Urban Water Research Association of Australia

# The Destruction of Cyanobacterial Peptide Toxins by Oxidants Used in Water Treatment

Research Report No [illegible]

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# The Destruction of Cyanobacterial Peptide Toxins by Oxidants Used in Water Treatment

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## FOREWORD

This report is based on UWRAA Research Project No. WS-60: 'Destruction of Algal Toxins by Oxidants Used in Water Treatment' which was undertaken during the period June 1993 to June 1994. Organisational responsibilities for the project were as follows:

Sponsoring Authority : South Australian Water Corporation

Research Agency : Australian Water Quality Centre, Adelaide, South Australia

Project Officer : Dr Brenton Nicholson, Australian Water Quality Centre

Principal Researcher : Joanna Rositano, Australian Water Quality Centre

The project was funded by the Urban Water Research Association of Australia and by the South Australian Water Corporation.

## SYNOPSIS

Initial studies carried out by the Australian Water Quality Centre into the effect of water treatment on the removal of cyanobacterial peptide toxins showed that some oxidants were effective in destroying peptide toxins. It was demonstrated that chlorine, permanganate and ozone rapidly oxidised the toxins under controlled conditions whereas monochloramine, hydrogen peroxide and peroxide/UV had very little, if any effect in reducing toxin concentrations. It was also found that the reactions of chlorine with the peptide toxins was pH dependant.

This project extended upon the work already covered to give a more detailed and complete study of oxidants which included chlorine, monochloramine, permanganate, ozone, peroxone, and hydrogen peroxide. Their effects on live material in particular were investigated along with the effect of pH on the reaction of the oxidants with the peptide toxins. For completeness, methods and results from the previous study are included in this report.

As with the reaction of chlorine it was found that pH also had an effect on the reaction of ozone with the peptide toxins. In contrast pH had little effect on reactivity involving potassium permanganate and hydrogen peroxide. Live cultures of toxic *Microcystis* required increased doses of chlorine and ozone to oxidise toxins due to the oxidant demand of the sample. Potassium permanganate was not effective in destroying toxins in samples containing live cyanobacteria as it could not readily access intracellular toxin, presumably because cell lysis by the action of permanganate was relatively slow.

A relative rate study of the oxidants under investigation resulted in the following order of oxidising ability: peroxone ≥ ozone >> permanganate > chlorine >> hydrogen peroxide or monochloramine.

# ACKNOWLEDGMENTS

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# 1. INTRODUCTION

The potential health hazards associated with toxin producing cyanobacteria in drinking water supplies have only recently been realised. Cyanobacteria produce a range of toxins including alkaloid neurotoxins and hepatotoxic peptides (refer to Table I). Those presenting the greatest threat to human health are the cyclic peptide hepatotoxins, microcystins and nodularin. This class of compounds has been identified as potent tumour promoters and liver toxins (Fujiki *et al* 1989, Nishiwaki-Matsushima *et al* 1991, 1992, Falconer 1991) capable of creating human health hazard through high-level short-term exposure as well as through long-term low-level exposure. Peptide toxins liberated to water as a result of chemical treatment of an algal bloom, or due to an old and dying bloom are relatively stable and have been shown to persist for weeks (Jones and Orr 1994, Berg *et al* 1987). Due to the stability and toxicity of these compounds, recent research in Australia has focussed on their removal from water.

Studies carried out on conventional water treatment processes such as coagulation/flocculation and filtration have shown that they are ineffective in removing these toxins from water (Keijola *et al* 1988, Himberg *et al* 1989, Hoffmann 1976). These studies suggested treatments such as granular activated carbon and ozonation, neither of which are commonly used in Australian water treatment plants, to be effective in reducing toxicity of toxic cyanobacteria laden waters. Recent work carried out in these laboratories has shown chlorination to be effective in the destruction of the peptide toxins in water (Nicholson *et al* 1994). This report contains the results of these studies, along with further studies carried out as an extension of this work to determine the effects of the major oxidants used in water treatment on the peptide toxins.

## 1.1 Project Aim

The aim of this project was to investigate the effectiveness of oxidants used in water treatment for the removal of cyanobacterial peptide toxins from drinking water. The oxidants investigated included:

- chlorine (including aqueous chlorine, sodium hypochlorite, calcium hypochlorite and monochloramine)
- hydrogen peroxide
- potassium permanganate
- ozone
- peroxone

Areas of investigation included:

- The effect of the oxidant on free toxin. This work was carried out using pure microcystin-LR, the most common and most toxic of the hepatotoxins, and algal extracts containing other variants of the peptide toxins.

- The ability of oxidants to remove toxicity from samples containing live *Microcystis aeruginosa.*

- The effect of pH on the oxidising capability of these oxidants with microcystins.

- A comparative rate study.

This report covers only work carried out on cyanobacterial hepatotoxins, however some preliminary oxidant studies were carried out on the cyanobacterial neurotoxins and are presented elsewhere. The effect of chlorination on anatoxin-a is reported in Rositano and Nicholson (1994). Ozone treatment of anatoxin-a was studied in collaboration with another research project and is reported in Pierrone (1993). Methodologies for the determination of paralytic shellfish poisons from cyanobacteria were not fully developed in these laboratories at the time of this study and only preliminary work was carried out. This work will be reported at a later date as part of an UWRAA funded project looking at the characterisation, and determination of PSP toxins in cyanobacteria and methods for their removal from water.

## 2. LITERATURE REVIEW

Increasing eutrophication of water bodies due to nutrient input from industry, sewage effluent and agricultural run off has caused an increase in cyanobacterial blooms worldwide (Carmichael 1992, Falconer 1988). As a direct consequence cyanobacterial blooms result in negative economic effects in terms of lost revenue from decreased recreational use of water bodies and increased cost of water treatment for drinking water.

Various cyanobacteria produce toxins (Carmichael 1992) which have been responsible for the death of livestock and wild and domestic animals which have drunk water infested with toxic cyanobacteria (Francis 1878, Codd and Poon 1988, Carmichael 1994). The main classes of toxins include hepatotoxic peptides, alkaloid neurotoxins and an alkaloid cytotoxin. Their structures are given in Figure 1 and the cyanobacteria which produce them are listed in Table I. Independent studies on the occurrence of toxic cyanobacterial blooms in countries around the world estimate that toxic blooms amount to 40 to 100 percent of all blooms surveyed (Baker and Humpage 1994, Sivonen *et al* 1990, Skulburg *et al* 1984, Pearson 1990, Carmichael *et al* 1988, Lanaras *et al* 1989).

Whilst there has as yet been no human fatalities due to cyanobacterial poisoning, toxic cyanobacteria has been implicated in a number of cases of human illness (Falconer *et al* 1983, Hawkins *et al* 1985). Probably the best demonstrated case of cyanobacterial toxin poisoning occurred in the United Kingdom when a group of army trainees undertook a canoeing exercise through water infested with toxic *Microcystis aeruginosa.* Some of the men ingested quantities of the water which resulted in severe gastrointestinal and pulmonary illness. This was directly attributed to the toxic cyanobacteria since no other factor could be attributed to causing these symptoms (Turner *et al* 1990). Pulmonary illness due to cyanobacterial peptide toxins has previously been reported by Slatkin *et al* (1983). A link between primary liver cancer and cyanobacterial peptide toxins has also been indicated. Yu (1989) reported an increased incidence of liver cancer in isolated rural communities in China which obtained their drinking water mainly from ponds often containing blooms of *M.aeruginosa.*

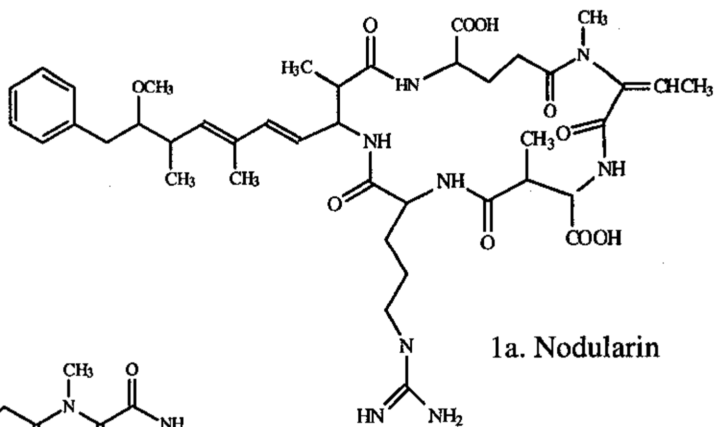
Cyanobacterial peptide toxins have been shown to inhibit protein phosphatase activity (MacKintosh *et al* 1990). This affects hepatocyte cell regulation which causes mass disruption of the cell and can lead to acute or chronic liver injury depending on the dose (Eriksson *et al* 1990, Runnegar *et al* 1993). Furthermore, it has been demonstrated that extremely low levels of the peptide toxins are potent liver tumour promoters (Nishiwaki-Matsushima *et al* 1992, Falconer and Buckley 1989). Of concern therefore, is the possibility of liver damage and enhanced susceptibility to the growth of liver tumours through the presence of low levels of peptide toxins in drinking water. This has emphasised the need for the development of sensitive assay techniques specific for hepatotoxins for monitoring drinking water supplies containing cyanobacterial blooms.

Currently available analytical techniques for the detection of the microcystin class of toxins are complicated by the large number of variants of peptide toxins produced by cyanobacteria. So far over 40 variants of microcystins have been identified and characterised (Carmichael 1992, Rositano and Nicholson 1994). The microcystins have the basic structure of Figure 1b and can have a range of amino acids at positions X and Y. Slight variations of functional groups on the basic ring structure and aliphatic side chain also give rise to variants.

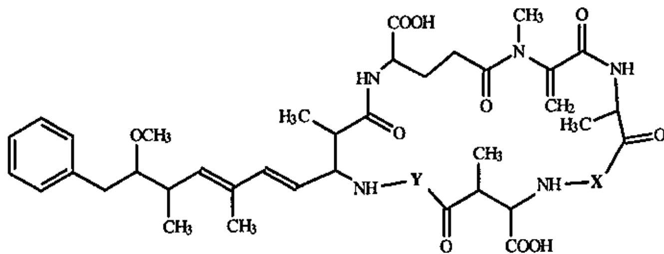
A number of biological assays have been tested by various laboratories for the initial screening of algal blooms in order to determine their toxicity. These include the brine shrimp assay, Microtox (Campbell *et al* 1994, Kiviranta *et al* 1991), *Drosophila melanogaster* bioassay (Swoboda *et al* 1994), and various cell line assays (Eriksson *et al* 1994). The Microtox, brine shrimp and *Drosophila melanogaster* bioassays were found to be unsuitable due to poor correlation with toxic strains of cyanobacteria or non-specificity for microcystin toxicity. The cell line assays proved to be effective only with freshly isolated liver cells and were specific for microcystin toxicity. However the hepatocyte isolation procedure was complicated and time consuming. The mouse bioassay for the initial screening of cyanobacterial toxicity is still the preferred technique.

For the determination of hepatotoxins in water, the ELISA technique has been assessed and found to be quantitative, sensitive and specific for the microcystin against which the antibodies have been raised (Chu *et al* 1990). An efficient polyclonal antibody which gives enough cross-reactivity with other microcystin variants has yet to be developed for this to be a viable technique.

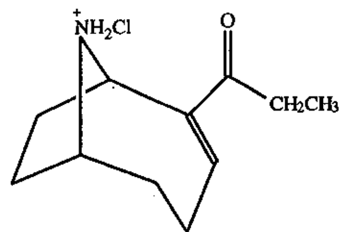
**Figure 1**



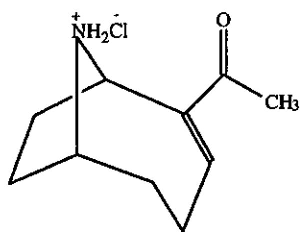

1a. Nodularin



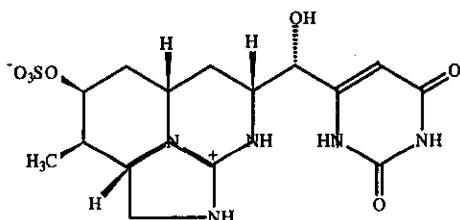

1b. Microcystin-XY



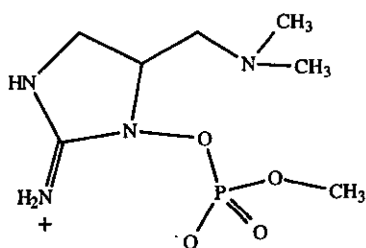

1c. Homoanatoxin-a



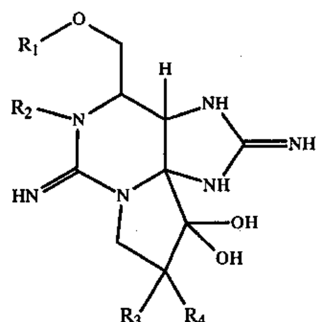

1d. Anatoxin-a




1e. Cylindrospermopsin




1f. Anatoxin-a(s)




1g. Paralytic Shellfish Poison Basic Structure

The phosphatase inhibition assay using a $^{32}P$ radiolabelled substrate has been assessed and used by various laboratories for the determination of microcystins in water (Lambert *et al* 1994, Edwards *et al* 1994, Sim and Mudge 1993). While this technique is extremely sensitive, the measurement of protein phosphatase inhibition allows the possibility for false positives due to other compounds with these properties which may occur in the sample. Inhibition of protein phosphatases also varies with different variants of microcystin resulting in quantitative errors (Eriksson *et al* 1990, Runnegar *et al* 1995).

TABLE I

CYANOBACTERIAL TOXINS AND THE CYANOBACTERIAL SPECIES WHICH PRODUCE THEM

| Toxin Class | Toxin Produced | Cyanobacterial Species* |
|---|---|---|
| Peptide Hepatotoxin | Microcystins | *Microcystis aeruginosa, Microcystis viridis, Microcystis wesenbergii, Anabaena flos-aquae, Oscillatoria agardhii, Oscillatoria agardhii var. isothrix, Nostoc sp., Anabaenopsis milleri* (Lanaras and Cook 1994). |
| | Nodularin | *Nodularia spumigena* |
| Alkaloid Neurotoxin | Anatoxin-a | *Anabaena circinalis, Anabaena flos-aquae, Anabaena lemmermannii, Anabaena spiroides, Oscillatoria sp., Aphanizomenon flos-aquae, Cylindrospermum sp.* |
| | Homoanatoxin-a | *Oscillatoria rubescens* |
| | Anatoxin-a(s) | *Anabaena flos-aquae* |
| | Paralytic Shellfish Poisons | *Aphanizomenon flos-aquae, Anabaena circinalis* (Humpage *et al* 1994) |
| Alkaloid Cytotoxin | Cylindrospermopsin | *Cylindrospermopsis raciborskii* (Ohtani *et al* 1992) *Umezakia natans* (Harada *et al* 1994) |

*references can be found in Carmichael (1992) except where indicated.

Protein phosphatase has been used in combination with other analytical techniques such as HPLC and capillary electrophoresis (Boland *et al* 1993, Lam *et al* 1995a, Jones and Orr 1994) to provide a more conclusive analysis. HPLC coupled to FAB mass spectrometry has also been used as a confirmatory method of analysis for cyanobacterial peptide toxins (Kondo *et al* 1992, Edwards *et al* 1993, Poon *et al* 1993) and has the potential to be a sensitive and specific technique for the detection of cyanobacterial toxins. However it requires sophisticated and expensive instrumentation dedicated to this analytical procedure and further developmental work.

In this study HPLC analysis with photodiode array detection was employed as the primary method of peptide toxin detection. It uses the principle that all peptide toxins have a characteristic UV spectrum and thus individual peaks in the HPLC chromatograph can be assigned as a microcystin variant and quantified with respect to a standard (usually microcystin-LR). Although this method cannot conclusively assign microcystin variant types to peaks unless the standards are available, it is used by most laboratories as the analytical method for peptide toxin analysis (Lawton *et al* 1994, Tsuji *et al* 1994).

Further to the monitoring of toxic cyanobacteria in recreational and drinking water supplies is the need for available technologies for water treatment where toxic cyanobacterial blooms are present. The common practice for controlling cyanobacterial blooms in Australian water bodies has been to dose with an algicide such as copper sulphate and cease all use of that water for a period of 7-10 days. This treatment causes lysis of the cells and in the case of toxic blooms release of the toxins (Kenefick *et al* 1993). Laboratory and field trials have shown that the peptide toxins can remain in solution for many weeks before toxin degradation occurs (Jones and Orr 1994, Berg *et al* 1987) posing a potential health risk to recreational users and consumers.

A number of laboratory and pilot scale water treatment studies have been conducted by various laboratories. Initial studies by Hoffmann (1976) and Falconer *et al* (1989) indicated that normal flocculation and chlorination processes did not remove toxicity caused by cyanobacteria but activated carbon was capable of removing the toxins. Keijola *et al* (1988) and Himberg *et al* (1989) also found this to be the case in addition to ozonation and to some extent slow sand filtration.

More detailed laboratory scale experiments later showed that only a few commercially available powdered activated carbons are capable of removing the hepatotoxins at contact times commonly

employed in water treatment facilities (Donati *et al* 1994, Jones *et al* 1993). Laboratory and pilot plant experiments undertaken by the Hunter Water Corporation (Craig *et al* 1995) indicated that granular activated carbon was effective for the removal of hepatotoxins in the short term, but the carbon was exhausted in a relatively short time period (less than five months) and therefore may not be economically viable. Biologically active filters followed by slow sand filtration were found to be effective in the removal of cyanobacterial cells and toxins in small scale water treatment facilities (Sherman *et al* 1995), and may be a viable option for servicing small communities.

Where previous pilot scale experiments have found chlorination to be ineffective for the removal of peptide toxins from water, laboratory scale experiments carried out in these laboratories found chlorine to be effective for their removal under controlled conditions and these results are documented in this report. UV irradiation at 254 nm was also found to remove toxicity (Rositano and Nicholson 1994) which is contrary to the findings of Tanaka *et al* (1993) who found microcystins to be unaffected by irradiation at 254 nm. However the experimental details were not outlined in their report and it is possible that the intensity of irradiation was too low to have any effect.

As part of studies on the potential health effects of algal toxins in drinking water supplies, preliminary laboratory studies on water treatment techniques for the removal of algal toxins were undertaken in these laboratories with funding from the South Australian Water Corporation (formerly the Engineering and Water Supply Department of South Australia). These studies included development of methods for the detection of peptide toxins in water, stability of the peptide toxins under various conditions and effects of oxidants and coagulants on the removal of the toxins. With funding from the Urban Water Research Association of Australia the study of oxidants for the removal of cyanobacterial toxins has been extended and the results are presented in this report.

