REMOVAL OF GEOSMIN AND 2-METHYLISOBORNEOL FROM DRINKING WATER THROUGH BIOLOGICALLY ACTIVE SAND FILTERS

BRIDGET MCDOWALL

School of Chemical Engineering
The University of Adelaide

A thesis submitted for examination for the degree of
PhD

May - 2008
1 INTRODUCTION
1.1 PROBLEM STATEMENT

The provision of adequate volumes of safe, clean drinking water to the world’s growing population is a continual and increasing challenge for water authorities. Although the health aspects of water are of primary focus, consumers generally judge the quality of water by its aesthetic value. The presence of adverse taste and odour often leads to the misconception that water is unsafe for drinking, leading to increased consumer complaints to water authorities and a decrease in the consumption of tap water.

2-methylisoborneol (MIB) and geosmin are the most common causes of musty odour in municipal drinking water supplies. These compounds can be detected by the human nose at part per trillion levels. The conventional treatment methods of coagulation, flocculation, sedimentation and chlorine-based disinfection are largely ineffective for removal of dissolved extracellular MIB and geosmin. Additional treatment processes that are robust and capable of removing the compounds to below the human odour threshold must be employed. Powdered activated carbon (PAC) is a common treatment method used to remove these compounds. It is advantageous as it can be added only as required, and has the ability to remove MIB and geosmin to below detection level of consumers. However, PAC usage becomes difficult when odour concentrations are high and more frequent. In addition, competition for carbon adsorption sites with the more abundant natural organic matter can decrease the effectiveness of PAC. An alternative method would be advantageous.

Recent research has shown promising results for the biological filtration of MIB and geosmin. Biological granular activated carbon filtration with pre-ozonation is considered to be the most promising alternative to PAC. However, this process is expensive and would require significant alterations to existing Australian water treatment plants. A process which can utilise existing operational methods with minor process changes is preferable. One such process is biologically active rapid sand (gravity) filtration.

A rapid sand filter may begin to operate biologically when no disinfectant residual is present. It has been shown that biological removal of MIB and geosmin can occur in biologically active rapid sand filters of selected water treatment plants, but the process is not well understood. A comprehensive study which incorporates engineering and biological aspects is necessary to ascertain the factors which lead to MIB and geosmin removal in biological rapid sand filters.
1.2 OBJECTIVES OF RESEARCH

The aim of this research is to develop a greater understanding of biological removal of MIB and geosmin through biologically active sand filters. While much study has been conducted on biological filtration of general natural organic matter (NOM) components including assimilable organic carbon (AOC), little of this has been used in the study of MIB and geosmin removal.

The major objectives of this work were,

- To validate the potential of biological rapid sand filtration for taste and odour removal;
- To screen two South Australian reservoir waters for MIB and geosmin degrading organisms;
- To discover the effect of pre-existing biofilm conditions on the start up periods required for biological degradation of MIB and geosmin in sand filters;
- To determine the impact of empty bed contact time on biological removal of MIB and geosmin in sand filters; and
- To determine methods to enhance the biodegradation of MIB and geosmin in biologically active sand filters
  - By inoculation with organisms capable of biodegradation of the compounds
  - By use of pre-ozonation to enhance biofilm formation.

The above aims were achieved by a range of full-scale, laboratory-scale and pilot-scale studies. The majority of experimental work was focused on sand media, although some work was conducted with anthracite.
2 LITERATURE REVIEW
2.1 TASTE AND ODOUR IN DRINKING WATER

The importance of the aesthetic qualities of drinking water can not be understated. The production of water of a high aesthetic quality is a major goal for water authorities as consumers judge the quality of drinking water by its taste, odour and appearance. The presence of adverse tastes and odours can give consumers the impression that the water is not safe for drinking, leading to increased consumer complaints (McGuire 1995).

A survey by the Australian Bureau of Statistics in 2007 (ABS 2007) found that 18.5% of Australians were dissatisfied with the quality of their drinking water. The problem was most notable in South Australia, with 25.8% of those surveyed being unhappy with their water to the extent that 8.7% would not drink tap water at all. Only 63.7% of South Australians reported drinking mains water as their primary drinking water source, compared to the national average of 80.8%. South Australia also showed the highest consumption in bottled water, with 13.4% of people relying on bottled water as the sole drinking water compared 8.3% nationally.

Of those Australians who expressed dissatisfaction with drinking water, 51.7% cited taste as the reason for their concern, while odour accounted for 12.1% (ABS 2007). Again, South Australia showed the most significant problem with 60.1% expressing dissatisfaction with taste and 14.5% having dissatisfaction with odour.

Taste and odour issues are a problem for water utilities world wide, with water authorities spending a large portion of their yearly budgets on taste and odour control. A recent study of over 800 utilities in the United States and Canada found that those utilities spent over 4.5% of their total budget on taste and odour control (Khiari et al. 2007).

There is no direct guideline for levels of taste and odour in drinking water. However, The Australian Drinking Water Guidelines (ADWG) (NHMRC 2004) state that “The taste and odour of drinking water should be acceptable to most people” while the 1994 World Health Organisation guidelines (WHO 1994) “require that taste and odour be acceptable to avoid consumer complaints”.

The most common causes of adverse odour in Australian drinking water are of biological origin. The ADWG (NHMRC 2004) state that “some algae can produce toxins and the detection of these algae by taste and odour provides a useful early warning of potential problems, although taste and odour do not necessarily indicate the presence of toxins.”

The major biological sources of taste and odour in Australian drinking water are the earthy-musty odours derived by algal predominantly by cyanobacteria and
actinomycetes in surface waters. These musty odours are primarily due to two non-toxic compounds, 2-methylisoborneol (MIB) and geosmin.

### 2.2 MIB AND GEOSMIN

MIB is a tertiary alcohol, also commonly known as 2-methylisoborneol. According to the International Union of Pure and Applied Chemists (IUPAC) it is called (1R-exo)-1,2,7,7,-tetramethyl-exo-1,2,7,7,-bicyclo-[2.2.1]-heptan-2-ol; 2-exo-hydroxy-2-methylbornane. MIB was first isolated from actinomycetes in the late 1960s (Gerber 1969; Medsker et al. 1969). Gerber (1969) was the first to give the compound its common name of MIB.

Geosmin is also a tertiary alcohol. It is known as trans-1, 10-dimethyl-trans-9-decalol (IUPAC). Geosmin was first isolated in 1965 and was named after the Greek ‘ge’ meaning ‘earth’ and ‘osmin’ meaning ‘odour’ (Gerber et al. 1965).

MIB and geosmin impart earthy-musty odours, similar to that which can often be noted in freshly turned soil. The odour threshold concentration (OTC) is the lowest concentration at which a compound can be detected by the human nose (Nerenberg et al. 2000). MIB and geosmin have OTCs at nanogram per litre levels (Young et al. 1996).

MIB and geosmin are reported to be non-toxic to humans. However, a study utilising the “Ames test”, a widely used assay for mutagenicity, found that MIB and geosmin inhibited growth of tester strains of Salmonella typhimurium at concentrations in the mg L⁻¹ range (Dionigi et al. 1993). The authors of this study suggested that MIB and geosmin may exhibit an antimicrobial activity similar to that reported for other terpene derived alcohols. Additionally, they were shown to inhibit the early development of sea urchins, with a toxicity mechanism similar to that shown towards the Salmonella test strains (Nakajima et al. 1996).

The physical structures of MIB and geosmin are shown in Figure 2-1.
A selection of physical and chemical properties of MIB and geosmin are shown in Table 2-1.

Table 2-1: Properties of MIB and geosmin (Pirbazari et al. 1992; Young et al. 1996).

<table>
<thead>
<tr>
<th></th>
<th>MIB</th>
<th>Geosmin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C₁₁H₁₂O</td>
<td>C₁₂H₂₂O</td>
</tr>
<tr>
<td>Molecular Weight (g mol⁻¹)</td>
<td>168.28</td>
<td>182.31</td>
</tr>
<tr>
<td>Boiling Point (°C)</td>
<td>196.7</td>
<td>270</td>
</tr>
<tr>
<td>Aqueous Solubility (mg L⁻¹)</td>
<td>194.5</td>
<td>150.0</td>
</tr>
<tr>
<td>Density (g cm⁻³)</td>
<td>0.929</td>
<td>0.949</td>
</tr>
<tr>
<td>Odour Threshold Concentration (ng L⁻¹)</td>
<td>6.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Physical form</td>
<td>White solid</td>
<td>Yellow oil</td>
</tr>
<tr>
<td>Henry’s Law Constant (Pa m³ mol⁻¹)</td>
<td>5.84</td>
<td>6.75</td>
</tr>
<tr>
<td>Vapour pressure (Pa)</td>
<td>6.76</td>
<td>5.56</td>
</tr>
</tbody>
</table>
2.2.1 Sources of MIB and Geosmin

MIB and geosmin are produced as secondary metabolites of a range of cyanobacteria and actinomycetes. About 70% v/v of Australian drinking water is sourced from surface water (Hoeger et al. 2004). Such waters are often affected by cyanobacterial and actinomycetes growth. Increased eutrophication from increases in nutrient loads from agricultural catchments means that concentrations of MIB and geosmin are likely to rise in fresh water bodies of all climates and conditions throughout the world (Dillon et al. 2002). Thus, water suppliers in many countries, including Australia, must now deal with increasing demand for effective removal of the metabolites. In addition, the prevalence of cyanobacteria increases in drought situations. This is a particular problem for Australia as it experiences severe drought conditions in the 21st century (MDBC 2007).

2.2.2 Cyanobacteria

Cyanobacteria are a group of prokaryotic oxygenic phototrophs, occurring commonly in fresh surface waters. They can also flourish in salty and brackish waters, and hot or cold environments (Chorus et al. 1999). Cyanobacteria produce a large range of secondary metabolites, ranging from toxic compounds such as the cylindrospermopsin and microcystin families, through to non-toxic taste and odour compounds such as MIB and geosmin (Codd 1995; Jüttner 1995b).

Cyanobacterial blooms often appear as a thick, blue-green scum on the surface of the water, giving the bacteria its common name of ‘blue-green algae’. A typical scum is shown in Figure 2-2. The deceased duck in the figure demonstrates the detrimental effects that cyanobacterial blooms can have on wildlife.
A large variety of cyanobacterial species, both benthic and planktonic, have been shown to be responsible for MIB and geosmin production. A selection of these species is shown in Table 2-2. Studies have shown that the ability of a species to produce MIB and geosmin may be specific to certain strains; with different strains of the same species exhibiting different odour-producing abilities (Suffet et al. 1995).
Table 2-2: A Selection of Cyanobacterial Species Responsible for MIB and Geosmin Production.

<table>
<thead>
<tr>
<th>Species</th>
<th>MIB</th>
<th>Geosmin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anabaena circinalis</em></td>
<td>YES</td>
<td></td>
<td>(Rosen <em>et al.</em> 1992; Lewis <em>et al.</em> 2004)</td>
</tr>
<tr>
<td><em>Anabaena scheremetievi</em></td>
<td>YES</td>
<td></td>
<td>(Izaguirre <em>et al.</em> 1982)</td>
</tr>
<tr>
<td><em>Phormidium tenue</em></td>
<td>YES</td>
<td></td>
<td>(Oikawa <em>et al.</em> 2004)</td>
</tr>
<tr>
<td><em>Pseudanabaena</em> (planktonic)</td>
<td>YES</td>
<td></td>
<td>(Izaguirre <em>et al.</em> 1999)</td>
</tr>
<tr>
<td><em>Oscillatoria f. granulata</em></td>
<td>YES</td>
<td>YES</td>
<td>(Tsuchiya <em>et al.</em> 1999)</td>
</tr>
<tr>
<td><em>Oscillatoria simplicissima</em></td>
<td>YES</td>
<td></td>
<td>(Izaguirre <em>et al.</em> 1982)</td>
</tr>
<tr>
<td><em>Oscillatoria curviceps</em></td>
<td>YES</td>
<td></td>
<td>(Izaguirre <em>et al.</em> 1982)</td>
</tr>
<tr>
<td><em>Oscillatoria tenuis</em></td>
<td>YES</td>
<td></td>
<td>(Izaguirre <em>et al.</em> 1982)</td>
</tr>
</tbody>
</table>

2.2.3 Actinomycetes

Another source of MIB and geosmin is the actinomycetes. This is a group of filamentous Gram-positive bacteria. They share some common features with fungi, specifically their growth in a branching network of filaments, known as a mycelium (Izaguirre *et al.* 1995). Actinomycetes are found in fresh and marine aquatic environments, and soil. Geosmin was first isolated in 1965 from the actinomycete *Streptomyces griseus* (Gerber *et al.* 1965). Other species shown to produce MIB and geosmin are *Nocardia* and *Microbispora* (Zaitlin *et al.* 2006).
2.3 TREATMENT OF MIB AND GEOSMIN

The treatment of taste and odour in drinking water is a complex issue ranging from control of odour producing organisms in the reservoir through to removal of the dissolved compounds and the intact algal cells through the water treatment plant.

