ABSTRACT

Cyanobacteria, or blue-green algae are recognised as major water contaminants that cause serious water quality problems for drinking water supplies in Australia and worldwide. They generate a number of metabolites that are responsible for a disagreeable earthy or musty taste and odour in water or toxins that pose health threats to human and animals. Warmer global temperatures, low river flows associated with drought and the increase in nutrient levels in water resulting from modern agricultural practices are all responsible for the proliferation of cyanobacteria in water resources. The increasing occurrences and severity of cyanobacterial blooms have placed enormous pressure on water authorities. There is a desperate need for the development of a range of strategies for treating cyanobacteria contaminated water to potable standards.

The fate of cyanobacteria in water treatment processes, particularly their impact on the recovery of water from waste streams, is not well understood. Reuse of this water is of increasing importance during drought periods in Australia. This study characterised the impact of conventional water treatment and waste management processes on a range of cyanobacteria species (specifically *Microcystis aeruginosa*, *Anabaena circinalis* and *Cylindrospermopsis raciborskii*) and determined the feasibility of recycling the water from sludge processing in the presence of cyanobacteria.

In this work, cyanobacteria cells were cultivated in the laboratory to produce artificial alum sludges that mimic those produced in water treatment plants in the presence of cyanobacterial blooms. By means of metabolite analysis and cell viability assays, it was shown that low pH stress (pH <5), which can occur during coagulation upon the addition of metal hydroxide coagulants, causes massive cell lysis and metabolite release. Avoidance of exposure to low pH was found to
maintain cell viability during coagulation, sedimentation, sludge centrifugation and filtration processes.

To identify the ease with which water can be recovered from cyanobacteria-laden waste stream, characterisation of dewaterability of cyanobacteria-laden sludges was carried out based on the fundamental dewatering theory developed by Buscall and White. Critical observations regarding the dewatering behaviour of these types of sludges in comparison to a range of biological and non-biological materials were drawn from this work. Similar to conventional alum rich water treatment sludges, cyanobacteria-laden alum sludge was difficult to compress to a high solids concentration. The sludge also exhibited a low permeability, making it significantly more difficult to dewater than conventional alum rich water treatment sludges. It is concluded that existing water treatment facilities will have difficulty handling cyanobacteria rich alum sludges.

This finding prompted a preliminary investigation on membrane filtration as an alternative technology for cell removal. The results demonstrated that cross-flow microfiltration is a promising process, particularly operating at low pressures. Filtration of cell-rich water at 50 kPa saw a 25% decrease in permeate flux but 95% of the initial permeate flux can be recovered after quick cleaning. The process was also found to cause negligible damage to the cells or any release of metabolites.

This thesis describes the novel developments in the management of cyanobacteria and metabolites in potable water production. The knowledge will assist water authorities to determine the most appropriate processing strategies for removing cyanobacteria from drinking water system.
DECLARATION

This is to certify that:

- This thesis comprises only my original work towards the PhD except where indicated in the preface;
- Due acknowledgement has been made in the text to all other material used; and
- This thesis is less than 100,000 words in length, exclusive of tables and bibliographies.

Feng Qian
December 2012
Some experimental work presented in this thesis was carried out in collaboration with the industry sponsor, the Australian Water Quality Centre, SA Water, South Australia. Due reference is made to this within the text.
The following paper was published during this work:

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Latin

$a$  Gravitational acceleration experienced at the inner base of the centrifuge tube (m$^2$s$^{-2}$)
$A$  Mass fraction of dissolved solids (-)
$A^*$ Filter cross sectional area (m$^2$)
$B$  Mass fraction of total solids including dissolved solids (-)
$c$  Mass concentration of solids (kg$\cdot$m$^{-3}$)
$C$  Cell density (cells$\cdot$mL$^{-1}$)
$C_0$ Initial cell density in the raw water (cells$\cdot$mL$^{-1}$)
$C_{\text{sup}}$ Cell density in the supernatant (cells$\cdot$mL$^{-1}$)
$d$  Length of an individual cell ($\mu$m)
$D_a$ Impeller diameter (mm)
$D_T$ Tank diameter (mm)
$D(\phi)$ Solids diffusivity (m$^2$s$^{-1}$)
$E_1$, $E_2$ Logarithmic cake compression fitting parameters in Equation 3.7 (-)
$g$  Gravitational acceleration (m$^2$s$^{-2}$)
$G$  Velocity gradient (s$^{-1}$)
$h$  Solid-liquid interface height (m)
$h_0$ Initial suspension height (m)
$h_{\text{base}}$ Base height of the LUMiFuge$^{\text{®}}$ cell (m)
$h_{\text{eq}}$ Equilibrium solid-liquid interface height (m)
$h_{\text{eq, predicted}}$ Predicted equilibrium interface height (m)
$h_i$ Equilibrium height for each $\phi_i$ calculated from Equation 3.14 (m)
$H$  Suspension or piston height in pressure filtration (m)
$H_0$ Initial suspension height (m)
$H_a$ Impeller height from bottom of tank (mm)
$H_{\text{eq}}$ Equilibrium height of cake in pressure filtration (m)
$H_l$ Liquid level (mm)

$J$ Permeate flux (L m$^{-2}$ h$^{-1}$)

$J_0$ Pure water flux (L m$^{-2}$ h$^{-1}$)

$k$ $P_y(\phi)$ fitting parameter in Equation 3.11 (-)

$l$ Total length of the filament (µm)

$l_{ave}$ Average length of a filament (µm)

$m$ Mass of permeate in cross-flow filtration (g)

$m_{dry}$ Combined mass of dried suspension and container (g)

$m_{empty}$ Mass of empty container (g)

$m_{liq,dry}$ Combined mass of dried filtrate and container (g)

$m_{liq,wet}$ Combined mass of filtrate and container (g)

$m_{wet}$ Combined mass of suspension and container (g)

$M_W$ Molecular weight (Da)

$[M_{extra}]$ Concentration of extracellular metabolites (µg L$^{-1}$ or ng L$^{-1}$)

$[M_{extra,0}]$ Initial concentration of extracellular metabolites (µg L$^{-1}$ or ng L$^{-1}$)

$[M_{total,0}]$ Initial concentration of total metabolites (µg L$^{-1}$ or ng L$^{-1}$)

$n$ $P_y(\phi)$ fitting parameter in Equation 3.11 (-)

$n^*$ Number of cells per filament (-)

$n_{ave}$ Average number of cells per filament

$N$ Average number of cells per square of the Sedgewick Rafter (-)

$N^*$ Total number of Sedgewick Rafter squares occupied by 100 filaments of $C. raciborskii$ (-)

$N_B$ Number of baffles (-)

$P_s$ Particle pressure (Pa)

$P_y(\phi)$ Compressive yield stress (Pa)

$\Delta P$ Applied pressure differential (Pa)

$q$ Impeller blade width (axial dimension) (mm)

$r$ Radial position of the solid-liquid interface (m)

$r^*$ Impeller blade length (radial dimension) (mm)

$r_0$ Initial radial position of the suspension meniscus (m)
\( r_{\text{max}} \)  
Radius measured from the centre of the centrifuge to the inner base of centrifuge tube (m)

\( r(\phi) \)  
Hindered settling factor (-)

\( R_{\text{max}} \)  
Maximum detection radius of LUMiFuge\textsuperscript{®} (m)

\( R(\phi) \)  
Hindered settling function (Pa s m\(^{-2}\))

\( s \)  
Length of impeller blade mounted on the central disk (mm)

\( t \)  
Time (s)

\( u(\phi) \)  
Settling velocity (m s\(^{-1}\))

\( V \)  
Specific filtrate volume (m)

\( V^* \)  
Filtrate volume (m\(^3\))

\( V_\infty \)  
Equilibrium specific filtrate volume (m)

\( W_B \)  
Baffle width (mm)

\( x \)  
Mass fraction of total solids excluding dissolved solids (-)

\( y \)  
Distance measured from the centre of the centrifuge (m)

\( y' \)  
Distance measured from the centre of the centrifuge, Equation 3.31 (m)

\( z \)  
Distance measured from the inner base of the centrifuge tube (m)

\( Z \)  
Function of \( z \), Equation 3.26 (m)

**Greek**

\( \alpha \)  
Specific resistance of cake to filtration (SRF) (m kg\(^{-1}\))

\( \beta^2 \)  
Filtration parameter in Equation 3.5 (m\(^2\) s\(^{-1}\))

\( \delta \)  
Correction factor in the calculation of \( P_s \), Equation 3.23 (-)

\( \delta^* \)  
Correction factor in the calculation of \( P_s \), Equation 3.25 (-)

\( \epsilon \)  
Correction factor in the calculation of \( \phi \), Equation 3.22 (-)

\( \epsilon^* \)  
Correction factor in the calculation of \( \phi \), Equation 3.24 (-)

\( \phi \)  
Solids volume fraction (-)

\( \phi_0 \)  
Initial solids volume fraction (-)

\( \phi_f \)  
Final or equilibrium solids volume fraction (-)

\( \phi_g \)  
Gel point in volume fraction (-)
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\phi_i$</td>
<td>Selected solids volume fraction between $\phi_0$ and $\phi_i$ (\text{-})</td>
</tr>
<tr>
<td>$\phi_\infty$</td>
<td>Equilibrium solids volume fraction (\text{-})</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Dynamic viscosity of the filtrate (Pa\text{s})</td>
</tr>
<tr>
<td>$\rho_{\text{liq}}$</td>
<td>Liquid/filtrate density (kg m$^{-3}$)</td>
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<tr>
<td>$\rho_{\text{sol}}$</td>
<td>Solids density (kg m$^{-3}$)</td>
</tr>
<tr>
<td>$\rho_{\text{susp}}$</td>
<td>Suspension density (kg m$^{-3}$)</td>
</tr>
<tr>
<td>$\Delta \rho$</td>
<td>Density differences between the solids and the liquid (kg m$^{-3}$)</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Angular velocity (rad s$^{-1}$)</td>
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</table>
CHAPTER 1 INTRODUCTION

1.1 Background

People have relied on surface waters as a drinking water supply for millennia. However, in the last few decades, the increasing occurrence of cyanobacterial blooms in surface waters used for potable water supply has limited access and increased the risk of using this resource. Cyanobacteria, more commonly known as blue-green algae, are a natural and widespread component of most aquatic systems. They have gained notoriety for their ability to impair drinking water quality. Some species produce secondary metabolites that impart disagreeable earthy or musty tastes and odour to water, whereas other species are capable of producing toxic metabolites that can potentially cause a serious threat to human and animal health through contact and consumption of water (Carmichael, 1997; Falconer, 2005).

The growth of cyanobacteria cells is favoured in water that is nutrient-rich, warm, well-illuminated and slow flowing. These conditions are easily achieved in the calm, stable water conditions of dams and reservoirs. While human actions and activities, such as fertiliser-rich agricultural runoff and effluent from sewage treatment, can help trigger blooms (Brookes et al., 2001), they are also influenced by climatic conditions. The natural climatic conditions of Australia assist with their proliferation nationwide. Records show that Australia has experienced toxic algal blooms for more than a century. The Murray-Darling outbreak in 1990-91 had a massive impact on inland Australia, reducing drinking water availability and resulting in the deaths of a large number of livestock (Falconer, 2005). Extended drought and the possibility of permanent climate change only serve to exacerbate such events. Water shortage not only leads to a higher risk of cyanobacterial blooms, but also forces governments and water authorities to turn to water sources traditionally ignored or discarded. This in turn...
leaves water authorities with no choice but to treat water containing cyanobacteria to safe standards.

Cyanobacterial blooms contain large numbers of cells. This leads to the possibility of high concentration of problem metabolites in the associated water. The bulk of cyanobacterial metabolites are stored internally within the cell (termed intracellular) until the cell dies (Lahti et al., 1997). Some species though, produce and release metabolites at all stages of their life cycle (Chiswell et al., 1999). Either way, any damage that may cause cell leakage will lead to an increase in extracellular (dissolved) metabolite concentration. The removal of dissolved metabolites from water requires advanced treatment processes, such as oxidation (chlorination, ozonation, etc.) or adsorption (powdered activated carbon or granular activated carbon). These processes can be both complex and expensive. Therefore, the most effective initial barrier to prevent problem metabolites from entering the drinking water system is to remove cells intact and without damage.

Conventional drinking water treatment processes, which are designed to optimise the removal of particulates and organic matter, have been shown to be effective in removing cyanobacterial cells (Velzeboer et al., 1995; Chow et al., 1999; Drikas et al., 2001a). The most critical steps within the processes for cell removal are coagulation and clarification. Coagulation involves the addition of metal hydroxide coagulants to the influent to cause particle aggregation whereas clarification separates the resultant aggregates from water through sedimentation or flotation. When cells are removed via these steps without damage, a large portion of the metabolites can also be removed. It is proposed herein that this significantly reduces the need and cost of treatment for dissolved metabolites.
Meanwhile, a waste (sludge) stream that contains high concentrations of cells and intracellular metabolites is generated during these separation steps. In conventional water treatment processes, the waste stream is typically concentrated via further dewatering processes. Water that is recovered from these processes is returned to the original water resource, i.e. the storage or the head of the plant. A key issue for consideration is that with higher numbers of cyanobacteria cells entering treatment plants during bloom situations, the management of cyanobacteria-rich waste becomes a major challenge. Any risk of cell lysis and metabolite release into the recovered water will cause the contamination of the entire plant when the water is recycled.

It is of vital importance to minimise the impact of cyanobacterial cells and metabolites during drinking water production so that cyanobacteria-laden water is treated to potable standards. In addition, water conservation consideration and ever-tightening waste discharge regulations have driven the necessity to use all available water, increasing the focus on recovering water, even from the cyanobacteria-rich waste stream. While there have been numerous studies on the viability of cyanobacteria in a range of environments and the associated release of intracellular metabolite, there is a lack of information in the literature concerning the fate of these cyanobacterial cells during drinking water treatment and cyanobacteria-rich waste management processes. This knowledge is crucial for water authorities as it can assist them to make optimal process choices that reduce the risk of cyanobacteria contamination to a minimum. The primary aim of this work is to address this knowledge gap.
1.2 Research Objectives

The research focused on determining the factors that influence the viability of cyanobacteria and the cause of metabolite release during conventional water treatment processes including sludge dewatering. A range of cyanobacteria species and metabolites were investigated via a range of well-established assay and characterisation techniques.

Another priority of the work was to determine the effect of cyanobacteria cells on the character and behaviour of the dewaterability of water treatment plant sludges. More specifically, the possible extent and rate of dewatering for these type of sludges was characterised using the fundamental dewatering theory developed by Buscall and White (1987). The knowledge is significant as it will help water authorities choose the most efficient approach and process operating conditions to recover water from a cyanobacteria-laden waste stream. More importantly, this information can be used to determine the feasibility and risk of recycling the waste stream from sludge processing in the presence of high concentrations of cyanobacteria and metabolites.

The use of membranes is increasing in the water industry. Exploring the possibility of using membrane filtration for cyanobacteria removal was a final objective. The impact of the process on cell viability and metabolite release was determined and the performance and efficacy of the process assessed.

This work is intended to provide water authorities with extensive knowledge to guide them on the best practices for handling cyanobacteria-laden water. It is hoped that the adaptation of the knowledge will assist the development of a best practice approach for the treatment of potable water in the presence of cyanobacteria.
1.3 Thesis overview

A description of each chapter in this thesis is given here.

In Chapter 1, an introduction to the issues concerning cyanobacteria and their metabolites is provided. The importance of this work is overviewed followed by a statement of the research objectives.

Chapter 2 provides an overview of cyanobacteria and their harmful metabolites and highlights the increasing threat that cyanobacterial blooms pose to public water supplies in Australia and worldwide. A review on different treatment options for the removal of cyanobacterial metabolites from potable water is provided, with an emphasis on conventional water treatment processes. The challenge and the importance of managing cyanobacteria-rich sludges produced from conventional processes are also discussed.

Chapter 3 presents an overview of dewatering theory and the sludge dewaterability characterisation used throughout the thesis. The characterisation techniques involved gravity settling, filtration and centrifugation.

In Chapter 4, the cyanobacteria species studied in this work are described. Details of the experimental methods employed to characterise the impact of conventional treatment and waste management processes on cyanobacteria are presented. The methods used for dewaterability characterisation of cyanobacteria-rich sludges are also described.

Chapter 5 investigates the factors that influence the viability of cyanobacteria and metabolite release during conventional water treatment and waste management processes. Mass balance of metabolites performed across the sludge production or cell removal process is provided to demonstrate the ratio of intracellular to extracellular metabolites during each treatment step.
Chapter 6 presents the dewaterability characterisation results for cyanobacteria-rich sludges. The difference seen between water treatment sludges, with and without cyanobacteria, is described and its implication to water treatment facilities is discussed. The comparison of dewaterability with other sludge types, both biological and non-biological, is also presented.

In Chapter 7, the results obtained from preliminary investigation on the performance of a microfiltration membrane process for cyanobacteria cell removal are presented. The impact of the process on cell viability and metabolite release is discussed.

Chapter 8 summarises the major findings from this work and provides the recommendations for further work.
2.1 Introduction

This study focuses on the removal of cyanobacteria and their metabolites from drinking water using conventional water treatment processes. The objective of this chapter is to review background knowledge that is critical to the successful removal of cyanobacteria and safe production of drinking water.

Section 2.2 overviews the characteristics of cyanobacteria and the occurrence of cyanobacterial blooms in surface waters. Section 2.3 introduces the most problematic metabolites cyanobacteria produce: cyanotoxins, their impact on humans and animals, and some guidelines regarding the safe exposure to cyanotoxins. Section 2.4 presents techniques that are commonly used to identify and quantify cyanobacteria and their metabolites. Section 2.5 reviews the efficiency of different treatment technologies for cyanobacteria and metabolites removal with a focus on conventional water treatment processes. Section 2.6 provides a discussion on the importance of the management of cyanobacteria-rich sludges produced from drinking water treatment processes.

2.2 Cyanobacteria

Cyanobacteria, commonly known as blue-green algae, are one of the most primitive living species on the earth. They have existed for more than 3.5 billion years and are found throughout a great variety of ecosystems ranging from the cold deserts of the Antarctic continent to the stones and sand of the world’s hot deserts (Falconer, 2005). The major locations of cyanobacteria are, however, in aquatic environments including streams, lakes, estuaries and the sea.
Cyanobacteria are photosynthetic prokaryotes, part of the bacterial domain, with no structured nucleus (Ladiges et al., 2005). However, unlike bacteria, they resemble algae and plants in that they contain chlorophyll a, which enables them to generate oxygen during photosynthesis and to provide the characteristic blue-green colour of many species. Some cyanobacteria strains containing heterocysts are also capable of nitrogen fixation (Ladiges et al., 2005).

Cyanobacteria are present in the form of single cells, single cells in filaments or single cells in colonies. They reproduce by asexual binary division (in unicellular forms). The individual cell has a spherical or cylindrical shape and a size range from 3 to 10 µm (Svrcek and Smith, 2004). Many contain an intracellular gas-vacuolate which regulates cell density and enables them to move up and down in the water column in order to fulfill their nutritional and photosynthetic requirements (Oliver, 1994). Some cyanobacteria strains possess additional surface structures outside the cell membrane, mainly of a polysaccharide nature (de Philippis and Vincenzini, 1998), which protects cells from stress in extreme conditions and leads to the formation of colonies which makes them a less preferred food in comparison with other organisms in the ecosystem.

In water, some cyanobacterial species are known to produce taste and odour (T&O) compounds that give water an earthy, musty flavour. In drinking water, the most common cause of T&O complaints are, 2-methylisoborneol (2-MIB) and geosmin. These volatile organic compounds are detectable to humans at less than 10 ng L⁻¹ (Watson et al., 2008). Cyanobacteria are the chief sources of these two compounds (Watson et al., 2008). Interestingly, their production is only limited to filamentous species (e.g. Anabaena circinalis, Aphanizomenon flos-aquae) (Juttner and Watson, 2007). More important, however, is the fact that a group of the genera, at least 40 species, have been identified to produce toxins that pose health threats to livestock and human beings (Carmichael, 1997).
While cyanobacteria are present in surface water supplies at all times, a serious issue for drinking water is during cyanobacterial blooms where the level of toxins and T&O compounds can be high. It has been theorised that changing environmental conditions and climate change are responsible for the abundance of cyanobacterial blooms (Brookes et al., 2001; Falconer, 2005; Paerl and Paul, 2012). Warmer global temperatures, calm steady water flow and changes in water quality associated with drought all favour the growth of cyanobacteria.

Cyanobacterial blooms have been reported all around the world. In Australia, the incidence of cyanobacteria blooms is increasing with recent blooms reported in South Australia, Victoria, Queensland, New South Wales and Tasmania. The most dominant species are *Microcystis aeruginosa*, *Anabaena circinalis* and *Cylindrospermopsis raciborskii* (Burch, 2001; Falconer, 2001). The major cyanobacterial outbreak recorded in the past decade in Australia was in the Warragamba Dam, New South Wales, during August 2007 (Sydney Catchment Authority, 2008). It persisted over three months and at one point, 700,000 cells mL$^{-1}$ of *Microcystis* sp. were reported. In order to avoid the cells entering the treatment plant, the local water authority was forced to lower the off-take depth from 10 metres to 48 metres below the surface. In another case reported in March 2008 (Department of Human Services, 2009), the raw water storage supplying Hopetoun, Victoria, was contaminated with a bloom of *A. circinalis*. The local water authority responded by taking the storage off line and treated the storage with copper sulphate until the bloom dissipated. These two actions are among the common responses to cyanobacterial blooms in Australia. Copper sulphate has found to cause cell lysis and subsequent release of toxic metabolites (Chow et al., 1999). This practice is no longer recommended. Furthermore, while the first option may be available at current water levels, long term run off trends suggest that reservoir levels will become lower over time and consequently, this will not always be an option. Also, complete closure of a reservoir in water stressed conditions is not a suitable alternative. It would be preferable to prevent cyanobacterial blooms occurring.
A major trigger for the proliferation of cyanobacteria in water is the input of nutrients resulting from modern agricultural practices (e.g. fertiliser application, waste from intensive animal farming, domestic and industrial waste water treatment effluent, and land clearing). The two main nutrient components are nitrogen and phosphorus. In an analysis by Smith (1983) of growing season data from 17 lakes throughout the world, it is claimed that cyanobacteria tend to dominate a water body when the mass ratio of total nitrogen to phosphorus (TN:TP ratio) is below 29. Modification of this ratio is therefore suggested as a way to manage blooms by sewage diversion, phosphorus removal from wastewater, or nutrient precipitation within the lakes themselves. This hypothesis was challenged by other researchers suggesting that the dominance of cyanobacteria is due to the increase in absolute phosphorus concentrations rather than a decrease in the TN:TP ratio (Scheffer et al., 1997; Xie et al., 2003; Falconer, 2005). A study of a *Microcystis* bloom in China indicates that a low TN:TP ratio is not the cause but actually a result of cyanobacterial blooms (Xie et al., 2003). While phosphorus has been viewed as a key factor in proliferation of cyanobacteria, excessive nitrogen loading in parallel with human population growth in watersheds is now considered to be as critical as phosphorus loading (Vitousek et al., 1997; Conley et al., 2009; Paerl and Scott, 2010).

Based on the review of the literature it is clear that our knowledge of the conditions in which cyanobacterial blooms will occur is currently incomplete. This lack of certainty makes the management practices required to prevent algal blooms in drinking water sources somewhat more complicated. It also makes prediction of algal blooms difficult. Although this is an interesting topic and is a precursor to any work into algal blooms, it will not be the focus of this study. However, the role of blooms in creating high toxin levels is important in understanding the way in which they will interact in a water treatment process and how effectively these toxins can be removed.
2.3 Cyanobacterial toxins

Cyanobacterial toxins or cyanotoxins are formed within the cells as secondary metabolites. They are not used for primary metabolism (cell division or energy production). They are formed at all stages of cyanobacteria growth (Pietsch et al., 2002). In nature, cyanotoxins are characterised by the state of their occurrence, intracellular (cell bound) and extracellular (outside the cell) respectively. The release of toxins in dissolved form can be caused by the natural biological process of cell lysis or by cell damage associated with the stress experienced in various treatment processes. The latter is of particular interest in drinking water production.

Pietsch and coworkers (2002) investigated the natural release of microcystin from the strain *M. aeruginosa*. They found that intracellular microcystin was produced mainly during the exponential growth phase of the culture. The concentration of extracellular microcystin started to increase towards the end of the exponential growth phase and a sharp rise of dissolved microcystin was observed in the decaying phase (A growth curve of *M. aeruginosa* is shown in Figure 2.1). The author also cited a few studies that showed similar results on some other species. For example, cyanotoxins were released at the end of the exponential phase for *Anabaena flos-aquae* and the middle of the stationary phase for *Plankotothrix rubescens*. Extracellular cylindrospermopsin, however, can be found at all stages of the life cycle (Chiswell et al., 1999).
Figure 2.1: A growth curve of laboratory cultivated *M. aeruginosa* consisting of exponential growth phase, stationary growth phase and decaying phase (adapted from Pietsch et al (2002)).

### 2.3.1 Cyanotoxins classification and their toxicity

Cyanotoxins can be classified into three groups: cyclic peptides, alkaloids and lipopolysaccharides. The most abundant cyanobacterial species that produce each toxin are summarised in Table 2.1.
Table 2.1 Cyanotoxins and producer organisms (adapted from Svrcek and Smith (2004)).

<table>
<thead>
<tr>
<th>Toxin group</th>
<th>Toxic effect</th>
<th>Producer cyanobacteria genera</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyclic peptides</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microcystins</td>
<td>Hepatotoxic</td>
<td>Anabaena, Anabaenopsis, Aphanocapsa, Hapalosiphon, Microcystis, Nostoc, Oscillatoria</td>
</tr>
<tr>
<td>Nodularins</td>
<td>Hepatotoxic</td>
<td>Nodularia</td>
</tr>
<tr>
<td><strong>Alkaloids</strong></td>
<td></td>
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<tr>
<td>Neurotoxic alkaloids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anatoxin-a</td>
<td>Neurotoxic</td>
<td>Anabaena, Aphanizomenon, Oscillatoria</td>
</tr>
<tr>
<td>Anatoxin- a (S)</td>
<td>Neurotoxic</td>
<td>Anabaena, Oscillatoria</td>
</tr>
<tr>
<td>Saxitoxins</td>
<td>Neurotoxic</td>
<td>Anabaena, Aphanizomenon</td>
</tr>
<tr>
<td>Cytotoxic alkaloids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cylindrospermopsin</td>
<td>Cytotoxic, Hepatotoxic, Neurotoxic, genotoxic</td>
<td>Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya</td>
</tr>
<tr>
<td><strong>Dermatotoxic alkaloids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alysiatoxin</td>
<td>Dermatotoxic</td>
<td>Anabaena, Aphanizomenon, Cylindrospermopsis, Umezakia</td>
</tr>
<tr>
<td><strong>Lipopolysaccharides</strong></td>
<td>Potentially irritates any exposed tissue</td>
<td></td>
</tr>
</tbody>
</table>
2.3.1.1 Cyclic peptides

The peptide toxins that have received most attention to date are microcystins and nodularins. They are named after *Microcystis* and *Nodularia*, the first genera from which the toxins were extracted. They are hepatotoxic, meaning they cause liver damage and can potentially promote tumor growth (Nishiwak-Matsushima *et al.*, 1992; Carmichael, 1997). The two toxins have similar structures (Figure 2.2 (a) and (c)). They have in common the amino acids 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid (abbreviated as Adda), D-Glucose, D-Methyaspartic acid and L-Arginine. The N-methyldehydrobutyrine (Mdhb) residue found in nodularin is usually replaced by an N-methyldehydroalanine (Mdha) in microcystin.

Among all the known toxins produced by cyanobacteria, microcystins have the most significant impact on human and animal health. Many livestock deaths caused by consumption of the toxins have been reported and the potential risk to human health from microcystin-contaminated drinking water is of most concern (Carmichael *et al.*, 2001; Falconer, 2005). The microcystin family has more than 65 heptapeptides with the common structure cyclo (D-Ala-L-X-DMeAsp-L-Z-Adda-D-Glu-Mdha), where X and Z are variable L-amino acids. For example, microcystin-LR, one of the most commonly found and most toxic variants in the family, contains leucine and arginine (shown in Figure 2.2 (b)). Microcystins have a molecular weight ($M_W$) between 900 to 1100 Da and size between 1.2 and 2.6 nm (Donati *et al.*, 1994). Due to their cyclic chemical structure, they remain stable under extreme conditions such as boiling and are resistant to chemical hydrolysis or oxidation at near neutral pH (Chorus and Bartram, 1999). Microcystins can be decomposed by intense ultraviolet light or oxidised by strong oxidising agents such as ozone. Moreover, biodegradation of microcystins can occur in the natural environment, but the toxins can persist for days before any significant degradation occurs (Antoniou *et al.*, 2005).
Acute microcystin intoxication leads to massive hepatic hemorrhagic shock and death (Carmichael, 1997; Falconer, 2005) whereas sub-acute cases can result in diarrhea, vomiting, weakness and pallor, all of which are undesirable outcomes from the drinking of water. Meanwhile, chronic ingestion of microcystin has found to induce primary carcinoma in rodents, and has been epidemiologically linked to the promotion of liver tumors and primary liver cancer in humans (Nishiwaki-Matsushima et al., 1992). The most prominent microcystin poisoning case occurred in a group of dialysis patients in Caruaru, Brazil in 1996 (Carmichael et al., 2001). After receiving haemodialysis treatment, 116 of 131 patients experienced visual disturbances, nausea and vomiting. Subsequently, 100 patients developed acute liver failure and of these, 76 died. The major contributing factor to death of the dialyses patients was the intravenous exposure to high concentration of microcystin-YR, -LR and -AR that was detected in the water supply to the dialysis centre.
Figure 2.2: Chemical structures of the cyclic peptide hepatotoxins. (a) General structure of microcystins, $M_W = 900-1100$ Da, $R_1$ and $R_2$ are H or CH$_3$ (b) Microcystin-LR, with the amino acid leucine in the X position and arganine in the Z position (c) Nodularin, $M_W$ approximately 800 Da.
2.3.1.2 Alkaloids

The alkaloid toxins are generally heterocyclic nitrogenous compounds with at least one nitrogen-carbon bond. This group contains toxins that are neurotoxic (nervous system damage), hepatotoxic (liver damage), genotoxic (disruption of cell genetic integrity) and dermatotoxic (skin irritants).

Neurotoxic alkaloids

The neurotoxins are toxins that have adverse effect on the nervous system. They can be classified into three groups: (i) anatoxin-a and its natural analogs, homoanatoxin-a, (ii) anatoxin-a (S) and (iii) saxitoxin. Most of them are fast and acute acting and have very low lethal doses. However, in terms of chronic toxicity, they do not appear to have the same degree of impact as cyclic peptides.

Anatoxin-a and homoanatoxin-a (Figure 2.3 (a) and (b)) have been isolated from *Anabaena*, *Aphanizomenon* and *Oscillatoria* (Nicholson and Shaw, 2001). The acetyl group in anatoxin-a is replaced by a propionyl group in homoanatoxin-a. Anatoxin-a has a $M_W$ around 165 Da. It can be degraded rapidly under sunlight with a half-life of 1-2 hours (Svrcek and Smith, 2004). Anatoxin-a (S) (Figure 2.3 (c)) ($M_W = 252$ Da) is not structurally related to anatoxin-a. It is the only naturally occurring organophosphate and has been extracted from *Anabaena flos-aquae* (Matsunaga *et al.*, 1989). Both toxins act immediately and cause symptoms including muscle overstimulation, convulsions and paralysis (Carmichael, 1997). Animal death due to respiratory arrest can also occur if the respiratory muscles are affected.