# 3. MATERIALS

All chemicals and solvents were of analytical or HPLC grade. High purity water was either reverse osmosis water further purified by distillation or water of equivalent purity prepared with a Milli-Q system (Millipore Corporation). Nitrogen gas was high purity obtained from BOC Gases.

## 3.1 Cyanobacterial Materials and Toxin Standards

Freeze-dried *Microcystis aeruginosa* Kütz. emend. Elenkin from Mt. Bold Reservoir, South Australia (10.2.88) and Lake Mokoan, Victoria (14.3.91), were used as the source of toxic material containing microcystins. The material from Mt. Bold had previously been shown to have an LD50 (mouse-ip) of 8 mg/kg and to contain microcystin-LR and -LA (Flett & Nicholson, 1991). A chromatogram is shown in Figure 2a. The chromatogram also contains a few minor peaks, some of which may be hepatotoxic as their UV spectra are typical of microcystins. However, these compounds have not been identified. *M. aeruginosa* from Lake Mokoan, Victoria had an LD100 of 10 mg/kg (mouse-ip). This material contained 2 major peaks and a large number of minor peaks as shown in Figure 2b. Standards were not available for toxin identification or precise quantification of toxin levels for this material.

Where these freeze-dried materials were used, toxin concentrations were based on the sum of the responses of the major peaks, i.e the sum of the responses of microcystin-LR and -LA in the case of the Mt Bold material and the sum of the responses of peaks LM1 and LM2 (Figure 2b) for the Lake Mokoan material, relative to a microcystin-LR standard. The concentration of all microcystins was thus given in microcystin-LR equivalents. Watanabe *et al* (1988) have shown that the UV responses of individual microcystins in HPLC analysis are very similar and hence this approximation should introduce very little error.

A toxic culture of *Microcystis aeruginosa* isolated from a bloom in Shepparton Victoria, (donated by Dr Gary Jones of the CSIRO Division of Water Resources) was grown in a modified ASM-1 medium (Gorham *et al* 1964) (10 L) at 25°C under continuous light of 60 $\mu M/m^2/sec$. The cells were allowed to grow for 10-15 days then centrifuged at 8 000 rpm for ten minutes to concentrate

the cells (Sorvall RC-5B refrigerated centrifuge). A direct cell count was carried out by staining with Lugols iodine solution after settling in a 1 mL Sedgwick-Rafter counting chamber. Differentiation between live and dead cells was not made. To the concentrated culture was then added 10 % v/v of 0.1 M potassium phosphate buffer to give a final pH of 7. The cyanobacteria contained two major toxic peaks, microcystin-LR and an unknown toxin. Again these toxins were quantified with respect to microcystin-LR standard. An HPLC chromatogram is shown in Figure 2c.

Microcystin-LR and nodularin standards were obtained from Calbiochem Novabiochem Corp. California

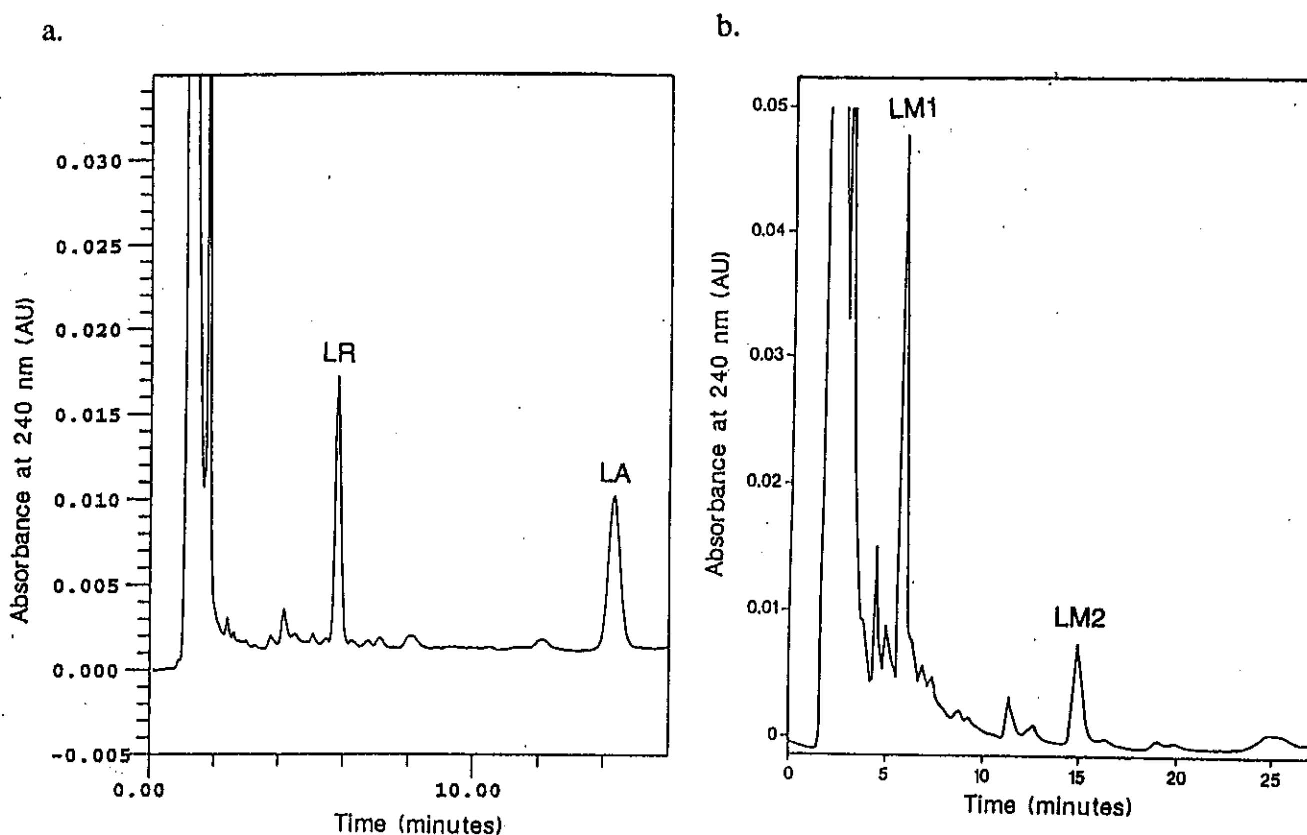



Figure 2. HPLC toxin profiles of *M.aeruginosa* extracts from a. Mt Bold reservoir (South Aust.) b. Lake Mokoan (Victoria).

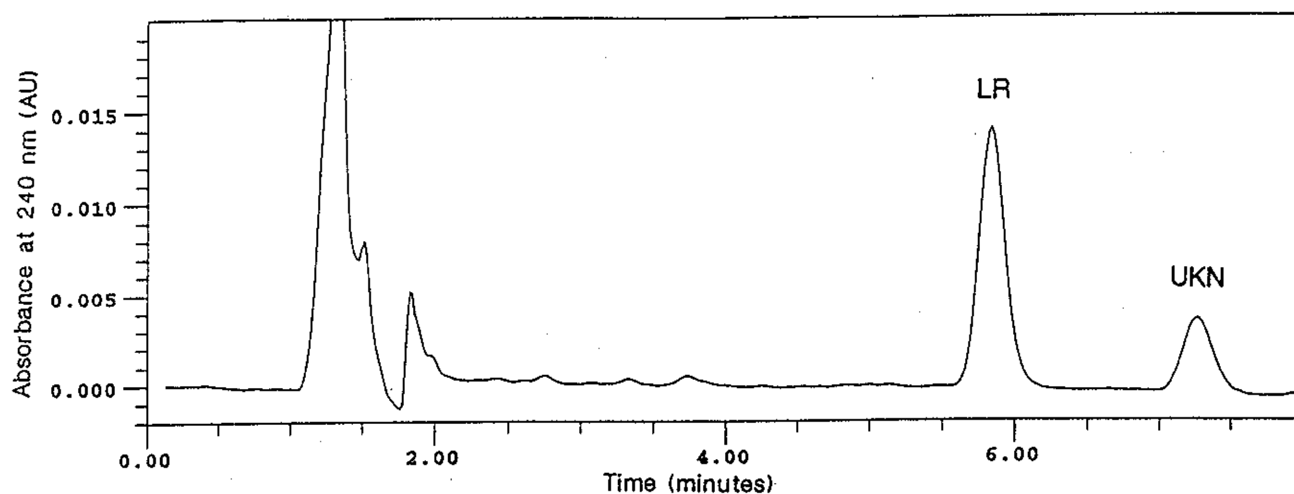



Figure 2c HPLC toxin profiles of *M.aeruginosa* extract from Shepparton (Victoria)

**3.2 Mouse Bioassays**

Mouse bioassays were carried out by the Institute of Medical and Veterinary Science, Adelaide, South Australia. 1mL physiological saline solutions were injected intraperitoneally into 20 g white female Balb/c mice. Death within 1-6 hours with the characteristic enlarged liver was used as the measure of hepatotoxicity.

## 4. METHODS

**4.1 Extraction of Toxins From Freeze-Dried Cyanobacteria**

Freeze-dried cyanobacteria was sonicated for 2 minutes using a Branson 250 Sonifier (duty cycle 40%, output 3) in Milli-Q water. The sample was then centrifuged at 10 000 rpm for 20 minutes using a Sorvall RC-5B refrigerated centrifuge and the supernatant decanted. The pellet was re-extracted and the supernatants combined.

## 4.2 Extraction of Toxins From Cultures of *Microcystis aeruginosa*

To determine the most efficient method for the extraction of toxin from live cells, quadruplicate samples of cultured *Microcystis aeruginosa* (100 mL) were treated in the following manner:

- Samples were sonicated for 3 minutes, centrifuged at 4 000 rpm for 20 minutes (Beckman GS-6), the supernatant decanted and 5 mL of water added to the pellet followed by re-extraction. The supernatants were combined and extracted by C18 solid phase extraction.

- Sodium dodecyl sulphate (10 mg) was added to each sample which was then extracted by sonication as described above.

- Samples were freeze-thawed then filtered through a Whatman GF/C and the residue washed with water and discarded. The filtrate was then extracted by C18 solid phase extraction.

## 4.3 Solid Phase Extraction and HPLC Analysis of Peptide Toxins

Waters 500 mg C18 Sep-Pak Vac cartridges attached to a vacuum chamber were primed with methanol (10 mL) followed by water (10 mL). The water sample or algal extract was passed through the cartridge at a rate of approximately 10 mL/min. The cartridge was then washed with water (10 mL), 10% methanol (10mL), and 20% methanol (10 mL) and the toxin subsequently eluted from the cartridge with 100% methanol (10 mL). The methanol fraction was then evaporated to dryness at 40°C under a stream of nitrogen and made up to 1.0 mL with 50% methanol/water. This was then filtered through a 0.45μm PVDF Gelman acrodisc and 50 μL injected onto an HPLC column.

Initial HPLC analyses were performed with a Waters Chromatography liquid chromatograph equipped with a model 501 solvent delivery system, a model 991 photo-diode array detector set at 240 nm and a model 917 autosampler. The column was either a Brownlee ODS 5 μm spheri analytical column, 4.7 mm x 220 mm ID or Waters symmetry C18, 5 μm 3.9 x 150 mm analytical column. This system was later upgraded to a Waters 996 photodiode array detector with Millenium software and 600E quaternary pump. The mobile phase was a solution of 27% acetonitrile / pH 7, 0.1M $KH_2PO_4$ buffer run at 1.0 mL/min (modified method of Meriluoto and Eriksson 1988).

### 4.4 Potassium Permanganate

For the following experiments a stock potassium permanganate solution (1000 mg/L) was prepared in distilled water and standardised by titration with sodium oxalate solution (Standard Methods 14th Ed p 187). Permanganate residuals were determined by DPD/FAS titration according to the method for chlorine residual determination (Standard Methods 14th Ed p 329) which gave a linear relationship between 0-7.5 mg/L.

#### 4.4.1 Reaction of potassium permanganate with microcystin-LR

Duplicate Milli-Q water samples (50 mL) containing microcystin-LR at 200 µg/L were dosed with potassium permanganate to give final concentrations of 0, 1, 2, 3, 4, 5, 7, and 10 mg/L potassium permanganate. The solutions were left standing for 30 minutes and then quenched with 0.1 M sodium thiosulfate (40 µL). The solutions were extracted and analysed for microcystin-LR.

#### 4.4.2 Reaction kinetics of potassium permanganate with microcystin-LR

To a closed reaction vessel containing a phosphate buffered solution (0.01M pH 7) of microcystin-LR (1 mg/L) and stirring flea in an inert atmosphere (high purity nitrogen) was added potassium permanganate solution to give a final dose of 2 mg/L (refer to Figure 3a). Samples of 15 mL and 1 mL were withdrawn at regular time intervals for permanganate residual determination and microcystin-LR analysis respectively. Reaction aliquots for toxin analysis were quenched with 0.1 M sodium thiosulfate solution (40 µL). This procedure was performed in duplicate.

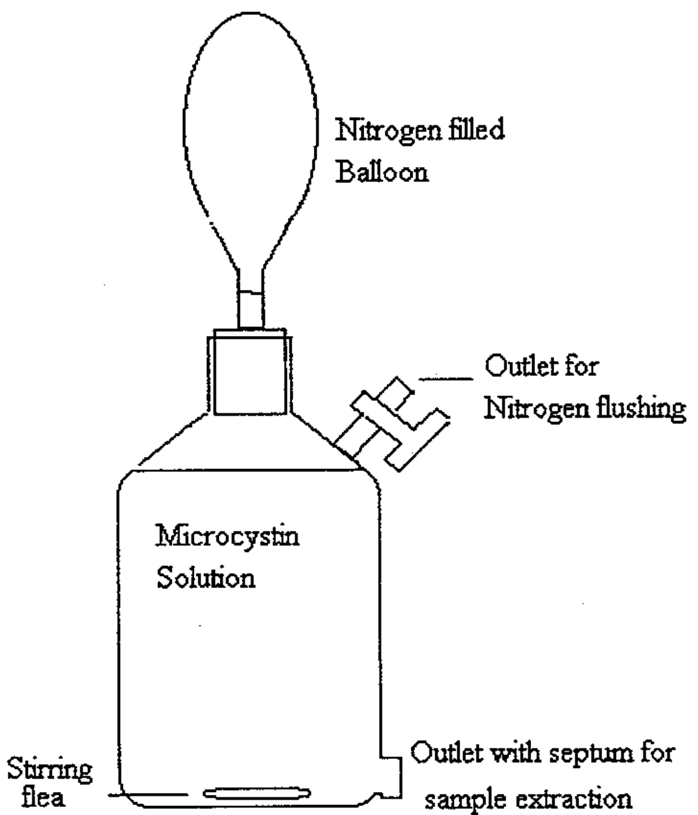



Figure 3a. Configuration of apparatus for macro kinetic studies.

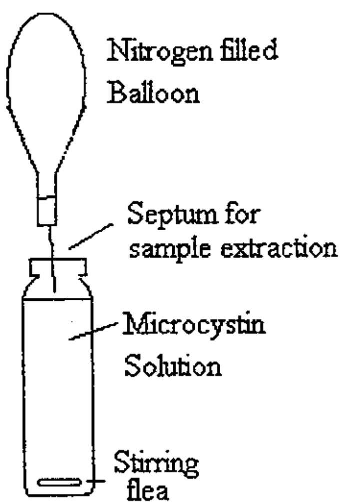



Figure 3b. Configuration of apparatus for micro kinetic studies.

### 4.4.3 Reaction of potassium permanganate with an *M. aeruginosa* extract

Duplicate Milli-Q water samples (50 mL) containing *M. aeruginosa* extract giving a total toxin concentration of 254 μg/L and having a DOC of 3.1 mg/L and pH 7 were dosed with potassium permanganate at 0, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, and 5.0 mg/L. The solutions were left standing for 30 minutes with occasional stirring and then quenched with 0.1 M sodium thiosulfate. The solutions were extracted and analysed for microcystins by HPLC analysis. Following HPLC analysis the remaining extract was diluted to 2.5 mL and sodium chloride added to give a physiological saline solution for analysis by mouse bioassay.

### 4.4.4 Effect of pH on potassium permanganate oxidation of microcystins

This experiment was similar to the previous experiment; however the permanganate dose rate remained constant at 3 mg/L and the pH was adjusted to 2.0, 4.0 7.2, 8.8, and 10.1 with dilute solutions of hydrochloric acid or sodium hydroxide. This experiment was run in duplicate.

### 4.4.5 Reaction of potassium permanganate with an *M. aeruginosa* culture

**Experiment 1**

A culture of *M. aeruginosa* was concentrated to give a cell biomass of 600 000 cells/mL. The culture had a TOC of 5.8 mg/L and a pH of 9.5. Culture solutions (100 mL) were treated with potassium permanganate at doses between 0, and 10 mg/L in duplicate in closed reaction vessels. After one hour permanganate residuals were determined with one set of solutions while the other set was quenched with sodium thiosulfate, extracted and analysed. This procedure was repeated with a culture having cell biomass of 330 000 cells/mL, TOC 3.6 mg/L and pH 7.4.

**Experiment 2**

A culture of *M. aeruginosa* having 490 000 cells/mL, TOC 3.1 mg/L, and containing 10 %, 0.1 M phosphate buffer pH 7 was dosed with potassium permanganate at 10 mg/L. This was allowed to react with occasional stirring for 4 h during which samples were taken every hour to determine microcystin concentration and permanganate residual. This experiment was repeated with a culture having 1.15 x $10^6$ cells/mL and TOC 10.4 mg/L over 8 h.

## 4.5 Chlorination

Stock chlorine solutions were prepared from commercially available reagents by dissolving or diluting reagents in Milli-Q water and determining the concentration of the solution as total free available chlorine by titration with DPD/FAS (Standard Methods 14th Ed p 329). All chlorine residuals were also determined using DPD/FAS titration. All reactions were carried out in closed reaction vessels to minimise the loss of chlorine to the atmosphere.

### 4.5.1 Reaction of chlorine with microcystin-LR

Milli-Q water samples (50 mL) containing microcystin-LR at 40 µg/L were dosed with chlorine water to give final concentrations of 0, 2, 4, 6, 8, and 10 mg/L chlorine. The solutions were left standing for 30 minutes and then quenched with sodium sulfite. The solutions were extracted and analysed for microcystin-LR.