In this section, the removal of MIB and geosmin through the conventional water treatment process is explored. The treatment process employed must be capable of targeting minute concentrations, due to the low odour threshold concentrations of MIB and geosmin. Additional treatment processes, such as adsorption by activated carbon, are often employed.

2.3.1 Conventional Treatment Processes

The conventional treatment process is generally comprised of coagulation, flocculation, sedimentation, filtration and disinfection.

An example of how the conventional water treatment process can be employed is shown in Figure 2-3.

![Figure 2-3: An example of the conventional drinking water treatment process.](image)

The conventional water treatment process may remove a large proportion of MIB and geosmin, provided that the compounds are contained within the algal cells (Ashitani et al. 1988; Ando et al. 1992). However, the process is ineffective in the treatment of dissolved MIB and geosmin (Bruce et al. 2002; Bruchet et al. 2004). This is in part due to their physical properties, as they are un-charged organic molecules, water soluble, moderately hydrophobic and quite volatile (Metz et al. 2006).

There are two major forms of sand filtration: slow sand and rapid sand filtration. Slow sand filtration is the oldest form of municipal water filtration, first developed in Paisley, Scotland in 1804 (Singley et al. 2000). More sophisticated technologies have since been developed; however, slow sand filtration is still a low cost and highly effective process. It is particularly useful for remote communities who need a reliable, easily built and maintained, cost effective method for treatment of water supplies.
Slow sand filtration is the traditional form of biological sand filtration. It involves the passage of water through a medium of high surface area, within which a series of physical, chemical and biological processes occur. After a period of time, an algal mat, known as the ‘schmutzdecke’ or ‘dirty layer’ forms on the surface of the filter. Biodegradable organic matter (BOM) is removed in the schmutzdecke by biological activity. Cleaning of the filter is accomplished by periodic scraping of the schmutzdecke and redistribution of the sand surface. Physical processes in the depth of the filter remove particles which were not removed in the top layer. Slow sand filtration has been shown to be capable of removal of geosmin, with the majority of removal occurring in the schmutzdecke (Jüttner 1995a).

Rapid sand (or gravity) filtration is the most common form of filtration used in Australian water treatment plants. It is generally preceded by coagulation, flocculation and sedimentation. In this process, water is passed downwards through the filter, either by gravity or pressure. Particle removal is primarily by means of two physical mechanisms: particle attachment and physical straining (Logsdon et al. 2002).

A large proportion of rapid gravity filters are pre-chlorinated or backwashed with chlorinated water in order to control the growth of biomass within the filter bed; a phenomenon that may affect particle removal and head loss development (Goldgrabe et al. 1993). However, biomass within a filter bed can be of great advantage for the removal of biodegradable compounds, and is known as biological filtration. This process will be discussed in more detail further on.

**Filtration Rates Associated with Sand Filtration**

Filtration rate is commonly classified by the hydraulic loading rate (HLR). This is calculated as the flow rate of the down flowing water ($Q$ [m$^3$/h]) divided by the surface area (m$^2$) of the filter,

$$H LR = \frac{Q}{Surface\, Area} (m^3 h^{-1})$$

(2.1)

Alternatively, filtration rate can be described by the empty bed contact time, EBCT. This is equal to the height of filter media (h) divided by the hydraulic loading rate (HLR),

$$EBCT = \frac{h}{HLR} (h)$$

(2.2)

Filtration rates and other significant design parameters associated with slow sand and rapid gravity filtration are shown below in Table 2-3.
Table 2-3: Conventional slow sand and rapid gravity sand filtration design parameters (adapted from (Rachwal et al. 1996)).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Slow Sand Filter</th>
<th>Rapid Gravity Sand Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtration (loading) rate (m h⁻¹)</td>
<td>0.1-0.3</td>
<td>5.0-30.0</td>
</tr>
<tr>
<td>Media Effective Size (mm)</td>
<td>0.2-0.4</td>
<td>0.5-2.0</td>
</tr>
<tr>
<td>Media Depth (m)</td>
<td>0.3-1.5</td>
<td>0.6-2.5</td>
</tr>
<tr>
<td>Media Contact Time (mins)</td>
<td>60.0-90.0</td>
<td>4.2-12.0</td>
</tr>
<tr>
<td>Media surface area (m²)</td>
<td>10,000</td>
<td>4,400</td>
</tr>
<tr>
<td>Filter run length (days)</td>
<td>20.0-200.0⁺</td>
<td>0.3-3.0</td>
</tr>
</tbody>
</table>

2.3.2 Adsorption Processes

Activated carbon has been shown to be an effective method for MIB and geosmin removal (Lalezary et al. 1986; Cook et al. 2000; Ridal et al. 2001; Ho et al. 2005). It is used in two forms, powdered activated carbon (PAC) and granular activated carbon (GAC). The most suitable form depends on the severity and duration of the odour episode.

**Powdered Activated Carbon**

PAC is a commonly used treatment method for MIB and geosmin. It can be used only as required, making it suitable in situations with isolated, seasonal odour episodes. The most common addition point is during the rapid mix stage of the coagulation step, as it allows for the PAC to be disposed of in the sludge waste (Ho et al. 2005).

To determine the required PAC dose, isotherm studies must be conducted utilising the chosen carbon and the source water (Suffet et al. 1995). Also, the type of carbon used will greatly impact on performance (Gillogly et al. 1999a; Tennant et al. 2007). Adsorption capacity is affected by factors including the presence of other compounds that compete for adsorption sites on the activated carbon; the presence of disinfectants which can oxidise the carbon’s surface; and water quality changes that can affect the form of the adsorbing compound (Suffet et al. 1995; Bruce et al. 2002). Other factors
include contact time, mixing conditions, point of dosing, and the presence of coagulants (Cook et al. 2000; Ho et al. 2005). Previous studies have shown that geosmin is more effectively adsorbed by PAC than MIB (Cook et al. 2000; Jung et al. 2004). One study suggested that the flatter structure and lower solubility of geosmin may be the reason for this difference, making geosmin more easily adsorbed into the pores of the GAC (Cook et al. 2000).

The disadvantages of PAC are numerous. Firstly, as the treatment is carried out in response to the outbreak, there may be a period of time where the odour reaches the tap of the consumer (Westerhoff et al. 2005). Difficulties with predicting required PAC doses can result in under-dosing, resulting in a breakthrough of MIB and geosmin to the distribution system and increased consumer complaints (Cook et al. 2000). Alternatively, over dosing, although it would result in acceptable water quality for consumers, leads to exorbitant costs to the water treatment authorities (Newcombe et al. 2002). On an operational level, PAC is a difficult substance to work with and can leave carbon residues throughout the treatment facility.

**Granular Activated Carbon**

Granular activated carbon is suitable in situations where odour episodes are more frequent, or required PAC dosages are high. GAC can be used as a filter-adsorber, where it achieves both particle removal and adsorption of the organic compounds, or as a post-treatment reactor. For taste and odour treatment, it is generally used in a post-treatment contactor, placed at the end of the treatment train (Suffet et al. 1995). This allows for the process to be specifically designed for the removal of the odour compounds with minimal competition by NOM.

A number of factors must be looked at when choosing GAC as the adsorptive media. After a finite period of time, the adsorptive capacity of the media will be exhausted and ‘breakthrough’ of the target compound will occur (Suffet et al. 1995; Mackenzie et al. 2005). At this time, the GAC must be replaced or regenerated. As with PAC, MIB and geosmin must compete for adsorption sites with the more predominant dissolved organic carbon (DOC), such as humic acids, fulvic acids, algal metabolites and algal detritus (Suffet et al. 1995; Mackenzie et al. 2005). Studies have shown that the lifespan of the adsorptive mode of GAC for MIB and geosmin is fairly short (Gillogly et al. 1999b; Ridal et al. 2001). To combat this problem, GAC is often coupled with ozonation and operated as a biological filtration system (Kainulainen et al. 1995; Graham 1999; Nerenberg et al. 2000). The effect of this process on MIB and geosmin removal is described in more detail in Section 2.5.
2.3.3 Oxidation Processes

The most common oxidation processes used in drinking water treatment are chlorine-based oxidation (including chloramination) and ozonation.

Chlorine-Based Oxidation

Chlorine-based oxidation, such as chlorination and chloramination, is commonly used as Australia’s primary disinfection method with its ability to control most waterborne diseases. Chloramine is often used in longer distribution systems, as it maintains the residual over long distances and residence times (Wilczak et al. 1996).

Dissolved MIB and geosmin are not affected by chlorination (Lalezary et al. 1986; Glaze et al. 1990). In fact, chlorination may increase the concentration of dissolved MIB and geosmin by lysing any remaining intact cyanobacterial cells, releasing the metabolites into the water (Ashitani et al. 1988).

Although chlorine does not oxidise MIB and geosmin, increases in free chlorine concentrations may mask the intensity of the earthy musty odour. This treatment can not be considered acceptable as once the chlorine residual is reduced, odours which were previously masked may reappear (Bruchet et al. 2004). A US taste panel study found that chlorine and chloramines did not change the panelists’ abilities to detect MIB and geosmin (Oestman et al. 2004). Additionally, chlorine itself is a taste and odour issue. Many complaints on the taste and odour of drinking water result from the swimming pool odour association with the use of chlorine and chloramines (Duguet et al. 1995).

Ozonation

Ozone (O₃) is often used in drinking water treatment for disinfection and for oxidation of organic compounds (von Gunten et al. 2003; Langlais et al. 1991). Studies on the removal of MIB and geosmin by ozonation have shown conflicting results.

The predominant mode of destruction of MIB and geosmin by ozone is by the hydroxyl radical, OH⁻ (Bruce et al. 2002; Bruchet et al. 2004; Ho et al. 2004). Higher pH, lower alkalinity and higher hydrogen peroxide levels may increase the formation of OH radicals and consequently increase the removal of MIB and geosmin (Bruce et al. 2002; Bruchet et al. 2004).

The character of the natural organic matter (NOM) can also affect the generation of hydroxyl radicals that are responsible for degrading the compounds, making it difficult to compare studies which were conducted in different waters. For example, one study found that only 50% removal of MIB and geosmin was achieved with an ozone dose of 33 mg L⁻¹ (Lalezary et al. 1986). However, this study was conducted in
highly purified water which would have prevented the formation of the hydroxyl radical. In contrast, Jung (2004) studied the ozonation of MIB in raw reservoir water. This study achieved 84.8% removal of MIB with a much lower ozone dose of 3.8 mg L\(^{-1}\) at a contact time of 6.4 minutes (Jung et al. 2004). A pilot scale study in Korea found that the use of ozone alone at a dose of 1 – 2 mg L\(^{-1}\) with a contact time of 10 minutes was not sufficient to reach the target MIB and geosmin concentrations of 15 ng L\(^{-1}\) (Ahn et al. 2007). Ho (2004) further investigated the effect of NOM character on the ozonation of MIB and geosmin. This study found increased removals in waters containing NOM fractions at the higher molecular weight range (Ho et al. 2004).

As with activated carbon treatment, studies on ozonation of MIB and geosmin have shown geosmin is more readily destroyed than MIB (Bruce et al. 2002; Ho et al. 2004).

In addition to the unpredictable nature of its effectiveness for MIB and geosmin control, the ozonation process can cause problems for downstream water quality. Ozonation of NOM results in formation of hydroxyl, carbonyl and carboxyl groups (Urfer et al. 1997). Ozone reacts with humic substances in the water, creating lower-molecular weight organics which are more readily biodegradable (Langlais et al. 1991). The lower molecular weight fractions are often referred to as biodegradable dissolved organic carbon (BDOC) or assimilable organic carbon (AOC). As carbon is the limiting substrate in the majority of drinking water situations, an increase in lower molecular weight NOM fractions can potentially result in greater biological instability in the water. For this reason, ozonation processes are often followed by a biological treatment step to ensure biologically stable water.

### 2.4 BIOLOGICAL PROCESSES

The structures of MIB and geosmin suggest that they may be susceptible to biodegradation, as they have structures similar to some biodegradable alicyclic alcohols and ketones (Rittmann et al. 1995). The low concentration of active bacteria in drinking water (oligotrophic environments) means that the most effective way to utilise biological activity is by biofilm attachment (Nerenberg et al. 2000).

In this section, the biological degradation of MIB and geosmin and its application to biological filtration will be discussed.

#### 2.4.1 Biological Degradation of MIB and Geosmin

Many studies have shown that MIB and geosmin can be biodegraded by naturally occurring aquatic organisms. A range of bacteria implicated in the degradation of both compounds are shown in Table 2-4.
Table 2-4: MIB and geosmin degrading bacteria (adapted from (Ho et al. 2006)).

