CHAPTER 1:

INTRODUCTION

Water bodies, both natural and man-made, are impacted by urban, industrial and agricultural activities. As a result, many aquatic ecosystems have become severely degraded and need to be restored to a level that can be permanently sustained through conservation and protection. The water quality targets should be in accordance with the quality of natural waters that are without the stress factors that cause degradation (Klapper, 2003).

Of the problems currently being experienced with natural and man-made water bodies, eutrophication is one of the most important. Eutrophication is the enhancement of the natural process of biological production in rivers, lakes and reservoirs, caused by an increase in nutrient levels, usually phosphorus and nitrogen compounds (Bartram *et al*., 1999). These increased nutrient levels usually result in an increased phytoplankton biomass, which is often dominated by toxic cyanobacterial species. The decay of the increased amount of organic matter may lead to the oxygen depletion in the water, which in turn can cause secondary problems such as fish kills from lack of oxygen. Eutrophication has a severe impact on the water quality and impairs the use of water for drinking, industry, agriculture and recreation (Carpenter *et al*., 1998).

Both the causes and effects of eutrophication need to be considered when restoring a eutrophic water body. The control of toxic cyanobacterial blooms remains a priority, but in treating the blooms in isolation, only a symptom of a greater problem is being addressed. Eutrophication management by reducing nutrient input as well as the internal source is the only feasible means of reducing the incidence of cyanobacterial blooms. It is important that the amount of nutrients entering eutrophic water bodies be drastically reduced. However, the time needed to restore eutrophic lakes and dams to their natural healthy state is longer than expected, as in many shallow lakes the phosphorus accumulated in the sediment may be many times greater than that in solution. This delays the effects of restoration measures through internal loading of phosphorus into the overlying water (Sondergaard *et al*., 2001; Lake *et al*., 2007).

Nutrient limitation through intervention is likely to be the most sustainable solution to eutrophication and its effects. Phosphorus limitation has been identified as being more achievable than nitrogen limitation, and there are chemicals available to achieve this, such as aluminium sulphate (alum), ferric salts (chlorides and sulphates), ferric

aluminium sulphate, clay particles and lime. These, however, have various disadvantages (Chorus & Mur, 1999; Lewandowski *et al*., 2003). In this study an environmentally friendly phosphorus removing product, Phoslock®, was reviewed, characterised and tested under both laboratory and field conditions.

Limiting the amount of phosphorus in a water body, and thus increasing the N:P ratio, is likely to affect the entire microbial community composition, not only that of the cyanobacteria and algae. The various methods available to investigate the structure of microbial communities were reviewed in this study. Denaturing gradient gel electrophoresis (DGGE) was the molecular tool chosen for this study to determine the effect of a reduced phosphorus concentration on the cyanobacterial and bacterial community composition in a field trial.

A bacterial species isolated from a eutrophic dam with cyanobacteriolytic capabilities was examined in the laboratory. The effect of combining this potential biological control agent with Phoslock® was investigated in order to determine whether the two agents could be used together to treat both the cause and symptoms of eutrophication simultaneously, with the Phoslock® treatment removing the phosphates released from the lysed cyanobacterial cells.

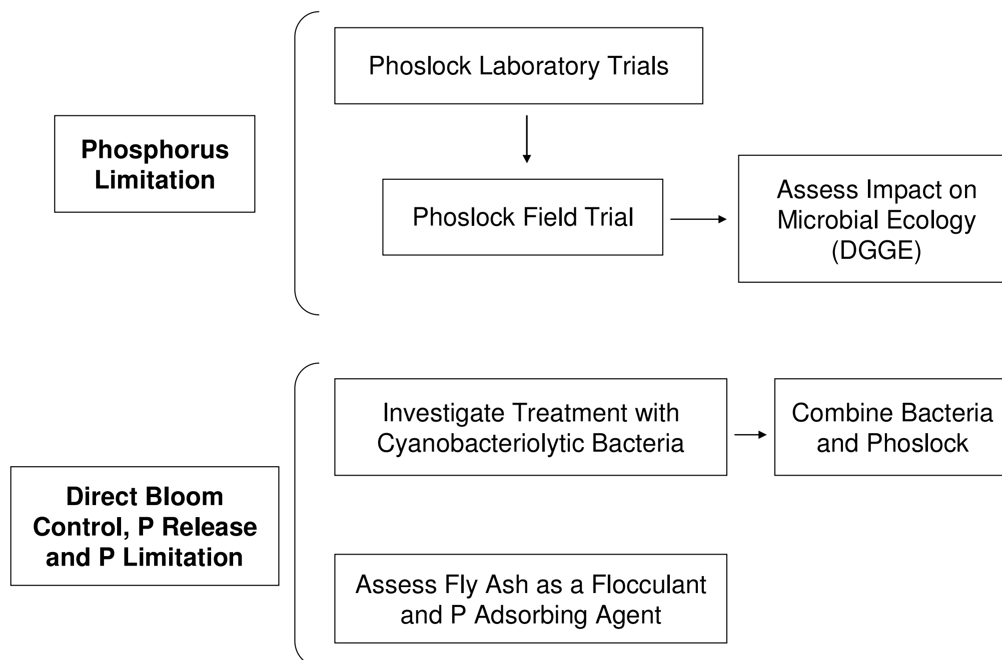
Various flocculants have been investigated for cyanobacterial removal in wastewater treatment as well as in natural water bodies. These include synthetic organic polyelectrolytes, chitosan, and various clays (Divakaran & Pillai, 2002; Sengco & Anderson, 2004; Pan, *et al*., 2006). In this study the use of fly ash, a waste product in the burning of coal for electricity generation, was investigated as a potential cyanobacterial flocculant, and its phosphate adsorbing capability was tested as an alternative to Phoslock®.

The objectives of this study were therefore:

- To characterise Phoslock® in the laboratory in terms of its kinetics, and the effect of pH and initial phosphorus concentration on the adsorption capacity.
- To determine the effectiveness of Phoslock® in water containing a high concentration of cyanobacteria with a high pH, in the laboratory as well as in a field trial.

- To assess the effect of a Phoslock® treatment and a reduction in phosphate concentration on the cyanobacterial and eubacterial species composition of a eutrophic water body using 16S PCR-DGGE.
- To identify a bacterial species isolated from Hartbeespoort Dam that appeared to have lytic activity towards *Microcystis aeruginosa*, and to determine the critical predator-prey ratio for treatment.
- To assess the possibility of combining the lytic bacteria with Phoslock® in order to produce a novel biological control product that can treat the cause of cyanobacterial blooms as well as the bloom itself, and remove the P released after bloom collapse.
- To characterise the chemical and physical properties of various fly ash samples, and test the samples for their ability to flocculate cyanobacteria and remove phophates from the water by adsorption.

The objectives can thus be illustrated as follows:



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CHAPTER 2:

LITERATURE REVIEW

1. Introduction

Eutrophication is the enhancement of the natural process of biological production in rivers, lakes and reservoirs, caused by an increase in nutrient levels, usually phosphorus and nitrogen compounds. Eutrophication can result in visible cyanobacterial or algal blooms, surface scums, floating plant mats and benthic macrophyte aggregations. The decay of this organic matter may lead to the oxygen depletion in the water, which in turn can cause secondary problems such as fish kills from lack of oxygen and liberation of toxic substances or phosphates that were previously bound to oxidized sediment. Phosphates released from sediments accelerate eutrophication, thus closing a positive feedback cycle. Some lakes are naturally eutrophic, but in many others the excess nutrient input is of anthropogenic origin, resulting from municipal wastewater discharges, industrial effluents and runoff from fertilizers and manure spread on agricultural areas (Bartram *et al*., 1999). Nutrient enrichment seriously degrades aquatic ecosystems and impairs the use of water for drinking, industry, agriculture and recreation (Carpenter *et al*., 1998).

Extensive cyanobacterial growth poses several severe implications on the general water quality as well as the maintenance of water treatment standards set for potable water. Massive cyanobacterial blooms can deplete the dissolved oxygen content resulting in fish kills and discolouration of the water by pigments released from the cells (Rae *et al.*, 1999). Because of their relatively small cell size, cyanobacteria easily penetrate and clog the fine sand filters and the primary coarse fast filters that are fundamental stages in drinking water purification (Botha-Oberholster, 2004). Biodegradation of cyanobacterial blooms contributes to the organic load of the water resulting in increased treatment costs. Non-toxic nuisance compounds such as geosmin and 2-methylisoborneol (2-MIB) that cause taste and odour problems in both dam and purified waters have been associated with cyanobacteria (Rae *et al.*, 1999).

Of greater importance is the fact that certain cyanobacteria produce toxic compounds, the consumption of which present severe health risks. Bloom-forming cyanobacterial genera with toxin producing members include *Microcystis*, *Anabaena*, *Anabaenopsis*, *Planktothrix*, *Aphanizomenon*, *Cylindrospermopsis*, *Raphidiopsis* and *Nodularia* (Codd *et al*., 2005). The genus of most concern as a toxin producer is *Microcystis*,

predominantly *M. aeruginosa* (Botha-Oberholster, 2004). Scum formation is particularly common with *Microcystis*, *Anabaena*, *Anabaenopsis*, *Planktothrix*, and *Aphanizomenon*, and less so with the remaining genera. Mat- and biofilm-forming genera with toxigenic members include *Phormidium*, *Ocsillatoria* and *Lyngbya* (Codd *et al.*, 2005).

The dominance of cyanobacteria in water bodies is a function of many contributing factors, all of which play a role in their superior competitive ability. Cyanobacteria have a unique physiology when compared with other phytoplankton, especially in terms of their nutrient biochemistry and buoyancy. Control of cyanobacteria is a challenge, and various methods have been employed in an attempt to reduce the severity of blooms. Some of these, including biological and chemical control, aim to treat the effects of eutrophication by treating the bloom, whereas other methods focus on managing eutrophication itself by curbing the nutrient import into lakes and reducing existing high nutrient levels.

Besides the phytoplankton, other members of the microbial community of a water body will be affected, either directly or indirectly, when the water chemistry and physics are altered. Shifts in the phytoplankton composition in response to treatment measures as well as other changes in the microbial community structure can be quantified and qualified using various methods, which are reviewed here.

2. Toxins

Mechanisms of cyanobacterial toxicity are very diverse and range from hepatotoxic, neurotoxic and dermatotoxic effects to general inhibition of protein synthesis. Toxicosis associated with cyanobacterial populations and their toxins affect wild and domestic mammals, birds and fish, with human cases ranging from mild to fatal (Codd *et al.*, 2005). Cyanotoxins fall into three broad groups of chemical structure, namely cyclic peptides, alkaloids and lipopolysaccharides (endotoxins).

2.1. Hepatotoxic cyclic peptides- microcystins and nodularin

2.1.1. Microcystins

Hepatotoxins have been most often implicated in cyanobacterial toxicosis (Codd *et al*., 2005). The hepatotoxic microcystins (MC) are produced by members of several cyanobacterial genera, including *Microcystis, Anabaena, Planktothrix* (*Ocsillatoria*), *Nostoc*, *Anabaenopsis* and *Hapalosiphon* (Carmichael, 1992). Microcystins are cyclic heptapeptides consisting of seven amino acids, including several D-amino acids and two unusual amino acids, namely N-methyldehydroalanine (Mdha) and a hydrophobic b-amino acid, 3-amino-9-methoxy-2-6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda) (Wiegand & Pflugmacher, 2005). The presence of Adda is essential for expression of biological activity (Gupata *et al*., 2003). There are over seventy different MCs, which differ mainly in the two L-amino acids at positions 2 and 4. The most common, and also the most extensively studied, are MC-LR, MC-RR and MC-YR (Jos *et al.,* 2005), with the variable amino acids being leucine (L), arginine (R) and tyrosine (Y). MC-LR has been found to be the most potent of the three toxins in mouse toxicity studies, followed by MC-YR and MC-RR (Gupta *et al*., 2003).

Microcystins are synthesized non-ribosomally by a multifunctional enzyme complex, consisting of both peptide synthetases and polyketide synthetases coded by the *myc* gene cluster (Oberholster *et al*., 2006). Microcystin synthetase genes *myc*A, *myc*B and *myc*C have been identified in *Microcystis* (Dittmann *et al*., 1996) and *Anabaena* (Meißner *et al*., 1996) as well as in microcystin producing strains of *Nostoc* and *Oscillatoria* (Neilan *et al*., 1999). Strains of other non-toxic cyanobacterial genera contain genes for similar peptide synthetase genes. Microcystin production appears to be linked to the presence of the *myc*B gene and to the occurrence of specific adenylation domains within the *myc*ABC region, although some non toxic *Microcystis* strains contain *myc*B (Tillet *et al*., 2001).

The primary effect on health is toxicity to liver cells, as a consequence of selective transport mechanisms, which concentrate the peptide toxins from the blood into the liver (Falconer, 1994). Microcystins accumulate in vertebrate liver cells due to active transport by a highly expressed unspecific organic ion transporter of the bile acid carrier

transport system. Death of vertebrate animals is mostly the consequence of severe liver damage which begins with cytoskeletal disorganization and can include cell blebbing, cellular disruption, liquid peroxidation, loss of membrane integrity, DNA damage, apoptosis, necrosis, intrahepatic bleeding and untimely death by hemorrhagic shock (Wiegand & Pflugmacher, 2005).

