the New England Tablelands, ranging in altitude from 1178m to 1505m (FSL). This area is comprised of undulating hills with terraces and benches also abundant, reflecting the volcanic origin of the district. There are three main geologic structures within the catchment, which provide the basis for the relatively fertile soils found here. The youngest and most prevalent is tertiary Alkali-olivine basalt, which is found over 91% of the catchment (Banens 1989). The average phosphorus levels found in this parent material are twice the world average with potassium also more than 70% higher (Wilkinson 1986). Permian Llangothlin Adamellite (Granite) comprises 8.2% of the catchment. Its intermediate age has resulted in it being overlain to a large extent by the tertiary basalt. Sandon Beds, ocean sediment laid down 300-400 million years ago comprises 0.5% of the catchment. This is mainly low-grade metamorphic chert and mudstone (Banens 1989).

2.1.3 Climate

Australia lies in the path of mobile high pressure systems which move eastwards at a mean latitude of 37° S in the late summer and about 29° S in late winter and spring, as a result of the seasonal displacement of the atmospheric circulation. Locations to the north of the anticyclones receive easterly or south-easterly winds; to the south, winds are predominately from the west. The New England Tablelands is located within this range of latitudes such that its climate is affected by both systems (USC 2002). In winter the climate in the New England Tablelands is dominated by the movement of cooler and usually much drier air from the continental interior or from the ocean to the south of the continent (cold

fronts). These cold fronts are responsible for the bulk of moisture during winter. In summer, easterly winds from the Tasman Sea dominate. These carry relatively more moisture and contribute to mainly summer rainfall maximum in the region.

The Malpas Catchment experiences around 105 rain days with an average of 890mm of rain annually falling mostly in the warmer summer months (Figure 2.2). Annual evaporation exceeds this at approximately 1219mm whilst average temperatures range from 10-23°C in summer and -1-10°C in winter (Figure 2.2) (USC 2002).

Figure 2.2 Mean monthly rainfall measured at Guyra Post Office, based on 112 years of data (Bureau of Meteorology cited in Wilson 2001:20).

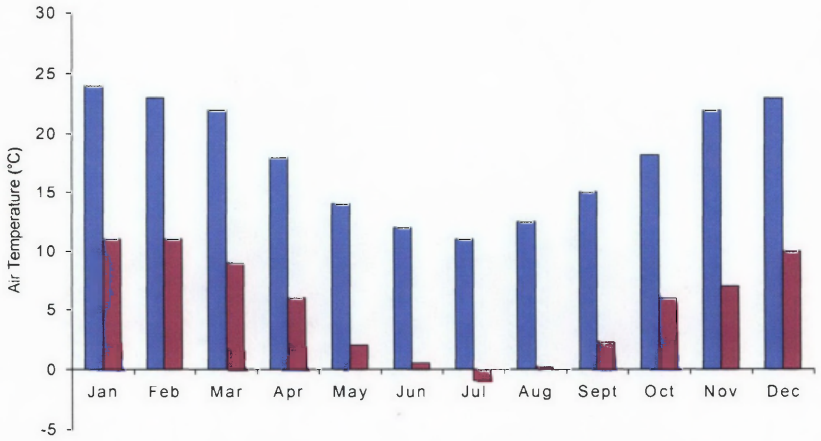


Figure 2.3 Mean daily maximum and minimum temperatures, measured at Guyra Post Office, based on 47 years of data (Bureau of Meteorology cited in Wilson 2001:21).

2.1.4 Vegetation

There are only three main vegetative communities present in Malpas Catchment due to extensive land clearing practices and location of suitable soil. These include:

- Closed grass land (majority of which is introduced pasture species),
- Woodland dominated by Snow Gums (E. *pauciflora*), black sallee (E. *stellulata*) and New England Peppermint (E. *nova angica*),
- Open Forests. Comprising similar species of trees as in the woodland community.

2.1.5 Hydrology

The majority of the Malpas Dam catchment (90%) is drained by two streams, the Gara River and Urandangies Creek. The Gara River flows from the north and varies dramatically in volume and timing of flows. The catchment covers an area of approximately 15000ha and extends from the northern part of the township of Guyra including the catchment north of Guyra Dam. The Department of Infrastructure, Planning and Natural Resources in Armidale have gauged Gara River since January 1997.

Urandangies Creek flows from the west into Malpas Dam and carries approximately one third of the flow typically carried by Gara River (Leys 1996). Its sub-catchment includes the eastern and south-eastern areas of the township of Guyra and is approximately 3000ha in size. The Department of Infrastructure, Planning and Natural Resources (DIPNR) in Armidale have gauged Urandangies creek since December 1998. The remaining 10% of the catchment, which includes the area immediately surrounding the dam and the small area to the northeast and east of the dam, is not gauged. Underground flow likewise has not been gauged or analysed.

2.2 Malpas Dam

2.2.1 Dam Statistics

Malpas Dam was built in 1968 and is located 25km northeast of Armidale on the New England Tablelands (30°15′S, 151°43′E), at an altitude of 1178m (FSL). It is a medium sized reservoir with a capacity of 13,000 ML with provision available to be increased to 26,000ML through the addition of storage gates. The dam wall is of earth and rockfill type located at the southern end of the dam near the off-take tower. The tower has multiple levels at which water can be sourced from the water column. Depths that can be utilised begin at the spillway level (water surface) and at six 3m intervals to a depth of 18m below the water surface. This allows Armidale Dumaresq Shire Council to select the level at which water quality is optimum. Basic data for Malpas Dam appear below (Table 2.1).