<table>
<thead>
<tr>
<th>MIB Degraders</th>
<th>Reference</th>
<th>Geosmin Degraders</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>(Egashira et al. 1992)</td>
<td><em>Bacillus subtilis</em></td>
<td>(Narayen et al. 1974; Yagi et al. 1988)</td>
</tr>
<tr>
<td><em>Flavobacterium multivorum</em></td>
<td>(Egashira et al. 1992)</td>
<td><em>Bacillus cereus</em></td>
<td>(Silvey et al. 1970; Narayen et al. 1974)</td>
</tr>
<tr>
<td><em>Flavobacterium sp.</em></td>
<td>(Egashira et al. 1992)</td>
<td><em>Arthrobacter atrocyaneus</em></td>
<td>(Saadoun et al. 1998)</td>
</tr>
<tr>
<td><em>Pseudomonas spp.</em></td>
<td>(Egashira et al. 1992)</td>
<td><em>Arthrobacter globiformis</em></td>
<td>(Saadoun et al. 1998)</td>
</tr>
<tr>
<td></td>
<td>(Tanaka et al. 1996)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus fusiformis</em></td>
<td>(Lauderdale et al. 2004)</td>
<td><em>Chlorophenolicus strain N-1053</em></td>
<td>(Saadoun et al. 1998)</td>
</tr>
<tr>
<td><em>Enterobacter sp.</em></td>
<td>(Tanaka et al. 1996)</td>
<td><em>Rhodococcus moris</em></td>
<td>(Saadoun et al. 1998)</td>
</tr>
<tr>
<td></td>
<td>(Tanaka et al. 1996)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>(Oikawa et al. 1995)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>(Yagi et al. 1988)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.4.2 Biodegradation Mechanisms for MIB and Geosmin

The mechanisms of the biodegradation of MIB and geosmin are not well understood. It has been suggested that due to its alicyclic alcohol structure, the biodegradation pathway of geosmin may be similar to that involved in the metabolism of cyclohexanol (Rittmann et al. 1995). In this process, cyclohexanol is oxidised to alicyclic ketone. The ketone is then converted to its corresponding lactone by monooxygenases, and subsequently broken down by ring cleavage to produce an acidic alcohol that can be oxidised to a diacid. It is noted that the process for geosmin biodegradation may be more complex, due to the tertiary alcohol group and its bicyclic structure.

One study looked at the biodegradation of geosmin using backwash water from a biological filter pilot plant at Lake Biwa, Japan (Saito et al. 1999). Contrary to the majority of studies, they reported that geosmin was more difficult to degrade than MIB. However the addition of ethanol greatly enhanced the biodegradation, leading to the assumption that the process involved co-metabolism. A partial biodegradation pathway was proposed, involving a dehydration step followed by allylic oxidation, resulting in a degradation product of enone.

MIB has a structure similar to that of camphor, a bicyclic monoterpane. For this reason it could be assumed that the biodegradation of MIB follows a similar path to that of the metabolism of camphor (Rittmann et al. 1995). In this mechanism the formation of unstable lactones follows sequential ring cleavage. Oikawa (1995) studied the biodegradation of MIB by the gene sequence responsible for the degradation of camphor, known as the \textit{cam} operon. The gene sequence was composed of \textit{cam}D, C, A, B and \textit{cam}R, and was isolated from \textit{Pseudomonas putida} PpG1 (Oikawa et al. 1995). The sequence was shown to degrade MIB in a similar pathway to that of the degradation of camphor.

Tanaka et al. (1996) identified two metabolic products when MIB was degraded by \textit{Enterobacter sp}. and \textit{Pseudomonas sp}. isolated from the backwash water of the Lake Biwa biological filter pilot plant. In both cases, MIB was degraded to 2-methylcamphene and 2-methylenebornane.
2.5 BIOLOGICAL FILTRATION

Biodegradation can be applied in the water treatment process for the removal of MIB and geosmin. Biological filtration is the process of using a biologically active film, biofilm, on the surface of a filter medium to remove BOM. The biofilm contains diverse colonies of heterotrophic bacteria which oxidise BOM, utilising it as an energy supply (Urfer et al. 1997).

Biological filtration has many advantages. It reduces the amount of biodegradable compounds in the water, such as BOM, ammonia and iron, thus increasing the biological stability of the water. The decrease in biological activity of the treated water lowers the chlorine demand of the water, thus reducing the formation of disinfection by-products (Huck et al. 2000). This is particularly important in systems using ozonation.

Huck (2000) states that the first step in implementing a biological process is to define the objectives. Managed biological filtration is optimising the process for BOM removal, while not compromising particle removal. If a conventional filter is to be made to operate biologically for the sole purpose of MIB and geosmin removal (thus being ‘managed filtration’), then it must be ensured that the primary filtration goal of particle removal is not compromised. The most workable strategy for the implementation of biological filtration without compromising finished water quality is background filtration, which is simply the operation of a conventional filter without continuous application of an oxidant (Huck et al. 2000). In this process, the filter is optimised for particle removal and any removal of BOM is considered a bonus. As explained earlier, rapid gravity filtration is often preceded by a chlorination step. Removal of the pre-chlorination step will result in background biological filtration. It is this process which is the focus of the study described in this thesis.

In biological filtration systems there is the potential that filtered water may contain increased numbers of bacteria, due to sloughing of the biofilm. The higher bacterial numbers in the product water are inactivated by the disinfection process, but the inactivated bacteria may contribute to higher particle counts and turbidity of the product water (Amburgey et al. 2005). A study on effluent water quality from biological filters found that turbidity was not notably different for filters that were non-chlorinated, backwash chlorinated and pre-chlorinated (Goldgrabe et al. 1993). Thus, biological filtration did not reduce filtered water quality in terms of turbidity. This was also observed in another study which found that biological filtration produced water with particle counts consistently below 0.1 NTU, regardless of temperature, media type and backwash protocol (Emelko et al. 2006).
The selection of filter media is of great importance when implementing a biological filtration process, for both cost and water quality issues. A major consideration is the choice between adsorptive versus non-adsorptive media. Granular activated carbon is often used for its ability to have both adsorptive and attached biological properties, while sand is an inert medium with only attached biological properties. Urfer et al. (1997) explained that anthracite/sand and GAC/sand filters can show similar average BOM removals. However, GAC/sand filters have been shown to establish biofilms more rapidly and to be more resistant to oxidant residuals (Huck et al. 2000). In a review of biological filtration studies it was suggested that little biomass growth occurs in the micropores of GAC due to their small size in comparison to the bacteria; 100 nm pore size compared with the larger bacteria size of 200 nm (Urfer et al. 1997). Thus the effective area for biomass attachment may be higher in sand particles than in GAC. However, some GACs have advantages of greater macroporous structure and therefore more protection from shear stresses. It also has adsorptive properties, meaning that additional compounds which may not be removed biologically will be removed by adsorption.

In a separate biofiltration study, it was commented that many studies have shown that biological granular activated carbon filters outperformed biological anthracite/sand filters at the same EBCT (Wang et al. 1995).

A number of studies have investigated the biological filtration of MIB and geosmin. A summary of some biological filtration studies using media other than sand for MIB and geosmin removal is shown in Table 2-5.
Table 2-5: Studies on biofiltration of MIB and geosmin with alternative media.

<table>
<thead>
<tr>
<th>Media</th>
<th>Main Areas of Research</th>
<th>Biofilm</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Namkung et al. 1987) 3-mm glass beads</td>
<td>Used an upflow reactor. Primary vs. secondary substrate usage. Achieved 55.4% geosmin removal; 43.6% MIB removal</td>
<td></td>
</tr>
<tr>
<td>(Hattori 1988) Honeycomb tube system</td>
<td>Removed 46% MIB; geosmin removed to below OTC</td>
<td></td>
</tr>
<tr>
<td>(Yagi et al. 1988) Bioactivated carbon filter seeded with Bacillus subtilis</td>
<td>Ability of Bacillus to degrade musty odour. Maximum removals of 56% MIB and 72% geosmin achieved.</td>
<td>Seeded media with Bacillus subtilis, found that both B. subtilis and B. cereus degraded geosmin</td>
</tr>
<tr>
<td>(Egashira et al. 1992) Porous ceramic, 4 – 5mm diameter</td>
<td>pH effects; water temp; SEM of biofilm surface</td>
<td>Isolated potential MIB degraders.</td>
</tr>
<tr>
<td>(Hrudey et al. 1995) Ozone/ GAC Ozone/ Sand</td>
<td>Use of flavour profile panel</td>
<td></td>
</tr>
<tr>
<td>(Hrudey et al. 1995) 3mm glass beads</td>
<td>First study to look at realistic ng/L levels with introduction of better analysis techniques. Concluded rapid sand filtration not suitable as transient episodes result in decreased removals.</td>
<td></td>
</tr>
<tr>
<td>(Sugiura et al. 2003) Honeycomb shaped plastic media, already existing facility</td>
<td>Study of biofilm removed from honeycomb tubing. Found decrease in removal with increased water pH</td>
<td></td>
</tr>
<tr>
<td>(Elhadi et al. 2004b) Fresh and exhausted GAC at EBCT 5.6 minutes</td>
<td>Good overview of removals in fresh and exhausted GAC, making assumptions that increases are corresponding to establishment of biomass.</td>
<td></td>
</tr>
<tr>
<td>(Terauchi et al. 1995) Porous ceramic, 4 – 5mm diameter</td>
<td>Soluble vs insoluble MIB/geosmin. 80% removal of MIB achieved.</td>
<td></td>
</tr>
</tbody>
</table>
2.5.1 Biological Sand Filtration for MIB and Geosmin Removal

Biological sand filtration would potentially require little retro-fitting of current water treatment facilities. However, biological removal of MIB and geosmin may not necessarily occur after the cessation of pre-chlorination. A long term, thorough study is required to understand the process.

The successful operation of a biologically active rapid sand filter is a delicate balance between the sustaining of conventional filtration goals such as particle removal and maximisation of filter run times, combined with the maintenance of a healthy biofilm capable of degradation of the target compounds.

Biological filtration of micro-pollutants such as MIB and geosmin requires a combination of factors to work effectively. Firstly, the degrading organisms must be present and active in the influent to the filter. Secondly, the filter operating conditions must be such that the bacteria are able to attach to the filter media and remain in the filter bed. There must also be sufficient contact between the target compounds and the degrading organisms in order for effective biodegradation to take place.

Studies on MIB and geosmin removal through sand filters are limited. In one case, a pilot plant study was conducted utilising sand taken from a full-scale rapid gravity filter (Summers et al. 2006). The sand had been in use for 2 years prior to the study. It was found that MIB removal of 12% was observed after the filter had been operating at room temperature with an EBCT of 7 minutes (hydraulic loading rate of 5 m h⁻¹) for a period of 2 months. This removal increased to 48% after a further 2 months of operation. In contrast, a sample of the sand was chlorinated to inactivate the biofilm. This filter was only capable of 7% MIB removal after 4 months of operation under the same conditions. A full-scale study at the Greater Cincinnati Water Works found that 80-90% removal of MIB was observed in a rapid sand filter operating with an EBCT of 6.0 – 7.4 minutes (HLR ~ 6.13 m h⁻¹) (Metz et al. 2006). This removal was sustained over the study period of 6 years. Complete removals of geosmin through a non-chlorinated rapid sand filter were also observed in a pilot plant study conducted in Japan (Ashitani et al. 1988).

Elhadi et al. (2006) looked at MIB and geosmin removal in a pilot-scale anthracite-sand dual media filter operated at 5.6 minute EBCT with no pre-existing biofilm. Taste and odour removals were low, at approximately 10% for MIB and 16% for geosmin. It is important to note that these filters were only operated for a period of 65 days. Taking this into consideration, these results are similar to that observed by Summers (2006) with the non-acclimated sand filter. These results show the importance of long term studies with well acclimated biological sand filters. It is
likely that significant periods of time are required to develop biofilms capable of MIB and geosmin removal.

Major parameters of concern when studying the removal of MIB and geosmin in sand biofilters include EBCT, backwashing regime, media particle sizing, influent water quality (including the effect of pre-ozonation) and biomass. The majority of biological filtration studies focus on the removal of general BOM rather than specific contaminants such as MIB and geosmin (Miltner et al. 1995; Wang et al. 1995; Urfer et al. 1997).

2.5.2 Filtration Rate and EBCT

In a review of biological filtration, it was noted that it is EBCT and not HLR that is the key parameter for BOM removal when operating within the range of EBCTs typically found in rapid sand filtration (Urfer et al. 1997). Typical filtration rates for rapid sand filtration range from 5 – 30 m h\(^{-1}\), corresponding to EBTCs in the range of 4 to 12 minutes (Rachwal et al., 1996). The use of a specific filtration rate is a function of the filter media, pre-treatment and the use of filter aid polymers (Kawamura, 2000). Dual-media filter beds may be capable of filtration rates as high as 15 to 20 m h\(^{-1}\) (Kawamura, 2000). The depth of the filter bed determines the EBCT. Filter depths are a function of media size. For mono-sand and dual-media filters, the following relationship generally applies:

\[
\frac{l}{d_e} \geq 1000;
\]

Where \(l\) is the filter depth (mm) and \(d_e\) is the effective size of the filter medium (mm) (Kawamura, 2000). Deeper beds may be required in order to achieve the required EBCTs.