One toxic mechanism of MC is the specific inhibition of protein phosphatases 1 and 2A (PP1 and PP2A) in both animals and higher plants. PP1 and PP2A are responsible for catalyzing the dephosphorylation of serine and threonine residues of phosphoproteins in eukaryotic cells, and have been shown to play an important role in the suppression of tumors in animal tissue (McElhiney, 2001). Inhibition of specific phosphatase enzymes results in hyperphosphorylation of proteins, which is exhibited by a breakdown of intermediate filaments of the cell cytoskeleton and a retraction of actin microfilaments. The cell distortion is such that the organizational structure of the liver itself is disrupted (Falconer, 1994). Chronic exposure to low concentrations of microcystins in drinking water may promote tumor growth in the human liver (Bourne *et al.*, 1996). It is the introduction of Adda into the hydrophobic groove at the catalytic site of the protein phosphatase that renders it inactive, and a covalent bond forms between the Mdha residue and the protein phosphatase molecule (Sivonen & Jones, 1999). Another toxic mechanism of MC-LR involves its binding to the β-subunit of ATP synthase, which may cause mitochondrial apoptotic signaling at high MC-LR concentrations (Wiegand & Pflugmacher, 2005). External signs of poisoning include weakness, pallor, cold extremities, heavy breathing, vomiting and diarrhea (Codd, 2000). Microcystins impair photosynthesis in aquatic plants, due to a significant decrease in chlorophyll a and b as well as carotenoids. The main fish organs affected by microcystins are the liver and kidneys, with symptoms similar to those described for mammals. Furthermore, the epithelial cells of the gills undergo degeneration and necrosis, and MC inhibits ion pumps such as Na^+-K^+, Na^+, HCO_3^- and Ca^{2+}-ATPases in fish gills (Wiegand & Pfugmacher, 2005).

Besides the above-mentioned toxic mechanisms, MC-LR enhances oxidative stress in animal cells, due to the formation of reactive oxygen species (Žegura *et al.*, 2004), loss of mitochondrial membrane potential and an increase in mitochondrial membrane permeability, all of which are steps to apoptosis. Similarly, in aquatic plants, exposure

to MC-LR enhances formation of hydrogen peroxide, thus increasing the oxidative stress on the plant (Wiegand & Pflugmacher, 2005).

The intracellular tripeptide glutathione acts as a co-substrate for the biotransformation enzymes glutathione S-transferases and for the antioxidative enzyme glutathione peroxidase. The hepatic glutathione content is a critical factor for preserving normal cellular redox balance and protecting hepatocytes from oxidative stress. In addition, cellular reduced glutathione is important for the regulation of cytoskeletal organization (Gupta *et al.*, 2003). Glutathione protects cells from the toxicity and oxidizing activity of MC-LR by binding to the α, β-unsaturated carbonyl group of Mdha. Binding to glutathione enhances the water solubility of the MC, aiding its excretion via the bile fluid, or, in the case of plants, deposition in the vacuole or binding to the cell walls (Wiegand & Pflugmacher, 2005). Microcystins cause depletion of hepatic GSH levels, with MC-LR causing a more significant depletion compared to MC-RR and MC-YR (Gupta *et al.*, 2003). Endotoxins, especially those of toxic cyanobacterial origin, reinforce the adverse effects of microcystins by inhibiting the activity of the glutathione S-transferases, which are the key enzymes in the detoxification of microcystins (Rapala *et al.*, 2002). It has been suggested that a similar detoxification mechanism occurs in plants, as the conjugation of MC-LR with glutathione has been demonstrated using glutathione transferases purified from the aquatic plant *Ceratophyllum demersum* (McElhiney *et al.*, 2001).

Rapala *et al.* (1997) indicated that external growth stimuli affect the levels of microcystins produced by certain cyanobacteria. Not only does the growth of *Anabaena* and *Microcystis* increase with increasing phosphorus levels, but the levels of intracellular microcystins also increase in a similar manner. Growth-limiting phosphorus concentrations decreased the concentration of microcystins in *Oscillatoria* species. This suggests that different cyanobacterial genera respond in a similar manner to the extracellular phosphorus concentration. High and low temperatures, compared to optimal growth temperatures decrease the toxicity or concentration of microcystins. MC-LR was detected more at lower temperatures and MC-RR at higher temperatures. Microcystin production is affected by light, as demonstrated by do Carmo Bittencourt-Oliveira *et al.* (2005) who analysed the presence of MC-LR in the cyanobacterium *Microcystis panniformis* Komárek *et al.* in different times during the light:dark (L:D)

cycle. Levels of MCs per cell were at least threefold higher during the day-phase than during the night-phase, with production peaking in the middle of the day phase. The same pattern was observed under a light:light (L:L) cycle, where the cellular MC content was twice as high as the L:D cycle. Therefore, in terms of toxin production, cyanobacteria express robust circadian rhythms that are independent of the cell division cycle.

Various algae-algae interactions have been observed in eutrophic systems through changes in the abundance dynamics of phytoplankton populations, and many of these interactions are attributed to microcystins (Wiegand & Pflugmacher, 2005). Kearns & Hunter (2001) showed that the presence of MC caused paralysis in the motile green algae *Chlamydomonas reinhardtii*, causing the cells to settle faster. *Microcystis aeruginosa* increased toxin production in the presence of a non-toxic culture of *Planktothrix aghardhii* (Engelke *et al.,* 2003). Singh *et al.* (2001) also found that MC-LR produced by *Microcystis aeruginosa* inhibited the growth and photosynthetic ability of non-toxic *Nostoc muscorum* and *Anabaena*, and increased cell lysis of these species. Interestingly, Hoeger *et al.* (2004) found that the highest microcystin levels were not found to coincide with the highest cell counts of *Microcystis aeruginosa*, but rather increased at the beginning of the exponential growth phase, when *M. aeruginosa* appeared to compete with *Anabaena circinalis* for the dominant position in a fresh water lake. After suppression of its competitor, microcystin levels decreased to previous low levels. These results seem to indicate that microcystins play a role in affording producers a competitive advantage in a eutrophic system.

McElhiney *et al.* (2001) indicated that terrestrial crop plants that were exposed to microcystins through contaminated irrigation water showed a greatly reduced crop quality and yield. In addition, the plants accumulated the microcystins in their tissues. The exposure of edible crop plants is a concern for human health, as the toxins may be carried through the food chain. Aquatic macrophytes also take up and accumulate microcystins. Saqrane *et al.* (2007) investigated MC-LR accumulation, detoxication and oxidative stress induction in the free-floating aquatic vascular plant *Lemna gibba* (Duckweed) by chronically exposing the plant to the toxin. Stress oxidative processes were determined by measuring changes in peroxidase activity and phenol compound content. Following MC exposure, a significant decrease of plant growth and chlorophyll

content was observed, and it was demonstrated that *L. gibba* could accumulate and bio-transform microcystins. Changes in the peroxidase activity and qualitative and quantitative changes in phenolic compounds were observed after 24h of exposure. Aquatic ecosystems where plants co-exist with toxic cyanobacterial blooms may suffer a negative ecological impact due to toxin bioaccumulation and biotransfer through the food chain.

The role of toxins in cyanobacteria is still not understood. Microcystins, as potent inhibitors of serine/threonine protein phosphatases, have been suggested to act as protective compounds against grazing zooplankton (Jang *et al*., 2003), as intracellular chelators inactivating free cellular Fe^{2+} (Utkilen & Gjolme, 1995), or to have some specific cell regulatory function (Rapala *et al.*, 1997). It has also been suggested that the natural function of toxic cyanobacterial secondary metabolites may be cell signaling and environmental signaling (Wiegand & Pflugmacher, 2005).

2.1.2. Nodularin

Nodularin is a cyclic pentapeptide with a structure closely related to that of microcystins, but showing less structural variation. Nodularin is composed of Adda and D-erythro-β-methyl-aspartic acid (D-MAsp) as well as *N*-methaldehydrobutyrine (Mdhb), which is similar to *N*-methaldehydroalanine (Mdha) in the microcystins (Wiegand & Pflugmacher, 2005). Nodularin-producing cyanobacteria of the genus Nodularia possess a microcystin synthetase gene orthologue, and therefore a similar biosynthetic pathway for toxin production (Neilan *et al*., 1999).

The mode of action of nodularin is very similar to microcystins, in that it inhibits the catalytic subunits of serine/threonine-specific protein phosphatases PP1 and PP2. Nodularin, however, does not bind covalently to the protein phosphatases (Sivonen & Jones, 1999). Nodularin has the same effects on plants and fish as microcystins (Wiegand & Pflugmacher, 2005).

2.2. Alkaloid toxins

The alkaloid toxins are diverse, both in their chemical structures and mammalian toxicities. Alkaloids, in general, are a broad group of heterocyclic nitrogenous compounds, usually of low to moderate molecular weight (Sivonen & Jones, 1999).

2.2.1. Saxitoxins

The saxitoxins (STX) are tricyclic, neurotoxic alkaloids, which are also known as paralytic shellfish poisons (PSPs) due to their occurrence and association with seafood. The name saxitoxin is derived from the mollusc from which it was first identified, *Saxidomus giganteus*, but the toxin is primarily produced by marine dinoflagellate planktonic species (Wiegand & Pflugmacher, 2005). In addition, saxitoxins are produced by some cyanobacteria, including *Aphanizomenon flos-aquae, Anabaena circinalis, Lyngbya wollei* and *Cylindrospermopsis raciborskii* (Sivonen & Jones, 1999). The toxin blocks neuronal transmission by binding to the voltage-gated Na^+ channels in nerve cells. By blocking the channel opening, the entering sodium flow is stopped which leads to muscle paralysis and death by respiratory arrest in mammals. The transport of STX through the food chain and bioaccumulation of these toxins is an important mechanism for the availability of these toxins to higher trophic levels (Wiegand & Pflugmacher, 2005).

2.2.2. Anatoxins

Three anatoxins have been described. Anatoxin-a (antx-a) and homoanatoxin-a are alkaloids described as secondary amines, whereas anatoxin-a(s) is described as a unique phosphate ester of a cyclic N-hydroxyguanidine structure. Anatoxin-a was first isolated from *Anabaena flos-aquae*, but has also been detected in other cyanobacterial species such as *Anabaena circinalis, Aphanizomenon* sp., *Cylindrospermum* sp., *Aphanizomenon* sp., *Planktothrix* sp. and *Microcystis aeruginosa* (Wiegand & Pflugmacher, 2005). Homoanatoxin-a is produced by *Oscillatoria* and anatoxin-(s) from *Anabaena flos-aquae* and *A. lemmermannii* (Sivonen & Jones, 1999).

The mode of action of anatoxin-a and homoanatoxin-a in birds and mammals is to mimic the neurotransmitter, acetylcholine, and by binding irreversibly to the nicotinic acetylcholine receptor. The irreversible activation of presynaptic acetylcholine receptors causes the sodium channel to be locked open. Muscle cells are over-stimulated by the inflowing sodium ions, and the depolarization causes a block in further electrical transmission, leading to paralysis. When respiratory muscles become affected, convulsions occur due to a lack of oxygen supply to the brain, and death by asphyxiation occurs. Anatoxin-a(s) is a potent acetylcholinesterase inhibitor, and is ten times more toxic to mice than anatoxin-a. Very few studies have shown anatoxin toxicity to aquatic organisms (Wiegand & Pflugmacher, 2005).

2.2.3. Cylindrospermopsin

Cylindrospermopsin (CYN) is a hepatotoxic cyclic guanine alkaloid (Codd, 2000), with a uracil moiety attached to a sulphated guanidine moiety. An intact pyrimidine ring is necessary for CYN toxicity. The toxin is produced by different freshwater cyanobacteria, including *Cylindrospermopsis raciborskii, Aphanizomenon ovalisporum*, *Umezakia natans, Rhaphidiopsis curvata* and *Anabaena bergii* (Wiegand & Pflugmacher, 2005).

In mouse studies, CYN causes liver, kidney, thymus and heart damage. Furthermore, CYN displays mutagenic and possibly carcinogenic activity. It induces DNA strand breakage and may disrupt the mitotic spindle, leading to chromosome loss (Wiegand & Pflugmacher, 2005). CYN does not inhibit protein phosphatases like microcystins, but is a significant and irreversible inhibitor of protein biosynthesis, probably inhibiting ribosomal translation (Hoeger *et al*., 2004). Uptake of CYN into cells seems to be by diffusion due to its small molecular weight, although small amounts may be taken up by bile carriers (Wiegand & Pflugmacher, 2005).

2.3. Lipopolysaccharides

Endotoxic lipopolysaccharides (LPS) are generally found in the outer membrane of Gram negative bacteria, including cyanobacteria, where they form complexes with proteins and phospholipids. They can elicit irritant and allergenic reactions in human

and animal tissues that come into contact with the compounds (Sivonen & Jones, 1999). LPS and its effects are well known from enteric bacteria, such as *Escherichia coli*, *Salmonella* spp., *Vibrio cholerae*, *Yersinia pestis* and *Pseudomonas aeruginosa* (Wiegand & Pflugmacher, 2005). Endotoxins have been associated with certain cyanobacterial genera, including *Synechococcus, Synechocystis, Microcystis, Anabaena, Phormidium, Oscillatoria* and *Schizothrix.* Of these, *Microcystis, Anabaena* and *Oscillatoria* pose the greatest threat because they often occur in great masses in nutrient rich water sources (Rapala *et al*., 2002).

