I am the author of the thesis entitled **ACID DRAINAGE, LIMNOLOGY AND BIOREMEDIATION OF WESTERN VICTORIAN COAL MINE LAKES** submitted for the degree of **DOCTOR OF PHILOSOPHY**

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Acid Drainage, Limnology and Bioremediation of Western Victorian Coal Mine Lakes

by

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A thesis submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

Deakin University

April 2008
I certify that the thesis entitled: **ACID DRAINAGE, LIMNOLOGY AND BIOREMEDIATION OF WESTERN VICTORIAN COAL MINE LAKES**

submitted for the degree of **Doctor of Philosophy**

is the result of my own work and that where reference is made to the work of others, due acknowledgment is given.

I also certify that any material in the thesis which has been accepted for a degree or diploma by any university or institution is identified in the text.

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(Please Print)

Signed ..............................................

Date .............................................. 3/2/2009
Abstract

In October 2000 a significant fish kill occurred in the Anglesea River estuary, as large volumes of acid water were flushed from the catchment. This event led Alcoa, who operate a brown coal mine and power station in Anglesea, at the bottom of the catchment, to investigate causes of such incidents.

Hydrogeological investigations and modelling showed that acid generation (from sulphide minerals associated with coal deposits) and transport in the catchment is associated with climate and groundwater recharge. Severe acid flush events are likely when years of low groundwater recharge are followed by years of high recharge.

Alcoa’s coal mining at Anglesea will result in a significant void (~ 50 million cubic metres) at the conclusion of the mine’s operation. Following mine closure, an acidic pit lake is likely to be created in the mine void, as water tables recover. The void’s geological and morphological characteristics, combined with the catchment’s naturally occurring acid drainage, are likely to retard the development of a “healthy” lake.

Nearby in Wensleydale, a 40 year old unremediated coal mine lake was studied to provide insights into the evolution of a coal mine lake. Wensleydale coal mine lake (~3000 ML) is still mildly acidic, with a pH ~5.0, and has very low productivity and biodiversity. The primary inhibitors of natural restoration were identified as ongoing acid inputs (adjacent springs have pH ~ 3.0), limited allochthonous inputs of carbon and nutrients, the large relative depth of the lake (mean depth 20.5 m) and limited area of littoral zone (~20% of aerial extent is < 5m depth).

Passive bioremediation of acid lakes, using sulphate reducing bacteria (SRB), is a preferred treatment technique based on environmental and economic criteria, but is
still at the research and development stage internationally. To test the potential of passive bioremediation techniques to improve the water quality in a coal mine lake, such as may be the case at Alcoa’s Anglesea site, 2 sets of bench experiments were followed by a 12 month field limnocorral deployment in the Wensleydale coal mine lake. Six mesocosms (limnocorral) 1.2 m diameter and 16 m long were installed to test the ability of a sediment substrate (a combination of biosolids from wastewater treatment, and chipped green waste) to stimulate SRB bioremediation.

The experiments showed that bioremediation of water quality can be achieved in a lake environment at viable application rates. In the field experiments an application of 2 g/L increased pH from 5 to approximately 6.5 and produced an average alkalinity of 8mg/L.

The limnological study of the Wensleydale coal mine lake has highlighted that water treatment via passive bioremediation techniques is just one aspect of a range of strategies that are applicable when planning for the successful rehabilitation of acidic warm monomictic lakes.
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Tim Tutt
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Region
1 Anglesea, Alcoa and Acid

1.1 Study Area

1.1.1 Anglesea and the Alcoa Coal Mine

Anglesea is located 150 km south west of Melbourne, Australia, on the coast and surrounding the Anglesea River estuary (Figure 1-1). The permanent population is approximately 2500, however as it is a popular weekend, holiday and tourist destination, with the famous Great Ocean Road starting nearby, the summer population increases to approximately 12,000. The Great Ocean Road Region attracts approximately 6.5 million tourists annually (Tourism Victoria 2007), many of whom travel through Anglesea.

Figure 1-1 Location of Anglesea Victoria (Source: www.whereis.com.au)
The Alcoa coal mine and power station, which commenced power production in 1969, is located only 2 km from the town shopping precinct, along the Anglesea River valley (Figure 1-2 and Figure 1-3).

Figure 1-2 Location of the Coal Mine and Power Station and the Anglesea Township. The Anglesea River Catchment is indicated by the dotted line, and the two sub-catchments of Salt and Marshy Creeks are shown. (Source: Australian Topographic Survey Geelong Victoria 1:100 000)
The mining operation extracts approximately 1 million cubic metres of brown coal annually from a 20-30 metre seam, below approximately 30-40 metres of overburden (Holdgate et al. 2001). Properties of the coal are given in Table 1-1.
Table 1-1 Anglesea Brown Coal Quality Characteristics (Holdgate et al. 2001)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-situ moisture content</td>
<td>44-48%</td>
</tr>
<tr>
<td>Specific Energy</td>
<td>13.2 MJ/kg</td>
</tr>
<tr>
<td>Sulphur content</td>
<td>3.8% (dry basis)</td>
</tr>
</tbody>
</table>

The power station withdraws approximately 3000 ML of groundwater from an unconfined aquifer below the power station (the same geological unit as the mine). This extraction plus mine dewatering has developed a cone of groundwater depression in the aquifer around the site, giving a water table lower than sea level (Sinclair Knight Merz 1998).

The Anglesea River catchment extends north westerly from the Anglesea township, and covers an area of approximately 125 km². At the head of the catchment some land has been developed for farming and gravel quarrying activities. Most of the catchment is comprised of pristine, heritage registered heath and woodlands that have remained protected from development by the mining lease itself. The Anglesea catchment has two sub catchments roughly equal in area, in which the tributaries Salt Creek and Marshy Creek intermittently flow (Figure 1-2). Average annual rainfall for Anglesea is approximately 600 mm (Alcoa rainfall data), and the climate is generally characterised by wet winters and hot dry summers (Pope 2006). The El-Nino weather phenomenon affects all of south eastern Australia including Anglesea, and results in unusual seasonal variability, with higher summer rainfalls through thunder storms, and drier autumns and winters.

1.1.2 Project Aims

In October 2000, a major fish kill occurred in the Anglesea River estuary (Pope 2006). The incident was associated with a large flow of acidic water from the catchment into the estuary, significantly altering water quality. The matter was reported in the local media, and both Alcoa (the major development in the
catchment, and suspected cause by some in the community) and local regulatory authorities began investigations into the incident. Monitoring records showed that acidic flows from the catchment frequently occurred, however the investigations could not satisfactorily explain how and why these flows were periodically severe enough to cause fish kills.

Alcoa realised there was a need to better understand the problem, both to determine whether its operations had contributed to the event, and to inform itself and the community of the cause. This instigated the current PhD project and led to the first of its two aims:

i. To determine the cause(s) and future likelihood of severe acid generation and transport events

Given the acid generating potential of the Anglesea catchment a future environmental issue for Alcoa is what will become of the mine void once operations end. Once mining and groundwater extraction cease at the Anglesea mine, the void created (a large portion of which will be below sea level) will fill with water, as the water table returns to pre-mining and power station extraction levels. The lake will be acidic due to acid mine drainage (AMD). This is not a desirable quality from an environmental, social or economic perspective, and lead to the second aim of the current PhD project:

ii. To investigate bioremediation strategies for the acid mine lake that is likely to form in the mine void, once the mine and power station cease operation

Bioremediation of the Anglesea mine lake has the potential to transform an environmental liability for Alcoa into an asset for the local community and greater region. Unfortunately knowledge about how to rehabilitate a whole lake is limited. Although anaerobic bioremediation using organic substrate is an established AMD treatment method (Acid Drainage Technology Initiative 1998; PIRAMID Consortium 2003), there are few examples of its application at a lake scale in Australia or worldwide (Murphy et al. 1999). Kleinmann (1998) outlines that what
is needed for acid mine lake rehabilitation are “...studies on available organics in
the laboratory, followed by mesocosm experiments and studies on the hydrologic
cycles in lakes, i.e. stratification, recharge rates, acidic inputs, etc...” (p. 429).
During the initial laboratory experiment phase of the project, it became apparent
that a key element was missing, in order to comprehensively investigate the
problem. Without a comparable water body to that which would form at Anglesea,
the project was limited to testing bioremediation at a relatively small scale, and
limnological processes could at best be conceptualised based on other studies of
acid mine lakes, and relatively nearby (~100km) volcanic crater lakes (De Deckker

Fortunately an old coal mine operation is located on the inland side of the Otway
Ranges, approximately 20km northwest of Anglesea, near the town of Winchelsea.
The Wensleydale open cut coal mine, now a lake, provided an excellent
opportunity to study the limnological processes and status of a 40 year old, 3000
ML acidic (pH ~4.8 - 5) mine lake. Wensleydale indicates that autochthonous
succession from a young acid lake to a healthy neutral lake in a time frame likely
to be acceptable to the community (eg: less than 10 years) is unlikely. An
understanding of how Wensleydale coal mine lake functions will ultimately help
understanding of how rehabilitation and bioremediation will function. It also
provided a site to conduct field mesocosm (limnocorral) bioremediation
experiments. Lastly, Wensleydale, in its current state, provides a tangible reminder
of what a future mine lake at Anglesea may look like, if research on rehabilitation
and bioremediation of acid mine lakes is not completed.

1.2 Acid Mine Drainage and Pit Lakes

Acid drainage is a term used to describe the acidic water generated by the
oxidation of pyrite (equation i) and related sulphur minerals, when exposed
through mining, or by natural processes such as the occurrence of the coal seam above the regional water table.

\[
\text{FeS}_2 + \frac{15}{4} \text{O}_2 + \frac{7}{2} \text{H}_2\text{O} \rightarrow \text{Fe(OH)}_3 + 2 \text{H}_2\text{SO}_4
\]

Acid Mine Drainage (AMD) is a significant environmental and economic challenge for the mining industry, in Australia and worldwide (International Network for Acid Prevention 2007; Murphy et al. 1999). A 1997 Australian Government report on AMD in Australia (Harries 1997) concluded that 54 sites are managing significant amounts (>10 Mt) of potential acid generating waste, and a further 62 sites are managing a smaller amount (<10 Mt). The cost of managing AMD was estimated to be in the order of $60 million per year. A more recent State of the Environment government report (Hamblin 2001) states that “…acid drainage is one of the most technically challenging problems facing the mining industry.” (p.110).

### 1.3 Combating Acid Mine Drainage

#### 1.3.1 The International Picture

Internationally, resources to address AMD appear to be allocated at various levels, ranging from large scale industry and government programs, to smaller scale initiatives such as the current research project. Some examples of large scale programs include the following:

- The International Network for Acid Prevention (INAP) is an organization of international mining companies dedicated to reducing liabilities associated with sulphide mine materials. It was founded in 1998 to become a global leader in meeting the acid drainage challenge. Its objectives are to facilitate networking and information sharing, technology transfer, and gap-driven research. Examples of its activities include the establishment and ongoing promotion of the International Conference of Acid Rock
Drainage (ICARD), described as the leading acid drainage event, held every 3 years. It also publishes a range of information, from newsletters to detailed technical publications. http://www.inap.com.au/

- In Europe the Passive In-situ Remediation of Acid Mine / Industrial Drainage (PIRAMID) Consortium was established in 2000 to undertake a 3 year technology development and dissemination project on AMD. PIRAMID was an EU wide project comprised of 11 universities across 6 countries with additional resources from a further 6 project subcontractors, made up of consultants, mining companies and institutes. The aim of the project was to produce a set of detailed engineering guidelines for design, construction and operation of Passive In-situ Remediation systems. These have been available on the web since September 2003. http://www.padre.imwa.info/objectives/index.html

- In Canada, the Mine Environment Neutral Drainage (MEND) program is a collaborative effort of mining companies, and both federal and provincial governments to reduce the environmental liabilities of acid drainage. Its budget was $17.5 million over 8 years and during this period the program has reduced the acid drainage liability by at least $400 million. Like the other programs information on all aspects of acid drainage is available from the MEND website, and it also organises regular workshops. http://www.nrcan.gc.ca/mms/canmet-mtb/mmsl-lmsm/mend/default_e.htm

- In America the Acid Drainage Technology Initiative (ADTI), is a government industry joint venture dedicated to the development and use of best science applications to the problem of acid mine drainage. In 1998 the group, through the National Mine Land Reclamation Centre at the West Virginia University, published the “Handbook of Technologies for Avoidance and Remediation of Acid Mine Drainage” (Acid Drainage Technology Initiative 1998). This comprehensive handbook reviews
current technology including passive bioremediation and anaerobic wetlands, as well as describing numerous case studies.  
http://www.unr.edu/mines/adti/  

- In 1994 the Australian Centre for Mine-site Rehabilitation and Research (now the Australian Centre for Mining Environmental Research (ACMER)) was established to be a major provider of technology transfer and collaborative environmental research to the mining industry, including in the area of AMD prevention and remediation. ACMER has run Australian workshops on acid drainage in 1998, 2000 and 2006, and has published the “Manual of Techniques to Quantify Processes Associated with Polluted Effluents from Sulfidic Wastes” (Garvie et al. 2000). In addition to its own work, ACMER promotes information transfer by having links to the above listed programs and their publications on its website. http://www.acmer.uq.edu.au/  

- Recently in Australia, The Centre for Excellence in Sustainable Mining Lakes (CSML) was established in Western Australia, through funding from The Australian Coal Association Research Program, the WA Government, Wesfarmers Premier Coal Ltd and Griffin Coal Mining Company Ltd. CSML is a partnership of Edith Cowan University, Murdoch University, the University of Western Australia and Curtin University of Technology. With funding of approximately $3.4 million over 3 years, CSML identified and developed 5 research areas covering a range of topics from passive anaerobic bioremediation treatment methods to aquaculture potential using mine waters. http://www.csml.curtin.edu.au/about.html  

1.3.2 Bioremediation.  
Modern water treatment utilises living organisms to bring about the destruction or transformation of waste organic and inorganic materials (Sawyer et al. 1994) Its application in treating acid mine drainage has been investigated as a more
sustainable alternate to direct chemical acid neutralisation treatment methods, for example wetland treatment has a low operational cost for long term management (Rose et al. 1998). As Klapper (2002) outlines in regard to succession of lake water quality “what special conditions have to be established...to allow the restoration...by in-situ purification?” (p. 75). The potential for in-situ purification therefore relies on the successful engineering of the right conditions to enhance microbial alkalinity production from anaerobic respiration, which can raise pH in the sediments up to neutrality (Wendt-Pothof et al. 1998).

“Bacteria are the most numerous of living organism on earth” (Tortora et al. 2007, p. 57) and the success of an organism or group of organisms in an ecosystem depends both on nutrient requirements (from carbon to trace minerals) and on tolerance of abiotic conditions (Atlas et al. 1993). The types and ranges of bacteria utilised in bioremediation will therefore vary and be dependant on the environmental conditions encountered or created (e.g. in engineered systems). In designing treatment of AMD, sulphate reducing bacteria are favoured, as sulphate is commonly in abundance. However SRB are by no means the only microbes present in a complex lake environment (Wetzel 2001).

Micro-organisms including bacteria can be categorised by their metabolic characteristics (i.e. the method of energy production and consumption within the organism). In a lake, there are two main environments (aerobic and anaerobic) defined by their oxygen status, and hence two main microbial types, defined by their method of respiration. In the engineered lake bioremediation process, organic additives aim to stimulate the microbial population as described by Langmuir (1997), “When decaying organic matter is introduced into a system that has been isolated from atmospheric oxygen, aerobic decay takes place first, depleting all free O₂, after which, as the Eh drops, anaerobic conditions are established, with NO₃⁻, NO₂⁻, and then SO₄²⁻ reduced” (p. 420).
Biochemical reactions in the different oxygen environments can be represented, where CH₂O represents organic matter, as:

**Respiration and aerobic decay**

\[ \text{CH}_2\text{O} \text{ (glucose) + O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O} \]

**Nitrogen reduction**

\[ \text{NO}_3^- + 2\text{H}^+ + 2\text{CH}_2\text{O} \rightarrow \text{NH}_4^+ + 2\text{CO}_2 + \text{H}_2\text{O} \]

**Sulphate Reduction**

\[ 2\text{CH}_2\text{O} + \text{SO}_4^{2-} + \text{H}^+ \rightarrow 2\text{CO}_2 + \text{HS}^- + 2\text{H}_2\text{O} \]

The microbial and bacterial remediation processes typically follow the general principle of succession in a given environment i.e. the fastest and maximum energy yielding reactions will commence first and be gradually replaced by slower and lower energy yielding processes. As Atlas et al (1993) explain, “from the pool of potential electron acceptors, the microbial community selects the one that maximises energy yield from the available substrate” (p. 339). The sulphate reduction process dominates in an acid mine lake as “nitrate and iron, usually scarce in the aquatic sediments, are rapidly depleted, leaving sulphate as the most oxidising electron acceptor” (Atlas et al. 1993, p. 340).

In the application of bioremediation, resident micro-organisms and bacteria are stimulated by engineering the nutritional and environmental conditions. A range of microbes and bacteria will be stimulated and the environment will initially be very dynamic, as key resources for productivity such as carbon and phosphorous are quickly utilised and may become very scarce (Klapper 2002). In the sediment, sulphate reducing bacteria should predominate and bioremediation (via acid neutralisation) should be ongoing, assuming that a constant or regular supply of substrate (e.g. from decaying algae) is also achieved.
1.3.3 Anaerobic Bioremediation of Acid Mine Drainage

Anaerobic bioremediation was suggested as a treatment method for acid drainage as early as 1969 by Tuttle et al. Research since this time has further developed understanding of the process. The technology has been applied extensively to treatment methods such as anaerobic wetlands and reactors that treat acid drainage before it is discharged to the environment. Knowledge regarding this treatment methodology is now well established as demonstrated by its inclusion in handbooks and detailed engineering guidelines such as the PIRAMID Guidelines (PIRAMID Consortium 2003).

In contrast, the treatment of acidic mining lakes using the anaerobic bioremediation process has had relatively less attention and is an area of ongoing research worldwide. Murphy et al. (1999) stated that for “…bioremediation in a pit lake...insufficient information is available to draw conclusions at present”. (p.13).

A review of relevant literature for this project suggests a number of factors have contributed to this relative lack of knowledge, including:

- the limnology of mining lakes varies significantly throughout the world, according to morphological characteristics, and also the geological, hydrological and climate setting
- the limnology of mine pit lakes is not well understood, as they have few natural counterparts
- the application is to a significantly different environment, compared to treatment of an acid stream flow
- the size and scale of the problem has hindered research and trials of the process
- there are few examples of its application
- treatment of AMD discharges to the environment received more attention
In an early study on anaerobic bioremediation of acidic mining lakes, Davison (1986) trialled a combination of lime and sewage sludge to promote reclamation in a small acid lake with an area of $3.6 \times 10^4$ m$^2$ and a mean depth of 1.5m. He concluded that the sewage acted as an acidity filter by both reducing sulphate, and by limiting oxygen supply to the pyritic sediment materials that were creating acidic groundwater inflows. Schiff and Anderson (1987), while studying the effects of atmospheric acidification on natural fresh water lakes, found that “the organic rich flocculent sediments...neutralise acid at a significantly higher rate than sandy sediments” (p.186). These examples demonstrate the role of organic rich sediment in combating acidity. They also highlight the fundamental rehabilitation problem of mine lakes in general; that they start life with no organic sediment and have limited means to create any, because they are low in productivity.

Brugam et al. (1995) tested the hypothesis that additions of organic matter could accelerate bacterial consumption of acidity. The experiment used 5 polyethylene mesocosms installed in an acid (pH ~ 3) coal mine lake, to which straw was added at varying dose rates. They found that the amount of acid neutralisation was related to the amount of straw added, and concluded that “…added organic matter causes the generation of acid neutralising capacity by sulfate reduction”. (p.158)

Lignite mining in Germany has produced extensive areas affected by AMD and created many mining lakes. The Lausitz region of East Germany alone has an estimated 1300 km$^2$ of area impacted by lignite mines (Charles 1998). It is reported that over 100 km$^2$ of large residual holes are present and that 259 lakes are forming with pH ranging from 2.2 to 3.4 (Lessmann et al. 2003). In 1995 an International Symposium on the “Abatement of Geogenic Acidification in Mining Lakes” was held in Germany. Information presented at the meeting was later collated and published in Geller et al (1998). Pienne and Peiffer (1998) investigated in-lake neutralisation processes in five adjacent lakes ranging in aerial
extent from 11 to 145 ha and from 10 to 45 m deep. Water and sediment testing revealed a lake that had received domestic wastewater "for several years" (p. 49) had recovered and was the least acidic of the lakes. In concluding, they suggested that "...sedimentation of organic matter initiates anaerobic alkalinity generating processes" (p. 61). Furthermore they suggested that an accumulated organic sediment layer would act as a barrier to oxygen-rich water entering the sulphidic aquifer and producing further acidity.

The microbiological aspects of the alkalinity generation process are discussed in detail by Wendt-Pothof and Neu (1998). They note that sulphate reduction is the most widely used approach in the treatment of acid mine drainage, and suggest that for sulphate reduction, a wide variety of organic carbon substrates can be utilised. They also note that other bacteria play a role in the process of degrading complex polymers, thereby consuming oxygen and generating the anaerobic environment and providing the substrate for sulphate reducing bacteria (SRB). The final point made by the authors is that a restoration strategy using organic additions needs to be tested in pilot applications, and that the economic feasibility of such a strategy has not yet been established.

The need for testing and research regarding methods for stimulating a strong anaerobic bioremediation process is further supported by Klapper et al. (1998). They state that "...ecological engineering may help develop mining lakes as new sustainable ecosystems" (p. 413). They also suggest that lakes themselves will be able to supply an ongoing organic load to the sediment for SRB acidity control.

At the 1995 Symposium on the "Abatement of Geogenic Acidification in Mining Lakes" various authors called for the testing of organics to stimulate SRB (Geller et al. 1998). Following this, Fyson et al. (1998) tested acidity removal by controlled eutrophication. Sediment and water were taken from the acidic Lake Koschen (pH 2.9-3.2) in the Lusatia region of Germany and placed in 20 L mesocosms (2.6m long, 10.5 cm diameter polycarbonate tubes) in a laboratory.
Phosphate rock and potatoes were added at various rates, then the water was circulated in the mesocosms for 9 months. The experiment found that a potato dose of 5.1 g (dry weight) increased pH of the water to above 6 and a dose of 34 g (dry weight) increased pH to above 8. Algae were also observed to grow in the mesocosms. Their conclusions were that organic carbon initiates anoxic sediment conditions, leading to a reduction in acidity, while at the same time algae may provide an ongoing organic supply to the sediment in the long term.