Increases in EBCT result in an increase in BOM removal up to a certain maximum. At this point, any more increase in EBCT results in negligible increases in BOM removal (Urfer et al. 1997). This observation was also noted by Huck (1999), who found that increased contact time will result in increased removal of BOM, but in a less than proportional way. Urfer et al. (1997) also noted that required EBCTs will vary for the compound being removed. A study on the effect of EBCT on MIB and geosmin removal showed that an increase in EBCT gave an increase in MIB and geosmin removals (Westerhoff et al. 2005). However, the range of EBCTs in this study was very narrow, from 2.1 minutes to 3.1 minutes.
2.5.3 Media Sizing

Media particle size is chosen depending on the particular characteristics of the plant. Dual-media filters with filtration rates in the range of 10 – 25 m h\(^{-1}\) generally utilise sand with an effective size of 0.45 – 0.65 mm, and a bed depth of 0.3; and anthracite with an effective size of 0.9 – 1.4 mm and a bed height of 0.45 m (Kawamura, 2000). Mono-media rapid sand filters with filtration rates in the range of 5 – 7.5 m h\(^{-1}\) generally utilise sand with an effective size of 0.45 – 0.65 mm and have a bed depth of 0.6 – 0.75 m (Kawamura, 2000).

Filtration theory suggests that greater particle removal would occur with smaller particle sizes, however head loss increases with smaller particle size (Goldgrabe et al. 1993). Smaller particle sizes give the optimum contact for biological activity (Huck 1999). A trade off between optimal biological and physical conditions must be found.

2.5.4 Backwashing

All rapid gravity filters must be backwashed periodically to control head-loss caused by accumulation of particles. The selection of the most appropriate backwashing system for a biological filter is of high importance. It is necessary to employ a backwashing process that gives optimal filter run times while not significantly affecting the biomass in the media. The presence of biomass in a filter can decrease filter porosity thus increasing head loss development rate and decreasing filter run times (Goldgrabe et al. 1993).

The major considerations of backwashing regimes are with or without air scour, and with or without chlorinated water. Water only backwash is the weakest process, with no abrasion of the grains. Air scour is often employed for more thorough cleaning. Collapse pulse backwashing is a combination of air plus water at sub-fluidisation velocity and has been shown to be the optimum method for cleaning of sand and dual media filters (Ahmad et al. 1998). Collapse pulse backwashing has been demonstrated to not have a negative effect on BOM removal in biological filters (Ahmad et al. 1998).

Backwashing with water containing an oxidant can negatively impact the fixed biomass within a biological filter (Servais, 2005). In some plants, the implementation of non-chlorinated backwashing may require significant retro-fitting of the plant (Goldgrabe et al. 1993). The presence of chlorine in the backwash water can result in a short term decrease in the biomass activity (Wang et al. 1995; Ahmad et al. 1998). One study showed that backwashing an anthracite-sand filter with chlorinated water resulted in an initial loss of 22% of the biomass at the top of the filter, but biomass levels recovered over the filter run (Miltner et al. 1995). Despite this recovery,
removals of MIB have been shown to be lower in filters that were backwashed with chlorinated water (Westerhoff et al. 2005).

2.5.5 Ozonation

As explained in Section 2.3.3, ozonation increases the amount of biodegradable organic substrates available for regrowth. Biological treatment can reduce the amount of biodegradable compounds which would otherwise contribute to decreased biological stability of the water (Langlais et al. 1991). This can in turn reduce the required disinfectant residuals, thus reducing the risk of THM formation and chlorine-odour production (Langlais et al. 1991). For this reason, ozonation and biological filtration is often considered to be a coupled process. The degree of BOM production by ozonation is dependant on a number of factors such as ozone dose, the nature of the organics in the water and other water quality parameters, such as alkalinity (Langlais et al. 1991; von Gunten, 2003). Servais et al (2005) conducted a review of BOM production by ozonation and found that optimum BOM production occurred when ozone was applied in the dose range of 0.5 to 1 mg O₃ per mg DOC. Servais et al (2005) also noted that significant BOM production occurred at lower ozone doses of 0.5 to 0.5 mg O₃ per mg DOC, the range typically use for reducing colour and improving coagulation/flocculation of raw water. A predictive model, known as the CHABROL model, has been developed that directly relates increases in BOM to increases in biomass in biological filters (Billen et al. 1992; Laurent et al. 1999).

A number of studies have looked at the advantages of employing ozonation followed by biological filtration of MIB and geosmin with GAC media. In a pilot plant trial involving GAC filtration with ozonation, a target ozone residual of 1.5 mg L⁻¹ gave a destruction of geosmin and MIB ranging from 40 – 70% (Ho 2004). Geosmin was consistently more easily destroyed by ozone than MIB. An ozone residual of 2 mg L⁻¹ gave higher MIB and geosmin removals, but at even higher doses the destruction was decreased (Ho 2004). Importantly, this study looked at the destruction of MIB and geosmin by the coupled process of ozonation and biologically active carbon. It was not known how much of the removal was attributed to biological removal alone.

It would be expected that application of ozone prior to an inert media such as sand or anthracite would result in lower biodegradation capacity than on GAC, due to the lesser surface area available for biomass attachment (Langlais et al. 1991). A pilot plant study showed that the biomass activity, as measured by a tetrazolium reduction assay, of a sand biofilter receiving ozonated water was 55% higher than an identical filter fed with non-ozonated water (Fonseca et al. 2001). The total biomass level, as measured by the phospholipid analysis, was 47% higher for ozonated biofilters than non-ozonated biofilters. The applied ozone dose was 1.3 ± 0.25 mg O₃ per mg DOC. Studies on the use of ozonation followed by biological sand filtration for MIB and
geosmin removal are limited. One study involved two anthracite-sand filters which were run in parallel and fed with de-chlorinated tap water spiked with some typical BOM components (Elhadi et al. 2006). One filter was spiked with low BOM concentrations, while the other filter was fed with higher BOM concentrations to simulate ozonated water. Removals were much higher for the high BOM level filters. Geosmin removals averaged 42% for higher BOM and 16% for low BOM levels, while MIB removals averaged 30% for the high BOM levels and 10% for the low BOM levels. A key limitation of this study was the use of synthetically ozonated water. It did not take into account water quality changes which result from ozonation, such as increased polarity and hydrophilicity, and increases in pH.

Theoretically, ozonation should have a beneficial impact on biofilters for MIB and geosmin removal by increasing the total biofilm accumulation (Nerenberg et al. 2000). However, it is not known if this increased biomass is capable of increased MIB and geosmin biodegradation (Nerenberg et al. 2000).

2.5.6 Biomass in Drinking Water Biological Filters

Biofilms are formed from suspended microbial cells transported in fluid flow and adsorbed onto solid surfaces. They grow, replicate and excrete extracellular polymeric substances (EPS), binding the cells together (Lewandowski et al. 2005). Biofilms can be patchy, discontinuous films such as those found in drinking water to thicker, continuous films such as those found in waste water treatment.

A number of different measurement techniques have been used to investigate the concentration or activity of biomass on biological GAC media. Many studies have utilised the phospholipid method (Miltner et al. 1995; Wang et al. 1995; Liu et al. 2001; Elhadi et al. 2006). The phospholipid method was based on the method of Findlay et al. (1989). In this method, the phospholipid content of a biomass sample is quantified by measuring the amount of phosphate in a sample (Findlay et al. 1989). Phospholipids are contained within living cell membranes and are thus an indication of the biomass concentration in a sample.

Other methods used have included the reduction of the tetrazolium salt, INT (Fonseca et al. 2001) and uptake of radio labeled glucose (Servais et al. 1991). A biomass respiration method, based on the change in dissolved oxygen through a biofilter, has also been used (Urfer et al. 2000).

The above mentioned methods can be time consuming and difficulties can occur with interpreting the data. A more rapid technique, which measures biomass activity, is preferable. Activity-based assays are preferable as only a small fraction of mature biofilms may be involved in substrate metabolism (Fonseca et al. 2001; Laspidou et al. 2004).
The measurement of adenosine triphosphate (ATP) is a good alternative as it is rapid and only requires the use of a plate reader to measure luminescence. It is also advantageous as it is an activity-based assay. ATP is the main energy carrier in all living cells, generated during exergonic (chemical reaction which releases energy) reactions and used to drive endergonic (reactions requiring energy input to proceed) reactions (Madigan et al. 2003). It is present in all living cells at a relatively constant concentration, approximately 2 mM in a growing cell. It also has a relatively short half life after cell death and lysis (Karl 1980). These factors mean that the ATP assay is an ideal method for the measurement of biomass activities in aquatic environments. In particular, ATP is ubiquitous to all living cells but not present in dead cells. Thus, quantification of the amount of ATP in a sample will give a good indication of the total amount of active biomass present. Also, it has a low detection limit of less than $10^{-12}$ mol. A study was conducted to assess the significance of ATP analysis for GAC filters used in water treatment plants (Magic-Knezev et al. 2004). In this study, the biomass was first removed from the GAC surface using high energy sonication at a power input of 40 W. Problems with this method is the possible inactivation of cells. However, the authors found good correlation between direct bacterial cell counts (by heterotrophic plate counts and acridine orange staining) and ATP results. Another problem is potential of interfering substances and variations in carbon to ATP ratio (nutrient starvation and other forms of environmental stress) (Karl 1980). In a study on the use of the ATP assay for biomass measurements in filters, it was noted that the time of sampling is important as the amount of ATP in a sample may vary depending on the current stage of the growth phase that the bacteria are in (Velten et al. 2007). This study also noted that the greatest error is in the non-homogeneity of the biofilm on the sand surface, and additional errors associated with the removal of biofilm from the media surface prior to analysis. Magic-Knezev et al (2004) conducted a study to determine the optimum treatment method to remove biofilm from GAC prior to the use of an ATP based assay. It was found that a series of sonication treatments could detach up to 90% of the attached biomass (Magic-Knezev et al. 2004). It may be preferable to carry out biomass analysis in-situ to avoid losses associated with the removal of biomass from the media surface.

A number of studies have investigated the vertical distribution of biomass within biological filters. Wang et al (1995) showed that the amount of biomass attached within a biofilter (as measured by the phospholipid method) decreased with increasing filter depth. In this study, it was shown that biomass decreased by approximately 50% at a filter depth of 15cm. This observation of decreasing biomass with increasing filter depth was also noted by Moll et al (1999).

All biological processes require both electron donor and electron acceptor substrates. Primary substrates are defined as those substrates that transfer the electrons from the
donors to the acceptors, thus providing the energy for the growth and proliferation of the bacteria (Rittmann et al. 1995). In the oligotrophic (nutrient poor) environment of drinking water, the primary substrate is composed of an aggregate of compounds such as hydrocarbons, carboxylic acids, humic substances and amino acids, broadly termed NOM.

Rittmann (1995) proposed the concept of the minimum substrate concentration for a compound to be considered a primary substrate, known as $S_{min}$. Substances which fall below this concentration do not provide the energy for bacterial growth, but can still be biodegraded. These compounds are known as secondary substrates. The low concentrations of MIB and geosmin in drinking water situations suggest that they are degraded as secondary substrates (Rittmann et al. 1995). Secondary substrates do not provide energy to the biological system and are reliant on the more predominant AOC to sustain the biological growth. Therefore, the biodegradation of MIB and geosmin is dependant on the concentration and chemical nature of the primary substrate. It also requires the presence of the degrading organisms and the expression of the genes and enzymes responsible for the degradation. The regulation of the enzymes responsible for the degradation of MIB and geosmin may require frequent exposure of the bacteria to the taste and odour compounds. This may be a problem in biofilters which are only exposed to seasonal MIB and geosmin occurrences.

The genes responsible for MIB and geosmin biodegradation have not yet been identified. This is the aim of another research project at the Australian Water Quality Centre (current as of 2008). Identification of the genes will enable the development of rapid genetic techniques to determine if a biological system is capable of MIB or geosmin biodegradation. However, as the genes are not yet identified, the first step in identifying if a system has the potential for biological filtration for taste and odour treatment is to determine if the bacteria responsible for degradation are present in the filter influent.

2.6 SUMMARY

This literature review has outlined the problems caused by the earthy-musty odorous compounds, MIB and geosmin. It has been shown that MIB and geosmin are difficult to remove by the conventional treatment process and common oxidants such as chlorine and ozone.

Biological treatment for MIB and geosmin removal has been presented as a promising treatment option. More research is required to determine the conditions under which biological removal of MIB and geosmin through rapid sand filters will be optimised.
This thesis will describe experiments carried out to study the removal of MIB and geosmin through biological sand filtration. A series of full-scale, laboratory-scale and pilot-scale studies were conducted.
3 MATERIALS AND METHODS
3.1 INTRODUCTION

This section outlines general analytical materials and methods used to achieve the aims of the research. A selection of experimental apparatus used in the laboratory studies is also presented. More specific experimental designs and methods are described in the relevant chapters. The pilot plant apparatus is presented in Chapter 7.