### 4.5.2 Reaction kinetics of chlorine with microcystin-LR

This experiment was identical to that for determining the reaction kinetics of microcystin-LR with potassium permanganate but using chlorine in place of permanganate. Reaction aliquots for toxin analysis were quenched with ascorbic acid.

### 4.5.3 Reaction of chlorine (as aqueous chlorine, sodium hypochlorite, and calcium hypochlorite solution) with an *M. aeruginosa* extract solution

Chlorination of an *M. aeruginosa* extract was carried out with aqueous chlorine, sodium hypochlorite and calcium hypochlorite solutions. High purity water samples (50 mL) containing an *M. aeruginosa* extract were dosed with the chlorine solutions at doses ranging between 0-2 mg/L (refer to Table 5 for total toxin concentrations). The stoppered flasks were left standing for 30 minutes with occasional stirring and then quenched with sodium sulfite, extracted and analysed for microcystins by HPLC. Following HPLC analysis the remaining extract was diluted to 2.5 mL and sodium chloride added to give a physiological saline solution for analysis by mouse bioassay. Mouse bioassay was not carried out for calcium hypochlorite.

### 4.5.4 Effect of pH on chlorine oxidation (as aqueous chlorine, sodium hypochlorite, and calcium hypochlorite solution) of microcystins.

High purity water was chlorinated to give a dose of 15 mg/L. The solutions were pH adjusted with dilute hydrochloric acid or sodium hydroxide solutions to give pH values of 3, 4, 7, 8, 9, and 10. To these solutions was added *M. aeruginosa* extract to give a total toxin content between 5.4 and 6.6 μg. The solutions were left standing for 30 minutes with occasional stirring then quenched with ascorbic acid, extracted and analysed. This experiment was carried out for all three chlorinating agents.

### 4.5.5 Reaction of chlorine with an *M. aeruginosa* culture

A culture of *M. aeruginosa* was concentrated to give a cell biomass of 412 000 cells/mL. The TOC was 4.7 mg/L and the pH was adjusted to 7 by the slow addition of dilute hydrochloric acid. The chlorine demand of the culture was determined to be 8.7 mg/L chlorine. A 1L culture was chlorinated at 10 mg/L aqueous chlorine and aliquots of 100 mL were withdrawn at various times over a 30 minute period, 20 mLs of this solution was used to determine chlorine residuals whilst 80 mL was quenched with ascorbic acid for extraction and microcystin analysis.

### 4.5.6 Reaction of chlorine with *N. spumigena*

*N. spumigena* cells from a very toxic fresh bloom (Campbell Park, Lake Albert, South Australia) were concentrated by allowing the naturally buoyant cells to accumulate at the surface, then removing the cells and reconstituting them in lake water. The final cell biomass was 2.5 x $10^5$ cells/mL and the TOC 9.6 mg/L. Subsamples (100 mL) were dosed with aqueous chlorine to give concentrations of 5, 10, and 15 mg/L in open vessels and 10 mg/L in a closed vessel. The solutions were stirred and 2 x 5 mL aliquots were taken at various time intervals to determine chlorine residual and toxin content by HPLC analysis. The fractions for HPLC analysis were quenched with sodium sulfite and ultrasonicated (1 minute) to release the toxin from any remaining intact cells. The solutions were then filtered (Whatman GF/C) and analysed by HPLC.

### 4.5.7 Chloramination

A concentrated solution of monochloramine was prepared from ammonium chloride and sodium hypochlorite at a ratio of 3.4 : 1 (w/w) chlorine to ammonia. The pH of the solution was 8.4, the optimum for monochloramine formation, and the total chlorine concentration was determined by DPD/FAS titration. High purity water samples (50 mL) containing an *M. aeruginosa* extract (Mt Bold) were dosed with monochloramine at a concentration of 20 mg/L. Solutions were allowed to stand for 30 minutes, 1 hour, 2 days and 5 days in stoppered flasks at room temperature in the dark. The solutions were quenched with ascorbic acid followed by extraction and HPLC analysis.

## 4.6 Hydrogen Peroxide

### 4.6.1 Determination of peroxide concentration

*Sodium Thiosulfate Method*

This method was used to determine the strength of the stock solutions. In a 500 mL Erlenmeyer flask was mixed 200 mL high purity water, 15 mL 10% potassium iodide

solution, 10 mL 1N sulphuric acid, 2 mL of a 50 g/L ammonium molybdate solution and 10 mL of the stock hydrogen peroxide solution. This mixture was titrated against 0.1M sodium thiosulphate and the concentration of peroxide calculated according to the following equation.

$$C \text{ (g/L hydrogen peroxide)} = \frac{\text{volume titrated x [thiosulphate] x 17}}{10}$$

*Titanium Sulphate Method*

The method of Eisenberg (1976). was used to determine hydrogen peroxide residuals in solution. Titanium sulphate reagent was made by digesting titanium dioxide (1 g) in concentrated sulfuric acid (100 mL) for 16 hours at 150°C. This was diluted with four parts high purity water and filtered through a glass fibre filter. (Whatman GF/C). Titanium sulphate reagent (5 mL) was reacted with 45 mL of the unknown peroxide solution and the absorbance measured at 400 nm using 40 mm cells. The concentration of peroxide was determined from a standard curve of peroxide concentration versus UV absorbance.

### 4.6.2 Reaction kinetics of hydrogen peroxide with microcystin-LR

This experiment was identical to that for the reaction kinetics of microcystin-LR with potassium permanganate using hydrogen peroxide in place of the permanganate with the exception that 10 mL reaction aliquots were reacted with titanium sulfate reagent for peroxide residual determination.

### 4.6.3 Reaction of hydrogen peroxide with microcystin-LR

High purity water containing 100 µg/L microcystin-LR was dosed with a stock solution of hydrogen peroxide to give duplicate 50 mL samples having final concentrations of 0, 1, 2, 5, 7, and 10 mg/L. The solutions were left to stand for 30 minutes with occasional stirring after which time one set of solutions was quenched with 0.1M sodium thiosulphate for toxin analysis and the second sample set was reacted with titanium sulphate solution for the determination of peroxide residual.

### 4.6.4 Reaction of hydrogen peroxide with an *M. aeruginosa* extract

This was conducted as for the previous experiment except that the toxin solutions contained an *M.aeruginosa* extract from Lake Mokoan at a total toxin concentration of 72 μg/L and solutions were left to stand for one hour. The solutions were dosed to give final peroxide concentrations of 0, 5, 10, 15, and 20 mg/L. The DOC concentration was approximately 6.3 mg/L and the pH was maintained at 6-7.

### 4.6.5 Effect of pH on peroxide oxidation of microcystins

Triplicate 50 mL solutions each containing 10 μg total toxin content from a Lake Mokoan extract were adjusted to pH levels of 1.5, 3.0, 6.0, 7.0, 8.5, and 9.5 with solutions of dilute hydrochloric acid or sodium hydroxide. The solutions were dosed with hydrogen peroxide to give final concentrations of 20 mg/L, the pH of the solutions readjusted if necessary and the solutions left standing for one hour. Two sets of solutions at each pH were quenched with 0.1M sodium thiosulfate and analysed for toxin content whilst the last set of solutions was reacted with titanium sulphate solution to determine the hydrogen peroxide residual. The DOC of the solutions was 9.8 mg/L.

### 4.6.6 UV/Peroxide treatment of microcystin-LR

High purity water samples (4 x 50 mL) containing 130 μg/L microcystin-LR were dosed with 20 mg/L hydrogen peroxide. They were placed in plastic petri dishes and the depth of the water sample was 1 cm. These solutions were subjected to UV irradiation at 140 $\mu W/cm^2$ for 30 minutes. Three controls were also run simultaneously under the following conditions:

i) microcystin-LR in distilled water placed in the dark
ii) microcystin-LR in distilled water + UV light (140 $\mu W/cm^2$)
iii) microcystin-LR in distilled water + peroxide placed in the dark

After 30 minutes peroxide residuals were determined where appropriate and the remaining solutions were quenched with 0.1 M sodium thiosulfate and extracted and analysed for microcystin content.

## 4.7 Ozone

Ozone was produced by a small home made ozonator fed by pure oxygen. All reactions were carried out in sealed vessels by the addition of pre-ozonated water (except where indicated in the methods). Pre-ozonated water was produced by constantly feeding a stream of ozone through high purity water in a 500 mL vessel equipped with a sparger (Figure 4). The ozone concentration of this solution was determined immediately preceding the addition of the stock solution to an experimental sample and immediately after addition to accurately determine applied dose rates. All ozone concentrations and residuals were determined by the indigo method.

### 4.7.1 Determination of ozone concentration

*Indigo Method*

Ozone concentrations and ozone residuals for ozone and peroxone experiments were determined by the indigo method (International Ozone Association - Standardisation Committee : 006/86 (F)). Ozonated solutions, and a control solution (90 mL) were reacted with a stock solution of indigo trisulphonate (Indigo blue)(10 mL) and the difference in absorbance measured at 600 nm using 5 cm cells on a Varian DMS 70 UV/Vis spectrometer. The concentration of ozone was calculated according to the following equation.

$$C\ (\text{mg/L ozone}) = \frac{\text{total volume} \times \Delta\ \text{absorption}}{\text{cell length(cm)} \times 0.42 \times \text{volume of water sampled}}$$

where 0.42 = proportionality constant
and Δ absorption = difference between absorption of sample and control

### 4.7.2 Reaction of ozone with microcystin-LR

Duplicate high purity water samples (100 mL) containing microcystin-LR at 166 μg/L were dosed with ozone to give final concentrations of 0, 0.07, 0.14, 0.21, 0.26, 0.33, 0.48, and 1.12 mg/L. The solutions were left standing for four minutes after which time one sample at each dose rate was quenched with 0.1M sodium thiosulfate and the toxin content determined. The other sample was reacted with indigo blue solution to determine the ozone residual.

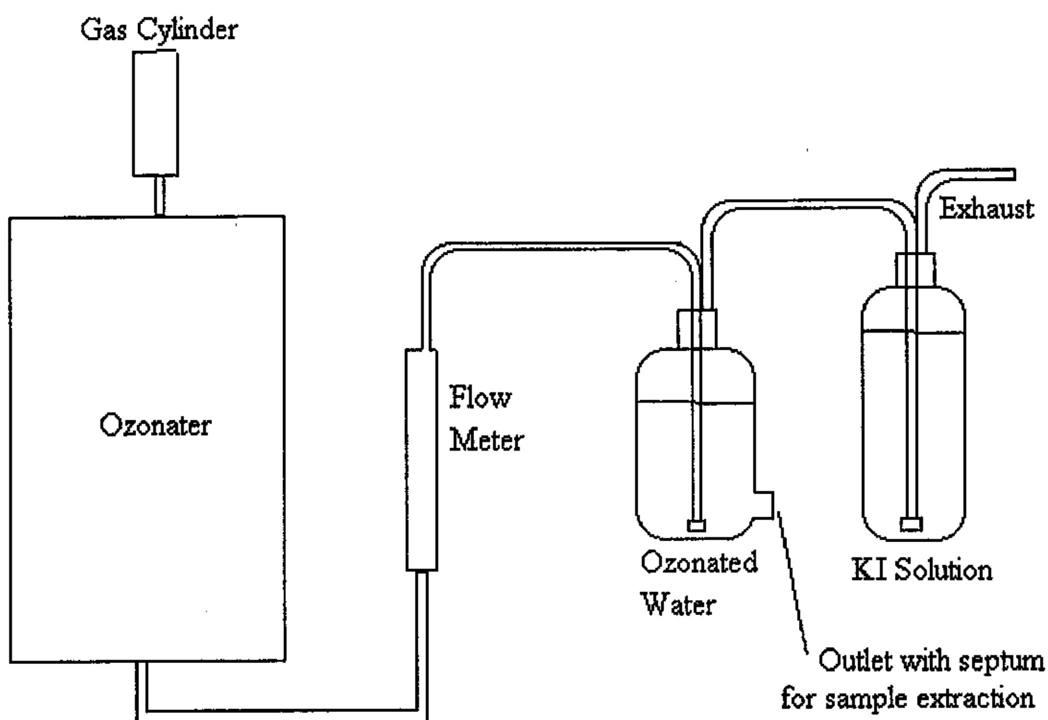



Figure 4. Configuration of apparatus for the generation of ozonated water.

### 4.7.3 Reaction kinetics of ozone with microcystin-LR

This experiment was identical to that for the reaction kinetics of microcystin-LR with potassium permanganate with the exception that a dose rate of 0.22 mg/L was achieved by the addition of ozonated water and 10 mL reaction aliquots were reacted with indigo blue

solution for ozone residual determination (run in duplicate). This experiment was scaled down from 200 mL to 20 mL utilising a smaller reaction vessel (Figure 3b) and identical conditions, however the dose rate was decreased to 0.02 mg/L (in triplicate). Sample aliquots for ozone residual determination were not taken in the scaled down experiment.

### 4.7.4 Reaction of ozone with an *M.aeruginosa* extract

High purity water (100 mL) containing an *M. aeruginosa* extract (Mt. Bold) to give a microcystin concentration of 220 μg/L and having a DOC of 8.5 mg/L and pH 6.8 was treated with various doses of ozonated water (refer to Table X). Ozone residuals were determined after five minutes and samples for toxin analysis were quenched with 0.1 M sodium thiosulfate (200 μL).

### 4.7.5 Effect of pH on ozone oxidation of microcystins

High purity water samples containing an *M. aeruginosa* extract (Mt Bold) were adjusted to pH 2.0, 3.5, 5.0, 7.3, 8.0, and 9.9 with dilute hydrochloric acid or sodium hydroxide solutions. Ozonated water was added to give a dose rate of 1.0 mg/L (range 0.9 - 1.1 mg/L); the samples had a final volume of 50 mL and had a resulting concentration of 250 μg/L microcystin and a DOC of 1.9 mg/L. After 5 minutes the reaction was quenched with 0.1 M sodium thiosulfate (200 μL) and the samples were extracted and analysed for microcystins. Two controls at pH 7 containing no ozone were also extracted and analysed.

### 4.7.6 Reaction of ozone with an *M. aeruginosa* culture

**Experiment 1**

A culture of *M. aeruginosa* was concentrated to give a cell biomass of 1.63 x $10^6$ cells/mL. The concentrated culture had a TOC of 8.0 mg/L and was buffered at pH 7 by the addition of 10% 0.1 M phosphate buffer. Concentrated culture samples (50 mL) were treated with ozonated water to give resulting doses of 0, 0.36, 0.70, 0.89, 1.0, 1.6, 1.9, 3.0, and 3.7mg/L, in triplicate in closed reaction vessels. After five minutes reaction time ozone residuals

were determined with one set of solutions while the other two sets were quenched with 0.1 M sodium thiosulfate (200 μL), extracted and analysed.

### Experiment 2

A culture of *M. aeruginosa* having 2.05 x $10^6$ cells/mL, TOC 11.4 mg/L, adjusted to pH 7 by the addition of 10% 0.1 M phosphate buffer was ozonated at approximately 8.0 mg/SL ozone (gas) for 20 minutes. Configuration of the apparatus is shown in Figure 5 and details of ozone flow rates are presented in Table XI. Samples for microcystin analysis and ozone residuals were withdrawn at various intervals over 20 minutes.

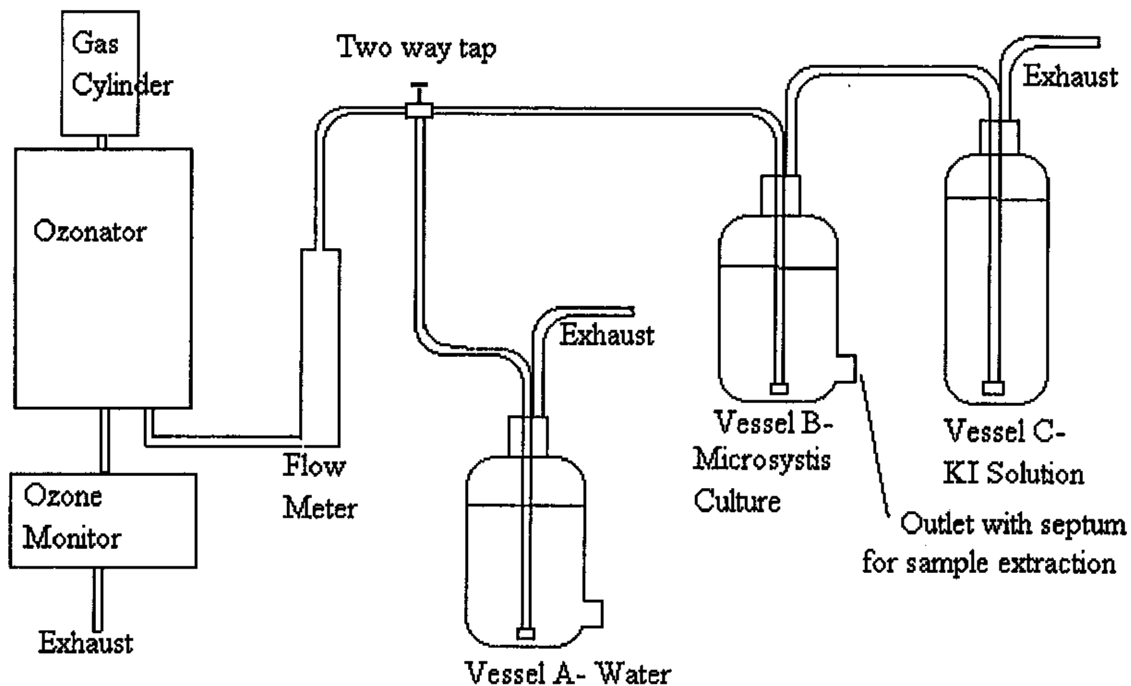



Figure 5.Configuration of apparatus for the ozonation of an *M. aeruginosa* culture.

## 4.8 Peroxone

A peroxone ratio (being the ratio of hydrogen peroxide concentration to ozone concentration) of 0.5 was attempted throughout these experiments, however this was not always achieved due to the rapidly changing concentration of the ozone stock solution. Peroxone dose rates are expressed as ozone doses. In all cases hydrogen peroxide was

added first. Individual residuals for hydrogen peroxide and ozone were determined wherever possible by the titanium sulfate and indigo methods respectively.

### 4.8.1 Reaction of peroxone with microcystin-LR

Duplicate high purity water sample sets (100 mL) containing microcystin-LR at a concentration of 200 µg/L, DOC of 2.5 mg/L, and pH 6.5 were treated with peroxone at ozone concentrations of 0, 0.02, 0.04, 0.07, and 0.09 mg/L. After 5 minutes one reaction set was quenched with 200 µL 0.1 M sodium thiosulfate, extracted and analysed by HPLC. Aliquots of 10 mL were withdrawn from each of the other samples and reacted with 10 mL indigo reagent for ozone residual determination whilst 45 mL aliquots were added to 5 mL of titanium sulfate reagent for peroxide residual determination.