Table 2.1 Malpas Dam Statistics

Maximum Depth (m)	20
Mean Depth (m)	9
Water Temperature Range (°C)	7-25
Water Surface Area (ha)	182
Mean Water Residence Time (days)	130

Malpas Dam is the principal source of potable water for Armidale residents (approximately 23,000). The water from Malpas Dam gravitates through a pipeline 35km to the water treatment facility located on the northern outskirts of Armidale where it undergoes traditional water treatment processes.

2.2.2 Aquatic Fauna

The comprehensive survey of algal populations in Malpas Dam conducted by Skinner (1973) identified 24 different species. The majority were green algae (Staurastrum) which are common in eutrophic water bodies and Trachelomonas (Euglenophyta) however diatoms were also abundant. Since that time a major source of pollution (Guyra abattoir) has been redirected from the Malpas Catchment and subsequently no longer contributes nutrients to the dam. This

may have altered the phytoplankton community structures however no recent information is available on this.

Zooplankton and invertebrate information for the dam is also sparse however common fish species present include the English Redfin (Perca *fluviatilis*) and Rainbow Trout (Salmon *gairdnerii*). It is known that the 'Armidale Fishing Club' regularly stock the dam with trout fingerlings for recreational fishing whilst there is also thought to be a stable population of freshwater eels living in the dam (M. Porter 1998, pers. comm., 3 October 1998). There are no European carp (Cyprinus *carpio*) present.

2.2.3 Sediments

The sediments of Malpas contain high concentrations of total phosphorus (>1588µg/g dry weight), which has been shown to be capable of becoming bio-available throughout the year in response to changes in water temperature, pH, and hypolimnetic dissolved oxygen (DO) concentrations (Boulton et al. 1995, Wilson 2001). Temperature is thought to act through its influence on biological processes within the dam whilst pH and DO affects and reflects bacterial action (Wilson 2001). Research indicates that phosphorus is readily released from both deep and shallow water sediments[3] (Boulton et al. 1995, Wilson 2001). Overall shallow water sediments have been found to release more Soluble Reactive Phosphorus (SRP) and Total Phosphorus and also contain more organic matter than deep-water sediments (Wilson 2001).

[3] In this study shallow was defined by a depth of less than 0.5m whilst deep included locations of between 4-6m in depth.

3.0 Cyanobacteria

3.1 Background

Cyanobacteria have been found in fossils from pre-Cambrian times of 3.5 billion years ago (Schopf 2000 cited in Whitton & Potts 2000:32). These fossils were found in stromatolites and oncolites which were formed in aquatic environments. It is commonly agreed that cyanobacteria were the first organism to release elemental oxygen and produce organic matter and as such represent the evolutionary step between bacteria and plants (Speer 1997, Speer & Wagoner 1997, Fay 1983).

With this long history it could be considered surprising that so much mystery surrounds these oldest of organisms (Humm & Wicks 1980). A typical example that reflects this lack of understanding is the variety of names they are given. These include: Blue-Green Algae, Cyanophyta, Myxophyta, Cyanchloronata, and Cyanobacteria (Fay 1983). Most of this confusion in nomenclature can be ascribed to the confusion in scientific circles regarding the classification of these organisms. Most names have been based on morphological characteristics which themselves can be environmentally determined (Fay 1983).

Recently cyanobacteria have derived their name from phycocyanin, a bluish pigment which they use to capture light for photosynthesis (Speer 1997), however the cell itself mainly utilizes chlorophyll-*a*, the photosynthetic pigment found in green plants. It is believed that the chloroplast in plants is actually a symbiotic cyanobacterium taken up by a green algal ancestor of the plants in the Pre-Cambrian period (Van Holden 1999).

Being prokaryotic organisms, cyanobacteria contain no membrane bound organelles. This effectively limits their rate of evolutionary change, so much so that today's cyanobacteria are almost unchanged from those of 3 billion years ago (Golubic & Hofmann 1976 cited in Whitton & Potts 2000:56). Cyanobacteria are classified into 5 subgroups: Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales, and Stigonematales (Castleholz & Waterbury 1989 cited in Whitton & Potts 2000:6).

Cyanobacteria have found many niches in the environment. They are responsible for a large part of nitrogen fixation in soils through their symbiotic relationship with legumes. They also co-exist with the aquatic fern species of azolla which is ubiquitous in water storages and tributaries in Australia (Fay 1983, Speer 1997). Another notable symbiotic relationship with fungi has produced complex organisms known as lichens which play a vital role in the colonization of bare sand and other inhospitable environments (Humm & Wicks 1980, Fay 1983, Speer 1997).

In addition the pleasing colours of coral, sponges, coral clams and the African Flamingo are due to cyanobacteria (Wood 1975) whilst some species of cyanobacteria are used for human and animal consumption. The cyanobacteria species Spirulina was an important component of the Aztec diet and is still popular constituent of oriental cuisine (Bushkin & Bushkin 2004). Non-toxic sources of Microcystis have also been grown on domestic waste and fed to fish in lagoons and fish ponds in India and Pakistan (Fay 1983, Speer 1997).

In everyday life cyanobacteria can be seen growing in fish tanks, salt marshes, trunks of trees, damp rocks and stones (Fay 1983, Van der Wall 1995). However, the most obvious manifestation of cyanobacteria is the ever increasing occurrence of dense cyanobacteria mats (blooms) which form on the surface of many water bodies throughout the world and which are a major concern to water users everywhere.

Although cyanobacteria have been present on the earth for 3.5 billion years the first documented case of a cyanobacteria bloom was by George Francis in 1878. Francis identified the cyanobacterium Nodularia *spumigena* near Adelaide, South Australia. Since that time 'algal blooms' have been recorded consistently throughout Australia (Van der Wall 1995, Long 1997).

Cyanobacteria blooms have the potential to greatly affect the economic and social well-being of communities as well as the health of aquatic ecosystems (Chapter 3.7). The biggest concern is the potential of certain bloom forming

species to produce toxic substances, which can be released into the water column and affect humans (Codd & Poon 1988, Carmichael 1989).