3.2 MATERIALS AND REAGENTS

All reagents were analytical grade. Solutions were made using Milli-Q water (Millipore Pty Ltd, USA).

3.2.1 MIB and Geosmin Stock Solutions

MIB and (+/-) geosmin were obtained from Ultrafine Chemicals, UK. Stock solutions were prepared by dissolving in Milli-Q water. MIB was received as a white crystalline solid and dissolved in Milli-Q water in a 200 mL volumetric flask. Geosmin was received as a yellow oil and was prepared in a similar fashion. Both flasks were sealed with stoppers and Parafilm ‘M’ (America National Can, USA) and wrapped in aluminium foil to avoid any degradation by light. The flasks were placed on a bench top shaker at room temperature for 7 days to dissolve all solids. The final stock solutions were transferred to 100 mL amber glass bottles and stored headspace free in the dark at 4 °C. Stock concentrations of 56.4 mg L⁻¹ MIB and 47.0 mg L⁻¹ geosmin were obtained.

3.2.2 Waters

Waters were sourced from the Morgan and Happy Valley Water Treatment Plants (WTPs) in South Australia. The waters were sampled in 10 L plastic containers and stored at room temperature for a maximum of 2 weeks before use.

Settled water was sampled from Morgan and Happy Valley WTPs prior to disinfection. Settled water refers to water which has been treated by coagulation, flocculation and sedimentation. Happy Valley reservoir water was sampled at the inlet to the WTP.

Water quality data is shown in Table 1. The data shown is an average of samples taken over the course of the studies, as dissolved organic carbon (DOC) and ultraviolet (UV) measurements vary seasonally. The DOC data indicate that Happy Valley and Morgan settled waters have similar concentrations of organic matter. However, the UV absorbance at 254 nm wavelength, and the specific UV absorbance
(SUVA) at 254 nm, indicate that the Morgan settled water has a higher degree of aromaticity.

Table 3-1: Water Quality Parameters.

<table>
<thead>
<tr>
<th></th>
<th>DOC (mg L⁻¹)</th>
<th>UV ₂₅₄nm (cm⁻¹)</th>
<th>SUVA (L/mg C m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Happy Valley Reservoir</td>
<td>4.8</td>
<td>0.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Happy Valley Settled</td>
<td>3.5</td>
<td>0.042</td>
<td>1.2</td>
</tr>
<tr>
<td>Morgan Settled</td>
<td>3.5</td>
<td>0.06</td>
<td>1.7</td>
</tr>
</tbody>
</table>

3.2.3 Sands

Three sands were used in this study, each with different biofilm conditions and particle size characteristics.

Two sands were sampled directly from the filter beds of two South Australian water treatment plants, Morgan and Myponga. Neither of these plants employs pre-chlorination of the filters; therefore both sands had previously had opportunity for biofilm growth. The sand sampled from the Morgan WTP had been in the filter since the commissioning of the plant in 1986. The sand sampled from Myponga WTP was taken from filter beds which are replaced every two years due to the formation of mud balls. The third sand was purchased new to the same size specifications as the Morgan sand. It was named ‘Happy Valley’ sand as it was used in the pilot plant studies conducted at Happy Valley WTP.

Sieve analysis was used to calculate size distributions of the sands. Effective size (ES) was calculated as the sieve size opening that will just pass 10% (by dry weight) of a representative sample of the filter material; that is, if the size distribution of the particles is such that 10% of a sample is finer than 0.45 mm, the filter media has an effective size of 0.45 mm. ES is also known as the d₁₀ value.

Media density, described as weight of media per cm³ filter volume, was measured by placing 50 mL of the wet media in a measuring cylinder. The known volume of media was then dried in a laboratory oven at 100 °C for 1 hour. The dry media was weighed and density was calculated as the dry weight divided by the volume. This value was used in the analysis of the ATP assay data.
Table 3-2: Summary of Filter Sand Properties.

<table>
<thead>
<tr>
<th></th>
<th>Morgan</th>
<th>Myponga</th>
<th>Happy Valley</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.S.</td>
<td>0.55</td>
<td>1.25</td>
<td>0.55</td>
</tr>
<tr>
<td>Length of Time in</td>
<td>26 years</td>
<td>&lt; 2 years</td>
<td>N/A (new)</td>
</tr>
<tr>
<td>Filter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media Density</td>
<td>1.54</td>
<td>1.62</td>
<td>1.46</td>
</tr>
<tr>
<td>(g cm(^{-3}) filter volume)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.3 ANALYTICAL METHODS

3.3.1 Dissolved Organic Carbon

Dissolved organic carbon (DOC) was measured using an 820 Total Organic Carbon Analyser (Sievers Instruments Inc, USA). Samples were filtered through 0.45 μm cellulose nitrate membrane filters (Schleicher and Schuell, Germany) before analysis to remove any particulate matter.

3.3.2 UV Absorbance

UV absorbance at 254 nm was measured on a UV/VIS 918 spectrophotometer (GBC Scientific Equipment Pty Ltd, Australia). Samples were filtered through 0.45 μm cellulose nitrate membrane filters (Schleicher and Schuell, Germany) prior to analysis. UV absorbance at 254 nm was measured in a 1 cm quartz cell and the absorbance was zeroed using Milli-Q water (Millipore Pty Ltd, USA). Specific UV absorbance (SUVA) was calculated as,

\[
SUVA = \text{absorbance} @ 254nm (m^{-1}mL^{-1}) / DOC (mgL^{-1})
\]

(3.1)

3.3.3 MIB and Geosmin Analysis

MIB and geosmin analysis was carried out by the Organic Chemistry Unit at the Australian Water Quality Centre, based on a previously published method (Graham et al. 1998). Sample concentration was achieved by solid phase microextraction (SPME) using a polydimethylsiloxane-divinylbenzene (PDMS-DVB) syringe fibre, (Supelco, Australia). Analysis was conducted on a Hewlett Packard 5890 Series II Gas Chromatograph with Hewlett Packard 5971 Series Mass Selective Detector (Agilent Technologies, Australia) against qualified labelled internal standards (Ultrafine Chemicals, UK).
3.3.4 Autoclaving

Autoclaving was used in a number of experiments for sterilisation. In each case, samples were autoclaved at 121°C for 15 minutes. In this thesis, unless otherwise noted, items referred to as ‘sterilised’ are those items which have been autoclaved.

3.3.5 Ozone Residuals

Ozone residual and stock concentrations were measured using the Indigo Colorimetric Method (APHA et al. 1998). The method is based on the rapid discolouration of the Indigo II reagent by ozone. The decrease in absorbance is directly linear to decreases in ozone concentration.

Absorbance was measured in a 5 cm quartz cell at 600 nm using a UV/VIS 918 spectrophotometer (GBC Scientific Equipment Pty Ltd, Australia). The spectrophotometer was zeroed and calibrated using Milli-Q water.

Ozone concentration was calculation according to the following equation,

\[
[O_3] = \frac{100 \times \Delta A}{f \times b \times V}
\]  

(3.2)

Where \([O_3]\) = concentration of ozone in aqueous phase, mg L\(^{-1}\); \(\Delta A\) = difference in absorption between sample and blank; \(f = 0.42\); \(b\) = path length of cell, cm (6 cm); \(V\) = volume of sample, mL (80 ml in this case).

The change in absorbance is taken as the difference between the absorbance of a non-ozonated sample and the absorbance of the ozonated sample of the same water. The \(f\) factor is based on a sensitivity factor of 20,000 cm\(^{-1}\) for the change in absorbance (600 nm) per mole of added ozone per litre. This corresponds to an adsorption coefficient for aqueous ozone, \(E = 2950/\text{M cm at 258 nm}\).

3.4 BIOMASS ANALYSIS

Quantitative and qualitative methods were used to study biofilms on the surface of the filter media. Scanning electron microscopy was used for the qualitative study. Quantitative data was obtained by two distinct methods: an ATP assay and a flow cytometric method. The ATP assay method was developed throughout the course of this study, and was not applied in the studies carried out earlier in the project. In these cases, flow cytometry was used.

The ATP assay was considered to be the preferred method over flow cytometry due to its ease of use and the fact that it is an activity based assay rather than a measurement of total biomass concentration. Additionally, inaccuracies with the flow cytometric
analyses may have occurred as the flow cytometer is designed to measure individual bacterial cells and not the clumps of bacterial cells which may be encountered in biofilm samples. These clumps of bacteria can potentially register on the flow cytometer as only one cell, resulting in inaccurate cell counts.

Biomass data obtained with flow cytometry cannot be used to compare with samples measured by the ATP method.

3.4.1 Qualitative Analysis - Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to provide a qualitative assessment of the biofilm growing in the biofilters. While SEM does not allow for the identification of particular organisms, it does show the diversity of biological growth within the biofilm. It is not possible to identify organisms from SEM images; however some assumptions can be made. In general, organisms of less than 2 µm may be bacteria, while organisms larger than 2 µm might include fungi or microalgae. A pre-treatment step is required to fix the biofilm to the sand surface. This pre-treatment involves a dehydration step which can affect the appearance of some micro-organisms, for example making spherical organisms appear collapsed. It may also result in the loss of some biomass from the media surface, further highlighting that this is a qualitative process only.

To prepare the filter media for SEM analysis, approximately 3 g of media was fixed over night in a solution of 4% Paraformaldehyde/1.25% Glutaraldehyde in PBS and 4% sucrose at a pH of 7.2. The media was then washed in washing buffer of PBS and 4% sucrose for two changes of 5 minutes each. The samples were postfixed in 1% osmium tetroxide (OsO₄) for 30 minutes on a rotator. To prepare for critical point drying, the samples were dehydrated using ethanol solutions of increasing concentrations: 70%, 90%, 95% and 100%. Each solution was used to wash the sample for 1 change of 10 minute, with the exception of 100% ethanol which was used for 2 changes.

The filter media particles were prepared for critical point drying by rinsing in 100% ethanol. Final dried samples were coated with platinum and mounted on stubs.

A Phillips XL 30 Field Emission Scanning Electron Microscope was used to obtain the SEM photographs.
3.4.2 Quantitative Analysis - ATP Assay

The samples were analysed using the ATPlite Luminescence ATP Detection Assay System (Perkin Elmer, USA). The ATPlite system is based on the production of light caused by the reaction of ATP with luciferase and D-luciferin, as shown in the following reaction.

\[ \text{ATP} + D - \text{luciferin} + O_2 \xrightarrow{\text{luciferase-Mg}^{2+}} \text{Oxyluciferin} + \text{AMP} + PPI + CO_2 + \text{light} \] (3.3)

The light emitted in the reaction is directly proportional to the concentration of ATP in the sample, and can be measured on a luminometer.

The sample preparation method supplied with the ATPlite kit was adapted for use on biofilm samples. The final method is outlined below. Details on the development of this method are presented in Appendix A.
3.4.3 ATP Assay Method

Sampling (use aseptic techniques)

1. Weigh 1.5 mL sterile eppendorf tubes
2. Backwash lab columns with RO water to remove excess suspended biomass
3. Scoop a small amount of filter media from the top 2 cm of the column into each tube, approximately 0.3-0.4 g, using a sterile stainless steel spatula
4. Add 200 µL Milli-Q water and invert gently three times (to removed suspended matter), then remove all water using sterile pipette
5. Weigh tubes and define the weight of media “wet weight”

Assay Protocol – Filter Media Preparation

1. Add 200 µL of lysis buffer to each tube
2. Vortex sand/lysis buffer mixture for 2 minutes
3. Draw up all liquid and transfer to new tube
4. Spin tube for 4 minutes at 10,000 x g to get pellet
5. Take 50 µL aliquots and transfer to new tubes (two for each sample)
6. Add 100 µL of sterile Milli-Q water to each tube
7. Add 50 µL enzyme substrate solution to each tube
8. Withdraw 200 µL of sample to each tube to luminometer plate

Summary of Filter Media samples:
- 50 µl of biofilm/lysis buffer solution
- 100 µL sterile Milli-Q water
- 50 µL enzyme substrate solution

Assay Protocol - Standard Preparation

To make standards for luminescence measurement

- Add 250 µL lysis buffer to eppendorf containing ~ 1 g of autoclaved media
- Vortex sand/lysis buffer mixture for 2 minutes
- Draw up all liquid and transfer to new tube
- Spin tube for 4 minutes at 10,000 x g to get pellet
- Take 50 µL aliquots and transfer to new tubes (two for each sample)
- Add 80 µL of Milli-Q
- Add 20 µL of chosen standard
- Add 50 µL of enzyme substrate solution
- (note that samples in wells are now further 1/10 dilutions)

Preparation of Standards

1. Add 1120 µL of sterile Milli-Q water to 11.2 µmol ATP standard to get 10mM standard
2. Make a series of 1 in 10 dilutions by aliquoting 100 µL of previous standard into 900 µL in new eppendorf
3. Make standards of 10^{-3} to 10^{-9}
4. Store in freezer at -20 °C for 3-4 months maximum
Analysis of Results

Samples were analysed in a 96 well Isoplate using the Wallac 1420 multilabel counter (Perkin Elmer, USA). The luminescence outputs were compared to ATP standard curves. Final ATP concentrations were multiplied by 4 to account for the use of the 200 μL of lysis solution used for the total weight of sand. This value was divided by the wet weight of the sand to obtain nmol ATP per gram filter media (wet weight).

