CHAPTER 9:

GENERAL DISCUSSION

In this study, phosporus limitation was examined as a possible means for cyanobacterial bloom control. The methods that were investigated focused on phosphorus reduction and the effect of this reduction on cyanobacterial and eubacterial community structures in a natural water body, as well on the treatment and removal of cyanobacterial blooms.

Phoslock®, a lanthanum-modified bentonite clay capable of removing phosphorus by adsorption, was first characterised in the laboratory in terms of its kinetics and the effect of initial pH and phosphorus concentration on the adsorption capacity. The product was also tested in cyanobacteria-containing lake water with a high pH value under laboratory conditions in order to gain understanding of the behaviour of Phoslock® in a natural water body. Phoslock® was most effective between pH 5 and pH 8, with a decrease in the adsorption capacity above pH 9. This was attributed to the formation of the hydroxyl species of the lanthanum ions on the clay surface above pH 8.35, which decreased the number of phosphorus binding sites available. Phoslock® settled more rapidly at higher pH values, which also reduced the contact time with the phosphorus in solution. The negative effects of high pH could not be overcome by increasing the Phoslock® dosage, however in a natural eutrophic water body, the pH of the sediment is lower than the overlying waterbody, so Phoslock® is expected to reach equilibrium in the sediment. Phosphorus remained bound to Phoslock® under anoxic conditions.

Phoslock® was then tested under natural conditions in a field trial at Hartbeespoort Dam from January to December 2006. The FRP (filterable reactive phosphorus) was reduced by more than 50% in the 24h following Phoslock® application. There was no change in the control area over this period, so it can be concluded that Phoslock® was responsible for removing the phosphorus from the water, despite the high pH of the surface waters. Phoslock® had no effect on the pH or nitrate concentration of the treated area, as the results were similar to those of the control throughout the trial. From August 2006 the water temperature increased, but the phosphorus concentration remained low in the treated site compared to the control, even after a large amount of nutrient containing water entered the site after the first rains. The cyanobacterial growth was visible from much earlier in summer in the control area, and the bloom was more severe throughout the summer months. The low phosphorus concentration in the water body and the reduced concentration in the sediment therefore effectively reduced the incidence and severity of the algal bloom in the treated site.

In order to assess the effect of the Phoslock® treatment on the cyanobacterial and bacterial species composition, a 16S PCR-DGGE analysis was performed on the field trial site. Samples were taken monthly from the treated and control areas from July 2006 until February 2007. Cyanobacterial specific 16S rDNA primers were used to amplify cyanobacterial DNA (Nübel *et al*., 1997), and general bacterial 16S rDNA primers (Muyzer *et al*., 1993; Fjellbirkeland *et al*., 2001) were used to amplify DNA from the entire bacterial population, including the cyanobacteria. DGGE profiles of each of the monthly samples were generated and analysed. It could be seen from the results that it was necessary to use cyanobacterial specific primers to analyse the cyanobacterial community composition by DGGE, as general bacterial primers did not give a detailed picture of the cyanobacterial species present in a sample. Using the 16S rRNA gene as a target was practical, as this sequence database is the largest. However, for the *Microcystis* spp., the resolution was low with this gene region, so it was concluded that DGGE of the rRNA-ITS region should be considered if a more detailed *Microcystis* profile is required (Janse *et al*., 2003). The lower phosphorus concentration in the treated area of the field trial encouraged the presence of diatoms, which are indicators of healthy species diversity. Unicellular cyanobacteria were present in both the treated and control areas, but there appeared to be a lag in the appearance of these species in the treated area. The different trophic levels of the treated and control areas affected the filamentous cyanobacterial population. Filamentous species were more prevalent in the treated area during the summer months than in the control area, and the treated area had a higher species diversity. The cyanobacterial species composition was thus affected by the Phoslock® treatment. As the cyanobacteria became more dominant in the treated and control areas from October, there appeared to be a shift in the bacterioplankton population. Species of Actinobacteria and Bacteroidetes were present in both the treated and control areas only until October, with one species of Actinobacteria only being present in the treated area. From November, the bacterioplankton population was dominated by β- and δ-proteobacteria. The Phoslock® treatment itself did not appear to affect the bacterial population, as the treated and control areas displayed similar patterns. For both the cyanobacteria and the bacterioplankton, the greatest effect on the species composition was in fact the seasonal change from winter to summer, as expected.