### 4.8.2 Reaction kinetics of peroxone with microcystin-LR

This experiment was identical to that for the reaction kinetics of microcystin-LR with ozone at a dose of 0.02 mg/L for volumes of 20 mL only (as in Figure 3b), and was carried out in triplicate.

### 4.8.3 Reaction of peroxone with an *M. aeruginosa* extract

Sample sets containing an *M. aeruginosa* extract (Mt Bold, 7 x 50 mL, 7 x 100 mL) giving a microcystin concentration of 87.5 µg/L and having a final DOC of 4.5 mg/L and pH 7.5 were treated with peroxone at ozone doses of 0, 0.04, 0.11, 0.18, 0.67, 0.91, and 1.48 mg/L. After 5 minutes, one reaction set (7 x 50 mL) was quenched with 200 µL 0.1 M sodium thiosulfate, extracted and analysed by HPLC whilst 10 mL aliquots were withdrawn from each of the other samples and reacted with 10 mL indigo reagent for ozone residual determination and 45 mL aliquots were added to 5 mL of titanium sulfate reagent for peroxide residual determination.

This experiment was repeated on the same extract material giving the same initial microcystin concentration at peroxone doses of 0, 0.05, 0.09, 0.26, 0.44, 0.68, and 0.96mg/L.

### 4.8.4 Reaction of peroxone with an *M. aeruginosa* culture

A culture of *M. aeruginosa* was concentrated to give a cell biomass of 620 000 cells/mL. The culture had a TOC of 4.5 mg/L and pH in the range 6-7. 50 mL Samples of the concentrated culture were treated with hydrogen peroxide and ozonated water to give resulting peroxone doses of 0, 0.11, 0.24, 0.55, 0.87, 1.12, 1.83, and 2.52 mg/L in duplicate in closed reaction vessels. After five minutes reaction time ozone residuals were determined with one set of solutions while the other set was quenched with 0.1 M sodium thiosulfate (200 µL), extracted and analysed.

The above experiment was repeated using an *M. aeruginosa* culture having 240 000 cells/mL, pH 8.5, and TOC 2.8 mg/L. Peroxone doses ranged between 0-2 mg/L (refer to Table XIV).

# 5. RESULTS AND DISCUSSION

## 5.1 Extraction of Microcystin from Live *Microcystis aeruginosa*

Three procedures were evaluated for the extraction of microcystin from live cultured material They were:

- Sonication
- Freeze-Thawing
- Sonication with a detergent - sodium dodecyl sulphate (SDS)

The results of these procedures are presented below (Table II). The total area of microcystin peaks from the HPLC analysis of a culture containing 700 000 cells/mL is given. Increasing peak area is indicative of increasing extraction efficiency.

TABLE II
COMPARISON OF EXTRACTION PROCEDURES FOR MICROCYSTIN FROM CULTURED *M. AERUGINOSA* (700 000 cells/mL).

| Procedure | Response (Peak Area) | Mean Response | Standard Deviation |
|---|---|---|---|
| **Sonication** | 0.01494, 0.01133 0.00794, 0.01255 | 0.01169 | 0.00292 (25%) |
| **Sonication + SDS** | 0.01735, 0.01551, 0.01652, 0.01499 | 0.01609 | 0.00105 (16.5%) |
| **Freeze-Thawing** | 0.02343, 0.02294 0.02267, 0.02334 | 0.02310 | 0.00035 (1.5%) |

The results clearly indicate that freeze-thawing of the sample prior to solid phase extraction was the most efficient of these procedures; in addition precision was also much better with this procedure. Consequently freeze-thawing was employed in the extraction of microcystin from cultured material in all experiments.

## 5.2 Potassium Permanganate

### 5.2.1 Method for the determination of permanganate residuals

Three methods for permanganate residual determination were assessed, these were:

i. Titration with sodium oxalate solution. (Standard Methods 14th Ed p 187)

ii. UV absorbance at 310 nm and 190 nm.

iii. Titration with DPD/FAS (as for chlorine residual determination, Standard Methods 14th Ed p 329).

The hot titration with sodium oxalate was cumbersome and was not sensitive or accurate enough at low permanganate concentrations as the end point colour change was difficult to determine. UV absorbance at 310 nm and 190 nm gave only weak responses; these were however linear having regressions of 0.989 and 0.981 respectively over the range of permanganate concentrations from 0.2 to 2 mg/L. The DPD/FAS titration technique proved to be the easiest and most accurate over a wider range of permanganate concentrations. The standard curve is given in Figure 6 and has a linear regression of 1. Residuals were determined by extrapolation from this line according to the equation $y = 1.07199x + .00787$.

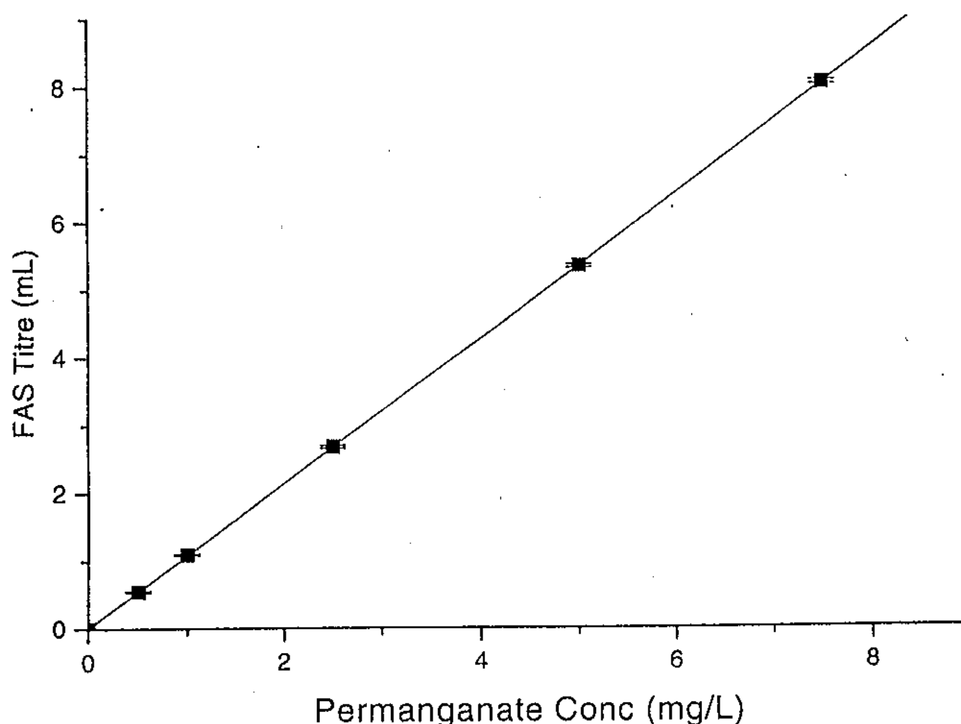



Figure 6. Standard curve for the determination of potassium permanganate residual by DPD/FAS titration.

### 5.2.2 Reaction of potassium permanganate with microcystin

As previously determined (Rositano and Nicholson 1994), permanganate proved to be effective in the oxidation of microcystin-LR. Permanganate treatment of an algal extract was carried out to determine its effectiveness in water having an organic loading and containing microcystin toxins other than microcystin-LR. Figure 7 shows the decrease in total microcystin for an algal extract (Mt Bold) containing predominantly microcystins LR and LA (254 μg/L total) and a solution of pure microcystin-LR (200 μg/L) after oxidation with potassium permanganate for 30 minutes. As can be seen in Figure 7, the oxidant demand increased due to the presence of oxidisable organic material (DOC 3.1 mg/L). It is also interesting to note at this point that microcystin-LA had a slower rate of oxidation in comparison to microcystin-LR and is shown in Figure 8

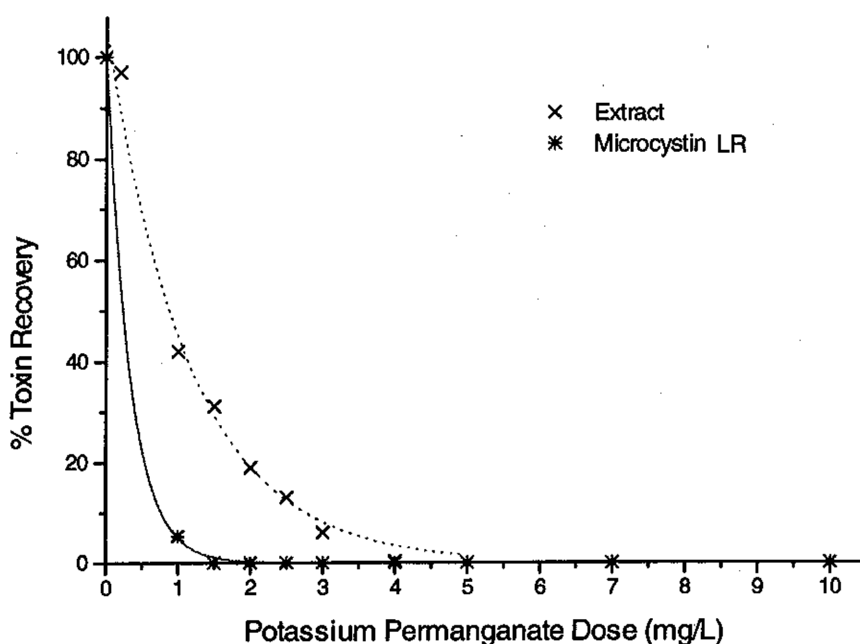



Figure 7. Toxin recovery after treatment of microcystin-LR with increasing doses of potassium permanganate. (Contact time = 30 minutes)

Mouse bioassays were carried out on the algal extract samples to determine acute toxicity after permanganate treatment. Destruction of the toxins as measured by HPLC corresponded well with loss of acute hepatotoxicity as determined by mouse bioassay.The results over the concentration range tested demonstrated the relationship of toxin removal measured analytically to progressive reduction of hepatotoxicity in the animal bioassay (Table III).

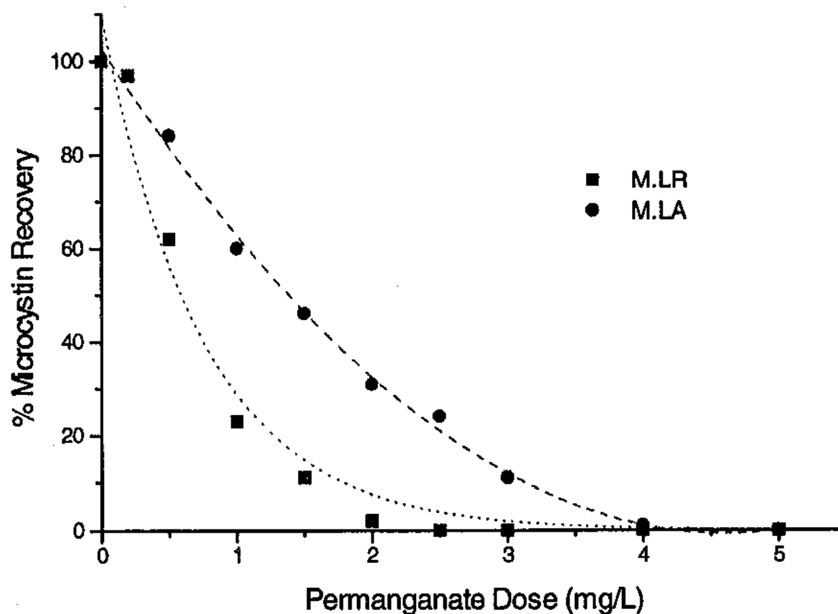



Figure 8. Oxidation of microcystin-LR and microcystin-LA with potassium permanganate.

TABLE III

MOUSE BIOASSAY RESPONSE OF PERMANGANATE TREATED ALGAL EXTRACTS.

| Permanganate Dose (mg/L) | Toxin quantity received by each mouse as determined by HPLC | Mouse Bioassay Response |
|---|---|---|
| 0 | 6.3 µg | + |
| 0.2 | 6.1 µg | + |
| 1.0 | 2.8 µg | + |
| 2.0 | 1.2 µg | # |
| 2.5 | 0.8 µg | # |
| 3.0 | 0.4 µg | - |
| 4.0 | 0.02 µg | - |
| 5.0 | 0 µg | - |

+ acutely toxic to mice, # not acutely toxic however mice showed signs of liver damage, - not acutely toxic

Extracts treated with up to 1 mg/L potassium permanganate were acutely toxic to mice whilst extracts treated with 2-5 mg/L permanganate were no longer acutely toxic. However animals sacrificed after 24 hours showed progressive liver damage corresponding to incomplete toxin removal (2 and 2.5 mg/L). This is also demonstrated in more detail in the work carried out with chlorinating agents (Table V). These results demonstrate the limitation of the mouse bioassay with death of the mouse as the end point in that it only determines toxins at levels sufficient to kill a mouse. Low levels of toxins may be present when a negative mouse bioassay result is obtained. This has been demonstrated previously (Flett and Nicholson 1991).

### 5.2.3 Effect of pH on potassium permanganate oxidation of microcystins

The recovery of microcystin remained relatively constant (between 1-5 %) for an extract solution containing 254 µg/L microcystin dosed at 3 mg/L permanganate over a pH range of 2 - 10.1. This is represented graphically in Figure 9. Permanganate has a standard oxidation potential of only 0.58 V under alkaline conditions according to Glaze (1990), however 'alkaline conditions' were not defined in this reference and it may require higher pH values than those tested to decrease the oxidising capabilities of permanganate. This would explain why the oxidation of microcystin occurs readily under the range of alkaline conditions tested.

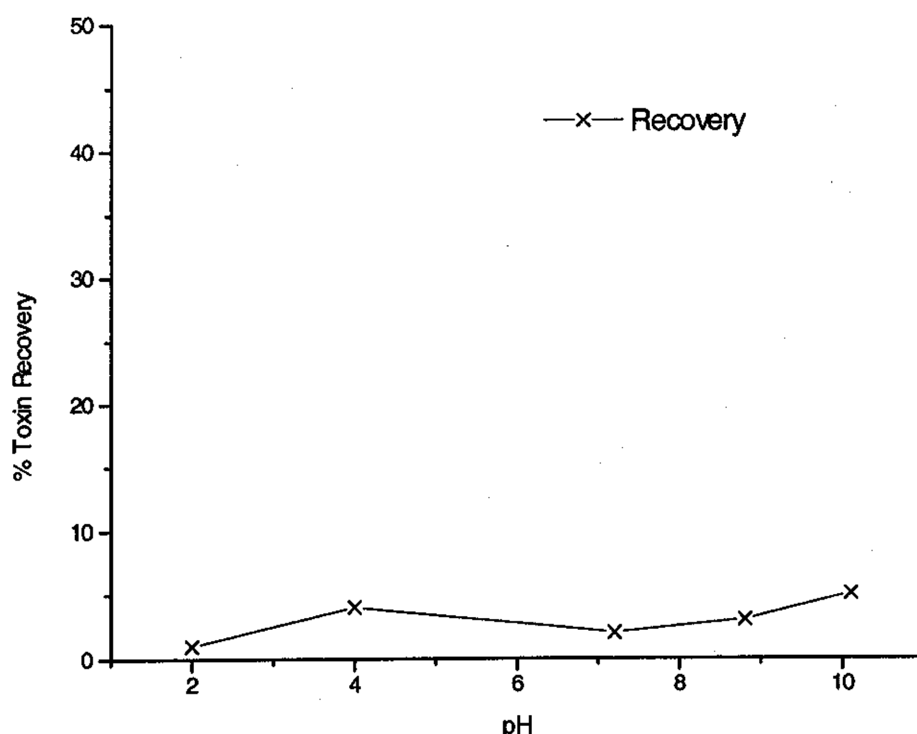



Figure 9. Microcystin recovery after treatment with potassium permanganate at various pH values.

### 5.2.4 Effect of potassium permanganate on an *M.aeruginosa* culture

Two cultures of *M. aeruginosa* were treated with potassium permanganate at various doses to determine the effectiveness of permanganate oxidation on intact cells. Despite the fact the cultures had different cell concentrations (experiment 1, 600 000, and experiment 2, 330 000 cells/mL) and pH values (9.5 and 7.4 respectively), the results were essentially the same. From the graphs in Figure 10 it is clear that permanganate had little if any effect on the toxin when present within cells after 1h contact time. At each dose the recovery of toxin was near 100 % and the permanganate residual close to 100 % of the dose applied. Hence it can be concluded that the permanganate was ineffective for toxin degradation at a contact time of 1 h due to its inability to successfully penetrate the *Microcystis* cell wall, or lyse the cells thereby releasing toxins to the surrounding solution where oxidation can occur.

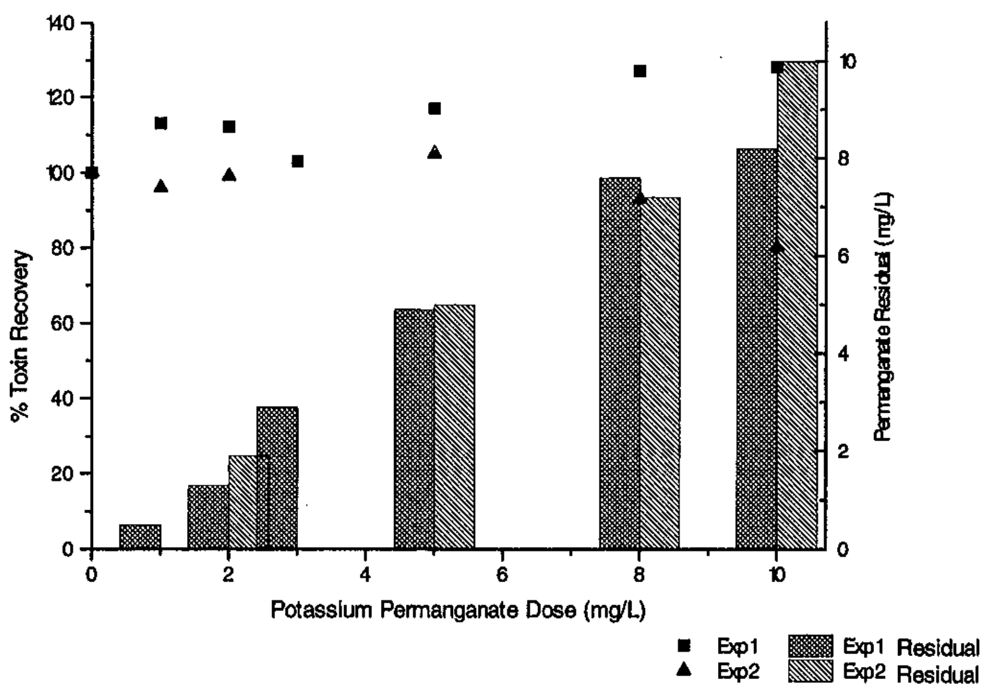



Figure 10. Microcystin recovery and permanganate residual from a culture of *M. aeruginosa* after treatment with potassium permanganate.