The data is presented as nmol of ATP per cm$^3$ filter media, as it gives a more representative of the actual biomass activity of a filter due to differences in densities of different filter media. The media densities were used to convert the weight values to unit filter volumes. Anthracite had a density of 0.69 g cm$^{-3}$ compared to the Morgan sand which had a density of 1.54 g cm$^{-3}$.

3.4.4 Bacterial Enumeration by Flow Cytometry

Flow cytometry was used to determine the number of viable bacteria in biofilm samples during the period when the ATP assay was in development. Flow cytometry was also applied to natural water samples. In each case, a final sample volume of 1 mL was required. Natural water samples were analysed at a 1:10 dilution, while biofilm samples were analysed with a 1:100 dilution.

Filter media samples were taken according to the ATP assay sampling method, up to Step 6 of the Assay Protocol - Filter Media Preparation. At this point, 1 mL of sterile Milli-Q water (Millipore Pty Ltd, USA) was added. The solution was shaken and vortexed for 1 minute periods for a total of 15 minutes. Supernatant samples of 10 μL were added to 990 μL of sterile Milli-Q to obtain a 1:100 dilution. The resultant solution was enumerated by flow cytometry. It is recognised that the detachment process may not have removed all bacterial species from the sand; however, the process allows for comparison between the two sands.

In cases where the entire biofilm was removed from laboratory columns, the sand was transferred to pre-weighed 50 mL centrifuge tubes and gently washed with Milli-Q water. The ‘wet weight’ of the sand was determined, and sterile Milli-Q was added to the sand up to a total volume of 50 mL. The sand was vortexed/shaken for 15 minutes and the supernatant was transferred to new centrifuge tubes and spun down at 4000 rpm for 10 minutes. The pellet was resuspended in 10 mL of sterile saline solution and cell numbers were enumerated using flow cytometry.

The analysis was based on a previous method, using the LIVE/DEAD® Baclight™ kit (Molecular Probes Inc, Eugene, USA) with subsequent enumeration by flow cytometry (FCM) (Hoefel et al. 2003).
Firstly, the LIVE/DEAD® Baclight™ kit was used to identify the bacteria that had retained the active process of maintaining membrane integrity (Hoefel et al. 2003). The kit consisted of two stains, SYTO-9 (3.34 mM; excitation 480 nm/emission 500 nm) in dimethyl sulfoxide (DMSO) and propidium iodide (PI) (20 mM, excitation 490 nm/ emission 635 nm) in DMSO. To determine which bacteria had maintained membrane integrity, equal amounts of SYTO-9 and PI were combined and vortexed briefly. An aliquot of 1 µL of the Baclight™ mixture was added to 1 mL of the sample to be analysed and incubated in the dark for 15 minutes, according to directions provided with the Baclight™ kit. During this time, the SYTO-9 penetrated the membranes of both permeable and intact bacteria, while the PI penetrated only the membrane-permeable bacteria. The SYTO-9 and PI then bind to the DNA. The intact, or active, bacteria contain only SYTO-9 and have green fluorescence under blue light excitation. Membrane permeable, or inactive, bacteria contain both SYTO-9 and PI and have a red fluorescence under blue light excitation.

3.5 LABORATORY METHODS AND APPARATUS

3.5.1 Bioreactor Studies

Bioreactors were used in a number of studies. The bioreactors consisted of pre-autoclaved 2 L Pyrex Schott bottles filled with waters and bacterial inoculums specific to each study.

The bioreactors were spiked at target MIB and geosmin concentrations of 100 ng L⁻¹ each and stirred constantly with sterilised magnetic stirring bars. Samples were taken aseptically and immediately filtered through 0.22 µm cellulose nitrate membrane filters (Schleicher and Schuell, Germany) to stop any further degradation.

3.5.2 Laboratory Column Apparatus

The experimental apparatus for the laboratory columns is shown in Figure 3-1.
Influent water was stored in pre-cleaned 20 L vessels, glass or plastic. MIB and geosmin stock solution was spiked directly into the vessels to the desired influent concentration. The flow rate of the influent water was controlled using an adjustable peristaltic pump (Gilson Minipuls 3, Australia). All tubing used was Tygon Lab tubing (Masterflex, USA). Feed water was stored at room temperature (20 ± 2 °C) and filtered through a 1 μm Polypure Capsule (Pall Life Sciences, USA) before being fed to the columns.

Details of the column apparatus are shown in Table 3-3, below.

Table 3-3: Laboratory Column Apparatus Characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reservoir Volume</td>
<td>20 L</td>
</tr>
<tr>
<td>Bed Height</td>
<td>15 cm</td>
</tr>
<tr>
<td>Column Internal Diameter</td>
<td>2.5 cm</td>
</tr>
<tr>
<td>Bed Volume</td>
<td>78.5 cm³</td>
</tr>
</tbody>
</table>
The relationship between EBCT and filtration rate for the columns is shown in Table 3-4.

Table 3-4: Laboratory Column Filtration Rates.

<table>
<thead>
<tr>
<th>Empty Bed Contact Time (min)</th>
<th>Filtration Rate (m h⁻¹)</th>
<th>Flow rate (mL min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>2.6</td>
<td>22.4</td>
</tr>
<tr>
<td>5</td>
<td>1.8</td>
<td>15.0</td>
</tr>
<tr>
<td>7</td>
<td>1.3</td>
<td>11.2</td>
</tr>
<tr>
<td>15</td>
<td>0.6</td>
<td>5.2</td>
</tr>
<tr>
<td>30</td>
<td>0.3</td>
<td>2.6</td>
</tr>
</tbody>
</table>

The columns were backwashed using reverse osmosis (RO) water. Each column was backwashed once per week unless stated otherwise. Water was allowed to flow upwards through the column at a sufficient rate to cause 10% expansion of the filter bed. The backwash duration was 2 minutes.

The water vessels were spiked with MIB and geosmin at concentrations of 100 ng L⁻¹ unless noted otherwise. It was difficult to maintain constant concentrations in the influent. This is a problem commonly encountered in studies using MIB and geosmin (Elhadi et al. 2004a; Westerhoff et al. 2005). The difficulties were attributed to volatilisation and losses due to adsorption to the glass bottle and tubing. The potential for biodegradation of MIB and geosmin in the vessels may also be a factor. To minimise this problem, the tubing and water vessels were cleaned weekly with hot tap water and rinsed with Milli-Q water.

3.5.3 Biofilm Removal Method

A number of the laboratory column studies required that biofilm be removed from the sand surface. To achieve this, autoclaved sand was sonicated for 30 minutes and rinsed well with RO water to remove particulates. The rinsed sand was transferred into 100 mL Pyrex Schott bottles and approximately 40 mL of a 2M sodium hydroxide solution was added. The bottles were topped up to 100 mL with Milli-Q water and agitated overnight on a mixer/roller. It was observed that the sodium hydroxide solution turned a dark brown colour. The dark brown sodium hydroxide solution was decanted and the sand was rinsed with RO water and the process was repeated. At this point, it was observed that there was no more colour coming off the
sand and it was considered to be clean. The sand was then rinsed thoroughly with RO water to ensure that all sodium hydroxide was removed. SEM was used to verify the presence/absence of biofilm on sand samples.
4 REMOVAL OF MIB AND GEOSMIN THROUGH BIOLOGICAL SAND FILTRATION - FULL SCALE STUDY WITH LABORATORY VALIDATION
4.1 INTRODUCTION

The Morgan WTP in South Australia has previously not required additional treatment, such as PAC, for taste and odour control despite receiving moderate to high levels of MIB and geosmin year round. Anecdotal evidence has pointed to the removal of MIB and geosmin occurring through the non-chlorinated rapid gravity filters.

In this chapter, a review of the MIB and geosmin water quality data from Morgan WTP is combined with a series of laboratory experiments to verify the hypothesis that the removal was occurring via biological activity in the rapid gravity dual media anthracite-sand filters of the plant.

4.2 MORGAN WATER TREATMENT PLANT

4.2.1 Treatment Plant Overview

Morgan WTP was built in 1986 and services a large portion of South Australia’s regional centres such as Whyalla, Port Pirie and Port Augusta (SAWATER 2007). The Morgan WTP takes its water directly from the River Murray and has a capacity of 200 ML per day.

A plant schematic is presented in Figure 4-1. The plant operates with conventional treatment comprised of coagulation, flocculation, sedimentation and filtration, with chloramine disinfection.

Figure 4-1: Morgan WTP schematic.
Morgan WTP has eight dual-media anthracite-sand rapid gravity filters. In contrast to many South Australian WTPs, the rapid gravity filters are not pre-chlorinated. The filter media have been in place since the commissioning of the plant in 1986.

Each filter has a design bed depth of 750 mm. It is likely that the media depth is now somewhat lower, due to media losses through backwashing over the last 21 years. The current depth has not been measured.

A summary of the filter properties is shown in Table 4-1.

Table 4-1: Filter Characteristics of Morgan WTP.

<table>
<thead>
<tr>
<th>Total bed height (mm)</th>
<th>750 mm (design depth)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>approximately 300 mm sand</td>
</tr>
<tr>
<td></td>
<td>approximately 450 mm anthracite</td>
</tr>
<tr>
<td>Filtration rate</td>
<td>10 m h$^{-1}$ operation at nominal flow</td>
</tr>
<tr>
<td></td>
<td>13.7 m h$^{-1}$ post backwash</td>
</tr>
<tr>
<td>Empty Bed Contact Time (approximate)</td>
<td>3 min</td>
</tr>
<tr>
<td>Sand Effective Size (mm)</td>
<td>0.55 mm</td>
</tr>
<tr>
<td>Anthracite Effective Size (mm)</td>
<td>1.0 mm</td>
</tr>
</tbody>
</table>

The Morgan filter run times can vary from 10 to 50 hours, depending on water quality and required plant output. At the time of writing the backwashing regime was under revision, but the conditions current as of April 2007 are as follows (pers. comm. John Knoblauch April 2007),

- Air scour for 5 minutes at approximately 36 m$^3$ m$^{-1}$,
- 2 minutes rest
- Water wash at 550 L s$^{-1}$ for 3 minutes
- Water wash at 850 s$^{-1}$ for 7 minutes
- Water was at 470 L s$^{-1}$ for 2.5 minutes
Product water from the filters is combined prior to being disinfected and sent to the filtered water storage tanks. As shown in Figure 4-1, the backwash water is sourced from the filtered water combined discharge sump. Ammonia, fluoride and caustic (sodium hydroxide) are also dosed in the filtered water combined discharge sump, resulting in these chemicals being present in the backwash water. The backwash pump is located at a point where the mixing of the dosed chemicals is poor, thus concentrations of the dosed chemicals in the backwash water are variable and unpredictable.

Ammonia is dosed to give a filtered water concentration of between 1.13 and 1.25 mg L\(^{-1}\) (Pers. comm. John Knoblauch May 2007). It is assumed that 20\% to 40\% of the dosed ammonia would be present in the filters during backwash, due to mixing within the sump and the location of the backwash pump. Sampling of the backwash inlet water on 18/06/07 gave an ammonia concentration of 0.074 mg L\(^{-1}\). This is a spot sample only; the ammonia concentration could vary significantly over time.

Caustic (sodium hydroxide) is added to achieve a filtered water pH of 8.8; this is an approximate dose of 20 mg L\(^{-1}\). Fluoride is dosed to achieve a filtered water concentration of between 0.5 to 0.9 mg L\(^{-1}\).

After leaving the sump, the filtered water is dosed with between 4.2 to 5.0 mg L\(^{-1}\) of chlorine to result in chloramine levels of between 4.0 to 4.8 mg L\(^{-1}\).

### 4.2.2 MIB and Geosmin Removal Through Morgan WTP

SA Water monitoring at Morgan WTP from 2000 to 2007 has shown an average of 11.7 (± 10.6) ng L\(^{-1}\) (n = 123) geosmin in the raw water. MIB levels are lower, averaging 3.5 (± 4.0) ng L\(^{-1}\) (n = 202) over the period 10/12/1997 to 26/07/2007.

On 17\(^{th}\) December 2004, the chlorine dosing point was moved to the filtered water sump. Chlorine was previously dosed just prior to the filtered water storage, as shown in Figure 4-1. The previous dosing point was causing problems with the chloramine residual monitoring, as there was insufficient distance between the chlorine dosing and the chloramine residual monitoring point to allow for sufficient mixing of the chlorine and ammonia. Thus, the chloramine residual monitoring was ineffective.