LPS consist of lipid A, core polysaccharides and an outer polysaccharide chain, known as the O-antigen. Cyanobacterial LPS differ to the LPS of enteric bacteria. They have a greater variety of long chain unsaturated fatty acids and hydroxy fatty acids, including the unusual fatty acid β-hydroxypalmitic acid which is found in the lipid A moiety. Cyanobacterial LPS often lack ketodeoxyoctonate, a common LPS component of Gram negative bacterial outer membranes, and contain only small amounts of bound phosphates when compared with enteric bacteria. The cyanobacterial O-antigen, on the other hand, is reminiscent of the *Escherichia coli* O-antigen, and is responsible for cyanophage adsorption and endotoxicity in aquatic environments. Lipid A is responsible for the toxic action, whereas the O-antigen is recognized by the immune system, leading to antibody production (Hoiczyk & Hansel, 2000).

LPS cause gastroenteritis and fever in mammals, and are involved in septic shock syndrome. This may aggravate liver injury induced by other cyanobacterial toxins such as microcystins and nodularins (Rapala *et al*., 2002; Wiegand & Pflugmacher, 2005). This is achieved by the release of inflammatory mediators such as tumor necrosis factor-α and interferon-γ. Additionally, LPS from cyanobacteria decrease glutathione S-transferase (GST) induction in the liver, in the same manner as noted in enteric bacteria. Since conjugation to GST is the start of detoxification of microcystins, inhibition of this enzyme system decreases the ability of the organism to metabolise these toxins. Cyanobacterial LPS from *Microcystis aeruginosa* was shown to be ten times less toxic compared to *Salmonella* LPS. However, the cyanobacterial LPS was found to act in a more potent manner than the LPS of enteric bacteria in suppressing GST activity (Wiegand & Pflugmacher, 2005).

2.4. Toxin stability

The four most important groups of cyanotoxins; microcystins, anatoxins, PSPs and cylindrospermopsins, exhibit different chemical stabilities in water.

2.4.1. Microcystins and nodularins

Being cyclic peptides, microcystins are extremely stable and resistant to chemical hydrolysis or oxidation at near neutral pH. Microcystins and nodularins stay potent even after boiling. In natural waters and in the dark, microcystins may persist for months or even years. Jones & Orr (1994) reported that after an algicide treatment in a small embankment of Lake Centenary, Australia, the total disappearance of microcystin-LR took more than three weeks. However, at high temperatures (40°C) and at elevated or low pH, slow hydrolysis has been observed, with 90% breakdown being achieved in approximately ten weeks. Rapid chemical hydrolysis occurs under conditions that are unlikely to be found outside the laboratory, for example 6M HCl at high temperatures. Microcystins can be oxidized by ozone and other strong oxidizing agents, and are degraded by intense UV light. These processes may be relevant for water treatment processes, but are unlikely to contribute to degradation in the natural environment.

In full sunlight, microcystins undergo slow photochemical breakdown and isomerisation, with the reaction being accelerated by the presence of water-soluble cell pigments, presumably phycobiliproteins. In the presence of these pigments, the photochemical breakdown of microcystin in full sunlight can take as little as two weeks for greater than 90% breakdown, or as long as six weeks, depending on the pigment and toxin concentrations (Sivonen & Jones, 1999).

2.4.2. Anatoxins

Anatoxin-a is relatively stable in the dark, but in pure solution in the absence of pigments it undergoes rapid photochemical degradation in sunlight. Breakdown is further accelerated by alkaline conditions. The half-life for photochemical breakdown is 1-2 hours. Under normal day and night light conditions at pH 8 or pH 10, the half-life

for anatoxin-a breakdown was found to be approximately fourteen days (Sivonen & Jones, 1999).

2.4.3. Saxitoxins

No detailed studies have been carried out on saxitoxin breakdown in sunlight, either with or without pigments. In the dark at room temperature, saxitoxins have been found to undergo a series of slow chemical reactions. The half-life for breakdown reactions are in the order of 1-10 weeks, with more than three months often needed to achieve greater than 90% breakdown (Sivonen & Jones, 1999).

2.4.4. Cylindrospermopsin

Cylindrospermopsin is relatively stable in the dark, with slow breakdown occurring at an elevated temperature (50°C). Pure cylindrospermopsin is relatively stable in sunlight, but in the presence of cell pigments, breakdown occurs rapidly, being more than 90% complete in 2-3 days (Sivonen & Jones, 1999).

2.5. Toxin removal

In response to the increase in health-related problems on a global scale, the World Health Organisation (WHO) has established safe guidelines for drinking water at 1.0µg MC-LR.l^{-1} (Jos, 2005). Additionally, a health alert should be published if the concentration of 10µg MCs.l^{-1} drinking water is reached, even for a brief period. Due to the lack of reliable data, no guideline value is set yet for concentrations of nodularins, cylindrospermopsins, neurotoxins such as saxitoxins and endotoxic lipopolysaccharides (Hoeger *et al*., 2004).

Until a bloom collapses or is otherwise affected by some treatment practice, the majority of toxins will be retained within the cells, making removal of intact cells a high priority. This is achieved using processes such as direct rapid filtration, to remove suspended particulate matter, and slow sand filtration. However, under bloom conditions, it is expected that a substantial proportion of toxin will be released into the water column, making the removal of toxins an unavoidable concern (Hrudey *et al*.,

1999). The three processes usually employed for the removal of microcystins from drinking water treatment include ozonation, chlorination, and adsorption by activated carbon (Rae *et al*., 1999).

Ozonation is the most efficient method for the complete destruction of both intra- and extra-cellular microcystins, as well as nodularin and anatoxin-a. The major consideration in the application of ozone is the ozone demanded by background DOC concentrations, as only after the DOC demand is satisfied, will the ozone show an effect on the toxins (Hrudey *et al*., 1999). The effectiveness of ozone in toxin degradation is also pH dependant, with a greater reduction in toxins at low pH values (Rae *et al*., 1999). Ozone also effectively removes the non-toxic odour causing compounds geosmin and 2-MIB. Although ozone is the most efficient method for removing toxins, the cost implications of the high ozone doses required and the highly specialized mass transfer techniques that are needed for treatment mean that this option is often not feasible for water purification (Strydom, 2004).

Chlorination is very effective at destroying microcystins and nodularin, but only under the correct treatment conditions: the free chlorine residue should be $0.5 mg.l^{-1}$ after 30 minutes contact time with a pH below 8. There is no discernable removal of anatoxin-a by chlorination (Hrudey *et al*., 1999).

Powdered activated carbon (PAC) can successfully remove microcystins and nodularins. It is recommended that this treatment method be combined with another, for example a pre-ozonation treatment, as in order to remove high amounts of toxins very high amounts of PAC are needed, and the presence of organic matter in the water interferes with toxin removal. Granular activated carbon (GAC) is more effective than PAC at removing toxins in the presence of organic compounds. Because GAC has also been shown to be more effective at adsorbing anatoxin-a than microcystin-LR, these two forms of activated carbon should be used in conjunction with one another to achieve maximum toxin removal (Hrudey *et al*., 1999). Adsorption and biodegradation mechanisms are known to be the predominant factors contributing to microcystin removal during the GAC filtration process. The presence of a biofilm within a GAC filter may increase its lifetime for the removal of problematic compounds such as the microcystins via biodegradation. Wang *et al*. (2007) demonstrated that biodegradation

was an efficient removal mechanism and that the rate of biodegradation was dependent upon temperature and initial bacterial concentration.

In terms of endotoxin treatment, the highest reductions occur during the early stages of water purification (coagulation, settling and sand filtration). Chlorination has been reported to decrease the endotoxin concentration, but activated carbon filtration was shown to increase it on some occasions. The removal of endotoxins is therefore dependant on the success of the water clarification steps (Rapala *et al*., 2002).

2.5.1. Biodegradation of toxins

Biodegradation is one of the safest means to remove cyanotoxins from water (Ishii *et al*., 2004). Jones *et al.* (1994) isolated a species of *Sphingomonas* that is capable of degrading both microcystin-LR and microcystin-RR, but not nodularin. The bacterium initiated ring opening of microcystin to produce linear microcystin as a transient intermediate. This compound was nearly 200 times less toxic than the parent toxin (Bourne *et al*., 1996). Ishii *et al.* (2004) demonstrated that *Sphingomonas* is capable of degrading microcystin-LY, -LW and-LF completely, as well as microcystin-LR. A strain of *Pseudomonas aeruginosa* isolated from a Japanese lake produced an alkaline protease that attacked the Adda side chain of microcystin-LR (Takenaka & Watanabe, 1997). A recent study by Meriluoto *et al*. (2005) demonstrated the potential use of the human probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* for microcystin removal. The fact that heat inactivated bacteria were more effective at removing microcystin-LR from solution than untreated bacteria indicated that bacterial metabolism was not involved in toxin removal, but rather that the toxin bound to the bacteria. Toxin binding to lactic acid bacteria may contribute, as one factor, to the lower oral toxicity of microcystins as compared to intraperitonial injection in mouse toxicity studies.

Little work has been undertaken on the biodegradation of anatoxins, saxitoxins or cylindrospermopsin. A *Pseudomonas* strain capable of degrading anatoxin-a at a low rate has been isolated (Sivonen & Jones, 1999).

3. Why cyanobacteria become dominant

Numerous hypotheses have been proposed to explain the success of blue green algae in eutrophic water bodies, identifying light, nitrogen or CO_2 as the limiting resource under eutrophy. The most convincing however, is that the nature of resource limitation changes during the eutrophication process, promoting cyanobacteria to a highly competitive position (Ferber *et al*., 2004).

3.1. Nutrient physiology and the importance of the N:P ratio

Because cyanobacterial blooms often develop in eutrophic lakes, it was originally assumed that cyanobacteria require high phosphorus and nitrogen concentrations, even though blooms often occur when phosphorus concentrations are at their lowest. Experimental data has shown that the affinity of many cyanobacteria for nitrogen and phosphorus is much higher than for many other photosynthetic organisms. This means that they can out-compete other phytoplankton organisms under conditions of phosphorus or nitrogen limitation. In addition to their high nutrient affinity, cyanobacteria 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 (Mur *et al*., 1999). Concentrations of phosphorus of below $0.1 mg.l^{-1}$ are sufficient to cause a cyanobacterial bloom (Bartram *et al*., 1999).

The nutrient physiology of cyanobacteria differs from that of other algae in that many species have heterocysts for nitrogen fixation in oxic well-lit waters, for example *Aphanizomenon* and *Nodularia* (Ferber *et al*., 2004). This ability allows nitrogen-fixing species to maintain high growth rates under conditions of nitrogen limitation, making them superior competitors. In addition, blue-green species that are not capable of nitrogen fixation, such as *Microcystis aeruginosa*, may be as abundant as nitrogen fixing species during times of nitrate deficiency. The low nitrogen: phosphorus (N:P) ratio hypothesis was first proposed by Pearsall (1932), and was later popularized by Smith (1983) who compiled and analysed data from 17 lakes worldwide, and observed a tendency for cyanobacterial blooms to occur when epilimnetic N:P ratios fell below about 29:1 by weight, and for blue-green algae to be rare when the N:P ratio exceeded this value. If the low N:P hypothesis is to be true, nitrogen fixing (heterocystous)

cyanobacteria overcome N shortage through fixation and would therefore always be dominant. However, this does not account for the dominance of non-heterocystous species when nitrogen is limited. Ferber *et al*. (2004) found that heterocystous cyanobacteria were dominant during periods of low nitrogen fixation (less than 2% of the required N), leading to the assumption that fixation is not the only means of heterocystous cyanobacteria outcompeting other algae. Nitrogen fixation is usually repressed at inorganic N concentrations greater than 7µM (Horne & Commins, 1987). Colonial and vacuolated non-heterocystous species such as *Microcystis*, *Oscillatoria* and *Planktothrix* are expected when the principal source of N is ammonium recycled within the water column, or a benthic ammonium source can be reached through vertical migration, and heterocystous species when neither of these sources is sufficient and fixation must be relied upon (Blomqvist *et al*., 1994; Hyenstrand *et al*., 1998). Ferber *et al*. (2004) therefore recommended that the low N:P hypothesis be modified to include the fact that both heterocystous and non-heterocystous vacuolated cyanobacteria will out-compete other species through the vertical migrations that give them superior access to nutrient sources, P as well as N. Several other factors have also been proposed to explain variation in cyanobacterial dominance below the N:P threshold, including light, temperature, pH and the effects of zooplankton (MacKay & Elser, 1998).