The effect of permanganate on intact cells was also studied over time. A toxic culture containing a cell concentration of 490 000 cells/mL and at pH 7.0, was dosed at 10 mg/L permanganate. Samples were analysed each hour for toxin concentration and permanganate residual. After four hours the toxin concentration had decreased by 46 % and the permanganate residual had steadily

decreased to 8.7 mg/L. This experiment was repeated over eight hours with a sample containing $1.15 \times 10^6$ cells/mL with similar results (Table IV).

TABLE IV

EFFECT OF POTASSIUM PERMANGANATE ON AN *M. AERUGINOSA* CULTURE WITH TIME.

| **Sample Time** | **% Toxin Recovery** | | **Permanganate Residual** | |
|---|---|---|---|---|
| | Exp1 | Exp2 | Exp1 | Exp2 |
| **0** | 100 | 100 | - | - |
| **1** | 91 | 70 | 10 | 9.2 |
| **2** | 86 | 66 | 9.7 | 8.6 |
| **3** | 79 | 66 | 9.3 | 8.1 |
| **4** | 54 | 66 | 8.7 | 7.4 |
| **5** | - | 58 | - | 7.1 |
| **6** | - | 55 | - | 6.1 |
| **7** | - | 47 | - | 5.6 |
| **8** | - | 42 | - | 4.2 |

These results imply that potassium permanganate is slowly causing lysis of the cyanobacterial cell allowing the release of toxin to water which is then oxidised by potassium permanganate. In a study by Lam *et al* (1995b) they monitored the release of microcystin-LR from a bloom sample of *M. aeruginosa* and *Aphanizomenon* into the water phase before and after dosing with various oxidants including potassium permanganate. It was found that the concentration of microcystin-LR in solution increased after treatment with potassium permanganate over two days and subsequently decreased, presumably due to the effect of permanganate and/or microbial activity.

### 5.2.5 Rate of reaction of microcystin-LR with potassium permanganate

The reaction of a 1 mg/L solution of microcystin-LR at pH 7 with 2.0 mg/L potassium permanganate was considered to be a first order reaction since the molar concentration of permanganate was much greater than that of microcystin-LR and remained essentially constant over the course of the reaction. This is depicted graphically in Figure 11.

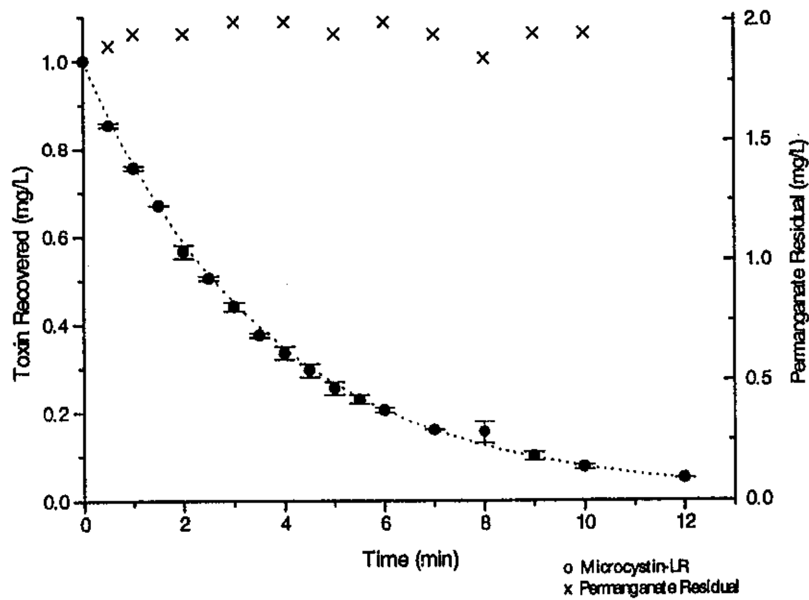



Figure 11. Decay curve of microcystin-LR on treatment with potassium permanganate

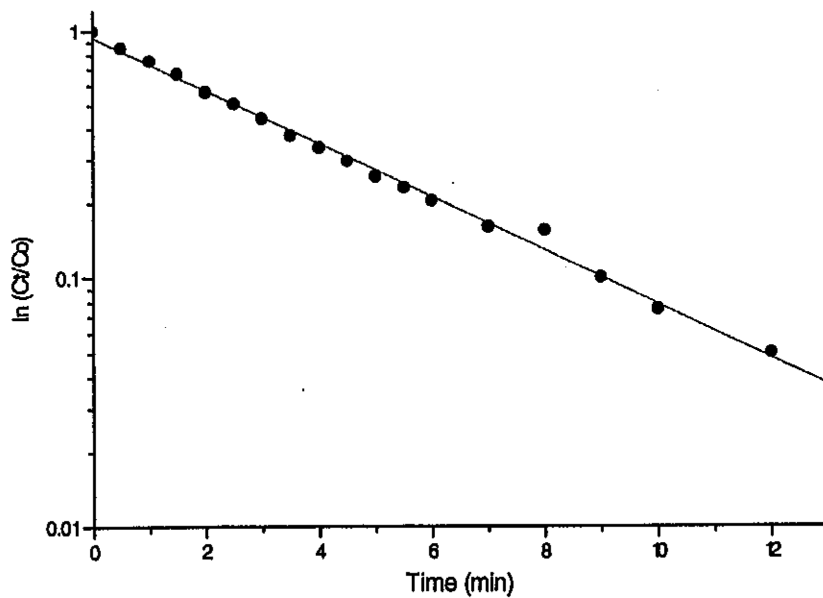



Figure 12. Log decay curve of microcystin-LR on treatment with potassium permanganate

The first order rate constant for the reaction of potassium permanganate at 2 mg/L with microcystin-LR at 1 mg/L and pH 7 was determined by plotting the log of the ratio of concentration of microcystin at time t over initial concentration ($C_t/C_0$) versus time and was found to be 0.108 $min^{-1}$ (Figure 12).

### 5.3 Chlorination

#### 5.3.1 Chlorination of microcystin-LR and an *M. aeruginosa* extract

Previous studies in these laboratories have shown that peptide toxins were rapidly destroyed by aqueous chlorine and calcium hypochlorite but less effectively removed by equivalent doses of sodium hypochlorite (Figure 13, Nicholson *et al* 1994). The results of these studies are presented.

A solution of microcystin-LR in Milli-Q water was initially chlorinated (chlorine water) with various doses of chlorine, the lowest being 2 mg/L. No microcystin was recovered from any of the samples. In further experiments with *M. aeruginosa* extracts, samples were dosed at less than 2 mg/L with various chlorinating agents and it was found that aqueous chlorine and calcium hypochlorite achieved over 95% removal of toxins over 30 minutes at doses around 1 mg/L. Sodium hypochlorite achieved approximately 40% removal at 1 mg/L and only 70-80% removal at doses of 5mg/L or greater. Initial toxin concentrations were in the range 130-300 µg/L which would be representative of highly contaminated water and would be 3-4 times the lethal dose to mice after the normal extraction / concentration procedure. The removal of toxin by chlorination was assessed by both HPLC and mouse bioassay.

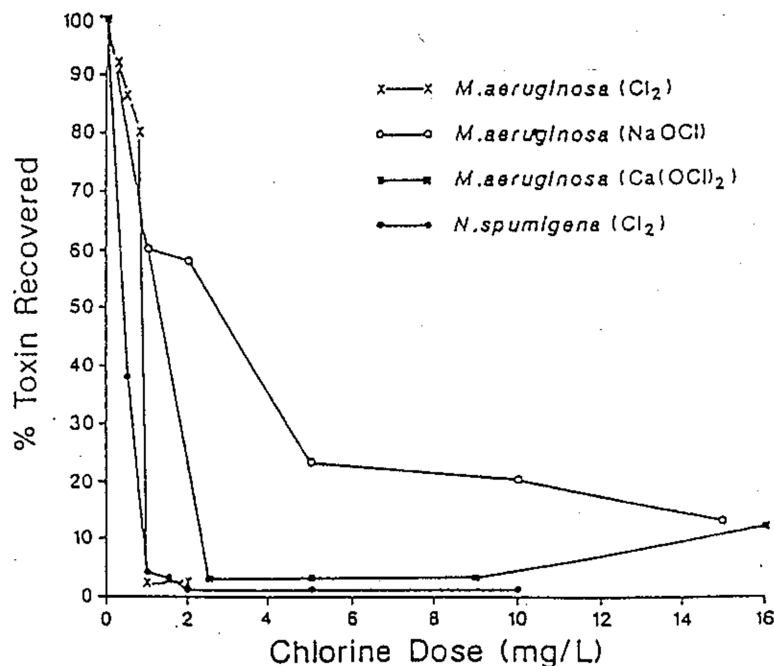



Figure 13. Microcystin recovery from algal extracts after treatment with chlorine at various doses Contact time = 30 minutes.

Destruction of toxins as measured by HPLC corresponded with loss of acute hepatotoxicity as determined by mouse bioassay (Table V). Acute toxicity was eliminated by treatment with doses of greater than 1 mg/L aqueous chlorine for microcystins and 0.5 mg/L for nodularin under these conditions.

Even though sodium hypochlorite was less effective than the other oxidants, the results once again demonstrated the relationship of toxin removal measured analytically to progressive reduction of hepatotoxicity in the animal (mouse) bioassay (Table V). Extracts treated with up to 2 mg/L sodium hypochlorite were still acutely toxic to mice while extracts from treatments of 5-30 mg/L were no longer toxic. Progressive liver damage corresponding to incomplete toxin removal was also observed. The data in Tables III and V indicate a mouse lethal dose of approximately 1-2 μg microcystins and nodularin which is in good agreement with that expected. The LD50 values for both microcystin-LR and -LA and nodularin are reported as 50 μg/kg (Carmichael *et al*, 1990). Assuming that a lethal dose for all mice tested is around 50-100 μg/kg, then the lethal dose for a 20 g mouse is calculated as 1-2 μg. Aqueous chlorine as the chlorinating agent was a more efficient oxidant for the destruction of the peptide toxins in comparison to calcium hypochlorite or sodium hypochlorite. This was demonstrated in subsequent work to be due to the effect of pH, sodium hypochlorite solutions being highly alkaline.

TABLE V

TOXIN CONCENTRATIONS AND MOUSE BIOASSAY RESULTS AFTER CHLORINATION WITH VARIOUS CHLORINATING AGENTS.

| **Chlorinating Agent** | **Chlorine Dose (mg/L)** | **Toxin Concentration (µg/L)** | **Mouse Bioassay Result* and Dose Injected (µg)** |
|---|---|---|---|
| *M aeruginosa* | | | |
| Aqueous Chlorine | 0 | 192 | + (3.8) |
| | 0.2 | 174 | + (3.5) |
| | 0.5 | 164 | + (3.3) |
| | 0.8 | 154 | + (3.1) |
| | 1.0 | 4 | - (0.08) |
| | 2.0 | 4 | - (0.08) |
| Sodium Hypochlorite | 0 | 298 | + (6.0) |
| | 1 | 180 | + (3.6) |
| | 2 | 174 | + (3.5) |
| | 5 | 70 | - (1.4) |
| | 10 | 58 | - (1.2) |
| | 15 | 38 | # (0.8) |
| | 30 | 50 | # (1.0) |
| Calcium Hypochlorite | 0 | 150 | not tested |
| | 2.5 | 4 | |
| | 5 | 4 | |
| | 9 | 4 | |
| | 16 | 18 | |
| *N. spumigena* | | | |
| Aqueous Chlorine | 0 | 128 | + (2.6) |
| | 0.5 | 48 | - (1.0) |
| | 1.0 | 6 | - (0.12) |
| | 1.5 | 4 | - (0.08) |
| | 2.0 | 2 | - (0.04) |
| | 5.0 | 2 | - (0.04) |
| | 10.0 | not detect. | - ( - ) |

contact time = 30 minutes.

* + = acutely toxic by mouse bioassay

- = non-toxic by mouse bioassay

# = not acutely toxic by mouse bioassay, however mice showed signs of liver damage

### 5.3.2 Effect of pH on chlorine oxidation of microcystins

The influence of pH over the range 3-10 on toxin removal for all three chlorinating agents while maintaining a constant chlorine concentration of 15 mg/L is illustrated in Figure 14. Toxin destruction was significantly reduced above pH 8-9 for all chlorinating agents.

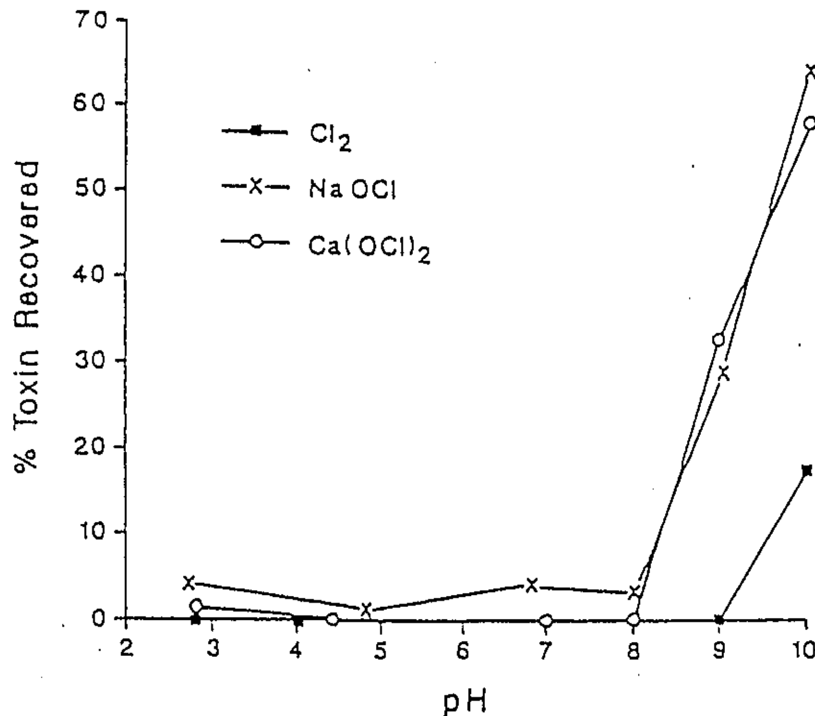



Figure 14. Microcystin recovery from algal extracts after chlorination at various pH values. Chlorine dose = 15mg/L. Contact time = 30minutes.

The reduction in toxin destruction with increasing pH above 8 can be explained in terms of the change in distribution of chlorine species with pH. This is illustrated in Figure 15 (White, 1992). Hypochlorous acid is a more powerful oxidising agent than the hypochlorite ion and with increasing pH, the percentage of hypochlorous acid decreases as the percentage of hypochlorite ion increases. If calcium or sodium hypochlorite were used for toxin destruction in natural waters the pH may not be greatly elevated due to the buffering capacity of natural waters. However if high dose rates of calcium or sodium hypochlorite are used resulting in pH values greater than 8 then pH adjustment would be necessary to ensure adequate toxin removal.

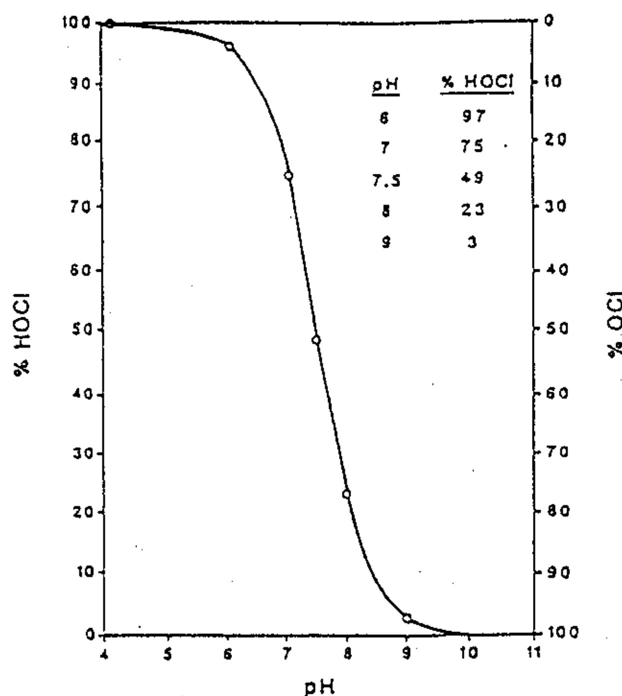



Figure 15. Distribution of chlorine species with pH. (White, 1992)

### 5.3.3 Chlorination of natural water containing *N. spumigena*.

The oxidation of nodularin by chlorine in a bloom of *N. spumigena* containing intact cells was also determined using aqueous chlorine as the chlorinating agent (see Methods section). The results are shown in Figure 16. A concentration of 10 mg/L free chlorine effectively removed 95% of nodularin (initial concentration 440 µg/L) with a chlorine residual of 2 mg/L after five minutes in both open and closed systems. In a closed system where the loss of chlorine to the atmosphere was prevented, complete destruction of nodularin occurred in 30 minutes. After this contact time the chlorine residual was 0.5 mg/L.

### 5.3.4 Reaction of chlorine with an *M.aeruginosa* culture

A similar experiment to that carried out with *N. spumegina* was also carried out on a culture of *M. aeruginosa* at a chlorine dose of 10 mg/L. Chlorine was effective in reducing the toxin content of the culture containing 412 000 cells/mL (46 µg/L microcystin) to less than 1 µg/L (>98% removal) after 30 minutes with a residual of 1.7 mg/L. These results are depicted in Figure 17.