In America, Castro et al. (1999) used laboratory (microcosm) testing of various organic waste products available near a former gold mine pit lake, to stimulate SRB and evaluate a full scale application. Of the two organics tested, waste potato peel was found to give better results than composted manure. Based on the experiments, the application of 0.2 g/L of potato waste would treat the mildly acidic (pH ~ 6) 200 ML lake (maximum depth 18m) and create near neutral water quality. The application rate of potato waste would be in the order of 50 dump trucks (20 tonnes) and could be met from the source over some months.

Similar studies of anaerobic bioremediation stimulated by additions of organics have been completed by Gibert et al. (2004), Fauville et al. (2004), Costa and Duarte (2005) and Totsche et al. (2003), specifically for lake applications. Recently in Australia through the Centre for Excellence in Sustainable Mining Lakes, McCulloch et al. (2006b) tested greenwaste and municipal sewage as potential organic sources to stimulate the anaerobic treatment process for application to a pit lake. Following a similar method to Fyson et al. (1998) the pH was increased from 2.4 up to 5.5 in 145 days using a combination of greenwaste and sewage at a dose rate of approximately 45 g/L.
1.3.4 In-situ and Lake Scale Experimentation and Trials

In-situ testing of organic additions to promote anaerobic bioremediation seems not to have been conducted to the extent of laboratory based testing. The practical difficulties of in-situ testing are likely to have limited its application.

In a study similar to Brugam et al. (1995), Koschorreck et al. (2002) installed 2 x 2 x 6.5 m mesocosms into Lake 111 in the Lusatia district of Germany. Packets of straw and straw plus Carbokalk (a by-product of sugar production) were placed on the sediment surface at 6.5m depth. Approximately 1.5 years later sediment cores from the packets were sampled and analysed. The study aimed to define the exact function of straw in the process as it was known to be beneficial from previous studies. The authors concluded that straw acted as a nutrient and carbon source for sulphate reduction in the sediment. However straw itself was felt to be a bad substrate for sulphate reducing bacteria as no colonisation was observed, even though the water inside the package was anoxic.

Another in-situ mesocosm experiment carried out in lake RL 117 (pH 3, $z_{max}=14$ m) in the Lusatia district, tested additions of soda ash, phosphate, and potatoes, both separately and in combination (Totsche et al. 2003). The study aimed to test chemical neutralisation from the soda ash, as well as microbial alkalinity production stimulated by the potatoes and organics (algae) generated by phosphorus stimulated primary production. In summary, the combinations of additives were assessed as being more successful, generating more promising results than an additive on its own.

In Canada, an in-situ mesocosm bioremediation experiment was conducted to reduce metal concentrations in pit lakes (Martin et al. 2003). In this instance acidity was not a significant issue, but the same bioremediation process of sulphate reduction was trialled by additions of ethanol and nutrients. Anoxic conditions were created in the bottom half of 12m deep limnocorrels and testing showed that
zinc and cadmium concentrations were reduced as a result of reacting with the hydrogen sulphide generated from sulphate reduction.

Few full scale lake bioremediation experiments have been conducted. In America the Anchor Hill Pit Lake in South Dakota was created to trial remediation technologies. Lewis et al. (2003) reported on the trials at the 6th International Conference on Acid Rock Drainage (ICARD) in 2003. The pit was approximately 265 ML in volume, rectangular in shape, had an aerial extent of 9.6 hectares, and a floor ranging in depth from 12m to 26m. An initial lime neutralisation process was followed by additions of organics. The initial organic addition was a proprietary mixture, specifically developed by a company for the application. Listed ingredients were methanol, “molasses” (proprietary manufactured product) and phosphoric acid. Approximately one year later, the lake, originally intended to have one organic addition, received its fourth organic addition, second sodium hydroxide addition and first application of woodchips. A number of unforeseen issues were experienced in the trial, such as the practicalities of mixing the large volumes of additions into the lake. Many of these issues would not have been encountered in either a laboratory or an in-situ mesocosm experiment. The importance of providing all microbial needs and not just food (molasses) is highlighted by the addition of woodchips to act as a substratum. The trial experienced at first the hand, the challenges of whole lake remediation, and a number of issues were detailed by the authors. These included:

- Physical profiling should be gathered frequently and if possible, gathered for at least one year prior to treatment
- Organic dosage should be based on recent water analysis
- Appropriate analysis of biological treatment such as bacteria counts should be performed.

At the Island Copper Mine on Vancouver Island Canada, the 400m+ deep mine was flooded with sea water as a strategy for acidity management. In addition Wilton and Lawrence (1998) reported that fish organics were added to stimulate
anaerobic bioremediation at depth. The lake’s unusual limnology (detailed in the following section) and great depth is likely to have inhibited further study of the effects of the organic addition and no further analysis of the bioremediation aspects of the mine has been reported.

1.4 Limnology of Mining Lakes

Lake characteristics and processes vary depending on a range of factors including size, shape, hydrological setting, water quality, climate, and stratification patterns (Wetzel 2001). Limnological processes must be given due consideration when planning a transformation from mine void to mine lake as they will be a major factor in determining what remediation strategies may be appropriate and may help to determine whether they will be successful or not.

The Island Copper Mine in Canada is an excellent example of tailoring AMD management strategies to suit specific environmental conditions. Its dimension, bed rock, and proximity to the sea, led to it being flooded with sea water to create a density isolated hypolimnion, on which a freshwater epilimnion layer lies. This strategy has effectively stopped any further oxidation of pyritic material in the sea water layer (Wilton et al. 1998).

Tasse (2003) found that stratification and mixing processes determined long term water quality of two adjacent stope lakes in Canada. In this instance the lakes became acidic after 30 years. The causes were identified as seasonal density inversions that carried oxygenated surface waters down to the sulphide minerals generating acidity, and exhaustion of carbonate minerals in the mine. Similarly a study of stratification in the Enterprise Pit Lake in tropical Australia suggested that the low pH values measured at depth were possibly due to transport by density entrained inflows of oxygenated water to active sulphide zones in the pit wall (Boland et al. 2002).
In lake remediation trials at the Anchor Hill Pit Lake in South Dakota USA, the importance of density considerations was experienced (Lewis et al. 2003). Two truck loads (43,630 kg) of sodium hydroxide were added to the lake with the intention of removing dissolved aluminium from the water column while at the same time increasing pH to aid bacterial processes. On addition however, the sodium hydroxide (with a specific gravity ~ 1.5) sank directly to the bottom of the lake with minimal mixing, and the anticipated pH rise was not observed. Monitoring of the trial also indicated that the lake was meromictic and it was suggested that this feature could be used as an advantage in the remediation process, as permanent stratification facilitates the maintenance of a permanent anaerobic layer.

1.5 Creating Lake Anglesea with Confidence

Large acidic pit lakes are uncommon in Australia and especially in Victoria, for various reasons, including Australia’s dry climate (Geller et al. 1998) and so knowledge and experience in their bioremediation is limited. This also appears to be the case worldwide. Given this, and the environmental, social and economic benefits that a healthy lake can provide, the need for the research is self evident (McCullough et al. 2006b).

Following the path outlined by Kleinmann (1998) the current research project undertook to answer the key question facing Alcoa regarding the transformation of a coal mine void into a healthy lake. This has involved:

- reviewing the current knowledge and technology on the subject
- laboratory based testing of potentially suitable organic materials to stimulate anaerobic bioremediation and identifying a preferred material or materials and optimum dosage rates
- studying Wensleydale coal mine lake to gain understanding of the limnology of a coal mine lake in southern Victoria,
• field testing the bioremediation process using preferred organic materials in-situ with 18,000 L mesocosms at Wensleydale coal mine lake

1.6 Thesis Outline

The Anglesea catchment geology and hydrogeology is analysed in Chapter 2, and a hypothesis and model explaining the spring 2000 fish kill and acid water draining from the catchment is presented.

The limnological examination of Wensleydale coal mine lake is presented in Chapter 3. This chapter discusses the history of the mine and then focuses on characterising the current lake. Some comparisons are made to morphologically similar lakes within the western district of Victoria, to place the behaviour observed in Wensleydale over two years, into a regional context. At the end of the chapter, the lake’s natural development is discussed and some hypotheses developed to explain its properties. In discussing Wensleydale early in the thesis, the intention is to further demonstrate why research into bioremediation is needed if long term benefits such as fishing and tourism are to be realised at Anglesea.

Two series of laboratory bench experiments, testing readily available organics for their ability to create anaerobic conditions and increase the pH of Anglesea acid mine water, are presented in Chapters 4 and 5. Chapter 6 presents the in-situ field mesocosm experiments and discusses the results while at the same time drawing some general conclusions. Chapter 7 applies the knowledge developed from the experimentation plus concepts developed throughout the course of the research, to a future mine lake scenario at Anglesea, and Chapter 8 summarises conclusions and recommendations from the project.
2 Acid Generation and Transport in the Anglesea Catchment

"The problem of acid drainage, traditionally referred to in Australia as acid mine drainage (AMD) and in North America as acid rock drainage (ARD), encompasses all issues associated with the actual and potential environmental effects of sulphide oxidation resulting from mining activities. Its significant potential for long term environmental degradation makes it one of the biggest environmental issues facing the mining industry" (Environment Australia 1997 p. 3).

Acid drainage is an adverse environmental phenomenon usually associated with mining sulphide rich ore deposits, and the development (usually by draining) of sulphide containing swamps or marshes for agriculture or other human purposes such as housing. Although human intervention is a common cause of AMD, it also occurs "naturally", without anthropogenic influence, and in fact naturally occurring acidic streams have been used as a first indicator of ore deposits by prospectors.

The Anglesea River catchment experiences acid drainage. To what extent the occurrence is natural or anthropogenic (primarily from the coal mine and power station operations at the bottom of the catchment) is unknown. In this chapter the geology, hydrogeology and hydrology is analysed to model acid drainage in the Anglesea Catchment.

2.1 Geology

Sinclair Knight Mertz (1994) found that “…the recent drilling program has highlighted the structural complexity that exists in the region” (p. 36) and reinforced previous studies’ findings, that the region is intensely variable in
subsurface geology (Hancock 1967). Within the complex geology, the primary unit of interest in relation to acid drainage is the Eastern View Formation (EVF). The formation comprises 3 main units - Lower, Middle and Upper. The Upper unit supplies water for Alcoa and contains lignitic deposits including the coal seams which are currently mined. The Upper EVF characteristics described by Sinclair Knight Merz (1994) are:

- inter-bedded grey and grey brown carbonaceous clay, silty clays, silts and fine to medium grained sand sequences, which contain minor fine gravel lenses
- brown coal seams are common towards the top of the sequence
- between Palaeocene or early Eocene in age
- sediments were laid down in fluviolacustrine conditions, with marginal marine influence at the base of the unit
- outcropping over an area of approximately 60 km²

### 2.2 Hydrogeology

The hydrogeology follows the main geological units in the Anglesea Basin, namely the Lower, Middle and Upper Eastern View Formation. The Upper unit is considered to be an unconfined aquifer, the middle unit an aquitard or leaky aquiclade and the lower unit a confined aquifer (Sinclair Knight Merz 1994). Sinclair Knight Merz (1994) estimate the Upper EVF to have an annual recharge of between 4,700 and 6,300 ML, and the Lower EVF to have an annual recharge in the order of 3,400 to 5,600 ML. They describe the properties of the Upper EVF aquifer as follows:

- it outcrops over approximately 60 km² in a zone NW of Anglesea and through the Bald Hills region
- its recharge is predominantly via direct infiltration
- its flow is considered to be down the valley syncline until the vicinity of Alcoa’s groundwater extraction is encountered
• that Alcoa’s large groundwater extraction has created a cone of depression around the mine site in the Upper EVF

A schematic representation of the hydrogeological setting is given in Figure 2-1.
Figure 2-1 Schematic Representation of Hydrogeology of the Anglesea Catchment (Sinclair Knight Merz 1994)
Figure 2-2 Anglesea Catchment showing approximate locations of bores drilled for Sinclair Knight Merz (1994) investigations of the hydrogeology.
From the 11 bores with lithology logs, the number of metres that a coal unit or a unit where coal is mentioned above the water tables was calculated (Table 2-1).

Table 2-1 Thickness of lithological units above water table that are listed as "coal" or "heterogenous units" mentioning coal

<table>
<thead>
<tr>
<th>Bore Number</th>
<th>Thickness of coal or heterogenous units mentioning coal (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B113002</td>
<td>24</td>
</tr>
<tr>
<td>B113003</td>
<td>18</td>
</tr>
<tr>
<td>B113004</td>
<td>18</td>
</tr>
<tr>
<td>B113470</td>
<td>51</td>
</tr>
<tr>
<td>B115867</td>
<td>12</td>
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<tr>
<td>B115868</td>
<td>37.4</td>
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<tr>
<td>B116458</td>
<td>42</td>
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<tr>
<td>B116460</td>
<td>40</td>
</tr>
<tr>
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<tr>
<td>B119349</td>
<td>0</td>
</tr>
<tr>
<td>B121768</td>
<td>70</td>
</tr>
</tbody>
</table>

Following the acid base accounting methods set out in Garvie et al. (2000), the acid potential (AP) at each of the 11 bores sites can be approximated by equation (iii), where AP is the acid potential and S is the sulphur content as a percentage:

\[
AP \text{ (kg H}_2\text{SO}_4/\text{tonne)} = 30.6 \times S \% \text{..........(iii)}
\]

Equation (iii), based on the stoichiometry of Equation (ii), states that for every 1 percent of sulphur in a tonne of material, 30.6 kg of sulphuric acid could be generated.

Given the amount of coal and lignitic units above the water table throughout the catchment (Table 2-1) the catchment has acid generating potential. The sulphur content (approximately 3.8%) in the coal mined at Alcoa’s open cut mine, and the
acidic water measured in the catchment (Hancock 1967; Hermon 2002; Pope 2006) strengthens the case for the catchment being naturally acid-generating.

2.4 Acid drainage and transportation

The hydrogeological system contributes to acid generation and transport in three ways:

- fluctuating water tables - dropping water tables introduce air into moist pore spaces of the unsaturated zone
- dissolved oxygen can be transported to pyrite by groundwater recharge
- groundwater flow, specifically in the vadose zone, transports any acid generated

These factors are schematically shown in Figure 2-3.
At Anglesea the Upper EVF aquifer has a complex stratigraphy, ranging from porous units bearing gravels and sands, to lignitic clays (Sinclair Knight Merz 1994). The hydrological cycle and seasonal groundwater processes for the Upper EVF aquifer and Anglesea catchment are schematically shown in Figure 2-4 and Figure 2-5 respectively.
The key processes of the cycle which directly influence acid generation and transport in the Anglesea catchment are precipitation, infiltration, percolation and groundwater to lakes and streams.
As depicted in Figure 2-5, some acid generation and transportation should theoretically occur every year. Over winter and spring, water infiltrates and percolates into the vadose zone oxidising pyrite and transporting acidic water down gradient. This water can either discharge as a spring flow to the surface, or mix with the existing groundwater.

Seasonal rainfall therefore directly influences the rate of acid generation and transportation. Variation to normal weather patterns, including extended dry periods, which may drop the water table regionally, will affect acid generation and transportation, potentially creating scenarios such as:

- low groundwater recharge, causing reduced generation and transportation of acid in the systems, and a potential build up of acid in pore spaces
high groundwater recharge, causing greater oxidation of pyrite plus increased transportation as percolating groundwater flushes pore water from the vadose zone

The proposed acid flush model can be semi-quantitatively tested using water balance equations. Anglesea climate data was used to model a catchment water balance with a focus on the spring 2000 acid flush event which caused a fish kill and closure of the estuary to recreation activities.

2.5 Analysis of Groundwater Recharge, Acid Generation and Transport

2.5.1 Rainfall Analysis

Monthly rainfall has been collated for the years 1990-2003 from Alcoa's records (Table 2-2). The years leading up to the acid flush and fish kill event in spring 2000 have been highlighted in bold and italics. The shaded cells indicate months when rainfall was higher than potential evapotranspiration.

Evapotranspiration (ET) describes water returning to the atmosphere from the two distinct processes of evaporation and transpiration, and is a key component of a water balance model. The figures presented for areal potential evapotranspiration were interpreted from Bureau of Meteorology (BOM) maps published in 2001, based on 30 years of climatic data. Explanatory notes on evapotranspiration from the BOM state that “...ET is a large component of the water balance; 90 per cent of the precipitation that falls on the continent is returned through ET to the atmosphere. However, despite its importance, ET is almost impossible to measure or observe directly at a meaningful scale in space or time” (BOM website). Areal Potential ET is defined in the BOM’s notes as “…the ET that would take place, if there was a unlimited water supply, from an area so large that the effects of any
upwind boundary transitions are negligible and local variations are integrated to an areal average” (BOM website).

Table 2-2 Monthly rainfall (mm) for years 1990 – 2004, Areal potential evapotranspiration and average and 90th percentile rainfall figures for the years 1968-2004

<table>
<thead>
<tr>
<th>Year</th>
<th>JAN</th>
<th>FEB</th>
<th>MAR</th>
<th>APR</th>
<th>MAY</th>
<th>JUN</th>
<th>JUL</th>
<th>AUG</th>
<th>SEP</th>
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<th>NOV</th>
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<td>33</td>
<td>30</td>
<td>78</td>
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<td>73</td>
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<td>68</td>
<td>71</td>
<td>56</td>
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<td>90%ile</td>
<td>91</td>
<td>122</td>
<td>91</td>
<td>125</td>
<td>121</td>
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<td>102</td>
<td>112</td>
<td>125</td>
<td>123</td>
<td>105</td>
<td>103</td>
<td>838</td>
</tr>
</tbody>
</table>

ET = Areal Potential ET (mm), Ave = Average, 90%ile = 90th percentile rainfall

In the 3 years prior to 2000, rainfall was well below average (670mm) - 1997 had 481mm, 1998 had 580mm and 1999 had 540mm. Analysis of monthly rainfall for the winter and spring of these years show that 9 out of 18 winter months (May to October) failed to record rainfall greater than areal potential evapotranspiration. This suggests that little or no groundwater recharge occurred. In 2000 however, rainfall in May, July, and October exceeded 90th percentile values and the other months (June, August and September) all had rainfalls that exceeded theoretical losses to evapotranspiration. The monthly rainfall data suggests 1997 to 2000 was
a dry period which ended in an unusually wet winter, and unusual rainfall patterns in spring.

2.5.2 **Ground Water Recharge - Water Balance**

Groundwater recharge occurs when a portion of precipitation infiltrates into the ground. A water balance equation is a type of mass balance equation used to measure inputs and outputs of a defined system or model unit. A water balance model (Figure 2-6) for the Upper EVF was developed using the following parameters:

- \( R = \) Rainfall
- \( A = \) area of recharge (area of outcropping upper EVF)
- \( E = \) Evaporation
- \( T = \) Transpiration – water used and or returned to the atmosphere by plants
- \( ET = \) Evapotranspiration (the combination of E&T)
- \( SR = \) surface run-off
- \( GD = \) Upper EVF aquifer outputs, where GDA = discharge to other aquifers or boundaries of the model eg the ocean, and GDS = surface waters, eg marshes and rivers
- \( GR = \) Upper EVF aquifer inputs, where GRA = recharge from other aquifers or boundaries of the model, eg northern plateau, or lower/middle EVF, and GRS = recharge from surface water eg marshes and rivers
- \( Ex = \) human extraction
Data for all the variables in the equation are not available for the EVF, and are unlikely to be obtainable with any confidence due to the complex nature of the geology described previously, as well as the difficulties of quantifying some of the parameters such as flow interactions between aquifers. To simplify the equation, the inputs of GD and GR were assumed to balance each other. This approach, although not ideal, is practical in this application as it is not possible to quantify these components.

The water balance equation (iv) is:

$$\Delta V \text{ (Upper EVF Aquifer)} = (R-ET-SR).A - Ex \quad ...(iv)$$

Other model limitations exist. For examples the proportions of surface run-off versus groundwater recharge is dependant on a number of factors including rainfall intensity, land use and initial percentage of soil saturation and cannot be inferred from historical monthly rainfall data. An approach commonly used is to estimate a percentage of rainfall which contributes to groundwater recharge (Schwartz et al. 2003; Sinclair Knight Merz 1994). In this application, rainfall less evapotranspiration has been called “available” rain for recharge or run-off. Of the
available rain, fifty percent was assumed to be contributing to groundwater recharge (Figure 2-7). By adopting these figures annual recharge volumes were generated that were comparable to those calculated by Sinclair Knight Merz (1994), which were 4700 to 6300 ML.

Figure 2-7 Rainfall (mm) contributing to recharge for the period 1968 to 2003

Based on the model assumptions so far, three successive years (1997, 1998 and 1999) of low recharge occurred, the only one in the data displayed. Figure 2-7 also shows that 2000 had the 3rd largest annual recharge rainfall from 1968 to 2003.

The volume of recharge is calculated by multiplying the recharge rainfall by the area of the aquifer catchment. The latter was previously calculated as between 45-60 km² (Sinclair Knight Merz 1994). The range is due to allowances being made for non-contributing areas, such as areas of clayey soil outcrops that have little or no infiltration. For this water balance the upper estimate of area (60km²) has been adopted. Lastly, Alcoa’s annual extraction from the upper EVF has been inputted into equation (iv) to complete an annual water balance. A summary of annual
water balance volumes, from 1968 to 2003, are presented in Table 2-3 and Figure 2-8.

Table 2-3 Upper Eastern View Formation annual water balances, assuming a surface area of 60km² for the catchment

<table>
<thead>
<tr>
<th>Year</th>
<th>Recharge (ML)</th>
<th>Alcoa (ML)</th>
<th>Balance (ML)</th>
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<tbody>
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<td>3336</td>
<td>5969</td>
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</tr>
<tr>
<td>1969</td>
<td>1370</td>
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</tr>
<tr>
<td>1970</td>
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</tr>
<tr>
<td>1971</td>
<td>2699</td>
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</tr>
<tr>
<td>1972</td>
<td>846</td>
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<tr>
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</table>
Table 2-3 and Figure 2-8 show that:

- The 3 years 1997, 1998, 1999 experienced very low groundwater recharge and when combined with Alcoa’s extraction produced a net withdrawal from the upper EVF system.

- 2000 was a high recharge year (the 3rd highest since 1968), and when combined with a low annual Alcoa extraction was in fact the largest net recharge year in the data set.

- Other low recharge years, in 1982 and 1994, were directly followed by good recharge years, in 1983 and 1995 respectively.