Xie *et al*. (2003) performed an enclosure experiment during the summer of 2001 in the shallow, subtropical Lake Donghu, China to examine the effect of TN:TP ratios and P-reduction on the occurrence of *Microcystis* blooms. The treatments were performed with an excess of N but with different amounts of P in the water column and sediment. *Microcystis* blooms occurred in the enclosures with higher concentrations of P with initial TN:TP <29 as well as TN:TP >29, indicating that the TN:TP ratio was not a deterministic factor for *Microcystis* blooms, at least in the highly eutrophic Lake Donghu. This is in agreement with Paerl *et al*. (2001), who suggested that the "N:P rule" is less applicable to highly eutrophic systems when both N and P loadings are very large and the N and P inputs exceed the assimilatory capacity of the cyanobacteria. The TP of the water in the enclosures with P-rich sediment increased dramatically after the bloom developed, with approximately 40% of the sediment P being released to the water column and assimilated by the *Microcystis*, leading to a decrease in the TN:TP ratios to about 10. The results therefore indicate that the low TN:TP ratio is not the cause of *Microcystis* blooms, but rather the result. No *Microcystis* blooms occurred in

the enclosures with low P concentrations in the water and the sediment, despite the presence of sufficient N, suggesting the effectiveness of P-reduction for the control of *Microcystis* blooms. In a comparative study of two water supply reservoirs situated in different climatic regions of Brazil, von Sperling *et al*. (2008) observed that in spite of the prevalence of high N:P values there was a clear trend in the dominance of cyanobacteria in the phytoplankton.

3.2. Effect of zooplankton

Zooplankton, especially *Daphnia*, are generally rare during a cyanobacterial bloom. It is unclear whether cyanobacteria predominate in lakes with low *Daphnia* grazing pressure because they exclude large effective grazers, or because grazer biomass is decreased due to other factors allowing cyanobacteria to dominate (MacKay & Elser, 1998). There are many reasons as to why cyanobacteria may exclude *Daphnia*; they have a low nutritional value, they can be toxic (MacKay & Elser, 1998) and their shape, especially that of filamentous cyanobacteria, may mechanically interfere with the filtering mechanism of grazers (de Bernardi & Giussani, 1990). Large grazers such as *Daphnia* promote high densities of inedible colonial cyanobacteria by selectively eating competitive phytoplankton (Haney, 1987). However, there is also evidence that high *Daphnia* densities exclude cyanobacteria completely. Although cyanobacteria typically dominated the phytoplankton community in Lake Trummen, there was one year when they did not dominate, despite a low N:P ratio. Smith (1983) attributed this to a change in the food web which likely led to an increase in *Daphnia*, as this year followed a winter fish kill that eliminated planktivorous fish. Gobler *et al*., 2007 suggested there may be a threshold density of *Microcystis* which is inhibitory to *Daphnia* grazing. When *Daphnia* were able to graze in Lake Agawam, mean densities of *Microcystis* and the percentage of *Microcystis* represented within the total algal community were both significantly lower than levels and percentages present when there was no grazing detected. *Microcystis* densities were also always below 6 x 10^4 cells.ml^{-1} when grazing by *Daphnia* was detected.

Compared with other zooplankton, *Daphnia* have a higher P content in their bodies (Anderson & Hessen, 1991), and therefore, because of their low body N:P ratio, recycle nutrients at a high N:P ratio that may adversely affect cyanobacterial populations

(Sarnelle, 1992). Because *Daphnia* returns N to the environment at a much higher rate than P, recycling theory predicts that the cyanobacterial population should become P limited in the presence of these low N:P grazers (Sterner, 1990). MacKay & Elser (1998) used a field experiment to test how the differential nutrient recycling by low N:P ratio *Daphnia* affects the physiological status of cyanobacteria, including rates of nitrogen fixation, when compared to a high N:P ratio species *Epischura* and a zooplankton-free control. The ammonium concentration in the *Daphnia* treatment was twice those of the *Epischura* treatment and control, making the N:P ratio the highest in the *Daphnia* treatment. This high N:P ratio caused the cyanobacteria to become P limited. Consistent with this, the rate of nitrogen fixation was 50% lower than in the other treatments. Thus, by differentially recycling NH_4-N relative to P, *Daphnia* reduce the advantage cyanobacteria have over other phytoplankton. Combined with the observation that *Daphnia* cannot survive in severe cyanobacterial blooms, they may be more effective at preventing the occurrence of cyanobacterial blooms than in controlling existing blooms.

3.3. Buoyancy in cyanobacteria

The ability of gas-vacuolate cyanobacteria to adjust their cell density and move up and down the water column is an important factor in cyanobacterial dominance. The advantages of buoyancy regulation include a reduction in the amount of cells lost by sedimentation, an improvement in light supply as the cells are nearer the well-lit surface water layers, and the ability to balance the supply of limiting resources by altering the cell position in the water column, for example to overcome the vertical separation in light and nutrient availability that occurs in stratified water bodies (Oliver, 1994). This is especially important in shallow lakes, where there is a short distance for vacuolated cyanobacteria to migrate to the bed, allowing the cells to spend much of the day on the lake surface photosynthesizing (Ferber *et al.*, 2004). Gas vesicles are exposed to intracellular turgor pressure generated by the difference in osmotic pressure between the cell cytoplasm and the surrounding medium, as well as hydrostatic pressure from the overlying water column. A high turgor pressure in the cell causes a collapse in a portion of the gas vesicles, thus reducing cell buoyancy (Oliver, 1994). Limitation of carbon, nitrogen and phosphorus have different effects on the gas vacuoles in cyanobacterial cells. When nutrients such as nitrogen and phosphorus are readily available and carbon

is not limiting, photosynthetic energy is used to synthesize cell constituents and carbohydrates do not accumulate. However, if growth is restricted by a limiting nutrient, energy capture exceeds the amount needed for growth and carbohydrates accumulate in the cell, causing an increase in turgor pressure and a decrease in buoyancy. Carbon limitation, in contrast, is expected to prevent the loss of buoyancy as the turgor pressure increase depends on carbon fixation (Klemer, 1991).

3.4. Recruitment of resting stages

Many phytoplankton species form resting vegetative stages when environmental conditions are harsh and these can survive for extended periods of time in the sediment. When environmental conditions are favourable again, they recruit to the water phase and continue growing (Ståhl-Delbanco *et al*., 2003). Many species of cyanobacteria, for example *Microcystis*, *Anabaena*, and *Aphanizomenon*, form resting stages and are, in addition, the most frequently occurring bloom-forming cyanobacteria (Willén & Mattsson, 1997). Takamura *et al*. (1994) found that the amount of *Microcystis* in the sediment can be much higher than the total amount of *Microcystis* in the water column, even during blooms. This means that a huge potential inoculum can be present in the sediment. Blooms are often formed rapidly (within days) and can often not be explained by growth of the existing pelagic population alone, suggesting that the rate of recruitment from sediment to water may be important to the formation of blooms (Ståhl-Delbanco *et al*., 2003).

Overwintering benthic cyanobacterial populations can only act as an inoculum if they remain vital and if they are able to leave the sediment. Verspagen *et al*. (2004) investigated the vitality and two possible recruitment mechanisms of benthic *Microcystis* colonies; passive re-suspension and an active increase in the buoyancy levels of the cells. They found that throughout the year benthic *Microcystis* populations were photochemically active and sufficiently vital to serve as an inoculum for the initiation of a bloom. Although *Microcystis* is able to survive under anoxic conditions by fermentation (Moezelaar & Stal, 1994), photosynthesis is sensitive to the high sulphide concentration found in the sediment. The photosynthetic vitality of *Microcystis* colonies found at the sediment surfaces of deeper parts of the lake was reduced, but colonies in the shallower parts of the lake were still in the euphotic zone and had the

highest photochemical vitality. Hence, colonies from the shallow sediments were better adapted physiologically to inoculate the water column. In terms of the mechanism of recruitment, changes in internal buoyancy seemed unlikely, as the carbohydrate content in benthic *Microcystis* is so low that a further decrease could not cause a buoyancy change, and there was no substantial increase in gas vesicle volume in spring. It was concluded that intense mixing of the water column was sufficient to re-suspend the sediment containing benthic *Microcystis*, and remove attached sediment so that buoyant colonies could enter the water column again. Bioturbation by macrofauna may also result in benthic *Microcystis* recruitment (Ståhl-Delbanco & Hansswon, 2002). Therefore, because the sediments of shallow parts of a water body are most frequently re-suspended and the vitality of colonies is the greatest in this zone, shallow areas play the most important role in cell recruitment and the development of algal blooms.

3.5. CO_2 concentration and pH

It was initially proposed by King (1970) that the onset of a cyanobacterial bloom results from low concentrations of CO_2 brought about by photosynthesis of algae early in the season, and that a low CO_2 concentration and high pH are a prerequisite for cyanobacteria to become abundant. Shapiro (1972) added to this by stating that cyanobacteria have better CO_2 uptake kinetics than green algae at low concentrations, and therefore reduce the CO_2 concentration to the degree that only they can photosynthesize and become abundant. When excess CO_2 and high nutrients (nitrogen and phosphorus) were added to an algal population containing green algae but dominated by cyanobacteria, the population shifted and was dominated by the green algae within 10d. A similar result was seen when nutrients were added and the pH lowered to 5 to make CO_2 more available. Addition of nutrients alone caused an increase in cyanobacterial growth and in the pH.

Shapiro (1997) disproved King's theory that low CO_2 and high pH are prerequisites for the formation of a cyanobacterial bloom, at least for the important species *Aphanizomenon flos-aquae* and *Anabaena flos-aquae* when he performed a whole-lake study in 1993. The south basin of Squaw Lake, Wisconsin, U.S.A. was artificially injected with CO_2 in an attempt to eliminate the massive blue-green algal bloom usually present in summer. The unmixed, un-injected north basin was the control. Despite a

great difference in the pH and CO_2 concentrations between the two basins, a cyanobacterial bloom began in both almost simultaneously and eventually reached the same size, with the predominant algal species in both basins *Aphanizomenon flos-aquae* and *Anabaena flos-aquae*. The statement that cyanobacteria do well at high pH because that is when free CO_2 concentrations are sufficient for them but not for other groups appears valid, as most of the green algae tested had a poorer ability to use low concentrations of CO_2 than the cyanobacteria.

3.6. Effect of trace metals

Trace metals are crucial for efficient carbon and nitrogen metabolism in cyanobacteria. Iron is important for photosynthesis as well as energy distribution within the cell. Addition of iron to a water body results in increased cyanobacterial photosynthesis, thus stimulating the growth rate and promoting blooms. In a study performed by Takeda *et al*. (1995), simultaneous addition of iron and nitrate stimulated algal growth more rapidly than the addition of nitrate alone. Both iron and molybdenum are involved in nitrate reduction and nitrogen fixation (Rueter & Petersen, 1987). Molybdenum enrichment of a Californian lake stimulated carbon fixation and the rate of nitrogen uptake, and had the greatest effect when nitrate was the dominant nitrogen source (Axler *et al*., 1980). Overall, three trace metal dependant processes may contribute towards dominance: efficient use of limiting light, nitrogen fixation and the production of extracellular iron binding compounds (Rueter & Petersen, 1987).

4. Methods of control

4.1. Biological control of cyanobacteria

It is important to know what controls cyanobacterial dynamics in their natural habitats. An alternative approach for the direct elimination of nuisance cyanobacteria involves the application of biological control agents. Changes in cyanobacterial populations have been attributed to a number of variables, including predation, nutrient depletion, light intensity, accumulation of metabolites, parasitism, and the pH and CO_2 content of the water. Despite the high abundance of bacteria and viruses in water bodies, the importance of lytic bacteria and viruses in regulating the population abundance of

nuisance cyanobacteria is seldom emphasized (Rashidan & Bird, 2000). Biological control has a number of advantages over chemical control. Biological control can be highly specific to the target organism, with no destruction of other organisms and no direct chemical pollution that might affect humans. Potential disadvantages include limited destruction of the target organism, limited survival of the agent or its removal by other organisms and problems with large scale production, storage and application of the biocontrol agent (Sigee *et al*., 1999).

Daft *et al*. (1985) proposed the following seven attributes that defined a good predatory bacterial agent: adaptability to variations in physical conditions; ability to search for or trap prey, capacity and ability to multiply, consumption of prey, ability to survive low prey densities (switch or adapt to other food sources), a wide host range and the ability to respond to changes in the host. In addition, Sigee *et al*. (1999) suggested that the microbial antagonists must be indigenous species of that particular lake environment, having not undergone any gene modification or enhancement.

The practice of introduction of foreign microbial agents has raised some concern with regards to environmental safety due to the so-called host specificity paradigm involving host switching (HS) and host range expansion (HRE) (Secord, 2003). The foreign microbial agents are able to reproduce naturally and may exploit the opportunities that are available in the new environment by shifting their host affinities to other host species (set of species) and/or add another target species other than the original target. The change in direction of the microbial antagonist is difficult to anticipate, and there is the possibility that the organisms may affect other economically important crops or organisms.

Viral pathogens would be ideal as biocontrol agents as they are target selective and specific for nuisance cyanobacteria. However, bacterial agents are considered more suitable than viruses as biological control agents because bacteria can survive on alternate food sources during non-bloom periods and the possibility of mutation within the host is not problematic, as bacterial predation is not reliant on unique attachment receptors (Rashidan & Bird, 2001).