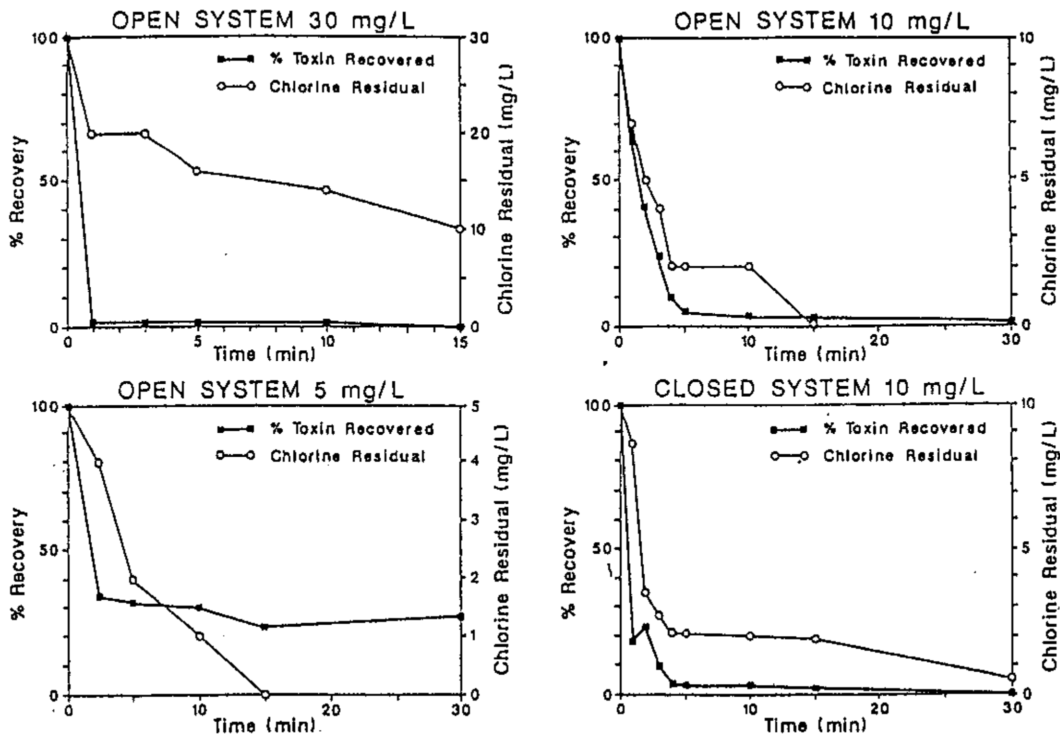



Figure 16. Toxin recovery following chlorination of Lake Albert water containing 2.5 x $10^5$ cells/mL in both open and closed systems.

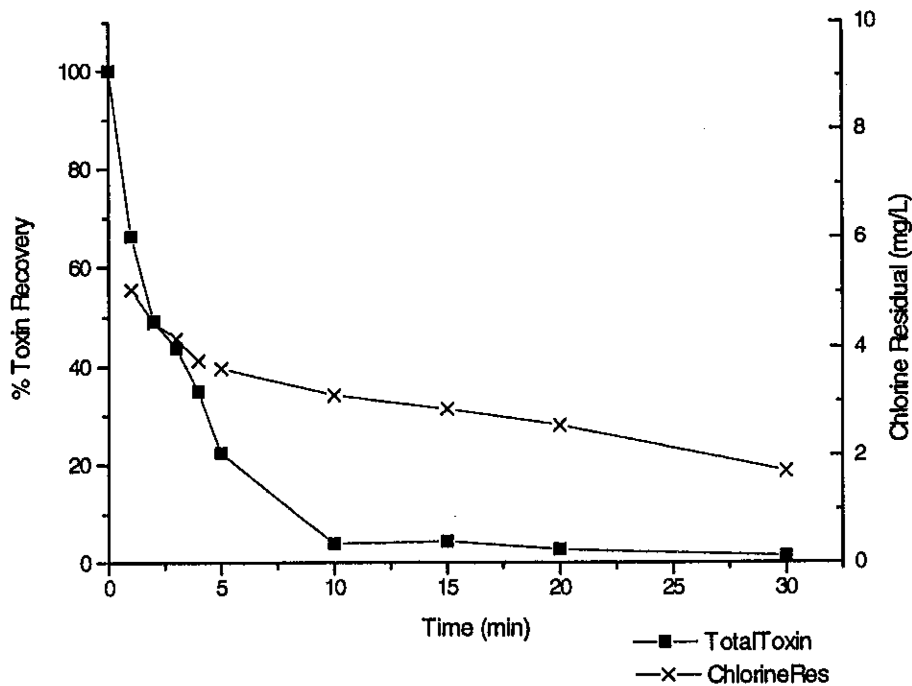



Figure 17. Chlorination of an *M. aeruginosa* culture. (Chlorine dose = 10 mg/L, Cell biomass = 412 000 cells/mL)

The effective destruction of the hepatotoxins by chlorine is contrary to results previously reported in the literature (Hoffmann 1976, Keijola *et al* 1988, Himberg *et al* 1989). In the case of Hoffmann (1976), a very low chlorine/toxin ratio (5 mg/L chlorine : 10 mg/L toxin) was used whereas in this study much higher chlorine/toxin ratios were used, e.g., in the case of sodium hypochlorite a ratio of 17:1 was required to reduce toxicity to below that detectable by mouse bioassay (calculated from 5 mg/L dose, Table V). It can also be calculated from the data of Hoffmann (1976) that at the chlorine and toxin concentrations used, assuming a toxin LD50 of 50 μg/kg (the LD50 of the most common microcystin, microcystin-LR (Carmichael *et al* 1990)) and a mouse weight of 20 g, toxin removal greater than 99.4% would have been necessary before a non-toxic response in mice was observed. The information presented is insufficient to estimate whether the use of calcium hypochlorite would have resulted in an elevated pH level which in turn would have also contributed to poor toxin removal.

The work described by Keijola *et al* (1988) and Himberg *et al* (1989) employed low chlorine concentrations (0.5 mg/L), open systems and sodium hypochlorite. The pH of the water increased only slightly after treatment (between pH 5.5 and 6.5)(Himberg *et al* 1989) indicating that the change in pH alone would not have been responsible for the poor removal of toxins. However the low doses used in open systems may have resulted in significant losses of chlorine to the atmosphere. In addition such low chlorine doses would be expected to be easily consumed by reaction with the naturally occurring humic material stated to be present (Himberg *et al* 1989). More recently, laboratory studies carried out by Jones *et al* (1993) and Lam *et al* (1995b) support our findings. Jones *et al* (1993) found that removal of microcystin at 20 μg/L from various waters by chlorine occurred where a total chlorine residual of 1.0 mg/L remained. Lam *et al* (1995b) reported complete destruction of microcystins over 4 days in bloom sample which had been treated with high doses of sodium hypochlorite.

### 5.3.5 Reaction kinetics of chlorine with microcystin-LR

The reaction of microcystin-LR with chlorine, as with the reaction of permanganate, followed first order reaction kinetics. The chlorine demand of a solution of microcystin-LR (1 mg/L) at pH 7 was less than or equal to 0.1 mg/L as can be seen from Figure 18. The rate constant was determined by plotting the log of the ratio of concentration of microcystin-LR at time t over the initial concentration ($C_t/C_0$) against time and was found to be 0.057 $min^{-1}$, approximately half that of permanganate (Figure 19) at the same dose rate and identical conditions. Considering the

molar concentration of chlorine at 2 mg/L is approximately three times higher than that of permanganate, permanganate is a much better oxidant of microcystin-LR than chlorine.

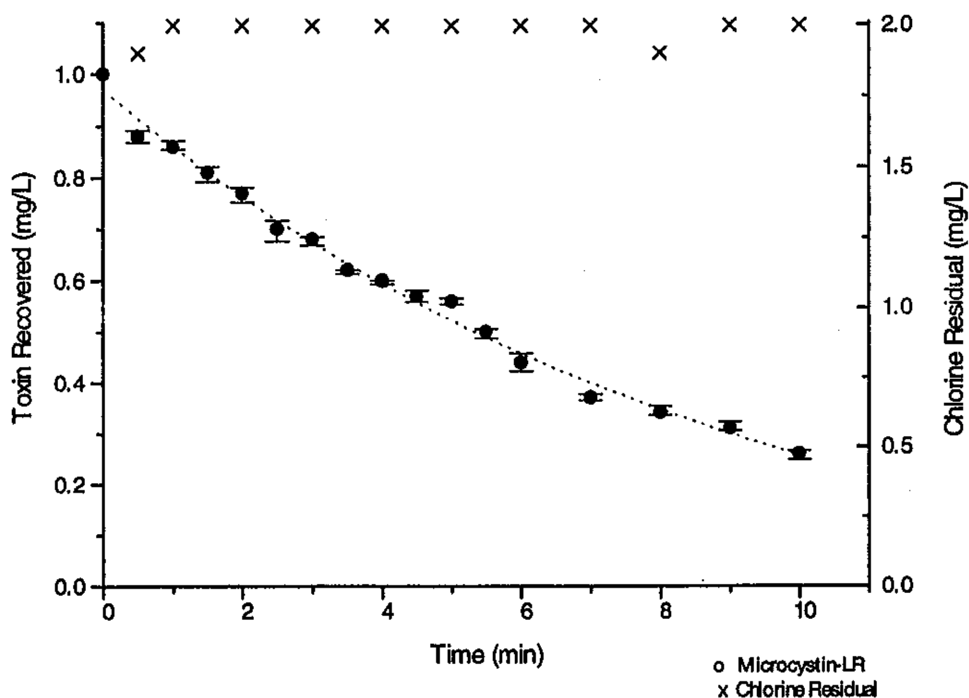



Figure 18. Decay curve of microcystin-LR with chlorine.

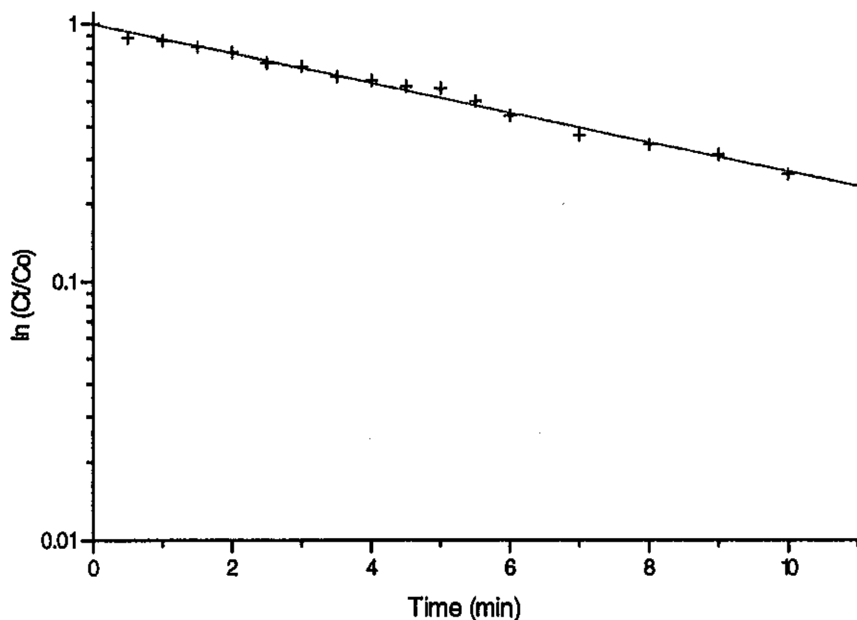



Figure 19. Log decay of microcystin-LR with chlorine.

### 5.3.6 Chloramination

Chloramination of dilute algal extract solutions at a concentration of 20 mg/L monochloramine did not have any significant effect on the toxins after a contact time of up to five days (Table VI). This is consistent with the weaker oxidising capability of monochloramine compared with both hypochlorous acid and permanganate (under acidic conditions).

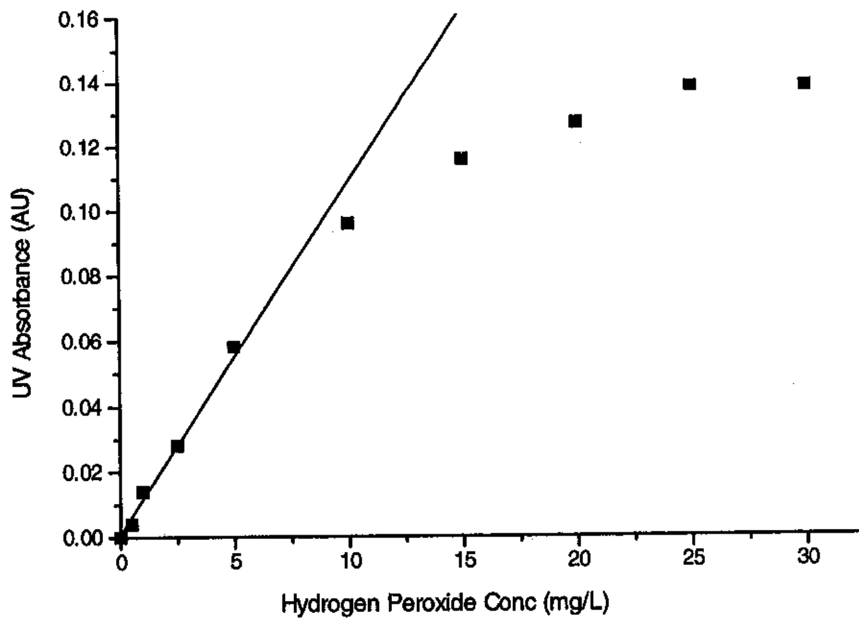
TABLE VI

RECOVERIES OF TOXINS FROM EXTRACTS OF *M. AERUGINOSA* TREATED WITH MONOCHLORAMINE (20 MG/L) OVER 5 DAYS.

| CONTACT TIME | RECOVERY OF TOXIN (%) |
|---|---|
| Control | 100 |
| 30 minutes | 90 |
| 1 hour | 89 |
| 2 days | 96 |
| 5 days | 83 |

## 5.4 Hydrogen Peroxide

The analysis of hydrogen peroxide concentration by the titanium sulfate method (Eisenberg 1976) was assessed. UV response using a 1 cm cuvette gave a linear response up to 20 mg/L (Figure 20) as opposed to analysis with a 5 cm cuvette which only gave a linear response to 10 mg/L.



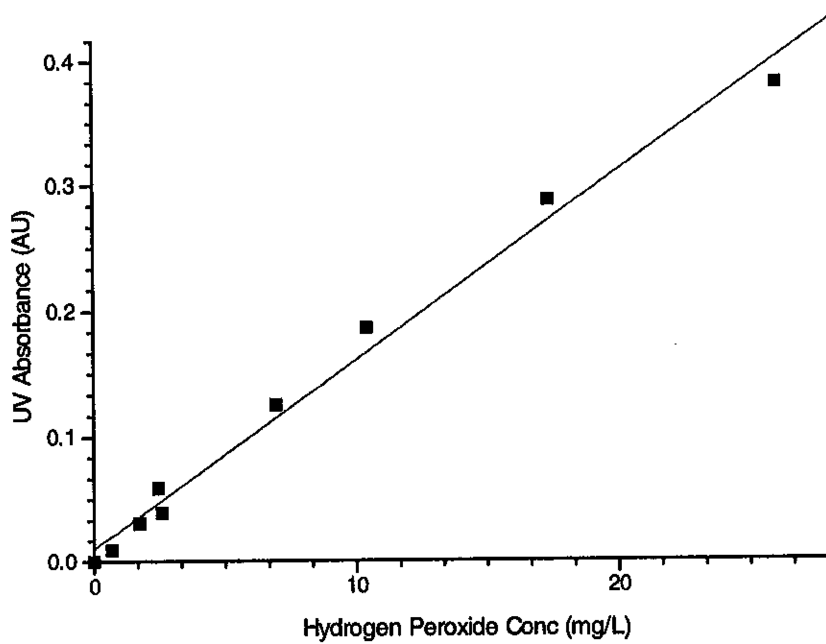




Figure 20. Standard curves for hydrogen peroxide via the titanium sulphate method using 5 and 1 cm cuvettes.

### 5.4.1 Reaction of hydrogen peroxide with microcystin-LR and an *M. aeruginosa* extract

Hydrogen peroxide was allowed to react with microcystin-LR in high purity water for 30 minutes. At a dose rate of 10 mg/L peroxide, 100% recovery of the toxin was obtained (Table VII). Higher dose rates and an increased reaction time of one hour were tested using a Lake Mokoan *M.aeruginosa* extract (Table VIII). This however made little difference to the rate of destruction of the toxin, a dose rate of 20 mg/L removed only 17% of the toxin with a peroxide residual of 11.2 mg/L after one hour.

TABLE VII

RECOVERY OF MICROCYSTIN-LR IN HIGH PURITY WATER AFTER TREATMENT WITH HYDROGEN PEROXIDE.

| **PEROXIDE DOSE (mg/L)** | 0 | 1 | 2 | 5 | 7 | 10 |
|---|---|---|---|---|---|---|
| **PEROXIDE RESIDUAL (mg/L)** | 0 | 0 | 0 | 1.5 | 1.8 | 3.3 |
| **TOXIN RECOVERED (µg)**<br>() = % | 5<br>(100) | 5<br>(100) | 4.2<br>(84) | 4.9<br>(98) | 5<br>(100) | 5<br>(100) |

Toxin concentration = 100 µg/L. Contact time = 30 minutes.

TABLE VIII

RECOVERY OF MICROCYSTIN FROM AN *M. AERUGINOSA* EXTRACT AFTER TREATMENT WITH HYDROGEN PEROXIDE.

| **PEROXIDE DOSE (mg/L)** | 0 | 5 | 10 | 15 | 20 |
|---|---|---|---|---|---|
| **PEROXIDE RESIDUAL (mg/L)** | 0 | 4.6 | 8.2 | 9.7 | 11.2 |
| **TOXIN RECOVERED (µg)**<br>() = % | 3.6<br>(100) | 3.3<br>(92) | 3.5<br>(97) | 3.7<br>(103) | 3.0<br>(83) |

Toxin concentration = 72 µg/L. DOC concentration = 6.8 mg/L. pH 6-7. Contact time = 1 hour.

### 5.4.2 Effect of pH on peroxide oxidation of microcystins

The oxidation potential of peroxide, like that of chlorine is pH dependent and is also lower at high pH. The effect of pH on the reaction of peroxide with microcystin is shown in Figure 21. However the effect of pH was not pronounced and oxidation was poor at all pH values. That peroxide was not a very effective oxidant for the removal of microcystin was initially surprising

since hydrogen peroxide is a strong oxidant and has a relatively high oxidation potential, especially under acidic conditions (1.78V). However the reaction is dependent on favourable chemical kinetics as well as favourable thermodynamics. Hydrogen peroxide is not used as a oxidant in water treatment because of its poor chemical kinetics (Glaze 1990). It appears therefore that the kinetics for the oxidation of microcystins is also unfavourable.

