ADSORPTION AND BIOLOGICAL FILTRATION
OF
MICROCYSTINS

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Abstract

For removal of microcystins (liver tumor promoting cyanotoxins) from drinking water granular activated carbon (GAC) filtration has been shown to be a very promising treatment option. It is not only an efficient adsorbent for microcystins but also can be operated as a biological reactor in which bacterial degradation of the toxins can occur extending the life-time of this application.

However, the competitive adsorption of coexistent natural organic matter (NOM) in all natural water sources would cause early breakthrough of microcystins and a lag-phase with uncertain length occurs prior to initiation of biodegradation. This project aimed to investigate the individual microcystin removal abilities by adsorption and biodegradation during GAC filtration in order to better understand the overall efficiency of this application. The simultaneous elimination of NOM in GAC filters was also investigated. In addition, to facilitate the biological removal of microcystins, the research aimed to identify the potential effect of key operational conditions on the degradation efficiency.

In this study microcystin removal in GAC filtration was divided into the adsorption and biodegradation phases. Effective adsorption of the toxins lasted only a short term in virgin GAC filters and the breakthrough behaviour was able to be modelled by the homogenous surface diffusion model (HSDM). The presence of biofilm on the surfaces of GAC resulted in a lower mass transfer coefficient ($K_f$) and lower adsorption kinetics of microcystins. In the biodegradation phase enhanced or complete removal was evident that was mainly due to biological metabolism. However, the highly efficient biodegradation of the toxins was difficult to predict in GAC filtration. Biodegradation was found to be easily affected by many operational factors.

NOM removal in GAC filters demonstrated specific features in this study due to the relatively high organic content in the Australian water. The adsorption efficiency decreased rapidly as the adsorption capacity became saturated.
Statement of Originality

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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There were also many other people who have facilitated completion of this project and the preparation of my thesis. I will forever grateful to Najwa Slyman, Edith Kozlik, Miriam Nedic, Rolando Fabris and Mary Drikas for their assistance and care at the Water Treatment Unit, AWQC. The School of Chemical Engineering at The University of Adelaide has also been a great place for me to do research, and the very helpful and kind staff there made my time during the two years of study stimulating and enjoyable.

Last but not least I would like to give my deepest thank to my parents who financially supported and encouraged my study overseas and made all of this possible.
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<td>Activated carbon</td>
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<tr>
<td>Adda</td>
<td>3-amino-9-methoxy-2,6,8-trimethyl-10 phenyldeca -4,6-dienoic acid</td>
</tr>
<tr>
<td>BGAC</td>
<td>Biological granular activated carbon</td>
</tr>
<tr>
<td>DOC</td>
<td>Dissolved organic carbon</td>
</tr>
<tr>
<td>EBCT</td>
<td>Empty bed contact time</td>
</tr>
<tr>
<td>GAC</td>
<td>Granular activated carbon</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<td>HPSEC</td>
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<td>HSDM</td>
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<td>$K_f$</td>
<td>Film mass transfer coefficient</td>
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<tr>
<td>m-LR</td>
<td>Microcystin-LR</td>
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<tr>
<td>MW</td>
<td>Molecular weight</td>
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<td>NOM</td>
<td>Natural organic material</td>
</tr>
<tr>
<td>PAC</td>
<td>Powdered activated carbon</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>UVA$_{254}$</td>
<td>Ultraviolet absorbance at 254nm wavelength</td>
</tr>
<tr>
<td>WTP</td>
<td>Water treatment plant</td>
</tr>
<tr>
<td>TCE</td>
<td>Trichloroethylene</td>
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<td>THMs</td>
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CHAPTER 1

INTRODUCTION

The aim of this research was to separate the mechanism of adsorption from biodegradation of microcystins in granular activated carbon (GAC) filtration. This enabled a comprehensive evaluation of the relative contributions of the physical and biological mechanisms for microcystin removal in GAC filters.

1.1 Background

1.1.1 Cyanobacteria and cyanotoxins

“Cyanobacteria” or “cyanotoxins” may not be as publicly well known as “algal blooms”, “green tides”, “red tides”, “inedible shellfish” or “fish kills” etc, but they have a common link–eutrophication.

Eutrophication is the enrichment of lakes, rivers and reservoirs with chemical nutrients (typically phosphorus, nitrogen or both) (APIS, 2005). This changing status of the aquatic environment, resulting in excessive growth of algal population, high levels of organic matter, oxygen depletion and specific unpleasant smells etc, causes specific health risks for communities who must rely on eutrophic waters as drinking water sources. One of the most severe health threats is derived from cyanobacteria, because approximately 75% of cyanobacterial blooms are accompanied by toxic metabolites, cyanotoxins, which have been implicated in both animal and human poisoning [Fott, 1971; World Health Organisation (WHO), 1998].
Cyanobacteria, also called cyanophyte or blue/green algae, are organisms capable of plant-type photosynthesis and have close relationship with both bacteria and algae (Eutrophication and Health, 2002). They belong to an ancient group of organisms that are approximately three billion years old (Yoo et al., 1995), and are perhaps the most primitive group of organisms living on Earth for they posses significant ecological advantages over other planktonic species (Haider et al., 2003). For example, many species of cyanobacteria possess gas vacuoles that allow them to adjust their position (depth) in the water column under varied nutrient conditions and they can also benefit from the nitrogen fixing capability in low nitrogen waters that increases their natural productivity.

Cyanobacteria are able to grow under a wide range of environmental conditions throughout the world. Recent studies showed that their habitats range from thermal springs to the cold oceans of Antarctica, and some species subsist in terrestrial environments, growing well in soil and on rocks (Yoo et al., 1995). Cyanobacteria can grow prolifically under favorable conditions that include ample sunlight; moderate to high concentrations of essential nutrients (phosphorus, ammonia, nitrate), which can be received from household, industrial and agricultural wastes; suitable water temperature (generally between 15 °C to 30 °C); and pH >6 (Skulberg et al., 1984). Therefore, cyanobacterial blooms typically occur in late summer or early autumn in eutrophic or hypereutrophic water bodies.

Since the first report of toxic cyanobacteria resulting in animal stock death was made in Australia in 1878 (Francis, 1878), these potentially hazardous compounds have been detected in fresh water in 27 countries and on all continents (Newcombe, 2002). With the development of more sensitive analytical techniques for the analysis of algal toxins over the past decade, the detection of cyanotoxins in drinking water has increased internationally.
During normal growth conditions, a number of species of cyanobacteria can produce one or more types of toxin, which are called cyanotoxins. Cyanotoxins are characterized by their existence state, either intracellular (cell bound) or extracellular (dissolved). In healthy cyanobacterial blooms, more than 90% of the total amount is bounded within the cells (Sivonen and Jones, 1999). However, when the population ages, the bound compounds will be released into the surrounding water and high levels of extracellular (dissolved) toxins will be generated (Kaebernick et al., 2000).

According to the current taxonomy, 150 genera with about 2000 species of cyanobacterium have been recognized, and at least 40 of them are known to be toxicogenic (Skulberg et al., 1993). Although the toxins produced are diverse, both in structure and function, they are generally separated into four distinct categories:

1. **Neurotoxins** (such as: saxitoxins, anatoxins)
   
   *Characteristics*: less common, potent nerve poisons; can cause muscle spasms, affect respiration, result in death, but no apparent chronic effects.

2. **Hepatotoxins** (such as: microcystins, nodularins)
   
   *Characteristics*: the most frequently observed cyanotoxins, inhibit specific enzyme systems and cause severe damage to the gut and liver, can lead to death within a few hours at acute dose exposure.

3. **General Cytotoxins** (such as: cylindrospermopsin)
   
   *Characteristics*: implicated in a number of diseases such as gastroenteritis, kidney damage, liver malfunction, and in particular, are carcinogenic.

4. **Endotoxins** (such as: lipopolysaccharides)
   
   *Characteristics*: may result in gastrointestinal upsets and skin irritations.

Among these known cyanotoxins, the hepatotoxic cyclic peptides (microcystins) are considered the most common (Sivonen and Jones, 1999), and investigations conducted on water treatment for the removal of cyanotoxins has been primarily focused on
microcystins (Donati et al., 1993; Yoo et al., 1995; Newcombe et al. 2002).

1.1.2 What are microcystins?
Microcystins are the largest family in the group of hepatotoxins (liver toxins) (WHO, 1998). They are monocyclic heptapeptide toxins commonly produced by the freshwater cyanobacterium, Microcystis aeruginosa (cell coenobium, Figure 1.1), from which the toxins derived the name. They can also be produced from other genera, namely, Anabaena, Nostoc and Planktothrix (Carmichael, 1992; Codd, 1995), and also a terrestrial (soil) species Haphalosiphon hibernicu, implying its widespread potential existence in the environment.

![Microscopic photo of the toxin producing Microcystis aeruginosa (cell coenobium) (Murphy et al., 2003)](image)

Microcystins have more than 60 structural variants, each with different toxicity (Hyenstrand et al., 2001). They all possess seven amino acids, with five non-protein amino acids (D-alanine, D-erythro-ß-methyl aspartic acid, D-glutamic acid, N-methyldehydroalanine and Adda (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca -4,6-dienoic acid)) and two variable L-amino acids (protein amino acids) at position X and Z respectively (Figure 1.2). Different structures in microcystins result in different lipophilicites and polarities, which could in turn affect toxicity (WHO, 1998). Microcystin-LR (m-LR) and -LA (m-LA), which were investigated in this study, have leucine (L), arginine (R) for m-LR and alanine (A) for m-LA in variable positions. They
represent two of the best characterised and the most toxic analogues of microcystin (Oudra et al., 2002; Oberholster et al., 2003).

![Diagram of microcystin structure with numbers indicating different amino acids.]

1 - D-Alanine  
2 - Variable L-amino acid  
3 - D-Methylaspartic acid  
4 - Variable L-amino acid  
5 - 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda)  
6 - D-Glutamic acid  
7 - N-Methyldehydroalanine

Figure 1.2 Representation of the molecular structure of microcystins (“The Toxic Cyanobacteria” website [http://lurac.latrobe.edu.au/~botbml/mictox.html])

The unusual Adda amino acid is an essential side chain used to study properties of microcystins. Variation of the Adda structure could potentially influence microcystin toxicity, as it is important in the binding to protein phosphatases where microcystins exhibit toxic activities (John and Charles, 1999; Mankiewicz et al., 2002). The stereochemistry and extent of methylation of Adda also greatly influences toxicity. Furthermore, the absorbance characteristics of Adda at wavelength 238nm are also utilized as a method for microcystin analysis.

1.1.3 Toxicity of microcystins

Microcystins can severely compromise human health. Although the mechanisms that mediate toxicity have not been fully understood, it is believed that uptake into
hepatocytes followed by the inhibition of serine/threonine protein phosphatases type 1 and 2A (PP1, PP2A) results in protein phosphorylation imbalance. The imbalance further causes disruption of the liver cytoskeleton, which leads to cytoskeletal damage, necrosis and pooling of blood in the liver, with a consequent large increase in liver weight and massive hepatic haemorrhage that leads to death (Honkanen et al., 1996; Eriksson et al., 1990; Romanowska-Duda et al., 2002).

Microcystin-LR has an LD$_{50}$ (the amount of a material, in a single dose, which causes the death of 50% of a group of test animals) value of 36-122 μg/kg in mice and rats by intraperitoneal injection or by inhalation (Stoner et al., 1989), which is comparable to the toxicity of chemical organophosphate nerve agents (Oberholster et al., 2003). Acute (short-term) exposure to high concentrations of microcystins can result in death from liver haemorrhage or liver failure, while chronic (long-term) exposure to low doses may encourage the growth of liver, kidney and other tumors (Jones and Orr, 1994; Codd and Bell, 1996; Jones and Negri, 1997). The observed symptoms, associated with microcystins, include diarrhea, vomiting, piloerection, weakness and pallor (Bell and Codd, 1994).

The global reports of the intoxication of human (Table 1.1) and many animals by microcystins at variable times and locations make it very difficult to investigate the health threat at which microcystin concentration is of concern. Although 1 μg/L has been recommended by the World Health Organization (WHO) as the maximum allowable microcystin-LR concentration in drinking water (WHO, 1998), a proposed value of 0.01 μg/L was suggested by Ueno et al. (1996) based on the occurrence of primary liver cancer and the presence of microcystins in the surface waters in certain regions of China.
1.1.4 Microcystin persistence in drinking water treatment plants

Microcystins have been detected in surface waters used for potable water sources internationally (Sivonen and Jones, 1999; Hitzfeld et al., 2000; Kabzinski et al., 2000), where the main hazardous effect on humans is through consumption of drinking water (Gupta, 1998). It is, therefore, vital for drinking water authorities world-wide to develop reliable water treatment strategies for the removal of cyanobacterial cells and associated toxins.

The traditional water treatment technologies (coagulation / sedimentation / filtration) have been documented to be insufficient at producing safe drinking water from microcystin contaminated sources (Hoffman, 1976; Keijola et al., 1988; Anselme et al., 1988; Lahti and Hiisvirta, 1989; Himberg et al., 1989). Microcystins, at varying concentrations, have been detected in product drinking water in a number of countries, including China (Wu et al., 2005), Australia (Bourke et al., 1983; Falconer et al., 1983), Finland (Lahti et al., 1997) and Latvia (Eynard et al., 2000). The presence of microcystins in drinking water is a major concern for water authorities. The main reasons for the microcystins recalcitrance to conventional treatment are as follows:

<table>
<thead>
<tr>
<th>Location and date</th>
<th>Symptoms</th>
<th>Consequences</th>
<th>Reference</th>
</tr>
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<tr>
<td>USA, 1931</td>
<td>Gastro-enteritis</td>
<td>No data</td>
<td>Tisdale et al., 1931</td>
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<tr>
<td>Canada, 1959</td>
<td>Gastro-enteritis, headaches, nausea, muscular pains</td>
<td>30 people affected</td>
<td>Chorus and Bartram, 1999</td>
</tr>
<tr>
<td>Australia, 1981</td>
<td>Gastro-enteritis, Liver injury</td>
<td>No data</td>
<td></td>
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<tr>
<td>Brazil, 1988</td>
<td>Gastro-enteritis</td>
<td>2000 people affected, 88 deaths</td>
<td></td>
</tr>
<tr>
<td>UK, 1989</td>
<td>Gastro-enteritis, vomiting, sore throats</td>
<td>20 people affected, 2 hospitalizations</td>
<td>Turner et al., 1990</td>
</tr>
<tr>
<td>Brazil, 1996</td>
<td>Acute hepatic failure</td>
<td>50 dialysis patients died</td>
<td>Jochimsen et al., 1998</td>
</tr>
</tbody>
</table>
First, the commonly applied conventional flocculation and filtration procedures are generally identified to be effective only in reducing microcystins that remain in healthy cyanobacteria, but do not remove the dissolved toxins that are known to be relatively stable compounds. When cyanobacterial cells lyse, either from natural decay or from artificial treatment, the intracellular toxins will be released into the water column. The hepatotoxins are “small” molecules with a molecular weight ranging from 800-1000 Daltons (Botes et al., 1982a, b) and most congeners are hydrophilic, so they are relatively soluble and stable in water. Moreover, they are non-volatile and resist extremes in pH (Wannemacher, 1989). Microcystins have been reported to withstand many hours of boiling and may persist for many years when stored dry at room temperature (Linda, 1999). Hence, once released to the water column, microcystins provide all the challenges of removing a water-soluble contaminant from water. Additionally, the conventional treatment methods may even cause release of intracellular toxins by system turbulence and pressure gradients during flocculation, as well as application of pre-oxidation (Chow et al., 1998) and consequently pose an even bigger challenge for microcystin removal. Therefore, a broad range of alternative treatment methods (Section 1.2) that could provide more efficient and reliable microcystin removal have been investigated.