Neurotoxic saxitoxins (general structure shown in Figure 2.3 (d), $M_W$ ranging from 280 - 450 Da) are commonly known as paralytic shellfish poisons (PSP). They were first identified in marine dinoflagellates (red tide) where they are responsible for paralytic shellfish poisoning after consumption of contaminated shellfish (Falconer, 2005). They disrupt the normal signaling between nerves and
muscles. *A. circinalis* is the major producer of PSP in fresh water in Australia. Toxic Australian isolates of *A. circinalis* produce PSPs exclusively and at high levels while isolates from American and Europe produce either anatoxin-a or anatoxin-a(S) (Brookes *et al.*, 2001). The factors causing this geographical localisation to Australian freshwater species have not been determined. A major poisoning event caused by saxitoxins occurred in Australia in the summer of 1990 where an outbreak of *A. circinalis* covered about 1000 km of the Darling River leading to the deaths of more than a thousand livestock and also contamination of the drinking water supply of several towns (Falconer, 2005).

**Cytotoxic alkaloids**

Cylindrospermopsin (structure shown in Figure 2.4, $M_W$ approximately 415 Da) is the most recent cyanotoxin identified (Falconer, 2005). It is now regarded as the most likely source of human illness through the drinking water supply along with microcystins. In fact, Australia’s worst case of human poisoning was attributed to cylindrospermopsin that resulted in severe hepatoenteritis among the children of an Aboriginal community in 1979 (Falconer, 2005). The toxin does not specifically target the liver (hepatotoxic) but causes damage in a range of organs (e.g. kidneys, heart, intestine and eye). Gastroenteritis and liver injury are the initial symptoms of the poisoning. There is also evidence to suggest that cylindrospermopsin is potentially genotoxic (Svrcek and Smith, 2004).
Cylindrospermopsin is produced mainly by *C. raciborskii*. It was originally a tropical and subtropical species found commonly in Northern Australia, but has appeared to spread into the Murray-Darling system in Australia in recent years (Baker and Humpage, 1994). Unlike *M. aeruginosa* and *A. circinalis* that tend to form scums accumulated on the surface of waters, *C. raciborskii* forms bands well below the surface. This may cause severe problems for water authorities as they are hard to identify and the bloom may occur at the depths where water intakes are located.
Dermatotoxic alkaloids
This type of alkaloid is commonly produced by benthic marine cyanobacteria, for example, *Lyngbya*, *Schizothrix* and *Oscillatoria*. They may cause severe skin irritation upon direct skin contact as well as oral and gastrointestinal inflammation (Chorus and Bartram, 1999).

2.3.1.3 Lipopolysaccharide (LPS) endotoxins
LPSs are characteristic components of the outer membrane of both cyanobacteria and Gram-negative bacteria (Rapala *et al.*, 2002). High endotoxin concentrations detected in blooms are more likely associated with the presence of Gram-negative bacteria that coexist with cyanobacteria. Studies have also found that the heterocysts of nitrogen-fixing cyanobacteria can attract heterotrophic bacteria and contribute to the rise of LPS (Wang and Priscu, 1994; Rapala *et al.*, 2002). Endotoxins are less toxic than the neuro- and hepatotoxins but have been implicated as possible agents for skin irritations, gastrointestinal disorders and respiratory allergy.
For Australian surface waters, based on the cyanobacterial species most commonly associated with blooms (\textit{M. aeruginosa}, \textit{A. circinalis} and \textit{C. raciborskii}), the main toxins of concern are microcystins, saxitoxins and cylindrospermopsin. While any processing and identification needs to able to deal with all possible scenarios, the literature suggests that to be representative of the Australian experience, the focus of this study should be on these compounds.

### 2.3.2 Toxin exposure routes and exposure limits for drinking water

Potential routes of exposure to cyanobacteria and their toxins include:

- Oral ingestion of contaminated water
- Inhalation of water and aerosols
- Direct contact with exposed parts of the body, including skin, eyes and ears
- Contaminated food (e.g. fish, mussels and zooplankton in which cyanotoxins can be bioaccumulated)
- Haemodialysis

The most common route for the uptake of cyanotoxins for animals and humans is the ingestion of water through drinking water or accidental ingestion during recreation.

In order to minimise the risk of adverse health outcomes caused by exposure to cyanobacteria in drinking water, guidelines have been developed in several countries. These countries are typified as being susceptible to cyanobacterial blooms (Carmichael, 1997). The World Health Organization (WHO) has set a provisional drinking water guideline level for total microcystin (as microcystin-LR toxicity equivalents) of 1.0 \( \mu \text{g L}^{-1} \). The National Health and Medical Research Council/Agriculture and Resource management Council of Australia and New
Zealand (NHMRC/ARMCANZ) derived a 1.3 μg L\(^{-1}\) guideline for microcystin. This is equivalent to a cell density of approximately 6500 cells mL\(^{-1}\) (Burch, 2001).

There is no recommendation for any of the other toxins at this time. Nevertheless, a guideline value of 1.0 μg L\(^{-1}\) for cylindrospermopsin was suggested based on a study on the observed adverse effect on organ weight, urine and serum parameters caused by the toxin (Humpage and Falconer, 2002). Likewise, a health alert level of 3.0 μg L\(^{-1}\) has been proposed for saxitoxin (Fitzgerald \textit{et al.}, 1999).

The WHO has also provided various alert levels (summarised in Table 2.2) for the management of cyanobacteria in source water for the application of drinking water production. Routine monitoring of water supplies for the presence of cyanobacteria is recommended.

From the onset of Alert Level 1, an assessment is required to determine, first, whether the cell numbers in the water supply intake can be reduced through offtake management and second, whether the water treatment system available is effective in reducing the concentrations of cells and toxins. Cell population in the water supply should to be monitored continuously and toxin testing needs to be initiated upon the identification of any toxic species that are present in sufficient cell numbers. At Alert Level 2 or above, the risk of adverse health effects are elevated significantly if alternative water supplies or advanced water treatment targeting cyanotoxins are not implemented. Effective water treatment systems are crucial at this stage and ongoing assessment of the system performance is important.
With these guidelines in mind, it is critical for water authorities to correctly identify the cyanobacteria species in water and to quantify the problem cyanobacteria and their metabolites. Based on the severity of the blooms, water treatment facilities can then determine the most suitable treatment strategies for successful removal of cyanobacteria and elimination of the problem metabolites.

### 2.4 Identification and quantification for cyanobacteria and cyanotoxins

Cyanobacteria are enumerated under a compound light microscope using a calibrated counting chamber. The cell counting results are typically expressed as cells per millilitre (cells mL$^{-1}$) of original source water. Using phase contrast microscopy, the morphological characteristics such as cell size, cell shape, structural features (filaments, colonies) and the presence and position of gas vacuoles, can be recognised. The method helps to identify any toxic species but cannot determine if the cells are currently producing toxins or at what concentration.

The toxicity of cyanobacterial blooms was traditionally determined by mouse bioassay. The method monitors the overall toxicity of the bloom but it lacks specificity for individual toxins. In addition, it could not detect toxins when the concentrations are low. Currently, a combination of high performance liquid chromatography (HPLC) with detection techniques such as UV adsorption, fluorescence and mass spectroscopy are used for toxin detection and quantification. For microcystin and saxitoxin determination, enzyme linked immunosorbent assay (ELISA) is also widely used. There are a number of commercial ELISA kits that allow fast and accurate measurement of toxin concentrations.
Table 2.2: A summary of the WHO guidelines for cyanobacteria in drinking waters (Adapted from Chorus and Bartram (1999)).

<table>
<thead>
<tr>
<th>Alert Level</th>
<th>Cell Density</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vigilance</td>
<td>200 cyanobacterial cells mL$^{-1}$</td>
<td>• Non-bloom conditions, cyanobacteria detected in the water sample at low numbers, weekly monitoring continuing</td>
</tr>
</tbody>
</table>
| 1           | 2000 cyanobacterial cells mL$^{-1}$ or 1 µg L$^{-1}$ chlorophyll-a with a dominance of cyanobacteria | • Trend towards increasing high numbers, or maintenance of moderate numbers, of cyanobacteria  
• Water may be unsuitable for drinking without appropriate treatment  
• In drinking water supplies, toxin testing to be initiated, particularly if the sample is predominated by a known toxic species - repeat on weekly basis  
• Low risk of skin irritation or gastrointestinal illness from primary recreational contact (swimming, water skiing etc.)  
• Continue weekly cyanobacterial counts and issue advisory notices to public |
| 2           | 100,000 cells mL$^{-1}$ or 50 µg L$^{-1}$ chlorophyll-a with a dominance of cyanobacteria | • Persistently high numbers of potentially toxic cyanobacteria widespread throughout the water source, and/or visible localised scums forming  
• Water may be unsuitable for drinking without appropriate treatment  
• In all storages, toxin testing to be initiated and/or continued on weekly basis  
• Switching to alternative supply should be considered if available  
• Weekly sampling for cyanobacterial counts to continue  
• Increasing risk of adverse health effects from primary recreational contact  
• More extensive media releases/advice to public |
Recently, research into the genetics and evolution of cyanobacteria has allowed the accurate and fast identification of toxic species of cyanobacteria in water even before the species has proliferated into a bloom. DNA based tests using polymerase chain reaction (PCR) have been developed to identify the toxic species *A. circinalis*, *C. raciborskii*, *L. majuscula*, *M. aeruginosa* and *N. spumigena* (Neilan, 2001).

It is clear that determination of cyanobacterial species and toxins can be a quite complicated process, generally requiring advanced biological techniques. These would not always be available to authorities in charge of water provision. While this could be outsourced, HPLC would generally be seen as the more appropriate technique in this situation. While the investigation of DNA-based tests and improvements to make them more widely acceptable would be of significant interest, it is not the focus of this work. For the purposes of this study, all analysis will be performed using HPLC and commercially available ELISA kits.

**2.5 Cyanobacteria and cyanotoxin removal technologies**

Monitoring water sources using the techniques described above ensures that water authorities can act immediately to the change of raw water quality associated with cyanobacteria blooms. While avoiding cyanobacteria intake (e.g. changing intake depth, adding a barrier upstream) should be the first response, it is not always possible particularly during drought when severity and occurrence of blooms are high. Moreover, a more serious challenge faced by water treatment facilities is the dissolved cyanotoxins that are already present in water. It is therefore of utmost importance to have comprehensive treatment strategies in place for both cyanobacteria and cyanotoxins. This section reviews technologies that are found to be effective for removing cyanobacteria and cyanotoxins. The methods range from physical removal of cells to chemical destruction of cells and toxins. As toxins are produced within the cells and remain intracellular until cell damage and lysis (with the exception of
cylindrospermopsin), it is reasonable to suggest that removing cells intact and without damage should always considered the highest priority.

2.5.1 Chemical processes

2.5.1.1 Chlorination

Pre-chlorination of water containing cyanobacteria cells is not recommended as the process has been found to cause cell lysis and the consequent release of intracellular toxins (Daly et al., 2007). Investigation of the application of chlorination for the removal of cyanotoxins was first carried out on microcystins. The initial work indicated that toxin destruction by chlorination was ineffective, even at high dosages (Hoffman, 1976). It was suggested that the efficacy of chlorination of toxins depends on the raw water quality, particularly the concentration of organic matter. Available chlorine was consumed by the organic matter, leaving insufficient amount of chlorine for microcystin oxidation. Nicholson et al. (1994) proposed that the chlorination of the cyclic peptide hepatotoxins also depends on pH. They showed that at pH below 8, both microcystin and nodularin were completely oxidised at aqueous chlorine dose of 1 mg L\(^{-1}\) or greater. The effect of chlorination was reduced with increasing pH above 8 due to the decreasing concentrations of hypochlorous acid. This is not considered an issue for many treatment systems, where the pH is typically less than 8.

Assuming an adequate dose is supplied, chlorination has shown to be effective in oxidising microcystin, nodularin, saxitoxin and cylindrospermopsin in various pH ranges (Nicholson et al., 1994; Senogles et al., 2000; Newcombe and Burch, 2002). Anatoxin-a, on the other hand, was found to be resistant to chlorination (Nicholson et al., 1994).
Limited knowledge of chlorination by-products is available. Although acute toxicity, as measured by the mouse bioassay, was effectively reduced during the chlorination process in general, it was shown that progressive liver damage could still be detected in the animals due to the incomplete toxin removal or to the formation of byproducts (Rositano et al., 1995). A study on the chlorination by-products of cylindrospermopsin cited by Svrcek and Smith (2004), has also shown liver damage and genotoxicity. An alternative to chlorination treatment is the use of chloramines. However, they appear to have little effect on microcystin and nodularin (Nicholson et al., 1994), suggesting that it may not be a suitable replacement in this case.

2.5.1.2 Potassium permanganate
Potassium permanganate is a strong oxidising agent that has been used to control taste, odour and biological growth in treatment plants. It has been reported to be effective in microcystin oxidation with low oxidant demand. However the process was found to contribute to an increase in residual manganese and turbidity in treated water (Petrusevski et al., 1996). A permanganate dose of 1-1.25 mg L\(^{-1}\) has been shown to reduce microcystin concentrations below the WHO guideline value of 1 µg L\(^{-1}\) in experiments performed with a surface water with initial toxin concentrations of 3.2, 7.1 and 1.1 µg L\(^{-1}\) for MC-LR, MC-RR and MC-YR (Rodriguez et al., 2007a). While it was suggested that permanganate was able to penetrate or lyse the cells (Lam et al., 1995; Schmidt et al., 2002), the dose must be carefully optimised in order to remove extracellular toxins without causing cell lysis and further release of toxins.

2.5.1.3 Ozonation
Ozonation has been used primarily for disinfection purposes or to remove colour and odour in water treatment. Two pathways for the oxidation of organic pollutants by ozone have been suggested: direct attack by molecular ozone via cycloaddition or electrophilic reaction, and indirect attack by free radicals
(primarily OH) formed by the decomposition of ozone (Masten and Davies, 1994). Ozone is highly reactive towards unsaturated bonds. In microcystin or nodularin, either of the double bonds in the Adda group (refer to Section 2.3.1.1) would be attacked rapidly leading to the elimination of the toxicity. It has been found that the level of microcystin-LR and nodularin (initial concentrations at 166 and 88 µg L⁻¹, respectively) was reduced to below the detection limits within 4 min and 15 sec respectively, after dosing with 0.22 mg L⁻¹ ozone (Rositano et al., 1998). The efficiency of ozonation with respect to the destruction of anatoxins and saxitoxins has not been well characterised. A study cited by Hitzfeld et al (2000) stated that anatoxin-a was more resistant to removal by ozone than microcystin-LR. The saxitoxins are recalcitrant to ozone even at a much higher ozone dose of 6.9 mg L⁻¹ (Rositano et al., 2001). The efficiency of ozonation is also shown to be pH dependent with alkaline conditions (pH >7.5) being less favourable due to the lower oxidising potential at high pH (Rositano et al., 1998). As stated previously, this is not an issue for most potable water supplies.

Competitive reactions with dissolved organic carbon (DOC) and alkalinity (i.e. carbonate and bicarbonate) are also major factors that affect the performance of ozonation of cyanotoxins. The presence of organics requires an increased dose rate to oxidise toxins due to competing reactions (Rositano et al., 1998). Shawwa and Smith (2001) observed that as the DOC concentration in water increased from 1 to 5 mg L⁻¹, there was a distinct lag phase before the destruction of microcystin-LR commenced. Moreover, ozonation of intact cells during pre-ozonation steps can cause cell lysis and increased ozone demand (Hart et al., 1998). Furthermore, a major ozone by-product, bromate formed by reacting ozone with bromide, can have potentially worse health effects on humans (Rodriguez et al., 2007b).
2.5.1.4 Hydrogen peroxide

Hydrogen peroxide (H$_2$O$_2$) can be an effective treatment of both cyanobacteria and their toxins and is considered an environmentally benign reagent because of its rapid decomposition into oxygen and water (Barrington and Ghadouani, 2008). The action of H$_2$O$_2$ on cells occurs primarily by the formation of hydroxyl radicals in solution. The hydroxyl radicals inhibit photosynthetic electron transfer and oxygen evolution and inhibit photosynthetic activity, hence inducing cell death. However, research has suggested that H$_2$O$_2$ alone is relatively ineffective in destroying microcystin-LR. The treatment of a 1 mg L$^{-1}$ microcystin-LR solution, with peroxide at 2 mg L$^{-1}$, showed little impact on toxin removal in 10 min (Rositano et al., 1998). Ozone or UV light is often used with H$_2$O$_2$ to enhance its effectiveness (Zhou and Smith, 2001).

2.5.1.5 Summary

The response of toxins to oxidation through chlorination, permanganate, ozonation or peroxide is complicated by the presence of organic matter. The dose requirement and dose rate may need to be adjusted according to the relative concentration of toxins and other organics in water, which requires constant monitoring in the water body. Pre-oxidation is generally not acceptable as it will cause cell lysis and the release of intracellular toxins. The risk increases if a treatment plant is not prepared to deal with the increased concentration of dissolved toxins. Adequate optimisation of upstream water treatment processes are required to ensure the removal of organics that would increase oxidant demand and thereby improve the efficacy of post-oxidation. Moreover, the by-products formed in oxidation processes can pose potential threats to human health.
2.5.2 Adsorption

One of the first applications of activated carbon adsorption in water treatment was for the removal of taste and odour compounds. They have now been used extensively for the removal of a wide range of contaminants, including pesticides, industrial chemicals and cyanotoxins. Activated carbon is available in two forms, granular activated carbon (GAC) and powdered activated carbon (PAC). GAC is typically used in flow-through columns, after conventional treatment and before disinfection. PAC can be added directly to water prior to coagulation, during chemical addition, sedimentation or prior to filtration. One of the initial studies on toxin removal (Hoffman, 1976) revealed that PAC was effective in removing two toxin extracts isolated from freeze-dried algal material which was harvested from an *M. aeruginosa* bloom. Filtration through GAC was also found to be capable of removing the toxins. Activated carbon has proven to be effective in adsorbing microcystins, anatoxin-a and cylindrospermopsin to different degrees (Lawton and Robertson, 1999).

Several studies have assessed the suitability of a range of carbons produced from different materials (wood, coal, peat and coconut). Wood-based PAC was found to be superior in effectiveness on toxin removal over coal and coconut based PAC (Donati *et al.*, 1994; Warhurst *et al.*, 1997). However, it has been proposed that surface characteristics (pore size, surface area) are the more important factor in determining the performance of activated carbon and not the parent carbon source (Lawton and Robertson, 1999). Yet it is important to note, the nature of the starting material and the mode of activation determines the surface characteristics and therefore sorption properties. Donati and coworkers (1994) proposed that the efficacy of microcystin adsorption was strongly dependent on the pore volume of activated carbon. Microcystin-LR has an estimated diameter of 1.2 to 2.6 nm meaning the molecules are considered too large to adsorb in micropores (diameter < 2 nm). Mesopores (2 - 50 nm in diameter), however, are of the appropriate size for adsorption to occur. In comparison, the molecular weights of the T&O compounds, geosmin and 2-MIB
are lower and can be adsorbed into the narrow range of micropores (Newcombe et al., 2010).

Similar to chemical process, the efficacy of activated carbon is complicated by the competitive adsorption of organic matter. Studies showed that the removal efficiency of toxins and T&O compounds declines due to competition for the binding sites on the carbon (Donati et al., 1994; Newcombe et al., 2002).

GAC has generally received more attention than PAC due to the fact that it is used in columns that can be easily regenerated and it can provide a constant barrier against unexpected episodes of toxins and T&O compounds. An additional advantage of using GAC is the development of biological activity on the GAC which allows further degradation of toxins. A pilot-scale GAC study cited by Svrcek and Smith (2004) shows more than 90% removal of microcystin when 7000 m$^3$ and 12000 m$^3$ treated water passed through two separate GAC filters. However, the efficiency declined to between 49 and 63% after this point due to saturation of GAC. Another study (Lambert et al., 1996) evaluated the performance of GAC in a water treatment plant, which had not been used for 5 months. It was revealed that adsorption capacity of used carbon is significantly reduced compared with virgin GAC of the same type. The life span of GAC for microcystin removal is relatively short, the best performing GAC, in a study by Hart et al (1998), gave a bedlife of at most, 30 days.

Although the use of activated carbon offers some success in removing toxins, the high cost associated with material replacement and regeneration is a huge disadvantage that hinders its application (Lawton and Robertson, 1999).
2.5.3 UV photolysis

Cyanotoxins are generally stable under natural sunlight whereas ultraviolet (UV) light has shown some promising results in decomposing the toxins (Tsuji et al., 1995). Several mechanisms of photolysis with UV light have been proposed for microcystin destruction (Lawton and Robertson, 1999): Photoisomerisation occurs when an electron in either of the unsaturated bonds of Adda (refer to Section 2.3.1.1) is promoted to a higher orbital, causing a rotation around the newly-formed single bond; an internal cycloaddition on the Adda group would be possible between unsaturated bonds and the aromatic ring. Both microcystin and anatoxin-a have been found to be effectively degraded at a UV radiation dosage from 1530 mJ cm\(^{-2}\) to 20000 mJ cm\(^{-2}\) (Tsuji et al., 1995). This technique is sometimes used together with H\(_2\)O\(_2\) or TiO\(_2\). The combination of TiO\(_2\) photocatalyst and UV radiation quickly decomposed microcystin with a half life of less than 5 min (Shephard et al., 1998). Again the efficiency of this process was largely dependent on the organic load of the water.

2.5.4 Biodegradation

Biodegradation is mediated by the development of biological activity in the filters due to attachment and growth of biofilm microorganisms. Bourne et al (2006) demonstrated that an endemic MJ-PV bacteria, isolated from a Sphingomonas sp. facilitated 90 to 95% of microcystin-LR degradation from an initial concentration of 50 µg L\(^{-1}\) within 2 days. Filters generally require a maturation phase that allows the microorganism population to establish in the filter and an acclimation period where the necessary biological species develop in the biofilm to an extent that the toxins can be completely and reliably removed. This could take weeks or even months. The establishment and maintenance of an effective microbial population can also be hindered by intermittent exposure to high levels of toxins due to irregular cyanobacteria blooms. The acclimation period is suspected to be dependent on the factors such as temperature, pH, predation by protozoa and conditioning or induction of an endemic microbial population.
capable of metabolising the microcystins. Although several bacterial species has been identified to date that have the capability to degrade microcystin, saxitoxin, cylindrospermopsin and T&O compound MIB and geosmin (Ho et al., 2012b), this technology has not been thoroughly investigated due to the difficulty in maintenance of the microbial population and a lack of control of or knowledge of the complex mechanisms. There is also concern that the process may produce toxic by-products. While several studies have shown that the by-products of microcystin degradation are non-toxic (Bourne et al., 1996; Ho et al., 2007), Kayal et al (2008) suggested that biotransformation from a less toxic to a more toxic saxitoxin variant may occur in biologically active filters.

2.5.5 Membrane filtration

Microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) are technologies that have emerged in recent years. There are a few studies on removal of cyanobacteria or cyanotoxins (specifically microcystins) by membrane filtration. The molecular weight cut-off (MWCO) for RO is 100 Da and between 200 and 1000 Da for NF. With most cyanotoxins having molecular weights below 1100 Da, both RO and NF are, in theory, capable of removing extracellular dissolved toxins. Low pressure UF and MF on the other hand use membranes with larger pore sizes (nominally 0.1 and 0.01 µm respectively) and ultimately are unable to remove dissolved toxins, meaning the focus for these techniques would be on the physical removal of intact cells.

Chow and coworkers (1997) assessed the efficiency of flat-sheet MF and UF membranes in removing the cells and toxins of a M. aeruginosa bloom. They concluded that 98% cells containing intracellular toxins were removed successfully by the membranes. The removal mechanism is physical separation as the pore sizes of both membranes were an order of magnitude smaller than the size of individual cells. Similar results were obtained in UF experiments with a culture of P. agardhii, where 98% of cell bound toxins were removed
(Gijsbertsen-Abrahamse et al., 2006). A small amount of cell-bound microcystin was released and transferred to the permeate, which is suspected to be caused by shear during pumping or the filtration process itself. The same study showed that over 96% rejection of microcystins and anatoxin-a was achieved by a single spiral wound NF membrane. Interestingly, adsorption of toxins onto UF and NF membranes has been documented in both studies and by others (Campinas and Rosa, 2010; Dixon et al., 2011b). For example, although the pores of UF membranes used were significantly larger than the size of microcystin, Gijsbertsen-Abrahamse et al (2006) observed a reduction of dissolved microcystin concentration in the permeate compared to the concentration in the feed.

Overall, membrane filtration technology has been shown to be a useful treatment process for removing both cyanobacteria and cyanotoxins present in water. It has been integrated into some conventional treatment processes together with other techniques to polish the treated surface water (Mouchet and Bonnelye, 1998; Dixon et al., 2011b; Dixon et al., 2011a). For example, Dixon et al (2011b) used an integrated membrane system combining coagulation, PAC and UF for removal of A. circinalis and the associated saxitoxin. Cells were removed completely using UF whereas dissolved saxitoxin was removed mainly via PAC adsorption. The hybrid system is thought to be ideal for simultaneous removal of cyanobacteria cells and their toxins.

Membrane fouling represents a challenge in the application of membrane filtration process. The attachment, accumulation or adsorption of cell materials onto the membrane surface and or within membrane pores can cause rapid permeate flux decline and increase of transmembrane pressure (Zhou and Smith, 2001; Kwon et al., 2005). While membrane permeability deteriorates due to the deposition of algal cells and subsequent formation of cake layer, extracellular organic matter (EOM) released by cyanobacteria is also found to be a contributor of membrane fouling (Henderson et al., 2008; Qu et al., 2012b). The
main components of EOM, polysaccharides and proteins, are thought to be the major foulants (Her et al., 2004). Several studies have demonstrated that a mixture of cells and EOM, which is typical for cyanobacteria-rich water, caused the most severe flux decline compared to filtration of cell only suspension (Hodgson et al., 1993; Qu et al., 2012a; Qu et al., 2012b). It was hypothesised that EOM fills the void between the cells during cake formation, hence increasing the cake resistance and lowering the permeability significantly.

High water recovery is always the primary interest of water authorities. The processes are traditionally operated at high pressure and high flux resulting in faster and more severe fouling. However, the cost associated with frequent cleaning and replacement of membranes has the potential to offset the efficiency of the process. This therefore warrants research into low pressure membrane processes. Flux decline and membrane fouling in high pressure (200 kPa) UF and MF processes of M. aeruginosa removal has been investigated previously (Chow et al., 1997). The study showed that the processes produced high initial flux but suffered significant fouling in less than an hour. There is little knowledge on the flux decline and fouling behaviour of MF/UF processes of A. circinalis and C. raciborskii removal. In particular, the low pressure processes on these three species has rarely been explored. Operating at low pressure has the advantage of slow fouling rate and this consequently extends the membrane life. It is worthwhile to investigate the low pressure MF/UF processes of cyanobacteria removal and their impact on cell damage and metabolite release as the knowledge in this area is limited.

2.5.6 Conventional water treatment processes

Most of the technologies described above focus on removing cyanotoxins in the dissolved form. The process can be quite complex and the high costs involved in some operations can be unfavourable for their further development. Moreover, the by-products produced by some of the processes will potentially still
compromise drinking water quality. A better option may be to remove toxins by physical removal of intact cyanobacterial cells. The conventional water treatment processes, which effectively removes the organics, microorganisms and large particulates in raw water is therefore a highly attractive option for removing cyanobacterial cells.

Conventional water treatment processes for the production of drinking water, as shown in Figure 2.5, vary in design but generally includes the addition of metal hydroxide coagulants to the influent stream to adsorb natural organic matter (NOM) and cause particulate aggregation followed by removal of resultant aggregates (clarification) by either sedimentation or dissolved air flotation (DAF) to a waste stream. The clarified water is typically passed to a sand bed filtration unit to remove particulates that were not removed in the previous step. The waste stream generated in the clarification process typically undergoes further treatment to recover water while generating a sludge concentrate. The water recovered from sludge treatment, which can be as much as ten percent of the influent, is commonly recycled back to the head of the process or to the reservoir. If the cyanobacterial cells containing intracellular toxins can be concentrated and removed intact and without damage it will minimise the quantity of cyanotoxins going forward through the process and hence avoid extra processes required to treat the dissolved toxins.
2.5.6.1 Coagulation

Several authors have investigated the removal of intracellular toxins via the removal of cells. Drikas and coworkers (2001b) examined the effects of copper sulphate and aluminium sulphate (alum) on *M. aeruginosa* cells. The results indicated that at the stoichiometric equivalent concentration of both copper and aluminium, alum had no impact on the number or viability of the cells. In contrast, the copper treated sample had a high dissolved microcystin concentration indicating significant cell damage and toxin release caused by copper. In another experiment (Chow *et al.*, 1999), it was shown that the presence of alum at concentrations used in water treatment does not appear to be toxic to *M. aeruginosa* over a 24 hour period. In addition, mechanical action (i.e. stirring during the coagulation process) did not cause any damage to the cells or additional release of toxins. Aluminium sulphate was also found to cause no damage to *A. circinalis* cells or additional release of geosmin (Velzeboer *et al.*, 1995). Similar results were observed with ferric chloride. The coagulation of cultured *M. aeruginosa* and *A. circinalis* with ferric chloride was shown to be
effective in aggregating the cells without damaging the cell membranes (Chow et al., 1998). On the other hand, the same studies revealed that none of the coagulants has assisted the removal of dissolved toxins. Nevertheless, cell-bound toxins can be separated from water effectively via coagulation followed by clarification, without causing cell lysis and toxin release into the system.

### 2.5.6.2 Clarification

The type of clarifier selected will impact on the efficiency of cell removal. It has been shown that the long flocculation times achievable in a sludge blanket reactor make it more effective than static settling (Mouchet and Bonnelye, 1998). However, the sedimentation efficacy may be impaired by auto-flotation of flocs. This has been observed when bubbles were formed within the flocs due to the generation of excessive oxygen by algae during photosynthesis (Jodlowski, 2002). In another study (Drikas et al., 2001a), the sedimentation process has shown to remove 100% of the intracellular microcystin by removing the settled *M. aeruginosa* laden sludge within 2 hours. But substantial decrease of cell viability (up to 90%) in the sludge was reported one day after coagulation while the cell number was reduced to half its initial value after two days. A significant release of intracellular microcystin was also observed during this period.

DAF process may perform better with cyanobacteria with higher buoyancy than sedimentation (Edzwald, 1993; Teixeira and Rosa, 2006). The process operates by dissolving air in water under pressure followed by releasing the air at atmospheric pressure. The released air forms tiny bubbles which adhere to flocculated particles and bring them to the surface. Previous studies have reported 94.5% of *Microcystis* sp. and 100% *Anabaena* sp. removal by DAF (Vlaski et al., 1996; Svrcek and Smith, 2004). Although the information on this topic is still limited, it has been suggested that the DAF process may have less impact on cells and the resultant water quality than sedimentation as the floating sludge is removed more easily and more frequently than settled sludge where cell lysis occurs over time.
2.5.6.3 Sand filtration

Sand filtration process can be applied either on an unclarified or clarified process stream, although it has been suggested that direct filtration without clarification is less successful for removing cyanobacterial cells (Lambert et al., 1996; Mouchet and Bonnellye, 1998). For instance, Lambert et al (1996) found poor removal efficiency of intracellular microcystin (14 to 60%) across a direct filtration step that followed alum coagulation. From an operational point of view, long filter runs with cells trapped in filter beds could lead to cell lysis and subsequent toxin release into the treated water. However, slow sand filtration has been found to be a useful technique for treating toxins by biodegradation. The filtration operates at lower loading rates and develops a biofilm above the filter that will allow for some biodegradation of dissolved toxins. Removal of microcystins has been reported by several authors (Grutzmacher et al., 2002; Bourne et al., 2006). Grutzmacher and coworkers (2002) found that over 95% of dissolved microcystins was biodegraded on or inside the filter bed during summer months whereas less than 65% was degraded during autumn months. However, another investigation on removal of saxitoxin variants by biologically active filters has seen a potential of increased toxicity of filtered water via biotransformation of saxitoxins (Kayal et al., 2008). Since the performance of sand filtration on cyanobacteria and toxin removal is debatable, there is a need for management strategies of cyanobacteria-rich waste generated from coagulation and clarification processes.