A bacterial species that was isolated from Hartbeespoort Dam that appeared to have cyanobacteriolytic activity was identified as *Bacillus cereus.* The cyanobacteriolytic nature of this species against *Microcystis aeruginosa* has previously been documented in the literature. Nakamura *et al*. (2003) found that the substance responsible for the lytic activity was produced in the stationary phase of growth, was non-proteinaceous, hydrophilic and heat stable, with a molecular weight less than 2kDa. It was thought that the bacteria attached to the surface of the cyanobacteria to first cause aggregation of cyanobacterial cells before lysis with extracellular products. The bacteria used in this study required contact for lysis, as with *B. cereus* in the studies performed by Nakamura *et al*. (2003) and Shunyu *et al.* (2006), but aggregation of the cyanobacteria was reduced in treated flasks. This may indicate that the strains were different, with the lytic substance and mechanism of lysis differing between these two organisms. The critical predator-prey ratio was 1:1 (cyanobacteria to predatory bacteria), as lower ratios of bacteria to *M. aeruginosa* did not cause the cyanobacterial population to decrease, although ratios of 1:10 and 1:100 kept the cyanobacterial population steady. A 1:1 ratio reduced the cyanobacterial population by 50% over a 14 day period, even though the bacterial population was seen to double in this time. A higher initial dosage may result in a higher degree of cyanobacterial cell death. *Bacillus cereus* was able to use *Microcystis aeruginosa* as its only nutrient source. This is of great importance in terms of the formation of a biological control product, as no addition nutrients will need to be supplied to the bacteria. No field trials have been performed to determine the effectiveness of this organism on a large scale, and laboratory tests cannot simply be extrapolated, especially because the predator-prey ratio appears to be important. The undertaking of field trials is therefore essential to determine the success of this organism as a biological control agent.

When Phoslock® and the cyanobacteriolytic bacteria were combined in a bacterial culture, Phoslock® had no effect growth rate of the bacteria. However, when the two agents were combined to assess the possibility of synergism, treatment with both Phoslock® and bacteria was no more effective than bacteria alone, and Phoslock® alone was more effective than either treatment with bacteria or with a combination of Phoslock® and bacteria. There is therefore no synergistic effect when these agents are used in combination, and Phoslock® was the most effective treatment method. The fact that the bacterial numbers increased to four times their original number in the

combination treatment, compared with only a doubling in number in the bacteria treated flask, may be due to the increased surface area for growth provided by the Phoslock®. Phoslock® could therefore be the vehicle for the bacteria as it does not affect the growth of the bacteria, and in fact promotes growth by providing a surface area for attachment. In addition, when used in combination, the phosphates released from the lysed cyanobacterial cells would be immediately adsorbed by Phoslock®, thus minimising any increase in the soluble phosphorus concentration in the water body and preventing further blooms.

Fly ash was an effective flocculant of cyanobacteria. Fly ash with a large amount of small particles was the most effective; in this study the ash from the Matla power station had the highest flocculation efficiency, and had an optimal dosage of 45g per m^2 of surface area and 1mm algal layer. The fly ash particles attached to the extracellular metabolites on the surface of the cyanobacterial cell colonies, causing them to become too dense to remain afloat. Four out of the seven fly ash samples tested caused cyanobacterial cell death after 36h. This was possibly related to the leaching of toxic elements, although only a small percentage of the total amount of trace elements were leached into solution, even at pH 2. The addition of fly ash to natural water bodies may not be hazardous, especially considering the added benefits of potential toxin removal from the water. As with the cyanobacteriolytic bacteria, field trials are necessary with the fly ash in order to determine the effect on a large body of water as well as whether the flocculation would be permanent in the turbulent conditions of a natural water body.

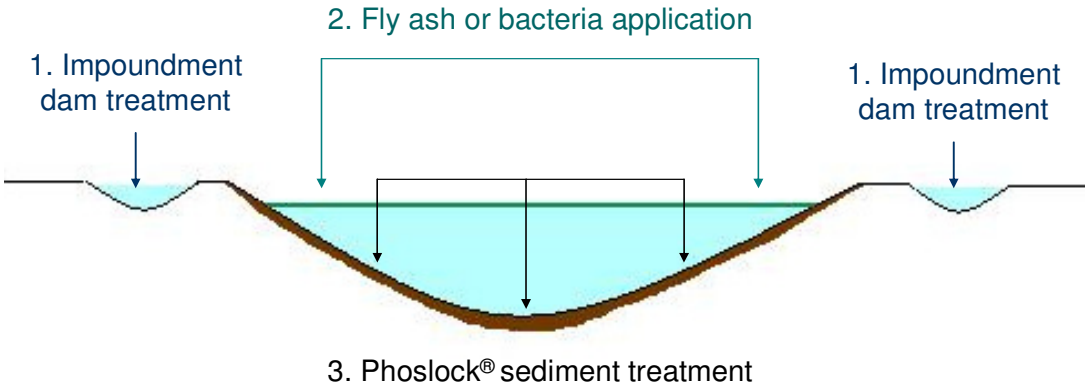
Phosphorus limitation using Phoslock® is a valuable tool for cyanobacterial bloom control. However, to treat a dam such as Hartbeespoort Dam with Phoslock® would be costly and logistically challenging. Combining Phoslock® with other control methods in an integrated manner may present a viable solution. The following treatment plan is therefore recommended for Hartbeespoort Dam:

1. Impoundment dams should be constructed at the mouths of each of the three rivers flowing into the dam, and the water in these dams treated with Phoslock® before being allowed to enter the dam. This will help to minimise further phosphorus input and prevent the problem from worstening.
2. Cyanobacteriolytic bacteria or fly ash should be applied to the surface cyanobacterial bloom in order to cause cell lysis, which will release the

phoshorus stored in the cyanobacterial cells. The maximum amount of phosphorus will therefore be available to be adsorbed during a Phoslock® application.