### 1.2 Available treatment technologies in removing or reducing microcystins in drinking water

There are several remedial technologies to compensation for the inadequate microcystin removal during conventional water treatment. These methods mainly include oxidation (chlorination, ozonation, potassium permanganate, UV, hydrogen peroxide and titanium dioxide); dissolved air flotation (DAF); membrane filtration, biodegradation as well as activated carbon adsorption.
1.2.1 Oxidation

Chlorination

Chlorine has been used as a disinfection reagent for water treatment since the 19th century (Lawton and Robertson, 1999) and it still is one of the most common chemicals used in modern water treatment facilities. However, its efficiency has been found to largely depend on the pH of the water. Chlorine gas dissolved in water forms hypochlorous acid (HOCl) following the reaction

$$ \text{Cl}_2 + \text{H}_2\text{O} \rightarrow \text{HOCl} + \text{H}^+ + \text{Cl}^- $$  \hspace{1cm} (Equ. 1)

Because HOCl is weak acid, under pH 5, chlorine solution is primarily hypochlorous acid which is an effective disinfecting agent. While above pH 5, hypochlorous acid starts to dissociate and form hypochlorite ions that are comparatively less effective, according to the reaction

$$ \text{HOCl} \leftrightarrow \text{OCl}^- + \text{H}^+ \quad \text{(pH} \leq 5) \hspace{1cm} \text{(Equ. 2)}$$

At pH values above 10, 100% dissociation of hypochlorous acid will occur as

$$ \text{HOCl} \rightarrow \text{OCl}^- + \text{H}^+ \quad \text{(pH} > 5) \hspace{1cm} \text{(Equ. 3)}$$

Application of chlorination for the removal of microcystins was carried out by Hoffmann (1976), Keijola et al. (1988), and Himberg et al. (1989), but all indicated the process was ineffective. This may be due to the fact that the pH at which the work was performed was unfavorable for the formation of hypochlorous acid (Lawton and Robertson, 1999). Contrary to the above results, chlorine has been demonstrated as an effective oxidant for the destruction of microcystins (Nicholson et al., 1994; Tsuji et al., 1997; Senogles-Derham et al., 2003). Nicholson et al. (1994) showed that microcystin-LR was effectively destroyed provided there was a chlorine residual of at least 0.5 mg/L with contact time of 30 minutes. Likewise, Tsuji et al. (1997) reported that 99% of microcystin-LR was removed with a chlorine dose of 2.8 mg/L after a contact time of 30 minutes. As in the results of Hoffmann (1976), the effect of pH on chlorination of microcystins was also shown by Nicholson et al. (1994), who reported
that the most efficient microcystin destruction occurred at pH values between 5 and 8 with a decreasing efficiency above pH 8.

In general, chlorination does appear to be an effective method for removing microcystins from drinking water, although this is largely dependent on the pH, dose level and maintenance of the residual chlorine in the water. Moreover, the products and the mechanism of microcystin decomposition still remain to be characterised; therefore harmful by-products may be as yet unknown.

**Ozonation**

Ozone, one of the most powerful oxidizing reagents, has been widely investigated for cyanotoxin removal in the last 10 years. Early studies conducted by Keijola et al. (1988) showed 100% removal of up to 60 μg/L microcystins was achieved with 1 mg/L ozone. Likewise, a 99% removal of microcystins after 15 seconds when treated with 0.05 mg/L ozone was found by Rositano et al. (1998). This extremely fast destruction of microcystins was corroborated in other Australian studies (Nicholson et al., 1993; Rositano and Nicholson, 1994; Rositano et al., 1998), which identified that ozone was more effective than chlorine, hydrogen peroxide and potassium permanganate for the destruction of microcystins provided residual ozone is guaranteed.

Use of ozone is sometimes unpredictable and its reliability to remove microcystins from water is highly dependent upon the quality of the water, which is noted to be the most significant parameter in the determination of the ozone dose required for the removal of the cyanotoxins. In particular, the nature and concentration of the dissolved organic material in the treated water body have been found to affect the oxidation reaction, and may result in incomplete toxin removal (Hart and Stott, 1993; Carlile, 1994; Rositano, 1996; Mouchet and Bonnellye, 1998; Rositano et al., 1998, 2001; Shawwa and Smith, 2001).
Potassium permanganate

Potassium permanganate, a powerful oxidizing agent capable of destroying organic compounds and microorganisms, has been widely applied since the 1960s. It is commonly used by organic chemists for the hydroxylation of alkenes for diol formation. Therefore, it should be effective in removing microcystins by attacking the unsaturated bonds.

Several studies have shown that permanganate was effective in removing microcystins. Carlile (1994) reported a removal of 76% microcystin-LR with a low dose of 0.7 mg/L permanganate, a higher removal of 88% with a dose of 1 mg/L, and a nearly 100% removal with doses greater than 1 mg/L. Similarly, Fawell et al. (1993) found that permanganate is efficient for microcystin removal in both raw and treated waters. They reported that a dose of 2 mg/L could destroy microcystins to below detection limit. Rositano et al. (1998) found that more than 90% of microcystins at 1 mg/L could be oxidized by a dose of 2 mg/L permanganate.

Potassium permanganate appears to show much promise as an oxidising agent, but preoxidation with permanganate followed by coagulation may cause algal cells to lyse, releasing additional amount of toxins, and also results in manganese being present in the treated water (Lam et al., 1995; Pietsch et al., 2002). Moreover, little is known about the character of the by-products from this decomposition.

Ultraviolet (UV) light

For the removal of some cyanotoxins, photo-oxidation has been shown to be a remarkably effective method. For example, 45-56% of microcystins, which are chemically and physically stable compounds (Tsuji et al., 1994), were destroyed after 8 hours of natural sunlight irradiation (maximum cumulative UV-radiation was about 30 W/m²). However, the dose requirement of this method often exceeds the range that is practical for water treatment application. In Carlile’s (1994) study, it was shown that a
dose of 22.5 W/cm\(^2\), which is two orders of magnitude greater than that needed for disinfection, was required to achieve 90% removal of anatoxins, and to remove 91% of microcystins-LR, a dose of 24 W/cm\(^2\) was required.

**Hydrogen peroxide**

In general, the use of hydrogen peroxide alone in water treatment is very limited. It has only been applied to the oxidation of phenolic wastewater and to the treatment of paper mill effluent, drilling muds and other types of organic wastewater (Lawton and Robertson, 1999).

Hydrogen peroxide is relatively ineffective in degrading microcystins. Only 17% cyanotoxin removal was obtained after 60-minute contact time with a dose of 20 mg/L hydrogen peroxide (Rositano and Nicholson, 1994). Researchers who investigated the effect of a 2 mg/L hydrogen peroxide solution on 1 mg/L microcystin-LR observed no toxin removal after 10 minutes. Although the oxidizing effectiveness of hydrogen peroxide seems to be enhanced by irradiating with UV or ozone, where all toxins are removed within 30 seconds (Lawton and Robertson, 1999), earlier research with UV alone showed similar destruction, suggesting a negligible contribution from the hydrogen peroxide. Therefore, this reagent appears not to be promising as an effective treatment method for microcystins.

**UV photocatalysis Titanium dioxide**

Titanium dioxide under UV photolysis has been found to be efficient for cylindrospermopsin removal at pH 9, where there would be greater production of hydroxyl radicals (Senogles et al. 2001). In contrast, Feitz et al. (1999) reported that the TiO\(_2\) catalyst rapidly degraded microcystin-LR at low pH 3.5, while at higher pH values, a distinct lag was observed before the toxin started to be degraded. This may be due to the adsorption of microcystin-LR to TiO\(_2\) at high pH, causing degradation by persistent organic radicals (Feitz et al., 1999).
No toxic by-products have been found during the TiO$_2$ induced photocatalytic oxidation of microcystin-LR, because the major mechanism of photocatalysis was to isomerize, substitute and cleave the Adda conjugated diene that is generally associated with the toxicity of microcystin (Liu et al., 2002). Recent investigations also found that the photocatalytic oxidation of microcystin-LR by TiO$_2$ and UV was enhanced with the addition of H$_2$O$_2$ (Cornish et al., 2000; Liu et al., 2002). They reported that the combination of H$_2$O$_2$/TiO$_2$/UV resulted in no detectable by-products, with a bioassay indicating that the toxicity of the treated water had been removed. However, in a study by Senogles et al. (2001), the efficiency of the photocatalytic degradation was greatly reduced at elevated concentration of dissolved organic carbon (DOC) between 15-32.5 mg/L. The authors attributed this to the decreased transmission caused by higher levels of DOC.

Although titanium dioxide seems to be an effective technology to remove microcystins, its removal efficiency can be easily affected by environmental conditions (such as pH and elevated concentrations of DOC).

1.2.2 Membrane filtration

Many types of membrane filtration have been reported to be effective in microcystin removal. Microfiltration (MF) and ultrafiltration (UF), for example, are both emerging membrane filtration technologies, which have been found very effective (>98%) in removing whole cells of *microcystis* (Chow et al., 1997). An 82-99% removal of dissolved microcystin-LR has also been reported by Simpson and Macleod (2002), as well as by Smith et al. (2002) who trialed eight different nanofiltration (NF) membranes. Similarly, three different reverse osmosis (RO) membranes have been shown to be able to efficiently remove m-LR and m-RR from potable water, with average removal rates of 96.7% and 99.9%, respectively (Neumann and Weckesser, 1998).
Generally, low pressure MF and UF membranes are better for removal of intracellular cyanotoxins, while high pressure membranes (NF and RO) may have the ability to remove extracellular dissolved cyanotoxins. However, when considering filtration, an important point is the lysis of cells. In the case of the above-cited studies, some damage to cells was observed (Chow et al., 1997).

### 1.2.3 Biodegradation

Biological treatment of contaminants in drinking water has been utilised by water suppliers worldwide for many years. It generally possesses many advantages; for example, 1) requires relatively little maintenance technology, 2) relates to relatively low infrastructure and running costs, 3) it does not require any additional treatment and only involves processes that remove contaminants without the addition of chemicals that in themselves may have potential health effects (Newcombe et al., 2001).

In recent years, specific attention has been given for the application of biodegradation for cyanotoxin removal, especially microcystin removal (Watanabe et al., 1992; Jones and Orr, 1994; Lam et al., 1995; Cousins et al., 1996; Tsuji et al., 1996, Christoffersen et al. 2002). Christoffersen et al. (2002) reported that a great number of microcystins in the environment are degraded by microorganisms, though they generally need a lag phase for adaptation. In the sediment infiltration study conducted by Holst et al. (2003), microcystins were detoxified by indigenous microorganisms in the sediment located in a water recharge facility, specifically at anoxic (<0.3% O₂) conditions.

It was believed that the high efficiency of biodegradation is due to specific aquatic microorganisms that effectively prey on cyanobacterial cells (Jones, 1994; Ho, 2004). Up to date, several strains of bacteria, such as *Pseudomonas aeruginosa* and *Sphingomonas sp.*, have been identified as microcystin degraders (Takenaka and Watanabe, 1997; Lahti et al., 1998; Park et al., 2001; Christoffersen et al., 2002; Saito et al., 2003; Harada et al., 2004 and Ishii et al., 2004).
The efficiency of microcystin removal by biodegradation is also affected by the selection of the media on which bacteria colonise. For example, more than 90% of microcystins were biologically degraded using a sand filter (Grützmacher et al., 2002; Ho et al., 2006). In a batch scale bank filtration experiment, complete microcystin removal took 10 days using soil containing high organic and low sand content, but 16 days using soil with low organic and high sand content (Miller and Hallowfield, 2001). The authors indicate that the difference could be due to insufficient nutrient availability for microorganism growth in low organics.

Successful removal of cyanotoxins employing biological filtration also depends on many other operational conditions, such as the presence and concentration of certain types of bacteria, the quality of the water being treated, the contact time and hydraulic loading of the filter. Furthermore, the delay (days to months) for biological degradation to occur, which is often referred to as the lag phase, is still a major hindrance for the confident application of biological filtration technology (Newcombe et al., 2002). Researches in the United Kingdom indicated a lag period of less than a week when m-LR at 10μg/L was used in reservoir water (Cousins et al., 1996). In the study conducted by Codd and Bell (1996), a lag phase of about 1 week was observed. In contrast, a lag phase of up to 21 days was observed by Jones and Orr (1994) when studies were carried out after cyanobacterial bloom treatment with algicide (i.e. copper sulfate).

1.2.4 Disadvantages from these treatment solutions

Although the previously discussed remedial treatment techniques can reduce microcystins relatively effectively, they usually exhibit many limitations that usually make them outside commonly adopted protocols. For example, oxidation by chlorine is a well established and effective one-step post-treatment technology, but for toxin removal a multi-barrier approach is desirable at the end of water treatment process.
Ozonation is a very rapid oxidation option but the high cost may prove to be prohibitive, particularly since the appearance of microcystins in water sources is typically seasonal and unpredictable. Ultraviolet (UV) light treatment appears to be promising, for it is a simple and clean technology, but required UV doses for the purpose of microcystin removal are significantly higher than those generally used for disinfection, also this technology is relatively new and thus remains to be seen how well it will perform. Moreover, further research must be carried out to examine the potential hazardous by-products associated with these oxidation methods. As for the option of filtration, in particular membrane filtration, the release of free toxins and the increase in the operational costs due to the high pressure drops make this type of treatment unsuitable for large-scale applications.

Therefore, it is vitally important to utilise an effective microcystin removal method, which should be cost-effective and sustainable, relatively easy to operate, and with minimal chance for generation of harmful by-products. Possessing all these traits, the use of activated carbon (Section 1.3), especially granular activated carbon filtration (Section 1.3.2), appears to be a very promising option.

### 1.3 Activated carbon

Activated carbon (AC) adsorption has been widely used in drinking water, wastewater treatment, as well as food, beverage, pharmaceutical and chemical industries. It has been recommended as one of the best available environmental control technologies by the US Environmental Protection Agency (EPA).

The initial application of AC by the water industry was to remove taste and odour problems. Recently, many of the studies relating to AC have been conducted on the removal of microcystins (Donati et al., 1993, 1994; Fawell et al., 1993; Craig and Bailey, 1995; Lambert et al., 1996; Cook and Newcombe, 2002). Results from most of
these studies indicate that the removal efficiency of AC filters is greatly dependant on the type of AC being used, and suggest that chemically-activated coal and wood-based ACs are the best options for microcystin removal (Donati et al., 1994; Hart and Stott, 1993; Lambert et al., 1996) due to their large volume of mesopores (2-50 nm), which are the appropriate size for the microcystin molecules (Donati et al., 1994).

There exist two forms of AC, namely, powdered activated carbon (PAC) and granular activated carbon (GAC).

1.3.1 Powdered activated carbon

PAC is commonly added directly to water prior to coagulation or filtration for microcystin removal. Although such application has been revealed to be effective by many studies, it has some disadvantages.

Firstly, its effective dosing rates are always very high. For example, in the investigation conducted by Lawton and Robertson (1999), a PAC dose of 800 g/m$^3$ was required to remove both the microcystin-LR and microcystin-RR. Moreover, Falconer et al. (1989) found that only very small percentages of the toxins could be removed by many types of the PACs in their investigation at a dosing level of 1 kg/m$^3$.

Secondly, the removal efficiency of PAC is dependent upon several operation conditions. One condition is the microcystin concentration in water being treated. At a full-scale Canadian treatment plant, a PAC supplement at 30 mg/L was monitored over 6 weeks (Lambert et al. 1996). The authors found that the average removal of all active microcystins ranged from 82% when the raw water microcystin concentrations were above 0.5 µg/L to only 31% when the concentrations were below this level.