In summary, conventional treatment processes have the potential for removing cyanobacteria cells and their intracellular toxins although they are not designed to remove dissolved toxins. The fact that infrastructure already exists means limited extra costs would occur in the implementation of cyanobacteria removal. Overall, however, there is a need to focus on the optimisation of the operations in order to minimise cell lysis and toxin release during the treatment processes. An important part of this is the recovery of water that may otherwise be removed as part of the sludge that forms during solid-liquid separation processes. The focus of this study is on these processes and maximum water recovery.
2.6 Management of cyanobacteria-rich sludge

While the separation events in conventional drinking water treatment process are capable of removing the majority of the cyanobacterial cells intact and without damage, the management of the highly concentrated cyanobacterial waste becomes problematic once the cells containing the intracellular metabolites are separated into the treatment sludge or membrane concentrate. Significant amounts of water can be recovered from further dewatering of sludge and it is common practice to recycle it back to the head of the plant. However, if the cells in the sludge lyse and cause the release of metabolites, the treated water will be contaminated and recycling can potentially contaminate the entire plant.

Time has proven to be a critical factor in sludge management. Drikas et al. (2001a) has shown that significant cell lysis in the sludge occurs within 2 days after the sludge has been produced. Another study on the fate of A. circinalis and C. raciborskii during sludge lagoon treatment has shown evidence of metabolite release over the 7 day period for both species (Ho et al., 2012a). There is currently a lack of knowledge on the timescale of dewatering of cyanobacteria-rich sludges. Hence, the priority of this project is to determine the dewatering characteristics of the cyanobacteria-rich sludge in order to find the optimum timescale to treat the sludge while preventing cell lysis.

Due to water scarcity in Australia, water providers cannot afford to lose the amount of water typically recovered from sludge. It is the aim of this work to identify the best operational strategies for sludge management in order to minimise cell lysis and metabolite release. Research into the release of metabolites during the treatment of waste streams (treatment sludge and membrane concentrate) and the potential risks posed by recycling the treated waste stream into the plant is thus considered paramount to achieving the aims of this project.
2.7 Summary

This review has provided some background information on cyanobacteria and highlighted the ever-increasing challenges associated with cyanobacteria and their released metabolites being faced by water authorities. Guidelines on the level of metabolite in water are available for treatment facilities which can help them decide the appropriate treatment methods. A review on the treatment technologies of cyanobacteria and their metabolites has recognised conventional water treatment processes to be the most attractive option. A knowledge gap exists on the fate of cyanobacteria once they are separated into the sludge stream and their impact on water quality. This forms the main aim of the study. Membrane filtration, as an alternative treatment technology, is also examined. The objective is to extend the knowledge in this area by addressing the issues of permeate flux decline and the impact of cyanobacteria on treated water.
CHAPTER 3  DEWATERING THEORY

3.1 Introduction

For water treatment plants handling high levels of cyanobacteria cells, the biggest challenge is to ensure that water is treated to safe standards and that the cell rich sludges are efficiently managed. A knowledge of the viability of cells and the release of metabolites by these cells as a function of time and treatment process type is therefore of the utmost importance. To obtain this information, a good understanding of the dewatering behaviour of this type of material is crucial, as is the response of the cells to the treatment conditions. There is currently little knowledge on the dewaterability of cyanobacteria-rich sludges. A characterisation tool is needed to comprehensively measure the rate and the extent of dewatering of these sludges in various water treatment and waste treatment processes and therefore accurately predict the dewatering behaviour of the sludges in each process. This will allow design and optimisation of each process in order to provide risk-free operating conditions that reduce the impact of cyanobacteria in water treatment to a minimum.

This chapter provides an overview of the fundamental dewatering theory applied for dewaterability characterisation in this work. Various characterisation techniques used to quantify the dewaterability of the sludges are also introduced.
3.2 Buscall-White dewatering theory

There are a number of laboratory techniques available to characterise the dewatering properties of particulate sludges. The two most well-known and commonly used measurements for dewaterability are specific resistance to filtration (SRF) and capillary suction time (CST). In SRF measurement, a sample is separated on filter paper under vacuum in a Buchner funnel. The filtrate volume is recorded as a function of time. The SRF is then determined from Darcy’s Law of flow through porous media:

\[
\frac{1}{A^*} \frac{dV^*}{dt} = \frac{\Delta P A^*}{\eta c V^*}
\]

where \( \alpha \) is the specific resistance of cake to filtration, i.e. SRF (m·kg\(^{-1}\)), \( V^* \) is the volume of filtrate (m\(^3\)), \( \Delta P \) is the applied pressure differential (Pa), \( A^* \) is the filter area (m\(^2\)), \( c \) is the solids concentration of suspension (kg·m\(^{-3}\)) and \( \eta \) is the dynamic viscosity of the filtrate (Pa·s).

SRF is a parameter that provides an average measure of the resistance to flow through a filter cake, assuming the filter cake is incompressible. For compressible materials, the parameter will show dependencies on the initial solids concentration and cake thickness and it is questionable whether the use of an average value is appropriate (de Kretser et al., 2008). As a consequence, for compressible filter cakes, differences in test procedures have been found to influence SRF results (Smollen, 1986). As such, SRF will not characterise dewatering behaviour during cake compression and in addition, provides no information about the extent of dewatering.

CST is determined by pouring a sample into a reservoir resting on a piece of chromatography paper. The filtrate is extracted from the reservoir by the capillary suction of the paper while a cake is formed at the bottom of the reservoir. The filtrate spreads outwards on the paper in a circular pattern and the time taken for the liquid front to move a specified distance is recorded. The CST measurement is also an empirical method that gives a good indication of filterability for sludge.
at a specific solids concentration (Scholz, 2005). It is necessary to specify the initial solids concentration before making a comparison of dewaterability. Similar to SRF, CST measurements do not account for cake compression or help determine the extent of dewatering. It has also been established that both methods can predict dewaterability on drying beds successfully, however, it is not accurate for mechanical dewatering processes (Smollen, 1986). Furthermore, neither SRF or CST can assist in the design and optimisation of dewatering devices from first principles (Harbour et al., 2001).

The limitations of SRF and CST have driven the need to develop a fundamental dewatering model that allows a comprehensive measure of dewatering behaviour irrespective of the dewatering devices or solids concentration. In 1987, Buscall and White developed a fundamental dewatering theory that uses two parameters to characterise the dewaterability of a particulate suspension (Buscall and White, 1987): the compressive yield stress, \( P_y(\phi) \) and the hindered settling function, \( R(\phi) \). They are material properties that describe the extent and the rate of dewatering of the suspension respectively and are applicable to compressible filter cakes. The solid-liquid separation group in the University of Melbourne has since developed a range of characterisation techniques to measure dewatering behaviour in terms of \( P_y(\phi) \) and \( R(\phi) \). These techniques are fast and robust and allow accurate measurement of dewaterability across a wide range of solids concentrations. The dewaterability information extracted can be used to model and optimise dewatering processes such as sedimentation, centrifugation and pressure filtration. These operations are of importance to a wide variety of industries. The Buscall and White model is used for characterisation of material dewaterability in this work.
3.2.1 Compressive yield stress, $P_y(\phi)$

The compressive yield stress relates to the extent of dewatering of the material. For a given particulate suspension, there exists a critical solids concentration (expressed as a volume fraction), or gel point, $\phi_g$, above which a suspension forms a continuously networked structure with a strength that resists compressive forces. This strength is referred to as compressive yield stress, $P_y(\phi)$. It is a physically measurable property that depends on the local solids volume fraction, $\phi$. Below the gel point, the compressive yield stress becomes zero as the solids concentration is too low to form a network.

$P_y(\phi)$ increases with increasing $\phi$, due to the greater number of inter-particle interactions. Applying a pressure that is higher than $P_y(\phi)$ on the suspension causes consolidation of particles and an increase in local solids volume fraction. The consolidation process continues until an equilibrium solids volume fraction is reached at which the suspension is able to withstand the applied pressure. An example of a compressive yield stress curve is displayed in Figure 3.1. It predicts the maximum solids concentration achievable for the suspension when applying a given pressure. This information in turn gives an indication of the most suitable dewatering devices required, based on the necessary pressures, to achieve a certain final solids concentration for this suspension. For example, from the figure, at 100 kPa, a maximum solids fraction of 0.4 could be achieved.
Figure 3.1: Compressive yield stress curve with gel point $\phi_g=0.1$, adapted from de Kretser et al (2005).

3.2.2 Hindered settling function, $R(\phi)$

The hindered settling function quantifies the resistance to flow of fluid through a suspension (or a solid bed). It determines the rate of dewatering of the suspension at a given concentration. It is derived from a hindered settling factor $r(\phi)$, proposed by Landman and White (1995), which models the hydrodynamic interactions between particles.
The settling rate of a suspension \( u(\phi) \) in m s\(^{-1}\), is related to \( R(\phi) \) by

\[
u(\phi) = \frac{\Delta \rho g(1-\phi)^2}{R(\phi)}
\]

where \( \Delta \rho \) is the density difference between the solids and liquid in the suspension (kg m\(^{-3}\)) and \( g \) is gravitational acceleration (m s\(^{-2}\)).

As the solids volume fraction of the suspension increases, each individual particle in the suspension will experience more hydrodynamic interactions as the suspension consolidates. The resistance to flow of the fluid also increases due to the reduction in fluid pathways between particles, hence an increase in \( R(\phi) \).

### 3.2.3 Solids diffusivity

Solids diffusivity \( D(\phi) \), first proposed by Landman and White (1994), serves as a single measure of dewaterability by combining the rate and the extent of dewatering. \( D(\phi) \) is related to \( P_y(\phi) \) and \( R(\phi) \) by the following relationship:

\[
D(\phi) = \frac{dP_y}{d\phi} \frac{(1-\phi)^2}{R(\phi)}
\]

Similar to the compressive yield stress and the hindered settling function, solids diffusivity is a material property that is independent of dewatering devices and is not expected to show dependencies on parameters such as the initial solids concentration of a test or process. A high value of \( D(\phi) \) suggests that the material dewateres more quickly. The solids volume fraction at which this \( D(\phi) \) value occurs gives an indication of the solids concentration achievable in the final product.

By measuring \( P_y(\phi) \), \( R(\phi) \) and calculating \( D(\phi) \), the compressibility, permeability and diffusivity of various sludges can be comprehensively characterised and directly compared. These parameters can also be used to design and optimise a range of dewatering processes. Characterisation of cyanobacteria-rich sludges has never been carried out before, and the outcome will allow us to determine if
the existing water treatment plants are capable of handling high levels of cyanobacteria cells. This process will also help to identify best ways to operate the treatment processes for minimum impact on the integrity of the cyanobacteria cells.

3.3 Characterisation techniques

3.3.1 Pressure filtration

Pressure filtration tests are used to characterise dewaterability of a material at high solids volume fraction. A sample, with initial solids volume fraction, $\phi_0$, is loaded into a dead-end filtration cell at an initial height, $H_0$. A constant pressure, $\Delta P$ is applied on the sample using a piston, causing the liquid to pass through the system via a permeable membrane at the base of the cell. This in turn leads to a reduction of suspension height in the cell. Meanwhile, a cake starts to form on the membrane. The cake formation process continues until the piston first comes into contact with the cake. At this point, cake compression begins and continues until equilibrium is reached.

The height of the suspension, $H$, is measured over time, $t$, until the equilibrium height, $H_{eq}$, is reached. The specific volume of the filtrate or volume of the filtrate per filter area, $V$ ($m^3 m^{-2}$ or m), can be determined from the reduction of $H$ over time. The filtration profile of a suspension under constant pressure filtration can be plotted as $t$ versus $V^2$. The plot is initially linear corresponding to the cake formation stage and is followed a cake compression region that approaches equilibrium.

For a material with traditional filtration behaviour (Stickland et al., 2005), the filtration profile consists of a long cake formation period followed by a short cake compression stage, as illustrated in Figure 3.2. This allows both the $P_f(\phi)$ and $R(\phi)$ data to be obtained rapidly via stepped pressure filtration tests.
Figure 3.2: Example of traditional constant pressure filtration behaviour (adapted from Stickland et al (2005)).

Stepped pressure filtration tests developed by de Kretser et al (2001) consist of a compressibility test and a permeability test. In the compressibility test, a sample at initial solids volume fraction \( \phi_0 \) is loaded into the filtration rig at initial height \( H_0 \). A constant pressure is applied on the sample until the equilibrium height \( H_{eq} \) is reached. The test is then stepped to a higher applied pressure until a new equilibrium is reached. This is repeated over several pressures. The equilibrium solids volume fraction, \( \phi_f \), for each applied pressure, can be determined using mass balance:
\[ \phi_f = \phi_0 \frac{H_0}{H_{eq}} \]  

3.4

This becomes one \( P_y(\phi) \) data point on the compressive yield stress profile. The stepped pressure compressibility test generates multiple data points at the applied pressures selected. This gives a complete \( P_y(\phi) \) profile at the high solids volume fraction region.

The data from the stepped permeability test is plotted as \( t \) versus \( V^2 \) and the gradient extracted (i.e. the \( dt/dV^2 \) value in the linear cake formation region for each applied pressure). Once a stable \( dt/dV^2 \) value is reached, the test is stepped to a higher pressure to obtain a new \( dt/dV^2 \) value and so on. The applied pressures are chosen to match the ones used in stepped compressibility test. A \( \beta^2 \) value is assigned as the inverse of the gradient of a plot of \( t \) versus \( V^2 \):

\[ \beta^2 = \frac{1}{\left( \frac{dt}{dV^2} \right)} \]  

3.5

Once \( \beta^2 \) is obtained for each \( \Delta P \), a power-law function is fitted to the \( \beta^2 \) versus \( \Delta P \) data, which enables the calculation of \( d\beta^2/d\Delta P \) and consequently \( R(\phi) \) using,

\[ R(\phi_f) = \frac{2}{d\beta^2} \left( \frac{1}{\phi} - \frac{1}{\phi_f} \right)(1-\phi_f)^2 \]  

3.6

Some materials, such as sludges of a biological nature show non-traditional filtration behaviour. This is represented by a short cake formation period and a long cake compression period (illustration in Figure 3.3) (Stickland et al., 2005). Unlike the methods used for calculating \( R(\phi) \) for traditional slurries, the gradient of a \( t \) versus \( V^2 \) plot cannot be extracted accurately due to the linear cake formation region being extremely short. Moreover, each constant pressure filtration step will take a long time which means there is a risk that the properties
of the suspension will change before equilibrium is reached. In this case, stepped pressure filtration tests are not useful. A different characterisation protocol is necessary to determine $P_y(\phi)$ and $R(\phi)$ information for these materials.

For non-traditional systems, a series of single constant pressure filtration tests are performed at a range of applied pressures. As discussed earlier, the time taken to reach equilibrium can be extremely long (more than 2 weeks) and as such, the test is often terminated before equilibrium is reached and before the sample starts to degrade or evaporation of the sample becomes significant. This allows the capture of sufficient data in the cake compression region for accurate data extrapolation without compromising the quality of the run.

![Diagram](image)

**Figure 3.3:** Example of non-traditional constant pressure filtration behaviour (adapted from Stickland *et al* (2005)).
The solids volume fraction $\phi$, over time $t$, can be calculated using a mass balance similar to Equation 3.4. A logarithmic functional is then fitted to the experimental $t$ versus $\phi$ data in the cake compression region. This allows estimation of equilibrium $\phi_\infty$, as supposed to the final solids volume fraction, $\phi_f$, determined by the user upon termination of the filtration run.

In the study by Landman and White (1997) on constant pressure filtration, they showed that the asymptotic behaviour during cake compression (illustrated in Figure 3.3) is given by a Taylor's series expansion that is logarithmic,

$$ t = E_1 - E_2 \ln(V_\infty - V) \quad 3.7 $$

where $E_1$ and $E_2$ are constants and $V_\infty$ is the volume of the filtrate at equilibrium.

In the dead-end filtration configuration, Equation 3.7 can be simply replaced by:

$$ t = E_1 - E_2 \ln(H - H_\infty) \quad 3.8 $$

where $H_\infty$ is the equilibrium height calculated from the $\phi_\infty$ estimate using Equation 3.4.

$E_2$ is given by:

$$ E_2 = \frac{4}{D(\phi_\infty)} \left( \frac{H_0\phi_0}{\pi\phi_\infty} \right)^2 \quad 3.9 $$

where $D(\phi_\infty)$ is the solids diffusivity at equilibrium.

By fitting Equation 3.7 to the experimental $H(t)$ data, the constant $E_2$ can be determined, and $D(\phi_\infty)$ is then calculated from Equation 3.9. The estimation methods are detailed in theoretical work presented by Stickland et al (2008). Meanwhile, a power-law function is fitted to $P_y(\phi_\infty)$ data at the estimated $\phi_\infty$. $R(\phi_\infty)$ is then calculated for each $\phi_\infty$, by rearranging Equation 3.3:

$$ R(\phi_\infty) = \frac{dP_y}{d\phi} \left( \frac{1 - \phi_\infty}{D(\phi_\infty)} \right)^2 \quad 3.10 $$
3.3.2 Gravity batch settling

In gravity batch settling tests, a well-mixed sample at an initial solids volume fraction $\phi_0$ that is less than the gel point, is loaded into a cylinder. As the suspension starts to settle, the height of the solid-liquid interface $h$ is measured over time until an equilibrium height, $h_{eq}$, is reached. The compressive yield stress of a suspension at low solids volume fraction, near $\phi_g$, can be determined by this technique. The analysis requires a function of $P_y(\phi)$ to be selected with parameters estimated, for example:

$$P_y(\phi) = k\left(\frac{\phi}{\phi_g}\right)^n - 1$$ \hspace{1cm} (3.11)

where $k$ and $n$ are estimated parameters.

The predicted equilibrium height $h_{eq,predicted}$ can be determined using:

$$h_{eq,predicted} = \frac{1}{\Delta \rho g} \int_{\phi_0}^{0} -\frac{1}{\phi(P_y)} dP_y$$ \hspace{1cm} (3.12)

where $h_0$ is the initial height of the suspension, and $\phi(P_y)$ is the inverse function of $P_y(\phi)$. Taking the equation for $P_y(\phi)$ shown in Equation 3.11, $h_{eq,predicted}$ is solved to yield:

$$h_{eq,predicted} = \frac{1}{\Delta \rho g \phi_g} \frac{k}{\left(1 - \left(\frac{1}{n}\right)^{\phi_0 \phi_g} + 1\right)} \left(\frac{\Delta \rho g h_0}{k} + \left(\frac{1}{\phi_g} - 1\right)\left(\frac{1}{n}\right) - 1\right)$$ \hspace{1cm} (3.13)

The experimental equilibrium height is then compared with the calculated value and the parameters $k$ and $n$ in Equation 3.11 are adjusted until the error between predictions and values from the experiments is minimised.

The batch settling technique is also used to estimate the hindered settling function of the suspension at low solids volume fraction up to the gel point ($\phi_0 < \phi < \phi_g$). The prediction method for settling rate at different solids concentrations using batch settling data was first proposed by Kynch (1952). The analysis was
later employed in a mathematical transform by Lester et al (2005) to determine \( R(\phi) \) for \( \phi_0 < \phi < \phi_g \) using \( h \) versus \( t \) data. It uses the key relation in Equation 3.2 to solve \( R(\phi) \).

A proprietary software package developed at the University of Melbourne (Batch Settling Analysis Method Software (BSAMS)) (Lester, 2002), is then used to transform raw settling data to dewaterability information. The inputs are the initial conditions \((\phi_0, h_0)\), material densities and \( h \) versus \( t \) data. The BSAMS program also takes the dewaterability data from pressure filtration and outputs functional forms of \( P_y(\phi) \) and \( R(\phi) \) that fits the data over an entire range of solids volume fractions from gravitational settling to pressure filtration.

### 3.3.3 Transient centrifuge settling

A full characterisation of permeability can be obtained by using the batch settling and pressure filtration techniques described above. However, a few studies have revealed that for some slurries, biological sludges in particular, this may not always be comprehensive (Studer, 2008; Wall, 2008).

It is often difficult to obtain batch settling data for biological sludges for a number of reasons. First, the wide particle size distribution means that the solid liquid interface is not always clear and, in some cases, multiple interfaces are observed. Second, the settling test can last more than 2 days due to a low permeability of the sludges. During this time, biological activity of the sludge causes the nature of the sludge to change. For example, in the preliminary investigation of this work, settled sludge was observed to float during extended batch settling tests, as the gas produced by cyanobacteria increased. When there is no batch settling data available, the analysis contained within the BSAMS software is unable to generate dewaterability properties over the entire range of solids volume fraction due to the lack of information at low solids volume fraction.
The studies above also showed that biological sludges have extremely low permeability (or high $R(\phi)$) at high solids volume fractions. This means that there exists a large gap in $R(\phi)$ at the intermediate solids volume fraction region, between batch settling and pressure filtration results. The interpolation methodology used within BSAMS to predict $R(\phi)$ is highly inaccurate due to a lack of data in this region. The interpolation is able to produce accurate results for non-biological materials in the same region because comprehensive settling data is able to overlap or approach the filtration data such that interpolation is either not required or is well defined.

The development of a transient centrifuge settling test allows the determination of $R(\phi)$ at low to intermediate solids volume fraction. This technique has become a useful alternative for dewaterability characterisation of sludges where traditional methods are not suitable.

In this test, a sample at $\phi_0$ is centrifuged at a constant rotational speed. As the solids start to settle under a centrifugal force, a solid-liquid interface forms and the height of the interface $h$ decreases over time. As before, $h$ is measured over time until an equilibrium height is reached. The traditional method requires manual measurement of $h(t)$ at a frequent interval, a new protocol developed by Studer (2008) allows automatic measurement of $h(t)$ in a LUMiFuge® Stability Analyser based on the difference in light transmission between supernatant and sediment.

Extraction of $R(\phi)$ values from transient centrifuge settling data is carried out via an algorithm developed by Usher et al (2012) and it involves the following steps:

1. A function to describe $P_y(\phi)$ is selected based on the $P_y(\phi)$ results at intermediate and high solids volume fractions as well as the equilibrium height obtained from transient centrifuge settling. The initial solids volume fraction, initial height, angular velocity of the centrifuge, centrifuge radius, and solids and liquid densities are used along with the functional form of
\( P_y(\phi) \) to predict the experimental equilibrium height. The parameters in \( P_y(\phi) \) function are then adjusted through iteration to the point where the predicted equilibrium height is within 0.1% of the experimental value.

2. Experimental \( h \) versus \( t \) data is filtered by 10% at the beginning and 1% at the end. This eliminates the start-up effects and errors associated with decreased sensitivity in the measurement of small sediment height towards equilibrium.

3. A set of initial \( R(\phi) \) values are estimated over the range of initial solids volume fraction \( \phi_0 \) and final solids volume fraction \( \phi_f \) from the truncated \( h \) versus \( t \) data, 20 solids volume fractions \( (\phi_i) \) are selected in equal increment between \( \phi_0 \) and \( \phi_f \). The equivalent height \( h_i \) for each \( \phi_i \) is calculated based on the mass balance,

\[
h_i = \frac{\phi_0 h_0}{\phi_i} \tag{3.14}
\]

\( R(\phi) \) is estimated using a method that is analogous to the gravity batch settling analysis by Kynch (1952). The analysis is modified to account for sedimentation under centrifugal force. The initial centrifuge settling rate \( \frac{dr}{dt} \) can be determined by modifying Equation 3.2 used in batch settling analysis:

\[
\frac{dr}{dt} = \frac{\Delta \rho \omega^2 r(1-\phi)^2}{R(\phi)} \tag{3.15}
\]

where

\[
r(t) = r_{\text{max}} - h(t) \tag{3.16}
\]

i.e. \( r \) is the radius measured from the solid-liquid interface to the centre of the centrifuge, \( r_{\text{max}} \) is the distance between the inner base of the centrifuge tube and the centre of the centrifuge and \( \omega \) is the angular velocity. Integrating Equation 3.15 with the initial condition \( r = r_0 \) at \( t = 0 \) gives:
\[ t = \frac{R(\phi)}{\omega^2 \Delta \rho (1 - \phi)^2} \ln\left( \frac{r}{r_0} \right) \]  

3.17

The \( R(\phi_i) \) value is estimated at each \( \phi_i \) such that the settling time \( t \) calculated from Equation 3.17 equals the experimental settling time for height \( h_i \). To estimate \( R(\phi) \) accurately, this requirement must be satisfied by all 20 solids volume fractions selected.

4. \( R(\phi) \) estimates are combined with \( R(\phi) \) at the high solids volume fraction determined from pressure filtration to generate a \( R(\phi) \) functional form.

5. \( P_y(\phi) \) and \( R(\phi) \) functional forms generated from step 1 and 4 are then used to predict the transient centrifuge settling data \( h \) versus \( t \).

6. \( R(\phi) \) estimates are refined by comparison between predicted and experimental settling data.

7. The \( R(\phi) \) estimates used in step 4 are now replaced by the new estimates and the analysis process continues. Steps 4 to 7 are repeated until the predicted settling profile matches well with the experimental values.

The complete analysis process outlined above is found to be computationally difficult and time consuming. However, the initial \( R(\phi) \) estimation described in step 3 alone has shown good agreement between experimental and predicted data (Usher et al., 2012). Therefore, characterisation of \( R(\phi) \) at low to intermediate solids volume fractions are carried out using Step 3 only in this work.
3.3.4 Multiple speed equilibrium sediment height technique

To improve the accuracy in generating a full compressive yield stress curve, compressibility information at intermediate solids volume fraction is beneficial. Two techniques are used commonly to characterise the compressive yield stress at intermediate solids volume fractions: the multiple speed equilibrium sediment height technique and the concentration profile technique.

The multiple speed method was first developed by Buscall and White (1987). A suspension is subjected to varying gravitational accelerations in a centrifuge by varying the rotational speed. The equilibrium solid-liquid interface height \( h_{eq} \) is measured for each gravitational acceleration, \( a \), experienced at the inner base of the centrifuge tube. \( a \) is calculated by the following equation:

\[
a = \omega^2 r_{max}
\]

It has been established by Buscall and White (1987) that for a flocculated suspension under compression, the compressive yield stress \( P_y(\phi) \) is a direct measure of the strength of inter-particle bonds or particle pressure \( P_s \), such that:

\[
P_y(z, t) = P_y[\phi(z, t)]
\]

where \( z \) is the distance measured from the inner base of the centrifuge tube.

At equilibrium, \( P_s(z, t) \) is independent of time \( t \), and therefore can be replaced by \( P_s(z) \). To determine \( P_s(0) \) and \( \phi(0) \) which are the particle pressure and solids volume fraction at the base of the centrifuge tube (i.e. \( z = 0 \)), an iterative or an approximate solution approach is used.

3.3.4.1 Iterative solution

The force balance equation relating the particle pressure \( P_s \) to gravitational acceleration \( a \), at equilibrium is:

\[
\frac{dP_s}{dz} = -\Delta \rho \phi a (1 - \frac{z}{r_{max}})
\]

and according to mass conservation:
\[ \int_{0}^{h_{eq}} \phi dz = \phi_0 h_0 \]

Manipulation of Equation 3.20 and Equation 3.21 allows \( P_s(0) \) and \( \phi(0) \) to be determined as:

\[
\phi(0) = \frac{\phi_0 h_0 (1 - \epsilon)}{(h_{eq} + a \frac{dh_{eq}}{da}(1 - h_{eq}) + \frac{h_{eq}^2}{2r_{max}})}
\]

\[
P_s(0) = \Delta \rho a \phi_0 h_0 (1 - \delta)
\]

where \( h_0 \) is the initial height of the suspension, \( \epsilon \) and \( \delta \) are correction terms derived from integral functions of the concentration and pressure profiles in the centrifuge tube, \( \epsilon^* \) and \( \delta^* \) (as follows), such that \( \epsilon = \epsilon^*(h_{eq}) \) and \( \delta = \delta^*(h_{eq}) \).

\[
\frac{d\epsilon^*}{dz} = \frac{\phi(Z(z) + (1 - \frac{h_{eq}}{r_{max}})a \frac{dh_{eq}}{da}}{\phi_0 h_0 r_{max} (1 - \frac{z}{r_{max}})^2}
\]

\[
\frac{d\delta^*}{dz} = \frac{P_s(z)}{\Delta \rho \phi_0 h_0 a r_{max} (1 - \frac{z}{r_{max}})^2}
\]

and \( Z(z) \) is defined as,

\[
Z(z) = (h_{eq} - z)(1 - \frac{h_{eq} + z}{2r_{max}})
\]

The iteration method begins with a curve fit for the raw \( h_{eq} \) versus \( a \) data followed by evaluation of its derivative, \( dh_{eq}/da \) at each value of \( a \). An initial estimate of \( P_s(0) \) is made for each value of \( a \) using the approximate solution described in the following part. The value is used to solve \( dP_s/dz \), \( d\epsilon^*/dz \) and \( d\delta^*/dz \) numerically. The correction terms \( \epsilon \) and \( \delta \) are then determined and substituted into Equation 3.22 and 3.23 to calculate \( \phi(0) \) and \( P_s(0) \). The initial estimate is compared with the calculated \( P_s(0) \) and adjusted for the next iteration. Iterations continue until the solution converges.
3.3.4.2 Approximate solution

By applying Buscall and White’s mean value theorem (1987) to the force balance Equation 3.20 and mass conservation Equation 3.21, an approximate solution can be obtained for \( \phi(0) \) and \( P_s(0) \):

\[
P_s(0) = \Delta \rho \phi \rho \left( \frac{h_{eq}}{2r_{max}} \right) \tag{3.27}
\]

\[
\phi(0) = \frac{\phi \rho h_b [1 - 2r_{max} (h_{eq} + a \frac{dh_{eq}}{da})]}{(h_{eq} + a \frac{dh_{eq}}{da})(1 - \frac{h_{eq}}{r_{max}}) + \frac{h_{eq}^2}{2r_{max}}} \tag{3.28}
\]

This method only requires a curve fit for the raw \( h_{eq} \) versus \( a \) data and evaluation of the derivative \( \frac{dh_{eq}}{da} \) at each \( a \). \( \phi(0) \) and \( P_s(0) \) can be then be determined directly from Equation 3.22 and 3.23. Examples of \( h_{eq} \) versus \( a \) profile and its derivative for a cyanobacteria-rich alum sludge were demonstrated in Figure 3.4 and Figure 3.5.

The error analysis on the approximate solution has revealed there is a good agreement between the approximate and iterative approaches. The error between the two is less than 3% (Green, 1997). Therefore, the approximate solution is a reliable method to determine the compressive yield stress of suspension at intermediate solids volume fractions in a simpler way, and it is the method used in this work.
The multiple speed experiments were originally developed for centrifuges of different size range (Green, 1997). They involved centrifuging a sample at multiple speeds and measuring the sediment height frequently at each speed until equilibrium was reached. One complete experiment often took more than a week. In this work, all the experiments were conducted in a LUMiFuge® Stability Analyser. The instrument allows rapid automated measurement of sediment height and most importantly, minimises manual handling and error encountered in the methods previously used. More details on the modified methods can be found in Section 4.8.5.
Another method to determine the compressive yield stress is through a concentration profile technique. This technique was developed by Green (1997), and there are a number of studies on the use of the technique based on equilibrium data (Buscall and McGowan, 1983; Auzerais et al., 1990; Bergstrom, 1992; Bergstrom et al., 1992; Burch, 2001; Falconer, 2001). The technique requires a sample to be centrifuged at a constant rotational speed until equilibrium is obtained. The gravitational acceleration $g$ varies with the distance from the centre of the centrifuge $y (y = r_{max} - z)$, such that

$$g = \omega^2 y$$  \hspace{1cm} 3.29
As the magnitude of the acceleration increases with \( y \), the particles at the bottom of the sediment bed experience a higher compression force than those at the top of the bed, resulting in a concentration profile across the sediment bed. The concentration profile can be determined by sectioning the sediment bed along a vertical axis into 5-6 slices with equal thickness and measuring the solids volume fraction of each slice.

The force balance Equation 3.20 can also be expressed in terms of \( y \),

\[
\frac{dP_s}{dy} = \Delta \rho \omega^2 y \phi(y) \tag{3.30}
\]

As discussed earlier, the compressive yield stress is equal to the particle pressure in the compression region (Equation 3.19).