- Four out of the first five (1968-73) years following the commencement of Alcoa’s mining operations saw a net extraction of groundwater, and resulted in the establishment of the cone of depression.
When considered in conjunction with the hypothesized process of acid drainage from the catchment, the water balance model does highlight the 1997-2000 period. It suggests little groundwater recharge occurred in 1997-1999 which is likely to have lead to an accumulation of acid in the system. In 2000, greater than average recharge occurred, producing a larger than usual spring flow into the Anglesea Catchment system and ultimately, into the Anglesea estuary. The scale of recharge in 2000 is made more distinct when compared to other similar periods of negative followed by positive water balances. 1982 showed a negative balance of 2027 ML, followed by a positive balance of 3878 ML in 1983. Although 1982’s negative balance is larger than the sum of the 3 negative balances for the period 1997, 1998 and 1999, this aspect of the model is only a precursor to an acid flush event. The recharge in 2000 was 5825 ML where as in 1983 it was 3878 ML, and this difference of almost 2000 ML (or 50% larger again), suggests that recharge volume may be the critical factor, or trigger point for an acid flush event.

The model presented, linking the source of the acidity (the coal’s pyritic compounds), with the hydrological cycle that in turn controls the rate of acid generation and its transportation, has been supported by water balance modelling. In spring 2000 the volume of acidic water entering the estuary was greater than most years, as indicated at the time by the fish deaths, and later in this study’s water balance model. More information regarding the water quality and ecotoxological aspects of the 2000 event, including high metal (aluminium ~ 89 mg/L, zinc 1.6 mg/L and iron 88.5 mg/L) concentrations due to acidity, that later precipitated out in the estuary, can be found in Pope (2006).
2.6 Analysis of Alcoa’s Mining Operations’ Influence on Acid Drainage, and Conclusions

Alcoa’s mining operations were postulated to have contributed to the 2000 fish kill. A number of factors suggest that Alcoa’s operations probably had little effect on the overall acid flush event. The area of the cone of groundwater depression, associated with the coal mine and power station extraction is conservatively estimated at 6 km$^2$ and it is located at the bottom of the catchment. Acidic water, some with a pH of 3, has been measured throughout the catchment at various times (Hancock 1967; Pope 2006), well beyond the cone of groundwater depression.

Acid water generated in the cone of groundwater depression (i.e.: the area directly influenced by the mine and extraction bores) should flow down gradient and theoretically be transported, into the mine or extracted by the groundwater bores. The aquifer at this point is approximately 50m below sea level and such water could not be expected to contribute to surface waters.

Some factors not specifically addressed in this chapter, that may also influence acidification are:

- The acid generating capacity of the marshes in the catchment (although given that they comprise only approximately 2% of the catchment area, the acid potential of the marshes is likely to be low, relative to the catchment as a whole)

- The effect of sand and gravel mining in the upper catchment, which may increase infiltration rates of both oxygen and water to coal that may be underlying the sand and gravel deposits. The works cover an area of approximately 60 hectares in comparison to 212 hectares of open cut mining area.
A seasonal water balance model has been used to explain the 2000 estuary acidification and fish kill event. The model is relatively simple, but provides a feasible explanation of acid drainage from the catchment. Use of seasonal water balances may provide the ability to predict potential acidification events in the estuary in future. The year 2000 and those preceding it could be used as a reference point, however a critical or trigger point for large acid flows from the catchment is still largely unknown.
3 Wensleydale - Limnological Examination

The Wensleydale coal mine lake has provided an excellent opportunity to study natural, “unmanaged” acidic mine lake evolution. The presence and history of Wensleydale has been fortuitous, in the sense that it has been possible to study the limnological processes of an acid lake in south west Victoria, after 40 years of predominantly autochthonous production. Kalin and Geller (1998) comment that “…no long term investigation (covering decades) is known for any mining lake. Whether young acid lakes become neutral by internal processes is still an open question … there are many examples of 40 year old acid lakes where no change had occurred” (p. 424).

The current limnological study of Wensleydale, conducted from July 2004 to November 2006, has also provided a sound basis for the prediction and management of acidity issues at a future Anglesea mine lake. The suitability of organic sediment bioremediation to treat acidity was also able to be tested at a field-trial scale at Wensleydale.

3.1 History and Background

The Wensleydale coal mine lake is located at the base of the north eastern end of the Otway Ranges, and is accessed from the road running between Winchelsea and Bambra (Figure 3-1). The mine lake is located in a gully approximately 150m above sea level, and 70 m above the level of the volcanic plains to the north. The creek’s catchment of approximately 1200 ha, stretches southwest into the Otway Ranges, and comprises both forested and cleared land of medium relief (Yttrup et al. 1995).
Coal was discovered at the site in 1914 when bores were sunk in the creek bed in search of groundwater. Further investigations estimated a coal reserve of 4,000,000 tons overlaid by a relatively thin overburden of 4.5m (15ft). Mining commenced in the early 1920’s almost 10 years after the coal was discovered (Houghton 1982). To access the coal, a coffer dam and two water diversion pipelines were constructed upstream of the mine site to divert creek flows around the mine. Overburden was deposited upstream of the mine, forming a considerable barrier to the original creek path (Figure 3-2). No records of water regime or quality at the time of the mine’s operations have been located.

Coal mining at Wensleydale ceased in the early 1960s, and very little historical information exists regarding mine operations and the 20 year period following. From the present condition of the site it is likely that little if any rehabilitation works occurred once operations ceased, and the mine void began to fill with water.
In 1985 some rehabilitation work was carried out by the current land owner (Figure 3-3). These works included:

- raising the level of the coal mine lake, by increasing the height of the earth bank at the lake outlet (the gully) to increase its recreational value
- constructing a channel between the upper coffer dam and the mine lake to allow safer passage of flood water (Yttrup et al. 1995), as the original diversion pipelines were no longer functional.

Since this time no further works have been carried out. Erosion of the overburden and mine walls continues, whilst revegetation is slowly occurring naturally. Water skiing is a popular lake activity over the summer months. The present owner has occasionally released fish into the lake in an attempt to stock it for recreational fishing. He reports this has not been successful.

Figure 3-2 – Aerial photo of Wensleydale Lake (tan colour) and surrounds. Coffer dam (olive colour) to the south, and overburden (white areas) in between the two.
Figure 3-3 Photos of Wensleydale Coal Mine Lake

a) Coffer Dam as seen from overburden pile, upstream of lake.
b) Gully (channel) connecting the coffer dam to the lake, created by water blasting in 1985.
c) Water course into the lake from the gully
d) Littoral Macrophytes
e) Lake from the overburden pile (looking north)
f) Beach at the southern end of the lake
3.2 Geology

Wensleydale is located at the north eastern end of the Otway Ranges. Formation of the Wensleydale coal seams took place during the Lower Nothofagidites asperus spore-pollen Zone, in the Middle Eocene, approximately 45 million years ago (Holdgate 2003).

Wensleydale coal quality (Holdgate 2003) is:
- moisture 50 - 55 %
- ash 1.6 - 4.3 % (dry weight basis)
- Calorific Value 23.7 - 25.8 ( mega joules /kilogram)
- Sulphur 1.2 - 2.7 % (dry weight basis)

The sulphur content of the coal is the source of acid drainage. At Wensleydale there are no significant areas of marsh above the mine that could be a potential acid source, as is the case at Anglesea.

3.3 Climate

3.3.1 Rainfall and Evaporation

Wensleydale has a similar climate to that of Anglesea, characterised by hot summers with low rainfall, and cool winters in which the majority of the average annual rainfall (737 mm) occurs (Bureau of Meteorology 2006). Colac Weather Station (approximately 30km west of Wensleydale) records have been used, as it is the nearest station to Wensleydale, on the northern side of the Otway Ranges.
Figure 3-4 Mean Daily Minimum (pink) and Maximum (blue) Temperature for Colac Weather Station

Figure 3-5 Monthly Rainfall – Observed (August 2004 – May 2006) and Long Term Averages for Colac Weather Station
The rainfall for the period August 2004 - August 2006 was 964.6 mm - 62% of the average rain total of 1561.6 mm. Of the 25 months in this time period, only 3 months (January and February 2005 and January 2006) recorded rainfall greater than long term averages. In February 2005 a summer thunderstorm delivered approximately 50 mm of rain in one day. Two distinct dry periods occurred in autumn 2005 and winter 2006.

Evaporation rates are highest over the summer months. Measured pan evaporation from the nearby Wurdiboluc Reservoir (~ 5km north) from October 2004 to March 2005 was 928 mm (Bureau of Meteorology 2006). This is the only published data for evaporation. The data is for the maximum evaporation period over the spring and summer months, and was probably collected to calculate water loss due to evaporation for the reservoir. It does show that over summer, almost 1m of evaporation occurs.

### 3.3.2 Wind

The maximum wind speeds for each month between August 2004 - May 2006 recorded at the Colac Weather Station are presented in Figure 3-6.

The mine lake is situated in a creek gully and is protected from winds from all directions by the surrounding well-vegetated hills, however the data shows that wind speeds of over 100 km/h are experienced in the district.
3.4 Wensleydale Acid Drainage

Mining occurred at Wensleydale before the importance of environmental protection was accepted. Acid mine drainage (AMD) from spoil heaps and unmined coal continues to effect the lake. A number of acidic springs (pH ~ 3) on the western lake bank discharge into the lake (Figure 3-7). After 40 years of unassisted or “natural” remediation, the lake is still mildly acidic, with a pH ~ 4.9.
No formal investigation has been completed into AMD at Wensleydale. Pyritic compounds in the coal are oxidised when exposed through mining, or by natural processes such as the occurrence of the coal seam above the regional water table. The other source of acidity identified at Wensleydale is generated from the oxidation of iron rich ground water (equation v).

$$4\text{Fe}^{2+}(\text{aq}) + 10\text{H}_2\text{O}(l) + \text{O}_2(\text{aq}) \leftrightarrow 4\text{Fe(OH)}_3(s) + 8\text{H}^+(\text{aq})$$

The upstream coffer dam does not appear to be affected by AMD, as it has a pH of ~ 6.5. The dam is significantly different from the mine lake - it is smaller and shallower, and has extensive littoral and emergent vegetation. The reasons it is not acidic include:

- it is located above the main coal seam and other sources of acid, such as overburden
- inflows of non acidic water from the catchment flush it efficiently
- its extensive littoral and emergent vegetation provide natural buffering processes
The coffer dam may also have played a role in keeping the mine lake acidic and oligotrophic in nature. The dam has acted as a trap for allochthonous matter, preventing the inflow of nutrients such as carbon, nitrogen and phosphorus to the mine lake. When water does travel from the coffer dam to the mine lake it is further filtered as it travels either through or over the mixture of gravel, sand, coal, silt and clay that comprises the overburden heaps.

### 3.5 Wensleydale Characteristics

#### 3.5.1 Methods

Monthly field trips to Wensleydale commenced in July 2004 and ended in earnest when the mesocosm experiment was dismantled and retrieved from the lake in November 2006. From July 2004 - February 2006 the lake’s physical profile and water samples were surveyed from approximately the middle of the lake. In August 2005 the mesocosm experiments commenced at the northern end of the lake (see Chapter 6), and physical profiling of the mesocosms and the lake adjacent to the mesocosms (the lake depth at this point is approximately 22m) was completed monthly until August 2006. Details of the methods used for physical profiling of the water column, water sampling and testing, phytoplankton and zooplankton sampling and analysis are presented in Section 6.5 or described in conjunction with data presented i.e. bathymetric mapping, sediment sampling and light profiling.
3.5.2 Morphometric Characteristics - Physical Dimensions

The morphometric characteristics of the lake (Table 3-1) have been calculated according to Wetzel (2001).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Length (m)</td>
<td>510</td>
<td>Middle of long axis</td>
</tr>
<tr>
<td>Maximum Width (m)</td>
<td>260</td>
<td>At the north east end</td>
</tr>
<tr>
<td>Surface Area (m$^2$)</td>
<td>$1.2 \times 10^5$</td>
<td>~ mean length $\times$ ~ mean width</td>
</tr>
<tr>
<td>Volume (m$^3$)</td>
<td>$2.46 \times 10^6$</td>
<td>Area $\times$ ~ mean depth</td>
</tr>
<tr>
<td>Maximum Depth (m)</td>
<td>38</td>
<td>greatest depth of the lake</td>
</tr>
<tr>
<td>Relative Depth (%)</td>
<td>9.72</td>
<td>Ratio maximum depth / mean diameter</td>
</tr>
<tr>
<td>Mean Depth (m)</td>
<td>20.5</td>
<td>Volume / Surface Area</td>
</tr>
</tbody>
</table>

A bathymetric map (Figure 3-8) and cross-sectional profiles (Figure 3-9) of the lake were developed using standard software (Surfer and Excel) from depth measurements made with an echo sounder (ROYAL Depth and Fish Recorder Model No. RF – 160R).
Excluding the 35m contour, which indicates the lake floor, the map shows small circular isolated contour lines, which are likely to be a product of the contouring software, and should be disregarded.
Figure 3-9 Cross Section Profile of Wensleydale along its longest axis (SW-NE).

The bathymetric map and cross section profile show a profile that is typical of mining lakes, and that is not dissimilar to the western district's volcanic crater lakes, such as Bullen Merri and Purrumbete (Herpich 2002; Mitchell et al. 1987). The walls are steep (ranging from 15 to 25 degrees) and the bottom is relatively flat. In the middle of the lake is a raised mound or spur from the east wall. This mound is approximately 10m high, relative to the lake floor and could be an access roadway or an embankment for the overhead cableway which transported coal in a north easterly direction to the old Wensleydale rail line.

Hypsographic and Depth-Volume Curves (Wetzel 2001) show the relationship of lake depth to its area and volume (Figure 3-10).
Lake productivity generally has an inverse relationship to lake depth, based upon the associated factors of volume to sediment area and volume to littoral zones (Mitchell et al. 1987; Wetzel 2001). It is in the shallow water productivity zones that nutrient recycling from the sediment to the water column is the most effective and where biological littoral activity can have significant input into the lake ecosystem. Over 80% of Wensleydale coal mine lake volume is found below 5m depth and 80% of its floor area also lies below the 5m depth (Figure 3-10). This is similar to Lake Purrumbete, a volcanic crater lake (~100km west of Wensleydale) that has approximately 80% of its total volume and approximately 90% of its aerial extent, below the 5m depth (Mitchell et al. 1987). Such lakes lack extensive areas of productive emergent or submerged macrophytes.

3.5.3 Lake Hydraulics

The creek flowing through Wensleydale has a catchment area of approximately 1200 ha, comprising both forested and cleared land of medium relief (NATMAP 1977; Yttrup et al. 1995). The creek flows into the coffer dam through a wetland area, and water leaves the coffer dam by an overflow outlet that discharges into the channel cut through the overburden pile. Creek flow into the lake from the channel.
is across the beach that has formed between the overburden and the lake (refer to photos in Figure 3-3c).

Flows in the creek have not been quantified, either previously or in this study. The mine lake water inputs include direct precipitation, surface water inflow from the creek, adjacent AMD spring inflow, and groundwater inflow. Outputs from the lake include evaporation, evapotranspiration by macrophytes and riparian vegetation which includes large trees, outflow to the creek, outflow to groundwater and human extraction (pumping). Observations made during the study indicate that lake levels remained fairly constant, fluctuating no more than approximately 30 cm.

Other hydrological observations include:

- the acidic springs on the west side of the lake, appear to flow all year round
- the inlet water course to the mine generally only flows after significant rain
- the inlet water course when flowing can range in pH from neutral to acidic indicating a number of sources of water can make up the inflow
- outflow is via overflow into two 750mm diameter concrete pipes set in the constructed earthen embankment at the northern end of the lake. The embankment is also a spillway, but the owner reports no overtopping of the embankment has occurred since 1985
- outflow occurs at times of significant inflow (usually in winter)
- reductions in lake level occur in summer when evaporation and extraction are greatest

While a detailed water balance for Wensleydale was beyond the scope of this study, the relatively constant water level in the lake indicates that the mine lake has substantial subsurface inputs. This conclusion is supported by electrical
conductivity monitoring over the study period which is further discussed in Section 3.5.5.

### 3.5.4 Thermal Stratification

Between July 2004 and July 2006 the lake was stratified for at least 10 months of the year. In this period the thermocline followed a seasonal pattern, establishing as water temperatures increased over spring, reaching its peak in the height of summer and then slowly breaking down over autumn. Mixing or destratification occurred in June or July, and water remained mixed into August (Figure 3-11, 3-12). The epilimnion reached a maximum temperature of \(\sim 22 \, ^{\circ}\text{C}\) in January, while the hypolimnion, below 12m depth, remained at 9-11 \(^{\circ}\text{C}\) for the entire period.

**Figure 3-11 Mid-lake water temperature profile of Wensleydale, July 2004 – December 2005**
Thermal stratification is the dominant physical process that drives many of the other environmental conditions of a lake. The stratification pattern is similar to that of volcanic lakes in Victoria's western district with similar morphological characteristics. These include Lake Bullen Merri, which has a maximum depth ($z_m$) of 66m, Lake Gnotuk ($z_m = 18.5$m) and Lake Purrumbete ($z_m = 45$m) (De Deckker et al. 1988; Herpich 2002; Mitchell et al. 1987). In volcanic lakes thermal stratification develops in spring and remains until late May-June. These studies confirm that deep lakes (>15m) in Western Victoria undergo an annual stratification cycle. It is predicted that a future lake at Anglesea mine would behave similarly.

Wind speeds in the region can reach over 100 km/h. The lake's largest fetch is approximately 500m and strong winds of the magnitude recorded in the region have the potential to cause some wave action and turbulence leading to water
column mixing. Because Wensleydale is located in a protected position and the lake has a relatively small surface area, the extent of wind disturbances on stratification would not be as significant, compared to regional volcanic lakes. In fact stratification monitoring does not show any significant disruption to the metalimnion curve during the monitoring period, indicating that wind-generated turbulence generally does not affect stratification at Wensleydale. In comparison, Lake Purrumbete (maximum length 3.21 km) experiences much more significant wind disturbance, leading to erosion of the thermocline and the development of multiple thermoclines (Mitchell et al. 1987). Herpich (2002) also observed fluctuations in Purrumbete's thermocline depths and the development of multiple thermoclines in January 1996. A stable hypolimnion that is not re-oxygenated with wind generated turbulence is preferable for a bioremediation process.

The stratification pattern observed in Wensleydale, is described as warm monomictic (Wetzel 2001) with minimum temperatures of ~9 °C observed in winter. This stratification pattern and temperature profile is favourable for an organic sediment bioremediation process, as for 10 months, oxygen flow to the sediment is limited and temperatures, although not optimal, are suitable for SRB activity (Zehnder 1988).

### 3.5.5 Electrical Conductivity (EC) Profiles

Water in the Wensleydale coal mine lake is relatively fresh, although an increase in the EC below the thermocline from ~600 µS/cm to ~750 µS/cm was measured in the period December 2004 to December 2005 (Figure 3-13).
The lake’s EC is determined by the relative portions and quality of water inputs and outputs, as outlined in section 3.5.2. Figure 3-13 shows a rise in EC at the very bottom of the lake, indicating likely inflow of more saline groundwater. Summer evaporation increases the epilimnion EC. Rainfall for 2005 was 499 mm, considerably less than the annual average of 737 mm and the western district did not receive significant winter rainfall to generate substantial run-off in 2005. Mitchell and Collins (1987) found evaporation and rainfall to be the main determinants of conductivity in Lake Purrumbete which ranged from 600 to 800 μS/cm. Given these observations, the increase in Wensleydale EC in general can be attributed to the balance between groundwater inflow, limited surface inflow and evaporation.

Converting Wensleydale EC data to total dissolved salts (TDS) concentration gives a lake range of approximately 0.36 to 0.45 g/L (using a conversion factor of 0.6, assuming the major ions are present in the same proportion as in seawater) which classifies the lake as freshwater (Wetzel 2001). Of the larger western
district lakes, only Lake Purrumbete is comparable, having a TDS range of 0.3 - 0.4 g/L, with Lake Bullen Merri (8 g/L) and Lake Gnotuk (55 g/L) being more saline (De Deckker et al. 1988).

**3.5.6 Dissolved Oxygen Profiles**

The surface layer remained well oxygenated between December 2004 - December 2005 while the hypolimnion oxygen levels reduced to approximately 60 - 80% saturated (Figure 3-14).

Figure 3-14 Dissolved oxygen (% saturation) profile in the centre of the Wensleydale coal mine lake, December 2004 - December 2005

In January 2005 anoxic conditions were detected at the sediment-water interface. In comparison, a more productive lake such as Lake Purrumbete, experiences severe hypolimnetic deoxygenation to the point of anoxia below the thermocline over late summer and autumn (Herpich 2002; Mitchell et al. 1987). Anoxic conditions or significant decreases in oxygen levels were never detected in the
hypolimnion at Wensleydale, and indicate little oxygen consuming activity such as microbial consumption in the remineralisation of organic matter.

The detection of very low oxygen levels at the water-sediment interface could indicate one or more of the following processes:

- oxygen is being consumed by microbial remineralisation of organic matter at the sediment interface
- the inflow of groundwater, which could have little to no dissolved oxygen, is being detected
- dissolved iron (as Fe$^{2+}$) in the groundwater is oxidising and depleting oxygen from the water (as described in section 3.4)

Evidence such as a small sediment thickness, low sediment volatile organic content and low water column chlorophyll a concentrations, does not support the hypothesis that the drop in oxygen concentration is attributable to organic matter remineralisation. As indicated by the EC results it is more likely that the inflow of groundwater to the lake is the cause of the low DO measurements at the sediment-water interface.

The lake’s low DO at the sediment, and reduction in hypolimnion DO over the stratification period is favourable for an anaerobic bioremediation sediment treatment, as outlined in sections 1.3.1 and 1.3.2.

Dissolved oxygen concentrations (in mg/L) at 5 and 20m depth, for the period December 2004 - February 2006, are presented in Figure 3-15.
Surface DO concentrations show the relationship between temperature and gas solubility. As temperatures increase, solubility decreases, and the decrease in DO concentrations in the summer period, from December to February, is clearly evident. In the hypolimnion however temperature remained more constant, and variations in DO concentration resulted from consumption of oxygen (as outlined) and no replenishment of oxygen across the thermocline. Figure 3-15 also clearly shows that as surface waters began to cool over autumn and winter, DO increased in the epilimnion while the hypolimnion DO, isolated by the thermocline, did not. It was not until destratification in July, that DO at 20m increased significantly.