A particular condition that can affect the removal rate of PAC is the concentration of natural organic material (NOM) in the source water. A study comparing PAC removal
efficiency for different water qualities was conducted (Lawton and Robertson, 1999), using both high purity waters and raw waters. The results showed that the toxin adsorption to PAC was greatly decreased by the high NOM concentration in raw water, due to competition for binding sites on the carbon.

Therefore, the efficient PAC dose rate for microcystin removal is related to the type of PAC being used, and the concentration of both the microcystins being investigated and the natural organic carbon in the water being treated. In contrast, the use of GAC filtration (Section 1.3.2), that has less of the disadvantages listed above, can be a very efficient process.

**1.3.2 Granular activated carbon filtration**

Granular activated carbon (GAC) is typically utilised in flow-through column reactors in water treatment processes to control various micropollutants such as taste and odor compounds, heavy metals and pesticide residue etc. It has also been shown to be one of the most effective options for removal of the cyanotoxins (Falconer et al., 1989; Lahti and Hiisvirta, 1989; Jones et al., 1993; Donati et al., 1993; Craig and Bailey, 1995; Lambert et al., 1996; Cook and Newcombe, 2002; Ho, 2004). The high toxin removal efficiency of GAC is attributable to the co-existence of adsorption and biodegradation mechanisms, both of which are significant contributing factors as microcystins are both adsorbable (Section 1.3) and biodegradable (Section 1.2.3). Of particular interest, biodegradation of microcystins could considerably extend the service life of GAC filters, which would reduce the cost of this technology and facilitate its practical application.

Firstly, GAC is an effective microcystin adsorbent. Previous studies with bench-scale treatment processes (including alum coagulation, sand filtration, GAC adsorption and chlorination) found that toxins from freeze-dried *Microcystis* and *Oscillatoria* blooms were essentially eliminated at feed concentrations between 30 and 56 µg/L (Keijola et al., 1988 and Himberg et al., 1989). Since the conventional treatment processes have
been reported to have negligible removal of dissolved microcystins, most of the achieved removal could be attributed to GAC adsorption.

In the above GAC studies by Keijola et al. (1988) and Himberg et al. (1989), the treated volume of the polluted water was only 10 bed volumes, thus the GAC capacity for toxin adsorption could not be seriously challenged and therefore complete removal of microcystins was observed. However, a study by Newcombe (2002) showed breakthrough of microcystins after a GAC filter was operated for 6 months with water that had a relatively high NOM concentration. The author pointed out that it may be due to the high loading of NOM in the water sample that reduced the adsorption capacity of the GAC filter. Similarly, research conducted on a full-scale GAC adsorber by Lambert et al. (1996) found that NOM may expend the adsorption capacity in GAC, which would reduce the removal efficiency for microcystins. Again, tests conducted on GAC that had been used in a water treatment plant for 5 months before evaluating its adsorption capacity for microcystins, demonstrated that 88% of the adsorption capacity of the GAC was reduced (Lawton and Robertson, 1999).

The findings presented above indicate that although GAC adsorption filters have been increasingly employed as an effective process for microcystin removal in modern treatment works, they may be easily saturated with NOM in normal water treatment circumstances resulting in toxin breakthrough after a relatively short life-time ranging from hours to hundreds of days (Falconer et al., 1983, 1989). This time uncertainty due to the adsorption exhaustion not only results in very high operating cost due to the frequent thermal regeneration required, but also makes it is difficult to estimate how long a GAC filter could last as an effective adsorbent to assure microcystin-safe water. It is, therefore, important to determine the microcystin adsorption capacity of GAC or the toxin breakthrough into the product drinking water.

Importantly, GAC packed columns could be used as a microcystin biodegradation
reactor. As documented previously (Section 1.2.3), microcystins can be decomposed by a wide variety of microorganisms in the environment, but the efficiency of this process will be affected by the selection of the substrate on which bacteria can attach. With GAC filters, given there is no disinfectant residual left in the treated water, a biological layer (biofilm) can be very quickly cultivated on the rough and porous surface of GAC particles (Miklas and Robert, 1998). Therefore, the overall toxin removal by the GAC column would be greatly enhanced and the overall lifetime of the GAC filter would also be extended.

However, the biodegradation process can only occur after a lag-phase of several days up to a few months (Jones and Orr, 1994; Lam et al., 1995; Cousins et al., 1996; Ho, 2004). The lag-phase is required for the microorganisms to acclimatize to the toxins as a carbon source (Jones and Orr, 1994). The length of the lag time depends on many operational conditions, such as the presence and concentration of certain types of bacteria, the quality of the water being treated, the contact time and hydraulic loading of the filter. Thus, it is uncertain when the biodegradation mechanism will occur under normal operating conditions for a wide range of treated water qualities. The variability of the lag-phase before onset of biodegradation, combined with the unknown lifetime of the filters functioning in adsorption mode, results in a serious uncertainty in the application of GAC for the removal of microcystins.

At any time during the use of GAC filters, it is unknown which mechanism, adsorption or biodegradation, takes the primary responsibility for microcystin reduction in GAC filters. Additionally, there is a lack of knowledge of the extent to which the remaining adsorption capacity contributes to toxin elimination after biodegradation of microcystins is triggered on in GAC filters.

Therefore, a comprehensive study that can separate the mechanism of adsorption from biodegradation of the toxins in GAC filters would be extremely beneficial for further
understanding of the removal principles during the filtration and would also make it possible to answer the above-mentioned problems.

1.4 Objectives of this research

The primary objective of this project was to determine the relative contributions of the physical adsorption and the biological process for the removal of microcystins in GAC filtration. This knowledge was obtained by designing and carrying out detailed GAC experiments that separated the two mechanisms.

In addition, the GAC experiment was also used to investigate simultaneous natural organic material (NOM) removal during GAC filtration, the disinfection by-products of which have been recognized as potential public health threats since the 1950s (Middleton and Rosen, 1956). As an effective NOM adsorber and potential biodegradation reactor, GAC filters have been extensively studied for effective elimination of NOM. However, these studies have rarely been conducted with the unique Australian waters that have high concentration of NOM, and did not separate the removal process regarding adsorption and biodegradation.

Finally, to facilitate the practical application of microcystin biodegradation in GAC filters, this study also aimed to identify the potential effects of different operational conditions on the behavior of the biological processes.
CHAPTER 2

EXPERIMENTAL METHODS AND MATERIALS

2.1 Experimental methods

The lab-scale, column experiment was designed to discriminate between the adsorption and biodegradation mechanisms for microcystin removal, which were expected to occur in GAC filters simultaneously.

2.1.1 Experiment setup

To separate the adsorption and biodegradation mechanisms during GAC filtration, three lab-scale glass columns (25 mm I.D. and 15 cm bed depth) were constructed and operated in parallel (Figure 2.1). These three columns included a non-sterile GAC column, a sterile GAC column and a sand column to simulate the combination of adsorption and biodegradation, solely adsorption and solely biodegradation, respectively. The sterile GAC was from the same source as the non-sterile GAC, but was maintained under sterile conditions, which was achieved by regular (weekly) autoclaving of the associated experimental apparatus (at 121 °C, 500 kPa for 20 minutes) for the duration of the experiment. Thus, biological activity and/or biodegradation was minimised in this column with only the adsorption mechanism occurring. The sand in the third column was non-absorbing and thus any microcystin removal in this column could be only attributed to biodegradation. A brief summary of the three columns is shown in Table 2.1.
Figure 2.1 schematic diagram of the experiment setup
Table 2.1 Operating parameters for the three columns (Figure 2.1)

<table>
<thead>
<tr>
<th>Component</th>
<th>Sterile GAC column</th>
<th>Sand column</th>
<th>Non-sterile GAC column</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packing material</td>
<td>Sterile virgin GAC</td>
<td>Non-porous fresh sand</td>
<td>Sterile virgin GAC</td>
</tr>
<tr>
<td>I.D. 1.0 – 1.4 mm</td>
<td>I.D. 1.0 -1.4 mm</td>
<td>I.D. 1.0 – 1.4 mm</td>
<td></td>
</tr>
<tr>
<td>Operating condition</td>
<td>Aseptic</td>
<td>Normal (non-aseptic)</td>
<td>Normal (non-aseptic)</td>
</tr>
<tr>
<td>Microcystin removal</td>
<td>Adsorption</td>
<td>Biodegradation</td>
<td>Adsorption and</td>
</tr>
<tr>
<td>mechanism(s)</td>
<td></td>
<td></td>
<td>biodegradation</td>
</tr>
</tbody>
</table>

2.1.2 Selection of the experimental design

Investigations concerning the separation of the adsorption and biodegradation mechanisms in GAC filters using sterile and non-sterile carbon beds have been previously carried out by Laat et al. (1985). The authors added silver sulfate (at Ag⁺ concentration of 0.5-1.0 mg/L) to maintain sterile condition in one of the filters. However, the silver sulfate adsorption on GAC (Jia and Demopoulos, 2002) could affect the adsorption efficiency of microcystins that are typically trace-level (micro-gram per litre) compounds. Therefore, the innovative experimental design (Figure 2.1) concerning autoclaving of the sterile GAC system was employed in this study.

There are a number of advantages in selecting non-porous sand as the packing material for assessing biodegradation rather than using spent GAC. Firstly, it could not be guaranteed that the saturated GAC would not retain any adsorption capacity for any of the adsorbable DOC constituents present in the water. The equilibrium of adsorption is a kinetic balance which can change in response to variable factors in the water such as pH and DOC. The non-porous sand, on the other hand, possesses no adsorbability itself, so it could exclusively simulate the biodegradation in GAC filtration. Secondly, a bio-regeneration effect, which could refresh the exhausted adsorption sites to a certain
degree, may occur once biological activities become significant on the GAC surface. In that case, the adsorption ability in the GAC would be re-exhibited.

2.1.3 Operation of the columns

During the experiment (Figure 2.1) microcystin (m-LR and m-LA) spiked water was transferred to a 20 L tank and mixed with a magnetic stirrer. The water was continually pumped through the three columns via a three-port peristaltic pump (Adelab, Australia) at an empty bed contact time (EBCT) of 15 minutes. Samples (250 mL) before and after each column were collected at regular time intervals for subsequent DOC and UV\textsubscript{254} measurement, as well as SPE extraction and HPLC analysis of the toxin concentration.

In order to minimise biological contamination of the sterile GAC column, a separate water reservoir and apparatus for collecting samples were constructed for the system; these are highlighted in dashed rectangles in the schematic diagram (Figure 2.1). The separate reservoir was a 20 L polypropylene autoclavable carboy with spigot (Southern Cross Science Pty Ltd, Australia). The water was sterilised in the carboy by autoclaving (at 121 °C, 500 kPa for 20 minutes) prior to use. To minimise any bacteria in the toxin spiking solution, the toxins were injected into the carboy via a 0.22 µm sterile filter disc (Gelman Sciences, Australia), which was then rinsed with 30 mL autoclaved Milli Q water to wash out the residual toxins into the carboy. The spiking procedure was conducted following aseptic techniques in a laminar flow cupboard.

To assess the sterility of the above procedures heterotrophic plate counts were employed. This method employed R2A agar plates (Oxoid Limited Corporate, UK) on which 100 µL of the effluent sample (taken from the system at daily intervals) was spread following aseptic methods. After incubation at 25 °C for 7 days, the number of bacterial colonies (count/mL) obtained on the plate was recorded.
2.1.4 Backwashing methods

The columns were backwashed weekly using distilled water for approximately three minutes at a 10% bed expansion (Figure 2.2). This was conducted to simulate WTP filters which are routinely backwashed to remove any algal growth and air bubbles that are trapped in the columns and to redistribute the media. Cleaning of the entire apparatus including tubing, connectors and feed tanks was also undertaken weekly to remove any algal or particle deposits.

![Figure 2.2 Demonstration of the backwashing technique](image)

The weekly backwashing procedure was also carried out for the sterile GAC column with non-sterile distilled water; however, autoclaving (at 121 °C, 500 kPa for 20 minutes) of the sterile GAC was performed after backwashing to maintain sterile conditions.

2.1.5 Scanning electron microscopy (SEM) study

To evaluate the development of biofilm on the surface of the GAC and sand particles, samples were taken from the columns for SEM analysis (Section 2.3.4) at two, four and six monthly intervals during the experiment. Samples containing approximately 20 particles were collected from the top portion of each column after backwashing.
2.1.6 Bacterial Enumeration

To quantify the bacteria concentration in the GAC and the sand columns when microcystin biodegradation was evident in both of the systems (day 220), GAC and sand samples of the same volume (5 cm$^3$) were withdrawn from the corresponding columns after backwashing. To detach the biofilm, the GAC and sand samples were separately vortexed in 20 mL glass test tubes containing sterilised Myponga water for 2 minutes. The resultant supernatant with bacteria was immediately decanted and collected to avoid any bacteria reattachment. The above procedures were repeated for five times for each of the samples. The collected bacterial solutions were mixed thoroughly using a magnetic stirrer. Bacterial concentration in these solutions was then analysed using flow cytometry (Becton Dickinson Biosciences, Australia), details of this method were presented by Ho (2004).

2.2 Reagents and materials

2.2.1 Reagents

All reagents used were high performance liquid chromatography (HPLC) grade or analytical grade. Aqueous solutions were made using deionised water (DOC $\leq$ 0.07 mg/L, 18 M$\Omega$ conductivity; Millipore Pty Ltd, USA) produced using a Milli-Q system.

2.2.2 Microcystin

Microcystin LR standard (purity $> 98.0\%$ by HPLC) was obtained from a commercial supplier (Sapphire Bioscience, Australia). The spiking microcystin solution which contained both m-LR and m-LA was extracted from *Microcystis aeruginosa* blooms that occurred in the Gippsland Lakes, Victoria, Australia. The isolation and purification of the toxins were conducted at the Australia Water Quality Centre. This procedure (Ho, 2004) involved freeze-thawing the bloom material in methanol and water three times in
order to lyse the algal cells and release the intracellular toxins. The resulting solution was sonicated for 30 minutes (FX 10 ultrasonicator from Unisonics Pty Ltd, Australia) and then centrifuged (Allegra 6 Centrifuge from Beckman Instruments, USA) for one hour. The separated toxin supernatant was decanted and filtered through Whatman GF/C filter membranes (Adelab Scientific, Australia).

The solid pellet obtained from centrifuging was resuspended in 75% methanol solution and treated following the same procedures as described above to extract any residual toxins. All the collected toxin solutions were combined and rotary-evaporated (Buchi Rotavap System from Medos Company Pty Ltd, Australia) to remove the methanol constituent. The final purification involved passing the toxin solution through preparative reverse phase flash chromatography and preparative HPLC. The received spiking solution of microcystins was stored in a freezer (~ 4 °C) prior to use.

### 2.2.3 Granular activated carbon (GAC)

A commercially available GAC, A6 (PICA, Australia) was selected for this study. It is a coal based, steam-activated GAC that has a relatively high micro- and meso-pore range. Additionally, the hardness of the GAC makes it suitable for regeneration. The physico-chemical properties of the GAC are listed in Table 2.2.

<table>
<thead>
<tr>
<th>Specifications</th>
<th>GAC A6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent Density (g/cm³)</td>
<td>0.40-0.45</td>
</tr>
<tr>
<td>Ash Content (% max)</td>
<td>10</td>
</tr>
<tr>
<td>Iodine Index (mg/g min)</td>
<td>850</td>
</tr>
<tr>
<td>Specific Surface Area B.E.T. (m²/g min)</td>
<td>1100</td>
</tr>
</tbody>
</table>

The desired GAC particle diameter of 1.0-1.4 mm was achieved by screening between 1.0 mm and 1.4 mm opening size sieves. The sieve tower was shaken for 15 minutes at
an angle of 15° to 20° and rotated 90° every 3 minutes. To minimize the potential
effects from the water soluble contaminants, the sieved GAC was then rinsed
thoroughly using deionised water and transferred to a 100 °C oven to dry overnight.
After the GAC reached a constant weight, it was then stored in a desiccator prior to use.