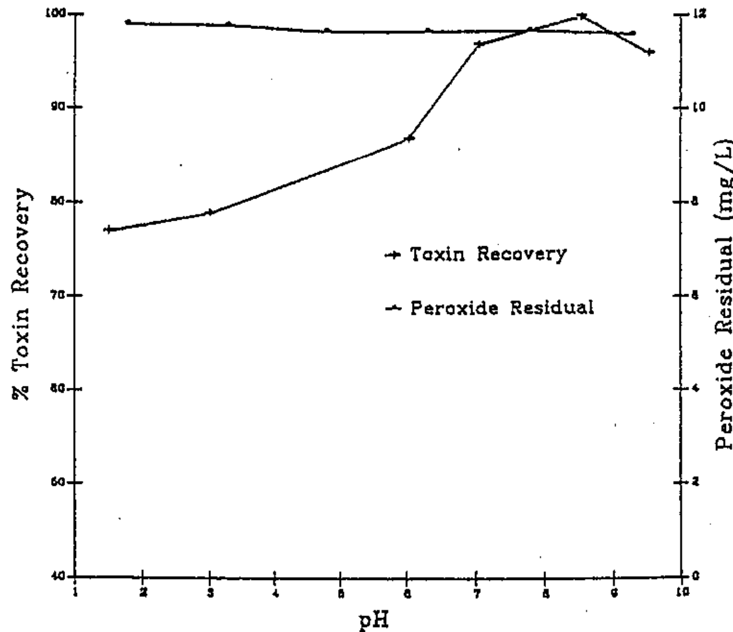



Figure 21. Effect of pH on hydrogen peroxide treatment of microcystin.

Hydrogen peroxide in combination with UV irradiation has greater oxidising capabilities than peroxide alone (Glaze 1990). An experiment was carried out to determine the combined effect of peroxide and UV irradiation on microcystin-LR. The removal of microcystin-LR was approximately 50% after a contact time of 30 minutes (Table IX). However UV irradiation alone, without peroxide effected a similar removal of microcystin. The decrease in toxin content was therefore due to the effect of UV irradiation alone and UV irradiation in combination with hydrogen peroxide offers no improvements in removal.

### 5.4.3 Reaction kinetics of hydrogen peroxide with microcystin-LR

Hydrogen peroxide (2 mg/L) was reacted with microcystin-LR (1 mg/L) as described. After 30 minutes the microcystin concentration had not decreased sufficiently to determine a rate constant. (Refer to Figure 30)

TABLE IX

EFFECT OF UV/HYDROGEN PEROXIDE TREATMENT FOR THE REMOVAL OF MICROCYSTIN-LR FROM DISTILLED WATER.

| TREATMENT | PEROXIDE DOSE (mg/L) | PEROXIDE RESIDUAL (mg/L) | TOXIN RECOVERED (µg) () = % |
|---|---|---|---|
| No UV, no peroxide (control) | - | - | 5.2 (87) |
| UV, no peroxide | - | - | 2.6 (43) |
| Peroxide, no UV | 20 | 11.7 | 5.9 (98) |
| Peroxide and UV | 20 | 11.7, 11.3* | 2.7, 2.1* (45, 35) |

Initial toxin content = 6 µg. Reaction time = 30 minutes.
* = replicate experiments

**Ozone**

In the reactions of ozone with *M. aeruginosa* extracts and live material it was noted that pigment material such as chlorophyll a and phycocyanin interfered with the analysis due to UV absorption. Residuals in each case could not be determined until these pigments had been sufficiently oxidised by ozone.

### 5.5.1 Reaction of ozone with microcystin-LR and an *M.aeruginosa* extract

Ozone is the most powerful oxidant used in water treatment having a standard oxidation potential of 2.07V. It is not surprising therefore that the reaction of ozone with microcystins was extremely fast. This was demonstrated in the reaction of pure microcystin-LR and ozone (Figure 22). A solution containing 166 µg/L microcystin-LR was completely oxidised by less than 0.2 mg/L of ozone. When other organic material is introduced into the reaction, such as with the reaction of ozone and an algal extract, competing reactions occur between the organic material and the toxin

for reaction with ozone and increased dose rates are required to oxidise any toxin present. This is shown in Figure 23 and Table X.

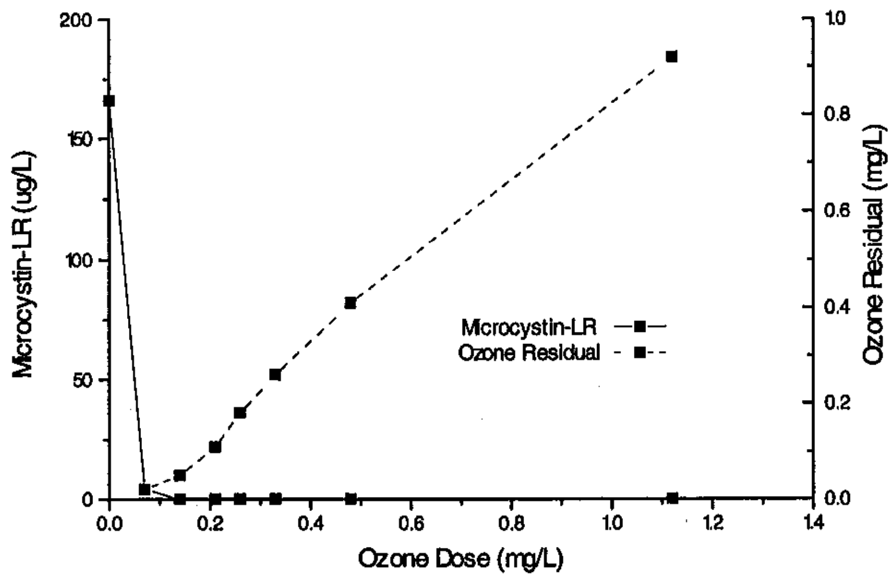



Figure 22. Reaction of ozone with microcystin-LR in high purity water. (Reaction Time = 4 minutes)

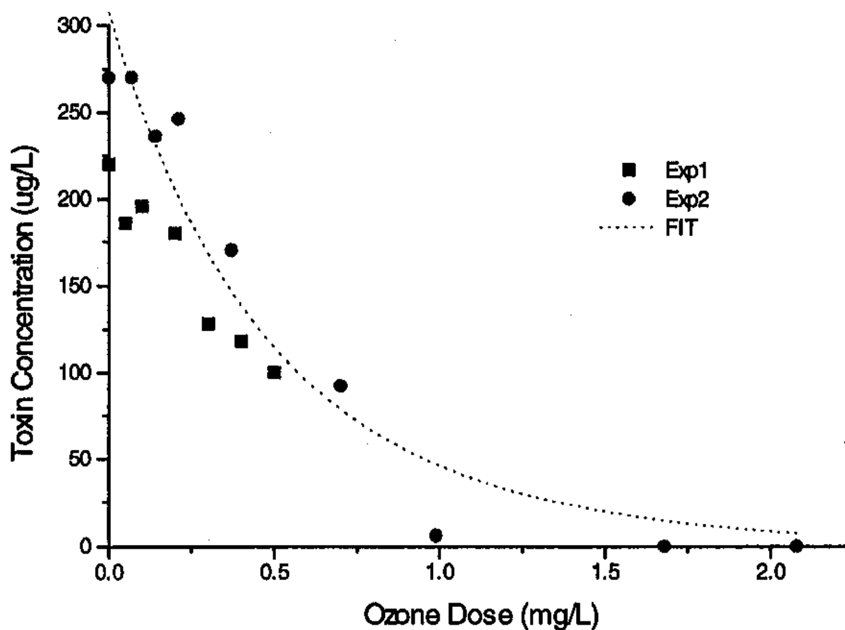



Figure 23. Reaction of ozone with an *M. aeruginosa* extract in high purity water. (Reaction Time = 5 minutes)

TABLE X

REACTION OF OZONE WITH AN *M.AERUGINOSA* EXTRACT IN HIGH PURITY WATER.

| **OZONE DOSE (mg/L)** | **RESIDUAL AFTER 5 MINUTES (mg/L)** | **TOXIN CONCENTRATION AFTER 5 MINUTES (µg/L)** | **TOXIN REMOVAL AFTER 5 MINUTES (%)** |
|---|---|---|---|
| **EXP 1** | | | |
| 0 | - | 220 | - |
| 0.5 | <0.01 | 186 | 16 |
| 0.10 | <0.01 | 196 | 11 |
| 0.15 | <0.01 | 172 | 22 |
| 0.20 | <0.01 | 180 | 18 |
| 0.30 | <0.01 | 128 | 42 |
| 0.40 | <0.01 | 118 | 46 |
| 0.50 | <0.01 | 100 | 55 |
| **EXP 2** | | | |
| 0 | - | 270 | - |
| 0.07 | <0.01 | 270 | 0 |
| 0.14 | <0.01 | 236 | 13 |
| 0.21 | <0.01 | 246 | 9 |
| 0.37 | <0.01 | 170 | 37 |
| 0.70 | <0.01 | 92 | 66 |
| 0.99 | <0.01 | 6 | 98 |
| 1.68 | <0.01 (0.12)* | <0.1 | 100 |
| 2.08 | <0.01 (0.05)* | <0.1 | 100 |

DOC 8.5 mg/L. pH 6.8

* Ozone residual after 2 minutes.

From these results one may surmise that once the ozone demand of the water has been met, complete removal of toxin can be achieved and an ozone residual should be detectable. However the efficiency and effectiveness of ozone as an oxidant in water treatment is dependent not only on the concentration of reactants but also on temperature, pH, and ionic composition of the water being ozonated.

### 5.5.2 Effect of pH on ozone oxidation of microcystins

An example of the effect of pH on the oxidation efficiency of ozone with microcystins is given in Figure 24. An algal extract solution containing 250 µg/L microcystin was treated with ozone at 1 mg/L at various pH values. At pH values greater than 7.5, microcystin was detected in the sample which was probably due to the accelerated rate of autocatalytic degradation of ozone under alkaline conditions.

Autocatalytic decomposition of ozone in water occurs via a complex radical chain process. The radicals produced in this process are extremely reactive and are probably the principal reacting species in the oxidation of solutes in water. Ozone decomposition is accelerated by the presence of hydroxide ions (or elevated pH) which act as radical initiators in a series of chain reactions (Figure 25). This increases the rate of consumption of ozone such that the availability of ozone for the oxidation of toxin is decreased.

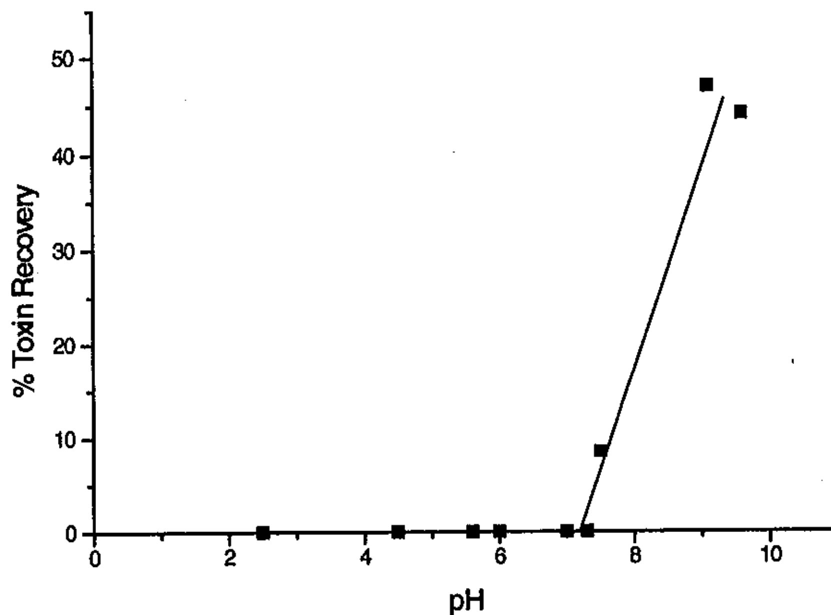



Figure 24. Effect of pH on the reaction of ozone with an *M. aeruginosa* extract. (Ozone Dose = 1 mg/L)

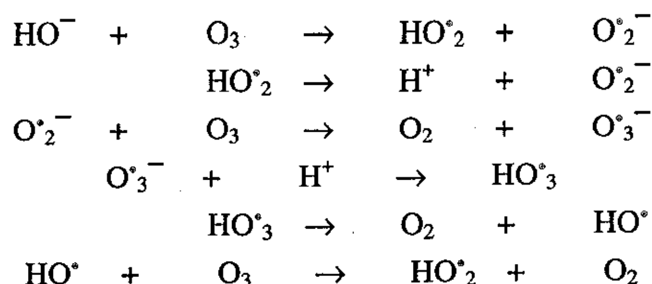



Figure 25. Mechanism for the decomposition of ozone under alkaline conditions. Decomposition is initiated by the hydroxide ion and propagated by the radicals $HO^{\bullet}$ and $O^{\bullet}_2{}^-$.

### 5.5.3 Reaction of ozone with an *M. aeruginosa* culture

Initial experiments of ozone with intact cells demonstrated that elevated doses of ozone would be required for the total destruction of microcystin from live *Microcystis* cells. A culture containing 1.63 x $10^6$ cells/mL at pH 7 was ozonated at various concentrations up to 3.7 mg/L ozone (Figure 26) and only 36% total microcystins was removed. Higher doses of ozone in our reaction vessel were cumbersome to produce as the concentration of the stock solution of ozonated water only ever reached around 12 mg/L. As one would expect in such a case there was no residual detectable after five minutes reaction time. A second experiment was designed to determine the ozone doses required to completely remove toxin from water containing *Microcystis* cells at concentrations equivalent to a bloom situation. A *Microcystis* culture containing 2.05 x $10^6$ cells/mL (250 μg/L total microcystin) was buffered at pH 7 and ozonated at a dose rate of approximately 2.5 mg/minute. Complete destruction required around 12 minutes contact time resulting in a total ozone dose of 29 mg (Figure 27). An ozone residual appeared after destruction of the toxin. This procedure was repeated another three times with consistent results, which are summarised in Table XI. In the event of a cyanobacterial bloom the pH of the water is often elevated to between 8-10. This experiment did not take into account variables such as increased pH and DOC which may occur in practice and which would likely have the effect of increasing the ozone dose and contact time required for toxin destruction.

This experiment clearly demonstrates that higher dose rates of ozone are required to remove toxicity from waters containing live cyanobacterial cells. Keijola *et al* (1988) and Himberg *et al* (1989) in their studies of water treatment processes for the removal of cyanobacterial toxins found processes containing ozonation to be effective under normal treatment plant operating conditions. Freeze-dried cyanobacteria or purified toxin added to lake water was used in their pilot and laboratory scale experiments in which case most of the toxin was freely and immediately available

for degradation as opposed to ozonation of whole cells which require destruction of the bacterial cell wall and lysis of the cell for release of toxin to the medium.

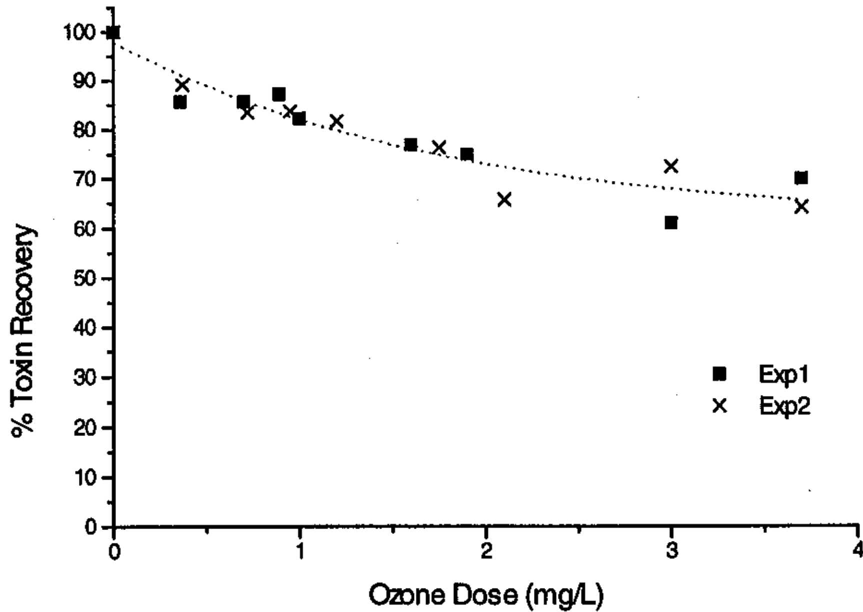



Figure 26. Microcystin recovery from a culture of *M. aeruginosa* after treatment with ozone. Cell biomass 1.63 x $10^6$ cells/mL, TOC 8mg/L, pH 7

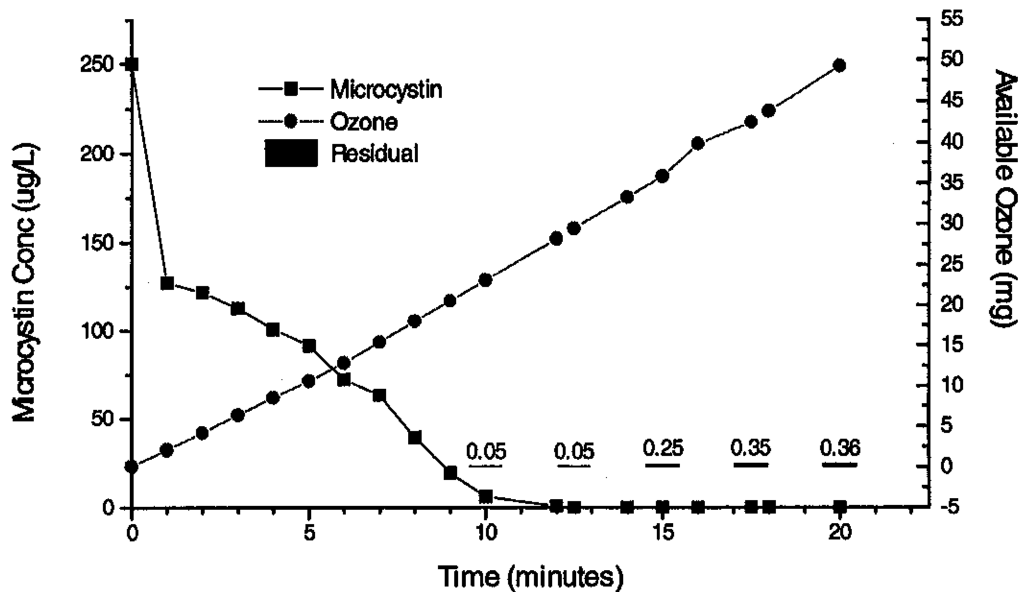



Figure 27. Microcystin decrease from a culture of *M.aeruginosa* during ozonation at 2.5 mg/min.