### 3.5.7 pH Profiles

The pH profiles (Figure 3-16) show that the bulk of the water body, below 10m depth, remained at a relatively constant pH, shifting only ~ 0.6 pH units over the monitoring period. In contrast, the surface water experienced greater variation in pH, ranging from 4.6 during destratification and increasing to a near neutral pH of
~ 6.4. The observed rapid increase in pH, over 1 month, followed a high rainfall event in February (see section 3.5.8 for supporting data) that is likely to have generated runoff. It is suspected that the input of the more neutral rain runoff was the cause of the initial increase in surface layer pH from February to March. The ongoing elevated pH may have been caused by increased phytoplankton activity and also riparian macrophyte growth, from allochthonous organic matter and nutrients delivered into the lake from the runoff input. The poorly buffered lake water is likely to be influenced by the photosynthetic process, generating alkalinity from respiration.

Figure 3-16 pH profile in the centre of the Wensleydale coal mine lake, December 2004 - December 2005

![Graph showing pH profile](image)

Wensleydale (pH ~ 5) is very acidic relative to the other lakes in the western district. Lake Purrumbete has a pH range of 6.8 - 9.1 and ten other western district lakes all generally had pH values > 8 (surface water) and varied by little more than 1 pH unit over a 12 month period (De Deckker and Williams, 1988). A lower pH
range such as is the case at Wensleydale should not, in itself, inhibit a bioremediation process (Johnson 2000).

3.5.8 Water Column Turnover (destratification)

Wensleydale’s stratification pattern is described as warm monomictic (Wetzel 2001). Turn over and water column circulation occurs for 2-3 months in winter. Stratification occurs in spring, summer, and autumn. This pattern and its influence on other water quality parameters are presented in Figure 3-17.

Figure 3-17 Temperature, Dissolved Oxygen (DO), pH and Electrical Conductivity (EC) measurements for 2005, at i) 5 m and ii) 20 m depth

i) Epilimnion (5m)
ii) Hypolimnion (20m)

The two graphs show the distinct layers of the epilimnion (graph i) which reached a maximum temperature of almost 22 °C in the height of summer before cooling to 10 - 11°C in July and August, and the hypolimnion (graph ii), which remained within a temperature range of 10 - 11°C. Also evident are the increasing EC levels in both layers. In the epilimnion this is probably due to net evaporation, and in the hypolimnion to groundwater inflow. The hypolimnion showed a steady decrease in DO levels at a rate of ~ 1 mg/L/month, which ceased at ~ 7mg/L following onset of destratification. This is consistent with a groundwater input, as typically groundwater has low or zero DO, but remineralisation of organic matter may also contribute. Lake pH also decreased slightly from May to July, which concurs with the groundwater inflow hypothesis. Lake turnover around July is identified by a change in hypolimnion DO, rising from 7 mg/L to almost 10 mg/L.
In relation to anaerobic sediment bioremediation, the turnover analysis suggests that the deep hypolimnion water, with increased alkalinity, nutrients and dissolved organic material, would mix through the entire water column during destratification. Ideally, this annual process would ensure that treatment is distributed through the lake, and that overall recovery is promoted by stimulating primary production.

### 3.5.9 Light Profiles

Visual inspection of water samples and glass fibre filters (GF/C 0.45 μm) following filtering of water samples revealed little particulate matter. Secchi depth readings (Table 3-2) indicate that the bottom of the photic zone extends to a maximum depth of approximately 11 m, assuming that photic depth = 2.7 x Secchi depth (Mitchell et al. 1987).

#### Table 3-2 Secchi Disc depth readings for Wensleydale coal mine lake

<table>
<thead>
<tr>
<th>Date</th>
<th>Depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept 04</td>
<td>0.98</td>
</tr>
<tr>
<td>Oct 04</td>
<td>0.95</td>
</tr>
<tr>
<td>Nov 04</td>
<td>1.20</td>
</tr>
<tr>
<td>Dec 04</td>
<td>1.86</td>
</tr>
<tr>
<td>Feb 05</td>
<td>0.40</td>
</tr>
<tr>
<td>March 05</td>
<td>1.00</td>
</tr>
<tr>
<td>May 05</td>
<td>2.80</td>
</tr>
<tr>
<td>Feb 06</td>
<td>2.90</td>
</tr>
<tr>
<td>April 06</td>
<td>4.00</td>
</tr>
<tr>
<td>July 06</td>
<td>2.80</td>
</tr>
</tbody>
</table>

The low February 2005 reading (0.4m) is significantly less than the other months, as heavy rain events had occurred for 2 weeks prior to the sampling date. The lake
appeared more coloured than usual on the sampling day and the Secchi readings confirmed this. The results also indicate that maximum surface water clarity occurs in the autumn months. Autumn weather in the district is often characterised by mild calm conditions, with low incidence of strong winds. The reduced wind conditions are the most likely explanation for increased clarity in the lake.

Photosynthetically Active Radiation (PAR) profiles were recorded on two occasions, in April 2006 and August 2006 (Figure 3-18), using a Kahlsico Model 268WA310 Radiometric Underwater Irradiometer. Ambient and underwater irradiance was measured and PAR values calculated as per manufacturers instructions. The photic depths calculated from the PAR results correlate well with the photic depth calculated from the Secchi disc readings, indicating that 1% of the surface light reaches to a depth of ~11 m. The maximum photic depth for Wensleydale is comparable to that of Lake Purumbete, where Mitchell and Collins (1987) recorded a mean Secchi depth of 5.6m and a PAR photic depth of 14m. Unlike Wensleydale however, light attenuation in Purumbete was due to colour associated with dissolved organic matter and suspended solids comprising organic debris, algae, and zooplankton. In Wensleydale it is more likely to be due to colloidal matter (too small to be retained on the 0.45 μm filter). This also gives the lake a blue colour due to Rayleigh light scattering.
Figure 3-18 Photosynthetically Active Radiation (PAR) profiles for Wensleydale in i) April 2006 and ii) August 2006

i) April 2006

ii) August 2006
3.5.10 Water Quality

A compilation and summary of water quality data from previous testing, consisting of 3 samples, conducted by the Rural Water Commission of Victoria (1990), as well as testing completed during this investigation (2004-2006) is shown in Table 3-3.

Table 3-3 Summary of Wensleydale coal mine lake water quality in 1990, and in 2004-06

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1990</th>
<th>2004-06</th>
<th>ANZECC* Lake &amp; Reservoir</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5</td>
<td>4.9</td>
<td>6.5 - 8.0</td>
</tr>
<tr>
<td>Electrical Conductivity μS/cm</td>
<td>637</td>
<td>700</td>
<td></td>
</tr>
<tr>
<td>Total Alkalinity, as CaCO₃ mg/L</td>
<td>4.3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Chloride, mg/L</td>
<td>160</td>
<td>~180</td>
<td></td>
</tr>
<tr>
<td>Sulphate, as SO₄ mg/L</td>
<td>39.7</td>
<td>37.2</td>
<td></td>
</tr>
<tr>
<td>Iron (undigested), mg/L</td>
<td>0.65</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Nitrate and Nitrite (NO₃), mg/L</td>
<td>&lt;0.15</td>
<td>0.31</td>
<td>0.01</td>
</tr>
<tr>
<td>Total Nitrogen mg/L</td>
<td></td>
<td>0.64</td>
<td>0.35</td>
</tr>
<tr>
<td>Filtered Reactive Phosphorus mg/L</td>
<td>&lt;0.01</td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>Total Phosphorus mg/L</td>
<td>0.01</td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>Chlorophyll a μg/L</td>
<td>0.5</td>
<td></td>
<td>5.0</td>
</tr>
<tr>
<td>DO (% saturation) Lower / Upper at the surface</td>
<td>90 - 110</td>
<td>90 - 110</td>
<td></td>
</tr>
<tr>
<td>Salinity mg/L</td>
<td>0.36 - 0.45</td>
<td>0.03 - 0.3</td>
<td></td>
</tr>
<tr>
<td>Turbidity</td>
<td>3 - 50</td>
<td>1 - 20</td>
<td></td>
</tr>
</tbody>
</table>

*ANZECC = Australian and New Zealand Conservation Council, default trigger values for physical and chemical stressors for south east Australia for slightly disturbed ecosystems (ANZECC 2000)

Comparison of the 1990 and 2004-06 data sets shows that over 15 years very little change in water quality has occurred. Natural processes in this instance do not appear to be operating at a rate which would allow succession to a more neutral lake to occur within decades. Wensleydale water quality meets ANZECC Guidelines for filtered reactive phosphorus, total phosphorus, chlorophyll a and dissolved oxygen. Guidelines are exceeded for nitrogen oxides, total nitrogen,
salinity, turbidity (on occasions) and pH. The lake has remained acidic, with low nutrients levels, and generally from the data set, water quality appears not to have changed greatly. Kalin and Geller (1998) note that in Germany there are many examples of 40 year old unremediated acid lakes where no change has occurred. Wensleydale appears to be following this path.

3.5.11 Nutrients

Samples were collected from the lake centre for nutrient analysis (Total Nitrogen (TN) and Total Phosphorus (TP)), on 4 occasions between July 2004 - January 2005 (Table 3-4).

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Date</th>
<th>July 2004</th>
<th>October 2004</th>
<th>December 2004</th>
<th>January 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP</td>
<td>TN</td>
<td>TP</td>
<td>TN</td>
<td>TP</td>
</tr>
<tr>
<td>0.1</td>
<td>0.04</td>
<td>0.66</td>
<td>-</td>
<td>-</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2 - 6</td>
<td>&lt;0.01</td>
<td>0.72</td>
<td>0.08</td>
<td>0.59</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>0.03</td>
<td>0.78</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>15</td>
<td>&lt;0.01</td>
<td>0.71</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>25 - 30</td>
<td>&lt;0.01</td>
<td>0.70</td>
<td>&lt;0.01</td>
<td>0.60</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>37</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*TP concentrations above the detection limit are highlighted in bold.
- indicates no sample was taken

The results show little variation in nutrient concentrations, either in the epilimnion or in the hypolimnion. The average ratio of TN:TP is 16.63 for the 3 samples that had phosphorus concentrations over the detection limit. These samples exceeded the ANZECC guideline trigger limit. For all other samples (TP < 0.01 mg/L) the ratio of TN:TP would be even higher (>54; Table 3-3). The results indicate that the
lake system is phosphorus limited (a ratio of TN:TP > 7:1 by mass represents P limitation (Herpich 2002)). The higher surface water phosphorus concentrations are probably caused by surface water inflows following winter rains. The lakes catchment of approximately 1200 ha comprises both native forest and cleared farmland. It also appears that phosphorus is utilised quickly in the lake as concentrations returned to below the detection limit in the December 2004 and January 2005 samples.

Total nitrogen concentrations range from 0.54 to 1.1 mg/L, exceeding the ANZECC trigger values for lakes and reservoirs by a factor of 2 to 3. From a regional perspective however, Wensleydale TN concentrations are comparable to those of Lake Purrumbete (0.58 -1.3 mg/L, (Herpich 2002)). Approximately 50% TN is NOx (Table 3-3). This may have been introduced by groundwater, which is commonly rich in NOx. When comparing TP concentrations however, Lake Purrumbete’s which has an extensively cleared and farmed catchment are substantially higher (0.07 mg/L to 0.21 mg/L) and phosphorus was not the limiting nutrient at Purrumbete (Herpich 2002).

The phosphorus results explain the low primary productivity observed in the Wensleydale. Low nutrient concentrations are common in mining lakes (Davison 1986; Geller et al. 1998) and increasing the concentrations is an integral part of the bioremediation processes, to stimulate primary productivity (Klapper et al. 1998).

### 3.5.12 Sediment Analysis

Sediment grab samples and cores were collected to investigate the historical productivity of the lake. Using an Eckman Grab and a Glew Corer, sediment samples were collected on two occasions (September and October 2004), from 8 locations around the lake (Figure 3-19).
The Glew Corer allowed undisturbed cores of sediment to be retrieved (Figure 3-20). Observation of the cores indicated that little organic matter had accumulated in the lake since its creation. Dark organic rich layers were not obvious in samples from any location. The majority of the samples revealed grey-brown clays and silts (Figure 3-20). There was an exception for samples collected at the southern end of the lake adjacent to the overburden dump and inlet water course, where sand was
encountered. Inspection of the samples revealed no obvious plant or other organism remains.

Figure 3-20 Photos of a Glew Corer sediment sample

Samples collected by both methods were placed in sealed containers and frozen on return from the field for later analysis. Sediment analyses (Table 3-5) were completed based on standard methods (American Public Health Association 2005a):

- samples were oven dried at 105 °C until constant weight
- sub samples were then fired in a muffle furnace at 550 °C to determine the volatile component
<table>
<thead>
<tr>
<th>Sample Location and Name*</th>
<th>Description</th>
<th>Sample Preserved</th>
<th>Volatile Solids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eckman Grab 1</td>
<td>Silt and Clay</td>
<td>Composite of grab</td>
<td>9</td>
</tr>
<tr>
<td>Eckman Grab 2</td>
<td>Silt and Clay</td>
<td>Composite of grab</td>
<td>8</td>
</tr>
<tr>
<td>Eckman Grab 3</td>
<td>Sand, Gravel and Silt</td>
<td>Composite of grab</td>
<td>5</td>
</tr>
<tr>
<td>Eckman Grab 4A</td>
<td>Silt and Clay</td>
<td>Gelatinous light brown layer on very top</td>
<td>10</td>
</tr>
<tr>
<td>Eckman Grab 4B</td>
<td>Silt and Clay</td>
<td>Composite of clays</td>
<td>7</td>
</tr>
<tr>
<td>Eckman Grab 5A</td>
<td>Silt and Clay</td>
<td>Gelatinous light brown layer on very top</td>
<td>9</td>
</tr>
<tr>
<td>Eckman Grab 5B</td>
<td>Silt, Clay and Gravel</td>
<td>Composite of grab</td>
<td>8</td>
</tr>
<tr>
<td>Glew Core 1A</td>
<td>Gelatinous brown orange layer</td>
<td>Top ~1.5 cm depth</td>
<td>10.3</td>
</tr>
<tr>
<td>Glew Core 1B</td>
<td>Silt and Clay</td>
<td>~1.5 cm to ~12m depth</td>
<td>6.3</td>
</tr>
<tr>
<td>Glew Core 1C</td>
<td>Silt and Clay</td>
<td>~12 to 22 cm depth</td>
<td>10.4</td>
</tr>
<tr>
<td>Glew Core 2A</td>
<td>Gelatinous brown orange layer</td>
<td>Top to ~2cm</td>
<td>10.1</td>
</tr>
<tr>
<td>Glew Core 2B</td>
<td>Silt and Clay</td>
<td>~2cm to ~4cm depth</td>
<td>9.2</td>
</tr>
<tr>
<td>Glew Core 2C</td>
<td>Silt and Clay</td>
<td>~4 cm to ~6cm depth</td>
<td>9.3</td>
</tr>
<tr>
<td>Glew Core 2D</td>
<td>Silt and Clay</td>
<td>~6cm to ~8cm depth</td>
<td>6.3</td>
</tr>
<tr>
<td>Glew Core 3A</td>
<td>Gelatinous brown orange layer</td>
<td>Top to ~2cm</td>
<td>8.2</td>
</tr>
<tr>
<td>Glew Core 3B</td>
<td>Silt and Clay</td>
<td>~2cm to ~4cm depth</td>
<td>7.8</td>
</tr>
<tr>
<td>Glew Core 3C</td>
<td>Silt and Clay</td>
<td>~6cm to ~8cm depth</td>
<td>7.5</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>8.3</td>
</tr>
</tbody>
</table>

*A, B, C, D are labels to distinguish samples.
Loss on ignition at 550 °C gave ~5% to ~10% (dry weight) volatile solids content (VSC) with an average of 8.3%. The measured volatile solids content can consist of organic matter, tightly bound water (eg. hydrated iron oxides) and mineral decomposition (eg. breakdown of carbonates). The data indicate that the upper most layer of sediment does have a slightly higher VSC than the lower layers, however given that no dark carbon based organics were observed, this result is more likely due to the other contributors of VSC, such as hydrated iron oxides.

In comparison Lake Purrumbete, which is highly productive, had a mean volatile solids content of 36.7 % (dry weight), from 4 sediment samples taken from the sediment of the open water area (Herpich 2002). This difference clearly indicates the low productivity of Wensleydale coal mine lake, relative to other district lakes.

### 3.5.13 Phytoplankton and Zooplankton

Sampling for phytoplankton (algae) by vertical and horizontal 5m drags of a 20 micron phytoplankton net in November 2004 found no algae. Chlorophyll a testing of two surface water samples from the north and south end of the lake at ~ 5m from the bank, returned low concentrations of 0.53 and 0.58 µg/L, respectively. In comparison, Lake Purrumbete’s mean chlorophyll a concentration has been reported as 6.97 µg/L (Mitchell et al. 1987), and in another study ranged from 3.33 to 13.24 µg/L (Herpich 2002).

Zooplankton and further algae sampling took place in December 2004. Again, virtually no algae or zooplankton were detected by eye or microscope from a lengthy (~1000m) surface trawl using a 20 micron phytoplankton net. Vertical trawling of the net from a depth of 5m did however reveal the presence of some zooplankton (Calanoid copepods and Rotifers). Algae were also detected in the vertically trawled samples (*Peridinium, Scenedesmus*, and *Mougeotia*). Abundances of all species were low, and therefore were not quantified.
3.5.14 Other Observations

Some macrophytes have established in small littoral colonies. Species identified included Baumea (which appears to be dominant), Triglochin, and a Juncus species. Sonar readings indicate that littoral areas are steeply sloped (e.g. the old mine wall), and are therefore not suited for macrophyte establishment.

Detailed investigation of macrophytes and aquatic plant life was beyond the scope of this study. No fish have been observed over the course of the investigations, although some ducks and other water birds have been sighted on a number of occasions.

3.6 Lake Evolution

Based on this analysis of Wensleydale Coal Mine Lake and its surrounding environment, some explanations are possible for its slow development toward a productive ecosystem. Wensleydale displays some characteristics that are common to many mining lakes, such as low pH and nutrient concentrations (Charles 1998; Geller et al. 1998). However Wensleydale also has some less common features such as an upstream coffer dam and overburden. These features inhibit allochthonous organics and nutrients entering the lake. Characteristics that have contributed to Wensleydale’s slow evolution are listed in Table 3-6.
Table 3-6 Characteristics influencing the development and rehabilitation of Wensleydale Coal Mine Lake

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Depth, Mean Depth, Depth to Volume and Area Ratios</td>
<td>Limited area of littoral zone and reduced sediment area in contact with photic zone reduces overall productivity</td>
</tr>
<tr>
<td>Cofferdam and overburden upstream of main lake</td>
<td>Inputs of allochthonous organics and nutrients from the catchment are retarded by the cofferdam and overburden.</td>
</tr>
<tr>
<td>Connection channel between cofferdam and lake</td>
<td>The channel is comprised of unconsolidated overburden materials (gravels, sands, silts, coal) and acts as a &quot;filter&quot; to further reduce allochthonous inputs to the lake</td>
</tr>
<tr>
<td>Ongoing Acid Inputs</td>
<td>Maintains the lake pH around 5</td>
</tr>
<tr>
<td>Water Quality - acidic</td>
<td>Low alkalinity and limited buffering capacity</td>
</tr>
<tr>
<td>Low Nutrient Concentration</td>
<td>Low Phosphorus concentrations limit productivity</td>
</tr>
<tr>
<td>Low organic content in sediment</td>
<td>Little organic matter in the sediment inhibits bacterial remineralisation activity, including potential sulphate reducing bacteria bioremediation</td>
</tr>
<tr>
<td>Low productivity</td>
<td>Little to no organic loading to sediment</td>
</tr>
</tbody>
</table>

The above factors all contribute to make Wensleydale a lake containing a depauperate flora and fauna. An understanding of these characteristics and their inter-relationships may enable better planning of rehabilitation strategies.
3.7 Wensleydale Conclusions

After approximately 40 years of natural succession or "do nothing" remediation, Wensleydale coal mine lake remains in an ecologically impoverished state, and is comparable to its adjacent unrehabilitated terrestrial post-mine landscape, where a few trees are starting to establish.

Investigations have revealed that its water is still acidic, and the lake has low productivity and biodiversity. They have provided insights into the processes controlling acid lake development, and following from this, a better appreciation of what measures could be taken to promote succession to a more neutral and "healthy" lake, at Wensleydale, at Anglesea in the future, or at other mining lakes.

The lake’s morphometric characteristics and thermal stratification pattern are comparable to nearby productive and diverse volcanic lakes, suggesting Wensleydale over time (albeit long) or with help, could support a more ecologically rich environment.

Bioremediation using organic sediment to stimulate anaerobic SRB activity seems compatible with the lake’s stratification pattern, water temperature, and general water quality.
4 Bioremediation Laboratory Experiments – “Material Scoping”

The task of creating a healthy mining lake at Anglesea will be a complex process and could incorporate a number of rehabilitation strategies and methods. All strategies will need to satisfy environmental, social and economic sustainability criteria. Given that the pH of the water in a future coal mine lake at Anglesea will be ~3.4, the successful scientific testing and demonstration of the bioremediation process to deal with acidity will be critical overall. The minimum desirable pH required for the maintenance of an ecosystem as per the ANZECC guidelines is 6.5. For successful acidic lake bioremediation, primarily driven by sulphate reducing bacteria (SRB) improving the pH to a more ecologically hospitable range (>6.5), an anaerobic sediment needs to be created (Tuttle et al. 1969). This sediment needs to be rich in organics, while also providing key growth requirements such as nutrients, trace minerals and microbial substrate or habitat.

The key questions regarding the creation of this anaerobic sediment are:
- what materials can be used?
- how much of the materials will be needed?

In searching for answers to the above questions, the types of materials that can be investigated are immediately constrained by the nature of the problem itself. The size and scale of the majority of mining projects, including Alcoa’s operations at Anglesea, are very large and therefore materials must be readily available nearby, and be “free” or low in cost. If this is not the case, economic feasibility of any bioremediation project may be immediately in doubt. The materials of choice are often labelled “wastes” or are managed by disposal rather than re-use, and therefore are likely to be free. They also include low cost materials that are usually produced in large quantities, such as straw or potatoes. For these reasons, at Anglesea, likely materials that could be used are similar to those identified in previous research (McCullough et al. 2006b), and include biosolids and
greenwaste. Norton (2004) tested the absorbent properties of biosolids to reduce metal concentrations in acid mine water (AMW). Using Anglesea Wastewater Treatment Plant biosolids, and a laboratory-prepared AMW equivalent solution, Norton found that biosolids reduced metal concentrations and increased the pH of the solution, by up to 2 pH units.