2.2.4 Sand
New sand (River Sands Pty. Ltd. Australia) of the same particle size range as the GAC
was selected for this study. The sand was non-porous, therefore, it was assumed to have
minimal adsorption capacity. Pre-treatment for the sand was performed following the
same procedure as the GAC.

2.2.5 Water samples
The water used in this study was obtained weekly from the Myponga water treatment
plant (WTP) (South Australia) and was transported to the laboratory in 25 L water
containers. The collected water had been treated via the processes of alum coagulation,
dissolved air flotation and rapid sand filtration. The water samples were taken after
these processes prior to disinfection with chlorine. The water had a comparatively high
DOC concentration of approximately 6-7 mg/L.

2.3 Analytical methods

2.3.1 Microcystin analysis
Water samples (250 mL) containing microcystins were concentrated by solid phase
extraction (SPE) using 500 mg Sep-Pak Vac 3cc C18 cartridges (Waters Pty Ltd,
Australia) following the methods given by Nicholson et al. (1994). Water samples were
filtered through 0.45 µm cellulose nitrate membrane filters (Schleicher and Schuell,
Germany) to remove any particulate matter prior to extraction by the cartridges, which
were pre-conditioned with 10mL methanol and 10 mL deionised water. During extraction, water samples of one in every five were spiked with 250 μL of 5 mg/L m-LR standard as an internal efficiency control. Samples were then forced through the cartridges by application of a vacuum chamber (Alltech Associates, Australia) at a flow rate of less than 10 mL/min. The cartridges were washed sequentially with 10mL of deionised water, 10% methanol and 20% methanol. After vacuum drying (for approximately 2 minutes), the concentrated toxins in the cartridges were eluted into 10 mL glass test tubes with 10 mL 100% methanol. The eluants were then dried in a Ratek Dry Block Heater (Adelab Scientific, Australia) at 40 °C under a nitrogen stream. The residues were then taken up with 500 μL of methanol and 500 μL of deionised water. The obtained 1mL concentrated extracts were finally filtered through 0.45 μm polyvinylidene fluoride (PVDF) filters (Gelman Sciences, Australia).

The high performance liquid chromatography (HPLC) methods described by Ho (2004) were used for the analysis of microcystins in this study. The HPLC was equipped with a Waters 717plus autosampler, which sequentially injected 50 μL sample from the 1 mL concentrated extracts. The flow rate was controlled by a Waters 600 pump controller at a flow rate of 1 mL/min; a Waters 996 photodiode array detector (Waters Pty Ltd, Australia) was set to the wavelength of 238 nm. The HPLC system was controlled by a computer equipped with the Waters Millenium 32 software, which also collected and stored the analytical data. The chromatography separation was carried out on a 150 x 4.6 mm I.D. column packed with 5 μm pore size Luna C18 (Phenomenex, Australia). The mobile phase consisted of a gradient mixture of solvent A (30% acetonitrile + 0.05% trifluoroacetic acid) and solvent B (55% acetonitrile + 0.05% trifluoroacetic acid).

### 2.3.2 Dissolved organic carbon and UV$_{254}$ absorbance

Dissolved organic carbon (DOC) and UV$_{254}$ absorbance (UVA$_{254}$) measurements were conducted using an 820 Total Organic Carbon Analyser (Sievers Instruments Inc, USA)
and a UV/VIS 918 Spectrophotometer (GBC Scientific Equipment Pty Ltd, Australia) at the wavelength of 254 nm. Samples were initially filtered through 0.45 μm cellulose nitrate membrane filters (Schleicher and Schuell, Germany). UVA$_{254}$ was measured in 1 cm quartz cells using deionised water (Millipore Pty Ltd, USA) as a reference.

### 2.3.3 High performance size exclusion chromatography

Prior to analysis samples were filtered through 0.2 μm cellulose nitrate membrane filters (Schleicher and Schuell, Germany) and stored at -18 °C until measurement. The HPSEC system consisted of a Waters 2690 Alliance System and built-in pumps, column heater and mobile phase degasser as well as a Waters 996 photodiode array detector UV-detection system (Waters Pty Ltd, Australia). The column was a Protein KW-802.5 column (diameter = 8 mm, length = 300 mm; Waters Pty Ltd, Australia). The mobile phase solution \([920 \text{ mM sodium di-hydrogen phosphate (NaH}_2\text{PO}_4) \text{ at pH 6.8}]\) and samples were injected at a flow rate of 1 mL/min. Results were analysed using polystyrene sulphonates (35000, 18000, 8000 and 4600 Daltons) and acetone as standards (Chin et al., 1994).

### 2.3.4 Scanning electron microscopy

SEM was used in this study for investigation and comparison of the biological growth on surfaces of both the GAC and the sand. The microscope was a Philips XL 30 field emission scanning electron microscope with an operating voltage of 10 kV. GAC samples taken from the column were immediately transferred into a 4 % paraformaldehyde/1.25 % glutaraldehyde solution in lead sulphide (PBS) + 4 % sucrose at pH 7.2 to fix overnight. The fixed samples were twice washed with PBS + 4 % sucrose solution followed by 30 minutes post-fix in 1% OsO$_4$ in PBS. The GAC particles were subsequently dehydrated with gradient ethanol (70%, 90%, 95% and 100%) solutions for 10 minutes, twice for each step, except the 100% solution which was used three times. A Balzers CPD 030 critical point dryer was then employed to dry the samples. Finally, the prepared GAC particles were mounted on stubs and coated
2.4 Validation of the experimental design - side effects from the sterilisation method of autoclaving

The autoclave process, which is defined as “an apparatus using superheated steam under high pressure in order to decontaminate materials or render them sterile” (Merriam-Webster Dictionary) was used in this study to sterilise the influent water, the GAC and the apparatus of the sterile GAC system. It was possible that the original characteristics of the autoclaved items could change and the adsorption capacity of GAC could be varied to some extent (Salvador and Jiménez, 1996; Shende and Mahajani, 2002). Thus, to ensure identical operating conditions between the sterile and the non-sterile GAC systems, the following procedures were performed to assess any side effects introduced during autoclaving.

2.4.1 Effects on the water quality

To determine any changes in the water quality and thus the adsorbability of the NOM due to autoclaving, duplicate water samples before and after this procedure were collected and analysed simultaneously in terms of DOC, UVA₂₅₄ and HPSEC.

2.4.2 Loss of organic carbon from GAC structure and apparatus

It has been documented that up to 10% of carbon could be lost from the original GAC structure at 650-850 °C during one set of thermal regeneration (Salvador and Jiménez, 1996). Although the autoclave temperature (121 °C) in this study was much lower, it was carried out frequently (weekly) compared to the regeneration process, which is normally undertaken every few months. Thus autoclaving of GAC may have led to carbon loss. Moreover, at the lab-scale study even a very small percentage loss of carbon would significantly affect the results. Therefore, it was necessary to quantify the
possible loss of carbon from the autoclaved GAC.

To assess the carbon loss, a new GAC sample from the same stock of GAC used in the column study was tested. It was autoclaved (at 121 ºC, 500 kPa for 20 minutes) for six times in 150 mL of fresh deionised water, which was retained after each autoclave run for UVA$_{254}$, DOC and HPSEC measurements.

The apparatus of the sterile GAC system, such as the tubing and connectors could potentially leach carbon during the autoclaving process. Thus, it was important to determine the extent to which the leaching could occur. To test for carbon leaching, all the apparatus were disconnected and separately autoclaved in individual Myponga water samples (Figure 2.3). One control sample containing just the water was also autoclaved. After autoclaving, the water in each sample was analysed for DOC concentration.

![Figure 2.3 Testing for carbon leaching from tubing and connectors belonging to the sterile GAC system during autoclaving](image)

2.4.3 Effects on adsorption capacity of the GAC

The adsorption capacity of the sterile GAC was examined before and after autoclaving. During this set of experiments, PAC A6 rather than the GAC A6 was examined. This was because PAC possessed more specific surface area than GAC and is thus more sensitive to any change. For the procedure, two identical slurry samples (10% w/v) of
50 mg PAC A6 were prepared in distilled water. One sample was autoclaved (121 °C, 500 kPa for 20 minutes) while the other was untreated. After the autoclaved sample was allowed to cool to room temperature, the two PAC slurries were individually added into Gator jars, each of which contained 2 L of Myponga WTP water spiked with microcystins. The stirring speed of the jar test was 100 rpm. Sub-samples of 100 mL were collected at time intervals of 0, 5, 10, 20, 30, 60 and 90 minutes for analysis of microcystins, DOC and UVA$_{254}$.

### 2.4.4 Effects on desorption

Studies on the effect of autoclaving upon the pre-adsorbed substances in the sterile GAC were carried out. It was considered important as under the high temperature of autoclaving, various processes or physical-chemical reactions could occur and potentially affect the compounds adsorbed on the GAC. For example, some volatile compounds were found to desorb from pre-loaded GAC at temperatures exceeding 100 °C (Salvador and Jiménez, 1996). Furthermore, the frequent (weekly) autoclave treatment in this study could have compounded the desorption effect to an extent where the inherent adsorption capacity for the trace levels of microcystins could be increased.

To determine the amount of organic carbon that could be desorbed during autoclaving, effluent samples (15 mL) were periodically collected from the sterile GAC column shortly after autoclaving. Effluent of the column prior to autoclaving was also taken for comparison. Collected samples were immediately filtered through 0.45 μm cellulose nitrate membrane filters (Schleicher and Schuell, Germany) and then analysed for DOC and UVA$_{254}$. 

To determine any desorption of microcystins from the sterile GAC due to autoclaving, microcystin analysis was carried out on the water sample in which the GAC was autoclaved (121 °C, 500 kPa for 20 minutes). These tests were carried out after the GAC had been operated for 2.5 months.
CHAPTER 3
LABORATORY-SCALE COLUMN
EXPERIMENT: MICROCYSTIN REMOVAL

3.1 Introduction

Although a few studies on microcystin removal using GAC filters have been undertaken (Hart et al., 1998; Bernezeau, 1994; Hart and Stott, 1993, Jones et al., 1993; Craig and Bailey, 1995), most of them put emphasis on its adsorption effect and not biodegradation. In the study of Ho (2004), biological degradation of microcystins in GAC filter was observed and considered a promising treatment option because of its high removal efficiency and ability to extend the service life of the otherwise very costly GAC filters. However, it was still unknown how long a newly established GAC bed could be used for effective microcystin removal or its efficiency as a function of the operation time. There is currently no information available on efficiencies of the two individual mechanisms, adsorption and biodegradation, which are likely to occur simultaneously in a GAC filter for microcystin removal.

The aim of the laboratory-scale experiment was to discriminate the relative importance of adsorption from biodegradation of microcystins in GAC filters. As shown in Section 2.1, three laboratory-scale columns, i.e. the sterile GAC, the sand and the non-sterile GAC columns, were operated in parallel for the replication of adsorption, biodegradation and the combination of both, respectively, in GAC filtration.

3.2 Results from the first set of experiments

Microcystin concentration in the influent and effluent of the three parallel columns (Section 2.1) was monitored for approximately 230 days during this study. Results
obtained from day 1 to day 65 were considered unreliable as an unsuitable SPE cartridge was used, and this part of the experiment was repeated (Section 3.3). Results after day 65 are presented in Figure 3.1 where concentration profiles of m-LR and m-LA in both of the inlet and outlet of the three columns (the sterile GAC, the sand and the non-sterile GAC column) are plotted against operation time. Two feed tanks supplying the toxin spiked water were used for this study to separately supply feed water to the sterile and the non-sterile GAC and the sand columns. Microcystin concentration in the two reservoirs was similar (Figure 3.1). The degradation of the two microcystin analogues (m-LR and m–LA) presented similar trends in the columns. Details of the degradation are described as follows regarding each of the three columns.

Figure 3.1 Influent and effluent concentrations of m-LR (A) and m–LA (B) of the columns during the first set of experiment
3.2.1 The non-sterile GAC column

A significant breakthrough of microcystins was evident in the effluent of the non-sterile GAC column between days 66 to 100 (Figure 3.2). Approximately 40% m-LR and 60% m-LA was detected in the column outlet. The breakthrough was not surprising as the GAC column had been operating for two months (days 1 to 65) with water containing relatively high concentration of NOM. Therefore, the GAC toxin adsorption capacity would have been partially depleted by the presence of NOM. Similar breakthrough results were also reported by earlier researchers including Craig and Bailey (1995) and Ho (2004) who studied adsorption of microcystins using two-month old GAC.

![Figure 3.2 Percent removal of m-LR and m-LA in the non-sterile GAC column](image)

**Figure 3.2** Percent removal of m-LR and m-LA in the non-sterile GAC column (the dotted circle indicates the reoccurred toxin breakthrough)

The adsorbability of m-LR was stronger than m-LA when GAC A6 was used for this study, as considerably higher percentages of m-LR were adsorbed compared to m-LA during the breakthrough period between days 66 to 100 (Figure 3.2). Similar observations were reported from research by Cook and Newcombe (2002), Newcombe *et al.* (2003) and Ho (2004), who investigated different types of GAC. However, this outcome was unexpected because m-LA is of lower molecular weight (MW) and higher hydrophobicity compared to m-LR, both of which are characteristics that would lead to greater adsorption. It is possible that the adsorbed m-LA molecules on the GAC surface presented strong electrostatic repulsion, which would negatively affect further
adsorption (Newcombe et al., 2003) and lead to the lower adsorption of m-LA.

After breakthrough, toxin removal rapidly increased to 100% at approximately day 100. This was attributed to biological degradation, as there was no significant change in the operation conditions and it was very unlikely that microcystin adsorbability of the GAC could be improved with time (Ho, 2004). It has also been shown that adsorption by dead cells or organic materials would not be significant (Lahti et al., 1997; Holst et al., 2003). The initial period of time prior to the occurrence of rapid biodegradation is known as the lag-phase. This phase is commonly observed in the application of the biological treatment technology and is a natural requirement as bacteria have to acclimatise to new carbon sources. Further discussion on the lag-phase phenomenon is given in Section 3.5.2 and Chapter 6.

Unexpectedly, toxin breakthrough reappeared (as indicated by the dotted line in Figure 3.2) after the initial biodegradation commenced at approximately day 100. Similar trends were not observed in Ho’s study (2004) even when the influent toxin concentration was nearly doubled. Explanations for the second breakthrough were unknown, although it could be attributed to the newly developed and less robust biofilm on the GAC surface, which could be easily affected by any variation of the operation conditions (temperature, water quality etc). Stable biological metabolism was reached from day 130 as no further toxin breakthrough was detected from then on (Figure 3.2).