Although stock deaths, fish kills and wildlife deaths have been attributed to toxic algal blooms, to date there have been no direct human fatalities (Francis 1878, McBarron & May 1966, Mulhearn 1959). There have however been cases of epidemics such as on Palm Island in 1979 which left 148 people (mainly children) suffering from hepatoenteritis (Byth 1980, Hawkins et al. 1985) and the death of 43 dialysis patients in Caruaru (Brazil) which are believed to be related to cyanobacteria toxins (Pouria et al. 1998).

The most common nuisance bloom forming species include (Jones 1994)[4]:

- Gomphosphaeria
- Coelosphaerium
- Microcystis
- Oscillatoria
- Spirulina
- Trichoesmium
- Anabaena
- Anabaenopsis
- Aphanizomenon
- Gloeotrichia
- Cylindrospermopsis

3.2 The Cyanobacteria Cell

3.2.1 Cell Structure

The typical prokaryotic cyanobacteria cell ranges from 2-6μm in diameter as in the case of the single cell species microcystis (Reynolds et al. 1981, Braby 2001). However filamentous forms may form colonies from cells and can grow up to 220μm in length (Whitton & Potts 2000). Cells appear homogenous in appearance due to the lack of membrane bound organelles. There are two distinct regions within the cyanobacteria cell (neucleoplasm); the central region and the peripheral region (Figure 3.1).

[4] Microcystis and Anabaena are the only species to date which have been recorded in Malpas Dam.

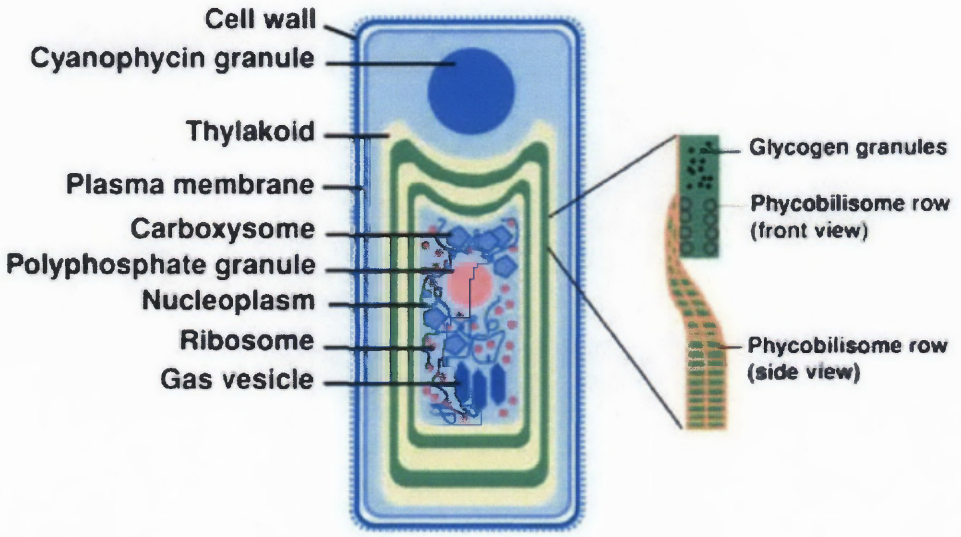


Figure 3.1 Cell structure of cyanobacteria (filamentous forms).

The central region contains the ribosomes, the circular DNA of the cyanobacterial cell and the photosynthesis apparatus for filamentous forms of cyanobacteria. The peripheral region comprises cell wall, the plasmalemma, and a gelatinous sheath. Within this is located the photosynthetic apparatus of single cell cyanobacteria

3.2.2 Reproduction and Colonial Formation

Cyanobacteria exist in single cell colonies or in filamentous strands. Single cell species of cyanobacteria may exist in unicellular form or in a loose colony (Plate 1) held together by a gelatinous matrix secreted by the growing colony. These are the simplest type of cyanobacteria (Whitton 1992). Most unicellular cyanobacteria cells reproduce by binary fission (trichome fragmentation) with the exception of the sub-groups Chamaesiphon and Pleurocapsales (Fay 1983). The more complex and more abundant taxa of cyanobacteria are the filamentous forms (Plate 2). These are distinguished by their mode of reproduction, which can be by multiple fission or binary fission or the production of akinetes (Plate 3).

Plate 1 Unicellular Cyanobacteria Cell (Mycrocystis *spp.*) (Purdue University 2002).

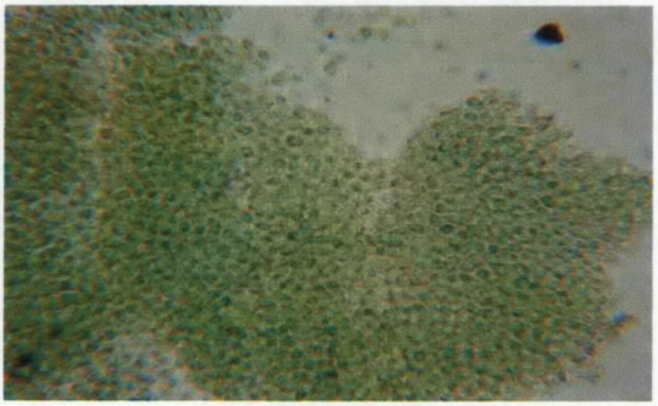
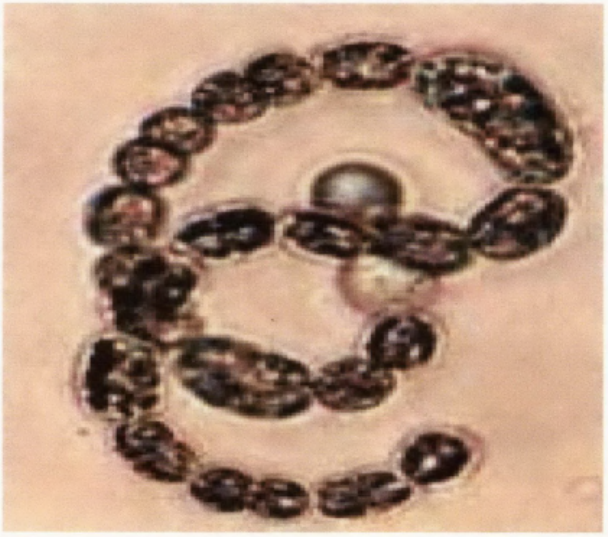