Two experiments were designed using biosolids to answer the key questions regarding what materials could be used to construct an organic sediment. The first “Material Scoping” experiments, discussed in this chapter, were designed to test the capacity of biosolids, and biosolids in combination with other possible materials, to increase the pH, and improve the overall water quality of acid mine water. The second phase of “Application Rate Determination” experiments were designed to determine the required application rate to achieve a satisfactory improvement in water quality. Details of the “Application Rate Determination” experiments are presented in Chapter 5.

4.1 Experiment Aim

To determine a preferred material or material combination, on the basis of its ability to stimulate SRB activity and so improve acidic water quality (specifically pH and nutrients), from a number of potential organic materials and substrates using laboratory bench experiments.

4.2 Materials

Acid mine water (AMW) was collected from the Alcoa Coal Mine at Anglesea. AMW was collected from a storage pond pump station in 20 and 25 L square plastic drums (HDPE) that were rinsed twice prior to filling.
Stabilised biosolids were collected from the South West Water Authority’s treatment and storage facility at Camperdown, approximately 70 km east of Warrnambool. Stabilised biosolids were collected from a stockpile using a shovel, and stored in sealed buckets. The term stabilised-biosolids refers to dewatered and composted biosolids from Warrnambool’s activated sludge wastewater treatment plant. The stabilised biosolids collected in April 2004 were processed and stockpiled over the 2001/02 summer.

Mulched (chipped) green waste was collected from a local Warrnambool waste transfer station. Green waste refers to garden and plant material collected by the operator or deposited by customers, and typically consists of grass, leaves, and branches of varying sizes.

Straw was collected from a local agricultural supply store.

Soil (a sandy loam) was collected from a farm near Warrnambool. The top layer of grass was removed with a shovel, and soil was taken from the 5-20cm soil horizon.

Anaerobic bacteria were collected from a local milk processing factory wastewater treatment plant. A dip sample was taken from the reactor, and transferred to a 500 mL HDPE plastic bottle for transit. The sample was used for seeding approximately 4 hrs later that day.

Duplicate samples of biosolids, straw, green waste and soil were analysed using standard methods (American Public Health Association 2005a), consisting of oven-drying samples at 103-105°C to determine water content, and firing in a muffle furnace at 550 °C to determine volatile content (Table 4-1).
Table 4-1 Water and volatile content (%) of organic materials used

<table>
<thead>
<tr>
<th>Material</th>
<th>Sample #</th>
<th>% Solid</th>
<th>% Water</th>
<th>% Ash</th>
<th>% Volatile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biosolids</td>
<td>1</td>
<td>66</td>
<td>34</td>
<td>77</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>68</td>
<td>32</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>Straw</td>
<td>1</td>
<td>85</td>
<td>15</td>
<td>8</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>83</td>
<td>17</td>
<td>6</td>
<td>94</td>
</tr>
<tr>
<td>Soil</td>
<td>1</td>
<td>83</td>
<td>17</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>84</td>
<td>16</td>
<td>93</td>
<td>7</td>
</tr>
<tr>
<td>Green waste</td>
<td>1</td>
<td>66</td>
<td>34</td>
<td>31</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>64</td>
<td>36</td>
<td>18</td>
<td>82</td>
</tr>
</tbody>
</table>

4.3 Methodology

4.3.1 Design and Construction

The experiment was set up on a laboratory bench under a fume hood (Figure 4-1).

Figure 4-1 Rectangular glass experiment vessels (fish tanks) assembled on the bench
Twenty-one glass tanks measuring approximately 25 cm long, 15 cm wide and 20 cm high, giving a maximum volume of approximately 7.5 litres, were labelled 1-21. The experiment treatment variables were designed as follows:

- 1-3 “Controls” - AMW only, no additions
- 4-6 “Biosolids - Seeded” - ~ 4 cm biosolids layer with 5 mL of anaerobic bacteria
- 7-9 “Biosolids (4cm)” - ~ 4 cm biosolids layer
- 10-12 “Biosolids (8cm)” - ~ 8 cm biosolids layer
- 13-15 “Biosolids + Straw” - ~ 4 cm biosolids layer and approximately 150 mL (one handful) of straw
- 16-18 “Biosolids + Soil” - ~ 4 cm biosolids layer and approximately 150 mL of soil
- 19-21 “Biosolids + Greenwaste” - ~ 4 cm biosolids layer and approximately 150 mL of green waste

Each batch of triplicate tanks was prepared at the same time. Control tanks were filled with 6 litres of acidic water. Tanks 4-21 were filled with their respective treatments by measuring out the weight (wet) of material then adding this to the tanks (Table 4-2). Once the treatment material was added, 4 L of acidic mine water was added to the tanks.
Table 4-2 Masses of organic substrates added to tanks for the material scoping experiment

<table>
<thead>
<tr>
<th>Tank</th>
<th>Treatment</th>
<th>Biosolids (g)</th>
<th>Other Materials (g)</th>
<th>Total Dose (Wet) g/L</th>
<th>Total Dose dry g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Controls</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>2</td>
<td>Biosolids - seeded</td>
<td>1060</td>
<td>5</td>
<td>266</td>
<td>178</td>
</tr>
<tr>
<td>3</td>
<td>Biosolids (~4 cm)</td>
<td>933</td>
<td>*</td>
<td>233</td>
<td>156</td>
</tr>
<tr>
<td>4</td>
<td>Biosolids (~8 cm)</td>
<td>1060</td>
<td>*</td>
<td>237</td>
<td>158</td>
</tr>
<tr>
<td>5</td>
<td>Biosolids + Straw</td>
<td>2427</td>
<td>*</td>
<td>607</td>
<td>407</td>
</tr>
<tr>
<td>6</td>
<td>Biosolids + Soil</td>
<td>920</td>
<td>26</td>
<td>237</td>
<td>160</td>
</tr>
<tr>
<td>7</td>
<td>Biosolids + Greenwaste</td>
<td>838</td>
<td>158</td>
<td>249</td>
<td>166</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>1217</td>
<td>205</td>
<td>356</td>
<td>237</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>1008</td>
<td>255</td>
<td>315</td>
<td>210</td>
</tr>
</tbody>
</table>

Once filled, tanks were left to stand for approximately 30 minutes, after which the final water height was marked on the side of each tank. Tanks were then randomly placed on the laboratory bench in a 7 by 3 grid. Insulation was placed between each tank and around the perimeter. Insulation was then also placed on top of the tanks and finally the insulated tanks were covered with black plastic, to exclude light (simulating the environment at the base of a lake) and inhibit algal growth (Figure 4-2).
4.3.2 Monitoring

The experiment commenced on the 3rd of May 2005 and basic water quality monitoring was initially undertaken at a relatively high frequency (5 times per week). This was reduced to once or twice a week after approximately 2 weeks and continued until the experiment was concluded on the 23rd of August 2005 (experiment duration was 115 days).

The following parameters were measured as part of the basic water quality monitoring process: temperature; pH; Electrical Conductivity (EC); Dissolved Oxygen (DO). Temperature and pH were measured using a WTW 330i pH meter,
EC was measured using a YSI Model 33 Temperature, salinity, Electrical Conductivity meter, and DO was measured using a YSI Model 57 Temperature Oxygen Meter.

Probes were rinsed with distilled water between tanks when necessary. Tanks were kept at their initial volume by adding distilled water.

Visual and sensory observations were made on each sampling occasion. Observations noted were the presence of gas/bubbles, presence of black precipitate and olfactory detection of hydrogen sulphide and other odours.

Detailed water quality testing was conducted by the Deakin University Water Quality Laboratory for the following parameters: sulphide; sulphate; chloride; Iron (filtered); Total Phosphorus (TP); and Total Nitrogen(TN). Samples for testing were collected at the conclusion of the experiment by a syringe that extracted a sample from approximately the middle of the free water. Prepared bottles (acid or extran washed) were then rinsed twice with sample water before filling.

Once all water samples were collected, remaining water was drained from the tanks prior to a basic visual inspection of the sediment remaining in the tanks.

### 4.3.3 Experiment Augmentation

After 52 days, tanks showing no sign of microbial activity had 10 ± 0.5 g of straw added to them and 1mL of inoculum/seed from tank 15. This intermediate step was based on the obvious microbial activity in tanks 13-15 and 19-21.
The experiment was concluded at day 115, when pH had reached or was approaching 8, and enough data had been generated to address the experimental aim.

### 4.4 Results and Discussion

Direct comparison of results from this experiment to other bioremediation bench experiments is limited, as acid water varies significantly between sources. General trends and percentage change can be compared between experiments however.

Table 4-3 lists the pH and EC of each tank at the start (day 3) and at the end (day 115) of the experiments. It also lists each tank's final water quality for the parameters of sulphide ($S^2$), sulphate ($SO_4^{2-}$), chloride ($Cl^-$), iron (Fe - filtered), total phosphorus (TP) and total nitrogen (TN).
At the conclusion of the experiment, and once water samples had been collected, a visual inspection of the sediment was completed. All sediments produced a composting organic smell when disturbed. In the “biosolids + straw” (13-15) and “biosolids + greenwaste” (19-21) tanks, a black sludge was evident and a strong hydrogen sulphide odour was noticed. Straw and greenwaste material was still
discernible in the tanks, indicating that not all of this matter had been broken down.

### 4.4.1 Temperature

Water temperatures ranged from 13.1 to 17.0 °C across all tanks, with an average of 15.2°C. Tanks on the outside perimeter of the array were slightly cooler on occasions than tanks in the middle, however no significant variation between tanks was detected, with all tanks remaining within 2 °C of each other.

### 4.4.2 pH

The average pH of the controls increased by only 0.11 during the experiment (Figure 4-3). An initial rapid increase in pH (ΔpH ~2.5) occurred across all treatments. The “biosolids + straw” and “biosolids + greenwaste” treatments continued to rise rapidly, reaching a pH of approximately 7 by day 7. After this time (day 7) the increase in pH for these treatments slowed, rising to just above 8 over approximately 100 days. The remaining treatments rose to pH ~ 6.5 by day 7, and reached a pH of ~ 7 at approximately day 20. No significant changes in pH then occurred in these treatments, until the addition of straw at day 52. Following this addition, a slight initial decrease was followed by a steady increase in pH until the experiment’s conclusion. At this point all treatment tanks had reached a pH greater than 7.5 with tank 15 (a “biosolids + straw” mixture) reaching the maximum final pH of 8.4.
The pH results indicated two key features of acidic water bioremediation:

- Biosolids alone have a net capacity for acid neutralisation
- Biosolids in combination with greenwaste or straw (both of which have a volatile content) increased the pH more than biosolids alone, and associated with this was more visually obvious microbial activity

The results also confirmed that bioremediation is applicable to acidic water at Anglesea. Similar Australian studies such as that by Harris and Ragussa (2000) using a sludge and rye grass mixture at a treatment rate of approximately 320 g/L obtained similar pH rises ($\Delta$pH = 4.95 after 140 days compared to $\Delta$pH $\sim$ 4.7 after 115 days). More recently in Australia, McCullough et al. (2006b) tested sewage and greenwaste, both individually and in combination, at an approximately 60 to 100 g/L dose rate. Over the 145 days that their experiment ran, pH increased in the greenwaste and sewage greenwaste combinations from 2.5 to 5.5.
Experiments on different organic materials to stimulate SRB remediation also achieved pH increases. Fyson et al. (1998) used whole potatoes in 20 L mesocosm experiments run over 9 months, and achieved pH increases ranging from 3.0-5.0. These increases were proportional to the dose rate of potatoes (5 and 34 grams (dry) per mesocosm, respectively). Costa et al. (2005) investigated the SRB treatment potential for AMD from an abandoned copper mine in Portugal. They tested sewage, anaerobic sludge and soil from the mining area in combination with lactose which was available in the effluent from a nearby cheese industry. Increases in pH from 2.3 to near neutral were obtained.

### 4.4.3 Electrical Conductivity

The electrical conductivity (EC) of the water varied little over the course of the experiment with the exception of the “biosolids - 8 cm” treatment (Figure 4-4). In some treatments EC rose over time, while in others it fell. The range of variations (excluding the “biosolids - 8 cm” treatments) from day 3 to 115 were between a minimum of 50 μS/cm for the controls, up to 266 μS/cm for the “biosolids + soil” mix. The “biosolids - 8 cm” dose increased an average of 1466 μS/cm from 3150 to 4616 μS/cm. Changes in total ion concentrations effect the electrical conductivity in the tanks. Remineralisation of organic matter would be expected to produce ions such as HCO₃⁻, PO₄³⁻, NO₃⁻, and NH₄⁺. The change in pH shows H⁺ concentrations decreased. Other ions may have been consumed by growing bacteria. The net change in ion concentration may therefore be small.
4.4.4 Nutrients - Total Nitrogen and Total Phosphorous

Acid Mine Water (AMW) is typically low in nutrients, and the concentrations measured in the controls confirm this for Anglesea AMW. The addition of biosolids and other materials significantly increased nutrient levels. The average concentration of total phosphorus (TP) was 0.90 (±0.41) mg/L in the controls, compared to the treatment tanks’ average of 7.7 mg/L (range of 4.2 - 10.5 mg/L) (Figure 4-5). The average total nitrogen (TN) concentrations was 7.07 (±3.98) mg/L in the controls, compared to the treatment tanks’ average of 26.7 mg/L (range of 6.6 - 50 mg/L) (Figure 4-6). Uncertainties represent one standard deviation.
Figure 4-5 Average Total Phosphorus concentrations at the conclusion of the material scoping experiment

Figure 4-6 Average Total Nitrogen concentrations at the conclusion of the material scoping experiment
Increases in nutrient concentrations occurred across all treatments. A direct relationship between biosolids application rate and increased nutrient concentration is not apparent, however similarities between TN and TP concentrations across treatments are noticeable. Higher nutrient concentrations were measured in tanks 4 to 12, which (with the exception of the “biosolids + soil” tanks) had a shorter period of noticeable microbial activity. In contrast, the “biosolids + straw” and “biosolids + greenwaste” variants had a longer period of microbial activity and have lower nutrient concentrations. Therefore lower nutrient concentrations or nutrient removal from the water column appears to be from uptake by bacteria, such as is the case of nitrogen in bacterially driven denitrification reactions. The exception is the “biosolids + soil” variants, where it is likely that a component in the soil such as calcium, may have absorbed or reacted with the nutrients.

Increases in nutrient concentrations are favourable in regards to AMD treatment, first to help stimulate SRB bioremediation, but also to enhance the establishment of other biological life, such as phytoplankton as a subsequent step in general lake rehabilitation. Of the other studies discussed in this section, only two measured nutrient concentrations. Harris and Ragussa (2000) measured phosphorus as it can complex and precipitate with metal ions such as aluminium and iron. They concluded that the main source of phosphorus came from decomposing rye grass, which increased concentrations up to a maximum of 25 mg/L, and overall, increased concentrations up to 10 mg/L. Fyson et al. (1998) specifically added phosphate rock and nitrate, as well as potatoes as a carbon source to stimulate SRB. They achieved peak increases of TP concentrations of 5-6 mg/L which then reduced to concentrations slightly higher than that of the controls. For comparison, this experiment recorded an average (across all treatments) total phosphorus concentration increase of approximately 6 mg/L, and total nitrogen increase of approximately 20 mg/L.
4.4.5 Sulphate

A reduction in sulphate concentration was observed in two of the three replicates for the "biosolids + straw" and "biosolids + greenwaste" treatments (Figure 4-7). This reduction, when taken into account with the other results and observations, indicates the presence and action of sulphate reducing bacteria. In all other treatments, sulphate concentrations increased to approximately double that of the control concentration.

Figure 4-7 Average Sulphate Concentrations at the conclusion of the material scoping experiment

In a study conducted by Harris and Ragussa (2000), the application rate of 320 g/L of sludge and ryegrass reduced sulphate by 85%, and McCullough et al. (2006b) reduced sulphate by 25%. In this experiment an average of 95% reduction was achieved in the four tanks (numbers 14, 15, 19 and 21) in which sulphate reduction was measured. It is unclear why sulphate reduction was not measured in all of the treatment tank replicates (i.e. in tanks 13 and 20 as well), especially as all other
data and visual and olfactory observations indicate otherwise. One possibility for these extraneous results is suspected to be laboratory error.

In regards to the other treatments, it is likely that although the tanks appeared to be anoxic or anaerobic for 20-30 days, sulphate reduction had not begun to reduce the concentration of sulphate in the water column.

4.4.6 Sulphide

Only Tank 14, a “biosolids + straw” treatment, recorded a sulphide concentration above the testing detection limit of 0.2mg/L. However hydrogen sulphide gas was detected by smell in all of the “biosolids + straw” and “biosolids + greenwaste” treatment tanks.

4.4.7 Chloride

Increases in chloride concentrations were approximately proportional to the amount of biosolids added. The mean control concentration was 390 mg/L compared to a mean of 440 mg/L for the “biosolids ~4cm” treatment and the maximum concentration of 580 mg/L in one of the “biosolids ~8cm” treatments (Figure 4-8).
Chloride is typically not involved in biological processes, and the results suggest that no significant reactions altering chloride concentrations occurred.

### 4.4.8 Iron

Iron concentrations were significantly reduced in all tanks except for the “biosolids + soil” treatment. The average iron concentration of the controls was 1.03 (±0.15) mg/L. The treatments other than the “biosolids + soil” tanks ranged in concentration from less than the detectable limit (0.02 mg/L) to 0.18 mg/L and had an average of 0.046 mg/L (Figure 4-9).
Iron concentrations were reduced in all treatments by ~90%, except in the case of "biosolids + soil", which experienced an increase of ~20%, up to ~1.2 mg/L. Similarly Harris and Ragussa (2000) achieved more than a 90% reduction in iron, with the sludge rye grass combination treatments. Reduction in iron is expected to occur, as iron hydroxides precipitate as pH increases above 5. McCullough et al. (2006), however, achieved no significant reduction in iron concentrations in the greenwaste and greenwaste-sewage combination treatments. This may have been due to pH increasing only to the range of pH 5-6, which may not have been high enough to cause precipitation, particularly if the iron was present in organic complexes, which can be retained in solution, even at higher pH levels. This phenomenon is perhaps evident in the current experiment, as the "biosolids + greenwaste" and "biosolids + straw" treatments showed slightly higher iron concentrations that the "biosolids only" treatments. The high iron concentrations of the "biosolids + soil" treatments are most probably due to the addition of extra iron, present in the soil.
4.5 Key Points and Conclusions

This first phase of “material scoping” bench experiments indicates the potential of a mixture of biosolids and greenwaste or straw to treat AMW through direct neutralisation and microbial bioremediation, primarily via sulphate reducing bacteria (SRB). While the application rates were higher than those used in other similar experiments, the purpose of this experiment was to identify preferred materials, and the issue of optimal application rates will be addressed in the following chapter.

The results of this experiment confirm that composted biosolids alone, with their combustible organic content of approximately 25%, are only able to improve AMD water quality by chemical neutralisation processes such as those studied by Norton (2002). Results established that without microbial available organics, sulphate reducing bacteria did not appear to establish in the AMW treated with biosolids alone. A key component in bioremediation therefore is the provision of “available organic” material, such as was the case in the addition of straw or greenwaste.

Both the rate, and the period of bioremediation seem to be dependant on the bioavailability of the organic materials and how quickly they are consumed in the process.
5 Bioremediation Laboratory Experiments - “Application Rate Determination”

The size and scale of modern mining operations limits the potential range of material application rates to what is realistically feasible. For example, a bioremediation material dose of 300g/L when scaled up to a 10,000 ML lake (dimensions 1 km² by 10 m deep) equates to a dose of 3 million tonnes which is both unrealistic, in terms of sourcing such a quantity of organic material, and unfeasible in terms of the logistics and costs of its application.

To determine the range of application rates to be tested in the experiments reported in this chapter, a number of guidelines were used:

- biosolids have a neutralisation capacity of approximately 1 mol H⁺/kg of biosolids (Garvie et al. 2000)
- regional natural lake sediment depths are typically 0.3-1 m range
- realistic constructed sediment depths are probably ≤ 5 cm (for an area of 1km² this equates to ~ 50,000 tonnes of sediment)

Based on the results of the “material scoping” experiments, along with considerations of economics and local availability, a combination of biosolids and greenwaste was chosen as the preferred bioremediation substrate.

5.1 Experiment Aim

To determine an optimal application rate of a biosolids and greenwaste bioremediation substrate by monitoring changes in water quality, and the ability to stimulate microbial activity in the form of anaerobic sulphate reducing bacteria (SRB), over time for a range of dose rates applied to Anglesea acid mine water.
5.2 Materials

Biosolids, greenwaste and acid mine water (AMW) from Anglesea coal mine were collected as described in the previous Chapter (Section 4.2). Sucrose (white sugar), which was later added to the experiments, was purchased from a supermarket.

5.3 Methodology

Experiments were set up in fourteen 20 L white buckets with lids, which served as the experiment vessels (Figure 5-1).