4.1.1. Cyanophages

Cyanophages are extremely widespread in both freshwater and marine environments. The rapid generation time of cyanophages makes them attractive agents for controlling cyanobacterial blooms. All the known cyanophages belong to three bacteriophage families: Myoviridae, Siphoviridae, and Podoviridae (Lu *et al*., 2001; Yoshida *et al*., 2006). These phages are morphologically and genetically diverse (Zhong *et al*., 2002). Despite their abundance and significance, few cyanophages have been characterized at the genome level. Examples of those characterised include P60, P-SSP7, P-SSM2, PSSM4, and S-PM2 (Chen & Lu, 2002; Mann *et al*., 2005; Sullivan *et al*., 2005). Liu *et al*. (2007) reported the complete genome sequence of the cyanophage, Pf-WMP4, which infects the freshwater cyanobacterium *Phormidium foveolarum* Gom.

Yan-Ming *et al*. (2006) investigated the spatial distribution and morphological diversity of virioplankton in Lake Donghu, China, which contains three trophic regions: hypertrophic, eutrophic and mesotrophic. High virus diversity was observed in the lake, with cyanophages representing a significant fraction of the virus community. Numbers appeared to be directly related to the concentration of chlorophyll a, and were higher in the eutrophic region. Most morphotypes belonged to *Siphoviridae*, *Myoviridae* or *Podoviridae*. It was concluded that cyanophages play an important role in the ecology of Lake Donghu.

There is a marked difference between unicellular and filamentous cyanobacterial hosts in the dependence of the cyanophage cycle on photosynthetic activity. Unicellular cyanobacterium-cyanophage systems show an absolute dependence for phage development on their host photosynthetic machinery, for example various *Synechococcus* strains and SM-1, AS-l and AS-1M cyanophages. In filamentous organisms the cyanophage cycle can proceed independently of host photosynthesis, for example in *Plectonema* sp. and *Nostoc* sp. /*Anabaena* sp., LPP and N-l phages. This difference may be due to the altered redox state of thioredoxin *m* in filamentous cyanophage-infected cyanobacteria (Teklemariam *et al*., 1990).

An important consideration in the potential use of cyanophages as biological control agents is the rapid appearance of host mutants. These may include changes in the algae

cell envelope, preventing phage adsorption. Cyanobacterial strains that are resistant to wild type phages may, however, be susceptible to attack by mutant cyanophage strains. The high degree of host specificity, occurrence of resistant host mutants and the effect of environmental factors all contribute to the unpredictability of cyanobacteria-phage interactions in the field. Difficulties involved with producing large amounts of active inoculum also present problems in the effective use of cyanophages as biological control agents in the lake environment (Sigee *et al*., 1999).

4.1.2. Predatory bacteria

In a report by Wright & Thompson (1985), volatile products released by various *Bacillus* species, including strains of *B. licheniformis, B. pimulis and B.subtilis* were inhibitory to cyanobacterial growth, particularly that of *Anabaena*. As was found in the study by Reim *et al*. (1974), the onset of marked detectable antagonism coincided with the sporulation of the majority of the *Bacillus* cells. Wright *et al.* (1991) identified one cyanobacteriolytic volatile product produced by the *Bacillus* species as isoamyl alcohol (3-methyl-1-butanol). Isoamyl alcohol is a volatile product of peptone metabolism in some *Bacillus* species. This compound may act synergistically with other complex volatiles to cause lysis of cyanobacterial cell suspensions. Contact is not required between the bacteria and the cyanobacteria in order for lysis to occur.

The culture filtrate of an atypical strain of *Bacillus brevis* lysed seven genera of cyanobacteria, including *Plectonema boryanum, Microcystis aeruginosa* and *Anabaena flos-aquae*. These bacilli produced two main classes of filterable substances that show biological activity, namely exoenzymes and polypeptide antibiotics. The heat stability and small molecular size of the diffusible inhibitory factor present in the culture filtrate suggested that the substance was of a non-enzymatic nature, and therefore was probably an antibiotic substance. *Bacillus brevis* produced two antibiotics, gramicidin S and tyrothricin. Gramicidin S was inhibitory to the growth of *Plectonema boryanum*, while tyrothricin caused no inhibition. Gramicidin S was therefore thought to be the cyanobacteriolytic substance produced by the atypical *Bacillus brevis* strain. Most extracellular antimicrobial products synthesized by the bacilli are sporulation related, and *Bacillus brevis* is no exception, as cyanobacteriolytic activity did not appear until the early stationary phase of growth (Reim *et al*., 1974).

Nakamura *et al.* (2003a) isolated a bacterium showing high lytic activity against *Microcystis*. The bacterium was identified as *Bacillus cereus*. *B. cereus* cells first attached to the surface of the cyanobacteria to induce cyanobacterial aggregation, and extracellular products of *B. cereus* subsequently lysed the cyanobacteria. The purpose of this two-step process of *B. cereus* may have been to lyse and assimilate cyanobacteria in a more effective manner. The cyanobacteriolytic activity of *B. cereus* gradually increased following the exponential growth phase, once again indicating that the cyanobacteriolytic activity involves sporulation in *Bacillus*. The cyanobacteriolytic substance was heat stable and hydrophilic. Proteinase-K treatment had no effect on activity, indicating that the lytic substance was non-proteinaceous, and it was less than 2kDa in size. No cyanobacteriolytic activity was observed under acidic conditions, but cyanobacterial cells were immediately lysed after shifting to alkaline conditions. This indicated that the cyanobacteriolytic substance was not denatured at acidic pH values, was not affected by pH shifting and was more effective in alkaline pH conditions. This is important, as cyanobacterial blooms often alkalise the aquatic environment because some cyanobacteria, including *Microcystis*, can use HCO_3^- more effectively than CO_2. At 30°C and 25°C, almost 100% of the cyanobacterial cells were lysed, with minimal activity at 3°C. Since the cyanobacterial membrane is considered quiescent at 3°C, the minimal activity may have been due to the low activity of the cyanobacteria. Optimum conditions for cyanbobacterial growth such as high temperature and alkaline pH may accelerate the active transport of the lytic substance to the cyanobacterial cells and subsequent rapid lysis. The cyanobacteriolytic substance produced by the isolated *B. cereus* strain was not linked to the enterotoxin or emetic toxin produced by pathogenic *B. cereus* strains. It also differed from the algicide gramicidin produced by *B. brevis* (Reim *et al*., 1974), indicating the possibility of a novel algicide. Shunyu *et al*. (2006) isolated *Bacillus cereus* from Lake Dianchi of Yunnan province, China, which was capable of rapidly lysing the bloom-forming cyanobacterium *Aphanizomenon flos-aquae* through cell-to-cell contact. The bacterium also showed lytic activity towards *Microcystis viridis*, *Microcystis wesenbergi*, *Microcystis aeruginosa*, *Chlorella ellipsoidea*, *Oscillatoria tenuis*, *Nostoc punctiforme*, *Anabaena Xos-aquae*, *Spirulina maxima*, and *Selenastrum capricornutum*.

A Gram negative, rod shaped motile bacteria thought to be a new species related to *Xanthomonas* was isolated that showed lytic activity towards select cyanobacteria,

including species of *Anabaena* and *Oscillatoria*. These cyanobacteria produce the compounds geosmin and 2-methylisoborneol (MIB), which cause off-flavours in commercially produced channel catfish. Most geosmin off-flavour has been attributed to species of *Anabaena*, whereas MIB off-flavours are associated with *Oscillatoria* species. This newly isolated bacterial species therefore represents an opportunity to selectively control the nuisance cyanobacteria. The lytic characteristics of the bacteria appeared to be associated with the living cells, as no lytic activity was associated with filtered broth (Walker & Higginbotham, 2000). The mechanism of lysis therefore differed from that seen with *Bacillus* species, all of which employed an extracellular agent to achieve cyanobacterial lysis.

Numerous strains of lytic gliding bacteria, mainly members of the *Myxobacteria* and *Cytophaga* groups, were isolated, which lysed cyanobacteria by attachment and secretion of diffusible lytic substances and therefore required direct contact with the host cell. They produced a variety of different exoenzymes capable of hydrolyzing the cyanobacterial cell wall, including proteases, glucosamidase and D-alanyl-N-lysine endopeptidase, as well as antibiotics. These anti-cyanobacterial substances resulted in the lysis of cyanobacteria and release of nutrients, which then may have been taken up by lytic bacteria for their own growth (Rashidan & Bird, 2000). The cyanobacterium *Phormidium luridum* was preyed upon by *Myxococcus* species, mainly *M. xanthus* and *M. fulvus.* These bacteria displayed entrapment capabilities, causing clumping in cyanobacteria prior to lysis, and seemed to be independent of any other nutritional requirement (Burnam *et al.,* 1981; Burnam *et al.,* 1984). Rashidan & Bird (2000) isolated two *Cytophaga* strains (C1 and C2), which demonstrated host specificity. One strain showed lytic activity only towards *Anabaena flos-aquae,* the other lysed only three *Synechococcus* species and *Anacystis nidulans*. *Cytophaga* are strict aerobic bacteria, and are dependant on organic matter for growth. They need a solid substrate for gliding, which explains why they preferred to be attached to cyanobacteria, although they can grow and reproduce in the absence of their host. No special attachment organelles existed on the surface of *Cytophaga* strains C1 and C2, but because contact was required for lysis, it seemed evident that surface lytic enzymes were involved in the lytic action of these bacteria, which was consistent with their host specificity.

There are reports of *Bdellovibrio* (Burnham *et al*. 1976) and *Bdellovibrio*-like bacteria (Wilkinson 1979; Caiola & Pellegrini 1984) causing cyanobacteria lysis. Burnham *et al*. (1968) demonstrated the endoparasitic behaviour of *Bdellovibrio bacteriovorus* on *Escherichia coli*. The *Bdellovibrio* irreversibly attached to the host*,* with the end of the cell opposite the sheathed flagellum, commenced a grating motion which lasted for several minutes, and entered the host's cytoplasm. Once inside the prey, *Bdellovibrio* inactivated the host's metabolism and fed off its nutrients (Yair *et al*. 2003). The exhaustion of cytoplasm contents triggered the *Bdellovibrio* to undergo multiple fission replications to produce progeny called bdelloplast. The bdelloplast, now flagellated, emerged after breaking the prey cell wall leaving behind ghost prey remnants. However, when *Bdellovibrio bacteriovorus* was added to an aqueous culture of *Phormidium luridum* it caused lysis of the cyanobacteria, but the mechanism of cyanobacterial lysis was not endoparasitic as expected; an extracellular substance was released that dissolved the cyanobacteria cell wall, allowing the bacterium to gain nutrients from the cyanobacterium (Burnham *et al*. 1976). The predation mechanism of *Bdellovibrio* was therefore prey-specific.

Recently, *Streptomyces neyagawaensis* was found to have lytic activity towards four cyanobacterial species, including *Microcystis aeruginosa*, *Anabaena cylindrica*, *Anabaena flos aquae* and *Oscillatoria sancta*. Results indicated that *S. neyagawaensis* did not secrete the anti-algal substance until the bacterium met the target alga, and that the anti-algal substance was present in the periplasmic fraction of the bacterial cell (Hee-jin *et al*., 2005).

Predatory bacteria have characteristics that make them more potent and valuable as control agents when compared to cyanophages. They can survive on alternate food sources during non-bloom conditions, and mutation to non-susceptible strains in the host is far less likely because there are no unique attachment receptors. Non-obligate predatory bacteria do not require the presence of prey cells for survival, but attack and destroy prey cells when nutrients in the environment become depleted (Rashidan & Bird, 2000).

4.1.3. Fungal pathogens of cyanobacteria

There have been various reports of predation of cyanobacteria by fungi. *Oscillatoria agardhii* was parasitized by the chytridiaceous fungus *Rhizophidium planktonicum*. However, this fungus is of limited use in the control of bloom-forming cyanobacteria because of the apparent obligate nature of these parasites and difficulties in their large-scale culture (Sigee *et al*., 1999). Fungal representatives of the genera *Acremonium*, *Emiricellopsis* and *Verticillium* lysed *Anabaena flos-aquae* and, in most cases, several other filamentous and unicellular cyanobacteria. Lysis of cyanobacteria by *Acremonium* and *Emericellopsis* sp. was associated with the formation of diffusible heat-stable extracellular factors (Sigee *et al*., 1999).

4.1.4. Field application of biological control agents

Although there are non-indigenous bacterial agents that have been isolated and characterised, it appears that the studies on application of biocontrol agents are rather limited, focusing mainly on the lysis of laboratory-cultured cyanobacteria. Before application of bacterial biocontrol agents to freshwater systems, information must be available on the anti-algal activity against target algae, the effects of bacteria on other organisms in the freshwater ecosystem and the prediction of the algal dynamics after removal of target algae (Choi *et al*. 2005). Another aspect of importance is agitation. Shilo (1970) and Daft *et al*. (1971) found that cyanobacterial lysis was ineffective if there was agitation, especially where contact lysis was involved. Under natural conditions, rapid mixing may favour the proliferation of cyanobacteria and discourage attachment of predatory bacteria.