Integrating Equation 3.30 gives the particle pressure at radial position \( y \),

\[
P_s(y) = \Delta \rho \omega^2 \int_0^y y' \phi(y') dy'
\tag{3.31}
\]

where \( y' \) is the distance from the centre of the centrifuge to the midpoint of the slice and \( \phi(y') \) is the concentration profile of the slices measured experimentally. \( P_s(y) \) can be solved computationally by using the trapezoidal rule. Alternatively, a function form of \( \phi(y') \) is generated to fit the experimental data and \( P_s(y) \) is then solved by numerical integration.

A potential difficulty with the use of algal materials using this technique is that there is a requirement that the equilibrium height at the end of the test is at least 10 mm thick. Sectioning a thin sediment bed into multiple slices of equal thickness is difficult and measurement of solids concentrations of these slices will introduce a large error. Both lab produced cyanobacteria alum sludges and harvested algae samples are at very low initial solids concentration. Preconcentration of the sample is therefore necessary before they can be used for this test. This sample preparation step is not only labour intensive but requires
a large quantity of raw materials (up to 30 L). Secondly, cyanobacteria alum sludge has a low compressibility. Even at the maximum speed achievable in the centrifuge, the equilibrium sediment bed contains a large amount of water. This makes the sectioning process extremely difficult. Collecting the slice from the centrifuge tube will either take part of the bottom slice or leave part of the top slice behind. For these reasons, the concentration profile technique is not used in this work.
CHAPTER 4 MATERIALS AND EXPERIMENTAL METHODS

This chapter provides details of the experimental methods used in the study. Section 4.1 introduces the cyanobacterial species being investigated. Section 4.2 describes the cell culturing techniques that were used to produce the majority of the cyanobacteria cultures. To characterise cells in suspensions or sludges and to examine the impact of water treatment processes on these cells, a range of analytical methods were employed including cell counting, metabolite concentration analysis and cell viability assay. They are described in Section 4.3 to 4.5.

Cyanobacteria-rich sludges were produced on a sufficient scale to provide sludge for dewatering characterisation. Jar testing described in Section 4.6 determined the optimum conditions for sludge production and Section 4.7 details the procedures of sludge production itself using these optimum conditions. Various dewatering characterisation techniques used in this study are explained in Section 4.8. Kinetic experiments of cell degradation and metabolite release during sludge production and dewatering processes are described in Section 4.9.

Methods for cross-flow membrane filtration are presented separately in Chapter 7.
4.1 Materials

Cyanobacteria species of most concern in Australia are *Microcystis aeruginosa*, *Anabaena circinalis* and *Cylindrospermopsis raciborskii* (Burch, 2001; Falconer, 2001). They have been identified in numerous rivers, reservoirs, water supply dams and lakes. There is a high record of occurrences of blooms associated with these species, mostly during drought seasons. *M. aeruginosa* produces variants of microcystins, including microcystin-LR and microcystin-LA. *A. circinalis* in Australia is the major producer of saxitoxin or paralytic shellfish poisons (PSP) as well as the taste and odour (T&O) compound, geosmin (Brookes *et al.*, 2001). *C. raciborskii* produces toxic cylindrospermopsin. The toxicity of each toxin produced from the three species has been discussed individually in Section 2.3.

This study has focuses on the three species, as their proliferation and the toxins and T&O compounds they produce have challenged not only water suppliers in Australia but worldwide. To provide a continuous supply of cell materials for the study, these species were cultured in the laboratory using artificial media. Three laboratory strains, *M. aeruginosa* (MIC309), *A. circinalis* (ANA188B) and *C. raciborskii* (CYP011k), shown in Figure 4.1 (a), (b) and (c) respectively, were supplied by the Australian Water Quality Centre, SA Water, South Australia. They were cultured to produce large quantity of cells for a range of experiments. The cell culturing procedures are detailed in Section 4.2.
Figure 4.1: Microscope imagine of laboratory strains of (a) *M. aeruginosa*, (b) *A. circinalis* and (c) *C. raciborskii*. 
4.2 Cell culturing

Cell cultures were grown in ASM-1 medium (Gorham et al., 1964) at 23°C under continuous illumination. The supplied culture strains adapted to a different level of light intensity, hence, they were each placed at the most suitable light condition in order to maintain their health and more importantly, a regular life cycle pattern. The optimum conditions were determined based on the cell count of each culture over a 35 day period. They were found to be 40, 15 and 10 mM photons m\(^{-2}\)s\(^{-1}\) for MIC309, ANA188B and CYP011K, respectively. Life cycles of the three strains at their optimum condition are shown in Figure 4.2. Cultures were harvested at the late exponential phase of life cycle. This corresponded to 15 – 22 days after inoculation. The harvested cultures were then scaled up at an inoculum to medium ratio of 1:9 (by volume). Oxygen in the form of air was supplied continuously to the cell cultures once the culture volume reached 500 mL.

ASM-1 medium was made by adding a set amount of nutrient stock solutions to Milli-Q water (water that is deionised and biologically inactive by passing town water through a 0.22 µm membrane). All stock solutions were prepared in Milli-Q water. The medium was then sterilised in a HS-60 vertical type steam autoclave (Hanshin Medical) and stored in the refrigerator at 4 °C. Glassware was sterilised in the autoclave before it was filled with various cultures. All the culturing work was performed in an AURA Mini laminar flow cabinet (Bio-Cabinet) to eliminate air-borne contamination.

To mimic the cell densities that have been recorded in natural blooms, the harvested culture was mixed with ASM-1 medium to make up cyanobacterial suspensions with cell densities between 300,000 and 1,000,000 cells mL\(^{-1}\).
4.3 Cell counting

Cell density, i.e. the population of cells in a cyanobacterial suspension, was determined by counting the number of cells present in a fixed volume of suspension under a compound microscope (Olympus BX51). A 1 mL sample of the suspension was first treated with one drop of *Lugol's iodine* solution (Fluka). Addition of *Lugol's iodine* solution helped to fix the cells and to make them settle. The sample was sonicated in a Transsonic TS540 sonication bath (Elma®) for approximately 10 seconds. Sonication eliminated cell buoyancy by reducing gas vesicles contained within the cells. The process also broke up filaments of *A. aeruginosa* into fragments of 1-4 cells for even dispersion and accurate counting.
Figure 4.3: Sedgewick-Rafter counting chamber.

The treated sample was transferred to a Sedgewick-Rafter counting chamber and allowed to fully settle for 10 minutes. To prevent formation of air bubbles due to sample evaporation, counting slides were placed in a humidity chamber. A humidity chamber was a sealed Petri dish filled with wetted tissues. After cells were settled onto the bottom plate of the counting chamber, the number of cells was counted under the microscope at 100x magnification with bright field illumination.

The Sedgewick-Rafter chamber, shown in Figure 4.3, has a central cell that holds 1 mL of sample with a dimension of 50 x 20 mm and 1 mm in depth. The base is ruled in a 1 mm² grid pattern. The number of cells contained in one square was counted. To achieve high precision, counting was conducted for at least 20 randomly selected squares. The cell density, \( C \), expressed as the number of cells per mL can be calculated using:

\[
C = N \times 1000 \quad 4.1
\]

\( N \) is the average number of cells in one square based on the total number of cells presented in the 20 squares being counted.
Counting became difficult when the cell population in one square exceeded 150. As such, dilution was sometimes performed by adding an appropriate amount of Milli-Q water to the sample.

**Counting C. raciborskii**

Filaments of *C. raciborskii* could not be broken into small fragments easily by sonication. As a result, there was often an uneven distribution of cells on the grids. Moreover, the cell walls in the filaments were not always distinguishable. To ensure accurate counting, a different method was used to count the *C. raciborskii* population, based on the relationship between the length of an individual filament and number of cells per filament:

**Step 1** The length of a clearly defined cell in a filament, as well as the length of the filament, were measured using DP2-BSW imaging software (Olympus). The number of cells in the filament, \( n^* \), can be estimated as:

\[
n^* = \frac{l}{d}
\]

where \( l \) is the length of the filament and \( d \) is the length of a single cell.

**Step 2** An average length of one filament, \( l_{ave} \), and the number of cells in the filament, \( n_{ave} \), were estimated by counting the total number of cells in 30 randomly selected filaments.

**Step 3** A continuous area was selected to count 100 filaments, the number of squares these filaments occupy, \( N^* \), were recorded.

The cell density of *C. raciborskii* was then determined using:

\[
C = \left( \frac{100 \times n_{ave}}{N^*} \right) \times 1000
\]
4.4 Cell viability assay

Cell viability assay assesses the integrity of cell membranes and detects metabolically active cells using fluorescence microscopy with the aid of two fluorochromes: fluorescein diacetate (FDA) is used to identify active cells and Sytox® Green (SG) is used to identify compromised or dead cells. A compound microscope (Olympus BX51) with a fluorescence attachment was used; the filter cube of the fluorescence unit is comprised of a 450 nm exciter filter, 500 nm diachronic beam splitter and a 515 nm barrier filter. A minimum of 100 cells were counted to achieve a precision greater than 10%. The sample preparation for FDA and SG staining is as follows.

4.4.1 FDA

FDA penetrates cell walls and is cleaved by intracellular esterases in metabolically active cells to yield green fluorescein that can be detected when excited by blue light (450 – 490 nm). Cells stained green indicates the presence of active esterase in the cells, and hence were considered metabolically active. Unstained cells were either dead or inactive.

A FDA (Sigma) stock solution was prepared at 10 mg\textbullet mL\textsuperscript{-1} in acetone and stored in freezer at -20 °C. To make a working solution, a stock solution was mixed with Milli-Q water in a volume ratio of 1 to 250 (stock to water). The working solution was prepared just before staining and microscopic examination, and it was preserved on ice to slow down degradation. 300 µL of the working solution was added to 400 µL of cell sample and mixed well before the sample was transferred to a Sedgewick-Rafter counting chamber. A 7 minute incubation period was required for the uptake of the stain. Cells were examined within 10 minutes as FDA was observed to damage A. circinalis cells over longer exposure.
4.4.2 Sytox® Green (SG)

SG penetrates compromised cell membranes characteristic of dead cells. It binds nucleic acids and exhibits bright green fluorescence when excited by blue light (450 – 490 nm). SG will not cross through intact membranes, therefore, cells that are not stained were considered alive and to have intact membranes.

The original stock solution (Invitrogen) was at a concentration of 5 mM. It was diluted subsequently in dimethyl sulfoxide to produce a working solution at 0.02 mM. The working solution was stored at 4 °C. A 20 µL working solution was added to 1 mL cell sample and mixed well before the sample was transferred to a Sedgewick-Rafter counting chamber. Each sample was incubated for 10 minutes before examination under the microscope.

4.5 Cyanobacterial metabolite analysis

Compared to the cell viability assay, metabolite analysis gives a direct indication of the sample quality in terms of its metabolite content. This was carried out in four stages: Sample collection, pretreatment, metabolite extraction and measurement. All the samples were collected and pretreated in the University of Melbourne laboratory while metabolite extraction and measurement were performed by the Australian Water Quality Centre. To minimise metabolite degradation during sample storage and transport, pretreatment was required immediately after samples were collected. The procedures for the analysis of each metabolite are detailed below.

4.5.1 Intracellular and extracellular metabolites

For samples containing cells, the metabolite concentration is made up of two components: intracellular and extracellular. Extracellular metabolite can be extracted and measured after cells were removed from a solution. To determine the concentration of intracellular metabolites, all the cells in the sample must be
lysed. The intracellular concentration was taken as the difference between the total extracellular concentration before and after cell lysis.

4.5.2 Microcystins

To measure the extracellular microcystins concentration, each sample was filtered with a GF/C filter (Whatman) followed by a 0.45 µm pore size syringe filter (Acrodisc®, Pall). This was done immediately after sample collection. It ensured that cells and other particles were removed from the sample and no further release of microcystins could contribute to the results.

Samples for total microcystins concentration underwent two cycles of microwave radiation (45 seconds each cycle) in a microwave oven (LG 2000 800 watt). This step lysed all the cells in the sample and resulted in the release of all the intracellular metabolites (Ho et al., 2009). The sample was then filtered with a GF/C filter and a 0.45 µm pore syringe filter.

All treated samples were placed in 270 mL PPE bottles that were pre-washed and rinsed with deionised water and stored at 4 °C. Samples were delivered overnight to the Australian Water Quality Centre on an ice pack in an insulated box.

Microcystins was extracted from each water sample using methods adapted from Nicholson et al (1994). The concentrations of microcystins were then determined by a high performance liquid chromatography (HPLC) system (1100 series, Agilent Technologies). The analytical methods were detailed in Ho et al (2006).
4.5.3 Saxitoxin

The same sample preparation methods were used for saxitoxin samples. A 2 mL sample was stored in a 10 mL centrifuge tube at 4 °C before transportation. Samples were then analysed using an enzyme linked immuno-sorbent assay (ELISA) (Abraxis, USA). Prior to the analysis, the samples were filtered with a 0.22 µm pore syringe filter. Each sample was then diluted in a solution provided by the supplier to the working range of the assay (0.02 – 0.40 µg L⁻¹). The concentrations of saxitoxins were determined by interpolation using a standard curve constructed for each run. The results were expressed as saxitoxin toxicity equivalents, STX-eq.

4.5.4 Geosmin

Due to the highly volatile nature of geosmin, extracellular geosmin samples were bottled as soon as they were filtered. To further prevent the biodegradation of geosmin, mercuric chloride (HgCl₂) was also added to the filtered sample. The final concentration of HgCl₂ in the sample was 4 mg L⁻¹.

To prepare samples for total geosmin measurement, the sample was dosed with HgCl₂. HgCl₂ not only lysed the cells but also stabilised geosmin. Boiling of the samples was avoided as the majority of geosmin in the sample would be lost through volatilisation.

Geosmin samples were stored in prewashed 60 mL amber glass bottles. Each bottle was filled with sample, leaving no headspace in the bottle. This was to keep geosmin loss to a minimum. All samples were stored at 4 °C.
Geosmin was extracted using a solid phase micro-extraction syringe fibre (Supelco, Australia) and analysed using a 7890 Gas Chromatograph System with 5975C VL Series Mass Selective Detector (Agilent Technologies) against internal standards (Ultrafine Chemicals, UK). The methods are detailed elsewhere (Graham and Hayes, 1998).

4.5.5 Cylindrospermopsin

Samples for cylindrospermopsin analysis were prepared using the same procedures as for microcystins analysis. All treated samples were placed in 270 mL PPE bottles and stored at 4 °C.

Cylindrospermopsin was extracted from the samples using solid phase extraction methods adapted from Wormer et al (2009). The concentration of cylindrospermopsin was analysed by a HPLC system using methods documented by Ho et al (2011).

4.6 Jar test

Jar tests were performed to establish the optimal conditions for sludge production with cell rich waters. The jar testing rig was a six beaker gang stirrer, with six-blade Rushton impeller. Each 2 L beaker is fitted with baffles. The beaker was first filled with water to 1.6 L. This set up was based on the ‘standard tank configuration’ illustrated by Holland and Chapman (1966) (Figure 4.4). Specifications of the configuration and jar testing setup are provided in Table 4.1.

Coagulant, Alum, prepared at 20 g L⁻¹ as Al₂(SO₄)₃·18H₂O, was added to each sample in different volumes (between 4 and 12 mL) while the sample was stirred at 330 rpm (velocity gradient, G = 278 s⁻¹). After alum addition, sodium hydroxide (NaOH) solution at 0.2 M was added to the suspension to correct the pH to between 6 and 7. Two minutes after chemical addition, the mixing speed was
reduced to 50 rpm \((G = 12 \text{ s}^{-1})\). Slow mixing was continued for 15 minutes. The samples were left to settle for 1 hour. The supernatant of each sample was collected after 1 hour for cell counting.

### 4.7 Sludge production

Experience in jar testing with 1.6 L cyanobacteria suspensions at cell densities typical of blooms suggested that large volumes of cyanobacteria suspensions were required to generate a sufficient amount of sludge for complete dewatering characterisation. A 60 L plastic cylindrical-shaped tank was used to perform these large-scale coagulation experiments. Similar to the set up in jar testing, the tank, pictured in Figure 4.5, was fitted with baffles and a Rushton turbine impeller in accordance with the standard tank configuration. All the dimensions are provided in Table 4.2.

![Diagram of tank configuration](image)

**Figure 4.4: Standard tank configuration based on Holland and Chapman (1966).**

- **\(D_a\)** - Impeller diameter
- **\(D_T\)** - Tank diameter
- **\(H_a\)** - Impeller height from bottom of tank
- **\(H_l\)** - Liquid level
- **\(q\)** - Impeller blade width (axial dimension)
- **\(r^*\)** - Impeller blade length (radial dimension)
- **\(s\)** - Length of impeller blade mounted on the central disk
- **\(W_B\)** - Baffle width
Table 4.1: Specifications of standard tank configuration and Jar test dimensions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Jar Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel Type</td>
<td>2 L beaker</td>
</tr>
<tr>
<td>Vessel diameter, $D_T$</td>
<td>127 mm</td>
</tr>
<tr>
<td>Liquid level, $H_I = D_T$</td>
<td>127 mm</td>
</tr>
<tr>
<td>Number of baffles, $N_B$</td>
<td>4</td>
</tr>
<tr>
<td>Baffle width, $W_B = D_T/10$</td>
<td>12.5 mm</td>
</tr>
<tr>
<td>Agitator type</td>
<td>Rushton impeller</td>
</tr>
<tr>
<td>Impeller diameter, $D_a = D_T/3$</td>
<td>42.3 mm</td>
</tr>
<tr>
<td>Impeller height from bottom of the vessel, $H_a = D_a$</td>
<td>42.3 mm</td>
</tr>
<tr>
<td>Impeller blade width, $q = D_a/5$</td>
<td>8.7 mm</td>
</tr>
<tr>
<td>Impeller blade length, $r^* = D_a/4$</td>
<td>10.1 mm</td>
</tr>
<tr>
<td>Length of impeller blade mounted on the central disk, $s = r^*/2 = D_a/8$</td>
<td>5.5 mm</td>
</tr>
</tbody>
</table>

The tank was filled to 48.4 L with cell suspension and left overnight at 25 °C under continuous illumination. Meanwhile, a cell-free sample containing only ASM-1 media was kept aside as a control.

To scale the coagulation process, the velocity gradient was kept constant with mixing time unchanged. Each water sample was mixed rapidly at 143 rpm ($G = 278$ s$^{-1}$) while being dosed with alum stock solution ($100$ g L$^{-1}$ as $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$) at a predetermined optimum dose. The pH of the suspension was corrected immediately to fall between 6 and 7 using $1\text{M NaOH}$ solution. The 2 minute rapid mixing was followed by 15 minutes of slow mixing at 19 rpm ($G = 12$ s$^{-1}$). The suspension was then allowed to settle for 4 hours before the supernatant was removed. The thin sediment at the bottom of the tank was transferred to a smaller container to settle overnight. This process usually generated between 500 mL and 1 L sludge. This was sufficient for full dewaterability characterisation of the material. To verify reproducibility, the process was repeated at least once.
Table 4.2: Dimension of mixing tank for large scale sludge production.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Jar Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel Type</td>
<td>60 L flat bottom, cylindrical tank</td>
</tr>
<tr>
<td>Vessel diameter, $D_T$</td>
<td>395 mm</td>
</tr>
<tr>
<td>Liquid level, $H_l = D_T$</td>
<td>395 mm</td>
</tr>
<tr>
<td>Number of baffles, $N_B$</td>
<td>4</td>
</tr>
<tr>
<td>Baffle width, $W_B = D_T/10$</td>
<td>42 mm, Rushton impeller</td>
</tr>
<tr>
<td>Agitator type</td>
<td>impeller</td>
</tr>
<tr>
<td>Impeller diameter, $D_a = D_T/3$</td>
<td>135 mm</td>
</tr>
<tr>
<td>Impeller height from bottom</td>
<td>135 mm</td>
</tr>
<tr>
<td>of the vessel, $H_a = D_a$</td>
<td></td>
</tr>
<tr>
<td>Impeller blade width, $q = D_a/5$</td>
<td>27 mm</td>
</tr>
<tr>
<td>Impeller blade length, $r^* = D_a/4$</td>
<td>33 mm</td>
</tr>
<tr>
<td>Length of impeller blade mounted</td>
<td>15 mm</td>
</tr>
<tr>
<td>on the central disk, $s = r^*/2 = D_a/8$</td>
<td></td>
</tr>
</tbody>
</table>
4.8 Dewatering characterisation techniques

A range of dewatering characterisation techniques were used in this work to fully characterise the dewatering behaviour of sludges containing cyanobacteria and algae species being investigated. This section describes the experimental methods for these techniques. As biological sludges can vary from sample to sample, it is important to first determine solid and liquid properties of each sludge accurately (i.e. dissolved and suspended solids concentrations, sludge liquid and solids densities) before dewaterability analysis and comparison can be made among them as well as with other materials.

4.8.1 Solids concentration measurement

4.8.1.1 Dissolved solids concentration of suspension

The sludge suspension was centrifuged in a bench-top centrifuge (Jouan CT422) at 3000 rpm for 5 minutes. The supernatant obtained from centrifugation was filtered through a 0.45 µm pore size syringe filter. The filtrate was then transferred into a pre-weighed sample container. The container was weighed again before being placed in the oven to dry at 60 °C. The sample was kept in the oven for two days. It was then removed from the oven and cooled to room temperature in a desiccator. The combined weight of dried sample and container was measured. This process was performed in duplicate. The mass fraction of dissolved solids, \( A \), was calculated using:

\[
A = \frac{m_{\text{liq,dry}} - m_{\text{empty}}}{m_{\text{liq,wet}} - m_{\text{empty}}}
\]

where \( m_{\text{liq,dry}} \) is the combined mass of dried filtrate and container, \( m_{\text{liq,wet}} \) is the combined mass of filtrate and container and \( m_{\text{empty}} \) is the mass of the empty container.
4.8.1.2 Total solids concentration of suspension

The suspension was poured into a pre-weighed container. After the combined weight of sample and container was measured, the container was dried in the oven at 60 °C for two days. The combined weight of the dried sample and container was measured after the container was cooled to room temperature in a desiccator. The process was performed in duplicate. Equation 4.5 was used to calculate the weight fraction of total solids, including dissolved solids, B.

\[ B = \frac{m_{\text{dry}} - m_{\text{empty}}}{m_{\text{wet}} - m_{\text{empty}}} \]  

where \( m_{\text{dry}} \) is the combined mass of dried suspension and container, \( m_{\text{wet}} \) is the combined mass of suspension and container.

The mass fraction of total solids in a suspension, \( x \), corrected for dissolved solids, now becomes:

\[ x = \frac{B - A}{1 - A} \]  

Using Equation 4.7, this can be converted to solids volume fraction, \( \phi \), if the solids density, \( \rho_{\text{sol}} \), and liquid density, \( \rho_{\text{liq}} \), of the material are known.

\[ \phi = \frac{1}{1 + \left( \frac{\rho_{\text{sol}}}{\rho_{\text{liq}}} \right) \left( \frac{1 - B}{B - A} \right)} \]  

4.8.2 Density measurement

Dewaterability analysis in this work uses solids volume fraction, \( \phi \), to express the solids concentration of a suspension. The following procedures were used to determine the solids and liquid densities of sludges so that volume fraction can be obtained accurately from Equation 4.7.
4.8.2.1 Density bottle calibration
The density of a suspension was measured using a 10 mL density bottle. The volume of the density bottle was calibrated prior to any density measurements. The dried, empty density bottle was weighed and filled with deionised water at room temperature. Air bubbles were removed by immersing the bottle in a sonication bath for 10 seconds. The bottle was refilled with water if necessary. The combined weight was measured to determine the mass of water in the bottle. Since density of water at room temperature is known, the volume of the bottle can be calculated.

4.8.2.2 Liquid density of sludge
Sludge suspension at room temperature was concentrated in a bench-top centrifuge (Jouan CT422) at 3000 rpm for 5 minutes. The supernatant obtained from centrifugation was filtered through a 0.45 µm pore syringe filter. Similar to the calibration step above, the density bottle was filled with filtered liquid and weighed. The liquid density was calculated from the known volume of the density bottle at room temperature and the mass of liquid that filled the bottle.

4.8.2.3 Solids density of sludge
The nature of sludges can change significantly from one sample to the other. The solids density of sludge therefore varies. The solids density for each sludge sample was determined through the measurement of suspension densities at different solids concentrations. The density bottle was filled with a sludge sample with known solids mass fraction (methods detailed in Section 4.8.1). Air bubbles or excess sample were removed from the bottle. The mass of the sample and its density can then be determined by weighing the combined mass. This density measurement was repeated on two more sludge samples at increasing concentrations. The higher solids concentration leads to a more accurate calculation of $\rho_{sol}$. Sludge samples at high concentrations were prepared by centrifuging the raw or settled sludge.
The density of a suspension can also be determined theoretically using Equation 4.8 if $\phi$ of the solids is known, giving:

$$\rho_{\text{susp}} = \rho_{\text{sol}}\phi + \rho_{\text{liq}}(1 - \phi)$$

If a value was assigned to $\rho_{\text{sol}}$, $\phi$ can be calculated using Equation 4.7. Substituting $\phi$ and the same $\rho_{\text{sol}}$ value into Equation 4.8 gave a theoretical $\rho_{\text{susp}}$ value (based on the assumption of $\rho_{\text{sol}}$ and experimental measurement of solids concentration). An accurate $\rho_{\text{sol}}$ can be found such that the difference between the theoretical and measured $\rho_{\text{susp}}$ was at minimum for all three suspensions tested.

### 4.8.3 Gravitational batch settling

Gravitational batch settling tests help to characterise the dewatering behaviour of sludge at low solids volume fraction, below the gel point, $\phi_g$ of the material. The tests were conducted in a 100 mL cylinder placed alongside a ruler with 1 mm graduations in order to accurately measure the height of the interface. Sludge with known solids volume fraction was poured into the container and the initial height of the sample was measured. As the solids in the sample started to settle, the height of solid-liquid interface was recorded over time. In the initial settling stage, the interface height was measured at short intervals (e.g. 30 seconds) as sample settled relatively fast. As the settling rate decreased, the measurements were taken less frequently. Data was recorded continuously until the interface height no longer changed with time. Section 3.3.2 details the analytical methods used to translate the settling data to the hindered settling function as a function of solids volume fraction.
4.8.4 Transient centrifuge settling

Transient centrifuge settling tests allowed characterisation of hindered settling function of sludge at low to intermediate solids volume fraction. This technique was useful in bridging the gap of dewaterability information between low and high solids volume fractions. More importantly, it provided a means to obtain dewaterability data when gravity batch settling data is not available, which is common for biological sludges. This technique was developed by Studer (2008). The theory has been discussed in Section 3.3.3.

The tests were carried out in a LUMiFuge® LF-100 Stability Analyser (pictured in Figure 4.6). This is a temperature controlled bench-top centrifuge fitted with a LED light source and a CCD sensor. The light source located above the rotor plate transmits light through sample cell that sits on the rotor plate. Meanwhile, the sensor located below the plate monitors light transmission through the cell in the radial axis. A schematic diagram of the operation of LUMiFuge® is shown in Figure 4.7. As there is a distinct difference in light transmission between the supernatant and the sediment, there exists a sharp drop-off of transmission at the solid-liquid interface when the sample starts to settle under centrifugal force. The LUMiFuge® allows the online capture of this unique profile for up to 8 samples simultaneously.
Figure 4.6: LUMiFuge® LF-100 Stability Analyser.

Figure 4.7: Schematic of LUMiFuge and its measurement principles (LUM-GmbH, 2005).
Sludges for transient centrifuge settling tests were often settled overnight under gravity and had the supernatant removed before they were loaded into LUMiFuge®. This pre-concentration was necessary to ensure that the final height of the sediment after centrifugation was sufficient for analysis. A small sediment height (less than 2 mm) will lead to a large error in subsequent data analysis. The sample was pipetted into a 10 mm square, flat-bottomed cuvette and centrifuged at a constant rotational speed at 20 °C. A suitable rotational speed was selected for each sample to ensure that settling of the sample occurred in a reasonable time frame allowing the instrument to record more gradual changes in the interface height over time. It also ensured that the equilibrium height was at least 2 mm. A sub-sample was taken for the initial solids volume fraction measurement. Transmission profiles over time were recorded on the SEPVView 5.1 software, at various intervals. Preliminary experiments were performed to determine the length and the sequence of the intervals in order to capture the complete settling behaviour of the sample during later experiments. The maximum number of profiles the program can capture is 255, multiple time intervals were employed in each test. Short time intervals (2 s) were used at the initial fast settling stage and long intervals (up to 300 s) towards the equilibrium stage at the end of the experiment. An example of the transmission profiles collected in one test is shown in Figure 4.8. Light transmission is plotted against the radial position from centre of the centrifuge.
Figure 4.8: Transmission profiles of a transient centrifuge settling test at 1000 rpm for an alum sludge containing *A. circinalis* cells.

To analyse the profiles and obtain interface height data in centrifuge settling, two initial parameters are required. They are the range and threshold, marked by the vertical and horizontal blue lines in Figure 4.8. Range defines the range of data to be analysed. The left boundary was placed at the meniscus level, indicated by a sharp downward spike on the profile due to the curvature of the air-liquid interface. The right boundary is placed at any point after all the transmission profiles are included. The threshold is the transmission value that indicates the solid-liquid interface position along the sample cell and was selected approximately midway between the transmissions immediately above and below the interface.

By defining these two parameters on the transmission profiles recorded over time, transient interface position data can be obtained. As illustrated in Figure 4.9, interface height, $h$, is determined from radial position of the interface, $r$, using Equation 4.9. $R_{max}$ is the maximum detection radius (130.98 mm) and $h_{base}$ is the distance from the internal base of the cell to the radius of the centrifuge (6.70 mm for 10 mm square cuvette cell with cell holder).
Determination of the hindered settling function from the interface height versus time data was carried out using Centrifugation Analysis Software – \( R(\phi) \). This program was developed by Usher (2010b) and all the calculations were derived from the theory detailed in Section 3.3.3.

\[
h = R_{\text{max}} - r - h_{\text{base}}
\]
4.8.5 Multiple speed equilibrium sediment height technique

The compressive yield stress function of sludge at low to intermediate solids volume fraction was obtained from centrifuge experiments using the LUMiFuge®. Eight rotational speeds were selected between 400 and 3200 rpm. The sample, at known solids volume fraction, was centrifuged at each rotational speed in the LUMiFuge® at 20 °C. The transmission profiles obtained from these tests gave an indication of the minimum time required for the sample to reach equilibrium height at each speed. The sample was centrifuged at these successive speeds, from the lowest to the highest, without any manual input. The SEPVView 5.1 software is limited to capture maximum of 255 transmission profiles and these were divided among the eight selected speeds. For each speed, a time interval was selected such that the total centrifuge time at that speed exceeded the minimum settling time obtained from these preliminary runs. Once the centrifuge cycle at the first speed was completed and equilibrium sediment height was reached, the centrifuge run was programmed to step up to the next speed until a new equilibrium height was established. The test continued until the last speed had been run. A set of transmission profiles for a typical stepped centrifuge test is shown in Figure 4.10. The equilibrium interface height for each speed was obtained using the same method described in Section 4.8.4. Calculation of compressive yield stress was carried out in Centrifugation Analysis Software – $P_y(\phi)$. The software was developed by Usher (2010a) using the theory outlined in Section 3.3.4.
4.8.6 Pressure filtration

Pressure filtration tests generate dewaterability data of material at higher solids volume fractions than centrifugation. All the tests were conducted in an automated piston driven filtration rig over a pressure range of 10 - 300 kPa. A schematic diagram of the rig is shown in Figure 4.11. During the filtration test, a pneumatic cylinder applies pressure to a sample contained in a stainless steel cylindrical cell, causing expulsion of liquid through a membrane (Millipore DVPP 0.65 µm) at the base of the cell that is supported by a permeable sintered disc. The applied pressure is monitored by a pressure transducer mounted on the piston face and is controlled by a feedback pressure controller. The filtration rate is monitored through the rate of downward movement of the piston using a linear encoder.
As discussed in Section 3.3.1, pressure filtration tests reveal the filtration behaviour of a material, either traditional (long cake formation followed by short cake compression) or non-traditional (short cake formation and long cake compression). If a material behaves traditionally, stepped pressure filtration tests are carried out. The test involves a series of constant pressures being applied to the sample progressively in an increasing order during one single run. The methods and dewaterability calculations are detailed in the work by de Kretser et al. (2001). Otherwise, several constant pressure filtration runs (20, 50, 100, 300 kPa) are performed for material exhibiting short cake compression and long cake formation filtration behaviour. The methods for calculating $P_y(\phi)$ and $R(\phi)$ for this type of material are summarised in Section 3.3.1.
4.9 Kinetic experiment of cell degradation and metabolite release

This experiment was conducted to determine the impact of conventional water treatment and waste handling processes on cell integrity and metabolite release for cyanobacteria-rich waters and sludges. The experiment was divided into two stages: sludge production and sludge dewatering.