There is poor mixing within the filtered water sump. Therefore, it is likely that the change in chlorine dose point would result in a mixture of free chlorine, free ammonia and chloramines in the backwash water. Prior to the change in chlorine dose point, the backwash water did not contain any disinfectants.

The change in chlorine dosing point directly coincided with an increase in geosmin and MIB levels in the product water. Figure 4-2 shows geosmin levels in the raw and product waters of Morgan WTP plant over the period 6/06/2004 to 6/02/2007. This
figure shows that until December 2005 the plant consistently produced water with between 0 to 1 ng L\(^{-1}\) of geosmin. At the time when the chlorine dose point was moved, significant geosmin breakthrough occurred. A similar trend is seen in Figure 4-3, with MIB levels increasing in the product water at this time.

The change in taste and odour removals through the plant was noted by researchers and operators. As the only major operational change during this period was the change in the chlorine dose point, it was believed that the chlorine in the backwash water was causing problems within the rapid gravity filters. The chlorine dose point was returned to its original location in August 2006.

Geosmin levels entering the plant increased markedly during this period as continuing drought conditions in the Murray-Darling basin resulted in poorer water quality in the River Murray, resulting in more frequent episodes of algal blooms. Despite 2007 providing the second highest average geosmin inlet level since data was collected, the data shows that after the chlorine was removed from the backwash water, the plant consistently produced water with geosmin levels of 5 ng L\(^{-1}\) and below. It must be noted that the analytical method for geosmin and MIB was changed during this period, with the new method having a minimum reporting limit of 4 ng L\(^{-1}\). Therefore, the effluent concentration of MIB and geosmin after the return to the previous backwashing regime was at or below reporting limit.

Although no validation was available, it has long been assumed that the geosmin removal was occurring through the rapid gravity filters. The lack of a disinfectant over the life of the filter would theoretically result in the growth of biomass within the filter media, which could potentially biodegrade the taste and odour compounds. The effect of chlorine/chloramine backwash on any biological activity was not considered as the filters were not deliberately being operated as biological filters.

In this thesis, backwashing with the chlorine-ammonia water will be referred to as chlorinated backwash although it is acknowledged that the presence of ammonia would result in a mixture of free chlorine, free ammonia and chloramines.
Figure 4-2: Geosmin concentrations (ng L\(^{-1}\)) in Morgan WTP, 2000 – 2007.

Figure 4-3: MIB concentrations (ng L\(^{-1}\)) in Morgan WTP, 1997 – 2007.
4.3 VALIDATION BY LABORATORY SCALE COLUMNS

Laboratory column experiments were conducted concurrently with the changes in backwashing conditions of the Morgan WTP to validate the contention that geosmin and MIB removal was occurring by biological activity in the rapid gravity filters.

4.3.1 Experimental Design

Laboratory experiments were conducted to investigate the ability of the filter media to remove MIB and geosmin biologically before, during and after the periods when the filters were backwashed with chlorinated water. In addition, the ability of the media to perform this function at a range of EBCTs was investigated.

Laboratory columns were set up as described in Chapter 3: Materials and Methods. Six columns were used in this study, detailed in Table 4-2.

EBCT was chosen as the primary filtration rate parameter, as the small volume of the columns meant that achieving loading rates similar to that of the full-scale was not feasible. The columns were run at a base EBCT of 15 minutes (HLR 0.6 mh⁻¹); however, a number of EBCT trials of 10 and 5 minutes were conducted to determine if the filter sand could degrade MIB and geosmin at EBCTs similar to that of the full-scale plant.

<table>
<thead>
<tr>
<th>Table 4-2: Laboratory columns containing Morgan filter media.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
</tr>
<tr>
<td>Column 2</td>
</tr>
<tr>
<td>Column 3</td>
</tr>
<tr>
<td>Column 4</td>
</tr>
<tr>
<td>Column 5</td>
</tr>
<tr>
<td>Column 6</td>
</tr>
</tbody>
</table>
4.3.2 Results of Column 1

The results of the MIB and geosmin removals through the laboratory column study with sand sampled prior to the chlorinated backwash (Column 1) are presented in Figures 4-4, 4-5 and 4-6. This sand had been in the filter since 1986 without exposure to disinfectants. The column was run for a period of 100 days at 15 minute EBCT and was challenged with reduced EBCTs of 5 and 10 minutes for short periods. MIB and geosmin concentrations in the influent and effluent of the columns are shown in Figures 4-4 and 4-5, while the percentage removals are shown in Figure 4-6. From the data presented in Figure 4-6 it can be seen that the percentage removals at day 0 were 96% and 97% for MIB and geosmin, respectively. Removal of both compounds to below analytical detection limit was achieved by the next sample point on day 8.

The EBCT was reduced to 10 minutes on day 58. No decrease in removal of either compound was observed. On day 56 EBCT was decreased to 5 minutes and removals remained high at 98% for MIB and 97% for geosmin.

This column showed that Morgan filter sand, prior to the chlorinated backwash period, was capable of removal of MIB and geosmin to below analytical detection limit with no lag period. High removals were achieved through the range of EBCTs commonly employed in rapid gravity filtration.

NOTE: This figure is included on page 50 in the print copy of the thesis held in the University of Adelaide Library.

Figure 4-4: MIB concentrations (ng L-1) through non-chlorinated laboratory column (figure adapted from (Bock 2006)).
Figure 4-5: Geosmin concentrations (ng L⁻¹) through non-chlorinated backwash laboratory column (figure adapted from (Bock 2006)).

Figure 4-6: Percentage MIB and geosmin removals through non-chlorinated backwash laboratory column (figure adapted from (Bock 2006)).
4.3.3 Results of Column 2

The results from the column study utilising sand sampled during the chlorinated backwashing regime (Column 2) are presented in Figures 4-7, 4-8 and 4-9. This column was run for a period of 26 days at an EBCT of 15 minutes with two spiking periods.

Figure 4-9 shows the percentage removals of MIB and geosmin through Column 2. Initial removals ranged from 14 to 19% for MIB and 30 to 34% for geosmin. After a cessation of spiking for 15 days, the removal increased to 36% for MIB and 70% for geosmin. It is likely that the initial low removals were due to background removals only, such as system losses and adsorption within the filter media. The increase in removal is due to the re-establishment of the biofilm in the absence of chlorine.

The results from Column 2 indicated that sand sampled during the chlorinated backwash period was initially not effective for MIB and geosmin removal, lending support to the hypothesis that the chlorine had reduced the biological activity within the filters. However, the biofilm was able to re-establish over the course of the study and biological removal returned. The re-established biofilm did not achieve removals of MIB and geosmin at the same level as the biofilm sampled prior to the chlorinated backwash.

![Figure 4-7: MIB concentrations (ng L\(^{-1}\)) through chlorinated backwash laboratory column.](image)
Figure 4-8: Geosmin concentrations (ng L\(^{-1}\)) through chlorinated backwash laboratory column.

Figure 4-9: Percentage MIB and geosmin removals through chlorinated backwash laboratory column.
4.3.4 Results of Column 3

Results from the laboratory column utilising sand sampled approximately 5 months after the cessation of oxidant in the backwash (Column 3) are shown in Figures 4-10, 4-11 and 4-12. Column 3 was run for a total of 176 days at a base EBCT of 15 minutes. EBCTs of 5 minutes were employed on day 99 and day 176 to challenge the filter.

The percentage removals of MIB and geosmin are presented in Figure 4-12. Removals on day 0 were markedly higher than for the chlorinated backwash column (Column 2), with 64% removal of MIB and 84% removal of geosmin. After a period of 18 days, the column was removing greater than 90% of both MIB and geosmin. These high removals are significantly above the background removals observed in Column 2, indicating that the biofilm had recovered from the damage caused by the chlorinated backwash.

After the initial spiking period, the column flow rate was reduced to 1 mL min\(^{-1}\) for 40 days to conserve water. After this time the column operation was returned to a 15 minute EBCT and spiking with MIB and geosmin was resumed on day 51. Removals of MIB and geosmin were decreased upon resumption of spiking, as indicated by the percentage removals presented in Figure 4-12.

An initial delay period before pseudo steady-state removal of the target compounds is achieved is common to all biological filters. The duration of this delay is dependent on the target compound, water quality and filter operating conditions (including media type) (Huck et al. 1995; Liu et al. 2001; Metz et al. 2006; Summers et al. 2006). For the purpose of this thesis it will be referred to as the acclimation period. It is possible that the MIB and geosmin degrading bacteria needed an additional period to become re-acclimated to MIB and geosmin biodegradation when spiking had been ceased for a period of time. This hypothesis was strengthened when decreased removals were observed when spiking was ceased for a further 15 days, from day 82 to 97, without changes in EBCT. On the resumption of spiking, removal had decreased to 54% for MIB and 79% for geosmin. The effect of the intermittent spikes is an important point to note, as the transient nature of algal blooms means that biological filters will often be challenged by sporadic contact with MIB and geosmin (Westerhoff et al. 2005).

The EBCT of the column was reduced to 5 minutes on day 99 and a marked decrease in removal occurred for both compounds. MIB removal decreased from 54% to 30% while geosmin removal decreased from 79% to 63%. Increasing the EBCT back to 15 minutes resulted in removals greater than 90% for geosmin and 80% for MIB. Here, it is seen that the impact of a decrease in EBCT was more pronounced than for Column 1.
The results of Column 3 indicate that the Morgan filter sand had recovered from the damage caused by the chlorinated backwash. However the biofilm was not as robust as it was prior to the backwash change. Decreases in removal occurred when spiking was stopped for any period of time. The effect of decreased EBCT was also more evident than for the pre-chlorinated backwash (Column 1) and more notable for MIB than for geosmin.

Figure 4-10: MIB concentrations (ng L\(^{-1}\)) through post chlorinated backwash laboratory column.
Figure 4-11: Geosmin concentrations (ng L⁻¹) through post chlorinated backwash laboratory column.

Figure 4-12: Percentage MIB and geosmin removals through post chlorinated backwash laboratory column.
4.3.5 Results of Column 4

The Morgan filters are dual-media, containing sand and anthracite coal. Anthracite was sampled from the surface of filter 1 in April 2007, 8 months after the cessation of oxidant in the backwash water. The column was run for 36 days at a base EBCT of 15 minutes. It was challenged by a 5 minute EBCT on day 24.

The results of the anthracite column are shown in Figures 4-13, 4-14 and 4-15. The percentage removals of MIB and geosmin through the column are shown in Figure 4-15. The column was initially run at a 15 minute EBCT for a period of 13 days. Removals of MIB over this period ranged from 64 to 75%, while geosmin removals ranged from 84 to 89%. A 9 day break in spiking did not have a negative impact on removals of either compound, with a measured MIB removal of 69% and a geosmin removal of 91% upon recommencement of spiking.

Decreasing the EBCT to 5 minutes on day 24 resulted in a decrease in MIB and geosmin removal to 25% for MIB and 61% for geosmin. This was similar to that observed for Column 3, where it was seen that biological removal in newer biofilms was negatively affected by decreases in EBCT. No anthracite column experiment was conducted prior to the chlorinated backwash to determine if the anthracite could remove MIB and geosmin at lower EBCTs during those timeframes.

This study did not determine the percentage contributions of anthracite versus sand to the total removal of MIB and geosmin through the filters at Morgan. However, this work shows that both filter media were able to support biomass capable of MIB and geosmin removal.
Figure 4-13: MIB concentrations (ng L\(^{-1}\)) through anthracite post chlorinated backwash laboratory column.

Figure 4-14: Geosmin concentrations (ng L\(^{-1}\)) through anthracite post chlorinated backwash laboratory column.
Figure 4-15: Percentage removals of MIB and geosmin through anthracite post chlorinated backwash laboratory column.
4.4 CONFIRMATION OF BIODEGRADATION IN LABORATORY COLUMN

The data collected from the Morgan laboratory column experiments indicated that MIB and geosmin could be effectively removed by Morgan filter media. The addition of chlorine and chloramines to the backwash water decreased MIB and geosmin removal, which was presumed to be due to inactivation of the microorganisms within the filter by the disinfectant. In the following sections, the case for biological removal is strengthened by conducting column studies utilising Morgan sand and anthracite with established biofilms that were inactivated by autoclaving.

4.4.1 Results of Column 5

The results of a column study utilising autoclaved Morgan sand sampled prior to the chlorinated backwash regime (Column 5) is presented in Figures 4-16, 4-17 and 4-18. The column was run for a total of 149 days to investigate the length of time required for biological activity to return in the column.

Shown in Figure 4-18, the removals of MIB and geosmin at day 0 indicate the percentage removal which would be attributed abiotic processes such as system losses within the column and associated apparatus. MIB removal at this stage was 9%, while geosmin removal was 21%. These results strongly suggest that the majority of removal observed in the non-autoclaved columns was by biodegradation.

Removal of MIB and geosmin increased rapidly, indicating that biological removals were returning. After a period of 29 days, geosmin removal had reached 83% while MIB removal reached 70%.