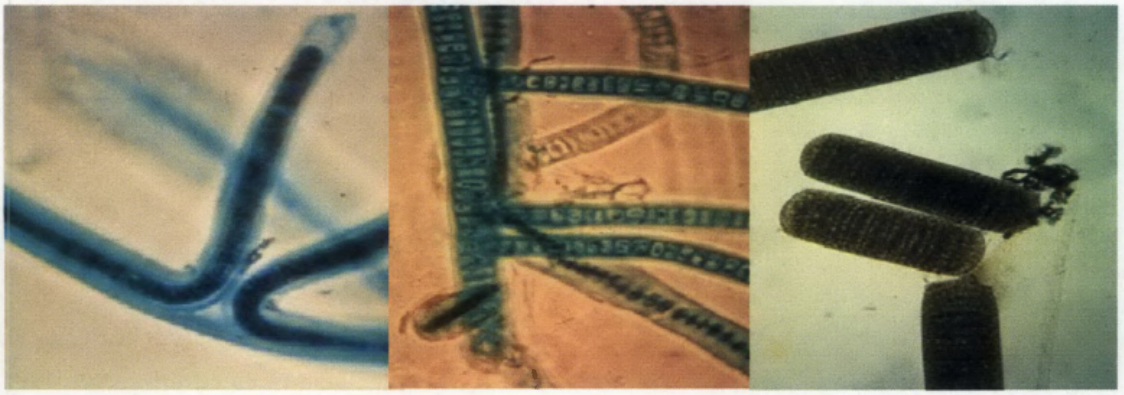
Plate 2 Filament of Anabaena circinalis cells (Anabaena *spp.*) (Purdue University 2002).



Trichome fragmentation may involve a single fragmented cell or segment fragments of between 5-15 cells of the main trichome. These fragments are known as hormogones (Plate 4). Oscillatoria, Rivularia, and Fischerella reproduce by this method. Trichomes are prone to branching (Plate 3). It can be false branching or true branching. False branching is when division in a trichome results in two separate independent trichomes whereas true branching occurs when the cells in a trichome have the potential for dividing in more than one plan (Whitton & Potts 2000).

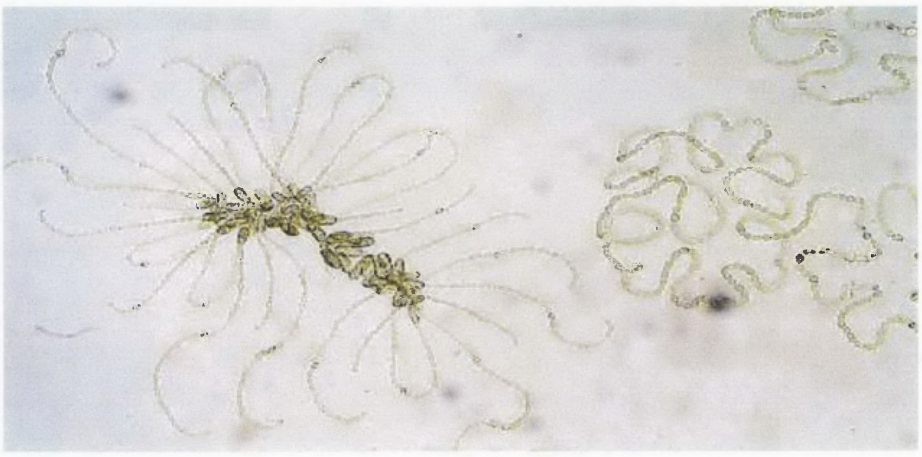
An aspect of akinete reproduction is that only 10-15% of the akinetes will germinate in the unfavourable environment in which they where sporned (Plate 4). Most will germinate at a latter date when conditions are more favourable (Garcia 2003). Some akinetes have been shown to be still capable of germination several years after being sporned (Fay 1983).

Plate 3 False branching (Scytenoema), true branching (Stigonema) and Hormogonia (Florida International University 2002).



Filaments covered by a protective mucilaginous sheath are known as trichomes. They generally contain three types of cells: vegetative, akinetes, and heterocyte cells. Environmental conditions determine the relative abundance of each.

Plate 4 Young filaments germinating from akinetes (Anabaena *spp.*) ((Florida International University 2002).



Vegetative cells are normal, photosynthetic cells formed under favourable growing conditions whilst akinetes are formed exclusively by heterocytoues

filamentous cyanobacteria from vegetative cells that have been transformed into spore like cells in response to unfavourable environmental conditions (Garcia 2003). During transformation the vegetative cell undergoes morphological-structural changes as well as changes in the bio-chemistry and the physiology of the cell. The location of akinetes in the trichome varies between genus of cyanobacteria, however there is some relationship between these cells and heterocyte cell location (Garcia 2003). Apart from this, there is very little known about the factors that induce akinete production (Whitton 1992). Given the varied means of reproduction there is associated variation in generation rates between species.