### 3.2.2 The sterile GAC column

Biological activity was absent in the sterile GAC column during the experiment as indicated by results of the regular agar assays (Appendix A). Therefore, adsorption was the sole mechanism for microcystin removal. Toxin breakthrough in the sterile GAC column was detected, which is presented in Figure 3.3. The data indicates that the adsorption capability for microcystins gradually decreased as the adsorption sites were depleted with time by NOM and microcystins.
The sterile GAC, which simulated only adsorption, should present the same toxin removal capacity as the non-sterile GAC before day 100 when adsorption was the predominant function (indicated in Figure 3.3). If there was any biological activity contributing to the removal, the non-sterile GAC column should show higher removal than the sterile GAC column. However, considerably lower removal rates were recorded for the non-sterile GAC (highlighted in Figure 3.3). Approximately 30% less of m-LR and 40% less of m-LA was adsorbed by the non-sterile GAC during the adsorption breakthrough period between days 66 to 100. Reasons for the different adsorption efficiency were unclear, although it was suspected that the biofilm that grew on the surface of the non-sterile GAC would have restricted the external pores thus decrease the diffusion efficiency or alternatively the weekly autoclaving treatment for the sterile GAC could affect the inherent adsorption capacity for microcystins. More details on these aspects are given in Section 3.5 and Chapter 5.

Compared with the microcystin removal in the non-sterile GAC column (Figure 3.2), the removal in the sterile GAC was highly variable. One reason for the variability could be the weekly autoclaving treatment where the GAC was sterilised. The microcystin adsorption capacity of the GAC was assumed unaffected by autoclaving at 121 °C, 500 kPa for 20 minutes as the carbon was activated at temperatures greater than 1000 °C.
However, outcomes from a trial, during which microcystin adsorption in the sterile GAC was measured daily between days 192 to 202 (Figure 3.4), indicate that adsorption was higher in GAC shortly after autoclaving on days 192 and 200 (underlined points in Figure 3.4). It was, therefore, proposed that the weekly autoclaving could be contributing to the fluctuation of toxin removal in the sterile GAC column.

![Figure 3.4](image)

**Figure 3.4** Impact of autoclaving on the effluent concentration of both m-LR (upper) and m-LA (lower) from the sterile GAC column – enhanced toxin adsorption after autoclaving

Reasons for the temporarily enhanced adsorption in the autoclaved sterile GAC column could be attributed to the partial desorption of pre-adsorbed substances (described in Section 5.3.4). Desorption from some of the pre-occupied adsorption sites could improve the adsorption capacity for microcystins. Importantly, it was shown that the amount of organic material released due to weekly autoclaving was not significant (<5%) relative to the amount that was adsorbed (Section 5.3.4). Hence, the interference caused by the autoclave treatment was a temporary phenomenon, and most of the data obtained for this study truly represented the adsorption trend of the sterile GAC column.

### 3.2.3 The sand column

Microcystin (m-LR) removal in the sand column is presented as toxin concentration in the influent and effluent water for this column (Figure 3.5). Microcystins were not eliminated in the sand column prior to day 211 as similar levels of the toxin were evident in the column inflow and outflow. However, a sharp decrease in the outlet toxin
concentration was observed from days 211 to 215 (highlighted in Figure 3.5), which indicates that biodegradation of microcystins was initiated during that time. The initiation of biodegradation in the sand column shows similarity with the degradation in the non-sterile GAC column at about day 100, which suggests that day 100 was the start of biodegradation in the non-sterile GAC, and the removal mechanism before this time was primarily adsorption.

![Figure 3.5](image.png)

**Figure 3.5** Removal of m-LR in the sand column (the dotted rectangular indicates the sharp decrease in the toxin outlet concentration when biodegradation commenced in the sand column)

The initiation time prior to biodegradation of microcystins was significantly different in the non-sterile GAC (approximately 3 months, Figure 3.1 or 3.2) and the sand column (greater than 7 months, Figure 3.5). This may be attributed to different biological growth patterns on the two substrates as indicated by SEM images (Figure 3.6). On the surface of the sand (image A), only sparse biological attachment and growth was evident after the column had been operated for approximately 3 months. In contrast, on the surface of the GAC (image B) where microcystin biodegradation had just been initiated at the time of imaging, a flourishing microbial community was well developed. After approximately 8 months (image C and D), the biofilm on both surfaces had matured. The sand surface (image C) still had much less biomass than the surface of GAC (image D), but it was comparable to the 3-months old GAC biofilm.
Biofilm growth (shown in Figure 3.6) occurred much faster on the virgin GAC particles than on the new sand. Reasons for the different biofilm growth rates were not obvious although it could be attributed to the different surface characteristics of the two substrate materials as shown in the SEM images in Figure 3.7. The surface of the sand (left) is rather smooth and nonporous, while the GAC (right) shows a much rougher surface with random widely distributed crevasses and ridges which provides additional surface area. Such structures of GAC could help protect newly attached bacteria from shear forces which could have been a major hindrance for biofilm development.
In addition to favorable surface characteristics of GAC for bacteria attachment, it is also possible that the external macropores or crevasses of the GAC, which lead to internal macropores, could result in further bacterial growth by providing inner surface area. To examine this possibility, SEM was used to view GAC granules that had been sliced in half to uncover the internal macropore surfaces of the GAC (Figure 3.8). It was evident that a negligible amount of bacteria grew in these pores as no biofilm was evident. Therefore, it is proposed that the biofilm only covered the outer surface of the GAC particles and no considerable extra area was provided by the open macropores or crevasses.
3.3 Results from the repeat experiment

Microcystin removal rates prior to day 66 were obtained from the repeat experiment that was conducted shortly after the initial study (Section 3.1). The employed apparatus and operational parameters were kept the same for the two experiments, except the sand column that was not re-commissioned as it was presumed that no biodegradation of microcystins occurred during the initial period of time because no removal was observed for 7 months (Figure 3.5).

Toxin concentration for the inflow and outflow from the columns was plotted against operation time (Figure 3.9). Toxin m-LR was completely adsorbed in both of the two GAC columns in the initial 9 days. Slight breakthrough was evident from the non-sterile GAC after day 10, but similar breakthrough was not observed in the sterile GAC column until day 35. From day 10, m-LR breakthrough from the non-sterile GAC column gradually developed and then stabilised at about 10% from day 18. After day 34 this decreased and after a further 4 days, no additional breakthrough was recorded. Therefore, biodegradation of microcystins was proposed to have started from day 34 in the non-sterile GAC column. In the sterile GAC column toxin breakthrough started from day 35 and then gradually increased.
Similar toxin removal behaviour was observed in the two experiments with respect to the difference in microcystin adsorption efficiency between the sterile and the non-sterile GAC column. Toxins were shown to be more readily adsorbed by the sterile GAC (no biofilm growth) than the non-sterile GAC (with biofilm growth) in both of the experiments. Further discussion regarding biofilm effects is given in Section 3.5.1.

Disagreement between the two studies was also evident. The length of the lag-phase prior to biodegradation of microcystins in the non-sterile GAC column was much longer in the first experiment (approximately 100 days) compared with the repeated work (only 34 days). This could be due to pre-filtration of the water source in the initial two months of the first study, which was not performed in the repeat work. A 0.2/0.8 µm capsule filter (Pall Life Sciences, USA) was used for pre-filtration of the water sample prior to use. This procedure had been proposed for removal of some of the microorganisms in the water, which may have caused pre-degradation of the toxins in the water reservoir before it was pumped to the columns as documented by a previous study also using Myponga water (Ho, 2004).

The pre-filtration through the 0.2/0.8 µm pore size filter potentially removed all bacteria that were responsible for microcystin metabolism. SEM images (Figure 3.10) were taken from the primary bacterial colonies that were extracted from the GAC column where biodegradation of microcystins was present. Five different types of bacteria

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**Figure 3.9** m-LR and m-LA concentration for the inflow and outflow from the columns in the repeated set of experiment

---
(unidentified) were dominant in respect to their morphology. It was evident that only two types (A and D) with short rod shapes were small enough to pass through the filter and be present in the filtered water, while the rest were relatively larger (B, C and E) and were likely to be trapped in the filter. Hence, there might be significantly less bacteria in the feed water that was supplied to the non-sterile GAC column in the initial two months of the first study, so this was probably the reason for the longer lag-phase before biodegradation occurred in the original non-sterile GAC column.

Figure 3.10 Scanning electron microscopic images showing the size of main bacteria species that were isolated from the non-sterile GAC column
3.4 Combined results

The dimensionless concentration of m-LR and m-LA in the three columns containing the sterile, the non-sterile GAC column and the sand column was determined (Figure 3.11) based on the first (Section 3.1) and the repeated (Section 3.2) experiments. Toxin breakthrough in the two experiments was well integrated with each other except for the different lag-phase periods prior to commencement of biodegradation in the non-sterile GAC column. It was considered acceptable to obtain different periods of initiation time as the GAC columns were studied at different times, and most importantly, the different lag-phase period does not affect the resultant toxin removal trend in the column. Therefore, the lag-phase for the repeated experiment is presented for discussion of the final results.
Each of the three columns (Figure 3.11) represented different microcystin removal mechanisms, i.e. adsorption (the sterile GAC column), biodegradation (the sand column) and the combination of the mechanisms (the non-sterile GAC column). Thus it is proposed that the partitioning of the relative importance of the two microcystin removal mechanisms in GAC filtration was successfully separated. The separated data provides essential information for further investigating and facilitating microcystin removal using GAC filtration. A mathematical model, which is able to predict the toxin removal efficiency in GAC filtration, could be developed by separately modelling the two removal mechanisms (adsorption and biodegradation) and then combining the results to calculate the overall efficiency.

### 3.5 Phases of microcystin removal in GAC filters

Microcystin breakthrough observed in the columns in this study is summarised in Figure 3.12 (A) based on the combined experimental results discussed previously and presented in Figure 3.11. The whole removal process in GAC filtration could be divided into two phases, i.e. Phase I (principally adsorption) and Phase II (principally biodegradation). A corresponding conceptual model describing the toxin elimination mechanisms in the two phases is also presented in Figure 3.12 (B).
Chapter 3 Laboratory-Scale Column Experiment: Microcystin Removal

Figure 3.12  A) Normalised percent breakthrough curves of microcystins in the sterile GAC, the non-sterile GAC and the sand column in the column experiment. Microcystin removal in GAC filtration is divided into Phase I (adsorption) and Phase II (biodegradation);

B) Conceptual model describing microcystin removal mechanisms by GAC before and after biofilm development. The corresponding removal rate by adsorption and biodegradation calculated from this study is also given.

Dimensionless concentration, \( \frac{C}{C_0} \)

<table>
<thead>
<tr>
<th>Operation Time</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-sterile GAC</td>
<td>Adsorption</td>
<td>Biodegradation</td>
</tr>
<tr>
<td>Sterile GAC</td>
<td>Adsorption</td>
<td>Biodegradation</td>
</tr>
<tr>
<td>Sand</td>
<td>Adsorption</td>
<td>Biodegradation</td>
</tr>
</tbody>
</table>

Adsorption

Rate:

\[ \geq 1.25 \, \mu g/m^2/d \text{ (m-LR)} \]

Biodegradation

Rate:

\[ \geq 1.06 \, \mu g/m^2/d \text{ (m-LR)} \]

\[ K_f' = K_f + K_{f,b} \text{ (biofilm effect)} \]

\[ K_f \]

\[ \text{Rate: } \geq 1.06 \, \mu g/m^2/d \text{ (m-LR)} \]

\[ \text{Adsorption} \]

\[ \text{Unknown rate} \]

Before (GAC) | After (GAC + Biofilm)
3.5.1 Phase I - Adsorption removal and biofilm effect

Adsorption is the primary mechanism for microcystin removal in a newly established GAC filter (indicated as Phase I in Figure 3.12) as the initiation of toxin biodegradation requires a period of time for development of biofilm in the GAC bed. For the present study, the highest microcystin (m-LR) adsorption rate in this phase was no less than 1.25 µg/m²/d (Appendix B).

The adsorption process is essentially the mass transfer process of toxin molecules from a liquid phase into the GAC porous structure followed by adsorption on the surface. The first transfer operation is usually described by mass transfer coefficient parameter $K_f$. Analytical models such as the plug flow Homogeneous Surface Diffusion Model (HSDM), is available from the scientific literature. This model is used widely to predict adsorption kinetics of organic compounds by activated carbon (Rosen, 1952; Weber and Liu, 1980; Yuasa, 1982; Sontheimer et al., 1988; Smith et al., 1987; Traegner et al., 1989; Roy et al., 1993 and Schmidt, 1994). The adsorption equilibria of HSDM can be described by the Freundlich isotherm equation (Freundlich, 1906):

$$ q = K_f C^{1/n} $$  \hspace{1cm} (Equ. 6)

Where $q$ is the equilibrium solid phase concentration (mg, adsorbate/g, adsorbent), $K_f$ = Freundlich isotherm constant (cm/s), $C$ = equilibrium liquid phase concentration (µg/L), and $1/n$ = Freundlich isotherm constant. The application of HSDM for modelling GAC adsorption of trace organic contaminants including microcystins from natural water has been previously validated (Ho, 2004). In the present study, the adsorption behaviour in the sterile and the non-sterile GAC columns was modelled using the HSDM (Figure 3.13).
The isotherm parameters ($K_d, K$ and $1/n$) applied in the HSDM model runs were obtained by minimizing the error between the experimental data and the curve fit. The values of these parameters (presented in Table 3.1) are reasonable when compared with those for other organic compounds cited in the literature, for example, 2-methylisoborneol (MIB) and m-LA adsorption using virgin P1100 and Picazine carbon in Ho’s (2004) study (shown in Table 3.1).

Table 3.1 HSDM input parameters ($K_d, K$ & $1/n$) describing m-LR adsorption onto the sterile and non-sterile GAC in the present study, and MIB and m-LA adsorption parameters cited in Ho’s (2004) study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Present study</th>
<th>Ho’s (2004) study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sterile GAC</td>
<td>Non-sterile GAC</td>
</tr>
<tr>
<td>$K_f$ (cm/s)</td>
<td>$3.0 \times 10^{-3}$</td>
<td>$1.2 \times 10^{-3}$</td>
</tr>
<tr>
<td>$K$ (ng/mg)(ng/L)$^{1/n}$</td>
<td>6.202</td>
<td>6.202</td>
</tr>
<tr>
<td>$1/n$</td>
<td>0.805</td>
<td>0.805</td>
</tr>
</tbody>
</table>
For the best fit of the non-sterile GAC adsorption data, the $K_f$ value needed to be lower than the value for the sterile GAC (Table 3.1). As mentioned previously (Section 3.2 and 3.3), this could be due to the biofilm that grew on the GAC surface causing reduction in surface diffusion of microcystins ($K_{f,b}$). Therefore, the toxin transfer efficient onto the GAC after development of biofilm ($K_{f}'$) is given by considering the interference of biofilm (conceptual model in Figure 3.12 B). Observation of the affected mass transfer rate by biological accumulation has also been documented by many other authors (Lowry and Burkhead, 1980; Schultz and Keinath, 1984; Bishop et al., 1995; Zhao et al., 1999; Lahav and green, 2001 and Song et al., 2006). For example, in Olmstead’s (1989) study the mass transfer coefficient ($K_f$) of trichloroethylene was found to decrease with increasing biomass growth on the GAC, but the Freundlich parameter ($1/n$), an indicator of the adsorption capacity of the adsorbent/adsorbate system, did not change significantly.

3.5.2 Phase II - Biodegradation removal and lag-phase

The transition from Phase I adsorption to Phase II biodegradation is very quick via a sharp decrease in the toxin breakthrough (Figure 3.12 A). This rapid transition from adsorption to biodegradation implies that the highly efficient biological removal of microcystins commenced in the non-sterile GAC column immediately after the bacteria acclimatised to the toxins. Therefore, biodegradation, as opposed to adsorption, became the primary mechanism for microcystin elimination in the biologically activated GAC column. Additionally, this finding also indicates that microcystins are highly biodegradable given appropriate operating conditions. In a previous GAC study conducted by Ho (2004) similar phenomenon of a sudden increase in microcystin removal was detected. The author attributed this to biological activities in the GAC filter, because significant breakthrough of the toxins reappeared in the filter outlet after the biofilm on the GAC was inactivated by sterilisation.