Figure 5-1 Experimental vessel positioned on laboratory bench and floor

Acid mine water that had been collected in 20L drums was homogenised in a 1000L container before 18L was transferred into each of the 14 buckets. Through the lid of the buckets, a plastic hose with attached bubbling stone was installed. A U-bend air lock was also fitted into the lid along with a rubber stopper hole to allow monitoring probes to be inserted (Figure 5-2).
A mixture of 10:1 biosolids:greenwaste by wet weight was prepared. This dose rate was between the ratios used in the scoping experiments of ~5:1 for biosolids:greenwaste, and ~35:1 for biosolids:straw. The mixture was then added to the buckets, at the application rates listed in Table 5-1.
Table 5-1 Experiment Application Rates tested in the 14 vessels (buckets)

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Treatment</th>
<th>Design Rate g/L</th>
<th>Applied Rate g/L</th>
<th>Dry Applied Rate(^*) g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ratio(^*) 100 to 1</td>
<td>5.00</td>
<td>5.06</td>
<td>3.54</td>
</tr>
<tr>
<td>2</td>
<td>Ratio(^*) 100 to 1</td>
<td>5.00</td>
<td>5.08</td>
<td>3.55</td>
</tr>
<tr>
<td>3</td>
<td>Ratio(^*) 200 to 1</td>
<td>2.50</td>
<td>2.46</td>
<td>1.72</td>
</tr>
<tr>
<td>4</td>
<td>Ratio(^*) 200 to 1</td>
<td>2.50</td>
<td>2.60</td>
<td>1.82</td>
</tr>
<tr>
<td>5</td>
<td>Mixture(^%) 2 g/L</td>
<td>2.00</td>
<td>2.05</td>
<td>1.43</td>
</tr>
<tr>
<td>6</td>
<td>Mixture(^%) 2 g/L</td>
<td>2.00</td>
<td>2.05</td>
<td>1.44</td>
</tr>
<tr>
<td>7</td>
<td>Mixture(^%) 1 g/L</td>
<td>1.00</td>
<td>1.04</td>
<td>0.73</td>
</tr>
<tr>
<td>8</td>
<td>Mixture(^%) 1 g/L</td>
<td>1.00</td>
<td>1.04</td>
<td>0.73</td>
</tr>
<tr>
<td>9</td>
<td>Mixture(^%) 0.5 g/L</td>
<td>0.50</td>
<td>0.52</td>
<td>0.36</td>
</tr>
<tr>
<td>10</td>
<td>Mixture(^%) 0.5 g/L</td>
<td>0.50</td>
<td>0.53</td>
<td>0.37</td>
</tr>
<tr>
<td>11</td>
<td>Biosolids 1 g/L</td>
<td>1.00</td>
<td>1.05</td>
<td>0.73</td>
</tr>
<tr>
<td>12</td>
<td>Biosolids 1 g/L</td>
<td>1.00</td>
<td>1.06</td>
<td>0.74</td>
</tr>
<tr>
<td>13</td>
<td>Greenwaste 1 g/L</td>
<td>1.00</td>
<td>1.04</td>
<td>1.04</td>
</tr>
<tr>
<td>14</td>
<td>Greenwaste 1 g/L</td>
<td>1.00</td>
<td>1.02</td>
<td>1.02</td>
</tr>
</tbody>
</table>

\(^*\)Ratio of water to sediment depth.

\(^%\) 10:1 Biosolids : greenwaste based on wet weight.

#Biosolids and greenwaste used had 30% moisture content.

The experiment commenced on 7th of September 2004 (T=0). Initially nitrogen was bubbled through the water in each bucket with the aim of inducing anaerobic conditions to promote early establishment of SRB. Nitrogen bubbling proved inadequate for consistently maintaining anoxic conditions in buckets across all the treatments however and after 77 days the experiment was altered, with the addition of a highly bioavailable organic substrate (sucrose) that was intended to serve two purposes. Firstly, the initial breakdown and consumption of the sucrose by aerobic microbial activity would consume all oxygen and create an anoxic anaerobic environment. Secondly the remaining sucrose and its derivates would provide more “food” to enhance SRB activity.
An unforeseen effect of the sugar was an increase in acidity as it was broken down into simple compounds such as pyruvic acid. To balance the acidity from the formation of pyruvic acid more biosolids were added to some of the treatments. Sugar and biosolids were added to the buckets at the following times and rates:

- On day 77, Buckets 5 to 14 received 2.9 and 1 g/L of sucrose to each treatment replicate respectively, e.g., bucket 7 had 2.9 g/L added and Bucket 8 had 1 g/L added,
- On day 90, Buckets 1 to 4 received 0.1 g/L of sucrose
- On day 90, a further 18 grams of biosolids (wet) was added to buckets 9 and 10, to neutralise pyruvic acid
- On day 92 another dosing of 18 grams of biosolids (wet) was added to buckets 7, 8, 9, 10, 11 and 12, to neutralise pyruvic acid

The final dose rates after sucrose and biosolids amendments are listed in Table 5-2.
Table 5-2 Final Dose Rates for the application rate experiment

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Original Design</th>
<th>Final Application Rate g/L</th>
<th>Final Application Rate g/L (Dry#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ratio* 100 to 1</td>
<td>5.16</td>
<td>3.64</td>
</tr>
<tr>
<td>2.</td>
<td>Ratio* 100 to 1</td>
<td>5.18</td>
<td>3.65</td>
</tr>
<tr>
<td>3.</td>
<td>Ratio* 200 to 1</td>
<td>2.56</td>
<td>1.82</td>
</tr>
<tr>
<td>4.</td>
<td>Ratio* 200 to 1</td>
<td>2.70</td>
<td>1.92</td>
</tr>
<tr>
<td>5.</td>
<td>Mixture% 2 g/L</td>
<td>4.94</td>
<td>4.32</td>
</tr>
<tr>
<td>6.</td>
<td>Mixture% 2 g/L</td>
<td>3.05</td>
<td>2.44</td>
</tr>
<tr>
<td>7.</td>
<td>Mixture% 1 g/L</td>
<td>4.93</td>
<td>4.32</td>
</tr>
<tr>
<td>8.</td>
<td>Mixture% 1 g/L</td>
<td>3.04</td>
<td>2.43</td>
</tr>
<tr>
<td>9.</td>
<td>Mixture% 0.5 g/L</td>
<td>5.41</td>
<td>4.65</td>
</tr>
<tr>
<td>10.</td>
<td>Mixture% 0.5 g/L</td>
<td>3.53</td>
<td>2.77</td>
</tr>
<tr>
<td>11.</td>
<td>Biosolids 1g/L</td>
<td>4.94</td>
<td>4.32</td>
</tr>
<tr>
<td>12.</td>
<td>Biosolids 1g/L</td>
<td>3.06</td>
<td>2.44</td>
</tr>
<tr>
<td>13.</td>
<td>Greenwaste 1g/L</td>
<td>3.93</td>
<td>3.62</td>
</tr>
<tr>
<td>14.</td>
<td>Greenwaste 1g/L</td>
<td>2.02</td>
<td>1.72</td>
</tr>
</tbody>
</table>

*Ratio of water to sediment depth.

% 10:1 Biosolids : greenwaste based on wet weight

# Biosolids and greenwaste used had 30% moisture content

Observations were conducted approximately weekly over the duration of the 273 day experiment, which concluded on the 7th of June 2005. Monitoring frequency varied in response to the rate of change of parameters measured, becoming more frequent when change was more rapid.
Water quality was measured using the same instruments as in the material scoping experiment detailed in Chapter 4:

- Temperature and pH were measured using a WTW 330i pH meter,
- EC was measured using a YSI Model 33 Temperature, salinity, Electrical Conductivity meter
- DO was initially measured using a YSI Model 57 Temperature Oxygen Meter, which was then found to be faulty. DO monitoring was ceased, and the data discarded.

Water quality testing for total nitrogen, total phosphorus, and sulphide was carried out by Deakin University Water Quality Laboratory. Room temperature ranged from 15 to 30 °C over the duration of the experiment, and as the experiment vessels were not insulated, the temperature of the reaction mixtures increased to a maximum of 23 °C.
5.4 Results & Discussion

Water Quality results are summarised in Table 5-3. Water temperature ranged from 15 to 23 °C and all vessels remained within one degree of each other on any given day.

Table 5-3 Results for application rate determination experiment, summarising initial (T=1 day) pH and electrical conductivity (EC) results, and final (T=273 days) pH, Electrical Conductivity (EC), Total Phosphorus (TP), Total Nitrogen(TN), and Sulphide.

<table>
<thead>
<tr>
<th>Vessel – Final Application rate mg/L (dry)</th>
<th>pH</th>
<th>EC (µS/cm)</th>
<th>TP*</th>
<th>TN*</th>
<th>Sulphide*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T=1</td>
<td>T=273</td>
<td>T=1</td>
<td>T=273</td>
<td>mg/L</td>
</tr>
<tr>
<td>AMD reference</td>
<td>3.27</td>
<td>N/A</td>
<td>1750</td>
<td>N/A</td>
<td>0.25</td>
</tr>
<tr>
<td>1. – Mixture 3.64</td>
<td>3.43</td>
<td>7.52</td>
<td>1750</td>
<td>1400</td>
<td>4.7</td>
</tr>
<tr>
<td>2. – Mixture 3.65</td>
<td>3.8</td>
<td>7.57</td>
<td>1750</td>
<td>1400</td>
<td>5.3</td>
</tr>
<tr>
<td>3. – Mixture 1.82</td>
<td>3.59</td>
<td>7.25</td>
<td>1750</td>
<td>1500</td>
<td>2.8</td>
</tr>
<tr>
<td>4. – Mixture 1.92</td>
<td>3.45</td>
<td>7.26</td>
<td>1750</td>
<td>1350</td>
<td>3.3</td>
</tr>
<tr>
<td>5. – Mixture 4.32</td>
<td>3.41</td>
<td>6.01</td>
<td>1750</td>
<td>1350</td>
<td>6.3</td>
</tr>
<tr>
<td>6. – Mixture 2.44</td>
<td>3.65</td>
<td>7.86</td>
<td>1750</td>
<td>1350</td>
<td>3.8</td>
</tr>
<tr>
<td>7. – Mixture 4.32</td>
<td>3.39</td>
<td>7.58</td>
<td>1750</td>
<td>1400</td>
<td>6.7</td>
</tr>
<tr>
<td>8. – Mixture 2.43</td>
<td>3.4</td>
<td>7.54</td>
<td>1750</td>
<td>1350</td>
<td>2.7</td>
</tr>
<tr>
<td>9. – Mixture 4.65</td>
<td>3.32</td>
<td>6.48</td>
<td>1750</td>
<td>1500</td>
<td>5.3</td>
</tr>
<tr>
<td>10. – Mixture 2.77</td>
<td>3.34</td>
<td>7.27</td>
<td>1750</td>
<td>1350</td>
<td>3.4</td>
</tr>
<tr>
<td>11. – Bio+Sug 4.32</td>
<td>3.36</td>
<td>7.83</td>
<td>1750</td>
<td>1400</td>
<td>6.8</td>
</tr>
<tr>
<td>12. – Bio+Sug 2.44</td>
<td>3.36</td>
<td>7.24</td>
<td>1750</td>
<td>1400</td>
<td>3.1</td>
</tr>
<tr>
<td>13. – GW+Sug 3.62</td>
<td>3.72</td>
<td>4.1</td>
<td>1750</td>
<td>1400</td>
<td>0.14</td>
</tr>
<tr>
<td>14. – GW+Sug 1.72</td>
<td>3.72</td>
<td>8.2</td>
<td>1750</td>
<td>1300</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Mixture = 10:1 Biosolids : Greenwaste substrate, plus sugar and biosolids additions

Bio+Sug = Biosolids only, plus sugar and biosolids additions

GW+Sug = Greenwaste only, plus sugar additions

*TP, TN and Sulphide concentrations at Time (T) = 273 days
5.4.1 pH

Increases in pH were measured in all treatments over the 273 day experiment. Figure 5.3 shows the variation of pH over time. The dramatic changes at 70-80 days, following the addition of sugar and biosolids are clearly evident in the graph.

Initial pH increases were proportional to dose rates, reflecting the chemical neutralisation capacity of the biosolids. After the addition of sugar to vessels 5-14, pH decreased rapidly as sucrose was broken down to simpler compounds such as pyruvic acid, by initial microbial degradation. A distinct sweet aroma and clouding of the water was noticeable at this stage. Vessels 1-4, which had sucrose added at much smaller rate also experienced the drop in pH but to a lesser extent.

Approximately one week after sucrose was added, all vessels except 5 and 13, exhibited a changing environment. The target sulphate reducing bacteria (SRB) appeared to establish, indicated by hydrogen sulphide odours. Accompanying this, increases in pH and a change in water colour were noted. The pH increase was initially rapid, with pH moving from 4 to 7 over ~ 30 days, and then slowed for the remainder of the experiment. In vessels 5 and 13 and to some extent in vessels 7 and 9, the generation of acidity resulted in the inhibition of microbial activity (Sawyer et al. 1994). In vessel 13 microbial activity appeared to cease and no further change in pH was observed for the remainder of the experiment. Figure 5-4 shows the cloudy water colour in vessel 13 and the dark black taint to the water of vessel 5, as it is in an active SRB anaerobic phase.

In general the range of final pH was comparable to that achieved in the material scoping experiments, and was achieved using much smaller application rates (in the order of 1-3% by weight) compared to those used in the material scoping experiments.
Figure 5-3 Variation of pH over time for the 14 treatments in the application rate determination experiment.
Figure 5-4 Vessel 1 full and empty, and vessels 13 and 5 at the conclusion of the experiment
5.4.2 Electrical Conductivity

Electrical conductivity (EC) decreased in all treatments from an initial 1750 μS/cm to an average of 1390 μS/cm, with a range from 1300-1500 μS/cm (Figure 5-5).

Figure 5-5 Electrical Conductivity at the start and conclusion of the application rate determination experiment

The decrease in EC measurements across all treatments is most likely due to the loss of ions, and precipitation of minerals from biological activity and chemical reactions, such as:

- Loss of sulphate and hydrogen ions
- Changes in the concentrations of carbonates, nitrogen oxides, phosphate oxides and ammoniums, as organic matter is remineralised, consumed or precipitated
- Loss of metals as metal sulphides or oxyhydroxides

At the conclusion of the experiment when the vessel lids were removed and water was drained, precipitate was clearly evident (Figure 5-4).
5.4.3 Nutrients – Phosphorus and Nitrogen

Total phosphorus (TP) concentration increased significantly in all biosolids treatments with the maximum concentration of 6.8 mg/L recorded in vessel 11 (Figure 5-6). TP concentrations reduced in the greenwaste plus sugar treatments by ~ 50% compared to phosphorus concentrations of the untreated acid mine water.

Figure 5-6 Total Phosphorus (TP) concentrations at the conclusion of the application rate determination experiment

Total nitrogen (TN) increased in ten of the fourteen treatments, with the average TN of all treatments being 2.42 mg/L, compared with 1.1 mg/L for the untreated AMW. Results ranged from a less than detection limit result of <0.01 mg/L for treatment 13, to the highest concentration of 8.5 mg/L for treatment 11 (Figure 5-7).
Figure 5-7 Total Nitrogen Concentration at the conclusion of the application rate determination experiment

The increase in the nutrient concentrations of total nitrogen and phosphorus in AMW is a desirable outcome, assuming that these high concentrations do not create adverse effects, such as eutrophication and algal blooms. The test results show more modest increases in nutrient concentrations than in the scoping experiments, which is proportional to the smaller application rates.

5.4.4 Sulphide

Sulphide above the analytical detection limit was detected only in vessel 5, however a hydrogen sulphide-like odour was noted by olfactory senses in all vessels except vessel 13, some time after the addition of sugar. Sulphide was detectable in Vessel 5, as at the time of sampling SRB were still active, as indicated by rising pH. In the other vessels SRB activity had slowed significantly and sulphide production had reduced as a result of this. In the case of vessel 13 (Figure 5-4), it appeared that SRB activity did not get under way, and thus a very low sulphide result would be expected.
5.5 Key Points and Conclusions

This experiment showed the initial remediation of water quality following the biosolids and greenwaste additions. The experiment also demonstrated that SRB bioremediation, which clearly occurred after amendments with sugar, can increase the pH of acidic water up to 8. Although the additions of sugar would be unlikely to be repeated in the field, the final application rates of the experiment remained in the range that could be feasible and realistic to achieve at full scale application. As Hockin and Gadd (2007) point out, “...the procurement of plentiful, cheap substrates is also something of a problem in achieving economic efficiency.” (p. 423).

The key question regarding “how much material will be needed to remediate an acid mine lake?” has been better defined in this experiment, with optimal rates being in the range of 2 - 4 g/L. This rate is much less than other bioremediation experiments conducted to date, such as application rates of 320 g/L (Harris et al. 2000) and 60-100 g/L (McCullough et al. 2006), although it should be noted that determination of application rates was not the primary aim in these experiments.

Some range in application rates is expected due to the multiple variables associated with acid mine water bioremediation. These variables include the nature of the acid water and organic materials, experiment methodology, and environmental factors such as temperature. McCullough et al. (2006) used “...dosing rates realistic of that able to be achieved....using regional sources” (p. 1181). In contrast, the study by Harris et al. (2000) on sludge and rye grass appears to be primarily a scoping study of the materials to determine what proportion of each would achieve the best results for application in a bioreactor rather than a lake environment. Fyson et al. (1998) used preliminary experiments to develop dosing rates for their phosphate rock additions and their “potato” dose rate was...
designed to "...determine the effects of decomposable organic matter addition..." on the process (p. 234).

Prior to the completion of this round of experimentation it was unclear if feasible application rates of biosolids and greenwaste SRB bioremediation would produce significant or even measurable improvements to acid mine water quality, and more specifically, increase the pH. After augmenting the experiment with sucrose additions, the experiment results support the potential of SRB bioremediation at feasible "real world" application rates. A key learning point of the experiment was the importance in supplying enough organics of a form that is suitable for microbial consumption, leading to the consumption of oxygen and the subsequent creation of anoxic conditions required for the SRB process.
6 Field Bioremediation Experiment

Following on from the two phases of laboratory-based experiments detailed in Chapters 4 and 5, it was decided that field or pilot trials should be conducted, to test the ability of biosolids and greenwaste to promote SRB bioremediation in in-situ conditions. Mesocosm experiments aim to replicate as best as possible, actual in-situ conditions, while remaining at a scale that allows replication, and that is feasible to manage.

Ideally, this phase of field experiments would have been conducted at the Anglesea mine site. At Anglesea, however, an acid mine lake does not exist, and the creation of a suitable site was not an option at the still-operational mine site. It was decided that the Wensleydale coal mine lake, given its similar geology and proximity to Anglesea, would be suitable for running field scale trials. The only negative aspect of using the Wensleydale coal mine lake as an experiment site was that its pH was ~ 4.5 – 5, whereas the initial pH of a lake at Anglesea is expected to be ~ 3 - 4. However as other researchers such as Brugam et al (1995), have successfully stimulated the bioremediation process in low pH environments, with out conventional neutralising agents, the positive aspects of the field experiment testing substrate and limnological factors was considered appropriate.

6.1 Aims

The primary aim of the field trials was to determine the effects of two application rates of bioremediation substrate (a mixture of 1:1 by weight of biosolids and green waste), in a deep water mine lake environment. The primary measures of the experiment would be changes in the water quality parameters of pH, alkalinity and nutrient concentrations.
A secondary aim of the trials was to monitor the effects of bioremediation on other water quality parameters including metal concentrations, and phytoplankton and zooplankton abundance and diversity.

### 6.2 Mesocosm and Limnocorrals use in research

Mesocosms (or as they are referred to in limnology, “limnocorrals”) are used across a range of science and research fields. They are generally used to conduct experimentation outside a laboratory, aiming to replicate “real life” or in-situ conditions as best as possible, while at the same time maintaining scalar practicality. They are also used for experimentation that may not be suitable in an open environment, such as where there may be possible adverse effects on the ecosystem being studied.

Bloesch et al. (1988) state that “…in-situ studies (limnocorrals), laboratory experiments and field investigations in lake systems are all necessary and complementary approaches to ecological problems. In large lakes, biomanipulation experiments cannot be performed but limnocorrals provide a practical alternative.” (p. 307). Caquet et al. (1996) supported this view, describing mesocosm experimentation as “…an intermediate level of study between laboratory and field” (p.125).

Limnocorrals have been used previously for AMD treatment research. Brugman et al. (1995) in their acid neutralisation mesocosm test state that “…experiments in enclosures have the advantage that adequate controls for the experimental manipulations can be designed. Enclosures are also more manageable logistically than whole lakes.” (p.154)

The use of mesocosms does, however, impose some limitations. Bloesch et al. (1988) notes that “…the use of mesocosms …is linked with problems of scaling. With respect to complexity of the enclosed system, our limnocorrals containing 70m$^3$ of lake water were much closer to lake conditions than to laboratory cultures.
However deviation of control limnocorals from the lake were caused by the relative small size of the enclosures preventing horizontal advection and decreasing vertical eddy diffusion.” (p.307)

Other complexities created by mesocosm experiments are increased variability. “The benefit of the limnocorral technique is at the same time its crux, because the system, while closer to nature, is very complex and therefore not easy to understand.” (Bloesch et al. 1988, p. 298).

Recently in AMD treatment trials, Martin et al. (2003) conducted limnocorral biomanipulations to test the effects of additions of algal nutrients and dissolved organic matter, on metal scavenging rates by biogenic particles.

In the current experiment, 8 limnocorals, were purpose-built tubular structures of 1.8m diameter, 12m long and made from polyethylene tarpaulin material. They were then supported from a pontoon platform, which was also used to mount a weather station and to undertake sampling from. A variety of mesocosm and limnocorral styles and structures have been used by researchers, and some examples are listed in Table 6-1.
<table>
<thead>
<tr>
<th>Researchers</th>
<th>Application</th>
<th>Description and characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brugam et al. (1995)</td>
<td>Neutralisation of coal mine lakes by addition of natural organic matter</td>
<td>• mesocosms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• surface to bottom of lake</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Cylindrical tubes 3m long and 1m diameter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• polyethylene</td>
</tr>
<tr>
<td>Edgar and Green (1994)</td>
<td>Calanoid copepod grazing on phytoplankton</td>
<td>• limnocorral</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• in situ lake environment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• cylindrical 4m long and 0.8m diameter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• polyethylene</td>
</tr>
<tr>
<td>Liber et al. (1997)</td>
<td>Persistence and fate of 2,3,4,6-tetrachlorophenol and pentachlorophenol in</td>
<td>• 12 in situ in lake environment</td>
</tr>
<tr>
<td></td>
<td>limnocorals</td>
<td>• 5x5m surface area, 4.5m deep</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• polyvinyl chloride walls with a polyethylene liner</td>
</tr>
<tr>
<td>Caquet et al. (1996)</td>
<td>Eco-toxicological studies</td>
<td>• Excavated ponds, 5 x 4m area, 1.5m deep 12m³ approx</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Lined with black PVC</td>
</tr>
<tr>
<td>Schiff and Anderson (1987)</td>
<td>Limnocorral studies of chemical and biological acid neutralisation in 2 freshwater lakes</td>
<td>• 29 limnocorals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 1m diameter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• installed in 1.2m of water with Styrofoam floats and a wooden support structure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• nylon reinforced polyethylene</td>
</tr>
<tr>
<td>Martin et al. (2003)</td>
<td>Bioremediation of water quality in pit lakes, specifically metal removal</td>
<td>• 16 limnocorals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 1.8 m diameter 12m long</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• polyethylene tarp material</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• supported in the lake from rafts.</td>
</tr>
</tbody>
</table>

In summary, mesocosm (limnocorral) field experiments are a logical step between laboratory and full scale trials or actual application of research findings. This intermediate step has advantages over the laboratory by testing research in more realistic conditions, but has the disadvantage of removing control, thereby adding more variables and uncertainties.
6.3 Experiment Design - Materials and Methods

6.3.1 Experiment Design

The design of the experiment was based on six mesocosms (a number determined by the project’s budget). The six mesocosms allowed three sets of duplicated application rates of organic sediment to be tested, and included:

- Mesocosms 1 and 2 - “Controls”
  - no substrate additions
- Mesocosms 3 and 4 - “Low application rate”
  - bioremediation substrate applied at rate of 0.2 g/L
- Mesocosms 5 and 6 - “High application rate”
  - bioremediation substrate applied at rate of 2 g/L

The application rates of 0.2 g/L and 2 g/L of a 1:1 biosolids + greenwaste mixture by weight, were determined based upon the results from the laboratory experiments described in Chapter 5, and also from consideration of practical limits of a full scale application (based on preliminary information, an application rate of 2g/L would require approximately 10 years of the region’s biosolids and greenwaste production).