Sludge production followed the procedures described in Section 4.7. Before the coagulation run, a sample was taken from the untreated suspension to analyse the initial concentrations of extracellular and intracellular metabolite as well as cell viability. During the coagulation run, samples were collected at different stages for extracellular or dissolved metabolite concentration and cell viability analyses. They were taken before alum addition, after alum addition, after NaOH addition and at the end of slow mixing respectively. For each chemical addition, the pH of the suspension was allowed to stabilise before sampling. During the sedimentation process, samples were collected at a 30 minute interval for dissolved metabolite concentration and cell viability analyses. After 4 hours of settling, supernatant was removed from the tank and the sediment was carefully transferred into a 5 L beaker. The sludge was allowed to settle overnight. The extra supernatant was removed and collected for dissolved metabolite concentration measurement. The remaining sludge formed the feed material of the sludge dewatering stage.

A small sample of raw sludge was taken for extracellular and intracellular metabolite measurement and cell viability assay. The sludge then underwent centrifugation in the bench-top centrifuge (Jouan CT422) at 3000 rpm for 5 minutes. Supernatant formed at the end of the centrifugation was collected for dissolved metabolite concentration analysis. Cell viability of the settled sludge was examined immediately.
The settled sludge was then mixed with the original sludge and filtered at pressures between 20 and 500 kPa in the piston driven pressure filtration rig (Section 4.8.6). Filtrate from each run was collected for dissolved metabolite concentration measurement. A small sample of the cake formed during filtration was taken and re-suspended in Milli-Q water on a vortex mixer for cell viability assay. Some samples, obtained from sludge in particular, were filtered and diluted in Milli-Q water to make up sufficient sample volume for metabolite concentration analysis.
CHAPTER 5  FATE OF CYANOBACTERIA IN CONVENTIONAL WATER TREATMENT PROCESSES

5.1 Introduction

The literature review (Chapter 2) proposed that the highest priority for treating cyanobacteria and their metabolites was to remove the cells without damage. The coagulation step in the conventional water treatment processes effectively aggregates cyanobacterial cells in water to form flocs for subsequent disposal. The most common coagulants, aluminium sulphate and ferric chloride, have been shown to cause no damage to cyanobacterial species *M. aeruginosa* and *A. circinalis* during coagulation (Velzeboer *et al.*, 1995; Chow *et al.*, 1998; Chow *et al.*, 1999). However, there has rarely been any systematic study in literature, on the impact of cyanobacteria across the entire water treatment process, particularly on the fate of those ending up in the sludge stream. Compromising the cell integrity during sludge dewatering processes can potentially lead to the release of intracellular metabolites into the recovered water. Returning this contaminated water to the head of the plant will pose a threat to the entire plant since processes such as coagulation, sedimentation and filtration have been ineffective in removing extracellular metabolites (Chow *et al.*, 1999). During periods of water shortage, water authorities cannot afford to discard the large amount of water recovered from sludge dewatering. Therefore, it is the aim of this work to determine the impact of conventional water treatment and specifically sludge dewatering processes on cyanobacteria and their metabolite release and to provide the best approaches to water treatment and sludge management targeting cyanobacteria.
5.2 Cyanobacteria in conventional WTP - Approach I

To examine the impact of sludge dewatering processes such as sedimentation, centrifugation and pressure filtration on the cyanobacteria species investigated in this study, a large volume of sludge was produced each time following the procedures in Section 4.7. As illustrated in Figure 5.1, flocs generated in the coagulation step settled to the bottom of the tank over time and formed a cyanobacteria-laden sludge. This sludge was allowed to settle overnight before it was dewatered further by centrifugation and pressure filtration. Samples were taken at each sampling point indicated on the diagram for total and extracellular metabolite analysis. A mass balance of metabolites was performed across the sludge production or cell removal process. The mass balance not only gives an indication of the level of accuracy achieved in the experiments but most importantly, demonstrates where the extracellular and intracellular metabolites were located throughout the process so that any factors that have an impact on the cells can be correctly identified.

Figure 5.1: Water treatment processes investigated in the study.
5.2.1 *M. aeruginosa*

Two coagulation experiments were conducted on *M. aeruginosa* rich waters at different cell densities. The alum doses used and the resultant percentage of cell removal were presented in Table 5.1. The percentage of cell removal is calculated using the following equation,

\[
\% \text{removal} = 1 - \frac{C_{\text{sup}}}{C_0}
\]  

where \( C_{\text{sup}} \) is the cell density in the supernatant that was collected after the 4 hour sedimentation process and \( C_0 \) is the initial cell density in the raw water.

In Experiment 1, *M. aeruginosa* rich water at 419,000 cells mL\(^{-1}\) was deliberately under-dosed with alum, resulting in incomplete removal of cells. A large portion of cells containing intracellular metabolites remained dispersed in the supernatant at the end of sedimentation and were removed with the supernatant. The under-dosed case aims to test the sensitivity and accuracy of metabolite measurement, which will be reflected on the mass balance of the system.

The initial concentrations of total and extracellular microcystin-LR (MC-LR) in raw water and the concentration of extracellular MC-LR immediately after coagulation process (i.e. at the end of the slow mixing step) are also presented in Table 5.1. In Experiment 1, there was no increase in the concentration of extracellular MC-LR over the coagulation process. In Experiment 2, 45% of MC-LR was extracellular prior to coagulation, indicating that the cells had aged. Nevertheless, only a small amount of increase in extracellular MC-LR was detected after the process. This suggests that regardless of the cell age, coagulation causes minimal damage to the cells. This is consistent with the findings by Chow *et al* (1999). In their study, coagulation experiments were conducted on *M. aeruginosa* rich waters at optimum alum dose as well as at half the optimum dose. It was shown that neither condition resulted in cell damage whereas copper sulphate at the equivalent concentrations has caused massive release of intracellular MC-LR.
Table 5.1: Impact of coagulation on *M. aeruginosa* and MC-LR release.

<table>
<thead>
<tr>
<th>Exp</th>
<th>Cell density</th>
<th>Alum dose as Al</th>
<th>% Cell removal</th>
<th>Initial total MC-LR</th>
<th>Initial extra MC-LR</th>
<th>Extra MC-LR after coagulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>419000 cells mL(^{-1})</td>
<td>8.1 mg L(^{-1})</td>
<td>71.4%</td>
<td>8.1 µg L(^{-1})</td>
<td>2.7 µg L(^{-1})</td>
<td>2.7 µg L(^{-1})</td>
</tr>
<tr>
<td>2</td>
<td>536000 cells mL(^{-1})</td>
<td>9.7 mg L(^{-1})</td>
<td>99.5%</td>
<td>8.6 µg L(^{-1})</td>
<td>3.9 µg L(^{-1})</td>
<td>4.2 µg L(^{-1})</td>
</tr>
</tbody>
</table>

The concentrations of extracellular MC-LR in supernatant were measured over the course of the sedimentation process after both coagulation experiments (shown in Figure 5.2). There is a small increase in extracellular metabolite in both cases (corresponding to a maximum of 9% of the intracellular MC-LR), but the majority of the cell bound metabolites have remained intracellular. This indicates that cells have maintained their membrane integrity during sedimentation. It also implies that the majority of metabolites in the settled sludge were intracellular. When these sludges underwent further settling overnight, the supernatant collected at the end had extracellular MC-LR concentrations of 3.1 and 4.3 µg L\(^{-1}\) for Experiment 1 and 2 respectively. This again shows that little cell lysis has occurred during overnight settling. Although the concentration of MC-LR in the clarified water was higher than the WHO guideline level of 1.0 µg L\(^{-1}\), the coagulation and sedimentation processes certainly have not worsened the water quality. On the contrary, if the optimum coagulant dose is applied (such as in Experiment 2), the processes will remove almost all the cells from the water. This will not only greatly reduce the need of using activated carbon or oxidation but also relieve the stress of subsequent treatment process such as sand filtration because cells can easily clog the filter and reduce the filter run time significantly (Henderson *et al.*, 2008).
A mass balance of MC-LR across the sludge production process for Experiment 1 is demonstrated in Figure 5.3. The following assumptions were made for the mass balance calculations:

- **No natural biodegradation of microcystins occurred during either coagulation or sedimentation process.** In natural environments, microcystins can be broken down via two pathways: microbial degradation and light (photolysis). The extent of photolysis of microcystin is much lower than microbial degradation (Chen et al., 2011). Unlike natural waters, waters used in these experiments consisted of a monoculture of cyanobacteria species and sterile artificial medium only. The experiments
were also completed within 2 days. Biodegradation was not likely due to the lack of metabolite-degrading organisms in water. Moreover, raw water or supernatant and sludge produced in the experiment was exposed to limited natural light, therefore, it can be assumed that there was minimal photolysis of toxins.

- **Cell growth was negligible.** The cell culture used in each experiment was harvested after the exponential growth phase, and was removed from the usual light source after being mixed with an artificial medium. The time interval between sampling the raw water and the sludge that was settled overnight was usually 25 hours. Cell counts were performed on the raw water samples during this period and showed only a very small increase in cell density. Therefore, it is reasonable to assume that minimal cell growth has occurred, and hence, no increase in intracellular metabolites during sedimentation.

- **Cells that were not coagulated remained uniformly dispersed in the supernatant throughout sedimentation.** The gas pockets in cyanobacterial cells make the density of individual cells significantly lower than that of water. As a result, cells that were not incorporated in the metal hydroxide flocs were unlikely to settle by themselves. They were then removed from the system when the majority of the supernatant was decanted after 4 hour sedimentation.

- **Cells that were incorporated in the metal hydroxide flocs settled and remained in the sludge.** This assumption may not be valid during sludge transfer. When sludge at the bottom of the large tank was poured to a smaller container, some cells could have escaped from the flocs. In this case, the supernatant produced overnight (Stream 4 in Figure 5.3) would have contained more intracellular MC-LR due to the increase in the number of uncoagulated cells. In fact, cell counts revealed that the number of cells in Stream 4 is 3 times higher than in Stream 2. However, the volume of the supernatant in Stream 4 was small (less than 4 L), the
increase in intracellular metabolites therefore causes only very small impact on the overall mass balance.

Based on these assumptions, the total mass of metabolites in the concentrated sludge stream (Stream 5) can be calculated. Figure 5.3 shows that the calculated value is close to the actual one with a 7.6% error. As expected, the actual mass is lower due to the loss of additional intracellular metabolites via Stream 4. In addition, systematic loss, such as occurred through sample transfer and evaporation, has inevitably contributed to the discrepancy. Mass balance calculations were performed on four experiments for *M. aeruginosa* including Experiment 1 and 2, giving an average error of 8.9%. This indicates that experimental techniques and toxin sample preparation methods are reasonably accurate and robust despite some losses.

In the under-dosed Experiment 1, where 71.4% cell removal was achieved, only 62.8% of the intracellular metabolites and 42.5% of the total metabolites in Stream 1 ended up in the sludge Stream 5. The rest was lost predominantly via the supernatant Stream 2. These losses would be significantly smaller if optimum alum dose was applied for complete cell removal, and extracellular metabolite concentration in the raw water was lower. Nevertheless, the mass balance calculation indicates that the majority of the cell-bound MC-LR in raw water, if not lost via supernatant, remains intracellular throughout the process and ends up in the sludge.
Figure 5.3: Mass balance of MC-LR throughout the sludge production process.
5.2.2 A. circinalis

Similar coagulation experiments were conducted on A. circinalis rich waters. The results of cell removal efficiencies, concentrations of saxitoxin (STX) and concentrations of the T&O compound geosmin, before and after coagulation are shown in Table 5.2. In Experiment 3, both total and extracellular geosmin concentrations in raw water were below the detection limit. This was due to sampling mistakes. Geosmin is a highly volatile and degradable compound but losses can be minimised by ensuring sample bottles do not have an air gap and by adding HgCl$_2$ solution to minimise biodegradation. However, in this experiment, no HgCl$_2$ was added to the geosmin samples and each sample was left with a large head-space in the sampling bottle. As a result, the majority of the compound had escaped the aqueous solution before the extraction procedures, causing very low or undetectable geosmin concentrations. The experimental methods were corrected in Experiment 4 giving an improvement in the results. Although loss of geosmin during the transfer of samples is inevitable, results in Experiment 4 showed that majority of geosmin (> 99%) in raw water was intracellular. In comparison, 54% and 33% of STX were intracellular initially in Experiment 3 and 4 respectively. However, it is noted that the initial extracellular STX concentration was below the health alert concentration, i.e. 3.0 $\mu$gL$^{-1}$ and the initial extracellular geosmin concentration is lower than the detection limit by most consumers (10 ngL$^{-1}$). A similar trend was observed by Ho et al (2012a) where they found that >98% of total geosmin and 50% of total STX were intracellular in untreated A. circinalis rich waters.

There was a noticeable increase in the extracellular STX concentration after coagulation in each experiment. This increase corresponds to 70% of the initial intracellular STX in Experiment 3 and 48% in Experiment 4. The increase in extracellular geosmin concentration during the coagulation was very small. However, in Experiment 4, a sample taken immediately after the alum dosing had an extracellular geosmin concentration of 131.0 ngL$^{-1}$, which corresponds to
40% of the intracellular geosmin in raw water. The experiment was conducted in the open tank with constant mixing, it is proposed that geosmin was dissipated into the air rapidly during this time, resulting in a significant decrease in the concentration. In the meantime, the sample taken after the alum dosing had an extracellular STX concentration of 1.9 µg L⁻¹, which equals 71% of the initial intracellular STX. Both the geosmin and STX results indicate massive cell damage immediately after alum dosing, but the damage appeared to be reduced after pH correction and slow mixing steps. Although the overall increase in extracellular geosmin concentration during coagulation is negligible, mainly due to its volatile nature, serious localised cell damage is evident after alum dosing. In Experiment 1, this rendered the water unsafe (above the 3.0 µg L⁻¹ STX limit) despite the near complete removal of cells. These results contradict the findings by the aforementioned studies (Velzeboer et al., 1995; Chow et al., 1999). The release of a large portion of intracellular metabolite indicates serious damage to *A. circinalis* cells during the coagulation process.

The results of extracellular STX concentration in the water during sedimentation are plotted in Figure 5.4. There was no increase in the concentration over time indicating that no further damage has been done to the cells in the sedimentation process. The geosmin results are not shown here. In Experiment 3, no geosmin was detected in most samples due to the lack of sample preservation. In Experiment 4, the concentration of extracellular geosmin remained below 5 ng L⁻¹ during sedimentation. Since cells were settled in an open tank, substantial loss of geosmin from the supernatant to atmosphere is expected over time. Nevertheless, further release of geosmin into the supernatant is unlikely based on the STX results.

The concentrations of extracellular STX in the supernatant after overnight sedimentation were 3.5 and 1.8 µg L⁻¹ in Experiment 3 and 4 respectively. This accounts for 20% and 9% of additional intracellular STX release. By comparison, the damage caused by coagulation is considerably greater.
Table 5.2: Impact of coagulation on *A. circinalis* and metabolite release.

<table>
<thead>
<tr>
<th>Exp</th>
<th>Cell density</th>
<th>Alum dose as Al</th>
<th>% Cell removal</th>
<th>Initial total metabolite</th>
<th>Initial extra metabolite</th>
<th>Extra metabolite after coagulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>302500 cells mL(^{-1})</td>
<td>8.1 mg L(^{-1})</td>
<td>95.6%</td>
<td>G: n.d.*</td>
<td>G: n.d</td>
<td>G: 7.2 ng L(^{-1})</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S: 3.7 µg L(^{-1})</td>
<td>S: 1.7 µg L(^{-1})</td>
<td>S: 3.1 µg L(^{-1})</td>
</tr>
<tr>
<td>4</td>
<td>140000 cells mL(^{-1})</td>
<td>6.1 mg L(^{-1})</td>
<td>81.6%</td>
<td>G: 325.6 ng L(^{-1})</td>
<td>G: 1.6 ng L(^{-1})</td>
<td>G: 4.4 ng L(^{-1})</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S: 2.1 µg L(^{-1})</td>
<td>S: 1.4 µg L(^{-1})</td>
<td>S: 1.7 µg L(^{-1})</td>
</tr>
</tbody>
</table>

* n.d.: below the detection limit, G: geosmin, S: saxitoxin

Figure 5.4: Concentrations of extracellular STX during sedimentation.
Figure 5.5: Mass balance of STX throughout the sludge production process.

1. **48.4 L A. circinalis water (302,500 cells mL⁻¹)**
   - Extra = 1.7μg L⁻¹ x 48.4L = 82.3μg
   - Intra = (3.7 - 1.7)μg L⁻¹ x 48.4L = 96.8μg
   - Total = 82.3μg + 96.8μg = 179.1μg

2. **Coagulation and Sedimentation 95.6% cell removal**
   - Extra = 3.0μg L⁻¹ x 44.15L = 132.5μg
   - Intra = 96.8μg L⁻¹ x (1 - 95.6%) x 44.15L + (44.15 + 3.25)L = 4.0μg
   - Total = 132.5μg + 4.0μg = 136.5μg

3. **4.25 L settled sludge**
   - Extra: extracellular saxitoxin
   - Intra: intracellular saxitoxin
   - Total: total saxitoxin

4. **Overnight sedimentation**
   - Extra = 3.5μg L⁻¹ x 3.25L = 11.4μg
   - Intra = 96.8μg L⁻¹ x (1 - 95.6%) x 3.25L + (44.15 + 3.25)L = 0.3μg
   - Total = 11.4μg + 0.3μg = 11.7μg

5. **3.25 L supernatant removed (assume 13,400 cells mL⁻¹)**
   - Total = 179.1μg - 136.5μg - 11.7μg = 30.9μg
   - Measured Total = 27.4μg L⁻¹ x 1.0L = 27.4μg
   - Measure Extra = 4.3μg L⁻¹ x 1.0L = 4.3μg
Similar to the *M. aeruginosa* experiments, a mass balance of STX across the sludge production process was performed. An example (Experiment 3) is shown in Figure 5.5. STX has been found to be recalcitrant to biodegradation in natural waters (Ho *et al.*, 2012a). In addition, the growth and decay rates of *A. circinalis* in the laboratory, as shown in Figure 4.2, are slower than those of *M. aeruginosa*. Therefore, the same assumptions used in the MC-LR cases can be applied here for the calculations of the STX mass balance. There was a 12.8% discrepancy between the calculated and the measured total STX masses in Stream 5. This can be attributed to the loss of some uncoagulated cells through Stream 4 as well as systematic loss via sludge transfer and evaporation, as discussed in Section 5.2.1.

Despite the high percentage of cell removal in Experiment 3, 76.2% of the total STX mass in water was lost through Stream 2 where the majority of it was extracellular. This ultimately leads to a low concentration of total STX in the sludge (Stream 5). The mass balance demonstrates clearly that most damage to the cells had been caused by the coagulation process, and although the majority of the cells were removed successfully, the process has caused massive release of intracellular STX to the supernatant and produced an ‘unhealthy’ sludge.

A mass balance of geosmin was not obtained from either experiment. In Experiment 4, the measured total mass of geosmin in Stream 5 was less than half of that calculated. This indicates significant loss of geosmin at various stages during the experiment. Nonetheless, the measured concentration of intracellular geosmin left in the sludge was still high (9887 ng L⁻¹). Therefore, it is important to develop the most appropriate methods for handling this sludge so that further release of intracellular geosmin can be minimised.
5.3 Identification of key factors for metabolite release

Based on the results in Section 5.2.2, one must first question the feasibility of using conventional water treatment process for cyanobacteria removal. Despite the previous findings, the coagulation process clearly causes serious damage to *A. circinalis* cells. Therefore, before an investigation on the fate of cyanobacteria in sludge can be completed, it is a priority to identify the actual cause of cell damage and metabolite release during coagulation. The conventional water treatment process would be unsuitable, even hazardous, if this cause was not eliminated.

The procedures of the coagulation process are illustrated in Figure 5.6. Raw water was mixed rapidly for a minute prior to dosing of alum. Alum caused the pH of the solution to decrease. In each of the experiments above, the pH decreased from 8 to as low as 3. The pH was then corrected to around 7 using NaOH solution. In Section 5.2.2, the metabolite release period was narrowed down to the rapid mixing stage, specifically before pH correction. During this period, three factors could possibly cause serious damage: shear stress, pH change and alum dose. Each factor was examined individually.

![Figure 5.6: Coagulation procedures used in Experiment 1 to 4.](image-url)
5.3.1 Shear

A jar testing rig was used to examine the impact of shear on different species. Cell rich waters were stirred at 330 and 50 rpm (G = 12 and 278 s\(^{-1}\)). These were the same velocity gradients used in the rapid and slow mixing stages of coagulation testing. Samples were taken after 3 min of constant mixing at each speed and the concentrations of the extracellular metabolites in each sample analysed. The percentage of metabolite release by shear was calculated using:

\[
\text{\% release} = \frac{[M_{\text{extra}}] - [M_{\text{extra},0}]}{M_{\text{total},0} - [M_{\text{extra},0}]} \tag{5.2}
\]

where \([M_{\text{extra}}]\) and \([M_{\text{extra},0}]\) are the final and initial concentrations of extracellular metabolites in water and \([M_{\text{total},0}]\), is the initial concentration of total metabolites. The percentage of geosmin and STX release at each mixing speed for \(A.\) circinalis cells and the percentage of cylindrospermopsin release for \(C.\) raciborskii cells are presented in Figure 5.7. Since negligible damage was done on \(M.\) aeruginosa cells during coagulation (as discussed in Section 5.2.1), the shear impact was not examined for this species.

For \(A.\) circinalis, less than 6% of the intracellular STX and less than 1% of the intracellular geosmin was released over the 3 min mixing time at both speeds. The percentage of release was minor compared to the overall release caused by coagulation, as was observed in Experiments 3 and 4. Therefore, it is concluded that shear, as utilised in conventional coagulation processes, is not the main contributing factor to cell damage.
Figure 5.7: Percentage of metabolite release caused by shear for (a) *A. circinalis* and (b) *C. raciborskii*.
The extracellular cylindrospermopsin (CYN) concentration appeared to increase when the cell suspension was mixed at 330 rpm. Although shear may have caused some cell lysis, natural release of CYN is also possible. Studies have not only shown that a large proportion of CYN can present in extracellular form, this proportion was also found to vary drastically throughout a *C. raciborskii* bloom mainly during the post-exponential or stationary growth phase (Chiswell *et al.*, 1999; Griffiths and Saker, 2003). In this experiment, 52.5% of CYN was extracellular initially. As the cells were harvested at the end of the exponential growth phase, it is possible that some natural release of CYN has occurred, even though the cells remained intact during the experiment.

### 5.3.2 pH

Experiments were performed between pH 3 and 11 on *M. aeruginosa*, *A. circinalis* and *C. raciborskii* using the jar testing rig. Each sample was mixed with an appropriate amount of 0.2M hydrochloric acid or 0.2M NaOH solution at 330 rpm to obtain the desired pH. pH 4 simulated the average water condition after alum dosing during coagulation. pH 11 was examined in order to determine the effect on cells if NaOH was added prior to alum dosing. Samples were taken from the water 30 s and 30 min after pH adjustment. The concentrations of extracellular metabolites in these samples were analysed. Cell viability of the water was examined at the end of each experiment. An experiment at pH 7 was performed by lowering the pH to 4 for 15 seconds followed by adjustment to neutral pH. Samples for extracellular metabolite measurement as well as cell viability were taken as before.
Figure 5.8: Effect of pH stress to A. circinalis cell: Percentage of STX and geosmin release after (a) 30 s and (b) 30 min. Initial pH of water: 8.1
From the extracellular metabolite concentration results, the percentage of metabolite release in each case was calculated, as presented in Figures 5.8, 5.9 and 5.10. Figure 5.8 (a) shows the STX and geosmin release 30 s after pH adjustment. At all the selected pH conditions, the percentage of geosmin release remained lower than that of STX. This is partially attributed to the loss of geosmin through vaporisation during mixing, sample preparation and analysis.

Another influencing factor is the potential for chemical conditions to introduce an error in geosmin analysis at acidic conditions. Hsieh et al (2012) investigated the effect of pH on the analysis of T&O compounds 2-Methylisoborneol and geosmin in water. The authors found that using gas chromatography and a mass spectrometric detector coupled with solid phase micro-extraction, the geosmin concentration obtained at acidic conditions was 31% lower than the one obtained at neutral pH. Dehydration of the tertiary alcohol, geosmin, was believed to be responsible for this variation. Although similar analytical methods were employed in this work (Section 4.5.4), the geosmin concentration was determined relative to deuterated internal standards (d5-geosmin), which would have also been affected by dehydration at low pH conditions. As a result, any loss of geosmin was likely to be corrected against the standards. However, to minimise possible errors, it is recommended in future work that pH be adjusted to neutral during sample preparation as the aforementioned study also found that the variation can be minimised by adjusting pH to neutral before analysis.

Apart from the two factors above, it is also possible that the transport mechanisms of geosmin and STX through the cell membrane are different. For example, geosmin may not have completely liberated from the cell constituents within 30 second and a portion of it was excluded through filtration of the sample. As a result, the measured percentage of geosmin release became lower than that of STX.
Although several factors may have influenced the accuracy of the geosmin measurement, the trend shown in Figure 5.8 suggests that the majority of the initial intracellular metabolites were released immediately after the cells were exposed to pH 3. By comparison, only a small proportion of the intracellular metabolites were released at the other pH conditions. In the pH 7 experiment, the pH was lowered to 4 for 15 s before being adjusted to 7. This period seems to be too short to cause any major cell damage. On the other hand, extended exposure to pH 3 and 4, as shown in Figure 5.8 (b), caused the release of all the intracellular STX, indicating that no cells survived below pH 5, over time. Therefore, it is proposed that exposure to low pH is the main cause of cell lysis and metabolite release for *A. circinalis*.

pH stress on cyanobacteria, to the best of our knowledge, has never been documented in the literature. There are studies on the survival of bacteria strains such as *Escherichia coli* and *Cupriandus metallidurans* under pH stress. For example, Baatout *et al.* (2007) studied an *E. coli* strain and a *C. metallidurans* strain after 1 hour of pH stress between 2 and 12. Membrane integrity assays revealed that for both strains, cell membranes of most of the cells were highly disrupted at pH 2 and 12. In another study on a food borne *E. coli* strain, the authors observed that the strain had a high acid tolerance (Jordan *et al.*, 1999). Significant number of cells survived even after 3 days exposure at pH 3. The acid tolerance was also found to be growth phase dependent.

pH is expected to have an effect on membrane integrity by affecting the proteins that make up a large portion of the cell membrane. By changing the solution pH, the amino acids, the building blocks of proteins, tend to stabilise pH by losing protons at high pH and gaining protons at low pH. This in turn affects the hydrogen bonding formed between the molecules and alters the overall shape of the proteins on the cell membrane. As a result, the membrane properties, such as selective permeability can be impaired. Cyanobacteria, however, appear to have a high alkaline tolerance. In fact, a high pH (> 8) environment favours the
dominance of cyanobacteria over eukaryotic algae (Paerl, 1996). *Microcystis* and *Anabaena* are among the rare species that are reported to be able to grow at a pH above 10 (Reynolds, 1986). This can possibly explain the relatively minor metabolite release at pH 11 observed in this study.

A similar trend was observed in *C. raciborskii* (Figure 5.9). At pH 4, most intracellular CYN was released within 30 minutes. The clear implication is that dosing alum first during coagulation is unsafe as it will potentially cause cell lysis and CYN release by decreasing the pH. Short term exposure to pH 5 did not cause any cell damage whereas a sizeable amount of intracellular CYN was released after 30 min. No increase in extracellular CYN concentrations was observed at pH 7 and 11. Therefore, for future experiments, it is proposed that increasing the solution pH first, before alum dosing, has the potential to avoid cell damage and massive metabolite release. Even though this may change the way that laboratory jar test and full scale plant operate, it is considered to be a worthwhile modification.
Figure 5.9: Effect of pH stress to *C. raciborskii* cell: Percentage of CYN release. Initial pH of water: 7.8.
Unlike *A. circinalis* and *C. raciborskii*, *M. aeruginosa* displayed a relatively high acid tolerance. Figure 5.10 indicates that 30 minute exposure to pH 4 induced less than 4% MC-LR release. This outlines why coagulation has caused minimal damage to the cells despite the low pH caused by alum dosing. It is possible that the *M. aeruginosa* have some attributes similar to *E. coli* that as noted earlier, have been found to have an increase acid tolerance (Benjamin and Datta, 1995).

Cell viability at different pHs was compared among the three species (Table 5.3). In the table, FDA +ve represents active cells and Sytox® Green +ve represents dead cells. Meanwhile, cells that were detected by neither stain, i.e. FDA –ve and Sytox® Green –ve, were considered to be inactive but have intact cell membranes. In each experiment, the untreated water (control) contained 100%
active cells and no dead cells. For *M. aeruginosa*, the percentage of active cells decreased with decreasing pH. However, no dead cells were detected at the various pH conditions. At pH 4, although only 1% of the cells were dead, FDA results reveal that no active cells were present in the water. This indicates that the pH 4 condition has caused stress to the cells but not sufficient to cause any permanent damage to the cell membranes. As a result, no significant release of MC-LR was observed under this condition or any other pH conditions, as suggested in Figure 5.10.

For the other two species, membrane integrity of most cells was impaired at pH 4, which mirrors the high percentage of metabolite release. Some cell lysis occurred at higher pH for *A. circinalis*, resulting in minor release of intracellular metabolites. Meanwhile, at least half of the cells remained active at higher pH. In comparison, no active cells were detected at pH 5 and 11 for *C. raciborskii* although cell membranes were intact at pH 11. Cell viability did not necessarily correlate the percentage of metabolite release in these experiments because the cell viability assays were generally performed 30 min after samples for extracellular metabolite concentration analysis were taken. More cell lysis was expected during this period particularly at low pH. Nevertheless, it is clear from both sets of results that low pH (<5) operation should be avoided.

| Table 5.3: Cell viability of cyanobacteria species at various pH. |
|--------------------------|--------|--------|--------|--------|
| pH | 4 | 5 | 7 | 11 |
| **M. aeruginosa** | FDA +ve | 0% | 25% | 55% | 78% |
| | Sytox® Green +ve | 1% | 0% | 0% | 0% |
| **A. circinalis** | FDA +ve | 0% | 84% | 63% | 52% |
| | Sytox® Green +ve | 100% | 27% | 14% | 13% |
| **C. raciborskii** | FDA +ve | 0% | 0% | 71% | 0% |
| | Sytox® Green +ve | 100% | 37% | 0% | 0% |
5.3.3 Alum dose

Since alum dosing is nominally accompanied by a decrease in pH, to examine the impact of alum dose alone, a low pH environment must be avoided. This was achieved by adding the necessary amount of NaOH solution first, such that the pH decreased to the neutral range after alum dosing. The amount of NaOH required varied depending on the raw water quality and alum dose. As a result, the pH after NaOH addition changed accordingly between 9.4 and 10.8. As discussed in Section 5.3.2, metabolite release at pH 11 was negligible. Therefore, cells were expected to remain viable until the addition of alum.