During Phase II, microcystin removal would be largely enhanced due to biodegradation. According to this study, the m-LR removal rate was shown increased from 380 to no less than 1.06 $\mu$g/m$^2$/d after biodegradation commenced in the non-sterile GAC column.
A test (Section 2.1.6) was undertaken to quantify the bacterial concentration in the non-sterile GAC and the parallel sand column, both of which were then in biodegradation phase. Results ($60 \times 10^7$ cell/cm$^3$ in the GAC and $1.37 \times 10^7$ cell/cm$^3$ in the sand) from this study showed that the biofilm that grew in the GAC and the sand column had very similar bacterial abundance. It thus indicates that the biofilm in the non-sterile GAC column was able to completely remove the microcystins under the employed operational conditions. Additionally, biodegradation occurs before adsorption as biomass is located on the surface of the carbon (Figure 3.12 B), and mass transfer through this biological layer would occur before adsorptive diffusion into the GAC solid occurs. Therefore, the removal of microcystins in Phase II was mainly due to biodegradation rather than adsorption (Figure 3.12 B).

Although biological GAC (BGAC) filtration seems to be a highly efficient option for microcystin treatment, the prerequisite lag-phase (Section 3.2.1) which may take days or months (depending upon the operational conditions) is a major obstacle for the practical application of this technology. Similar lag periods of varying length have been widely reported from a large spectrum of biological studies. It has been found to be very difficult to predict the lag-phase, as its occurrence and length relates to many factors, such as previous exposure to the substances (Mort and Deanross, 1994; Mrgain et al., 1995; Gotvajn and Zagorckoncan, 1996; Mort et al., 1999; Guieysse et al., 2001); concentration of the substances (Gotvajn and Zagorckoncan, 1996; Thomas et al., 2002); existence of enzyme inducers (Yassir et al., 1998); reactions between genes involved in degradation (Churchill et al., 1999); the volume of test medium (Ingerslev et al., 2000); and the amount and concentration of specific degrading microorganisms (Ingerslev et al., 2000; Preuss et al., 2002; Bending et al., 2001).

The lag-phase period prior to microcystin biodegradation is hard to predict. In Ho’s (2004) GAC column study, the lag period for microcystin degradation decreased and in one instance disappeared upon intermittent re-dosing of microcystin. Observations from the batch tests conducted in the present study (Chapter 6) indicate that the length of delay could also be affected by a diverse range of operation conditions, such as the temperature, the quality of water, the concentration of bacterial cells and the aeration level in the
solution. Since microcystin biodegradation is extremely important for GAC filtration processes, further investigation to minimise the lag-phase is necessary.

3.6 Conclusions

The results from this long-term column experiment demonstrate that the relative contribution of adsorption and biodegradation for the removal of microcystins in GAC filtration have been successfully separated. Relevant information on the toxin adsorption, biodegradation and the combination of the two mechanisms is represented by the results of the sterile GAC column, the sand column and the non-sterile GAC column, respectively (Figure 3.11). The whole microcystin removal process in GAC filtration could be divided into two phases, i.e. Phase I (mainly adsorption) and Phase II (mainly biodegradation).

The GAC adsorption capacity for microcystin would be gradually depleted by natural organic matters in the source water and the toxins themselves. Generally, adsorption of the two analogues of microcystin (m-LR and m-LA) both follows a typical breakthrough curve, but they are of different adsorbability. Higher adsorbability was evident for m-LR in this study (Figure 3.3). The proposed reason for this difference was that the adsorbed m-LA molecules (-2 charge) which have higher negative charge than m-LR (-1 charge), may present stronger electrostatic repulsion and thus inhibit adsorption due to hydrophobic interaction with the surface (Newcombe, 2003).

The behavior of microcystin adsorption in the sterile and the non-sterile GAC column could be modelled using the HSDM (Figure 3.13). The value of the mass transfer coefficient, $K_f$, was found to be lower for the non-sterile GAC than for the sterile GAC as the adsorption of microcystins was higher in the sterile GAC compared with the non-sterile GAC (Figure 3.3). This is proposed to be attributable to the biofilm that blocked the external pores of the GAC thus decreasing the surface diffusion of microcystins into the internal adsorption sites (Figure 3.12 B).

Transition from Phase I adsorption to Phase II biodegradation is very quick (Figure 3.12
A) and therefore difficult to predict. In Phase II, microcystin removal would be enhanced due to the initiation of biodegradation, which also becomes the primary removal mechanism rather than adsorption as in Phase I. The high removal efficiency in the biodegradation phase is preferred but it usually occurs after an unpredictable lag-phase period. For confident practical application of this technology, further investigation aiming to eliminate this delay period should be a priority.

Finally, biodegradation of the toxins occurred much earlier in the GAC column than in the parallel sand column. The possibility that GAC supplied extra surface area that was provided by the open macropores or crevasses in GAC was excluded as the biofilm was found to exist mainly on the outer surface of the GAC particles (Figure 3.8). The reason for the difference in rate of biodegradation between the columns is attributed to the distinctive surface characteristics of the two materials (Figure 3.7), where GAC appears to be a better substrate for biofilm attachment and development than the sand in this study (Section 3.2.3).
CHAPTER 4

REMOVAL OF NATURAL ORGANIC MATTER
IN GAC AND SAND COLUMNS

4.1 Introduction

Although the major aim of this project was to identify the relative contributions of adsorption and biodegradation for the removal of microcystins in GAC filtration, an additional aspect for the application of these processes is the simultaneous removal of Natural Organic Material (NOM). NOM in drinking water is usually a complex mixture of organic compounds that are formed from natural decomposition of animal and plant materials; industry, agriculture and domestic effluents as well as urban and rural rainfall runoff. The composition of NOM typically ranges from small hydrophilic acids, proteins and amino acids to relatively larger humic and fulvic acids (apparent molecular weight usually between approximately 500 and 30,000) which is the predominant constituent of the comparatively high NOM level waters in Australia (Choudry, 1984).

Health problems associated with NOM have been emphasised since the discovery of its disinfection by-products (DBP), which have been recognized as potential threats for the public wellbeing since the 1950s (Middleton and Rosen, 1956). For example, there is particular concern with chlorine DBPs, such as trihalomethanes (THMs), some of which are carcinogenic and may cause series of ailments varying in character and intensity (Sontheimer, 1988). High levels of NOM can also impact human health by promoting intensive biological regrowth in drinking water distribution systems (Yavich et al., 2004). Therefore, water treatment processes that are able to decrease the NOM
Adsorption, which concentrates organic substances on the inner surfaces of solid adsorbents, is the most effective process for removal of NOM in water treatment plants (Sontheimer, 1988). Activated carbon has large internal surface areas (1,000,000 m$^2$/kg) that are non-polar, and is the most common adsorbent used by water authorities (Orshansky and Narkis, 1997). Activated carbon can remove a wide range of organic compounds from water including some health-concerning chemicals such as trihalomethanes, pesticides, industrial solvents, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons.

GAC filtration, which has been extensively used for water treatment processes since the 1930’s offers an effective option for NOM removal due to its high adsorption capacity and rapid adsorption kinetics (Guerine and Boyd, 1997; Rangel-Mendez and Streat, 2002; Song et al., 2006). To optimise the design and the practical operation of this application, extensive studies concerning characterisation of NOM adsorption have been carried out. However, different water sources have different NOM constituents and thus exhibit different adsorbability during GAC filtration (Aiken and Cotsaris, 1985; Najm et al., 1990; Summers, 1989). Consequently, it is difficult to predict the adsorption effect of NOM in water treatment plants. To address this problem, many on-site studies have been conducted for specific water environments (Vahala et al., 1999; Wietlik et al., 2002), but research has been restricted in Australian situations, which usually exhibit high NOM concentrations due to the unique climate and soil conditions.

Application of GAC filters in water treatment systems can also stimulate biodegradation of various target NOM compounds (Mollah and Robinson, 1996; Xu et al., 1997; Jones et al., 1998 and Jung et al., 2001) given that an appropriate biofilm is developed on the GAC grains. This biological process is considered important as it can potentially accelerate the NOM removal and prolong the life-time of GAC filters compared to
solely adsorptive GAC units (Jonge et al., 1996). However, there is no literature available relating to the separation of adsorption and biodegradation for removal of NOM in long-term GAC filters.

The objectives of the research reported in this chapter were to characterise the NOM removal profiles in GAC filtration based on Australian reservoir water, and to separate the adsorption and the biological reduction of these organic compounds.

### 4.2 Methods

The experimental design as presented previously for the microcystin study (Chapter 2 and Chapter 3), which consisted of three columns, i.e. the sterile and the non-sterile GAC and the sand column (Section 2.2), was also used for the NOM investigation. Separated adsorption (the sterile GAC column) and biodegradation removal (the sand column) and the combination of the two mechanisms (the non-sterile GAC) are represented by the three columns. The NOM breakthrough profile was chosen as the measurement criteria for the performance of GAC columns in this study. Influent and effluent samples of the three columns were regularly sampled for NOM analysis. The analytical instruments used to obtain experimental data were: a dissolved organic carbon (DOC) analyser, a UV spectrophotometry and a High Performance Size Exclusion Chromatography (HPSEC) unit.

DOC is the most commonly applied parameter used to quantify NOM. It represents the fraction of total organic carbon (TOC) that passes through a 0.45 μm pore size filter, and is a heterogeneous mixture of various complex organic materials ranging from macromolecular humic substances to small molecular weight hydrophilic acids and various hydrocarbons (Thurman, 1985). Breakthrough of substrates that are precursors of the carcinogenic THMs in GAC units is often similar to that of the DOC (Sontheimer et al., 1988). Samples were pre-filtered through 0.45 μm cellulose nitrate membrane filters (Schleicher and Schuell, Germany) and measured using an 820 Total Organic
Carbon Analyser (Sievers Instruments Inc, USA). The determined DOC value from the analytical instrument is based on the oxidation of the organic matters to CO₂, which is measured and converted to the amount of DOC.

UV absorbance, as a surrogate for DOC measurement, is usually applied as it is quick, straightforward and uses only spectrophotometric instrumentation. Many organic compounds in raw water sources possess the property of absorbing light in the ultraviolet region (10-400 nm) and the visible region (400-800 nm) (Sontheimer, 1988). Absorption at 254 nm (UVA₂₅₄) correlates well with DOC although it tends to reflect only the more complex NOM structures especially the aromatic and unsaturated carbon bonds (–C=–) (Traina et al., 1990), including humic substances, aromatic compounds, tannins and lignins. Pre-filtered (0.45 μm) water samples were measured using a UV/VIS 918 Spectrophotometer (GBC Scientific Equipment Pty Ltd, Australia) at 254 nm in 1 cm quartz cells using Milli-Q water as a reference.

HPSEC is a reliable and informative technique. The use of HPSEC allows characterisation of UV absorbing NOM constituents according to their molecular weight, so it is useful in determining what range of size molecules are removed from the columns during this study. HPSEC analysis was conducted to assist with interpretation of the obtained DOC and UV₂₅₄ data. HPSEC samples were filtered through 0.22 μm cellulose nitrate membrane filters (Schleicher and Schuell, Germany) and analysed using a Waters 2690 Alliance System (Section 2.3.3).

4.3 Results and Discussion

The removal profile of NOM in the three columns (the sterile and non-sterile GAC, and the sand column) is discussed below.
4.3.1 Non-sterile GAC column

![Graph showing DOC concentration over time for non-sterile GAC column](image)

**Figure 4.1** DOC concentration of the influent and effluent of the non-sterile GAC column (the dotted circle indicates the initial NOM breakthrough)

![Graph showing UV absorption at 254nm over time for different columns](image)

**Figure 4.2** UV₂₅₄ absorbance of the influent and effluent of the three columns containing the sterile and non-sterile GAC and the sand column (the dotted circle indicates the initial NOM breakthrough)

NOM removal in the non-sterile GAC column for the duration of this study was determined by analysis of DOC and UVA₂₅₄ in the influent and effluent of the column (Figure 4.1 and 4.2). Generally, the removal of NOM in this column followed a typical breakthrough curve (Sontheimer *et al.*, 1988). During the initial period, approximately 1 week, a low concentration of NOM appeared in the effluent (as indicated by the dotted line in Figure 4.1 and 4.2), which could be attributed to the less adsorbable or non-adsorbable proportion of NOM in the water. HPSEC analysis was carried out to
determine the molecular weight distribution of UV absorbing NOM in the influent and effluent of the non-sterile GAC column (at day 5) (Figure 4.3). It is evident that almost all the UV absorbing organic matter was removed in the initial period of operation of the GAC column except a small proportion of NOM that may be of extremely low or no adsorbability (highlighted in Figure 4.3) (Roberts and Summers, 1982; Roberts et al., 1984 and Sontheimer et al., 1988). This immediate breakthrough was found to be strongly related to the concentration of the non-adsorbable NOM in the water being treated and the EBCT condition used in the operation (Sontheimer et al., 1988).

![Figure 4.3 HPSEC chromatograph showing NOM adsorption in new GAC (day 5)](image)

After the initial period (approximately 7 days), NOM breakthrough in the effluent of the non-sterile GAC column immediately increased at a high rate as determined by the DOC concentration (Figure 4.1) and UVA$_{254}$ (Figure 4.2). This part of the breakthrough profile corresponded to the rapid decrease in the available adsorption sites as the adsorption zone reached the end of the column, and the extent of this rapid breakthrough largely depended on the EBCT of the filter (Hyde, 1980) and the adsorption capacity of the GAC for strongly adsorbing compounds (Sontheimer et al., 1988).
1988). Regarding the DOC concentration in this study, NOM adsorption continued until day 60 when the breakthrough rate reached up to 80%, while after approximately 5 months (150 days), only a low removal (~10%) was evident (Figure 4.1). The reason for the final minor removal was attributed to the mechanism of biodegradation in the filters. In practise, operation of GAC units is often terminated before the low adsorption phase is reached (Sontheimer et al., 1988). Conclusively, the breakthrough profile observed herein was in good agreement with the literature (Roberts et al., 1984) in relation to DOC removal in drinking water plants.

4.3.2 Sterile GAC column

Removal of NOM in the sterile GAC column was also determined in terms of UVA$_{254}$ (Figure 4.2) and DOC concentration (Figure 4.4), and the resultant NOM breakthrough curve was compared with that of the non-sterile GAC column. It was observed that the removal of NOM in the sterile column appeared highly variable. A similar fluctuation was evident in the microcystin concentration (Chapter 3), and it was attributed to the weekly autoclaving of the sterile GAC column, which was proposed as a possible cause of the observed variability (Section 3.3.2). Further studies on this aspect are presented in Chapter 5. Ignoring the fluctuation, DOC removal in the sterile column (Figure 4.4) exhibited very similar behaviour with the non-sterile GAC column throughout the experiment, details of which were presented in Section 4.3.1.

![Figure 4.4 DOC concentration of the influent and effluent of the sterile GAC column](image-url)
After operation of 3 to 6 months there was still up to 20% adsorption of DOC occurring in the sterile GAC column (Figure 4.4). Part removal of the UV absorbing NOM constituents was also observed with the HPSEC analysis (Figure 4.5) which was conducted on both of the influent and effluent water samples of the sterile GAC column after 5.5 months operation. It was evident that some adsorption capacity remained in the 5.5 months preloaded GAC especially for the NOM consisting of low range of molecular weight. From this evidence it is proposed that the low percentage (~10%) removal of NOM that is usually shown in GAC filters after several months’ operation can contribute in part to residual adsorption capacity rather than biodegradation as usually assumed (Sontheimer et al., 1988).