The column was subjected to two trials where the EBCT was sequentially decreased. The first trial where EBCT was sequentially decreased was conducted on day 58. Geosmin removals decreased to 83, 66 and 64% for 10, 5 and 3.5 minutes respectively. MIB removals during this period were 72, 56 and 50%.

The column was returned to the 15 minute EBCT until day 99, when spiking was ceased and the column was run at a 27 minute EBCT for 20 days. Upon recommencement of spiking, MIB and geosmin removals remained high, in the range of 85 to 90% for MIB and 91 to 94% for geosmin.

A second trial where EBCT was sequentially decreased was conducted on day 138. Decreases in EBCT to 10 and 5 minutes again showed decreases in MIB and geosmin removals. Geosmin removals decreased to 88 and 59% for 10 and 5 minutes respectively, while MIB removals decreased to 78 and 38%.
These results show that the removal of MIB and geosmin in the acclimated Morgan sand was predominantly by biological degradation. The trials where EBCT was sequentially decreased indicated that although the biofilm recovered and could remove the compounds at the baseline 15 minute EBCT, the re-established biofilm was not as effective at the lower contact times.

NOTE: This figure is included on page 61 in the print copy of the thesis held in the University of Adelaide Library.

Figure 4-16: MIB concentrations (ng L⁻¹) through autoclaved Morgan sand laboratory column (see Figure 4-18 for EBCT details) (adapted from (Bock 2006)).
Figure 4-17: Geosmin concentrations (ng L⁻¹) through autoclaved Morgan sand laboratory column (see Figure 4-18 for EBCT details) (adapted from (Bock 2006)).

Figure 4-18: Percent removals of MIB and geosmin through autoclaved Morgan sand laboratory column (adapted from (Bock 2006)).
4.4.2 Results of Column 6

Column 6 contained anthracite sampled 8 months after the cessation of the chlorinated backwash. The anthracite was subsequently autoclaved to investigate the removal of MIB and geosmin that could be attributed to background removals. The influent and effluent concentrations of MIB and geosmin, and the percentage removals of each compound through the column, are shown in Figures 4-19, 4-20 and 4-21, respectively.

The column was spiked on day 0, immediately after autoclaving. Initial removal of geosmin was 32%. Initial MIB removal was lower at 9%. This is the level of removal through the anthracite that can be attributed to background removals.

The column was continually spiked for a period of 36 days at an EBCT of 15 minutes. Geosmin removal of up to 80% was obtained by day 24, which is similar to that observed for the autoclaved sand. MIB removal did not return as rapidly as it did for the sand, and removals of MIB remained low throughout the study. The maximum MIB removal during this study was 35%, occurring on day 36.

The EBCT was decreased to 5 minutes on day 65. Geosmin removals decreased from 70 to 38%. MIB removals were less affected; however, the low MIB removals throughout this study were not considered to be above background physical losses.

Figure 4-19: MIB concentrations (ng L⁻¹) through autoclaved anthracite laboratory column. Circled data points indicate 5 minute EBCT.
Figure 4-20: Geosmin concentrations (ng L⁻¹) through autoclaved anthracite laboratory column. Circled data points indicate 5 minute EBCT.

Figure 4-21: Percentage removals of MIB and geosmin through autoclaved anthracite laboratory column. Circled data points indicate 5 minute EBCT.
4.5 BIOMASS ANALYSIS

The ATP assay was used to study the biomass activity of the filter media in the post chlorinated backwash column. The assay was not applied to the other columns in this study, as the method was in development while those studies were being carried out.

4.5.1 Post Chlorinated Backwash Columns

Results of the ATP assay conducted on the post chlorinated backwash sand and anthracite columns (Columns 3 and 4) are shown in Figures 4-22 and 4-23, respectively. ATP monitoring was started on day 70 of the study due to delays in the development and validation of the assay.

![Figure 4-22: Biomass activity of post chlorinated backwash sand (nmol ATP per unit filter volume). Error bars indicate standard deviations from triplicate analysis.](image-url)

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The assay showed that there was a higher biomass activity on the anthracite than the sand. There was less variation in biomass activity for the sand than for the anthracite. Average ATP levels on the sand were 529 (SD 30) nmol ATP per cm$^3$ filter volume. Average ATP levels on the anthracite prior to autoclaving were 931 (SD 45) nmol ATP per cm$^3$ filter volume. As discussed previously, an issue with the ATP method is that the ATP content of a cell varies depending on the growth phase of the cell (Karl 1980). This may account for variations in the ATP levels obtained. Despite these variations, the anthracite showed consistently higher biomass levels than the sand.

It is difficult to compare these ATP values to those in previous studies. One study looked at ATP concentrations in 9 different rapid sand filters which had been operating for a range of 820 to 7300 days (Magic-Knezev et al. 2004). ATP concentrations observed in this study had a large range from 16 – 2592 ng cm$^{-3}$. The author is not aware of any other studies looking at the ATP concentrations on anthracite-sand filters, however a number of studies have looked at ATP on GAC (Magic-Knezev et al. 2004; Velten et al. 2007).

The literature does not give adequate comparison between general biomass levels in anthracite versus sand. One study compared the biomass concentration, as studied by phospholipid content, at the top of anthracite-sand to sand only filters. This study found that the anthracite-sand filter contained half the biomass of the single media
filter (Urfer et al. 1997). Another study also noted that anthracite sand filters contained approximately 54% less biomass than sand filters (Wang et al. 1995). However, both these studies looked at media sampled from the top of the filter. Actual biomass densities in biological filters vary with the depth of the filter (Urfer et al. 1997). In a study looking at an anthracite-sand filter it was found that the biomass concentration decreased by 30% and 40% at depths of 15 cm and 30 cm respectively (Wang et al. 1995). The Wang et al. (1995) study did not distinguish between the differences in biomass on the anthracite or sand portions of the filter.

The exact depth from which the sand was sampled from the Morgan filters is not known, due to inherent difficulties with sampling of media from operating rapid gravity filters. However, the anthracite was sampled directly from the top of the filter while the sand was sampled below the top of the sand layer. Following the results of the Wang et al. (1995) study, it would be expected that the sand layer would have a lower biomass level than that of the anthracite due to its lower depth in the filter.

The final ATP value on Figure 4-23 shows the biomass activity of the autoclaved anthracite column (Column 6) 30 days after autoclaving. The ATP level of the anthracite had decreased to 175 nmol ATP per cm$^3$ filter volume at this time. Interestingly, despite this low biomass activity, the column was removing greater than 80% of the influent geosmin at that time.

These results suggest that biomass activity is not a good indication of the MIB and geosmin degradation potential of a biofilm. The anthracite and sand biofilm columns both exhibited geosmin removals of over 90% and MIB removals of over 70% at 15 minute EBCT, despite the anthracite exhibiting higher ATP levels. It has been noted previously that there may be some minimum concentration above which biomass is not limiting, and that would be specific for the target compounds and the temperature of the system (Urfer et al. 1997). It is more likely that the biodegradation process is governed by the concentration of the specific MIB or geosmin degrading bacteria, and the ability of these organisms to form biofilms on the filter media. General biomass measurements, whether by ATP, phospholipid or other total biomass measurements, are not adequate for predicting the taste and odour degradation potential of a biofilm. These methods take into account the biomass activity of the entire community and the results are not an indication of the activity of the degraders on their own.
4.6 SEM ANALYSIS

SEM was conducted on each of the sand samples while they were in use in the laboratory columns. In the following pictures, the biofilm appears in some places as a ‘mat’ composed of a variety of different organisms and extracellular matter. It must be noted that the SEM process is qualitative, not quantitative. The SEM images show that the biofilm is quite patchy and non-heterogenous. This is common to biofilms developed in oligotrophic environments (such as drinking water), which may consist of a sparse coverage of cells with little structural complexity (Stoodley et al. 2002).

Scales for each SEM image are shown on the data bar at the bottom of the scan.

4.6.1 SEM of Sand Prior to Chlorinated Backwash

A selection of SEM pictures taken of Morgan filter sand prior to the chlorinated backwash is shown in Figures 4-24 through 4-25. The images show a thick biofilm composed of many different organisms including bacteria, micro algae and extracellular biofilm matter.

Figure 4-24: SEM image of sand prior to chlorinated backwash, scan 1.
Figure 4-25: SEM image of sand prior to chlorinated backwash, scan 2.

4.6.2 SEM of Sand During Chlorinated Backwash

SEM pictures of sand during the chlorinated backwash are shown in Figures 4-26 and 4-27. It can be seen that there is much less biofilm on the sand during this time, but some filamentous matter remains.

Figure 4-26: SEM image of sand during chlorinated backwash, scan 1.
4.6.3 SEM of Sand After Chlorinated Backwash

The sand sampled post chlorinated backwash had a biofilm similar in appearance to that of the pre chlorinated backwash sand. SEM images of this sand are shown in Figures 4-28 and 4-29. The presence of biofilm demonstrates how the biofilm had recovered after the cessation of the chlorinated backwash.
4.6.4 SEM of Anthracite After Chlorinated Backwash

SEM scans of the anthracite taken after the cessation of the chlorinated backwash are presented in Figures 4-30 to 4-32. These scans show that there is a diverse biofilm present on the media surface.
Figure 4-31: SEM image of anthracite, scan 2.

Figure 4-32: SEM image of anthracite, scan 3.
4.7 GENERAL DISCUSSION AND CONCLUSIONS FROM CHAPTER 4

The results presented in this chapter demonstrate that Morgan WTP was operating biologically for MIB and geosmin removal prior to the implementation of a chlorinated backwashing regime. The laboratory scale study proved that biological MIB and geosmin removal was occurring through both the anthracite and sand media. This is the first study of which the author is aware that has shown the potential of biological rapid anthracite-sand filtration for MIB and geosmin removal by a long term full-scale study with laboratory validation. The results presented have provided important information for the water treatment industry as they show that a conventional water treatment plant can achieve cost effective MIB and geosmin removal with minimal changes to plant operation.

The results of the laboratory columns demonstrate that the Morgan sand had recovered from the damage caused by the chlorinated backwash. However the biofilm was not as robust as it was prior to the change in backwash regime. Decreases in MIB and geosmin removal were observed when spiking stopped and recommenced for any period of time. It has previously been demonstrated that chlorinated backwash does not always result in complete destruction of drinking water biofilms. In one case, backwashing a biological anthracite-sand filter with chlorinated water resulted in a 22% loss of biomass (as measured by the phospholipid method) (Miltner et al. 1995). This decrease in biomass concentration was accompanied by a decrease in removal of formaldehyde, acetaldehyde, methyl glyoxal and AOC-NOX. Over the course of the filter run time, removal of these compounds increased as the biomass levels recovered. Miltner et al. (1995) also showed that water only backwash of a non-chlorinated biological anthracite-sand filter showed no biomass loss through abrasion. Additionally, Wang et al. (1995) found that a pilot anthracite-sand filter backwashed with chlorinated water had a mean biomass concentration of 10 nmol lipid-P/g media. An identical column backwashed with non-chlorinated water showed a biomass concentration of 55 nmol lipid-P/g media, indicating the losses in biomass associated with chlorinated backwash regimes.

The laboratory study showed that a well established biofilm could remove MIB and geosmin at EBCTs representative of that found in full-scale rapid gravity filtration. However, biofilms that had re-established after damage due to chlorination or autoclaving were not as effective at removing MIB and geosmin at these EBCTs. Sand that was sampled approximately 5 months after the chlorinated backwashing was ceased was able to remove up to 100% of the influent MIB and geosmin at a 15 minute EBCT. However, reduction of the EBCT to 5 minutes resulted in a decrease in MIB and geosmin removals. This suggests that an extended period of time is required...
for biomass within a biological rapid gravity filter to be sufficiently dense and acclimated to perform at filtration rates in the range of rapid gravity filtration.

Columns 2 and 5 provided information on approximate levels of background (or abiotic) removals in the laboratory sand filters. Background losses are those removals of MIB or geosmin through the laboratory columns not caused by biological activity. Observed background removals at 15 minute EBCT were 15% for MIB, and 34% for geosmin.

The ATP assay showed that biomass activity did not give an indication of the MIB and geosmin degradation potential of a biofilm. Biomass activities in the anthracite media sampled after the cessation of chlorinated backwash had higher biomass levels than the sand sampled within the same period. However, both columns showed similar MIB and geosmin removals. It is likely that the biodegradation process is governed by the presence of the specific degrading organisms and the subsequent attachment of these organisms to the surface of the filter media, not by total biomass activities. However, there may be some minimum level of biomass required before biodegradation occurs, as evidenced by the decline in MIB and geosmin removals after chlorination and autoclaving.

The following chapters address questions that arose from this study. In particular, the length of time required for a filter to become capable of MIB and geosmin biodegradation and how these newer biofilms respond to various EBCTs is investigated. Also, it is determined if a system which is not performing adequately for MIB and geosmin removal can be enhanced. Two methods of biofilm enhancement were studied: seeding with MIB and geosmin degrading organisms, and application of pre-ozonation.