The extracellular metabolite concentrations 2 minutes after alum dosing were determined. For \textit{A. circinalis}, no extra release of geosmin was detected while only 7.1\% of the intracellular STX was released. This is a great improvement from 70\% and 48\% release obtained in Experiments 3 and 4. The results now agree with the findings by Velzeboer \textit{et al} (1995). In their study, \textit{A. circinalis} culture was mixed with natural water to make up a suspension with cell density at around 200,000 cells m\textsuperscript{-1}. The alum dosed was less (4.8 mg L\textsuperscript{-1} by Velzeboer \textit{et al} (1995) compared with 8.1 mg L\textsuperscript{-1} in the current work) which in turn caused a smaller decrease in pH (8.7 to 6.7 observed by Velzeboer compared with 8.0 to 3.9 in the current work). Since the pH of the water in Velzeboer’s study was maintained close to neutral, no severe damage to the cells was observed.

Similar to \textit{A. circinalis}, no extra release of CYN was detected after alum dose for \textit{C. raciborskii}. An experiment on \textit{M. aeruginosa} was not performed as it had been established earlier that coagulation only had a minor impact on the cells even though the pH of the water decreased to 4 after alum dosing.
5.4 Cyanobacteria in conventional WTP – Approach II

The study to date has shown that alum dosing to cyanobacteria rich suspensions only causes minor cell damage and metabolite release for *A. circinalis* and *C. raciborskii*, provided the cells are not exposed to a low pH environment. Based on these observations, the coagulation procedures used in Experiment 1 to 4 were modified. Since all three species have shown to have high tolerance to alkaline conditions, the order of chemical dosing during the rapid mixing stage of the coagulation process was reversed (as shown in Figure 5.11). Before the large scale experiments, a series of jar tests were performed to determine the approximate amount of NaOH that was sufficient to compensate the pH decrease after alum dosing. The impact of coagulation and sedimentation processes on *A. circinalis* and *C. raciborskii* under the new approach will be discussed in the following sections. The results on *M. aeruginosa* are not presented here as changing the coagulation procedure was not shown to make any difference to metabolite release.

![Figure 5.11: Modified coagulation procedure.](image-url)
5.4.1 A. circinalis

Larger scale sludge production was now carried out using the modified coagulation procedure. The impact of the new approach during two experiments for A. circinalis (Experiment 5 and 6) is demonstrated in Table 5.4. Compared to the previous method (Experiments 3 and 4), the percentage of release of STX during the new coagulation approach was minimal. Geosmin results are not shown here. Due to its volatile nature, the concentrations of extracellular geosmin in water after coagulation were even lower than before. By adjusting the order of the chemical dosing, water was kept above neutral pH at all times. As a result, cells were aggregated without any damage. Cell viability assays on the samples taken before and after the process also revealed that 98% of the cells were active and membrane integrity of all the cells remained intact during the modified coagulation process.

A mass balance of STX was performed across the sludge production processes as before. An example of the mass balance (Experiment 6) is illustrated in Figure 5.12. Since no cell lysis or STX release occurred during coagulation and sedimentation, there was no increase in the mass of extracellular STX in Stream 2, as opposed to that observed in Experiment 3 (Figure 5.5). The concentration of extracellular STX in the supernatant generated from settled sludge increased to 3.0 µg·L⁻¹ overnight. However, in terms of the total mass of STX in the settled sludge, the increase was negligible.
Table 5.4: Impact of coagulation on *A. circinalis* and STX release using the new coagulation approach.

<table>
<thead>
<tr>
<th>Exp</th>
<th>Cell density</th>
<th>Initial total STX</th>
<th>Initial extra STX</th>
<th>Extra STX after coagulation</th>
<th>%STX release</th>
<th>Exp</th>
<th>%STX release</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>477400 cells mL⁻¹</td>
<td>12.1 µg L⁻¹</td>
<td>2.4 µg L⁻¹</td>
<td>2.6 µg L⁻¹</td>
<td>2%</td>
<td>3</td>
<td>70%</td>
</tr>
<tr>
<td>6</td>
<td>390000 cells mL⁻¹</td>
<td>4.8 µg L⁻¹</td>
<td>2.5 µg L⁻¹</td>
<td>2.6 µg L⁻¹</td>
<td>4%</td>
<td>4</td>
<td>48%</td>
</tr>
</tbody>
</table>

The mass of intracellular STX in Stream 5 was measured as 96.8 µg, which was 87.0% of the mass in the raw water (Stream 1). This is a large enhancement from the 23.9% obtained in Experiment 3. Although some loss through the system occurred via supernatant removal and sample transfer, it has been demonstrated that the majority of cells were removed effectively from the raw water. More importantly, without exposure to low pH, most of the intracellular metabolites in the raw water maintained inside the cells and ended up in the sludge stream. Moreover, a reasonable mass balance was obtained across the system. The predicted total STX mass in Stream 5 is only 3.4% higher than that measured.
**Figure 5.12:** Mass balance of STX throughout sludge production process using the new approach.
Table 5.5: Impact of coagulation on *C. raciborskii* and CYN release.

<table>
<thead>
<tr>
<th>Exp</th>
<th>Cell density</th>
<th>Initial total CYN</th>
<th>Initial extra CYN</th>
<th>Extra CYN after coagulation</th>
<th>%CYN release</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>378900 cells mL⁻¹</td>
<td>29.4 µg L⁻¹</td>
<td>7.6 µg L⁻¹</td>
<td>8.0 µg L⁻¹</td>
<td>1%</td>
</tr>
<tr>
<td>8</td>
<td>828800 cells mL⁻¹</td>
<td>47.7 µg L⁻¹</td>
<td>18.2 µg L⁻¹</td>
<td>19.3 µg L⁻¹</td>
<td>4%</td>
</tr>
<tr>
<td>9</td>
<td>641300 cells mL⁻¹</td>
<td>26.3 µg L⁻¹</td>
<td>8.1 µg L⁻¹</td>
<td>7.2 µg L⁻¹</td>
<td>-5%</td>
</tr>
<tr>
<td>10</td>
<td>390000 cells mL⁻¹</td>
<td>23.9 µg L⁻¹</td>
<td>7.6 µg L⁻¹</td>
<td>7.7 µg L⁻¹</td>
<td>1%</td>
</tr>
</tbody>
</table>

5.4.2 *C. raciborskii*

Large scale coagulation of *C. raciborskii* cells using the new approach was also investigated. The results are summarised in Table 5.5. Similar to *A. circinalis*, the *C. raciborskii* cells remained intact during coagulation, provided that the pH of the water was maintained above 5.

The concentration of extracellular CYN in water during sedimentation was monitored over time. The data is plotted in Figure 5.13. In these experiments, flocs generated by coagulation were allowed to settle in the large coagulation tank for 5 hours. At t = 5 h, the supernatant was removed and the sludge was transferred to a 5 L beaker for further settling. As shown in Figure 5.13, during the first sedimentation stage, there was only a slight variation in the extracellular CYN concentration. A small rise in the concentration was observed in all four experiments after the sludge was transferred. It is possible that a small degree of cell lysis has occurred during sludge transfer resulting in the release of a small amount of intracellular CYN. Meanwhile, it is noted that the volume of the cell suspension has now reduced to more than a tenth of its initial and the volume of the supernatant generated from further settling was even smaller. As the cells were concentrated in the sludge stream, any release of CYN into the supernatant, no matter how minuscule, would be magnified and cause a
noticeable increase in the extracellular CYN concentration in the supernatant. Nevertheless, after this initial increase, there was barely any further increase during overnight sedimentation.

Overall, the coagulation and sedimentation processes have not caused any major damage to the cells. If cells are removed efficiently, most intracellular CYN should travel with the cells and finish up in the sludge stream. The mass balance of CYN across the system was calculated using the assumptions described in Section 5.2.1. The mass balance of CYN for Experiment 9 is shown in Figure 5.14. There was a 24.6% discrepancy between the calculated and the measured total CYN mass in Stream 5. In fact, the discrepancy obtained for the four experiments range from 19.8 to 24.6%. The error is significantly larger than the average achieved from the MC-LR and STX mass balance. This implies that in addition to the systematic loss, CYN degradation may have occurred during the process. A study by Chiswell et al (1999) found that CYN degraded rapidly under natural sunlight with a half-life of 1.5 h. However, the compound was relatively stable under the artificial light sources that have been used in this study. The cause of the large error is not well understood, nevertheless, in Experiment 9, the mass of intracellular CYN measured accounted for 76.6% of the initial mass in the raw water. It means that most of the intracellular CYN was separated successfully from the raw water without deteriorating the water quality. Meanwhile, it is worth noting that the concentration of extracellular CYN in the raw water already exceeded the suggested guideline level of 1.0 µg L⁻¹. Further treatment of water on extracellular CYN removal is necessary, although by removing the majority of the cells and intracellular CYN using the conventional water treatment processes, the cost of subsequent treatments will be reduced significantly.
Figure 5.13: Concentrations of extracellular CYN during sedimentation.

Decant supernatant and transfer sludge
Figure 5.14: Mass balance of CYN throughout sludge production process using the new approach.
5.5 The fate of cyanobacteria in sludge dewatering

Conventional water treatment processes have been shown to be capable of separating cyanobacteria cells effectively from water without causing any major damage to the cells. In the meantime, a waste stream or sludge generated from the processes would contain high concentrations of cells and intracellular metabolites. Drinking water sludges are commonly transported and deposited into landfill or sent to sewer. The water industry each year spends millions of dollars on sludge disposal (Burris and Lough, 1996). In addition, with ever-tightening environmental regulations and legislations, companies are required to minimise the quantity of wastes produced. Moreover, in times of water stress, there is a high incentive to recover and reuse the water from sludge dewatering. However, treating sludge laden with cyanobacteria and metabolites presents a processing challenge. Any damage to cells caused by the sludge dewatering processes would contribute to the release of harmful metabolites to the treated water. There have been a limited number of studies on the effect of sludge dewatering processes, specifically lagoon treatment and sand filtration, on cyanobacteria and the subsequent water quality (Himberg et al., 1989; Chow et al., 1999; Ho et al., 2012a). Drikas et al (2001a) observed that the number of healthy *M. aeruginosa* cells in an alum sludge dropped by more than 90% after a day and a large quantity of intracellular MC-LR was released to the sludge supernatant within two days. In another study on cyanobacteria-laden alum sludges in lagoon treatment process (Ho et al., 2012a), the authors found a significant rise of extracellular saxitoxin in the supernatant after 3 days for a *A. circinalis sludge* and the extracellular CYN concentration was more than doubled after 5 days for a *C. raciborskii* sludge. If this is a result of ageing, then faster and more efficient sludge dewatering processes, such as centrifugation and pressure filtration may be beneficial. There is no information in literature on how cyanobacteria cells in sludge handle the pressure applied in a centrifuge and/or a filter press. In this section, the impact of these processes on cyanobacteria and the subsequent metabolite release is investigated.
5.5.1 *M. aeruginosa* alum sludge

A cell rich alum sludge generated from the coagulation and overnight sedimentation processes was concentrated in the bench-top centrifuge at 3000 rpm for 5 min. A schematic of the mass balance around the procedure is outlined in Figure 5.1. The concentration of extracellular metabolite, in this case, MC-LR, in the supernatant collected upon completion was compared to the initial concentration of extracellular MC-LR in the sludge. The results from different batches of sludge samples are summarised in Table 5.6. No release of MC-LR was observed during the centrifugation experiment. In addition, cell viability assay on the concentrate did not detect any cells with damaged membranes. Therefore, it can be concluded that the process caused no impact on the cells.

During each experiment, the magnitude of compression experienced by the cells in the centrifuge was calculated to be less than 1 kPa. The exposure time was also relatively short. In comparison, pressure filtration tests applied constant pressures ranging from 20 and 300 kPa. The run time varied between 2 and 11 hours. The concentrations of extracellular MC-LR in the filtrate produced from *M. aeruginosa*-rich Sludge A, B and C at different pressures are plotted in Figure 5.15. The figures at 0 kPa represent the initial concentrations of extracellular MC-LR in the sludges.

<table>
<thead>
<tr>
<th>Sludge sample</th>
<th>Initial total MC-LR</th>
<th>Initial extra MC-LR</th>
<th>Extra MC-LR after centrifugation</th>
<th>%MC-LR release*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>335.7 µg L⁻¹</td>
<td>9.6 µg L⁻¹</td>
<td>7.3 µg L⁻¹</td>
<td>&lt; 0</td>
</tr>
<tr>
<td>2</td>
<td>133.1 µg L⁻¹</td>
<td>3.5 µg L⁻¹</td>
<td>1.3 µg L⁻¹</td>
<td>&lt;0</td>
</tr>
<tr>
<td>3</td>
<td>523.9 µg L⁻¹</td>
<td>9.7 µg L⁻¹</td>
<td>11.3 µg L⁻¹</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>4</td>
<td>369.2 µg L⁻¹</td>
<td>29.0 µg L⁻¹</td>
<td>32.7 µg L⁻¹</td>
<td>1%</td>
</tr>
</tbody>
</table>

* The supernatant produced was more than 90% of the initial volume of the sludge. The calculation was simplified using Equation 5.2.
There was a slight increase in the concentration of extracellular MC-LR during the high pressure runs. However, the increase was negligible compared to the total MC-LR concentrations in the raw sludges. The total MC-LR concentration in Sludge A was significantly lower than the other two because it consisted of aged cells (coagulation Experiment 1). The majority of MC-LR in the raw water was extracellular and had been removed during the sedimentation process. The data at 20 kPa was not obtained. Nevertheless, the high pressure filtration process did not cause any significant damage, even when the cells in the sludge were aged.
It was difficult to sample the filter cake for cell viability assays due to its small and compact volume. A number of samples were collected and showed that none of the cells in the resuspended cake were FDA active, although some of them were dead. Unfortunately, the number of cells present in these samples was extremely low (< 1000 cells mL⁻¹). Therefore, it may not be representative of the population in the whole filter cake. However, based on the toxin results, *M. aeruginosa* cells are shown to have survived the pressure filtration process and the release of intracellular MC-LR was minimal. Moreover, the favourable results mean that high pressure runs not only can reduce the sludge volume significantly, the time cells spent in the device was also reduced, hence decreasing the risk of natural cell lysis over time.

### 5.5.2 *A. circinalis* alum sludge

The extracellular concentrations of STX and geosmin in the supernatant after centrifugation are presented in Table 5.7. Sludge samples 1 and 2 came from coagulation Experiments 3 and 4 (refer to Table 5.2). Both experiments used the old coagulation methods that incurred a pH decrease to 4. As a consequence, the majority of the intracellular metabolites had been released during coagulation alone, resulting in an ‘unhealthy’ sludge. The increase in the concentrations of extracellular metabolites over the centrifugation process was more noticeable in sample 2 than in sample 1. For example, the percentage of STX release was 10.4% in sample 2 compared to 1.7% in sample 1. Cell age could be a contributing factor. As presented in Table 5.2, the ratio of extracellular to intracellular metabolites in the raw water used in Experiment 4 is higher than that used in Experiment 3. This suggests that the cells in sample 2 were possibly older than in sample 1 and hence more susceptible to damage.
Sludge samples 3 and 4 were produced using the modified coagulation procedures. The increase in the concentrations of extracellular metabolites over the centrifugation process was negligible. Cell viability assays also showed that at least 80% of the cells remained FDA active and no dead cells were detected in the sludge.

Pressure filtration tests were also performed on the *A. circinalis* rich alum sludges. The concentrations of extracellular STX and geosmin in the filtrate were measured. An example for sludge sample 4 is shown in Figure 5.16. The figures at 0 kPa represent the initial concentrations of extracellular STX and geosmin. There was no clear trend on the release of metabolites with increasing pressure. However, the percentage of metabolite release was generally less than 0.7% for both geosmin and STX, except for geosmin release at 200 kPa which was 2.4%. It is worth noting that the absolute concentrations of geosmin in the filtrate were at least two orders magnitude higher than the 10 ng L\(^{-1}\) consumer detection limit. However, the volume of the sludges used in each pressure filtration run was only 80 mL. The high concentration of extracellular geosmin in the filtrate is not likely to cause any noticeable impact on the raw water quality if it were to be recycled.

<table>
<thead>
<tr>
<th>Table 5.7: Effect of centrifugation on the release of STX (S) and geosmin (G).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sludge sample</td>
</tr>
<tr>
<td>Initial total</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Initial extra</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Extra after centrifugation</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Figure 5.16: Concentrations of extracellular STX and geosmin in the filtrate, produced from pressure filtration of *A. circinalis* alum sludge.

The pressure filtration results on sludge samples 1 to 3 are not shown here, however the impact of the process was even smaller in these sludges than in sample 4. Therefore, it is believed that pressure filtration also has negligible effect on the *A. circinalis* cells in the sludges, regardless of the cell age or the sludge nature.
5.5.3 *C. raciborskii* alum sludge

The concentration of extracellular CYN in the supernatant after centrifugation is presented in Table 5.8, with the percentage being minor, except for sample 1. The cause of this aberration is unknown. Cell viability assays revealed no increase in the percentage of dead cells in the concentrate from that in the raw sludges, including sample 1. Therefore, the process does not appear to have caused any cell lysis or induced CYN release for *C. raciborskii* cells.

Pressure filtration tests on two concentrated *C. raciborskii* sludges (Sludge D and E) were carried out at 20, 150 and 500 kPa. The results of the concentrations of extracellular CYN in the filtrate are shown in Figure 5.17. The concentrations in the filtrate were similar to the initial concentration (0 kPa) at all the pressures tested. It indicates that the *C. raciborskii* cells in the sludges were able to endure high pressures.

**Table 5.8: Effect of centrifugation on the release of CYN.**

<table>
<thead>
<tr>
<th>Sludge sample</th>
<th>Initial total CYN</th>
<th>Initial extra CYN</th>
<th>Extra CYN after centrifugation</th>
<th>%CYN release</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>826.4 µg L⁻¹</td>
<td>20.9 µg L⁻¹</td>
<td>91.0 µg L⁻¹</td>
<td>8.7%</td>
</tr>
<tr>
<td>2</td>
<td>1349.3 µg L⁻¹</td>
<td>25.1 µg L⁻¹</td>
<td>44.6 µg L⁻¹</td>
<td>1.4%</td>
</tr>
<tr>
<td>3</td>
<td>633.9 µg L⁻¹</td>
<td>22.6 µg L⁻¹</td>
<td>16.9 µg L⁻¹</td>
<td>&lt;0</td>
</tr>
<tr>
<td>4</td>
<td>475.8 µg L⁻¹</td>
<td>18.1 µg L⁻¹</td>
<td>19.3 µg L⁻¹</td>
<td>0.3%</td>
</tr>
</tbody>
</table>
Figure 5.17: Concentrations of extracellular CYN in the filtrate produced from pressure filtration of \textit{C. raciborskii} alum sludges. The initial concentrations of total CYN in Sludge D and E were 671.7 and 966.6 µg L\(^{-1}\) respectively.

5.6 Conclusions

This study has allowed us to gain a better understanding on the impact of conventional water treatment and sludge dewatering processes on cyanobacteria cells. The risk of cell lysis and metabolite release during coagulation, sedimentation, centrifugation and pressure filtration processes has been assessed using metabolite analysis and cell viability assays.

It has been demonstrated that low pH stress (pH < 5) can cause massive cell lysis and metabolite release in some species during the coagulation with alum. Although the exposure time to low pH is usually brief during coagulation, it was shown that a pH below 5 can potentially impair water quality permanently through
serious localised cell damage. Therefore, it is recommended that water treatment facilities ensure that a low pH is avoided at all times and that a good pH buffer system is in place.

Without exposure to low pH, cells have been shown to maintain their viability throughout the coagulation and sedimentation processes without significant release of metabolites. Moreover, upon the effective removal of cells using these processes, the majority of the intracellular metabolites were separated from the raw water. This approach has the potential to significantly reduce requirements for, and the cost of, subsequent water treatment.

Furthermore, centrifugation and pressure filtration were shown to cause minimal impact on the cells in the resultant sludge. Cells were able to resist high pressures during these further dewatering processes while sludge volume was reduced significantly. However, it is worth noting that the majority of cyanobacteria-rich water samples used in this study were obtained by harvesting the laboratory cultures at the end of the exponential growth phase. During natural bloom events, raw water may contain cells at a range of ages, and aged cells (i.e. at decaying phase) may show different behaviour. Therefore, further investigation on samples taken at different points throughout the growth phase would be necessary to ensure the behaviour seen here would be mimicked in a bloom condition.

Nevertheless, these findings will help water treatment plant operators and owners to make decisions on how to treat cyanobacteria-laden waters and manage the resultant sludges safely and effectively.
CHAPTER 6  DEWATERING OF CYANOBACTERIA-RICH SLUDGES

6.1 Introduction

The dewatering properties of a wide range of materials are well documented in the literature. These materials include biological sludges (e.g. dairy sludge and sewage sludge) and non-biological materials (e.g. water treatment plant (WTP) sludge, mineral tailings) (Green et al., 1998; Harbour et al., 2004; Stickland et al., 2008; Wall, 2008). However, the dewatering behaviour of cyanobacteria-rich WTP sludge is not yet well understood. This chapter provides outcomes of the dewaterability investigation of this sludge. The dewatering properties of laboratory-produced WTP sludge containing high concentrations of cyanobacterial cells were characterised in terms of compressive yield stress, $P_y(\phi)$ and hindered settling function, $R(\phi)$. This information is useful because it not only identifies the ease with which water can be recovered from the sludge but also, in this case, the information was augmented with metabolite release data. This helps to determine whether water recovered from various dewatering processes is safe for recycling.

6.2 Dewatering characteristics of freshwater and marine algae

While cyanobacterial cell cultivation was in progress, several algal cultures were obtained from Biomax Fuels, Melbourne to assist in developing and trialing characterisation techniques. These were freshwater samples of Scenedesmus pleiomorphus and Chlorella vulgaris. A marine sample was also provided that consisted of a number of species including diatoms. Due to the commercial-in-
confidence nature of the work, most species, in the latter sample remained unidentified. As cyanobacteria share many physical characteristics with algae, it was proposed that the dewatering characterisation of these species would be similar.

6.2.1 Dewaterability characterisation at low solids volume fraction

To characterise the dewatering behaviour of algal cultures at low applied pressures and by implication, low solids volume fraction, a transient gravitational batch settling test was performed. All the algal samples tested were untreated raw materials, freshly harvested from the bioreactors. During settling, three distinct regions of suspension were observed for each sample, as shown in the example in Figure 6.1. Large flocs of algal cells settled rapidly over the course of a few hours. These formed an initial sediment bed and left a suspension of fine particles. Over the course of several days the fine particles settled to produce a clear supernatant at the top.

The presence of both rapidly-settling (self-flocculated) and slowly-settling (unicellular) algae complicated analysis, with two interfaces moving simultaneously. Ignoring either interface would not truly represent the whole system. Moreover, the rate of movement of the lower interface could be influenced by the settling behaviour and cake formation of fine particles. This makes the analysis of settling data extremely difficult, and indeed, outside of current theoretical capabilities. Therefore, the dewatering properties of algae at low solids volume fraction could not be determined using this test.
For a mixed-culture freshwater sample, the height of the interface between the clear supernatant and the fine particle region of the suspension was monitored. This data is presented in Figure 6.2. The plot consists of two linear sections with a sharp transition between the two. Lester et al. (2005) developed an analytical method to extract the hindered settling function, $R(\phi)$, from batch settling tests. The characteristics of the settling curve in Figure 6.2 were described in their work as sedimentation mode 1 or unhindered settling. Fine particles in the suspension were well-dispersed, un-networked at extremely low solids fraction, which means that minimal interactions are expected between settling particles, resulting in this unhindered settling behaviour. The lack of a non-linear transition region on the settling curve limits the extraction of $R(\phi)$ over the low range of solids fraction, from the initial solids volume fraction, $\phi_0$ to the gel point, $\phi_g$. As a consequence of the multiple interface settling and mode 1 settling of the finer fraction, transient batch settling tests were deemed unsuitable for characterisation of unflocculated algal materials.
Figure 6.2: Plot of the height of interface (between the supernatant and fine particle layer) versus time for a freshwater algae in transient gravitational batch settling, $\phi_0 = 0.00057$ v/v.
6.2.2 Dewaterability characterisation at high solids volume fraction

Pressure filtration techniques facilitate characterisation of suspensions at high solids volume fraction. The suspension loaded into the filtration cell was always at or above the gel point. Moreover, to ensure accuracy in the analysis, the suspension being tested must contain a sufficient amount of solids such that the filter cake obtained at the end of the filtration run has a height of no less than 2 mm. Because the raw algal samples had very low solids volume fraction, they were pre-concentrated in a bench-top centrifuge at 3000 rpm for 5 minutes. The solids volume fraction for the algal suspensions loaded in the filtration cell ranged from 0.0138 to 0.0235 v/v.

Single pressure filtration tests of both freshwater and marine algal samples were conducted at 50 kPa. The gradient of a $t$ versus $V^2$ plot (shown in Figure 6.3, between $V = 0.01$ to 0.04 m) revealed that for freshwater samples there is a non-linear dependence between $t$ and $V^2$. This indicates that freshwater algae have the characteristics of a typical biological sludge (Stickland et al., 2005). From the same plot, the marine algae sample displayed a long linear cake formation region, which is typical of non-biological materials, such as mineral suspensions (Green et al., 1998).
Based on the results from the single pressure filtration tests, a series of constant pressure filtration runs (pressure ranged from 20-300kPa) was conducted on the freshwater algae sample and the dewatering information was obtained using a biosludge characterisation protocol (Stickland et al., 2005). Conversely, marine algae displayed a long cake formation region and therefore their dewatering characteristics were determined rapidly by stepped pressure filtration tests (de Kretser et al., 2001). The calculated dewaterability data for freshwater and marine algae are presented in Figures 6.4 and 6.5.
Figure 6.4: Compressive yield stress as a function of solids volume fraction showing a comparison between freshwater and marine algae at pressures between 10 and 300 kPa.

The marine algae culture attained a higher solids concentration at a given pressure relative to the freshwater algae samples (shown in Figure 6.4). The results suggest that the particle (cell) nature and/or the inter-particle interaction between cells are very different in these two cases.

\( R(\phi) \) data for the three samples is shown in Figure 6.5. The data represents the drag in the system and a lower value indicates faster dewatering. The resistance to flow in these tests for freshwater algae was up to three orders of magnitude higher than for the marine algae. There are three phenomena that are expected to contribute to this observation.
First, the dispersed nature of the marine algae is expected to have caused the cells to form a more compact network. Dispersed systems represent the simplest case for inter-particle interactions when the particles only interact through hydrodynamic and Brownian diffusion forces (Zhou et al., 2001) and under compression, the system can achieve a maximum packing fraction that is close to random close packing. The high solids concentration obtained in the marine algal culture could be partially attributed to the presence of the diatom species. The rigid cell wall is likely to give the cells similar attributes to a hard sphere. As a result, a more compact filter cake is expected for such a system.
Second, the high salt concentration is expected to screen repulsive electrostatic interactions between the algal cells (diatoms) and cause slight flocculation at high solids. Whilst this is expected to decrease the compaction of the filter cake at a fixed solids concentration, it is likely that the permeability of the cake will improve. For example, Zita and Hermansson (1994) found that lowering ionic strength decreased flocculation and caused an increase in the non-settling fraction of an activated sludge. Conversely, increasing ionic strength has been shown to increase re-flocculation of dissolved floc fragments. This partially explains why bioflocculation in wastewater treatment plant deteriorates during heavy rainfall or snow melting periods, causing poor effluent water quality (Hermansson, 1999). In the case of marine algae, it is possible that the high salt content is beneficial to the rate of dewatering.

The third phenomena of interest to the dewatering behaviour of these systems is that of cation bridging (Higgins and Novak, 1997), whereby cations, particularly divalent cations, are incorporated within the cell-extracellular polymeric substance (EPS) network, creating a floc matrix. As marine algae are maintained in a brine environment, the aggregation of cells is likely to be enhanced by the cations present in the suspension, thus achieving a higher extent of dewatering compared to freshwater algae. However, in order to have a better understanding of the different dewatering behaviours of freshwater and marine algae, investigation on a broad range of species would be important.

### 6.2.3 Dewaterability characterisation at low to intermediate solids volume fraction

Due to the difficulty encountered in elucidating dewatering information from transient batch settling, dewaterability data at lower solids volume fraction could not be obtained. Therefore, transient centrifuge settling tests were performed in a LUMiFuge® Stability Analyser. The method of extracting \( R(\phi) \) values at a range of low to intermediate solids volume fraction from the LUMiFuge test is detailed in
Section 3.3.3. Similar to the pressure filtration test, if the equilibrium sediment height was less than 2 mm after the suspension underwent centrifugation, errors in the analysis became significant. As a consequence, pre-concentration of raw suspensions was performed when necessary.

The $R(\phi)$ data calculated for freshwater and marine species is presented in Figure 6.6. The results were in good agreement with those generated from pressure filtration tests (Figure 6.5). The marine algae dewatered at a faster rate than the three freshwater samples tested. Within the freshwater samples, pure *Scenedesmus* sp. exhibits poor rate of dewatering properties compared to the mixed cultures of freshwater species (which contains predominantly *Chlorella vulgaris*). Pure *Chlorella* sp. has the highest permeability of the three. It is postulated that this trend is caused by the structural difference between the species. *Scenedesmus* (Figure 6.7) is a colonial green alga consisting of cells aligned in a flat plate. The colonies most often have two or four cells, but may have 8, 16, or very occasionally 32 cells. The size of the colonies range from 10 to 50 $\mu$m. Typically the end cells each have two long spines up to 200 $\mu$m in length protruding from their outer corners. The spines of *Scenedesmus* can increase the interactions between each other and hinder compaction. This is reflected on the high $R(\phi)$ values for *Scenedesmus*. *Chlorella vulgaris* is a small spherical green algae (3-10 $\mu$m), existing in unicellular form. The degree of interaction between the cells is much lower than for *Scenedesmus*. As a result, both the lower interaction and less asymmetric nature of the cell colony (almost spherical in this case) leads to lower $R(\phi)$ properties.
Figure 6.6: Hindered settling function at low to intermediate solids fraction. Values derived from transient centrifuge settling data.

Figure 6.7: General structure of *Scenedesmus* sp.
6.3 Dewaterability of cyanobacteria-rich sludge

6.3.1 Optimum dose selection

The slow settling behaviour of algal and cyanobacterial materials is suggestive of the need for coagulation if separations are to be manifest at large scale and on a reasonable time frame. Prior to large scale sludge production, coagulation experiments were investigated at small scale to determine the optimum alum dose. Jar testing was performed on *M. aeruginosa*, *A. circinalis* and *C. raciborskii* suspensions at cell densities between 50,000 and 850,000 cells mL\(^{-1}\). The coagulation mechanism employed here was sweep coagulation. In sweep coagulation, hydrolysing coagulants such as aluminium and ferric salts form amorphous hydroxide precipitates and particles in water become enmeshed in the growing hydroxide precipitate and settle to form sludge (Packham, 1965). The minimum solubility of aluminium hydroxide occurs at coagulation pH between 6 and 7 (Duan and Gregory, 2003), therefore all the coagulation experiments were conducted within this pH range. Outside this range, alum started to become more soluble and eventually the amount of precipitate present in the suspension was insufficient to drive coagulation, especially using the sweep mechanism and at an industrially relevant rate.

The optimum alum dose was identified as the condition at which the cell count in the supernatant after one hour settling was minimum, i.e. when the highest percentage of cell removal was obtained. The optimum alum dose for each specimen at various cell densities is given in Figure 6.8. At the optimum doses, 94 to 99% of the cells were captured and settled to the base of the coagulation vessel, producing a relatively cell-free supernatant. For each specimen, there was a strong linear correlation between cell density and optimum alum dose. This provided a simple and reliable way of estimating optimum dosage at a given cell density. When large-scale coagulation experiments were performed (48.4 L), operating at optimum conditions ensured that cells were effectively removed from the suspensions to form cell-rich sludges.
Figure 6.8: Optimum alum doses for suspensions of *M. aeruginosa*, *A. circinalis* and *C. raciborskii* at various cell densities. Coagulation pH was between 6 and 7.