During field trials performed by Wilkinson (1979) and Caiola & Pellegrini (1984), a *Bdellovibrio*-like bacterium caused lysis of *Neofibularia irata, Jaspis stellifera* and *Microcystis* cells respectively. The bdelloplast were localised within the cell wall and cyanobacteria cytoplasm membrane. The infecting bacterium was similar in size and appearance to previously described *Bdellovibrio's*. These observations, though not replicated under controlled laboratory conditions, indicated the possibility of endoparasitism of the cyanobacteria by *Bdellovibrio*-like bacteria. The *Bdellovibrio*-like bacteria are an attractive biological control agent because in some cases they penetrate

the host cells specifically, exhaust host cell contents and replicate to form bdelloplasts, which attack further cells.

Nakamura *et al*. (2003b) immobilised *Bacillus cereus* N-14 in floating biodegradable plastic carriers, at a cell concentration of 3 x 10^7 cells/ml per 1g dry weight of starch-carrier float. This was used as an effective *in situ* control of natural floating *Microcystis* blooms, eliminating 99% of floating cyanobacteria in 4 days. The bacteria utilized the starch as a nutrient source and amino acids were derived from the lysis of *Microcystis*. The floating carrier enabled immobilized bacteria to be directed to floating cyanobacteria blooms.

4.2. Chemical control of cyanobacteria

Algicides have been used widely in some regions to control prevailing cyanobacterial blooms (Chorus & Mur, 1999). Examples of the chemicals most often used include copper sulphate ($CuSO_4$), Reglone A (diquat, 1,1-ethylene-2,2-dipyridilium dibromide) Simazine (2-chloro-4,6-bis(ethylamino)-s-trizine) alum (($Al_2(SO_4)_3 14H_2O$) and lime ($Ca(OH)_2$). In water treatment plants, potassium permanganate ($KMnO_4$) is applied to control phytoplankton related odour problems. Of these chemicals, both alum and lime remove phosphorus from the water. The remaining chemicals remove cyanobacteria by disrupting cell functions such as new cell wall synthesis, photosynthesis or other enzymatic reactions.

Before chemical treatment, MC-LR is present in high amounts in the cyanobacterial cells, but is at a low concentration in the water. After treatment with copper sulphate, Reglone A and Simazine, there is a substantial increase in the toxin concentrations in the water due to cell lysis. Thus, improving the aesthetic value of a lake by chemically removing a toxic cyanobacterial bloom could increase the potential health risks. Treatment with alum and lime causes coagulation of the cyanobacteria, resulting in flocculation. The exocellular concentrations of MC-LR when treated with alum and lime are consistently lower than with other chemical treatments. Treatment with lime showed no increase in exocellular toxin concentrations when compared to a control, but alum shows a three-fold increase. It has been suggested that the aluminium ions in alum may cause cell lysis, but to a lesser extent than other chemicals. Lime or alum treatment

represents a more favourable treatment of toxic cyanobacterial blooms, because of their ability to remove cells with minimal toxin release (Lam *et al*., 1995).

4.3. Control using turbulent mixing

Stability of the water column is a prerequisite for bloom formation, and mixed conditions prevent bloom formation and arrest the growth of colony forming cyanobacteria such as *Microcystis* and *Aphanizomenon*. However, green algae such as *Scenedesmus* spp. tend to dominate during periods of more intensive mixing (Ibelings, *et al*., 1994). Naturally available photosynthetic photon flux densities (PPFD) for phytoplankton fluctuates as a complex function of the daily passage of the sun, weather conditions, wave action and mixing over the underwater light gradient. One of the causes of reduced growth of cyanobacteria under mixed conditions may be the reluctant acclimation of cyanobacteria to changes in the PPFD (Collins & Boylen, 1982). Ibelings *et al*. (1994) made a direct comparison between the cyanobacterium *Microcystis aeruginosa* Kützing emend. Elenkin and the eukaryotic green alga *Scenedesmus protuberance* Fritsch with respect to their acclimation to fluctuations in PPFD by simulating the conditions induced by wind mixing over the underwater light gradient in lakes. *Microcystis* exhibited a more reluctant acclimation to the fluctuating PPFD when compared to *Scenedesmus*, whose growth rate was higher in all light regimes. This implied that if *Scenedesmus* was not subject to sedimentation losses (Visser *et al*., 1996b), it would outcompete *Microcystis* in lakes. These results emphasized the importance of buoyancy regulation in cyanobacteria for increasing their daily light dose.

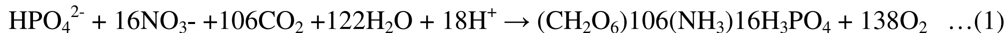
Artificial mixing by means of air bubbling was installed in Lake Nieuwe Meer, Amsterdam, using a system of seven perforated air tubes installed just above the lake sediment in an attempt to reduce the extensive growth of *Microcystis* in the lake. The following seasons showed a shift from cyanobacterial dominance to flagellates, green algae (mainly *Scenedesmus*) and diatoms (mainly *Cyclotella* and *Stephanodiscus*). The total phosphorus and total nitrogen concentrations were not affected by the mixing and remained high, leading to the conclusion that light limitation was responsible for the shift in the phytoplankton composition (Visser *et al*., 1996a). A distinction must be made between colony forming and filamentaous cyanobacteria in terms of their floatation velocity. Colonies have a much higher floatation velocity than filamentous or

single celled cyanobacteria, and the mixing velocity therefore needs to be high enough to keep the colonies moving in the turbulent flow (Visser *et al.*, 1997; Huisman *et al.*, 2004). Artificial mixing may temporarily reduce the amount of cyanobacterial growth, but it does not solve the problem of eutrophication.

4.4. Eutrophication management

4.4.1. Nutrient limitation

The significance of phosphorus in eutrophication has resulted in the development of many remediation plans, based on the management of the phosphorus concentration. It is accepted that phosphorus control is more achievable than that of nitrogen, because, unlike nitrogen, there is no atmospheric source of phosphorus that is bio-available. In addition, the general equation for photosynthesis (Equation 1) (Hereve, 2000) shows only one gram of phosphorus is required for every seven grams of nitrogen for the formation of the organic matter created in the process.



$$HPO_4^{2-} + 16NO_3- + 106CO_2 + 122H_2O + 18H^+ \rightarrow (CH_2O_6)106(NH_3)16H_3PO_4 + 138O_2 \quad \ldots(1)$$

This indicates that a small degree of phosphorus reduction can achieve a much greater degree of growth reduction of cyanobacteria than a reduction of a similar magnitude in the nitrogen level. This fact, together with the availability of gaseous nitrogen to N-fixing organisms, makes phosphorus reduction strategies far more effective alternatives in eutrophication management.

In South Africa, the orthophoshate standard for effluents discharged into water bodies is $1mg.l^{-1}$ (NIWR, 1985). However, even if this standard is complied with, it may take years for phosphorus levels to decline below the threshold effective for controlling cyanobacterial biomass in dams that are already in a eutrophic state. This is due to the fact that hypertrophic aquatic ecosystems have specific positive feedback mechanisms, which stabilize the trophic state and cyanobacterial dominance. An example of this is the anoxic sediments typical of hypertrophic waters, which have a high capacity for phosphorus storage. The retention time of the dam is also an important consideration, as dams, lakes and reservoirs with high retention times will show extremely slow declines

in phosphorus concentrations, even after external inputs have been reduced to levels which should ensure a mesotrophic or oligotrophic state (Chorus & Mur, 1999). It is clear that a reduction in the phosphorus inputs into the eutrophic water body will not necessarily result in dam remediation.

4.4.2. Chemical removal of phosphorus

A solution may be to reduce the internal phosphorus concentration. Precipitation of phosphorus from the water to the sediment can be a successful measure, provided it is undertaken so that phosphorus remains permanently bound to the sediment. Experiments with precipitation of phosphorus have been undertaken with aluminium sulphate (alum), ferric salts (chlorides and sulphates), ferric aluminium sulphate, clay particles and lime as $Ca(OH)_2$ and $CaCO_3$. Ferric salts are effective in precipitating phosphorus, but are difficult to handle because of their acidity. Furthermore, the iron-phosphorus complex is stable only under oxic conditions, which means that phosphorus may be released from the anoxic sediments of eutrophic waters. In addition, iron may be a limiting micronutrient in some systems, and, in such situations, treatment with ferric salts may actually stimulate cyanobacterial growth. Hydrogen ions are liberated when alum is added to water bodies, especially lakes with a low or moderate alkalinity, leading to a sharp decrease in pH. This may consequently lead to the formation of toxic species of aluminium such as Al^{3+} and $Al(OH)^{2+}$ (Cooke *et al.*, 1993). An increase in the pH of a water body above pH 8 may result in re-release of the phosphorus from the aluminium flocs (Lewandowski *et al.*, 2003). Lime, as described previously, is used for the flocculation of intact cyanobacterial cells. Lime has also been shown to function as a longer-term algal inhibitor, as it is able to precipitate phosphorus from the water. $Ca(OH)_2$ precipitates phosphorus more efficiently than $CaCO_3$. The dose rates are quite high for sufficient phosphorus precipitation, which limits the use of this technique in large lakes and dams (Chorus & Mur, 1999). There is therefore a need for a treatment method that can bind phosphorus in a stable manner and remove it from eutrophic waters under both anoxic and oxic conditions, as well as over wide pH ranges.

4.4.3. Physical sequestering of nutrients

Asaeda *et al.* (2001) installed two vertical curtains having depths that covered the epilimnion thickness of Terauchi dam in Japan. The purpose of the curtains was to curtail the nutrient supply from nutrient rich inflows to the downstream epilimnion of the reservoir. There was a marked reduction in cyanobacterial blooms downstream from the curtain in spring and summer. The curtain prevented the direct intrusion of nutrients into the downstream zone. Epilimnion algal concentrations were higher in the upstream zones. Thus, within the upstream zone the algae consume large amounts of the inflow nutrients, reducing the nutrient supply to the downstream zone of the reservoir. Floating curtains such as these may be used to segregate *Microcystis* algal blooms, minimising turbulence. This would allow the introduction of microbial antagonists, and afford the predator ample time to attach to the prey and initiate the lytic process.

4.4.4. Phoslock® as a eutrophication management tool

Lanthanum is a rare earth element (REE) that is relatively abundant in the earth's crust compared to other REEs. Lanthanum compounds have been used in water treatment processes, as they are cheaper than those derived from other rare earth elements and the point of zero charge of lanthanum oxides is higher than that of other well-known adsorbants (Woo Shin *et al.*, 2005). Examples include use of lanthanum salts for precipitative removal of Arsenic (As) ions (Tokunaga *et al.*, 1997; Tokunaga *et al.*, 1999), the use of lanthanum impregnated silica gel for removal of arsenic, fluoride and phosphates (Wasay *et al.*, 1996a) and lanthanum oxide and lanthanum impregnated alumina for adsorptive arsenic removal (Wasay *et al.*, 1996b). According to Douglas *et al.* (2000), lanthanum was highly efficient at removing phosphorus with a molar ratio of 1:1 (Equation 2), compared with sodium aluminate ($NaAlO_2$), which is relatively inefficient with a molar ratio of 7:1 needed to achieve a similar phosphorus uptake.

$$La^{3+} + PO_4^{3-} \rightarrow LaPO_4 \qquad \ldots(2)$$

Ning *et al.* (2008) developed a La(III)-modified zeolite adsorbent (LZA), and examined its phosphate adsorption capacity in sewage plant effluent, in the presence of other

anions such as sulfates, bicarbonates, and chlorides. The LZA showed good selectivity for phosphate removal, and the authors were able regenerate the LZA for re-use.

Lanthanum is toxic, depending on its concentration and application rate. It can react with cell components such as nucleoproteins, amino acids, enzymes, phospholipids and intermediary metabolites. This is because lanthanum has many physical and chemical characteristics in common with calcium. Its action is mainly mediated by the replacement or displacement of calcium in different cell functions and its high affinity for the phosphate groups of biological molecules, resulting in toxicity or impaired function. Lanthanum is considered only slightly toxic to mammals. It is, however, highly toxic to species of *Daphnia* in both acute and chronic tests (Barry & Meehan, 2000). The potential toxicity of lanthanum ions has been overcome by incorporating it into the structure of high exchange capacity minerals, such as bentonite. This lanthanum-modified bentonite, known as Phoslock®, was developed by the Australian CSIRO, and forms a highly stable mineral known as rhabdophane ($LaPO_4.nH_20$) in the presence of oxyanions such as orthophosphates (Douglas *et al*., 2000). Rare earth- anion products are stable, due to their low solubility (Firsching, 1992). The incorporation of the lanthanum ions into bentonite is obtained by taking advantage of the cation exchange capacity of clay minerals. This exchange capacity is a result of a charge imbalance on the surface of the clay platelets, which is balanced by surface adsorbed cations exchangeable in aqueous solutions. During the preparation of Phoslock®, lanthanum ions are exchanged with these surface adsorbed exchangeable cations (Douglas *et al*., 2000). As the rare earth element is locked into the clay structure, it can either react with the phosphate anion in the water body or stay within the clay structure under a wide range of physiochemical conditions. In low ionic strength water, the lanthanum remains strongly bound to the clay silicate plates, but under conditions of high ionic strength (saline water) there is a possibility of re-exchange of the bound La^{3+} for ambient Na^+ or Ca^{2+} ions. This is not a possibility in fresh water, but may present a problem in estuaries. Any lanthanum released under these conditions is not expected to remain free, but to become strongly associated with natural humic material in the water and sediments through interaction with carboxylate groups in humic and fulvic acids (Geng *et al*., 1998; Dupre *et al*., 1999). Specific formulations of Phoslock® are used under estuarine/saline conditions to minimize lanthanum release. Phoslock is capable of

removing dissolved P under anoxic conditions, as well as over a wide pH range (pH 5-11), making it a unique water treatment product (Douglas *et al*., 1999).