6.3.2 Mesocosms

The design of the mesocosms was developed in conjunction with the manufacturer, with reference to mesocosms used in trials at the Equity Silver Mine near Houston, Canada (Martin et al. 2003). A critical factor in the design was the mesocosms’ length. To ensure an environment that is representative of a deep lake, the mesocosm base needed to be situated in the hypolimnion, below the thermocline, for the entire annual stratification cycle. Monitoring of Wensleydale coal mine lake (Chapter 3) indicated that the thermocline reached depths of ~12m,
so it was decided that the mesocosms should be 16m long. A prototype mesocosm was constructed and installed, to test its suitability. Following the successful deployment of the prototype, some minor changes were made and the final mesocosm specifications and features (Figure 6-1) were:

- 16m long, 1.2m diameter, cylinder shape, closed at the base and open at the top
- constructed from green Canvacon 5000E, that was sewn and heat welded
- fibreglass battens were inserted into sleeves at 2m intervals, to ensure a cylindrical shape was maintained
- a flotation collar was incorporated into the top of the mesocosm
- tether points were attached to the base and top of the mesocosm

**Figure 6-1 Photos of mesocosm stretched out during assembly, and the fibre glass batten installed in the batten sleeve**

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### 6.3.3 Mesocosm Installation

A site at the northern end of the Wensleydale coal mine lake was selected for the experiment because it was removed from the high-use beach area at the southern end, and had a suitable depth of water ~ 20m. A floating support frame was
constructed to hold the mesocosms in place. It consisted of 150mm diameter PVC pipe and fittings glued into a rectangle approximately 4m by 3m. Additional support and buoyancy was added to this frame using treated pine and hardwood timbers, 25 L plastic drums, and 90mm diameter PVC pipe. The frame was held in location by a mooring line (weighted by bricks and metal chain) to the south, and 2 anchor lines fixed to trees on the lake’s northern shore (Figure 6-2).

Figure 6-2 Mesocosm experiment location indicated by white box. Black lines indicate mooring lines
The method of mesocosm deployment (Figure 6-3), using two boats and four people, was completed as follows:

- The mesocosm was towed, floating, towards the experiment site from the beach at the southern end of the lake
- Approximately 50m from the experiment site, the mesocosm opening was held partially under the water to commence drag filling until a point where it became physically impracticable
- Small weights were attached to the bottom of the mesocosm, to assist in sinking, and to align the mesocosm vertically in the water column
- The mesocosm was slowly manoeuvred into place and secured to the support frame
- The mesocosm was then completely filled, using a fire fighting pump (sucking water from ~5m depth) to transfer water into the mesocosm until it overflowed
Figure 6-3 Photos of the mesocosm deployment sequence
6.3.4 Biosolids and Greenwaste Substrate Application

Biosolids and greenwaste were gathered by the same method as for the material scoping experiment described in Section 4.2. The biosolids were oven-dried to remove all moisture and to ensure destruction of pathogenic organisms. Dried biosolids were accurately weighed into application amounts and transferred into containers for transportation. Samples of greenwaste were oven-dried to determine their moisture content. Greenwaste, as collected, was then accurately measured into dry weight application amounts and transferred into containers for transportation.

Based on a mesocosm volume of ~18,100 L, the following total dry weights were added to the mesocosms:

- M3 and M4 – “Low application rate”
  - 1.81 kg of biosolids and 1.81 kg of greenwaste
- M5 and M6 – “High application rate”
  - 18.10 kg biosolids and 18.10 kg of greenwaste

Dosing was completed on 5 July 2005 after the initial (T=0) monitoring of each mesocosm was completed (Figure 6-4). The biosolids sank immediately when applied, while approximately 50% of the greenwaste appeared to sink below the surface while the remainder floated partially submerged or on the water’s surface.

Assuming the combined mixture had a bulk density of 0.5 g/cm³ the thickness of the created sediment layers were:

- M3 and M4 ~ 0.6 cm
- M5 and M6 ~ 6.0 cm
Figure 6-4 Photos of the application of biosolids (top left) and greenwaste (top right) into mesocosms, and all 6 mesocosms 30 minutes after dosing (bottom)
6.4 Review and modification of methodology

Field experiments such as at Wensleydale are acknowledged to reduce control and increase exposure to external variables (Bloesch et al. 1988). The experimental design had some limitations that enabled some external variables to affect the experiment, including birds perching on the mesocosms and waves overtopping the rim of the mesocosms.

On each monitoring occasion, the equipment was assessed for integrity and evidence of any external influences. The first remedial action concerned the prototype design. After installation of the prototype mesocosm (M1), a number of changes were made, the most significant being increasing the size of the flotation collar, which had initially provided little freeboard to prevent lake water incursion from wave overtopping.

The second occasion on which problems were noted was in August 2006, one month into the experiment. The mesocosms, which had been floating with acceptable freeboard in July (at the time of dosing), had lowered significantly in the water. Mesocosms M5 and M6, that had the most weight added (approximately 37 kg), were particularly low in the water. The top of their collars was sitting just above the surface of the lake, and the boat wake was observed to wash into them as it was docked against the support frame. Additional flotation was added to the structure in the following week.

In February, a decision was made to augment the experiment, by adding a “super” dose to M4 (original dose 0.2 g/L). An additional application of 4g/L (~ 75kg) of 1:1 biosolids and greenwaste was added. During the March monitoring trip the additional weight was observed to have lowered the whole structure in the water and M4 had only 2-3 cm of freeboard. Within the next month more flotation was again added, to raise the structure and mesocosms higher out of the water.
Other external factors observed during the experiment included:

- continued lake water incursion, as a result of reduced freeboard, wave action in storms, and from water skiing
- bird excrement and feathers entering the mesocosms as the structure became a perch (perching was discouraged with physical barriers such as nails.)
- filamentous algae colonised the mesocosm material, both internally and externally

These external factors were not evenly applied to all mesocosms. Mesocosms adjacent to the main body of the lake received more water incursion and the birds had favourite perching positions on certain area of the support frame and mesocosms.

On each monitoring occasion, observations such as whether bubbles were generated when the mesocosm or sediment were disturbed (the boat docking or from the monitoring probes) were noted. Also the presence of hydrogen sulphide odour was tested for when water samples were collected.

Overall the experimental setup performed satisfactorily, achieving the intended goal of replicating the lake environment, particularly its thermal stratification pattern.
6.5 Monitoring Program and Methodology

6.5.1 Monitoring Program

The monitoring program was designed to measure physical, chemical and biological changes in the mesocosms. A judgement was made on the frequency that each parameter should be monitored, based on its expected rate of change and the importance of the measurement in answering the experiment’s primary aims.

Some alterations to the initial monitoring program occurred during the course of the experiment. The alterations and the reasoning behind them were:

- ceasing chlorophyll a analysis. Colonising algae were primarily of the filamentous type attached to the walls of the mesocosm. Water column chlorophyll concentrations were extremely low except in instances where filamentous algae were also collected in the water column sample. On this basis, the sampling process was deemed unrepresentative and a suitable replacement was not determined.

- removal of sulphide analysis. From previous experiments a smell test for presence or absence of hydrogen sulphide was considered appropriate

The completed monitoring program is presented in Table 6-2.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Details</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature, Dissolved Oxygen, % Dissolved Oxygen, Turbidity, pH and Electrical Conductivity</td>
<td>Instrument - YeoKal 611 Water column profile</td>
<td>Monthly</td>
</tr>
<tr>
<td>Alkalinity as CaCO$_3$ equivalents.</td>
<td>E &amp; H* Field Titration to pH 4.8 using standardised HCl (0.01 M)</td>
<td>Monthly</td>
</tr>
<tr>
<td>Total Nitrogen, Oxidised Nitrogen(NO$_x^-$), Total Phosphorus, and Soluble Reactive Phosphorus(SRP),</td>
<td>E &amp; H* NO$_x$ and SRP field filtered</td>
<td>Monthly</td>
</tr>
<tr>
<td>Metals - various</td>
<td>E &amp; H* Total and filtered</td>
<td>Bi-monthly</td>
</tr>
<tr>
<td>Sulphate and Chloride</td>
<td>E &amp; H*</td>
<td>Bi Monthly</td>
</tr>
<tr>
<td>Phytoplankton diversity</td>
<td>Composite sample 1-5 m depth with Lund Hose Pipe. Grab samples at 9 &amp; 13 m depth with Horizontal Sampler</td>
<td>Quarterly</td>
</tr>
<tr>
<td>Zooplankton diversity and abundance</td>
<td>Sampled with a Schindler Trap at, 0m, 6m and 12m depths.</td>
<td>Quarterly</td>
</tr>
</tbody>
</table>

* E & H = Epilimnion (samples ranged from 2-6m depths) and Hypolimnion (samples ranged from 10-15m depths) water layers in the mesocosm.
6.5.2 Physical Profiles

A YeoKal 611 Intelligent Water Quality Analyser (manufactured by Yeo-Kal Electronics Pty Ltd Australia) was used to physically profile the water column in each mesocosm, as well as the water in the lake itself, adjacent to the mesocosms. The instrument was checked and calibrated before each monitoring occasion. The meter probe was kept away from the walls of the mesocosm during data collection. Intervals between data collection depths varied on each field trip, but were in the range of 1-4m, with the exception of the metalimnion in the thermocline, and the anoxic zone at the oxycline, which were monitored at 0.5 m intervals.

6.5.3 Water Samples

All water samples were collected using a WILDCO horizontal depth sampler attached to a rope which was calibrated at 1m intervals. Single samples were collected from the upper section of the mesocosm (between 2-6 m depth) and in the lower section (between 12-15m depth) on each field trip. When the lake was stratified, samples were collected from the epilimnion and hypolimnion water layers (Table 6-3).

Samples were then dispensed from the sampler into bottles prepared appropriately (e.g. acid washed for nutrients and trace metals) for the parameters to be analysed. Samples for filtered metals, nitrate, nitrite and soluble reactive phosphorus (SRP) were filtered through a 45 μm glass fibre paper, and all metal samples were acidified with reagent grade nitric acid (0.1 mL acid per 50 mL sample). SRP is also referred to as filterable reactive phosphorus (FRP), however as some colloidal particulate matter may pass the filter, SRP has been used throughout this thesis. Samples were kept cold on ice in an esky prior to delivery to the laboratory, and were then frozen for later analysis. All water testing was completed by Deakin University Water Quality Laboratory, Warrnambool.
Table 6-3 Water Sample depths (in m) for the period July 2005 – August 2006

<table>
<thead>
<tr>
<th>Date</th>
<th>Upper</th>
<th>Lower</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Epilimnion)</td>
<td>(Hypolimnion)</td>
</tr>
<tr>
<td>5 July - 05</td>
<td>8*</td>
<td>8*</td>
</tr>
<tr>
<td>10 Aug - 05</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>6 Sept - 05</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>5 &amp; 25 Oct – 05</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>7 Nov – 05</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>12 Dec - 05</td>
<td>2.5</td>
<td>12</td>
</tr>
<tr>
<td>14 &amp; 31 Jan - 06</td>
<td>2.5</td>
<td>12</td>
</tr>
<tr>
<td>14 Feb - 06</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>20 Mar - 06</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>20 Apr - 06</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>10 May – 06</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>7 Jun - 06</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>2 Aug - 06</td>
<td>4</td>
<td>14</td>
</tr>
</tbody>
</table>

*Single sample taken – water column not stratified

6.5.4 Alkalinity

A field burette, standardised HCl (0.01 M) and a WTW pH meter were used to titrate samples to a pH end point of 4.8. Titrations were usually completed within 6 hours of sample collection and all titrations were completed within 24 hrs of collection (American Public Health Association 2005b). Results are reported as mg/L (CaCO₃ equivalent).
6.5.5 Phytoplankton

Phytoplankton samples were collected by two methods for species diversity analysis. A composite sample of 0-5m depth was collected using a Lund hose pipe (approximately 1.5 L sample) and then drained into a clean bucket. The sample was then concentrated using an algae net (20 micron) into a 60 mL specimen jar. The second method of sample collection involved lowering a WILDCO horizontal water sampler (~2.5 L) to depths 9m and 13m to capture samples. The same procedure of draining the sample into the bucket and concentrating the sample using the algae net was used to concentrate the samples into 60 mL specimen jars.

On the first occasion the samples were preserved with Lugol’s Iodine, for later species analysis. On subsequent trips, preservative was not added, but sample analysis was completed within two days, as identification was considerably more efficient with fresh samples. Standard microscope analysis and reference material were used for identification (Entwisle et al. 1997).

6.5.6 Zooplankton

Zooplankton samples were collected using a Schindler Trap (approximately 24 L) with an outlet strainer screen of 80 microns. The handle of the trap (approximately 60 cm above the base of the trap) was lowered to 3 sample depths of 0m, 6m and 12m. Once drained, the sample collected on the strainer was carefully washed into 60 mL specimens jars. On return to the laboratory, samples were preserved with ethanol. Analysis of the type and number of zooplankton was completed by homogenising (hand agitation) the samples made up to 60 mL, and sub-sampling 5mL using a syringe. The sub-sample was then emptied onto a counting tray for identification and quantification of species. Repeat analyses were randomly conducted for approximately 1 in 3 samples.
6.6 Results

6.6.1 Thermal Stratification

Thermal stratification is a key process in creating the anoxic hypolimnion that is required to stimulate sulphate reducing bacteria (SRB) activity. The thermal stratification pattern of the lake adjacent to the mesocosms (Figure 6-5) correlated well to the previous year’s pattern, which had been monitored in the lake centre.

Figure 6-5 Thermal stratification of the lake adjacent to the mesocosms, July 2005 to August 2006.

Stratification commenced in September, with warming of the top 1m of the lake. Warming of the epilimnion continued until a maximum temperature of ~ 22 °C was reached in January 2006. From this date, the epilimnion cooled gradually and turnover or mixing occurred between June and August 2006. Throughout the
course of the experiment, there was little variation between the thermal profiles of the lake and the mesocosms. On any month the difference in temperature between the mesocosms and the lake at any given depth was generally less than 0.2 °C. Figure 6-6 shows typical temperature profiles of the lake and the 6 mesocosms.

Figure 6-6 Temperature profiles of water columns in the 6 mesocosms and in the adjacent lake (March 2006)

The temperature profiles of all mesocosm at important stages of the annual stratification pattern are presented in Table 6-7.
Figure 6-7 Thermal stratification in the 6 mesocosms in a) July 2005, b) October 2005, c) January 2006, d) May 2006, e) August 2006

a) July 2005

b) October 2005

c) January 2006
Maximum surface water temperature exceeded 22 °C in January 2006 (Figure 6-7c). The thermocline at this time extended from approximately 5m to 10-12m. The hypolimnion showed negligible change in temperature, from 10.3 to 10.5 °C over the duration of the experiment. The metalimnion reached a maximum depth of approximately 14m in autumn (May) at which time the epilimnion temperature was ~13.7 °C and the hypolimnion was ~10.4 °C (Figure 6-7d). Turn over of the water column was estimated to have occurred shortly after the June monitoring trip when the water column temperature range was only 11.5 to 12 °C. By the August monitoring trip, the water column temperature ranged from ~10 °C at 16m, to ~10.4 °C at the surface (Figure 6-7e).
This stratification pattern was similar to the previous year’s (2004-05) monitored in the middle of the lake. In 2004-05 the maximum epilimnion temperature reached slightly above 22 °C and the metalimnion reached a maximum depth of ~10m in May.

Thermal stratification is favourable for the bioremediation process. The establishment of a thermocline in September 2005 created a hypolimnion that was isolated from re-oxygenation until at least June 2006. In this 9 month period of anoxic conditions, assuming that other bioremediation necessities were met, the bioremediation process using enhanced sulphate reducing bacteria (SRB) activity should have established.

### 6.6.2 Bioremediation Indicators – Dissolved Oxygen, pH and Alkalinity

The process of bioremediation requires the establishment of a suitable anoxic environment for sulphate reducing bacteria (SRB). The thermal stratification of the lake described in the previous section isolated the hypolimnion layer, hindered reoxygenation, and marked an initiating step in creating the desired anoxic environment. The other key elements for promoting SRB activity are the provision of both a substrate for colonisation, and nutritional requirements. This was achieved by the addition of a rich organic sediment, made up of biosolids and greenwaste at a ratio of 1:1 by dry weight. M3 and M4 were treated at a low application rate (0.2 g/L), M5 and M6 were treated at a high application rate (2 g/L), and no additions were made to the experimental controls, M1 and M2.

Throughout this section, results for DO, pH and alkalinity are considered together. Results are then reported, in terms of the water quality, in the four following sections:

- the adjacent lake
- the control mesocosms (M1 and M2)
- the low application rate (M3 and M4)
- the high application rate (M5 and M6).

### 6.6.2.1 Adjacent Lake

In the adjacent lake water column, dissolved oxygen (DO) (Figure 6-8) and pH (Figure 6-9) showed similar behaviour to that of the previous year’s monitoring in the middle of the lake. These profiles provided a baseline for comparison to the experimental mesocosms.

**Figure 6-8** Dissolved Oxygen (% saturation) profiles of the lake water column adjacent to the mesocosms

DO in the epilimnion remained over 80% saturated throughout the year (Figure 6-8). The hypolimnion experienced a gradual reduction in DO to ~ 60% saturation during its isolation. Lake pH remained in the range of 4.7 to 5 for the experiment period (Figure 6-9).
6.6.2.2 Control Mesocosms (M1 and M2)

The purpose of control mesocosms is to enable any influence of experimental design and external factors other than the variables of interest to be detected. The control mesocosms (M1 and M2) provided a baseline for assessment of the effect of the experiment variables, the organic sediment dose application rate.

External factors that may have influenced the experiment included lake water incursion (specifically to M1), filamentous algae growth, inputs to the mesocosms of bird feathers and excrement, and wind or rain transported materials such as dust.

Water quality parameters of dissolved oxygen (DO), pH and alkalinity are profiled for M1 and M2 in 3-month periods throughout this section. Results for the first 3 months of the experiment are shown in Figure 6-10.
At the start of the experiment (July) both mesocosm profiles were similar. DO was at approximately 90% saturation, and pH was ~ 5 to 12m. Below 12m, M1 showed a decrease in oxygen levels to ~ 40% saturation, and pH ~ 6 at 15.5m depth. M1 had been deployed as a pilot in the lake for approximately 2 months prior to July. The cause of DO reduction in M1 may be attributable to the reduced mixing of the water column induced by the thermocline (Figure 6-7), and also in part by the long narrow structure of the mesocosm. Replenishment of DO through mixing was hindered, and oxygen present was being used in microbial breakdown of dead algae in the accumulated sediment. The pH increase supports this explanation, and suggests that a natural bioremediation process had established in M1 in a period of approximately 2 months.
Alkalinity results for samples taken near the surface (4m) and at depth (12m) ranged from 0.3 to 1.3 mg/L during the period.

Figure 6-11 shows profiles for the next 3 months of the experiment (October to December 2005).

Figure 6-11 M1 and M2 dissolved oxygen (a) and pH (b) profiles for October to December 2005

During this time period, the effect of thermal stratification became more evident in the profiles below the thermocline (~ 5m). A decline in DO accompanied a slight increase of 0.1 - 0.2 in pH. The profiles of M1 and M2 were very similar for this period with the exception of the mesocosm bottom (below 15m). By December the M2 water profile below 14m began to replicate M1. The establishment of reduced DO and higher pH in M2 below 14m occurred over a slightly longer time frame than for M1 (~ 5 months). DO concentrations were reduced to less than 20%
saturation and associated with this, the pH increased to almost 6.5 at the bottom of both mesocosms.

Alkalinity showed no increase from the previous period. Samples taken in the epilimnion (2.5 and 4 m) and in the hypolimnion (12 and 14 m) ranged from 0.5 to 1.6 mg/L.

The profiles of M1 and M2 in mid to late summer (January to March 2006) are shown in Figure 6-12.

**Figure 6-12 M1 and M2 dissolved oxygen (a) and pH (b) profiles for January to March 2006**

In mid to late summer the influence of stratification was clearly reflected in the DO and pH profiles. DO in the epilimnion was ~ 90% saturated, while pH remained relatively constant in each mesocosm, at approximately 5.3 and 5 for M1 and M2 respectively. The drop in M1 pH to 4.8 – 5, first evident in December,
corresponded to the start of water skiing on the lake. Given that surface water pH in M1 is comparable to that of the lake, it is believed that waves resulting from water skiing overtopped M1 (the prototype mesocosm with lowest freeboard) causing a pH reduction. Further lake water incursion also occurred to M1 after M4 was given an additional biosolids and greenwaste dose in February. This lowered the mesocosms’ support frame, again reducing the freeboard of M1.

Below the thermocline, DO reduced from ~ 80% to ~ 60% saturated at 15m (Figure 6-12a). In the bottom 1m of both mesocosms DO reduced to very low oxygen levels (< 30% saturated). A corresponding increase in pH of ~0.5 occurred below the thermocline. In both mesocosms a pH of 6 was reached during this time period.

Alkalinity results again showed little change in the January to March period. Samples taken in the epilimnion (4 m) and in the hypolimnion (13 and 14m) ranged from 0.1 to 1.5 mg/L.

Profiles for the last 3 months of the experiment, April to August 2006, are shown in Figure 6-13.
Profiles over autumn continued to show the influence of the thermocline on DO and pH levels. As the thermocline weakened and deepened, so did oxygenated water of the epilimnion (~90% saturation). The hypolimnion in autumn had been isolated for over half a year and DO concentrations reduced to below 20% saturation in the bottom 1m of the mesocosms.