Under optimum conditions most species of cyanobacteria generate in 12-36 hours (Mori et al. 1996, Ouyang et al. 1998). Anabaena is the exception to this with a mean generation time of only 4.3 hours whilst Mycrocystis *spp.* generate on average every 2 days under optimal conditions. The fastest of all cyanobacteria is the thermophlic Synechococcus (Anacystis *nidulans*) which generates every 2 hours (12 generations per day). It has been shown that generation rates increase when more reduced forms of carbon and nitrogen are supplied (like sugars and ammonia) (Fay 1983)[5]. Blooms usually only last for 5-7 days (Van der Wall 1995).

3.3 Photosynthesis

Cyanobacteria are mostly photoautotrophs in that they depend primarily on light as the source of energy for photosynthesis (Van der Wall 1995) and carbon dioxide (CO_2) as the source of carbon. Heterotrophic and photo-heterotrophic cyanobacteria also exist although there are comparatively fewer species. The habitat for these species tends to be extreme such as the bottom of lakes in the euphotic zone, digestive tracks of animals (Van der Wall 1995), attached to coral sponges (Singapore Zoological Gardens Docents 2000) and in arctic permafrosts (Friedmann 2003).

[5] See chapter 3.0 for conditions conducive for bloom formation

Photosynthesis carried out in aerobic environments produces oxygen (Equation 1), whilst under anaerobic conditions other substances such as H_2S are produced (Fay 1983).

Equation 1

$$6CO_2 + 12H_2O + light\ energy \Rightarrow C_6H(12)O_6 + 6O_2 + 6H_2O$$

Oxygenic photosynthesis in cyanobacteria is a two phase operation. Phase I is known as the 'Light Phase' and incorporates two photosystems (I & II). The light phase is schematically referred to as the Z-diagram (Figure 3.2). This phase provides the energy required to reform the high energy compounds Adenosine triphosphate (ATP) and Nicotinsmide adenide dinucleotide phosphate (NADPH). These compounds then act as electron donors in Phase II or 'Dark Phase' reactions of the Calvin Cycle. The Calvin Cycle produces glucose and other carbohydrate molecules from CO_2 and energy provided by the ATP and NADPH from 'light phase' reactions through phosphorylation and oxidation (Equation 2) (TLPCF 2003).

Equation 2

$$6CO_2 + 12NADPH + 12H_2O + 18ATP \Rightarrow C_6H_{12}O_6 + 12NADP^+ + 18ADP + 18P_i$$

Two primary photo-reactions occur in cyanobacteria photosynthesis. Both reactions utilize chlorophyll-*a* (the only type of chlorophyll found in cyanobacteria) with varying dependence on other proteins such as phycocyanin and phycobilin (Photosystem I relies mostly on chlorophyll-*a* whilst Photosystem II is dependent on phycobilin proteins).

Chlorophyll-*a* has two major adsorption peaks (corresponding to red light, 420nm and blue light, 662nm) and four lesser peaks. *C*-phycocyanin adsorbs at 275 and 365nm in the ultra violet range and also at 615-620nm (yellow light) (Humm & Wicks, 1980). Phycobilin pigments ionize at wavelengths at which

chlorophyll a is not sensitive and subsequently provide the chemical energy at times of poor light quality, or light limited conditions. This provides an advantage over other forms of algae, which are dependent solely on chlorophyll-*a* for energy harvesting.

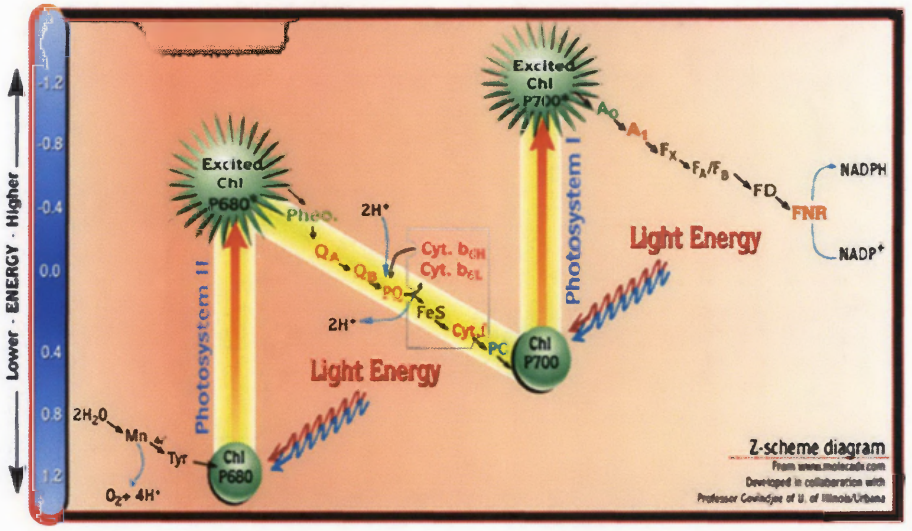


Figure 3.2 **The Z-diagram (Light Phase reactions): The electron transport pathway from water (H_2O) to NADP+ (the Nicotinamide Adenine Dinucleotide Phosphate, oxidized form)[6](Govindjee 2003).**