As the lanthanum exchange process is carried out in solution, Phoslock® was originally prepared as slurry. However, the disadvantages of the transport of the excess water and the presence of excess residual lanthanum ions from the manufacturing process led to the formation of the granular form of Phoslock®. One of the essential features of this granular Phoslock® is that it should disperse into fine particles in water that have a similar particle size distribution to that of the parent slurry. This is necessary to ensure that the maximum number of lanthanum sites are exposed to the phosphate ions.

Two full-scale Phoslock® applications were undertaken in the summer of 2001/2002 in the impounded riverine section of two estuaries subject to cyanobacterial blooms along the coastal plain of south-western Australia. Phoslock® applied as a slurry from a small boat reduced the dissolved P in the water column to below detection limits within a few hours, and substantially reduced the amount of P released from the sediment throughout the course of the trial. The effect of the reduction in the P concentration on phytoplankton growth was clear, with the chlorophyll a concentrations of the treated areas being significantly lower than the control areas (Robb *et al*., 2003).

5. Microbial community analysis

Manipulation of the chemical and physical elements of a water body is likely to affect the microbial dynamics, both directly and indirectly. It is important to be able to describe these changes, both quantitatively and qualitatively.

According to Dejonghe *et al*. (2001), microbial diversity can be described by two components: the species richness or abundance, which is the total number of species present, and the species evenness or equitability, which is the distribution of individuals among those species. The richness component of diversity has been determined by methods such as plating, fluorescence and light microscopy and, more recently, DNA and RNA analysis (Dejonghe *et al*., 2001; Duineveld *et al*., 2001). For years, the most popular technique for investigating microbial diversity was plating (cfu). However, it is difficult to culture bacteria from environmental samples due to the selectivity of growth

media and conditions (Sekiguchi *et al*., 2002). Only 1-10% of global bacterial species are culturable (Duineveld *et al*., 2001; Von Wintzingerode *et al*., 2002). The relative proportion of bacteria growing on agar plates to those counted by fluorescence microscopy varies from 0.1% to 10%, which implies that investigations based on bacterial isolates may only include a small part of the total bacterial diversity (Amann *et al*., 1990). Although microscopic techniques can be used to obtain information about bacterial numbers and special distribution, these techniques lack the ability to assess diversity and distinguish between microbial populations (Duineveld *et al*., 2001).

The introduction of molecular methods to microbial community analysis provided a means to more accurately determine species richness within diversity. A first attempt to study unculturable as well as culturable species in an environment involved cloning random fragments of environmental genomic DNA and then sequencing clones that contained rRNA genes (Dejonghe *et al*., 2001; Fromin *et al*. 2002). However, this process is laborious and time consuming, and is therefore not suitable for the study of successional population changes in a microbial community. Hybridization techniques that make use of specific oligonucleotide probes are more suited to studying population dynamics, but probes rely on sequence data, and may be either too specific, targeting one population only, or too general, overlooking closely related but ecologically different populations (Muyzer, 1999). Because of the laboriousness of cloning, researchers began to use PCR to selectively amplify these rRNA genes from total microbial community DNA. This technique uses different primer sets to amplify the ribosomal genes of all types of organisms (Archaea, Bacteria or Eukarya) present in an environmental sample (Dejonghe *et al*., 2001). The DNA fragments obtained from this technique can be sequenced, or separated and visualized by various fingerprinting techniques (Dejonghe *et al*., 2001; Duineveld *et al*., 2001). Fingerprinting techniques enable the analysis of the diversity of different populations in natural ecosystems, and offer the potential to monitor community behavior over time (Muyzer, 1999).

5.1. Culture independent assessment of microbial communities

Methods for microbial community analysis that are culture-independent involve the extraction and analysis of signature biochemicals from environmental samples (Blackwood *et al*., 2003). Extracted genomic or ribosomal nucleic acids analysed using

molecular genetic techniques enables microbial community analysis to be coupled with a phylogenetic framework (Amann *et al*., 1995). The uncultured diversity of a sample reflects species that are closely related to culturable organisms as well as well as species from virtually uncultured lineages (Blackwood *et al*., 2003).

Molecular methods usually involve the separation of PCR amplicons on the basis of DNA sequence differences. These include denaturing gradient gel electrophoresis (DGGE), ribosomal intergenic spacer analysis (RISA), single strand conformation polymorphism (SSCP), terminal restriction fragment length polymorphism (T-RFLP) and amplified ribosomal DNA restriction analysis (ARDRA) (Blackwood *et al*., 2003). These methods only reveal diversity if the community is relatively simple, as only a small fraction of the species indicated by DNA rehybridisation rates or clone library sequence analysis can be seen on a gel (Nakatsu *et al*., 2000). However, these methods provide a rapid means to determine the relative abundance of common species in a sample in a manner independent of culture constraints, and are valuable for testing hypotheses based on the comparison of samples (Blackwood *et al*., 2003).

Ribosomal RNA (rRNA) molecules are most often used as molecular chronometers because they are highly conserved in terms of structure and function (Kent & Triplett, 2002). Ribosomes are the organelles in which translation of RNA to proteins takes place. The relative size and density of ribosomes and their subunits is expressed in Svedberg units (S), on the basis of its sedimentation rate in a sucrose density gradient during centrifugation. Prokaryotic ribosomes consist of two subunits made up of RNA and proteins, a 30S subunit and a 50S subunit. The 30S subunit of a prokaryotic ribosome consists of a 16S molecule of rRNA, which is coded by the 16S gene of the bacterial genome, and 21 proteins (Nester *et al*., 2001). Certain domains within rRNA molecules undergo independent rates of sequence change, and are known as hypervariable regions (Yu & Morrison, 2004). Phylogenetic relationships can be determined by analysing these sequence changes over time (Kent & Triplett, 2002). Currently, 16S rDNA sequences constitute the largest gene-specific data set, and the number of entries in generally accessible databases is continually increasing, making 16S rDNA-based identification of unknown bacterial isolates more likely (von Wintzingerode *et al*., 2002). However, the taxonomic resolution of 16S rRNA genes is sometimes insufficient for the discrimination of closely related organisms. As a result,

research has also focused on the rRNA 16S to 23S internal transcribed spacer (rRNA-ITS). This region may enable high resolution analysis due to its greater degree of sequence heterogeneity when compared to 16S rDNA, as well as the considerable number of published rRNA-ITS sequences (Janse *et al*., 2003).

5.1.1. Single-strand conformation polymorphism

SSCP was developed for the detection of mutations, mainly in human genetics (Swieger & Tebbe, 1998). The method involves the separation of single strands of PCR-amplified rRNA genes with the same length but different conformational structure in a polyacrylamide gel (Lee *et al*., 1996). Under non-denaturing conditions, single stranded DNA molecules will fold into specific secondary structures according to their sequences and physicochemical environment (Swieger & Tebbe, 1998). SSCP can be used in combination with an automated sequencer to differentiate between species based on the PCR products of 16S rRNA genes (Widjojoatmodjo *et al*., 1995). A major limitation of SSCP for the analysis of community DNA is the high rate of reannealing of single stranded DNA after initial denaturation during electophoresis, especially at the high concentrations of DNA that are often required for analysis of high diversity communities. Another disadvantage of SCCP is that more than one band is detectable on a gel from a double stranded PCR product following electrophoresis. Three bands are typically visible, two single strands and one double-stranded DNA molecule. However, several conformations of one product may coexist in one gel leading to multiple bands. Also, conformations of products might be similar, resulting in the detection of fewer than three bands per organism. Finally, PCR products with similar sequences may adhere to each other, forming heteroduplex molecules (Swieger & Tebbe, 1998).

5.1.2. Terminal restriction fragment length polymorphism

T-RFLP can effectively discriminate between microbial communities in a range of environments (Blackwood *et al*., 2003). This technique uses PCR of 16S rRNA genes, in which one of the two primers used is fluorescently labeled (Dejonghe *et al*., 2001). The amplified PCR product is then cut with a restriction enzyme. Terminal restriction fragments (T-RFs) are separated by gel electrophoresis and visualized by exciting the

fluorescent label (Blackwood *et al*., 2003). With this technique, a pattern of bands is obtained, with each pattern corresponding to a different species (Dejonghe *et al*., 2001). T-RF sizes can be compared to a theoretical database derived from sequence information (Blackwood *et al*., 2003), thus providing information about changes in the community structure as well as an idea of the microbial richness of an ecosystem (Dejonghe *et al*., 2001). T-RFLP profiles have the advantage of being relatively robust to variability in PCR conditions (Blackwood *et al*., 2003).

5.1.3. Amplified ribosomal DNA restriction analysis

ARDRA is another DNA fingerprinting technique based on PCR amplification of rRNA genes in combination with restriction of the amplified fragments (Dejonghe *et al*., 2001). This technique appears to give too many bands per species to provide reliable genotypic characterisation of communities, but it is capable of monitoring specific populations within microbial communities and is useful for analysing bacterial diversity (Torsvik *et al*., 1998).

5.1.4. Reverse transcription PCR

A picture of the metabolically active members in a system can be obtained by extraction of RNA instead of DNA, followed by reverse transcription PCR (RT-PCR) (Dejonghe *et al*., 2001). The first step involves the production of complementary DNA (cDNA) from a messenger RNA (mRNA) template, employing the use of dNTPs and an RNA-dependant reverse transcriptase at 37°C. In the second step, double-stranded DNA is produced using a thermostable transcriptase and a set of upstream and downstream DNA primers. After approximately 30 cycles of PCR, the original RNA template is degraded by RNase H, leaving pure cDNA in solution. Exponential amplification using RT-PCR provides a highly sensitive technique that can detect very low copy number RNAs. The technique is widely used in genetic disease diagnosis, and the quantitative determination of RNA in a cell or tissue gives an indication of gene expression levels.

5.1.5. Denaturing gradient gel electrophoresis

Muyzer *et al*., (1993) introduced DGGE as a new approach for directly determining the diversity of complex microbial populations. DGGE relies on the sequence variation of a specific amplified region to differentiate between species, thus enabling the evaluation of genetic diversity, the monitoring of succession in microbial communities, and the determination of the dominant communities in a sample (Cocolin *et al*., 2001; Koizumi *et al*., 2002; Stamper *et al*., 2003). DGGE can also be used to determine the purity and uniqueness of isolated strains. A portion of DNA is suitable for analysis using DGGE if it can be amplified specifically from the target organism, if it has enough sequence heterogeneity for the desired resolution and if it is part of a gene for which a large amount of sequence information has been deposited in sequence databases (Janse *et al*., 2003).

The procedure is based on electrophoresis of PCR-amplified 16S rDNA fragments in polyacrylamide gels containing a linearly increasing gradient of denaturants (Muyzer *et al*., 1993). The denaturants most commonly used are constant heat (60°C) formamide (0-40%) and urea (0-7M). The double stranded DNA fragments of 200-700 basepairs are equal in length but differ in basepair sequences (Ferris *et al*., 1996; Nakatsu *et al*., 2000, Kawai *et al*., 2002). Separation in DGGE relies on difference in the mobility of a partially melted DNA molecule during electrophoresis in polyacrylamide gels when compared with that of the completely helical form of the molecule (Muyzer *et al*., 1993). Initially, the fragments move according to their relative molecular mass. However, when a sufficiently high denaturant concentration is reached, strand separation occurs (Curtis & Craine, 1998). The “melting” of fragments proceeds in discrete “melting domains”, which are portions of the DNA fragment which require the same concentration of denaturants in order to separate. Once the melting domain with the lowest denaturing concentration requirement reaches that position in the DGGE gel, a transition from helical to partially melted molecules occurs, and migration of the molecule will practically come to a halt (Muyzer *et al*., 1993), forming a discrete band in the gel. The base pair composition and more importantly the sequence of the fragment determines the denaturant concentration at which this occurs, and analysis of a complex microbial community therefore results in a ladder of bands on the gel (Curtis

& Craine, 1998; Ferris *et al*., 1996; Wu *et al*., 1998). The technique is sensitive enough to detect single base pair differences in sequences (Myers *et al*., 1985).

The electrophoresis bands can either be probed with diagnostic oligonucleotides to identify particular sequences (Muyzer *et al*., 1993), or the bands can be excised from the gel, reamplified using PCR and then sequenced (Ferris *et al*., 1996). Alternatively, markers can be constructed using known species sequences, and the marker run alongside test samples to determine the identity of bands within the sample. This method was employed by Theunissen *et al*. (2005) for the analysis of probiotic organisms from yoghurt and lyophilized capsule and tablet preparations. Two markers consisting of the PCR-product of known lactobacilli and *Bifidobacterium* were run adjacent to test samples, and band patterns could be used for rapid species identification. Keyser *et al*. (2006) developed a marker to identify the dominant *Archaea* in upflow anaerobic sludge blanket bioreactors. They concluded that the DGGE marker holds great potential for the molecular monitoring of individual microorganisms as well as population shifts that may occur in anaerobic bioreactors.