6.3.2 Cyanobacteria-rich sludges and their solids densities

Each large scale coagulation experiment generated a sufficient amount of sludge to carry out measurement of solids density of the sludge and dewaterability characterisation. Information of solids density is important as it is used to determine the solids volume fraction of a sample. This is incorporated into the dewaterability analysis. As a result, direct dewaterability comparisons among different materials of different density are made possible.
As alum dose varied according to the cell density of the original cell suspension, every sludge sample produced from the large scale coagulation experiment had a unique composition. Therefore, the solids density of each sludge sample varied. The solid phase densities of the sludges, $\rho_{sol}$ were measured and are summarised in Table 6.1.

Alum sludges without cells contained only precipitated alum and as such, all had a similar solids density, around 2300 kg m$^{-3}$. This was regardless of the alum dose. Sludges containing cells had lower solids densities. The value varied between 1880 and 2180 kg m$^{-3}$. Cyanobacterial cells typically contain intracellular gas vesicles and water. The density of individual cells is comparable to the density of water and significantly lower than that of alum sludge. Therefore, the addition of cells to alum sludge always caused a reduction in the overall solids density. This is also reflected by the general decreasing trend of solids density with the increasing cell number in the sludge for all three types of cyanobacterial sludge.

<table>
<thead>
<tr>
<th>Sludge</th>
<th>Cell density (cells mL$^{-1}$)</th>
<th>Alum dose as Al (mg L$^{-1}$)</th>
<th>$\rho_{sol}$ (kg m$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alum</td>
<td>0</td>
<td>8.1</td>
<td>2350</td>
</tr>
<tr>
<td>Alum</td>
<td>0</td>
<td>8.9</td>
<td>2330</td>
</tr>
<tr>
<td>Alum</td>
<td>0</td>
<td>12.2</td>
<td>2370</td>
</tr>
<tr>
<td>M. aeruginosa + Alum</td>
<td>345000</td>
<td>8.1</td>
<td>2100</td>
</tr>
<tr>
<td>M. aeruginosa + Alum</td>
<td>466500</td>
<td>8.9</td>
<td>2040</td>
</tr>
<tr>
<td>M. aeruginosa + Alum</td>
<td>848750</td>
<td>12.2</td>
<td>1880</td>
</tr>
<tr>
<td>A. circinalis + Alum</td>
<td>350250</td>
<td>8.1</td>
<td>1990</td>
</tr>
<tr>
<td>A. circinalis + Alum</td>
<td>390000</td>
<td>8.9</td>
<td>1850</td>
</tr>
<tr>
<td>C. raciborskii + Alum</td>
<td>378900</td>
<td>8.1</td>
<td>2180</td>
</tr>
<tr>
<td>C. raciborskii + Alum</td>
<td>588750</td>
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<td>2130</td>
</tr>
<tr>
<td>C. raciborskii + Alum</td>
<td>828800</td>
<td>12.2</td>
<td>2091</td>
</tr>
</tbody>
</table>
6.3.3 Dewaterability under high pressure conditions

Pressure filtration tests were conducted to characterise dewaterability of cyanobacteria-rich sludge at higher solids volume fractions. For accuracy of measurement, the settled sludge was often pre-concentrated in the centrifuge before it was loaded into the filtration cell. Typical solids volume fractions of the sludge samples loaded in the filtration cell were between 0.002 and 0.005 v/v.

As an example, a single pressure filtration experiment was conducted on an *A. circinalis* alum sludge at 10 kPa. The gradient of filtration time, $t$ versus the square of specific filtrate volume, $V^2$ is plotted in Figure 6.9. It shows a long linear cake formation region followed by a short cake compression region. This is characteristic of typical non-biological materials (Stickland *et al.*, 2005). The same behaviour was observed in all cyanobacteria-laden sludges as well as pure alum sludges. This suggested that despite the high concentration of cells present, the alum precipitates in the sludge were dominant to the dewatering behaviour, causing a resemblance to a non-biological material such as a water treatment plant sludge (Harbour *et al.*, 2004). Due to the ‘traditional’ filtration behaviour (i.e. long cake formation time) (Stickland *et al.*, 2005), dewaterability of cyanobacteria-laden sludge at high solids volume fraction is able to be characterised rapidly using stepped pressure filtration tests.
Figure 6.9: $t$ versus $V^2$ plot for an *A. circinalis* alum sludge in a constant pressure filtration run at 10 kPa. (cell density: 390,000 cells mL$^{-1}$, alum dose: 8.9 mg L$^{-1}$ as Al, $\phi_0 = 0.00425$).

Figure 6.10 shows a plot of the compressive yield stress, $P_y(\phi)$, as a function of solids volume fraction for *M. aeruginosa*, *A. circinalis* and *C. raciborskii* alum sludges (MIC, ANA and CYP respectively) at the same alum dose of 8.1 mg L$^{-1}$ as Al. Results for pure alum sludge at the same dose (control) are also presented.
There was little difference in compressibility between the sludges produced from the three cyanobacterial species and the control sample. It appeared that once the sludges were compressed to a certain volume fraction (in this case, above 0.03), their compressibility became dominated by hydroxide precipitates, a type of sludge that is generally difficult to compress beyond 15-20 %v/v. Increasing the applied pressure had little impact on the sludge and the amount of water present in the final product remained high (greater than 85 %v/v). A study by Dixon et al (2004) found that hydrolysed alum has a very low solids concentration at the gel point ($\phi_{gel} = 0.003$) and the material failed to consolidate significantly by compression. The authors attributed this poor dewatering nature to its low density and small particulate dimensions. For a given volume fraction, the
Compressive yield stress has been shown to have an inverse squared dependence on the particle size (Zhou et al., 2001). As the particle size decreases, the total surface area of the system increases as well as the degree of inter-particle bonding, thus the total network strength of small particle system is higher than that of a large particle system at the same solids concentration. As a result, a much higher applied pressure is required in order to increase the solids content of the system. Moreover, the effect of the non-zero size of the electrical double layer around the small alum precipitates is expected to be more pronounced, resulting in a larger ‘effective’ particle size. As a consequence, the system starts to form a network at very low solids concentration (gel point) and is more recalcitrant to compression. The $P_y(\phi)$ profiles shown here indicates that the presence of alum precipitates had an adverse effect on the possible extent of dewatering for cyanobacteria-rich sludges.

Similar compressibility behaviour was observed at different alum doses (shown in Figure 6.11) with a small and expected variation in compressibility at increasing alum dose, assuming that at these doses, alum precipitates dominate the sludge compressibility.
Figure 6.11: Compressive yield stress of laboratory produced cyanobacteria-laden alum sludges at high solids volume fraction (alum doses 8.9 and 12.2 mg L\(^{-1}\) as Al).

Hindered settling function data for cyanobacteria-rich sludges and a pure alum sludge (control) of the same alum dose (8.1 mg L\(^{-1}\) as Al) are presented in Figure 6.12. Results for a high dose alum sludge and a low dose sludge, without the presence of cells, are also included on the plot (Verrelli et al., 2009). These two sludges were previously produced by performing coagulation on raw water collected from Sugarloaf Reservoir, Melbourne, using similar experimental methods and apparatus. They define the limits of \(R(\phi)\) for a typical WTP alum sludge (Verrelli et al., 2009).
Figure 6.12: Hindered settling function of laboratory produced cyanobacteria-laden alum sludges. *Results of the two laboratory produced WTP alum sludges are also provided for comparison.

At a fixed volume fraction, lower $R(\phi)$ values indicate a faster dewatering rate. Conversely, it means that to achieve the same throughput, the required size of the dewatering device is larger. At the higher solids volume fractions (> 0.05 v/v), the difference in $R(\phi)$ between the cyanobacteria-rich sludges and pure alum sludge was significant. At comparable $\phi$, the $R(\phi)$ for sludges containing cells was up to 5 times higher than that of a pure alum sludge. The values were of an order of magnitude higher than that of the low dose alum sludge, which clearly had the highest permeability. Although sludges may be dominated by alum precipitate, the result indicates that the materials also show some biological characteristics. Such sludges are typically highly impermeable beyond a certain
concentration (Stickland et al., 2008; Wall, 2008). In comparison to alum sludges, they contain high molecular weight and cross-linked biomolecules, resulting in a more compactable floc structure (Tiller and Kwon, 1998; Anderson et al., 2002). In a study comparing the dewaterability of the sludges produced from alum and from polymer coagulants, Harbour et al (2004) demonstrated that by replacing the open gelatinous nature of the alum hydroxide flocs with polymers, a higher solids content was attained. However, the sludge took much longer to dewater. Moreover, investigations on the dewaterability of dairy and sewage sludges also showed that the presence of high molecular weight extracellular polymeric substances (EPS) was responsible for the poor permeability of the materials (Studer, 2008; Wall, 2008). It is evident from the $R(\phi)$ data that the presence of the cyanobacterial cells has had an adverse impact on the permeability of the alum sludge, most likely due to the EPS fraction.

Among the cyanobacteria-rich sludges, one containing $C. raciborskii$ had the highest $R(\phi)$ (i.e. the lowest permeability) whereas $A. circinalis$ sludge has the highest permeability. Though both $A. circinalis$ and $C. raciborskii$ exist in long filaments in their natural form, filaments of $A. circinalis$ cultured in the laboratory were considerably shorter, consisting of only 2 to 8 cells per filament. They were sensitive to shear and easily split into unicellular form. $C. raciborskii$, however, had much longer filaments (20 cells per filament on average) and these long filaments remained after coagulation. As a result, the flocs in the sludge were expected to have a more open structure, providing extra hindrance to dewatering processes. The evidence suggests that the $R(\phi)$ values of this sludge are higher because of the greater particle asymmetry and greater number of inter-particle interactions.

$R(\phi)$ values of cyanobacteria-rich sludges at different alum doses are presented in Figure 6.13. At an alum dose of 8.9 mg L$^{-1}$ as Al, the $M. aeruginosa$ rich sludge had a lower permeability compared to the control sample (without cells). On the other hand, the difference diminished between the $R(\phi)$ values of the $A. circinalis$
rich sludge and those of the control. At an alum dose of 12.2 mg L\(^{-1}\) as Al, the permeability of \textit{C. raciborskii} rich sludge was still significantly lower than that of the control, indicating that the asymmetrical particle structure continued to play a role here. However, the permeability of the \textit{M. aeruginosa} rich sludge started to improve as the alum dose increased. The cell density of \textit{M. aeruginosa} for coagulation nearly doubled at the higher alum dose. One would expect a lower permeability in the resultant sludge as the number of cells increases. The opposite however was observed here. It appears that by increasing the alum dose, alum precipitates dominated the dewatering behaviour of this sludge, making it more permeable but difficult to compress at the same time.

![Figure 6.13: Hindered settling function of laboratory produced cyanobacteria-laden alum sludges (alum doses 8.9 and 12.2 mg L\(^{-1}\) as Al).](image-url)
More $R(\phi)$ data are shown in Figure 6.14. The plot demonstrates the effect of alum dose on the permeability of a cyanobacteria-rich sludge. At a given solids volume fraction, for *A. circinalis* sludge, $R(\phi)$ was higher than that of the control sample at an alum dose of 8.2 mg L$^{-1}$. As the dose increased to 8.9 mg L$^{-1}$, $R(\phi)$ values were reduced and became comparable to those of the pure alum sludge at the equivalent dose. For *M. aeruginosa*, this trend was not followed immediately when the dose was increased. The permeability of the sludge continued to decrease as the dose increased from 8.2 to 9.3 mg L$^{-1}$. This trend reversed and the permeability of the sludge improved significantly at an alum dose of 12.2 mg L$^{-1}$. The data indicates that it will take more than 6 times longer to dewater a sludge containing *M. aeruginosa* cells. Therefore, the presence of cells had a significant impact on the sludge dewatering behaviour, although alum precipitate was the dominating component in the sludge.
6.3.4 Dewaterability under low pressure conditions

The dewaterability of cyanobacteria-rich sludge at lower solids volume fraction was characterised using a LUMiFuge® Stability Analyser. The compressibility and permeability results are shown in Figure 6.15 and Figure 6.16 respectively.
Figure 6.15: Compressive yield stress of laboratory produced cyanobacteria-laden alum sludges at low applied pressures.

Sludges, with or without the presence of cells, showed a similar $P_y(\phi)$ profile as a function of solids concentration, although the *A. circinalis* rich sludge appeared to attain a slightly higher final solids concentration at any given pressure. This was also observed in the higher pressure filtration data. In the $P_y(\phi)$ characterisation experiment, sludge samples were dewatered in a LUMiFuge® Stability Analyser, at increasing rotational speeds, up to 3200 rpm. Despite these rotational speeds, the data in Figure 6.15 shows that the amount of solids in the settled sludge after centrifugation was less than 3 %v/v. This highlights the poor dewaterability of these sludges.
Figure 6.16: The hindered settling function of laboratory produced cyanobacteria-laden alum sludges at low applied pressures.

The $R(\phi)$ data shown in Figure 6.16 reveal that at low solids fraction, the dewaterability of cyanobacteria-rich alum sludges deteriorate rapidly with increasing solids volume fraction. At least a two order of magnitude increase in $R(\phi)$ is observed over a 0.02 volume fraction increase for all sludges. This suggests that the sludge, initially a dilute suspension, compresses very quickly to an impermeable network, which is extremely slow to dewater. The *A. circinalis* sludge had the highest permeability. This is consistent with the trend found in $R(\phi)$ at high applied pressure.
6.4 Comparison of dewaterability of a range of materials

The dewaterability of two industrial cyanobacteria sludge samples (a water treatment plant (WTP) A. circinalis alum sludge and a dissolved air flotation (DAF) M. aeruginosa sludge) were examined in this work. They were compared with the laboratory produced sludges. The comparative data is shown in Figures 6.17 and 6.18. Data for zirconia, sewage sludge, dairy sludge and WTP alum sludge obtained in other studies are also presented (Green et al., 1998; Studer, 2008; Verrelli, 2008; Wall, 2008).

The $P_f(\phi)$ results in Figure 6.17 show that across the solids volume fraction range tested, the pressure required to consolidate the material to a given solids volume fraction is the highest for alum sludges. Moreover, the difference of compressibility between cyanobacteria rich and cyanobacteria free alum sludge is small at the higher solids volume fractions, suggesting that alum precipitate is the dominating component in the cell-laden sludge and is the main reason these materials are difficult to compress.

At any chosen applied pressure, the two industrial cyanobacteria-containing samples were able to compress to a higher final solids volume than the laboratory produced samples. The difference is most noticeable in the DAF sludge. The DAF sludge contained predominantly M. aeruginosa cells with polymeric flocculant and alum, the latter at a very low dose. It is likely this very low dose when compared to the WTP sludge means that the impact of alum precipitates on the extent of dewatering is less and the DAF sludge can be dewatered to a greater extent.

The marine algae sludges demonstrate a significantly higher extent of dewatering relative to other biological sludges, and are similar to a zirconia slurry. As discussed in the previous section, the hard sphere-like diatom species present in the marine algae culture is likely to aid their dewaterability relative to soft particulates such as cyanobacteria cells and other bacteria and cells that...
produce a large amount of EPS. However, the extent of diatom presence in the mixed culture is unknown. The high ionic strength in marine samples could also be a contributing factor in making the flocs more compact and easy to compress due to aggregation at high salt.

Figure 6.17: The compressive yield stress of different materials.
Figure 6.18: The hindered settling function of different materials.

Figure 6.18 shows that $R(\phi)$ values for both sewage and dairy sludges are up to four orders of magnitude larger than for inorganic material at an equivalent particle/cell size such as zirconia. As discussed in Section 6.3.3, the primary cause of the intractable dewatering nature of these materials sludges is assumed to be the presence of EPS. Most cyanobacteria alum sludges produced from the laboratory show higher $R(\phi)$ values than pure alum sludge under high pressure conditions. However, these values are still considerably lower than that of sewage and dairy sludges, suggesting alum precipitates are still the dominating component in the sludges. Although the dewatering properties of pure cyanobacteria cells were not fully characterised, a constant pressure filtration run
at 50 kPa on a dense *M. aeruginosa* culture (φ around 0.01) indicated that cyanobacteria cells were extremely slow to dewater. The time taken to reach an equilibrium dewatered state was more than a week, compared to one day for alum coagulated samples. Thus, it is believed that a sludge dominated by cyanobacterial cells will show similar behaviour to that of sewage and dairy sludges. The DAF *M. aeruginosa* sludge proved this to be the case. It had a low permeability at low solids concentration but became highly impermeable as it was compressed to higher solids. The $R(\phi)$ value eventually reached the same magnitude as that of dairy and sewage sludges (bacteria rich sludges). The WTP *A. circinalis* sludge, on the other hand, was more permeable. This sludge was produced from raw water that had a cell density below 30,000 cells mL$^{-1}$ (at least 10 times lower than the concentration in laboratory prepared suspensions). Moreover, the sludge contained a large amount of powdered activated carbon, which was dosed to the raw water before the coagulation stage. These carbon particles are likely to have enhanced the permeability remarkably, causing a two orders of magnitude decrease in $R(\phi)$. As a result, this material dewatered much faster than the other WTP sludges.

### 6.5 Conclusions

The dewatering behaviour of cyanobacteria-rich sludges produced from *M. aeruginosa*, *A. circinalis* and *C. raciborskii* have been characterised in terms of their extent and rate of dewatering. These types of sludges demonstrated poor dewaterability. They were similar in compressibility to pure alum sludges. The final products had a low solids content despite the application of a high pressure. Their rate of dewatering declined extremely quickly as the solids volume fraction increased. Sludges become highly impermeable under high pressure conditions and the time required to reach equilibrium during dewatering increased from hours to days. This is expected to cause major problems for drinking water production facilities. Existing dewatering equipment in treatment plants will not be able to handle sludges containing a high percentage of cyanobacterial cells.
More importantly, when the cells start to lyse in the sludge, during processing, water recovered from the process may contain high concentrations of toxic metabolites or unpleasant T&O compounds, rendering the water unusable.

With $P_y(\phi)$ and $R(\phi)$ data, the residence time of sludges in a range of dewatering processes can be predicted accurately. With the metabolite release data (discussed in Chapter 5) incorporated into the timescale of sludge dewatering, an inter-relationship between sludge processing and metabolite release can be established. This allows the selection of suitable treatment processes and optimum operation conditions and ensures that the risk of cyanobacterial contamination is at minimum.
7.1 Introduction

It has been shown (Chapter 6) that water treatment plant sludges containing cyanobacteria cells have poor dewaterability and as such, it is highly likely that existing facilities in conventional water treatment processes may not be able to handle a high inflow of cyanobacteria cells. Other ways for cell removal were then considered.

In the past two decades, membrane filtration has emerged as a promising technology for the production of high water quality and it is also an effective way of removing biocolloids. Chow et al. (1997) studied the efficiency of membrane filtration in cross-flow and dead-end (DE) modes on M. aeruginosa cells using flat-sheet microfiltration (MF) and ultrafiltration (UF) membranes. They found that more than 98% of the cells were removed successfully by the process and no significant increase of microcystins was observed in the permeate. Similar observations have been documented in several other cross-flow filtration studies (Mouchet and Bonnelye, 1998; Gijsbertsen-Abrahamse et al., 2006; Campinas and Rosa, 2010).

The aim of this study is to evaluate the removal efficiency of laboratory grown M. aeruginosa, A. circinalis and C. raciborskii cells via cross-flow filtration at different transmembrane pressures. Special attention is given to MF membranes as they have larger pore size compared to UF membrane and therefore lower membrane resistance. The MF process is also operated at lower pressures than UF, requiring lower energy. A sound understanding of cross-flow filtration behaviour and membrane fouling with cyanobacterial cells is important as it ensures the maximum cell removal efficiency and safe water quality. The knowledge can also
benefit other applications, for example, *Cryptosporidium* and *Giardia* removal in drinking water treatment (Gijsbertsen-Abrahamse *et al.*, 2006) and marine algae removal in desalination (Ladner *et al.*, 2010). In the latter case, seawater was found to cause severe reverse osmosis fouling due to the presence of dinoflagellate phytoplankton. Pretreatment of seawater using cross-flow MF and UF was recommended to remove these problematic algal species. The impact of the MF process on cell damage and metabolite release to the treated water is also examined herein. The outcomes of the study will help determine the most suitable operating conditions for efficient cell removal and safe water production by cross-flow MF processing.

### 7.2 Experimental methods

#### 7.2.1 Cyanobacteria suspensions

Feed suspensions were prepared by mixing laboratory cultures of *M. aeruginosa* (MIC), *A. circinalis* (ANA) and *C. raciborskii* (CYP) with ASM-1 medium. Cultures were taken for experiments at the late exponential growth phase. 5 L of feed suspension was made for each filtration run one day in advance and was maintained at 25 °C under continuous illumination before the experiment.

#### 7.2.2 Cross-flow filtration experiments

The experiments were performed with a lab-scale cross-flow unit (Sterlitech SEPA CF Membrane Element Cell) with an active filtration area of 140 cm². A schematic diagram of the system is provided in Figure 7.1. The unit consists of a filtration cell and a cell holder. The cell was inserted in the cell holder and was pressurised by supplying compressed air through a fitting on the side of the cell holder at 600 kPa. The feed stream was pumped from the feed tank by a positive displacement pump with variable speed (650 Series, SSD Drives) and entered at the bottom of the filtration cell. The stream flowed parallel to the membrane and generated shear forces at the membrane surface. Particles rejected by the
membrane were carried away with the flow and exited through a circular opening at the bottom end of the cell. The concentrate stream, also known as the retentate, was recirculated back to the feed tank and mixed with the feed suspension. The permeate passed through the membrane and exited through the permeate outlet located at the top end of the filtration cell. The permeate was collected in a beaker placed on an electronic balance. The mass of permeate over time was measured with the balance and was recorded by LabVIEW software at 3 second intervals. Pressures at the feed and the retentate sides were monitored and the transmembrane pressure (TMP) of each run was adjusted via the back pressure regulator located on the retentate side.

Within the cell, the shim (a thin stainless steel spacer), foulant (feed) spacer, and permeate carrier were assembled together with the membrane in the order shown in Figure 7.2. The shim and the spacer provided channels for the feed suspension to be transported from the feed to the retentate end of the cell. The spacer also helped to create turbulent flow against the membrane surface and hence promotes resistance to fouling. The permeate carrier directed the flow towards the permeate outlet.

Polyethersulfone flat sheet membranes (Koch membranes) with a nominal pore size of 0.1 µm were used for all experiments. Each membrane was cut to fit the filtration area of 140 cm² (14.6 cm in length and 9.5 cm in width) provided by the filtration cell. The membranes were rinsed to remove chemical preservatives and soaked in Milli-Q water overnight prior to experimentation.

Experiments were conducted at a constant TMP of 50 and 150 kPa and cross-flow velocity of 0.5 m·s⁻¹. Filtration under dead-end (DE) filtration mode was also examined. This was achieved by increasing the TMP via the back-pressure regulator until flow of the retentate stream stopped in the unit. A TMP of 270 kPa was obtained in DE mode for the chosen cross-flow velocity. Before each experiment, the pure water flux was measured by feeding deionised water to the
system at the selected TMP and cross-flow velocity for 10 minutes or until steady state was reached. The feed was then switched to cell suspension. The experiments ran for 2.5 hours. At the end of each experiment, the membrane was cleaned thoroughly while still in the cell. This clean-in-place (CIP) method involved 3 cycles in the following order:

- Clean with deionised water for 20 minutes at a cross-flow velocity of 0.8 m\(\cdot\)s\(^{-1}\)
- Clean with NaOH solution for 10 minutes at the same cross-flow velocity, solution pH between 10.5 and 10.8
- Clean with deionised water for 10 minutes at the same cross-flow velocity

To examine the cleaning efficiency, the pure water flux was then measured at the experimental TMP and cross-flow velocity.

Figure 7.1: Schematic of laboratory cross-flow filtration system (PI: pressure gauge, PC: data logging computer).
7.2.3 Cell viability and metabolite analysis

Cell viability as well as total and dissolved metabolite concentrations were determined in the feed suspension prior to each experiment. The analytical methods are detailed in Sections 4.4 and 4.5. At the end of the experiment, cell counting was conducted on the permeate samples and the dissolved metabolite concentration in the permeate was measured. In addition, cell viability and dissolved metabolite concentration were determined in the retentate. A small sample was also taken for cell viability assay from the rinse water that was collected for 5 seconds after the first cleaning cycle was commenced. This water should contain any cells that had been trapped in the fouling layer.
7.2.4 Long term filtration trials

The long-term cross-flow filtration behaviour of cyanobacteria suspensions was examined in a series of 75 hour filtration runs. The experiments were conducted in recirculation mode where the retentate stream was continuously returned to the feed tank. Each experiment was operated at a TMP of 50 kPa and cross-flow velocity of 0.5 m \( \text{s}^{-1} \). The permeate collected over the first 28 hours was poured back to the feed tank without interrupting the run. This was repeated at \( t = 53 \) h. Samples were taken periodically from the permeate for dissolved metabolite analysis throughout the run. At the end of the run, the CIP technique employed in the short experiments was also used here. The pure water flux was measured after CIP operations.

7.3 Cell removal

For all three species examined, no cells were detected in the permeate collected over each filtration run regardless of the cell densities in the feed water. The results are consistent with previous studies (Gijsbertsen-Abrahamse et al., 2006; Campinas and Rosa, 2010; Dixon et al., 2011b). The pore size of the membrane was at least one order of magnitude smaller than the cells, therefore the MF process was able to remove cells completely.

In comparison, the performance of cell removal via conventional treatment processes is dependent on the raw water quality. As the cell population in raw water varies each time, coagulation with an insufficient amount of coagulant will result in poor performance in cell removal. The presence of other components in raw water such as natural organic matter (NOM) will also complicate the coagulation process and increases the coagulant demand. Moreover, as discussed in Section 2.5.6.3, direct sand filtration is not able to remove cells and intracellular metabolites completely even though the filter can sometimes act as a biological media to degrade metabolites.
MF is a reliable process for cyanobacteria removal and can potentially be used as a pretreatment step to reduce the amount of cells and metabolites that conventional processes have to handle. However, the efficacy of the process is largely determined by membrane fouling behaviour and the resultant flux decline profiles. These will be discussed in the following sections.

7.4 Flux decline at different TMPs

An example of the permeate flux versus time profiles for *M. aeruginosa* at different TMPs is given in Figure 7.3. The initial flux increases with increasing TMP. A sharp flux decline is observed within 30 minutes of operation followed by a slower decline for both 150 kPa and 270 kPa (DE mode). The change in flux is more gradual at 150 kPa. No flux decline is observed at 50 kPa over the course of the filtration run.

In membrane filtration, flux decline is most usually caused by the development of a resistive layer either on or in the pore structure of the membrane. In cross-flow filtration, the deposition of solids to the membrane surface is competing against shear generated from the cross-flow. At 50 kPa, it is postulated that the shear effect dominates over the deposition of cells on the membrane. As a result, a constant permeate flow is obtained as cells are continuously swept away from the membrane surface. In fact, this suggests that the filtration is operated close to the so-called ‘critical flux’. Howell (1995) proposed the concept of a critical flux in MF and defined it to be the flux below which there is no deposition of particles on the membrane. Flux decline is expected to be insignificant at this operation condition. This critical flux has also been observed by other authors (Field *et al.*, 1995; Li *et al.*, 1998). Li *et al* (1998) developed a method that allowed continuous, in situ, direct observation of particle deposition on membrane surfaces. The experiments were conducted on yeast cells and latex beads. The microscopic image of the membrane during the 30 minute filtration run confirmed that there exists a critical flux, below which particle deposition on the membrane
is negligible. Their operation conditions of filtration of yeast cells (mean diameter 5 \( \mu \)m) are similar to the ones used here (a flux of 20 L\( \cdot \)m\(^2\)\( \cdot \)h\(^{-1}\) and cross-flow velocity of 0.74 m\( \cdot \)s\(^{-1}\)). However, in this study, cells were detected in the water that went through the first cleaning circle. Although cells were trapped in various locations within the system (e.g. pump and piping), it is possible that some cell deposition has occurred during the 50 kPa run.

![Flux-time relationship at various TMPs.](image)

**Figure 7.3**: Flux-time relationship at various TMPs. MIC cell density: 900,000 cells\( \cdot \)mL\(^{-1}\), cross-flow velocity: 0.5 m\( \cdot \)s\(^{-1}\).
At higher TMP, i.e. 150 kPa, the filtration rate is initially higher than the one obtained in the low pressure run. By applying higher pressure, more permeate is forced through the membrane pores. However, higher pressure is also accompanied by a higher transport of solids towards the membrane surface. The hydrodynamic effect of cross-flow is reduced and cells deposited on the membrane cannot be sheared off as easily and quickly as at lower TMP. As a result, membrane fouling occurs via pore blockage and cake build up, leading to a reduced permeate flow. In DE mode, the initial permeate flux is the highest. However, without shear, cells deposit onto the membrane continuously and a cake layer keeps growing. The growing cake layer provides an increasing resistance in addition to the membrane resistance, causing a more rapid flux decline.

The same set of experiments generate mass of permeate over time data. The comparison between different pressures is shown in Figure 7.4. At the beginning of the filtration run, the permeate is accumulated more quickly at the highest TMP due to the higher filtration rate. As the membrane starts to foul, the production of permeate slows. 50 minutes into the run, the amount of permeate produced at 150 kPa surpasses that at 270 kPa and remains higher throughout the rest of the run. This result demonstrates the clear advantage of cross-flow filtration over DE filtration. Although fouling is observed at both pressures, it occurs at a slower rate in the cross-flow mode with the aid of shear. At 50 kPa, the initial permeate flux is significantly lower than at the other two pressures. As a result, the mass of permeate collected during the experiment is lower. Meanwhile, the flux remains constant, indicating that minimal fouling has occurred at the low pressure. Although the permeate production is initially higher for the high pressure runs, a crossover is anticipated over extended filtration time due to severe membrane fouling encountered at the higher pressures.
Figure 7.4: Accumulation of permeate over time at various TMPs. MIC cell density: 900,000 cells mL⁻¹, cross-flow velocity: 0.5 m s⁻¹.

The flux-time relationship in Figure 7.3 can be replotted as permeance versus time (as shown in Figure 7.5). If TMP dictates the energy demand of the process, it is clear from the permeance decline profiles that operating at low TMP has a great advantage. Despite the higher initial values obtained at 150 kPa and 270 kPa, this only lasted briefly (less than 30 min) and permeance obtained at 50 kPa remains far superior than at high pressures for the rest of the run.
More importantly, Figure 7.5 indicates that the impact of pressure on membrane fouling is not linear. This simple exercise does not represent fouling quantitatively and one must consider the fact that higher TMP gives higher cake resistance. However, if particle deposition and cake formation are the only contributors to membrane fouling, the permeance decline profiles are expected to be closer. It is thought that other factors may have also caused fouling.
As discussed in Chapter 6, the presence of cyanobacteria caused a decrease in permeability of alum sludge. EPS is believed to be responsible for this observation. The permeability of pure cyanobacterial sludge is predicted to be lower than for the alum rich case. To give an example, the dewaterability data of untreated freshwater algae presented in Chapter 6 (Figure 6.17 and 6.18) are curve-fitted and re-plotted as \( R(\phi) \) versus \( P_y(\phi) \) (Figure 7.6). Overall, more than four orders of magnitude decrease in permeability, as demonstrated by \( R(\phi) \), was noted from low to high pressures. More specifically, when the pressure is increased from 50 to 270 kPa, the permeability deteriorates by a factor of 15. This trend in dewatering characteristics mirrors the permeance decline behaviour observed here. It is most likely that EPS has played a role in membrane fouling, escalating flux decline at high pressures. The detrimental impact of extracellular organic matter (EOM) on membrane fouling has been documented by other authors (Her et al., 2004; Henderson et al., 2008; Qu et al., 2012a; Qu et al., 2012b). High operating pressure will lead to an increase in solid flux towards the membrane surface and consequently faster cake development on the membrane. Based on the dewatering behaviour observed from freshwater algae, it is postulated that the process will suffer a significant reduction in the production rate due to severe decrease in permeability, suggesting that low pressure operation may be more favourable.
7.5 Comparison of flux profiles of different species

MIC, ANA and CYP have different cell morphology (as shown in Figure 4.1), though the size of the individual cells are comparable. Laboratory cultured MIC are unicellular spherical cells while the other two species are filamentous. Connell et al (1999) found that particle shape had an influence on cross-flow filtration flux because of the structure of the cake formed on the membrane. The study compared the flux profiles of a range of materials including spherical and irregular, branched particles. A slower flux decline and higher pseudo-equilibrium flux were observed for the latter. The scanning electron microscope images of
the membranes provide evidence that spherical particles such as silica deposit closer together on the membrane in an evenly distributed manner and form a compact cake layer whereas irregularly shaped particles, e.g. graphite, are bridged further apart, forming loose and a less evenly distributed cake layer that leaves larger gaps for the passage of permeate. As a result, the overall resistance of permeate flow is lower in the latter case, leading to a higher flux.