![Figure 4.5 HPSE chromatograph showing NOM adsorption in the preloaded sterile GAC (5.5 months)](image)

Adsorption of the UVA254 characterised NOM in the sterile GAC column (Figure 4.2) was higher in comparison with the DOC characterised NOM (Figure 4.4) as there was still approximately 30% UVA254 removal after 6 months when the adsorption capacity for DOC was shown to be almost saturated. Similar preferential adsorption of UV
absorbing organic matters was reported by other researchers (Sontheimer et al., 1988; Åsa et al., 1997; Li et al., 2003). Reasons for this phenomenon are unknown although it could be attributed to the relatively small MW (<1000 Daltons) of the UV absorbing NOM in the Myponga water (Figure 4.3 and 4.5), because smaller molecules are more readily adsorbable than large MW compounds. Åsa et al. (1997) reported the same conclusion when interpreting a similar adsorption preference that was observed in a pilot-plant GAC study. Another possible reason for the observed higher adsorption rate of the UV absorbing NOM could be due to the different analytical methods used. As mentioned previously (Section 4.2), the DOC analyser measures organic compounds that can be oxidised to CO₂ during analysis, the amount of which is consequently correlated to the amount of DOC. Since it can not be ensured that all the carbon constituents in the water sample are oxidisable, this approach could be less sensitive than the analysis by UV absorbance. Therefore, the significantly decreased removal efficiency of NOM at the end of the adsorption was difficult to be concluded from the DOC determination (Figure 4.4) compared with the more sensitive UV absorbance analysis (Figure 4.2).

4.3.3 Sand column

Removal of NOM in the sand column was determined as UVA₂₅₄ (Figure 4.2) and DOC concentration (Figure 4.6) in the influent and effluent of the column. Generally, there was no observable removal of NOM in this column throughout its operation for approximately 11 months (Figure 4.2 and 4.6). It is, therefore, concluded that the adsorption of NOM was absent in the sand column.
Observable biological removal of NOM was also not evident in the sand column. A HPSEC analysis (Figure 4.7) conducted on water samples prior to and after the sand column that was operated for approximately 10 months, showed very similar MW distribution of the organic matters in the two samples. Reasons for the absence of biodegradation of NOM in the sand column could be attributed to the EBCT (15 minutes, to be consistent with the parallel GAC column) employed in this study, which is low compared to the EBCT of 30–60 minutes that is usually used in a biological sand column.
4.4 Conclusions

The removal behaviour of NOM in GAC (A6) filtration was studied using Australian reservoir water. Because of the relatively high NOM concentration in the water, some specific features were observed from the DOC breakthrough curve. First, the adsorption efficiency of the GAC decreased rapidly after the initial breakthrough, and the removal capacity was shown to be almost saturated after a very short time (about two months). Moreover, the preferred high rate, steady-state adsorption phase (a time span of nearly constant effluent concentration) that usually occurs after the initial breakthrough was not observed in this case. All these features could be due to the high load of the organic compounds in the water, which exhausted the adsorption sites rapidly.

Low percentage (~10%) removal of NOM that is usually observed in commercial GAC filters after several months operation was also observed in the experimental GAC columns during this study. Since any removal of DOC in the sterile GAC column was due to adsorption, the small reduction rate during this time period could be contributed in part to the residual adsorption capacity of the GAC rather than biodegradation, as is usually assumed (Sontheimer et al., 1988).

Finally, the favoured adsorption of UV absorbing NOM compounds compared to the DOC constituents during GAC filtration, which was previously reported by other investigators, was also observed in the sterile GAC column in this study. Two probable reasons could explain this phenomenon. One is the relatively small MW (MW<1000 Daltons) of the UV absorbing NOM in the water, which could be more easily adsorbed on to GAC. Secondly, it was assumed that the DOC analyser used in this study was less sensitive than the UV analyser due to the possible non-oxidising nature of some NOM compounds.
CHAPTER 5
EVALUATING THE VALIDITY OF THE EXPERIMENTAL DESIGN

5.1 Introduction

To quantify the relative importance of the toxin removal by adsorption and biodegradation in a GAC filter, three columns were commissioned: the sterile and the non-sterile GAC column and the sand column. The toxin removal properties of each column were adsorption only for the sterile GAC, adsorption and biodegradation for the non-sterile GAC and biodegradation only for the sand column. To enable the comparison between the columns, it was essential to ensure that the operation conditions for the columns were consistent throughout the experiment. However, the sterilisation treatment (autoclaving) that was conducted prior to application of the sterile (GAC) column may induce a potential difference in operational conditions between the two GAC columns.

The sterilisation treatment, described in Chapter 2, was achieved by autoclaving (121 °C, 500 kPa for 20 minutes). It is a high temperature and pressure process during which various physical or chemical reactions could be promoted in the water and GAC sample, or even in the apparatus involved in the sterile system. Relevant information on this aspect was not available in the literature. Moreover, removal of the microcystins (m-LR and m-LA), which were at trace levels (μg/L), could easily be affected by any change in
the water and GAC characteristics. It was proposed that the fluctuation in the results obtained from the sterile GAC column (Figure 3.4) could be attributed to the weekly autoclaving.

It was, therefore, considered essential to validate the experimental design for this study. In particular, it was necessary to determine the possible side-impacts that could result from autoclaving the sterile GAC system.

5.2 Experimental methods

To investigate possible impacts from autoclaving, a series of experiments (Section 2.4) were carried out on the equipment items involved in the sterile column system that were regularly autoclaved including source water, GAC and associated apparatus. These autoclave studies consisted of the following four experiments: 1) water quality before and after autoclaving were measured for change in terms of DOC, UV\textsubscript{254} and HPSEC; 2) increase of the carbon concentration in the water due to carbon leaching from the GAC structure and tubing during autoclaving was quantified by autoclaving the items separately in deionised water, which was consequently subjected to carbon analysis; 3) variation of GAC adsorption efficiency due to autoclaving was examined in a jar test by comparing toxin adsorption kinetics of a PAC sample (the identical type of carbon A6 as the GAC) before and after autoclaving; and 4) desorption of the pre-adsorbed organic carbon from the GAC during autoclaving was quantified by periodically analysing the carbon in the effluent stream from the GAC column that had been autoclaved.

5.3 Results and Discussion

5.3.1 Effects on water quality

HPSEC results from the treated water before and after autoclaving are shown in Figure
5.1. No variation was observed in molecular weight distribution of the NOM constituents in the water after autoclaving. This was supported by the results from DOC and UVA\textsubscript{254} analysis (indicated in Figure 5.1), which were additional parameters used for determination of the water quality. Thus, there was no observed effect on water quality during autoclaving in regard to the HSPEC analysis.

![Figure 5.1](image)

**Figure 5.1** Molecular weight (MW) distributions, DOC and UVA\textsubscript{254} results of Myponga treated water before and after autoclaving determined by high performance size exclusion chromatography

5.3.2 Loss of organic carbon from GAC structure and apparatus

A virgin GAC A6 sample, which was the same amount as used in the column experiments, was repeatedly autoclaved in deionised water (DOC \leq 0.07 mg/L) to quantify carbon that may be released from GAC during autoclaving. Results shown in Figure 5.2 indicate the amount of carbon lost during autoclaving characterised by DOC and UVA\textsubscript{254}. Approximately 0.8 mg DOC (or 0.002 cm\textsuperscript{-1} UV absorption) was released from the GAC structure during a single autoclaving run, which accounted for approximately 0.4% of the carbon treated by the GAC column (based on a flow rate of
4.9 mL/min and DOC of the water at ~6 mg/L).

![Graph showing leaching of DOC and UV absorption at 254 nm](image)

**Figure 5.2** Carbon leached from the original GAC structure during six sets of autoclaving as determined by DOC and UV abs @ 254 nm

HPSEC results plotted in Figure 5.3 show the MW distribution of organic carbon in the water, which was autoclaved with the virgin GAC. The HPSEC and DOC/UVA$_{254}$ results were consistent as a negligible amount of carbon was released from the GAC after autoclaving (pink curve) compared to the source water (blue curve). Therefore, it was concluded that the weekly sterilisation treatment via autoclaving did not result in significant carbon loss from the GAC structure compared to the total amount of carbon treated through the GAC column.
In addition to the GAC structure, carbon release could occur from autoclaving the experimental apparatus, especially the silica tubing and connectors. The data plotted in Figure 5.4 show the amount of carbon released from the apparatus after autoclaving. The results are based on the area of each tube that was in contact with the water. Although higher concentrations of carbon were evident in water samples that were autoclaved with tubing, minimal carbon, approximately 3 mg in DOC, was released during one autoclaving run, which accounted for less than 1% of the carbon treated by the system in one week. It was, therefore, concluded that the possible confounding effects from autoclaving experimental apparatus of the sterile GAC system was assumed to be insignificant, although to fully quantify this observation repeated experiment would be required to give statistic significant results.

Figure 5.3 Comparison of the carbon released from the GAC structure during autoclaving and the carbon content of the source water. Determined by high performance size exclusion chromatography
5.3.3 Effects on GAC adsorption efficiency

Alteration of GAC (A6) adsorption efficiency after autoclaving was examined using the methods described in Section 2.4.3. The adsorption behaviour of PAC A6 was analysed from a sample before and after autoclaving. The adsorption rate was measured with respect to removal of microcystins (m-LR and m-LA) and NOM (DOC and UVa254) from the Myponga treated water (Figure 5.5). The adsorption rate for both the toxin and NOM constituents were identical in the autoclaved and non-treated PAC samples. Thus, it was concluded that autoclaving GAC A6 did not significantly affect adsorption capacity for both microcystins and NOM constituents in the source water.
Figure 5.5 Comparison of the adsorption behaviour of PAC A6 before and after autoclaving as determined from microcystins (m-LR and m-LA) and NOM (UVA$_{254}$ and DOC) in the Myponga source water

5.3.4 Effects on desorption during autoclaving

The pre-adsorbed NOM and microcystins could be desorbed from the sterile GAC during autoclaving (Section 2.4.4), and consequently result in greater adsorption capacity for microcystins.
The desorption of NOM from the autoclaved GAC was measured using DOC concentration and $\text{UV}_{254}$ absorption (Figure 5.6). It was evident that desorption of the pre-adsorbed carbon occurred from the autoclaved GAC as the concentration of NOM (characterised by DOC and $\text{UV}_{254}$) was elevated in the effluent water of the column. Approximately 4 times higher DOC concentration was shown in the effluent than in the influent water when the column was operated immediately after autoclaving. Subsequently the desorption of DOC decreased rapidly and ceased after approximately
500 mL of treated water had passed through the column. The UV$_{254}$ absorbance from the outlet of the GAC column was almost doubled immediately after autoclaving. This elevated absorbance then gradually decreased and vanished after about 5 L water had flowed through the column (Figure 5.6).

![Graph showing MW distribution](image)

**Figure 5.7** MW distribution of the UV sensitive organic carbons that were desorbed from GAC during autoclaving and that were present in the source water (determined by HPSEC).

The UV sensitive organic carbons that were desorbed from the GAC were analysed using HPSEC (Figure 5.7). The MW distribution of the desorbed organic carbon indicated that the desorption did not peak at the MW range that would potentially affect the adsorption of microcystins (>1000 Daltons). Rather, the desorbed UV sensitive organics showed a similar MW distribution to the organics in the source water except with the organics at MW of approximately 500 Daltons (indicated in Figure 5.7). Moreover, the desorbed organics possessed relatively lower aromaticity than the source water as shown by the specific UV absorbance (SUVA = UVA$_{254}$ (m$^{-1}$) / DOC (mg/L)) parameter (Figure 5.8), which indicates that aromatic compounds are less likely to be desorbed from the GAC.
Figure 5.8 Specific UV absorbance (SUVA) of the released organic carbons from GAC during autoclaving, and the SUVA of the source water

Quantitatively, however, the amount of NOM desorbed was not significant compared to the amount adsorbed. The total amount of desorbed organic carbon could be calculated using the trendline equations of the desorption results (indicated in Figure 5.6). The calculation showed that approximately 2 mg of DOC or 0.004 of UV$_{254}$ absorbing carbon was desorbed from the GAC during one autoclave run, while during one week, approximately 50 mg of DOC or 1.75 of UV$_{254}$ absorbing carbon was adsorbed by the GAC at the time when the test was conducted (2.5 months from start up). Additionally, microcystins were not detected in the water in which the preloaded GAC was autoclaved. Thus desorption of the pre-adsorbed microcystins was less likely to occur during autoclaving of GAC. The results demonstrated that the sterilisation treatment via autoclaving did not result in considerable desorption of the pre-adsorbed substances from the sterile GAC.

5.4 Conclusion

The possible impact from autoclaving the sterile GAC system was quantified by a series
of experiments that covered various aspects including water quality, GAC adsorption efficiency and organic detachment from GAC during autoclaving. It was concluded that no significant impact occurred due to the regular autoclaving treatment of the GAC. The operation conditions for the sterile and the non-sterile GAC columns were verified and thus, the experimental method used in this project was considered valid.
CHAPTER 6

BIODEGRADATION OF MICROCYSTINS AND THE OPERATIONAL CONDITIONS

6.1 Introduction

Granular activated carbon (GAC) has been shown to be a successful option for mitigation of microcystins, which are recalcitrant compounds to conventional water treatment. Adsorption of GAC plays a critical role in microcystin removal in a freshly established filter, but the adsorption capacity and therefore the removal efficiency would be quickly reduced by the excess loading of NOM. The subsequent biodegradation of microcystins could be expected in GAC filters, which is able to enhance the removal efficiency. Observations in the long-term column experiment in this study (Chapter 3) concluded that biodegradation was the primary microcystin removal mechanism once it is initiated in GAC columns.

To date, many investigations have been carried out on microcystin biodegradation, but most focus on identification and isolation of the degrading bacteria, or on determination of the detoxification process. Only a few biodegradation studies have been conducted to identify possible impacts from the operational conditions. Christoffersen et al. (2002) investigated the microcystin biodegradation behaviour in cultures of different DOC properties (by adding lysed algal materials that were collected from different algal bloom sources). The biodegradation process, in this study, was found to be not affected by the DOC properties as almost the same reduction rates were evident in the studied algal
cultures. In the study by Holst et al. (2003) microcystins were biodegraded in the sediment of a water recharge facility under oxic, microaerophilic or anoxic conditions. The authors reported that the degradation was significantly accelerated under the last condition, suggesting that the process was affected by the abundance of electron acceptors in the reaction solution. For the impact of other operational conditions on the microcystin biodegradation process, however, there was no available information in the literature.

To facilitate the application of microcystin biodegradation in GAC filters, it was required to identify the potential effects from different operational conditions. In this study, an attempt was made to record the degradation behaviour under varied temperature, light irradiation and water quality conditions as well as different initial bacterial concentrations.

**6.2 Methods**

The bacterial source used for this biodegradation study was derived from the non-sterile GAC column that was operated for about 6 months (when biodegradation of microcystins was evident in the column) in the long-term column experiment (Chapter 3). The biological GAC column was subjected firstly to backwashing for removal of any interfering contaminants such as algal growth and particle deposits, then, 5 g of the GAC sample was withdrawn from the column. Bacteria on the GAC particles was detached from the carbon surface by repeating the following step 5 times: the GAC was vortexed with deionised water in a 20 mL glass test tube for 2 minutes and the supernatant was immediately decanted and collected. The collected bacterial solutions were then mixed thoroughly using a magnetic stirrer, and kept well mixed prior to the experiments, thus it was assumed that the bacteria were evenly distributed within the culture.