[6] **"Abbreviations used are (from left to the right of the diagram):** Mn **for a manganese complex containing 4 Mn atoms, bound to Photosystem II (PSII) reaction center;** Tyr **for a particular tyrosine in PSII;** O_2 **for oxygen;** H+ **for protons;** P680 **for the reaction center chlorophyll (Chl) in PSII: it is the primary electron donor of PSII; Excited (Chl) P680 for** P680* **that has the energy of the photon of light;** Pheo **for pheophytin molecule (the primary electron acceptor of PSII; it is like a chlorophyll *a* molecule where magnesium (in its center) has been replaced by two "H"s);** Q_A **for a plastoquinone molecule tightly bound to PSII;** Q_B **for another plastoquinone molecule that is loosely bound to PSII;** FeS **for Rieske Iron Sulfur protein;** Cyt. f **for Cytochrome f;** $Cytb_6$ **(L and H) for Cytochrome b_6 (of Low and High Energy);** PC **for copper protein plastocyanin;** P700 **for the reaction center chlorophyll(Chl;actually a dimer, i.e., two molecules together) of PSI; it is the primary electron donor of PSI; Excited (Chl) P700 for** P700* **that has the energy of the photon of light;** Ao **for a special chlorophyll a molecule (primary electron acceptor of PSI);** A_1 **for a phylloquinone (Vitamin K) molecule;** F_X, F_A, and F_B **are three separate Iron Sulfur Centers;** FD **for ferredoxin; and** FNR **for Ferredoxin NADP oxido Reductase (FNR). Three major protein complexes are involved in running the "Z" scheme: (1)** Photosystem II**; (2)** Cytochrome bf complex **(containing $Cytb_6$; FeS; and Cytf) and (3)** Photosystem I**. The diagram does not show where and how ATP is made.**

Environmental conditions such as the quality of light, nitrogen, phosphorus, and iron concentration can influence the relative abundance of the photosynthetic pigments within cyanobacteria cells (Humm & Wicks 1980). This is known as complementary chromatic adaptation (CCA) and is found in those cyanobacteria, which synthesize phycocerythin. CCA allows for a higher degree of light harvesting and occurs mainly in the phycobilisomes, the light antenna of the cyanobacteria (Oliver & Ganf 2000:172).

3.4 Nitrogen Fixation

Many species of cyanobacteria can fix elemental nitrogen (Table 3.1). They represent the only photosynthetic oxygen-producing plants capable of nitrogen fixation. These nitrogen fixing species have the simplest external nutritional requirements of any living thing and are divided into three groups (Stal 2000:97):

I. Heterocystous[7] cyanobacteria.
II. Anaerobic N_2-Fixing non-heterocystous cyanobacteria
III. Aerobic N_2-Fixing non-heterocystous cyanobacteria.

Table 3.1 Common nitrogen fixing species of cyanobacteria (adapted from Stal 2000: 97)[8].

Type I	Type II	Type III
Anabaena	Plectonema boryanum	Gloeothece
Nostoc	Oscillatoria limnetica	Oscillatoria
Aphanizomenon	Synechococcus sp.	Trichodesmium
Nodularia		Lyngbya
Calothrix		Microcoleus
Scytonema		

[7] Heterocytes are the site of nitrogen fixation in filamentous cyanobacteria which develop in response to nitrogen limitation (Fogg et al. 1973, Howarth et al. 1998 cited in Oliver & Ganf 2000:177). This has given rise to speculation that these species of cyanobacteria become dominant in conditions where nitrogen stress provides them an advantage over other species (Whitton & Potts 2000).

[8] Many other species of cyanobacteria are capable of fixing nitrogen. This list presents some of the more common species.

Heterocytous cyanobacteria developed heterocytes (specialized cells) in response to the increase in atmospheric O_2 and to protect themselves from the O_2 evolved in their own photosynthetic action (Fay 1983). Heterocytes are the site of most nitrogen fixation in those species that have them. These species have no Photosystem II (Table 3.1). By retaining Photosystem I, heterocytous cyanobacteria are capable of generating reducing power and ATP in cyclic photoelectron transport coupled with photo-phosphorylation. They are also able to meet cell requirements, though less efficiently, in O_2 dependent dark respiratory metabolism (Stal 2000: 97).

Non-heterocytes species are able to fix nitrogen only in low exogenous concentrations of O_2. This process is sometimes aided by the presence of nitrogenase (an enzyme required for nitrogen fixation), which may be produced by the action of a few cells located in the centre of filaments which lack PSII (Humm & Wicks 1980).

The process of nitrogen fixation is a high energy consuming process (Equation 3). To reduce one N_2 molecule requires 16 molecules of ATP.

Equation 3

$$N_2 + 8e^- + 8H^+ + 16MgATP \Rightarrow 2NH_3 + H_2 + 16MgADP + 16P_i$$

Although requiring much energy this aspect of cyanobacteria provides an advantage over non-nitrogen fixing algae and other planktonic competitors during nitrogen limited conditions. Under reduced oxygen tension vegetative cells may also fix nitrogen (Fay 1983).

3.5 Nutrition

3.5.1 General Nutritional Requirements

The nutritional requirements for cyanobacteria are more diverse than the basic green plant. Whereas most green plants have similar nutritional needs, cyanobacteria tend to differ between species. However, all cyanobacteria must

obtain their nutrients in the dissolved form (Wood 1975). In the case of nitrogen cyanobacteria preferentially assimilate ammonia (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), and nitrogen (N_2) (Tandeau & Houmard 1993 cited in Oliver & Ganf 2000:176).

Cyanobacteria may utilize essential and beneficial nutrients (Wood 1975). Essential nutrients are those that are needed to complete the cells life cycle, are not replaceable by another element, and participate directly in cell metabolism. Beneficial nutrients are replaceable and will not hinder cell metabolism in their absence or replacement (Table 3.2) (Wood 1975).

Table 3.2 General nutrient requirements of cyanobacteria[9].

Essential Nutrients	Beneficial Nutrients
carbon	sulfur
copper	calcium
iron	potassium
manganese	boron
molybdenum	chlorine
nitrogen	chrominium
phosphorus	cobalt
	copper
	silicon
	sodium
	titanium
	vanadium
	zinz

3.5.2 Response to Nutrient Limitation

Nutrient deficiency stimulates the storage of excess non-limiting nutrients of both essential and beneficial types in the cell due to their relative abundance (Stal 2000:175). These stores can later be used when other nutrients become available (Kromkamp et al. 1989). Often the limiting nutrients tend to be phosphorus and nitrogen which are integral components in the photosynthetic functioning of the cell (P) and the production and regulation of gas vacuoles within the cell and hence buoyancy (N) (Oliver & Ganf 2000:176).