3. Ideally, a full Phoslock® dosage should be applied at this point, to remove the soluble phosphorus from the water body as well as to form a sediment cap to prevent future recycling of phosporus from the sediment. However, in order to minimise cost, it is recommended that Phoslock® be applied to the sediment only, by means of submerged pipes at sediment level. Although this will not be as effective as treating both the water and the sediment, recycling of phosphorus will be minimised. This strategy, combined with the prevention of new phosphorus inflow using impoundment dams, should cause the dam to move towards a more mesotrophic state.

This remediation plan can be illustrated schematically:



References

Fjellbirkeland, A., Torsvik. V. & Øvreås, L., 2001. Methanotrophic diversity in an agricultural soil as evaluated by denaturing gradient gel electrophoresis profiles of *pmoA*, *mxaF* and 16S rDNA sequences. *Antonie van Leeuwenhoek*. 79:209-217.

Janse, I., Meima, M., Kardinaal, W.E.A. & Zwart, G., 2003. High-resolution differentiation of cyanobacteria by using rRNA-internal transcribed spacer denaturing gradient gel electrophoresis. *Appl. Environ. Microbiol*. 69:6634-6643.

Muyzer, G., De Waal, E.C. & Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol*. 59:695-700.

Nakamura, N., Nakano, K., Sugiura, N. & Matsumura, M., 2003. A novel cyanobacteriolytic bacterium, *Bacillus cereus*, isolated from a eutrophic lake. *J. Biosci. Bioeng*. 95(2):179-184.

Nübel, U., Garcia-Pichel, F. & Muyzer, G., 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria. *Appl. Environ, Microbiol*. 63:3327-3332.

Shunyu, S., Yongding, L., Yinwu S., Genbao, L, Dunhai, L., 2006. Lysis of *Aphanizomenon flos-aquae* (Cyanobacterium) by a bacterium *Bacillus cereus. Biological Control.* 39:345-351.

CHAPTER 10:

CONCLUSION

The rehabilitation of a eutrophic water body cannot involve the use of a single strategy. Instead, an integrated rehabilitation plan is essential in order to ensure that the immediate problem of high nutrient concentrations and toxic algal blooms are dealt with as well as the long term goal of limiting nutrient input. The methods of eutrophication control that were discussed in this study target various stages of such an integrated eutrophication management plan.

Phoslock® treatment of a eutrophic water body should form an integral part of rehabilitation, as it resets the ecological clock by returning a water body to its natural, mesotrophic state. This is essential, as limiting point sources is simply not enough; it will take many years for a highly eutrophic water body to improve even if all incoming nutrient sources are stopped completely, as the sediment itself acts a source. Phoslock® removes soluble phosphorus from the water body, and forms a layer on the sediment preventing release of phosphorus back into the overlying water. The positive effect of Phoslock® was demonstrated in the field trial at Hartbeespoort Dam, where the soluble phosphorus remained significantly lower in the treated area than in the control. The reduced phosphorus availability also affected the cyanobacterial growth, which was reduced in the treated area and began later in the summer.

The DGGE results confirm that the nutrient status of the Phoslock® treated area versus that of the control affected the cyanobacterial population composition. More filamentous species were present in the treated area than in the control area, where only unicellular species were present. The lower phosphorus concentration in the treated area encouraged the presence of diatoms, which are indicators of a healthy ecosystem as they are sensitive to the N:P ratio.

Biological control with cyanobacteriolytic bacteria and flocculating the cyanobacteria with fly ash are methods that focus on treating the symptoms of eutrophication. Neither of these treatments are capable of completely removing an algal bloom, but both provide possible solutions to the immediate aesthetic problem. Fly ash can potentially adsorb the toxins produced by many bloom-forming cyanobacteria, and therefore may have the added benefit of improving the water quality while removing the algae. Fly ash may also be used in waste water treatment for this purpose.

Treatment of a cyanobacterial bloom with cyanobacteriolytic bacteria or fly ash will cause lysis of the cyanobacterial cells, resulting in the release of stored phosphorus. Therefore, it would be useful to apply one or both of these agents to a cyanobacterial boom before a Phoslock® treatment, to ensure that the maximum amount of phosphorus in available in solution for adsorption onto the Phoslock® surface.

Future research goals arising from this research include the following:

- The reason for cyanobacterial death when treated with certain fly ash samples needs to be determined.
- Field trials need to be performed with the cyanobacteriolytic bacteria as well as with the fly ash, as laboratory data cannot safely be extrapolated to large scale conditions.
- The effect of fly ash leaching in a large water body needs to be clarified.
- The effect of fly ash on the cyanobacteriolytic bacteria should be examined if these two agents are to be used in conjuction, as the fly ash may have a killing effect on the bacteria as observed in the cyanobacteria treatments.
- The potential use of Phoslock® as a vehicle for the biological control agent should be investigated.
- The potential ability of fly ash to adsorb cyanobacterial toxins such as microcystin must be tested, as this may be a promising alternative to activated carbon in water treatment.