**6.2.1 Microcystin biodegradation under varied temperature, light irradiation and water quality conditions**

Seven sterilised glass bioreactors (2 L) were each filled with 1.5 L of sterilised water and
continuously mixed with sterilised magnetic stirrers. The water contained in all five reactors was Myponga treated water. In the other two reactors Myponga treated water was diluted with deionised water at volume ratio of 1:1 and 1:9 resulting in 50% and 10% of the original NOM concentration. Microcystins were added into these reactors at a target concentration of 5 μg/L m-LR. After allowing complete mixing of the toxins in the reactors, bacteria were introduced by adding 5 mL of the prepared bacterial solutions into each of these bioreactors.

For determination of temperature effects, four of the bioreactors containing non-diluted Myponga water were covered by aluminium foil (no light irradiation) and put under different temperature conditions. Three of them were placed into incubators with temperature settings of 25 °C, 33 °C and 40 °C respectively, and the fourth reactor was run at ambient temperature of 22 °C.

To identify the effect of light irradiation, the fifth reactor containing non-diluted Myponga water was also placed under ambient temperature (22 °C) conditions but without covering (i.e. natural light irradiation allowed). The degradation profile in this reactor was compared with the covered fourth bioreactor.

The last two reactors filled with diluted Myponga water, were used to determine the effect of water characteristics. They were studied under ambient temperature (22 °C) without covering. Therefore the biodegradation behaviour in these two reactors could be compared with the fourth bioreactor as described above.

Microcystin degradation in these bioreactors was monitored for approximately 10 days. Subsamples of 150 mL were taken from each of the containers at regular time intervals. Microcystin concentration in these samples was determined.
6.2.2 Microcystin biodegradation in cultures of different bacterial concentration

In this test, another seven sterilised 2 L glass bioreactors were each filled with 1.5 L of sterilised Myponga treated water. Microcystin solutions were then added into these reactors with a target m-LR concentration of 10 μg/L, and the reactors were then mixed continuously using magnetic stirrers. Different volumes (5 mL, 2.5 mL, 1.0 mL, 0.5 mL, 0.2 mL and 0.1 mL) of the prepared bacterial solution [corresponding to 75.5 x 10^6, 38.0 x 10^6, 15.1 x 10^6, 7.6 x 10^6, 3.0 x 10^6 and 1.5 x 10^6 cell/mL determined by flow cytometric analysis (Becton Dickinson Biosciences, Australia)] were added into six of the bioreactors respectively. One reactor was used as a control where bacteria were not introduced. Samples (150 mL) were taken from the bioreactors at regular intervals and analysed for microcystin concentration using HPLC.

6.3 Results and Discussion

6.3.1 Effects from temperature

The concentration of m-LR in the bioreactors that were placed under different temperature conditions (Section 6.2.1) was monitored (Figure 6.1). Biodegradation at 25 °C and 33 °C was very similar and presented the highest degradation rate after the initial lag period of about 50 hours. Complete removal of the toxin occurred after approximately 75 hours in these two bioreactors. The reactor at ambient temperature (22 °C) had the same lag-phase (~50 hours) but the subsequent removal rate was much lower than observed in the reactors at 25 °C and 33 °C. Toxin could not be detected after 150 hours in this bioreactor. In contrast, decrease of m-LR was not evident in the 40 °C reactor during the experiment (~10 days), and the bacteria died as observed during a subsequent agar plate assay.
It is indicated from this test (Figure 6.1) that the temperature under which microcystins are biodegraded had a considerable effect on the degradation rate. Temperatures ranging from 25 °C to 33 °C favour this particular biological process, whilst high temperature conditions (40 °C) did not suit the growth of bacteria and the biodegradation of microcystins ceased. Similar observations of these temperature effects were also evident during biodegradation of many other organic contaminants (Dibble and Bartha, 1979; Cho et al., 1994; Cadiz, 2002; Timothy, 2003 and Altaf et al., 2005).

### 6.3.2 Effects from light condition

Microcystin LR degradation in both of the coated (no light) and the non-coated (natural light) bioreactors (Section 6.2.1) is shown in Figure 6.2. The lag period prior to the rapid biodegradation process was similar in the two reactors. However the reduction rate was more than doubled in the reactor with natural light irradiation compared with the one without light. Total toxin removal occurred after approximately 100 hours (natural light) and 150 hours (no light) in the reactors.
Therefore, for this particular biological study (Figure 6.2) the lag-phase prior to microcystin biodegradation was not considerably affected by the light irradiation condition, but the subsequent biological removal process was significantly accelerated by it. The impact of exposure to light has been previously investigated on biodegradation of various pollutant compounds, but different findings were reported. For example, Jie and Wang (2005) has investigated biodegradation of dibutyl phthalate under illumination conditions, and found the degradation rate slightly decreased. The author pointed out that illumination could affect the proliferation of algae and bacteria, so retarded the biodegradation. On the contrary, a research conducted by Hung and Liao (1996) found that organophosphate was degraded more rapidly when UV irradiation was applied, while growth rate of biomass was reported reduced. According to the authors, this outcome was most likely attributed to an increase in membrane permeability to the substrate, the process of which was found to be the rate-limiting step in the biodegradation. For the current microcystin biodegradation study, reasons that were attributable to the light enhanced degradation rate are still unknown, and further investigation is needed to quantify the effects.

### 6.3.3 Effects from water quality

Degradation of m-LR in water samples of different NOM concentrations (100%, 50% and
10%) was determined (Figure 6.3). As described above, a lag-phase of about 50 hours was observed in the undiluted (100% NOM) Myponga water. In contrast, no observable lag-phase occurred in the diluted 50% NOM sample (Figure 6.3). However, in the highly diluted water, i.e. the 10% NOM, the 50 hour lag-phase reappeared. So, the water sample featured with medium NOM abundance in this study had significantly decreased the lag period prior to microcystin degradation.

![Figure 6.3](image)

*Figure 6.3* Biodegradation of m-LR in bioreactors of undiluted (100% NOM), 50% (NOM) diluted and 10% (NOM) diluted Myponga treated water

NOM concentration of the water sample also showed impact during the toxin biodegradation phase. The 10% NOM sample presented a similar degradation rate (~0.1 μg/hour) with that in the undiluted (100% NOM) water sample, whilst in the 50% diluted solution the degradation was at a much lower rate (~0.04 μg/hour, Figure 6.3). In extremely low NOM water, the activity of bacteria was restricted by nutrient supply shortage and thus affected the metabolism efficiency of microcystins. The microorganisms in the 10% NOM water died at approximately 75 hours as shown in a subsequent agar plate assay, and consequently the degradation of microcystins halted at that time (Figure 6.3). This is in agreement with the observation from the study by Bengtsson and Zerhouni.
The originally slow biodegradation process of polycyclic aromatic hydrocarbons (PAHs) in low organic-content soil cultures was found to be accelerated by increasing the NOM in the cultures. The authors attributed this phenomenon to the restricted microorganic activity by the initial limited nutrient supply.

The finding of restricted biodegradation of microcystins under extremely low NOM concentrations is essential for practical application of biological GAC filtration. It indicates that if extremely low nutrient levels are present in the water that is being treated to eliminate microcystins, alternative treatment profiles, rather than biodegradation must be considered.

### 6.3.4 Effects from bacterial cell concentration

Microcystin LR degradation in bioreactors with a series of different bacterial concentrations (Section 6.2.2) is shown in Figure 6.4. The degradation behaviour, especially the lag-phase in each of the reactors was monitored. As expected, the higher the concentration of the initial bacterial cells, the shorter the lag period and the quicker the complete degradation. A lag time of approximately 100–150 hours was taken for the first three highest cell concentration reactors, but of more than 200 hours was required for the other three low bacterial concentration reactors (Figure 6.4).

![Figure 6.4 Biodegradation of m-LR in bioreactors of different bacterial cell concentration](image-url)
Although different lag period was evident (Figure 6.4), the biodegradation rate of m-LR was shown to be very similar in all bioreactors. Total removal of m-LR was realised after approximately 50 hours in all biological reactors after the lag-phase. It was thus demonstrated that the initial bacterial concentration would have limited effect on the biodegradation rate after the initial lag period.

### 6.4 Conclusions

During these batch tests, an attempt was made to identify the effect of selected operational conditions on the biodegradation of microcystins. It was concluded from the outcome of these studies that the toxin biodegradation process was easily affected by the tested parameters (temperature, light irradiation, NOM water characteristics and the initial bacterial concentration) in respect to the lag-phase and the degradation rate. Based upon the observations reported, the lag period varied under different water characteristics (NOM concentration) and different bacterial concentrations; while the operational conditions of temperature, light irradiation and NOM affected the rate of biodegradation that occurred after the lag period.

The results observed from this study were reasonably predictable, and for a full understanding of the effect of varying these operational conditions further work is necessary. Variation of operating parameters is unavoidable in practice and reasonably unpredictable in large-scale water treatment facilities, so it would be difficult to model and estimate the application of microcystin biodegradation under real world operating conditions.
CHAPTER 7

CONCLUSIONS

7.1 Conclusions

Treatment of cyanobacterial toxins, in particular microcystins, in contaminated water has become an ever-increasing problem for drinking water utilities internationally due to the increasing occurrence of these potentially hazardous compounds. Granular Activated Carbon (GAC) filtration, which has been readily adopted in many developed countries for removal of problematic micropollutants, is a promising option for treatment of microcystins due to the ability of GAC to accommodate mechanisms of both adsorption and biodegradation. However, insight into the individual toxin removal from the two mechanisms that determine the effective lifetime of a GAC filter is required to optimise the application of this technology. Valuable information is provided from this study in which the partitioning of the relative importance of adsorption and biodegradation of microcystins in GAC filtration was successfully demonstrated.

The two microcystin reduction mechanisms in GAC filtration were successfully separated in a lab-scale, column experiment, which was validated (Chapter 5) and operated continuously for 11 months. In this study, GAC A6 columns with an empty bed contact time (EBCT) of 15 minutes were used for treatment of m-LR and m-LA at a total inflow concentration of 10-20 μg/L in Myponga treated water. The whole time-course of GAC operation was divided into adsorption and biodegradation phases.

In the initial adsorption phase, competition from the natural organic matter in the source water resulted in a very short lifetime (7-10 days or 900–1400 bed volumes) of the
virgin GAC bed for effective microcystin removal (<1 μg/L m-LR in effluent). Adsorption of both m-LR and m-LA followed a typical breakthrough curve, but higher adsorbability was evident for m-LR during this study. The rate of adsorption during this period decreased from ≥ 520 to 280 μg/m²/d (m-LR). The plug flow homogeneous surface diffusion model was used for modelling the microcystin adsorption in the sterile and the non-sterile GAC columns, where the mass transfer coefficient, $K_f$, was shown to be lower for the latter column. This was attributed to the biofilm that blocked the external pores and decreased the surface diffusion of microcystins into the internal adsorption sites.

Transition from the adsorption phase to the biodegradation phase occurred quickly and was difficult to predict. In the later phase, biological removal of microcystins greatly enhanced the behaviour of the GAC columns and biodegradation became the primary mechanism. The dosed toxins were completely biodegraded, which led to a high elimination rate of 440 μg/m²/d (m-LR). In addition, GAC appeared to be more favorable as a substrate for biofilm attachment and development than the sand with the same particle size used during this study. This was attributed to the distinctive surface characteristics of the two materials and the ability of microorganisms to colonise on them.

The highly efficient biodegradation of microcystins in GAC filtration was difficult to predict, because this process could be easily affected by a series of operational conditions such as temperature, light irradiation, NOM water characteristics and bacterial concentration. For development of a mathematic model to estimate this biological application in water treatment facilities, further studies are necessary to fully understand the effect of these operational variances.

Natural organic matter removal in the GAC columns was also investigated. Several specific features were observed from their breakthrough curves due to the relatively
high NOM concentration in the water. The adsorption efficiency was shown to decrease rapidly and the adsorption capacity was almost saturated in a very short time (about two months). The preferred high efficiency steady-state adsorption phase was not observed because the NOM adsorption zone rapidly reached the end of the column.

Low percentage (~10%) NOM removal in preloaded GAC filters (several months) is frequently observed in practical applications. The mechanisms that led to this removal were unknown and usually assumed to be biodegradation. During this study similar removal was evident in the adsorption GAC column (the sterile GAC), which indicated that the minimal NOM reduction rate could be contributed, in part, to the residual adsorption capacity of the GAC rather than biodegradation as is generally assumed in the literature.

### 7.2 Future work

It was concluded from this study that biodegradation is most likely to be the primary function for maintaining efficient microcystin reduction in GAC filters, thus, it is of vital importance to facilitate this process. However, the lag-phase for the growth of microorganisms during acclimitisation is unavoidable and currently there is limited understanding on how to shorten or remove the lag-phase prior to the initiation of biodegradation, which is hindering the implementation of this technology. Further investigation is required to identify optimal growth conditions to minimise the lag-phase.

Additionally, a validated mathematical model that could predict the efficiency of a GAC filter for microcystin removal as a function of operation time would be of tremendous help and enable practical application of this technology. The model could be developed based on the two functioning mechanisms, adsorption and biodegradation, the removal efficiency of which was successfully obtained from this study.


Jochimsen E. M., Carmichael W. W., Cardo D. M., Cookson S. T., Holmes C. E. M.,


Lowry J. D. and Burkhead C. E. (1980) The role of adsorption in biologically extended


Treatment, Second Edition, AWWA Research Fundation, USA.


### Appendix A

#### Bacterial Colony Count

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Appendix B

Calculation of Microcystin Adsorption Rate in the Non-Sterile GAC Column

Assuming the GAC particles used in this study were spherical thus total surface area, \( S \) (m\(^2\)), that was presented in the non-sterile GAC column can be calculated as following,

\[
V = \left(\frac{\pi}{4}\right) * D^2 * H \quad (1)
\]
\[
v = \left(\frac{\pi}{6}\right) * d^3 \quad (2)
\]
\[
s = \pi * d^2 \quad (3)
\]
\[
S = V * s / v \quad (4)
\]

Where:

- \( V \) – volume of the GAC bed, m\(^3\);
- \( D \) – diameter of the GAC column, m;
- \( H \) – height of the GAC column, m;
- \( v \) – volume of one GAC particle, m\(^3\);
- \( d \) – diameter of the GAC particle, m;
- \( s \) – surface area of one GAC particle, m\(^2\);
- \( S \) – total surface area in the GAC column, m\(^2\).

Since the highest adsorption rate normally occurs at the beginning of operation with a virgin GAC filter, so in this study, the highest microcystin adsorption rate in the non-sterile GAC column could be calculated based on the toxin removal results during the initial period of time. This calculation only applies to the microcystin dosage in this study, the highest of which was approximately 6.5 \( \mu \)g/L m-LR in the beginning of the repeated
set of experiment (Figure 3.9). Therefore, the detected microcystin (m-LR) adsorption rate in the non-sterile GAC column, R (µg/m²/d), was

\[ R = C_{m-LR} \ast v \ast 60\text{min} \ast 24\text{hr} \ast 1000 / S \]  

(5)

Where:

R – the detected m-LR adsorption rate in the non-sterile GAC column;

\( C_{m-LR} \) – the highest m-LR concentration that used in the initial complete adsorption period, 6.5µg/L;

\( v \) – water flow rate of the non-sterile GAC column, mL/min.

Substitute the following operating parameters to the equations (1) – (5),

\( D = 2.5 \ast 10^{-2} \text{ m}; \)
\( H = 15 \ast 10^{-2} \text{ m}; \)
\( d = 1.2 \ast 10^{-3} \text{ m} \) (the average value of the GAC particle size, 1.0 – 1.4 mm);
\( v = 4.9 \text{ mL/min}; \)

the observed adsorption rate is calculated,

\[ R = 1.25 \mu\text{g/m}^2/\text{day}. \]

Note: The maximum adsorption rate of microcystins was not measured in this study.