[9] It should be noted that even though sulphur, potassium and calcium are required by all algae they are replaceable and are thus considered beneficial nutrients (Wood 1975).

3.6 Mobility

Mobility of cyanobacteria is not limited by their lack of flagella[10] or cilia, which other algae utilize. They overcome this deficiency by gliding/undulation, rotation, jerking, flicking and swaying of cells in contact with a solid surface (Brahamsha 1999). These forms of movement do not promote dispersion but do allow cyanobacteria to seek favourable environmental conditions within the water column and provide a competitive advantage over less motile algae which are subject to the force of gravity and tend to settle out of the water column during stable conditions (Fay 1983). Movement is influenced by temperature, photon flux density, quality of light, pH, and the chemical composition of the medium (Fay 1983). Utilising these forms of movement speeds of up to 25μm/s have been reported for Synechococcus in sea water (Brahamsha 1999).

Possibly the most important type of movement cyanobacteria exhibit is due to the presence of gas vacuoles found within the cell. These expand and contract (inflate and deflate) in response to light intensity, nutrient availability and subsequent cell metabolism. In this fashion cyanobacteria can move vertically within the water column to attain nutrition and favourable light exposure (Fay 1983).

3.7 Deleterious Impacts of 'Cyanobacteria Blooms'

There are many problems associated with blooms of cyanobacteria. Possibly the most hazardous is the ability of some species to produce toxins. Other issues relate to polysaccharides produced during cell growth and carcinogenic trihalomethane by-products produced from the chlorination of cell material. In addition, objectionable taste and odours in drinking water are common aspects of blooms whilst increased treatment costs of this water and decreased recreational utility and aesthetic value of the water body further add to the overall negative impact of blooms. Another aspect is the large amounts of oxygen required for

[10] Cilia and flagella are microtubal projections from the cell. They are motile and designed either to move the cell itself or to move substances over or around the cell. The primary purpose of cilia in mammalian cells is to move fluid, mucous, or cells over their surface. Cilia and flagella have the same internal structure. The major difference is in their length.

the biological breakdown of cell material following the decline of a bloom (Hawkins & Tansitt 1995). This increased biological oxygen demand (BOD) can lead to anoxic conditions at the sediment/water interface which in turn favours hydrogen sulphide production as well as reduction (resolublisation) of iron and manganese and possible concurrent release of bound nutrients (Boulton et al. 1995). From this arrises further issues regarding taste and odour, and eutrophication of the water as well as the possibility of fish kills due to hypoxia.

3.7.1 Toxicity

In Australia a dense bloom of cyanobacteria has an approximate 60% chance of being toxic (Blackburn et al. 1997). However the causes and reasons why this is the case are still largely conjecture. In the past apparently similar blooms have shown dissimilar toxin characteristics with sudden and dramatic changes even occurring within the same bloom.

Numerous researchers have attempted to elucidate the causes of this, however it is only recently with improvements in technology that reliable results have been obtained. Environmental factors such as nitrate, phosphate, temperature and radiance have been shown to affect toxin concentrations by up to an order of magnitude however they are not responsible for 'triggering' toxin production within a bloom (Sivonen 1990, Codd & Bell 1995, Blackburn et al. 1997). The most promising evidence to date attributes toxin production to genetic factors within and between species with some influence from geographical location also (Jones 1994, Blackburn et al. 1997).

Once toxins are produced by cyanobacteria they become a significant health concern due to their potentially fatal toxicity and continued persistence. Each toxin type: neurotoxin, hepatotoxin, endotoxin and cytotoxin behave differently in aquatic systems.

Neurotoxins have been shown to have three modes of action on the nervous system of affected animals, however the result is the same in each case, death by paralysis of the peripheral skeletal muscles, then the respiratory muscles then respiratory arrest (Reesom et al. 1990). The neurotoxins (anatoxin-a, anatoxin-

a(s), homoanatoxin, aphanotoxins I/II) have LD_{50}[11] values of 10-250µg/kg body weight. Ageing of blooms results in an increase in toxicity as the toxins change to the more toxic dx-GTX toxins (Blackburn et al. 1997). Degradation of minor amounts through the action of micro-organisms (2.4-30µg/mL) may occur over short periods of time however the main mechanisms for breakdown (chemical deactivation) may take up to 90 days to completely neutralise all neurotoxins present (Kirvanta et al. 1991, Blackburn et al. 1997).

Hepatotoxins breakdown the cell-cell connections and intracellular architecture in the liver of the affected animal (Falconer et al. 1983a, 1983b, Galey et al. 1987, Beasley et al. 1989). The animal generally dies due to a massive blood haemorrhage in the liver. Hepatotoxins (microcystin and nodularin) have an LD_{50} of 60-200µg/kg body weight and can act at the acute and chronic levels. The toxicity is variable depending on the method of dosing, the species affected, and the toxin variant present. Degradation of microcystins in natural water is primarily due to the action of micro-organisms (Pseudonomas *spp.*) and bacteria (Spingomonas), which can degrade up to 500µg/L per day (Codd & Bell 1995b). Degradation will generally proceed soon after toxins are produced but may proceed only after an initial lag phase of 1-4 weeks if the water source has little history of bloom formation (due to the absence of already established microorganisms suitable to aid breakdown) (Rapala et al. 1994). Once degradation has begun, most (95%) of the toxin will be deactivated within 3 weeks (Jones & Orr 1994).