The resolution of DGGE can be enhanced by incorporating a GC-rich sequence into one of the primers to modify the melting behavior of the fragment, allowing the detection of virtually all possible sequence variations (Curtis & Craine, 1998; Ferris *et al*., 1996; Muyzer *et al*., 1993). A GC clamp attached to the 5' end of the PCR product also prevents complete melting of the fragment during separation in the denaturing gradient (Heuer *et al*., 1997). Sheffield *et al*. (1989) attached a 40 base pair GC clamp to one end of amplified DNA fragments that encompass regions of the mouse and human β-globin genes. The clamp increased the number of mutations detectable by DGGE from 40% of all possible single base changes to close to 100%. In some cases, the attachment of a GC clamp alters the melting behavior of domain in such a way that the choice of which denaturant conditions to use is simplified. When a DGGE fragment with two or more melting domains is separated by electrophoresis on a DGGE gel, the fragment will be arrested at the position in the gel where the denaturant concentration dissociates the fragment at its lowest melting domain (Wu *et al*., 1998). Therefore, if the fragment has more than one melting domain a GC clamp may not be necessary. Wu *et al*. (1998) found that GC-clamped products with a perfect melting curve often resulted in smears or diffuse bands, whereas fragments containing a high melting domain run without a

GC clamp provided sharper bands and thus better results. They concluded that if the melting analysis of a short fragment (<200bp) predicts a high melting domain <40bp in size located at the end of the fragment and differing by not more than 5°C in melting temperature, then the fragment is suitable for DGGE analysis without a GC clamp.

5.1.5.1. Community diversity analysis using DGGE banding patterns

The variations between DGGE profiles were classically described visually on a single gel by the disappearance, appearance or the changes in the intensity of specific bands. However, an increasing number of studies propose statistical analysis of DGGE banding patterns, and employ various software packages to lead to more refined results (Fromin *et al*., 2002). Banding patterns on DGGE gels can be normalised using gel image software, using a reference pattern consisting of known type strains. By including a reference pattern consisting of six different type strains every six lanes on a gel, Temmerman *et al*. (2003) were able to normalise the gel patterns from probiotic products, enabling the comparison of different DGGE gels. For each known probiotic species, the band position of the corresponding type strain was determined and stored in a database, allowing individual bands in future gels to be rapidly identified. Normalisation software also allows images to be compared when samples are collected and analysed over a period of time, making it possible to monitor changes in community structure (van Hannen *et al*., 1999). Banding pattern similarity can be compared using dendrograms, which can identify outlier clusters and show the degree of intra-group similarity (Stamper *et al*., 2003).

Because DGGE makes it possible to screen multiple samples, it enables monitoring of fluctuations in microbial communities during seasonal and environmental changes in their habitat (Muyzer, 1999). Ward *et al*. (1998) were among the first to use DGGE of 16S rDNA fragments to study population changes in microbial communities. They examined the seasonal distribution of community members in a hot spring microbial mat community. More recently, Pierce *et al*. (2005) studied variation in the bacterioplankton community structure of three Antarctic lakes of different nutrient status subject to extremely rapid environmental change during the seasonal transition from winter to summer. Their results indicated that the changes in nutrient input and

duration of ice-cover lead to marked changes in the structure and stability of the bacterioplankton community.

DGGE fingerprint interpretation assumes that the band intensity is directly related to the species abundance, where each band represents a single species. Various software packages capable of calculating the relative band densities are employed to determine diversity indices (Fromin *et al*., 2002; Stamper *et al*., 2003). Most microbial diversity indices are based on indices developed for plant and animal studies, for example the Shannon Weaver and Simpson indices. There is some difficulty in applying these indices to microbial communities, as a clear definition of species and an unambiguous identification of each individual is necessary. An ideal bacterial index should ideally satisfy the following conditions (Watwe & Gangal, 1996): (i) the index should encompass three important dimensions of diversity, namely the species richness or number of different biotypes, their relative abundances and the differences or taxonomic distances between biotypes, (ii) it should be based on a statistically justified parameter and should not be sensitive to small changes in this parameter, (iii) possible errors or test result variability should not disproportionately affect the index, and (iv) since samples of microbial communities are small in comparison to the ecosystem, the index should not be overly sensitive to sample size. The Shannon index incorporates aspects of both species richness and species evenness, weighting individual classes by their relative abundances, and is the most common diversity index used by microbial ecologists. Nübel *et al*. (1999) quantified the diversity of oxygenic phototrophs (cyanobacteria, diatoms and green microalgae) in hypersaline microbial mats. The number of bands visible in the DGGE gels provided an estimate of richness, and the relative band intensity allowed for the calculation of the proportional abundance ("evenness") of each population and the Shannon-Weaver indices.

5.1.5.2. Limitations of DGGE

As with any molecular method, DGGE has certain limitations. More than one species may be represented by a single band on the gel, either as a result of phylogenetically related species sharing analogous sequences in the amplified area, or of similar melting profiles between phylogenetically unrelated species. The co-migration of non-related sequences to an identical point in the gel is especially a problem in complex microbial

communities (Fromin *et al*., 2002). For closely related organisms, the relationship between nucleotide sequence, phylogeny and the melting point is not well established. The retardation of a fragment in the gel matrix may therefore not properly indicate phylogenetic relatedness at a high resolution, such as the species level (Kisand & Wikner, 2003).

Jackson *et al*. (2000) used site directed mutagenesis to create *E. coli* 16S rDNA fragments differing by 1-4 base pairs. Migration on a DGGE gel was able to consistently distinguish single base changes, however, fragments with multiple base changes proved more difficult to resolve. Ferris & Ward (1997) detected artificial bands when analyzing complex banding patterns, which were most likely a result of heteroduplex molecules. Multiple bands may also be produced from a single species as a result of molecules produced by different rRNA operons of the same organism (Muyzer, 1999). It is generally accepted that only populations representing more than 0.1-1% of the target organisms in terms of relative proportion are displayed in a DGGE profile, and as a result not all populations present in a habitat appear on the gel (Fromin *et al*., 2002; Muyzer, 1999). Some of these limitations, such as the presence of heteroduplex molecules are not commonly found, whereas other limitations such as the limited sensitivity can be improved by hybridisation analysis or by the application of a group specific PCR (Muyzer, 1999). It is possible to analyse DNA fragments up to 1000 base pairs using DGGE, but larger fragments are not suitable. Large fragments migrate very slowly in polyacrylamide gels, and the degree of resolution between mutant and wild-type fragments decreases with size due to the melting of multiple domains in larger fragments (Sheffield *et al*., 1989).

It is important to note the pitfalls of molecular ecological approaches when studying microbial diversity. Each physical, chemical and biological step involved in the molecular analysis of the environment is a source of bias which may lead to a distorted view of the microbial community structure. The method of sample collection and preservation is crucial for the subsequent analysis steps (von Wintzingerode *et al*., 1997). The importance of sample handling procedures was illustrated by Rochelle *et al*. (1994). There was significant variation in 16S rRNA gene types and diversity from anaerobic deep marine sediment samples. Samples stored aerobically for 24h before freezing contained mainly beta and gamma Proteobacteria, whereas samples stored

anaerobically at 16°C contained mainly sequences representing alpha Proteobacteria. Samples taken anaerobically and frozen within 2h had the highest species diversity. von Wintzingerode *et al*. (1997) recommend releasing and stabilizing the nucleic acids immediately after sample collection. Lysis of microbial cells from environmental habitats to release the cell contents represents a crucial step in a PCR-mediated approach. Insufficient or preferential cell disruption will bias the view of microbial diversity; however, rigorous conditions may result in sheared DNA fragments which increase the formation of chimeric molecules during PCR. Contaminants must be removed from nucleic acid preparations, as certain molecules inhibit downstream reactions. Humic acids from soils strongly inhibit *Taq* polymerases.

PCR amplification has become the method of choice for obtaining rRNA sequence data from microbial communities. Although the method is routine for pure cultures, several problems arise when the method is applied to environmental samples. Co-extracted contaminants can inhibit amplification, differential amplification may occur and artefactual PCR products may form. These include chimeric molecules, which are composed of parts of two different but homologous sequences, deletion mutants due to stable secondary structures and point mutants due to misincorporation of nucleotides by DNA polymerases (von Wintzingerode *et al*., 1997). Amplified DNA can only qualitatively reflect species abundance if the efficiency of amplification is the same for all molecules. This requires several assumptions: (i) all the molecules must be equally accessible for primer hybridisation, (ii) hybridisation of the primer to the template must occur with equal efficiency, (iii) the DNA polymerase must extend with equal efficiency for all templates, and (iv) limitations imposed by exhaustion of substrate must affect the extension of all templates equally (Suzuki & Giovannoni, 1996). Lyautey *et al*. (2005) amplified the same DNA extract with three different PCR reactions. When the replicate amplicon was loaded onto the gel, dissimilarity between amplicons was only 3% of the detected bands. They confirmed that amplification is therefore not the step that introduces much variability into the analysis process. Contaminating DNA containing the specific target sequence of the PCR reaction can lead to amplification in the negative control without external DNA being added, and co-amplify in the experimental reactions. One also has to consider that 16S rRNA sequence variations due to *rrn* operon heterogeneity unavoidably lead to a biased reflection of the microbial diversity (von Wintzingerode *et al*., 1997).

Despite the limitations of DGGE, it is a well-established molecular tool in environmental microbiology and is reliable, reproducible, rapid and inexpensive. DGGE allows the simultaneous analysis of multiple samples, making it possible to monitor changes in microbial communities over time (Fromin *et al*., 2002; Muyzer, 1999).

6. Conclusion

Toxic cyanobacterial blooms have many implications for human health, as well as water quality. Eutrophication of water sources remains a problem that as yet has not been solved, mostly due to a lack of compliance with standard regulations, as well as the increasing human population. There is a need for a safe and effective treatment for eutrophic water bodies. Research into developing further understanding of the human health significance of cyanobacteria and individual cyanotoxins is a priority, and safe guideline values for toxins other than microcystin need to be established. Information concerning the efficiency of cyanotoxin removal in drinking water sources is limited. Simple, low-cost techniques for cyanobacterial cell removal, such as slow sand removal, should be investigated and developed further.

Biological control of toxic algal blooms, especially with bacteria, is an attractive solution. To date, however, the focus has been on laboratory studies when the efficiency of these agents in lysing cyanobacteria has been investigated. Although laboratory studies have an important part to play in biological control work, results obtained should be viewed with caution if they are to be interpreted in the lake context (Sigee, 1999). Laboratory data cannot simply be extrapolated to the freshwater environment. In cases where a biological control agent is shown to be effective, environmental testing as well as full-scale field trials need to be conducted.

Chemical control mechanisms have been employed often in the past to control cyanobacterial blooms, but they often lead to the release of toxins through cell lysis. Flocculants such as alum and lime result in less toxin release, but the introduction of these chemicals into aquatic ecosystems is often unfavourable. Turbulent mixing of a water body will reduce the cyanobacterial growth by giving green algae a competitive edge, but does not address the problem of eutrophication itself, only the symptoms.

Eutrophication management is the only feasible means of treating the cause of cyanobacterial blooms. It is important that the amount of nutrients entering eutrophic water bodies be drastically reduced, although highly eutrophic bodies make take many years to return to a mesotrophic state. Nutrient limitation through intervention may be the solution. Phosphorus limitation has been identified as being more achievable than nitrogen limitation, and there are various chemicals available for this purpose. The disadvantage of many of these however, is that they will release P under certain conditions of pH and anoxia, and some are toxic. Phoslock® is stable over a wide pH range, does not release P under anoxic conditions, and is non-toxic and environmentally friendly. Because P is permanently locked away and is not bioavailable, Phoslock® appears to be a viable means of eutrophication control.

Altering the chemistry of a water body by limiting certain nutrients is likely to affect the microbial community composition. Limiting P will result in an increase in the N:P ratio, and thus a shift in the algae population from cyanobacteria to green algae is expected, as well as the cyanobacterial species composition itself. Various methods for investigating microbial communities have been reviewed here. DGGE is a reliable, reproducible and well-established molecular tool in environmental microbiology that allows the simultaneous analysis of multiple samples, making it possible to monitor changes in microbial communities over time. It is the method of choice for many community studies.

Future research and goals

- More work needs to be done to determine the effects of cyanotoxins on human health, and safety guidelines for all such toxins in drinking water need to be set. The establishment of such guidelines will in turn increase the need for an effective and inexpensive toxin removal system. The use of bacteria for this purpose has shown great possibility, and more research needs to be conducted in this regard.
- The potential for the use of biological control agents in cyanobacterial bloom control needs to be investigated further. The bacterial species that are the most effective on a laboratory scale need to be applied in more large scale tests, as little data is available on the effects of up-scaling laboratory trials.

- Products and processes that focus on the removal of phosphorus rather that treatment of the cyanobacterial blooms need to be developed and improved. Treatment of the causes of eutrophication, rather than its symptoms may be the only way to remediate eutrophic water bodies.
- It is essential that the orthophoshate standard of 1mg.l^{-1} be complied with. Monitoring of industrial and sewage effluent is necessary, and there is a need for authorities to punish non-compliant offenders.

7. References

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