Experiments on spherical cells (MIC) and long filaments (ANA and CYP) were expected to generate different results. Flux data for each cyanobacteria species at three TMPs is presented in Figure 7.7. Feed water was prepared at the same cell density. At 50 kPa, no flux decline occurs for all three species. The deposition of cells on the membrane is negligible during the filtration run, as discussed in Section 7.4. At 150 kPa, flux decline is observed in both spherical cells and filaments. MIC filtration experiences more rapid decline and the flux remains lower than the other two throughout the run. The porous cake layer formed by the filamentous species is believed to be responsible. This observation is also made by Dixon et al (2011a) in UF of M. flos-aquae and A. circinalis from natural waters. Removal of DOC and colour through UF membrane was greater in a water sample with M. flos-aquae present. The cake layer formed by A. circinalis is less dense than by M. flos-aquae, allowing a higher penetration of fine particles through the system.
Particle size is another factor that can contribute to the different flux decline behaviours. Baker et al. (1985) uses a Scour Model to explain the influence of particle size on flux decline. The model was first proposed by Fane (1984). The scouring motion generated by the tangential flow preferentially dislodges large particles from the cake. It is proposed here that long filaments of ANA and CYP that protrude above the cake surface are therefore more susceptible to scour, preventing the development of cake layer resulting in slower flux decline.
In DE filtration, the flux decline in spherical cells is faster in the beginning. At $V = 0.05$ m (around 40 minutes), the flux starts to approach those of the filamentous species. Although spherical cells are packed closely on the membrane and provide a higher resistance, large filaments also build up on the membrane continuously. In the absence of cross-flow, no particles can be swept away and larger particles provide additional resistance through a thicker cake layer. Moreover, higher TMP also causes the cake to be packed closer regardless of the particle sizes. As a result, the difference in flux between the three species diminishes over time.

### 7.6 Effect of cell density of feed water

Two cell densities in feed water were selected to examine the effect on flux decline: 500,000 and 900,000 cells mL$^{-1}$. Normalised flux profiles obtained for MIC and CYP are presented in Figure 7.8 and Figure 7.9 respectively. Flux $J$, is normalised to the pure water flux ($J_0$) obtained prior to each experiment.
Figure 7.8: Effect of cell density on MIC filtration. Cross-flow velocity: 0.5 m s\(^{-1}\).
Increasing the solids concentration of feed water has generally shown to lower the permeate flux (Riesmeier and Kroner, 1987; Wakeman and Tarleton, 1991; Chang and Hwang, 1994; Tarleton and Wakeman, 1994). As the concentration increases, the flux of solids towards the membrane surface increases leading to higher probability of solids deposition and cake formation. This effect is not observed at 50 kPa TMP for both species, indicating that nearly doubling the cell density here is not sufficient to cause membrane fouling as shear generated by the cross-flow velocity is able to sweep all the particles back into the bulk suspension. The effect of solids cell density in feed water is more pronounced at higher pressures. Cell deposition occurs at both 150 kPa and 270 kPa and
membrane fouling is exacerbated by the increase of solids concentration. By introducing higher solid flux towards the membrane surface, the cake layer grows more rapidly resulting in an increased resistance to filtration. A less remarkable difference on flux profiles between two cell densities is observed in CYP. This is in agreement with related work by Connell et al (1999) where they found that the flux of branched carbon particles was not affected by the increasing concentration. CYP filaments are expected to form a loose, porous cake at high pressures and provide a lower resistance to filtration, despite a possible increase in the cake mass due to an increase in cell density. More importantly, the elongated shape and large particle size also means that they are more susceptible to scour and are more easily removed from the existing cake layer by cross-flow. In DE filtration mode, however, the effect of concentration is smaller for both species. The desired hydrodynamic cross-flow effect is removed in this case, causing rapid cake build up at both cell densities. MIC results show a slightly bigger difference indicating that the flux decline is more responsive to an increase in cake mass as the spherical cells are packed more closely on the membrane compared to long filaments.

It is noted that due to the difference in the size and shape of the individual cell between the two species, the total biovolume of the species will be different at the same cell density. This can introduce possible error when comparing the flux decline profiles at the selected cell densities. However, the difference in biovolume is small and is not likely to cause any significant impact on the flux profiles.
7.7 Effect of cross-flow filtration on cell viability and metabolite release

The most important factor that determines the application of cross-flow filtration technology on cyanobacteria removal is its effect on cell viability and the subsequent release of problematic metabolites. A number of studies have addressed this topic (Chow et al., 1997; Gijsbertsen-Abrahamse et al., 2006; Campinas and Rosa, 2010), but there is limited knowledge on the impact of applied TMP on cell damage. Selecting a suitable operating pressure for cross-flow filtration is vital as it not only gives the optimum flux profile but most importantly, minimises the impact of cyanobacteria on the water quality. To help determine the effect of filtration process at various pressures, the dissolved metabolite concentration in feed suspension at the beginning ($t = 0$) and the end ($t = 150$ min) of the filtration run were measured as well as the dissolved metabolite concentration in the permeate at $t = 150$ min. The cell viability of feed suspension was examined at $t = 0$ and 150 min. The viability of the cells that were washed off in the first 5 seconds of the cleaning cycle was also determined. The results for different cyanobacterial species are presented in the following sections.

7.7.1 *M. aeruginosa*

Dissolved microcystin-LR (MC-LR) concentrations in feed suspension at $t = 0$ (feed) and $t = 150$ min (retentate) and permeate at $t = 150$ min are plotted in Figure 7.10. The dissolved MC-LR concentration in the permeate is lower than that in the feed. MC-LR has a molecular weight around 1000 Da (approximately 3 nm) while membranes used in this work have a pore size of 0.1 µm. The membrane is able to reject cell-bound MC-LR via cell removal but not dissolved extracellular toxins. The decrease in metabolite concentration in permeate is most probably due to adsorption of MC-LR to the membrane. This adsorption behaviour has been documented by several studies (Gijsbertsen-Abrahamse et al., 2006; Campinas and Rosa, 2010; Dixon et al., 2011a). The investigation by
Gijsbertsen-Abrahamse et al. (2006), on the removal of cell-bound microcystins using UF revealed the same trend on the dissolved toxin concentrations of feed and permeate. In a study of UF on _M. aeruginosa_ cultures at different age, Campinas and Rosa (2010) attributed similar behaviour to adsorption of microcystin to the membrane and to the algogenic organic matter (AOM). AOM contains polysaccharides and proteins that are capable of forming an adhesive layer and causes serious membrane fouling (Her et al., 2004; Lee et al., 2006; Dixon et al., 2011b). Comparison of microcystin absorption between 2-month old and 4-month old _M. aeruginosa_ samples revealed that higher microcystin rejection by UF membrane was achieved in parallel to cell damage in the older sample. Dissolved microcystins are postulated to aggregate or be enmeshed onto AOM. More adsorption occurs in the older sample because _M. aeruginosa_ is found to produce increased amount of AOM with ageing (Pivokonsky et al., 2006). Moreover, the nature of the membrane can be a factor in enhancing microcystin adsorption. Hydrophobic polyethersulfone (PES) membranes were used in this work. A study by Lee and Walker (2008) found that MC-LR adsorbed to PES membranes due to the hydrophobic interaction of MC-LR with the membrane. MC-LR has a hydrophobic nature in aqueous media (Lee and Walker, 2006).

The effect of TMP on MC-LR release to permeate cannot be determined at 50 kPa and 150 kPa as it is masked by adsorption. In high pressure dead-end mode, however, the dissolved MC-LR concentration in permeate is higher than in the feed. This indicates that as the MC-LR adsorption sites on the membrane become saturated, breakthrough of the toxins occurs. Although the effect of TMP cannot be quantified easily with the presence of adsorption, high pressure has clearly shown to cause a greater damage to the cells throughout the process.
The dissolved MC-LR concentrations in retentate at 50 and 150 kPa are both higher than the feed concentration. The concentration is higher at 150 kPa than at 50 kPa. This suggests that increasing the TMP has a stronger effect on metabolite release as the cells deposited on the membranes experience more compression. Moreover, shear generated by the cross-flow velocity is presumed to be partially responsible for the release of MC-LR. It is worth noting that the water recovery of the filtration process, which is defined by the ratio between permeate and feed flow-rates, is very low (0.75% at 50 kPa and 3.3% at 150 kPa) in the laboratory unit. This means that the number of passes for individual cells to be circulated through the system is higher than that expected at an industrial scale. Hence, the amount of shear experienced by these cells is more significant, causing more cell damage. The low water recovery in the laboratory unit also means that cells have a longer residence time in the system which leads to a higher risk of intracellular metabolite release through cell ageing and lysis.

To further validate the observations above, a cell viability assay was also carried out via the double staining method. The results are presented in Table 7.1. At $t = 0$, 95% of the cells in the feed water were active (FDA +ve) and no dead cells were detected (Sytox® Green –ve). For both 50 kPa and 150 kPa, the percentage of active cells in the retentate at $t = 150$ min decreased slightly from that in feed suspension at $t = 0$. Meanwhile, cells circulating through the system remained alive based on the Sytox® Green results. Tests on cells that came from the rinse water in CIP saw a greater decrease in active cells at all three pressures. A significant amount of dead cells were detected in DE filtration mode. This agrees with the dissolved MC-LR concentration results for DE discussed earlier and demonstrates again the strong impact of high pressure on the cells deposited on the membrane. In comparison, cells that were retained on the membrane have maintained their integrity in cross-flow mode at both TMPs. Therefore, it is concluded that cross-flow filtration causes no damage to the cells within the experimental period.
It is noted that the cell viability results on rinse water samples can only be used as an indication of the trend observed here instead of a direct measure of cell damage caused by the filtration process. This is because each rinse water sample contained a small amount of feed or retentate that was purged out of the system initially during CIP. In general, this water contained predominantly healthy active cells and minimal dead cells. The results are therefore influenced by the presence of these cells. For this reason, the cell viability in rinse water will not correlate strictly with the percentage of metabolite release in permeate or retentate. However, the results will complement the latter to give an indication of the impact of the process on cells.

![Graph](image)

**Figure 7.10:** Concentration of dissolved MC-LR in the feed, permeate and retentate during filtration runs with MIC cells. Cell density: 900,000 cells mL\(^{-1}\), cross-flow velocity: 0.5 m s\(^{-1}\), total MC-LR concentration in the feed: 25.1 µg L\(^{-1}\).
Table 7.1: Cell viability of MIC at various TMPs. MIC cells in the feed: 95% FDA +ve and Sytox® Green +ve nil.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Stain</th>
<th>50 kPa</th>
<th>150 kPa</th>
<th>270 kPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retentate</td>
<td>FDA +ve</td>
<td>90%</td>
<td>91%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Sytox Green +ve</td>
<td>1%</td>
<td>&lt;1%</td>
<td>-</td>
</tr>
<tr>
<td>Rinse water*</td>
<td>FDA +ve</td>
<td>75%</td>
<td>76%</td>
<td>71%</td>
</tr>
<tr>
<td></td>
<td>Sytox Green +ve</td>
<td>1.1%</td>
<td>&lt;1%</td>
<td>18%</td>
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</tbody>
</table>

*water collected for 5 seconds after commencing the first cleaning cycle in CIP operation and containing any cells that were trapped in a removable fouling layer.

### 7.7.2 A. circinalis

Results of dissolved metabolite concentrations and cell viability are presented in Figures 7.11 and 7.12 and Table 7.2 respectively. It is noted that geosmin concentration is often underestimated due to the volatile nature of the compound. Although HgCl$_2$ has been added to minimise geosmin degradation when the samples were bottled, a significant loss of extracellular geosmin is inevitable in both permeate and retentate throughout the experiment before samples were taken. To reduce the geosmin loss, the feed tank and permeate collection beaker were both covered. Nevertheless, the geosmin results, used alongside the saxitoxin results, give an indication of the impact of the filtration process on ANA.
Figure 7.11: Concentration of dissolved geosmin in the feed, permeate and retentate during filtration runs with ANA cells. Cell density: 900,000 cells mL$^{-1}$, cross-flow velocity: 0.5 m s$^{-1}$, total geosmin concentration in the feed: 2380 ng L$^{-1}$. 
Figure 7.12: Concentration of dissolved saxitoxin in the feed, permeate and retentate during filtration runs with ANA cells. Cell density: 900,000 cells mL\(^{-1}\), cross-flow velocity: 0.5 m s\(^{-1}\), total saxitoxin concentration in the feed: 10.4 µg L\(^{-1}\).

At 50 kPa, dissolved geosmin and saxitoxin concentrations in the permeate are similar to those in the feed. Adsorption of metabolites to the membranes cannot be overlooked. In particular, adsorption of saxitoxin has been observed by Dixon et al (2011b) in a study of UF removal of A. circinalis. The results nonetheless provide some information on the overall impact of the process. The dissolved geosmin concentration in the retentate was not obtained, but no increase in dissolved saxitoxin concentration in the retentate is observed over the duration of the experiment. Therefore, it is concluded for these cells that low pressure processes cause no harm to ANA cells. This is further indicated by the cell viability results. The percentage of active cells present in the rinse water sample is low. This sample was likely to contain any cells that were part of the fouling
layer of the membrane. The low proportion of active cells is probably due to the movement of cells deposited on the membrane being restricted. As a result, cells experience more stress. However, no dead cells were found throughout the system and as such, there was minimal metabolite release.

Filtration at higher pressures appears to have a stronger impact on the cells. Both permeate and retentate have elevated concentrations of geosmin and saxitoxin. If all the extracellular metabolites in the feed are assumed to pass through the membrane and end up in the permeate, i.e. no adsorption of metabolites to the membrane has occurred, it can be estimated that 4.8% and 37.7% of the intracellular geosmin would be released into the permeate at 150 kPa and 270 kPa (DE) respectively. Meanwhile, 23.9% of the intracellular saxitoxin is released at 150 kPa and 67.4% is released at DE mode. The release is expected to be higher if adsorption is taken into account. Cell viability results also revealed significant cell lysis in the rinse water sample in DE mode, whereas no dead cells have been detected in the 150 kPa system.

Operating at high TMPs for ANA generally causes higher risk with more cell damage and intracellular metabolite release. The results are in agreement with the finding by Matsushita et al (2010). In the study of geosmin release from cells during MF, the authors observed geosmin release at increased TMP as a result of cell breakage. The possibility of geosmin adsorption to MF membrane was also proposed by the authors.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Stain</th>
<th>50 kPa</th>
<th>150 kPa</th>
<th>270 kPa</th>
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<td>&lt;1%</td>
<td>-</td>
</tr>
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</tr>
<tr>
<td></td>
<td>Sytox® Green +ve</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>13%</td>
</tr>
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</table>
7.7.3 *C. raciborskii*

The results on dissolved cylindrospermopsin (CYN) are illustrated in Figure 7.13. At 50 kPa, the dissolved CYN concentration in the permeate is lower than that in either the feed or retentate. Since the size of CYN is orders of magnitude smaller than the membrane pore size (CYN molecular weight 165 Da), there is a strong indication of CYN adsorption to the membranes. Compared to MC-LR, there appeared to be a greater degree of adsorption. The dissolved CYN concentration increases at higher TMPs, implying that more CYN is released. The breakthrough of CYN occurred eventually once the adsorption sites on the membrane were saturated. These results prove again that high pressure operation incurs a higher risk of toxin release and therefore should be avoided. Shear effects are also likely to contribute to the release of intracellular CYN but this cannot be observed due to the decrease of dissolved CYN concentration in the permeate. Furthermore, cell viability results presented in Table 7.3 showed that cross-flow filtration at both TMPs causes no damage to the cells. Cells in the retentate and the rinse water samples remained active during the experiments.
Figure 7.13: Concentration of dissolved CYN in the feed, permeate and retentate during filtration runs with CYP cells. Cell density: 900,000 cells mL\(^{-1}\), cross-flow velocity: 0.5 m s\(^{-1}\), total saxitoxin concentration in the feed: 41.3 µg L\(^{-1}\).

Table 7.3: Cell viability of CYP at various TMPs. CYP cells in the feed: 100% FDA +ve and Sytox\textsuperscript{®} Green +ve nil.

<table>
<thead>
<tr>
<th>Sample</th>
<th>FDA +ve</th>
<th>50 kPa</th>
<th>150 kPa</th>
<th>270 kPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retentate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FDA +ve</td>
<td>100%</td>
<td>100%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sytox\textsuperscript{®} Green +ve</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Rinse water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FDA +ve</td>
<td>100%</td>
<td>95%</td>
<td>91%</td>
<td></td>
</tr>
<tr>
<td>Sytox\textsuperscript{®} Green +ve</td>
<td>0</td>
<td>&lt;1%</td>
<td>11%</td>
<td></td>
</tr>
</tbody>
</table>
7.8 Long term cross-flow filtration trials

Results in the previous section indicate that it is safer to operate the cross-flow filtration process at low pressure in order to minimise cell damage and metabolite release. The other benefit of low pressure operation, as discussed previously, is low membrane fouling. In the 2.5 hour period, flux remains constant and negligible cell damage occurs for all three species. However, the long term behaviour is not known. To address this, 75 hour filtration trials were performed on MIC and CYP suspensions at 50 kPa. The flux decline profiles for the two trials are plotted in Figure 7.14. The flux after each refill (permeate return to the feed tank) is plotted in a new colour.

![Figure 7.14: Long term flux-time relationship at 50 kPa. Cell density: 500,000 cells mL⁻¹, cross-flow velocity: 0.5 m s⁻¹.](image-url)
Flux declines are observed for both species over time. MIC causes a faster and greater decline than CYP. This observation is consistent with the results of these species at high pressure operations discussed in Section 7.5. Filaments of CYP are more susceptible to shear and form a cake with high porosity when compared to spherical MIC cells. As a result, membrane fouling is faster and more severe for the latter. Moreover, a noticeable increase in flux occurs at $t = 20$ h for CYP, before any permeate refill. This indicates that a fraction of the cake layer may be sheared off, possibly re-exposing some membrane area and reducing the overall resistance to flow. The cell density of the feed water increases over time. When the cell density is lowered by adding all the permeate back to the feed, the MIC run responds with a greater flux recovery at both refill times whereas the addition of permeate has barely had any impact on the flux, particularly at the second refill in the CYP run. This suggests filtration of MIC has a stronger dependence on feed cell density. These results agree well with the findings in Section 7.6. Both trials appeared to have reached a steady-state flux towards the end of the first and second refill periods. This indicates that equilibrium may be achieved between the rate of cell deposition and the rate of cell removal by the tangential flow.

To examine the long term impact of the low pressure cross-flow process on cell damage and subsequent metabolite release, toxin analysis and cell viability assays were conducted. The dissolved MC-LR concentration in the permeate over time is shown in Figure 7.15. For comparison, the extracellular MC-LR concentration in the feed at the beginning of the run is also presented on the plot (at $t = 0$). In the early stage ($t < 14$ h), the dissolved MC-LR concentration in the permeate is only slightly higher than in the feed. Although adsorption of MC-LR to the membranes needs to be taken into account, the overall impact of the process on permeate quality is negligible. The concentration starts to increase gradually after $t = 14$ h. At $t = 75$ h, where the dissolved MC-LR concentration nearly doubles the initial value. This increase corresponds to 34.7% of the intracellular MC-LR concentration in the feed at $t = 0$. This value is expected to
be higher if adjustments for MC-LR adsorption are made. Since no major cell damage and toxin release is observed in the 2.5 hour experiment, the large increase in dissolved toxin concentration in permeate over the long term is most likely due to prolonged exposure of the cells to shear. The time effect can also be important. A study has shown that a significant amount of intracellular metabolites was released to dissolved form towards the end of the life cycle due to cell ageing and lysis (Pietsch et al., 2002). However, as mentioned before, the water recovery in the laboratory system is considerably lower than on an industrial scale. The number of passes that individual cells are circulated is extremely high and the time that cells stay in the system is a lot longer. Therefore, commercial units are expected to generate much better permeate quality.
Figure 7.15: Dissolved MC-LR concentration in the permeate measured over time. Cell density: 500,000 cells mL⁻¹, cross-flow velocity: 0.5 m s⁻¹, total MC-LR concentration in the feed: 11.5 µg L⁻¹.

Figure 7.16 presents the dissolved CYN concentration of the permeate in the CYP run. Compared to MC-LR, the concentration of dissolved CYN remains relatively constant throughout the run except for a small increase at \( t = 70 \) h. This suggests that the long term impact of the process is small on CYP cells, although adsorption of CYN to membrane is expected to have enhanced the CYN rejection.
Figure 7.16: Dissolved CYN concentration in the permeate measured over time. Cell density: 500,000 cells mL$^{-1}$, cross-flow velocity: 0.5 m s$^{-1}$, total CYN concentration in the feed: 31.0 µg L$^{-1}$.

### 7.9 Membrane cleaning

The CIP method has been shown to be effective in removing fouling. The initial pure water flux on the new membrane is recovered completely in all the short term experiments. In comparison, 93% and 95% of the initial water flux are obtained after cleaning, in the long term MIC and CYP trials respectively. This suggests that membrane fouling formed over long time periods becomes partially irreversible.
7.10 Implication of laboratory results

The most important factors to consider for the industrial application of cross-flow filtration are water recovery rate and plant footprint. High pressure processes give a faster water recovery and smaller footprint but greater membrane fouling. The capital cost is lower in high pressure process due to the small footprint but the operating cost increases with the increasing energy input.

In this laboratory-scale study, the flux generated at low TMP is very small. This suggests that larger membrane area is required in order to obtain the same throughput achieved at high TMP. This will cause a significant increase in the capital cost. However, flux decline is a lot slower in low TMP operation. This means that membrane cleaning is required less frequently at low pressure process and the longevity of the membrane is extended. For high TMP operation, more rigorous cleaning regimes are necessary to restore the initial flux. Operating costs associated with membrane cleaning and membrane replacement cost can therefore be substantial. Although a high water recovery rate is the primary interest for industry, membrane fouling that limits the process performance must not be overlooked. Hence, low pressure operation is likely to be more attractive. Moreover, water recovery at low pressure can potentially be improved by changing the membrane properties as well as increasing the pore size (provided that there is no penetration of cells through the membranes).

In terms of cell damage and metabolite release, this study shows that the low TMP process has minimal impact compared to the higher TMP. High pressure DE operation is not recommended as cell lysis occurred causing metabolite release into the treated water. This becomes another vital benefit for low pressure processes as safer water quality will be obtained.
Overall, low pressure processes appear to be more favourable than high pressure processes for cyanobacterial cell removal. In particular, the process will become economically viable if existing infrastructure can be used. For example, similar operating conditions can be found in a membrane bioreactor (MBR) system. Integration of MBR into the treatment processes should be considered if plants are frequently coping with large quantity of cells.

MBR technology is used extensively in wastewater treatment. The process produces good effluent quality with a small footprint. Membrane filtration occurs either within the bioreactor (submerged) or externally through recirculation (side stream). In submerged configuration, the filtration is driven by a pressure difference across the membrane through a hydraulic head. The aeration within the bioreactor provides a turbulent cross-flow velocity across the membrane surface. In the side stream configuration, the pressure drop across the membrane is higher as it is driven by pumping. Although a lower flux is obtained in submerged MBR resulting in a larger footprint, laboratory results on MF suggests that less cleaning is required for the low pressure system, which can lower the operating cost significantly. Moreover, in the side-stream configuration and high pressure operation, the extra energy cost associated with pumping is substantial and the pumping process may increase the risk of cell damage and metabolite release. Therefore, despite the larger footprint, low pressure, submerged MBR may be more favourable for cyanobacterial cell removal.

The technology has been trialed by a number of treatment plants in South Australia for cyanobacteria removal. One of the major concerns, however, is the solids retention time (SRT) of cells in the system. For wastewater treatment application, high SRT is generally observed to result in lower membrane fouling rates in MBRs and SRT is typically between 10 to 50 days (Van den Broeck et al., 2012). Depending on the state of the cyanobacteria bloom, this timeframe may not be acceptable for the purpose of cyanobacteria removal. If aged cells are not removed from the system in time, they will start to release metabolites
and cause contamination of the finished water. Therefore, any prolonged detention of cells is not recommended and for that reason, waste management in the MBR also needs to be considered.

Furthermore, it is recommended that cross-flow filtration is incorporated in addition to processes such as activated carbon adsorption in order to achieve a complete removal of cyanobacteria cells and their metabolites.

**7.11 Conclusions**

This study has demonstrated that cross-flow MF is a promising technology for cyanobacteria removal, particularly at low operating pressures. The process removes cells completely from water using size exclusion. Flux decline is negligible at low pressure as sufficient shear is generated by the cross-flow velocity to remove cells from the membrane surface. Long term experiments observed a flux decline of up to 25% within 75 hours, but CIP technology was shown to provide excellent cleaning. Operating at low pressures also gives low risk of cell damage and metabolite release. Overall, a low pressure process such as the one used in submerged MBR is recommended. However, it is important to test the technology on natural bloom samples. For example, natural organic matter has found to complicate the process by increasing the flux decline in *M. aeruginosa* filtration (Kwon et al., 2005). Furthermore, MF membranes appear to adsorb dissolved metabolites as well as reject cell-bound metabolites. To gain a better understanding on the behaviour of metabolite adsorption to membranes, further investigation at pilot or full scale is recommended.
Cyanobacterial blooms have been a long-standing problem for drinking water supplies in Australia and worldwide. Due to the increased occurrence and severity of blooms in recent years, drinking water authorities are faced with the challenge of treating cyanobacteria-contaminated water to safe standards. Therefore, a knowledge of reliable treatment approaches, for both the cells and the problem metabolites they produce, is of crucial importance to the industry.

This work has demonstrated the benefits of using conventional water treatment processes for cyanobacteria and metabolite removal. By monitoring the cell viability and metabolite concentration, various processes in the treatment train were evaluated for their influence on cyanobacteria cells and subsequent water quality. The dewaterability of cyanobacteria-rich sludges were characterised and the results enable the identification of the difficulties that may be encountered in sludge management.

The impact of conventional treatment processes on cyanobacterial species *M. aeruginosa*, *A. circinalis* and *C. raciborskii* has been examined using metabolite analysis and cell viability assays. Low pH stress (pH <5), which can occur during coagulation upon the addition of coagulant, was observed to cause massive damage to *A. circinalis* and *C. raciborskii* cells. This resulted in the release of intracellular metabolites (saxitoxin, geosmin and cylindrospermopsin) and impaired the quality of the treated water. Based on this finding, it is recommended that low pH operating conditions should always be avoided during treatment processes when a high number of cyanobacteria cells is present in water.
After eliminating the exposure of low pH conditions in coagulation, it was shown that cells remained viable during coagulation and subsequent sedimentation processes. The mass balance of metabolites across these separation events demonstrated that conventional water treatment processes are capable of removing cyanobacteria as well as intracellular metabolites from water. As a result, the requirement for subsequent treatment processes such as activated carbon adsorption and oxidation that target dissolved metabolites will be significantly reduced and hence lower the treatment cost.

The sludge stream produced from the above processes is rich in intact cells and intracellular metabolites. There is very limited knowledge on the fate of cyanobacteria during sludge dewatering processes and their impact on the water quality. Sedimentation, centrifugation and pressure filtration processes were investigated in this study. The results show that they do not cause any major damage to the cells in the sludge stream. Negligible increase in dissolved metabolites was detected in the treated waters. With these processes, sludge volume can be reduced effectively without compromising water quality. It also means that water recovered from these processes is safe to recycle to the head of the plant, with activated carbon adsorption or advanced oxidation as a secondary barrier.

Another major outcome of the study is the comprehensive dewaterability characterisation for cyanobacteria-rich sludges. Accurate prediction of the dewatering behaviour of these sludges provides vital information for development of the best strategies for sludge management. Through the testing of a range of artificial water treatment sludges containing different cyanobacterial species, the effect of cyanobacteria on the dewatering character of the sludge was determined. A comparison of dewaterability of cyanobacteria-rich alum sludges to that of pure alum sludge revealed that these sludges showed similar compressibility profiles. They failed to compress to high solids concentrations even at high applied pressures (up to 300 kPa). Moreover, the presence of cells
was shown to impair the dewatering efficiency in terms of the rate of dewatering. The sludges exhibited low permeability, particularly at high pressure conditions. The rate of dewatering was predicted to be up to 5 times slower than that of pure alum sludges.

From a processing perspective, the major implication for the drinking water industry is that the size of the dewatering device required will be significantly larger in order to achieve the same throughput. Existing facilities in the treatment plants are thought to be incapable of handling sludges containing a high concentration of cyanobacteria cells, such as those encountered during bloom seasons. In terms of impairing water quality, risk of cell lysis and metabolite release increases when sludge takes longer to dewater. As a consequence, the amount of water recovered from the processes will need to be limited.

The analysis of treatment options based on the knowledge base produced indicates that membrane technology is a likely alternative method for cyanobacteria removal. Preliminary investigations were undertaken to evaluate the performance of microfiltration at different operating pressures. Experiments with a cross-flow filtration unit showed that a microfiltration membrane with a pore size of 0.1 µm was sufficient to remove cells completely from water. Processes with unicellular *M. aeruginosa* experienced faster permeate flux decline than the filamentous species (*A. circinalis* and *C. raciborskii*). It was demonstrated that operating the process at low pressures (e.g. 50 kPa) is beneficial because membrane fouling occurs at a very slow rate under this condition. Fouling was seen to accelerate at high pressures. Moreover, minimal cell damage and metabolite release was observed under low pressure conditions. The results suggest that cross-flow microfiltration has a good potential for cyanobacteria removal and safe water production.
Overall, this work has advanced our knowledge of the fate of cyanobacteria and metabolite release during drinking water treatment and sludge management. The knowledge gained on the dewaterability of cyanobacteria-rich sludges can be utilised in process equipment modeling to predict the performance of sludge dewatering equipment such as thickeners, centrifuges and filter presses. The modeling enables prediction of the timescale profiles of the sludges within each of the processes and allows process optimisation and improvements in equipment design. In summary, the outcomes of this study will help water providers to select the most appropriate processing route for treating cyanobacteria-rich waters to potable standards and to make suitable decisions on the return rather than the disposal of water recovered from sludge dewatering to the drinking water treatment process.

The findings and outcomes from this study implies the need for further work, in terms of:

- In natural blooms, cells will be at a range of ages. The majority of experiments conducted in this work used cells towards the end of the exponential growth phase. Aged cells may give a different response to the treatment processes examined in this work. Similarly, the resultant sludges may exhibit a different dewatering character. Therefore, testing of samples taken at different stages of the life cycle is needed to understand a full range of scenarios.

- Testing on synthetic cyanobacteria-rich water which consisted of laboratory cultured species and synthetic culturing media provide useful information for prediction of the behaviour when dealing with natural waters containing a high concentration of cells. However, as cells may show different behaviour in the natural environment and the composition of natural water and natural blooms are more complex, it is critical to source and examine natural bloom samples in order to validate the results obtained from the laboratory-scaled experiments.
• The knowledge gained on the dewaterability of cyanobacteria-rich sludges can be utilised in process equipment modeling to accurately predict the timescale profiles of sludges within dewatering equipment such as thickeners, centrifuges and filter presses. This will become a powerful tool for water treatment facilities in process design and optimisation and will ultimately assist the development of the most suitable methods for managing cyanobacteria-rich sludges.

• Preliminary studies on cross-flow microfiltration have shown the attractive aspects of membrane technology. It is recommended that the work be continued to fully explore the practicality of the process. It would be important to test a range of operating conditions (transmembrane pressure and cross-flow velocity), different membrane types and membrane cleaning routines. This will help to determine the most economical way to operate the process.
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