Endotoxins have LD_{50} values in the order of mg/kg body weight and are a natural component of the outer cell membrane of the cyanobacterial cell; as such they will be present for the duration of a bloom. Endotoxins produce skin irritations, vomiting, diarrhoea and other minor maladies (Baker & Fawell 1990, Codd & Bell 1995b). There is also some evidence to suggest that they also act on other algae to increase the competitiveness of cyanobacteria.

[11] Dose inducing 50% mortality in test subjects of intraperetonealy dosed mice.

The cytotoxin characterised from Cylindrospermopsis raciborskii is a subtle toxin with death occurring days after poisoning due to general changes in most organs of the affected animal (Falconer et al 1993a). Literature on its degradation and resistance is not available.

The persistence of cyanobacterial toxins and their continued threat is increased through the action of bioaccumulation. A number of aquatic organisms are capable of this including fish, filter feeders (muscles, oysters) and zooplankton (rotifers etc.) Consumption of these organisms should be avoided if taken from environments that feature regular toxic blooms.

3.7.2 Treatment and Removal of Toxins from Freshwater

Removal of toxins from potable water sources has been achieved, however, this has generally involved the use of technology exceeding the provisions of most water treatment plants. Traditional methods such as slow sand filtration, alum flocculation and sedimentation have shown no success (Hoffmann 1976, Keijola et al. 1988, Himberg et al. 1989, Falconer et al. 1989). Procedures such as flocculation can actually exacerbated the problem by lysing cells and releasing intracellular toxin into the water to be treated (Baker & Fawell 1990). Initial trials with different forms of chlorine proved unsuccessful however recent studies have shown that it is very capable of deactivating hepatotoxins depending upon the type and dose of chlorine used (Brenton et al. 1997). It was however unsuccessful at removing neurotoxins. At first glance this is an attractive option for treatment plants that already have provisions for chlorination and have only hepatotoxic blooms, however the residual chlorine concentration of 1.0mg/l that is required in the treated water can lead to the formation of carcinogenic by-products.

Powdered activated carbon has shown wide application and success in removing microcystins however this procedure also has its operational problems (Falconer et al. 1983b, Jones et al 1993). The efficiency of activated carbon adsorption of toxins is dependant on the initial concentration of toxin, form of the activated carbon, the type of carbon used, time period between carbon dosing and flocculation, as well as the contact time (Falconer et al 1983b, Himberg et al.

1989, Jones et al. 1993). In water treatment plants this information is not always available at the required time, which may lead to ad-hoc procedures in carbon dosing, and contact time.

Most oxidants have shown some ability to neutralise cyanobacterial toxins, their ability being directly proportional to their oxidative potential. The most promising albeit initially expensive procedure involves oxidation utilising ozone. Ozonation has been shown to detoxify all cyanobacterial toxins (Himberg et al. 1988, Keijola et al. 1988). Not unlike PAC the efficiency of ozonation is also dependant on the initial concentration of toxin as well as dosage and contact time of the ozone. However, one significant advantage is that the by-products of ozonation are of a non-toxic nature (Jones & Fawell 1991).

3.7.3 Polysaccharide Production

The polysaccharides produced by cyanobacteria are considered to be the same as the non-toxic polysaccharides produced by plants and have been shown to be resistant to degradation and can persist in water for some time. It is uncertain whether these polysaccharides are released during growth or during decay. Some of their many functions include adhesion and immobilisation of the organism, protection against desiccation, protection from grazing and protection from toxic substances (Decho 1990 cited in Stal 2000:89).

The main problem associated with these harmless products is that they cause foaming in the water treatment process and can also foam when badly affected water is boiled. This is a particular problem for softdrink manufacturers as it causes precipitation in carbonated softdrinks. Typically a white colloidal floc is produced when the pH is around 2-3.

3.7.4 Trihalomethane Precursors

Trihalomethane (THM) precursors are formed from the chlorination of organics in raw water. Algal biomass can contribute to this load of organics resulting in high levels of THM. These are thought to be carcinogenic (Florida International University 2003).

3.7.5 Taste and Odours

There are two main types of odours associated with cyanobacteria: earthy or musty smells due to the presence of geosmin or 2-methylisoborneol (2-MIB) (a substance produced during growth of cells), and a foul rotten smell which is caused by sulphurous compounds (such as H_2S, rotten egg gas) produced during anaerobic bacterial decay of the cell material (Lanciotti et al. 2003).

Geosmin and 2-MIB have extremely low odour threshold capacities (OTC) of 0.004-0.2μg/L and 0.009-0.1μg/L respectively. One teaspoon of geosmin in an Olympic sized pool is detectable by human sensors. Products such as metabolites (beta-cyclonitral) or organic compounds (fatty acids, hydrocarbons etc) produced by cyanobacteria may also contribute to taste and odour (Lanciottie et al. 2003).

3.7.6 Treatment Plant Operation

Cyanobacterial blooms affect both the physical and the chemical side of the water treatment process. The physical presence of excessive algal cells increases turbidity, reduces the efficiency of disinfection and can block filters and sprinkles leading to increased maintenance costs (M. Porter 1998 pers. comm. 3 August 1998). The chemical impacts of algae are associated with their ability to diurnally change the pH of the water they inhabit. This may lead to changes in the form and toxicity of heavy metals and ammonia as well as altering chemical reactions involved in the water treatment process (Queensland Water Quality Task Force 1992).

3.7.7 Recreational and Aesthetic Uses

In Australia and abroad many water storages and rivers are closed to the public for extended periods due to the presence of cyanobacterial blooms. The reasons for this are those already mentioned, namely toxins and disagreeable tastes and odours. This can result in significant financial loss to resorts and holiday destinations as well as to manufacturers of food and drinks which rely on contaminated water as their main supply.