TABLE XI
OZONE FLOW RATES, RESIDUALS AND MICROCYSTIN RECOVERIES FROM THE OZONATION OF A *MICROCYSTIS* CULTURE.

| Sample Time | Ozone Conc (mg/SL Gas) | Gas Flow (L/min) | Available Ozone (mg/min) | Ozone Residual (mg/L) | Microcystin Conc (µg/L) |
|---|---|---|---|---|---|
| 0 | - | - | - | - | 250 |
| 1 | 7 | 0.3 | 2.1 | 0 | 127 |
| 2 | 7.2 | 0.3 | 2.1 | 0 | 122 |
| 3 | 7.4 | 0.3 | 2.2 | 0 | 113 |
| 4 | 7.1 | 0.3 | 2.1 | 0 | 101 |
| 5 | - | - | - | - | 92 |
| 6 | 7.5 | 0.3 | 2.2 | 0 | 72 |
| 7 | 8.2 | 0.3 | 2.5 | 0 | 63 |
| 8 | 8.4 | 0.3 | 2.6 | 0 | 50 |
| 9 | 8.2 | 0.3 | 2.5 | 0 | 19 |
| 10 | - | - | - | 0.05 | 6 |
| 12 | 8.2 | 0.3 | 2.5 | - | 1 |
| 12.5 | - | - | - | 0.05 | 0 |
| 14 | 8.2 | 0.3 | 2.5 | - | 0 |
| 15 | - | - | - | 0.25 | 0 |
| 16.5 | 8 | 0.3 | 2.6 | - | 0 |
| 17.5 | - | - | - | 0.35 | 0 |
| 18 | 8.2 | 0.3 | 2.7 | - | 0 |
| 19 | 8.4 | 0.3 | 2.7 | - | 0 |
| 20 | - | - | - | 0.36 | 0 |

### 5.6 Peroxone

A peroxone ratio of 0.5 (concentration of peroxide / concentration of ozone) was aimed for in each of the following experiments, however this was not always achieved due to the variable concentration of the ozone stock solution. Peroxide residual determinations of *Microcystis* extracts and live cellular material are not presented, either because the dosing levels were too low

for our analytical technique, or the results were invalid due to interfering UV absorbance from pigment material.

### 5.6.1 Reaction of peroxone with microcystin-LR and an *M. aeruginosa* extract

Results of the reaction of peroxone with microcystin-LR and an *M.aeruginosa* extract are given in Tables XII and XIII respectively. The results clearly demonstrate peroxone, at a ratio around 0.5 to be an efficient oxidant for the removal of cyanobacterial peptide toxins which have been liberated to water.

TABLE XII
REACTION OF PEROXONE WITH MICROCYSTIN-LR

| Ozone Dose (mg/L) | Peroxide Dose (mg/L) | Peroxone Ratio | Microcystin-LR Conc (µg/L) | Ozone Residual (mg/L) |
|---|---|---|---|---|
| 0 | 0 | 0 | 200 | 0 |
| 0.02 | 0.01 | 0.50 | 22 | <.01 |
| 0.04 | 0.02 | 0.50 | 4 | <.01 |
| 0.04 | 0.03 | 0.75 | 0 | 0.02 |
| 0.07 | 0.04 | 0.67 | 0 | 0.03 |
| 0.09 | 0.05 | 0.55 | 0 | 0.05 |

TABLE XIII

REACTION OF PEROXONE WITH AN *M. AERUGINOSA* EXTRACT.

| Ozone Dose (mg/L) | Peroxide Dose (mg/L) | Peroxone Ratio | Microcystin-LR Conc (μg/L) | Ozone Residual (mg/L) |
|---|---|---|---|---|
| 0 | 0 | 0 | 89 | 0 |
| 0.04 | 0.025 | 0.62 | 88 | <.01 |
| 0.05 | 0.025 | 0.5 | 85 | <.01 |
| 0.09 | 0.05 | 0.55 | 69 | <.01 |
| 0.11 | 0.05 | 0.45 | 79 | <.01 |
| 0.26 | 0.12 | 0.46 | 16 | <.01 |
| 0.29 | 0.17 | 0.43 | 51 | <.01 |
| 0.44 | 0.25 | 0.57 | 6 | <.01 |
| 0.68 | 0.37 | 0.54 | 0 | 0.04 |
| 0.91 | 0.37 | 0.36 | 0 | 0.05 |
| 0.96 | 0.50 | 0.52 | 0 | 0.13 |
| 1.48 | 0.5 | 0.35 | 0 | 0.07 |

### 5.6.2 Reaction kinetics of ozone and peroxone with microcystin-LR

An attempt was made to determine the rate constants for the reactions of ozone and peroxone with microcystin-LR. However the reactions were so fast that this was not possible using the simple technique employed in this study.

A solution of microcystin-LR (1 mg/L) was treated with ozone at 0.22, and 0.02 mg/L under the conditions described. The higher dose resulted in zero recovery of microcystin-LR after 15 seconds.The treatment of a microcystin solution at 0.02 mg/L ozone and peroxone resulted in a microcystin-LR recovery of 63 ± 2 % for ozone and 46 ± 3 % for peroxone. This is represented in Figure 28.

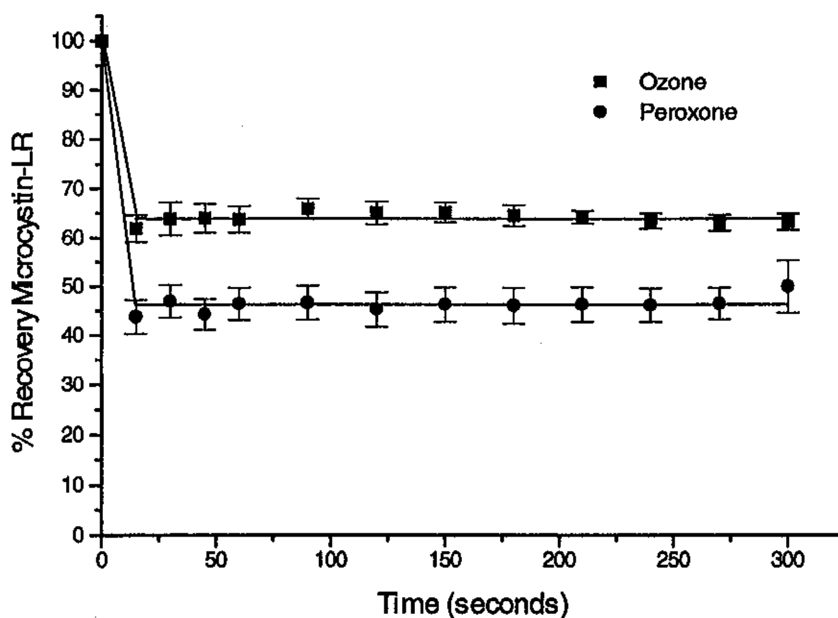
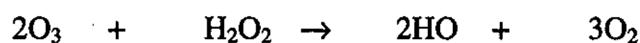



Figure 28. Reaction of microcystin-LR with 0.02 mg/L ozone and peroxone

The recoveries reflect the reactivity of each oxidant implying a more complete and possibly faster rate of reaction of peroxone with microcystin-LR. The reaction of ozone with hydrogen peroxide, as described by the following equation, produces the hydroxy free radical which is non-selective and more reactive than ozone. This would account for the increased rate of reaction of peroxone with microcystin-LR.



$$2O_3 \quad + \quad H_2O_2 \rightarrow \quad 2HO \quad + \quad 3O_2$$

### 5.6.3 Reaction of peroxone with an *M. aeruginosa* culture

The reaction of peroxone with *Microcystis* cultures did not result in a steady decrease of microcystin as in previous reactions with microcystin-LR and *M. aeruginosa* extracts. As illustrated in Figure 29, the results still follow the general trend of decreasing toxin recovery with increasing ozone dose, however microcystin recoveries are inconsistent at low dose rates.

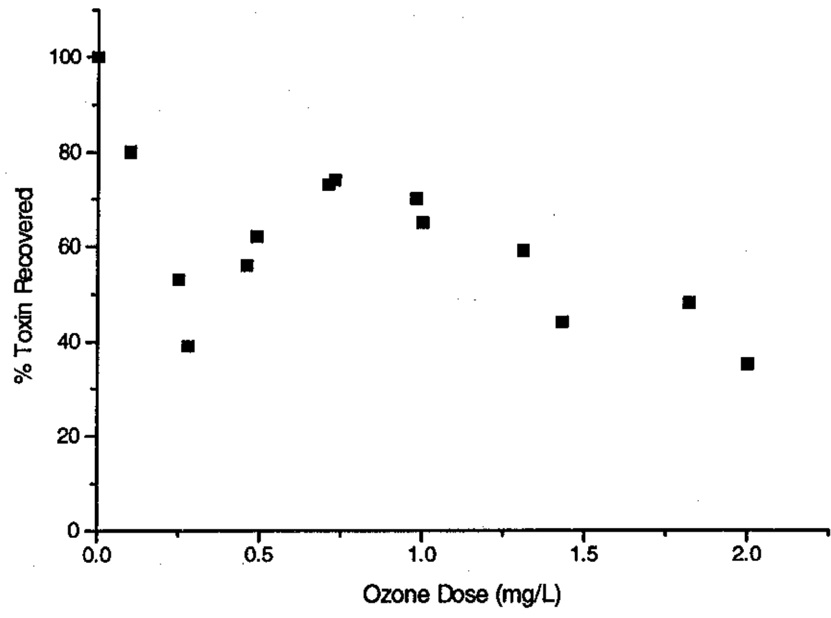



Figure 29. Reaction of peroxone with a culture of *M. aeruginosa*. Contact time = 5 minutes. (Results given are for experiment 2, Table XIV)

TABLE XIV
REACTION OF PEROXONE WITH *M. AERUGINOSA* CULTURES.

| Ozone Dose (mg/L) | Peroxide Dose (mg/L) | Peroxone Ratio | Microcystin-LR Conc (μg/L) | Ozone Residual (mg/L) |
|---|---|---|---|---|
| Experiment 1 (TOC 4.5 mg/L, 620 000 cells/mL, pH 6-7) | | | | |
| 0 | 0 | 0 | 75 | 0 |
| 0.11 | 0.05 | 0.47 | 65 | <0.01 |
| 0.24 | 0.125 | 0.52 | 36 | 0.02 |
| 0.55 | 0.25 | 0.46 | 24 | 0.04 |
| 0.87 | 0.375 | 0.43 | 37 | 0.08 |
| 1.12 | 0.5 | 0.45 | 53 | 0.08 |
| 1.83 | 0.575 | 0.41 | 30 | 0.10 |
| 2.52 | 1 | 0.4 | 24 | 0.18 |
| Experiment 2 (TOC 2.8, 240 000 cells/mL, pH 8.5) | | | | |
| 0 | 0 | 0 | 24 | 0 |
| 0.11 | 0.05 | 0.45 | 19 | <0.01 |
| 0.25 | 0.125 | 0.5 | 13 | <0.01 |
| 0.28 | 0.125 | 0.47 | 9 | <0.01 |
| 0.46 | 0.25 | 0.54 | 13 | 0.04 |
| 0.49 | 0.25 | 0.51 | 15 | - |
| 0.71 | 0.375 | 0.52 | 18 | 0.02 |
| 0.73 | 0.375 | 0.51 | 18 | - |
| 0.98 | 0.5 | 0.51 | 17 | - |
| 1.0 | 0.5 | 0.5 | 15 | 0.08 |
| 1.31 | 0.75 | 0.57 | 14 | - |
| 1.43 | 0.75 | 0.52 | 11 | 0.09 |
| 1.82 | 1.0 | 0.55 | 11 | - |
| 2.0 | 1.0 | 0.49 | 9 | 0.09 |

Contact time - 5 minutes

# 6. SUMMARY AND CONCLUSIONS

Chlorine (as chlorine gas dissolved in water, sodium hypochlorite, or calcium hypochlorite), ozone, peroxone and potassium permanganate were all capable of destroying cyanobacterial peptide toxins when present in water under neutral / acidic conditions. The respective oxidation potentials of these oxidants are higher under acidic conditions than under basic conditions (Glaze 1990), and this was reflected by the poorer oxidation efficiencies of chlorine and ozone under basic conditions. At pH values above 7, chlorine exists predominantly as the hypochlorite ion which is a weaker oxidising species than hypochlorous acid. This resulted in incomplete oxidation of microcystin under basic conditions in comparison to acidic conditions at the same chlorine doses. Ozone is an extremely reactive species but undergoes autocatalytic degradation which is enhanced under basic conditions. Hydroxide ions react with ozone to produce radical ions which are quickly consumed by their reaction with other ozone molecules or with other solutes present in the medium. As pH increases, hydroxide ion concentrations increase which accelerates the degradation of ozone such that the complete oxidation of microcystin is not achieved.

Potassium permanganate did not display any significant decrease in oxidising capabilities at the alkaline pH values investigated even though, according to Glaze (1990) the oxidation potential under alkaline conditions is lower than that for hypochlorite.

Monochloramine and hydrogen peroxide were both ineffective at removing microcystins from water. Monochloramine is a weaker oxidant than those mentioned previously. This is reflected in its much lower oxidation potential (0.75) compared with those for chlorine, ozone and permanganate (1.49, 2.07 and 1.68 respectively). It is not surprising therefore that the reaction with microcystin is not favourable. Hydrogen peroxide on the other hand is a strong oxidant (oxidation potential 1.78 V under acidic conditions) and yet was relatively ineffective. This is probably due to unfavourable reaction kinetics of this oxidant.

The relative reactivities of the oxidants chlorine, potassium permanganate, hydrogen peroxide, ozone and peroxone with microcystin-LR are depicted graphically in Figure 30. The rates of oxidation of microcystin-LR by ozone and peroxone were too fast to be determined using the simple procedure employed in this study. The percentage recovery of microcystin-LR after

oxidation with peroxone is less than that after oxidation with ozone which implies a more efficient and possibly faster oxidation rate with peroxone. The reactivity of potassium permanganate with microcystin-LR is approximately two times faster than that with chlorine at a dose rate of 2 mg/L (permanganate oxidised 50 % microcystin-LR in 2.5 minutes compared with 5.5 minutes for chlorine). On a molar basis potassium permanganate oxidation is essentially four times faster than aqueous chlorine at pH 7. Under these experimental conditions, the reactivity of hydrogen peroxide with microcystin-LR was negligible.

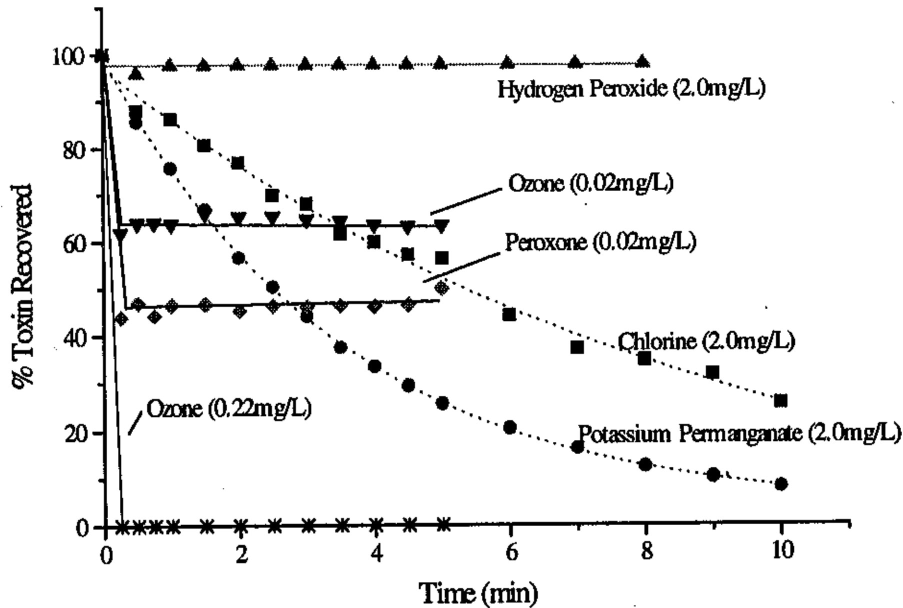



Figure 27. Comparative rates of oxidation of microcystin-LR (1 mg/L) with various oxidants.

The reaction of potassium permanganate with intact *Microcystis* cells over one hour had little effect on removing the toxicity of the sample although with pure microcystin-LR or *M.aeruginosa* extracts the toxins were rapidly destroyed. It would appear that unlike ozone or chlorine, potassium permanganate does not readily cause lysis of the cyanobacteria and release of toxins to the surrounding medium. This is critical in order to reduce the toxicity of a bloom sample or live culture. This was also demonstrated by Lam *et al* (1995b) who found that sodium hypochlorite induced higher microcystin release to the water from a bloom sample of *M. aeruginosa* and *Aphanizomenon* than did potassium permanganate. Both chlorine and ozone are potent bactericides (White 1992), upon cell death and lysis the toxin released to the medium is readily oxidised and consequently toxicity is removed from the *Microcystis* cultures. However higher dose rates were required to meet the oxidant demand of the sample. Thus results with pure toxin or freeze-dried material (in which the cells are already lysed) cannot necessarily be extrapolated to

live material. With live material the ability of the oxidant to lyse the cells and release toxins to the surrounding medium is a critical first step before oxidation can occur.

Even though chlorine is effective in the removal of peptide toxins from water containing live cells, it may not be acceptable to use pre-chlorination for waters containing a toxic cyanobacterial bloom. Chlorinating at low levels would have the effect of lysing the cells, liberating the toxins to the water but not necessarily removing toxicity. Chlorinating at high levels to meet the chlorine demand and ensure toxin removal will increase concentrations of undesirable disinfection by-products (Van Steenderen *et al* 1988). Ozonation would be the most effective measure in removing toxicity as chlorinated disinfection by-products would not be an issue. The use of chlorine during the final stages of water treatment for maintaining a residual in the distribution system would ensure complete removal of cyanobacterial peptide toxins. The extent of chlorinated by-product formation would depend on the extent of oxidation of by-product precursors by ozone.

## 7. RECOMMENDATIONS

The studies presented in this report assess the possibility of using oxidants for the removal of cyanobacterial peptide toxins from water. Further studies are required to evaluate the efficiency of oxidants such as chlorine, ozone and peroxone in particular on the removal of cyanobacterial toxins under conditions likely to be encountered in natural waters. Pilot plant studies to confirm the successful application of the laboratory studies are especially important.

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