The pH of the water at the bottom of M1 was similar to that of its surface water in April and May, and this may have been due to lake water incursion. By June however, the pH below 14m had increased. In M2, pH in the epilimnion increased by approximately 0.2 pH units over autumn, and the water below 14m continued to show an abrupt increase in pH.
By June, breakdown of stratification was imminent, and by August the temperature difference between the surface and bottom water was less than 0.5 °C. Post mixing, the DO and pH profiles still varied with depth, unlike profiles for temperature, reflecting inefficient mixing within the mesocosms. DO decreased from ~ 80% saturated at 14m to almost anoxic just above the base of the mesocosm. pH increased to over 6.5 within the same depth range.

Alkalinity showed no significant increases in the autumn period, with one exception. Results still ranged from 0.1 to 1.5 mg/L in both M1 and M2, however in June, the M2 hypolimnion sample, taken at 15m, had an alkalinity of 4.2 mg/L. The M1 sample from the same depth had an alkalinity of 1.1 mg/L. DO and pH profiles indicated an abrupt water quality change below 14m and this was also reflected in the M2 result.

DO, pH and alkalinity profiles indicate that some bioremediation activity had developed at the base of the mesocosms. When the mesocosms were removed from the lake, in September 2006, a black sediment layer had accumulated on the base of both mesocosm (Figure 6-14) supporting this interpretation of the profiles.

Figure 6-14 Black sediment in the base of Mesocosm 1 on completion of the experiment
The control studies in M1 and M2 show that “natural” processes in the mesocosms were capable of initiating changes in water quality. This included changes due to SRB activity in the very bottom waters of the mesocosms. The control mesocosms also suggest that:

- some autochthonous generation of organic matter was stimulated by the provision of a substrate in the photic zone for algae colonisation
- sedimentation rates in the mesocosms were approximately 2mm per year, from algae, water and wind transported particulates and possibly bird excrement
- alkalinity did not change significantly in the main water column during the experiment

6.6.2.3 Low Application Rate (M3 and M4)

A low application rate of 0.2 g/L (~ 3.6 kg biosolids/greenwaste mixture total), was added to M3 and M4. As was done for the control mesocosms, results for DO, pH and alkalinity in M3 and M4 are presented in 3-month periods throughout this section. Figure 6-15 shows profiles for the first quarter (July – September 2005).
M3 and M4 were ~95% saturated with DO, and had a pH of 5 over the entire water column at the commencement of the experiment (July 2005). An effect of the treatment was seen in both mesocosms in August. The pH increased to approximately 5.4 between the surface and 15m, and an increase in pH to 6.5 was measured in the bottom 1m. DO reduced to ~ 80% saturated below 8m. By September 2005 low DO levels (<40% saturation) were measured below 15m, and pH had also increased to ~ 6.5.

Alkalinity results for the period ranged from 0.5 to 1.2 mg/L in samples taken from the both the epilimnion and the hypolimnion.
In spring (October to December 2005), very low oxygen concentrations were found at the base of the mesocosm, and the oxycline between the hypolimnion and the epilimnion strengthened (Figure 6-16).

Figure 6-16 M3 and M4 dissolved oxygen (a) and pH (b) profiles for October to December 2005

DO profiles remained similar for the period, with good replication between M3 and M4. In December the thermocline and oxycline coincided between 4 and 6 m. Below the oxycline, DO reduced gradually from 80% saturated to ~ 60% at 14m. Below 14m, a sharp reduction in DO occurred, with anoxic conditions present at the base of the mesocosm. Corresponding to this DO decrease was a sharp pH rise (from ~ 5.5 to ~ 6.5). The bulk of the water column remained at pH 5.5, except in the top 2 m of the water column, where pH increased to 6 and 6.5 for M3 and M4 respectively. The increase was probably due to algal photosynthesis decreasing dissolved CO₂.
A difference between the epilimnion and hypolimnion alkalinity started to become apparent in this time period. Epilimnion alkalinity ranged from 0.8 to 1.3 mg/L and from 1.3 to 1.9 mg/L in the hypolimnion.

After monitoring in February, an additional dose of organic sediment was added to M4. By March a dramatic effect was seen (Figure 6-17).

Figure 6-17 M3 and M4 dissolved oxygen (a) and pH (b) profiles for January to March 2006

Over the summer months of January and February, DO continued to fall in the hypolimnion. pH increased at 15m - 16m depth for both mesocosms. The epilimnion DO continued to range from 90 - 100% saturated, and pH increased in the top 6m of the water column, reaching 6.6 in M3 in February, most likely as a result of algae photosynthesis. Alkalinity of the epilimnion was 1.3 mg/L indicating that little buffering capacity was added by algae. Other epilimnion
alkalinity results for the period ranged from 0.5 to 2.2 mg/L. Alkalinity results in the hypolimnion ranged from 1.2 to 3 mg/L, excluding M4 in March. One month after the additional dose, M4 hypolimnion alkalinity was 27.5 mg/L. Corresponding changes in DO and pH profiles were also found in M4 in March (Figure 6-17). The hypolimnion below 9m became anoxic, and pH increased to ~ 6.5. Epilimnion pH in M4 was reduced to ~ 5.2, probably as a result of lake water incursion.

In autumn (April – August 2006) as the thermocline deepened, a corresponding shift in DO and pH profiles was evident in M3 and M4 (Figure 6-18).

Figure 6-18 M3 and M4 dissolved oxygen (a) and pH (b) profiles for April to August 2006

The DO profile of M3 remained at ~ 80% saturation in the epilimnion as it deepened from May to June, while the pH was ~ 6.2. In the hypolimnion, the large DO decrease and pH increase were still present.
M3 (like M2) recorded its highest alkalinity (6.5 mg/L) for the June sample taken at 15m. Other results ranged from 2 to 3.4 mg/L for both the upper and lower water column samples. Following turnover in August, alkalinity was reduced to 1.4 mg/L and 1.2 mg/L in the upper and lower water column respectively.

M4 remained strongly influenced by its additional dose of biosolids and greenwaste through autumn and into winter. A reduction in epilimnion oxygen concentrations was observed each month and by June an increase in the epilimnion pH was apparent. In August the pH from the surface to 10m was ~ 6.2 with a further increase to ~ 6.8 close to the sediment. Alkalinity results in the upper section increased from 1.3 mg/L in April to 3.6 mg/L in August. Samples from the hypolimnion indicated strong microbial activity with alkalinity increasing from 35 mg/L to 43 mg/L to 107 mg/L, for the months April, May and June respectively. In August a reduction in alkalinity to 37 mg/L occurred after lake turnover.

The results indicate that the low application rate of 0.2 g/L of organic sediment induced an anoxic environment at the sediment-water interface. Increases in pH were initially caused by the neutralisation capacity of the biosolids, with subsequent changes resulting from microbial activity.

The additional dose of 4 g/L of biosolids and greenwaste to M4 had a proportionally large effect on the system. The dose suggested there was a eutrophication risk associated with the addition of the organic sediments, as outlined by Wendt-Potthof and Neu (1998), who stated that “…adding organic substrates…would have to be planned carefully to avoid permanent eutrophication of the lake.” (p. 276).
6.6.2.4 High Application Rate (M5 and M6)

A high application rate of 2 g/L of the biosolids and greenwaste mixture was added to M5 and M6 (~36 kg total). As in previous sections, results are presented in three-monthly periods, and Figure 6-19 shows profiles of dissolved oxygen (DO) and pH for the period July to September 2005.

Figure 6-19 M5 and M6 dissolved oxygen (a) and pH (b) profiles for July to September 2005

One month after dosing, profiles showed a significant reduction in DO concentrations below approximately 6m. pH increased to ~6 below 8m, and in the deepest 1m increased to ~6.5. In the upper section of the mesocosms, incursion of lake water caused pH to reduce to ~4.8, similar to that of the surrounding lake.
By September, anoxic conditions had developed at the base of both mesocosms. DO concentrations in the hypolimnion were ~40%, and a sharp oxycline developed at the thermocline region. In both mesocosms the epilimnion DO remained well saturated (90 – 110%). pH in the upper water column of M6 rose in September, approximately 2 weeks after extra flotation was added, however the pH in the upper section of M5 did not. Below 8m, pH in M5 and M6 showed good replication, and reached ~6.5 at the sediment–water interface. Alkalinity in the hypolimnion increased from less than 1 mg/L to 5.3 mg/L in M5 and 5.8 mg/L in M6. Alkalinity in the upper section of the water column remained less than 1 mg/L.

Between October and December 2005 (Figure 6-20) an oxycline extended from 4m to 15m, where water was anoxic.

Figure 6-20 M5 and M6 dissolved oxygen (a) and pH (b) profiles for October to December 2005
Below 14 m, pH remained between 6 and 6.5 in both mesocosms for the period. The reason for the decrease in pH between 9 m and 14 m in M6 in November is not known. By December the entire water column above 10 m showed a similar low pH. The effect could be due to frequent lake water incursion. M6 appears to have been the most affected by the waves because it was closest to the centre of the lake. It therefore received the brunt of the waves, and may have partially shielded the other mesocosms from the effects of lake water incursion. An alkalinity drop to 0.5 mg/L in the hypolimnion in M6 coincided with a drop in its pH in November. This supports the hypothesis that a large volume of lake water entered the mesocosm. By December alkalinity in the hypolimnion of M6 had increased to 1.9 mg/L. M5 hypolimnion alkalinity ranged from 4 to 5.3 mg/L in the period while in the epilimnion it ranged from 0.5 to 1.3 mg/L.

In the January to March quarter (Figure 6-21), an oxycline remained in the hypolimnion. Anoxic conditions persisted at the base of each mesocosm and DO rose to 80-90% saturation at the bottom of the epilimnion (~8 m). Just above the sediment, pH increased to ~6.7, and the pH profiles of each mesocosm remained similar for the 3 months. The pH of M6 was consistently lower than that of M5. Alkalinity remained low (< 1.5 mg/L) in the epilimnion for both mesocosms, but continued to increase in the hypolimnion, reaching 10.3 mg/L in M5, and 11.7 mg/L in M6 by March.
As the thermocline deepened in autumn so too did the well-oxygenated water of the epilimnion (Figure 6-22). At the same time, a general increase in water column pH was observed. Alkalinity in the hypolimnion rose to a maximum in June of 90 mg/L in M5 and 50 mg/L in M6. Alkalinity in the upper water columns in June were 4.4 mg/L in M5, and 2.5 mg/L in M6.
In August 2006 (post-turnover) the bottom waters remained anoxic, and a sharp oxycline from 0% saturated at 14m to 70% saturated at 12m was observed. The bulk of the water column between 0 and 12m was just under 80% saturated for both mesocosms. After mixing, M5 had a pH of approximately 6.4 from the surface to 12m depth, the pH then increased to almost 6.8 at the sediment. M6 had a pH of approximately 6.2 from the surface to 12m depth, which then increased to 6.6 at the sediment. Alkalinity in August was 4.3 mg/L and 12.6 mg/L in M5, and 3.9 mg/L and 12.1 mg/L in M6, for samples taken from 4m and 14m respectively.

Over 12 months, the high application rate of 2g/L of biosolids and greenwaste allowed microbial activity to create anoxic conditions at, and adjacent to the sediment, but not in the entire hypolimnion water layer. Alkalinity and pH were increased in both replicates.
6.6.3 Application Rate and Temporal Analysis of SRB Bioremediation

Suitable conditions were created in a relatively short time frame in mesocosms M3, M4, M5 and M6, in order for the targeted anaerobic SRB bioremediation process to establish. Following its retrieval in August 2006, the substrate mixture of M6 displayed dark black tainting (Figure 6-23) with a strong odour characteristic of anoxic sediments and hydrogen sulphide.

Figure 6-23 Photo of residual biosolids and greenwaste substrate in M6 in August 2006

Alkalinity (Figure 6-24), pH (Figure 6-25), and dissolved oxygen (Figure 6-26), measurements from the upper (epilimnion at 4m depth) and lower (hypolimnion 14 m depth) portions of the 6 mesocosms’ water columns for the experiment period, July 2005 to August 2006 are shown below.
Figure 6-24 Alkalinity of the epilimnion and hypolimnion of each mesocosm, August 2005 – August 2006
Figure 6-25 pH of the epilimnion and hypolimnion for each mesocosms, August 2005 – August 2006

Figure 6-26 Dissolved Oxygen (%) of the epilimnion and hypolimnion for each mesocosm, August 2005 – August 2006
Within 2 to 3 months, the addition of a biosolids and greenwaste mixture, in combination with the annual lake stratification process, created conditions that were favourable for the SRB bioremediation process. The results show that there is a general trend between alkalinity increases and substrate application rate. A gradual increase in alkalinity over time post substrate addition is also evident, which suggest a suitable environment has been maintained and sufficient nutritional requirements have not been exhausted for the various microbial processes, as outlined by Wendt-Potthof and Neu (1998).

Figure 6-24 shows that increases in alkalinity occurred in the hypolimnion. The difference between control mesocosms and low application rate although not as dramatic as high dose rates is discernable. The correlation of the graphs showing increases in pH and alkalinity, associated with decreases in dissolved oxygen levels, are consistent with sensory observations of hydrogen sulphide gas, made in the field when collecting water samples, and confirm that SRB contributed to bioremediation.

The sulphate reduction process is summarised by the following chemical equation:

\[
2(\text{CH}_2\text{O}) + \text{SO}_4^{2-}_{\text{(aq)}} + 2\text{H}^+_{\text{(aq)}} \rightarrow 2\text{CO}_2 + \text{H}_2\text{S} + 2\text{H}_2\text{O}
\]

(where CH₂O is a simplified representation of organic matter)
The reduction of sulphate consumes hydrogen ions and also generates carbon dioxide. The carbonate alkalinity relationship in natural waters, which also relates to algae photosynthesis is summarised by the following chemical equations:

\[ \text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+ \]

\[ \text{M(HCO}_3\text{)}_2 \leftrightarrow \text{M}^{2+} + 2\text{HCO}_3^- \]

\[ \text{HCO}_3^- \leftrightarrow \text{CO}_3^{2-} + \text{H}^+ \]

\[ \text{CO}_3^{2-} + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{OH}^- \]

(where M represent a positive metal ion)

From the sulphate reduction equations, stoichiometry indicates that increasing the supply of organic substrate will consume more acidity (H\(^+\)) and generate alkalinity in the forms of HS\(^-\) and carbonate equilibrium (Figure 6-27).

![Figure 6-27 Effect of application rate on mean alkalinity of the water in August 2006 (at the conclusion of the mesocosm experiment)](image-url)
Bacteria are ubiquitous (Odom et al. 1993), and so activation of sulphate reducing bacteria (SRB) should occur rapidly, given the right conditions. The high dose of 4 g/L of biosolids and greenwaste in M4 rapidly effected a large portion of the hypolimnion. An initial alkalinity increase occurred due to the neutralisation capacity of the additional organic material, however subsequent sensory observations indicated SRB activity. The increase in alkalinity from March to June 2006 demonstrated how effectively the SRB bioremediation process worked in the mesocosm environment.

### 6.6.4 Electrical Conductivity

The Wensleydale coal mine lake’s electrical conductivity (EC) is approximately 750 μS/cm. Water column profiling was conducted to monitor potential increases in EC due to the application of biosolids and green waste. The EC of the mesocosms and the adjacent lake water profiles are shown in Figure 6-28 for defining months in the experiment.
Figure 6-28 Electrical Conductivity (EC) Profiles of the lake and mesocosm in September 2005, January 2006, March 2006 and August 2006
At the commencement of the experiment in July 2005, the EC of all mesocosms was ~760 µS/cm. In September 2005, two months after dosing, the low application rate had minimal effect on EC. A change was only measured at the sediment-water interface, where EC increased to 800-900 µS/cm. The high application rate however, increased EC in the lower portion of the mesocosm (below 8m) to 800 µS/cm.

By January 2006, the effect of thermal stratification was evident. The hypolimnion EC of all mesocosms was in the range 700 - 740 µS/cm, with M5 and M6 lying at the high end of that range. In M5 and M6, EC had reduced in comparison to September 2005 levels, possibly due to a nett uptake of ions in metabolic reactions, neutralisation of H⁺ ions, and settling of particles containing absorbed ions. Below 14m, the increase in EC was proportional to the treatment dose rate. The epilimnion EC remained at ~ 750 µS/cm in all mesocosms.

In March 2006, a sharp rise in EC was measured in M4, following the biosolids and greenwaste dose augmentation, which occurred in February 2006. The hypolimnion EC increased from ~ 720 µS/cm to 840 µS/cm.

In August 2006, at the conclusion of the experiment, the EC of the water column between the surface and 10m for all mesocosms and the surrounding lake was within a range of ~15 µS/cm. M1 = 715 µS/cm, M2 = 705 µS/cm, M3 = 715 µS/cm, M4 = 720 µS/cm, M5 = 715 µS/cm, M6 = 715 µS/cm, and the Lake = 705 µS/cm. Below 10m however, EC had increased as a result of the treatment additions. Generally, the increases were proportional to the application rate, with higher doses of organic materials resulting in higher EC readings.

Values of EC measured at depths > 15m were often influenced by disturbance of the sediment by the probe. In general, at the sediment-water interface, EC ranged from ~ 750 to 900 µS/cm, but once sediment was disturbed EC could range from
1000 to 1500 \( \mu \text{S/cm} \). The maximum EC value recorded when the probe was resting on sediment was 2000 \( \mu \text{S/cm} \).

The results suggest an application of biosolids and greenwaste will increase EC, however the effect will generally be localised, adjacent to the sediment. In comparison, the lake water EC adjacent to the sediment has also been measured to increase to approximately 800 \( \mu \text{S/cm} \) (Section 3.5.5).

### 6.6.5 Nutrients

Increasing the nutrient concentrations in acid mine water (AMW) is a key part of the rehabilitation process. Stimulation of primary production in the lake is fundamental to its ecological restoration. Primary production will provide an ongoing organic load to the sediment, promoting ongoing SRB bioremediation and thus controlling acidity.

#### 6.6.5.1 Nutrient Concentrations

During the mesocosm experiment, the biosolids and greenwaste treatment is most likely to have provided the largest input of nutrients to the mesocosms. Biosolids have approximately 21 g/kg total nitrogen (TN), 20 g/kg Kjeldahl nitrogen (N), and 14 g/kg total phosphorous (TP), on a dry weight basis (personal communication South West Water Authority 2006). The greenwaste used in this experiment was not tested for nutrients, however McCullough (2006) reports greenwaste (dry) to have 0.02 g/kg Kjeldahl N, and 0.002 g/Kg TP. Throughout the experiment, other nutrient inputs into the mesocosms included wind and rain transported matter such as dust, water incursion, and bird excrement. All but the latter are likely to have been negligible sources.

Concentrations of nutrients were expected to proportionally increase, relative to the treatment application rate. Concentrations were also expected to vary over
time, due to differences in generation and consumption rates, such as remineralisation from the substrate, and uptake by phytoplankton. Table 6-4 lists the total nitrogen (TN) and oxides of nitrogen (NOx), and Table 6-5 lists total phosphorus (TP) and soluble reactive phosphorus (SRP) concentrations in July 2005 (commencement), and August 2006 (conclusion) and the range of nutrient concentrations from monthly monitoring. At commencement of the experiment, when the water columns were assessed as being homogenous, only 1 sample was taken, at 8m. In August 2006, although temperature profiling indicated no thermal stratification, other physical profiles of DO and pH indicated changing water quality with depth, and hence 2 samples were taken, one at 4m and the other 14m.

<table>
<thead>
<tr>
<th>Mesocosm</th>
<th>TN mg/L</th>
<th>NOx mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T=0*</td>
<td>T=13*</td>
</tr>
<tr>
<td>M1 Epi</td>
<td>0.67</td>
<td>0.68</td>
</tr>
<tr>
<td>M1 Hypo</td>
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<td>0.84</td>
</tr>
<tr>
<td>M2 Epi</td>
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<tr>
<td>M2 Hypo</td>
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<td>0.77</td>
</tr>
<tr>
<td>M3 Epi</td>
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<td>0.72</td>
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<tr>
<td>M3 Hypo</td>
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<td>1.1</td>
</tr>
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<td>M6 Epi</td>
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</tr>
<tr>
<td>M6 Hypo</td>
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</table>

* = months
* = of monthly analysis
Epi = Epilimnion   Hypo = Hypolimnion
Detection Limit = 0.01 mg/L

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Table 6-5 Total phosphorus (TP) and soluble reactive phosphorus (SRP) concentrations at the commencement (T=0 months) and conclusion (T=13 months) of the field mesocosm experiments

<table>
<thead>
<tr>
<th>Mesocosm</th>
<th>TP mg/L</th>
<th>SRP mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T=0*</td>
<td>T=13*</td>
</tr>
<tr>
<td>M1 Epi</td>
<td>&lt;0.01</td>
<td>0.15</td>
</tr>
<tr>
<td>M1 Hypo</td>
<td>0.04</td>
<td>&lt;0.01 - 0.12</td>
</tr>
<tr>
<td>M2 Epi</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>M2 Hypo</td>
<td>0.06</td>
<td>&lt;0.01 - 0.06</td>
</tr>
<tr>
<td>M3 Epi</td>
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</tr>
<tr>
<td>M3 Hypo</td>
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</tr>
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<td>0.05</td>
</tr>
<tr>
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<td>0.02 - 0.62</td>
</tr>
<tr>
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<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>M6 Hypo</td>
<td>0.1</td>
<td>0.01 - 0.33</td>
</tr>
</tbody>
</table>

* = months
Epi = Epilimnion  Hypo = Hypolimnion
Detection Limit = 0.01 mg/L,
+ = of monthly analysis

6.6.5.2 Application Rate and Temporal Analysis of Nutrients

The largest increases and ranges of nutrient concentrations were measured in the high application rate mesocosms (M5 and M6), and also in M4 after its additional dose of 4g/L of biosolids and greenwaste (Table 6-4 and Table 6-5). M4, which received the highest application rate of the biosolids and greenwaste substrate, had the largest increases in nutrient concentrations.

Epilimnion and hypolimnion nutrient results for all mesocosm have been averaged (0.005 mg/L has been used in calculations to represents <0.01 mg/L) and further averaged into 3 distinct periods of the experiment. These time periods include:
"Winter 2005" (July - September 2005), "Stratified" (October 2005 - April 2006), and "Winter 2006" (May - August 2006). This manipulation of the data summarises the general trends of nutrient concentrations for each mesocosm, and results for Total Nitrogen (Figure 6-29), Nitrogen Oxides (Figure 6-30), Total Phosphorous (Figure 6-31), and Soluble Reactive Phosphorus (Figure 6-32) are presented below.

Figure 6-29 Average Total Nitrogen Concentrations per mesocosm for "Winter 2005", "Stratified" and "Winter 2006" time periods
Figure 6-30 Average Nitrogen Oxide concentrations per mesocosm for “Winter 2005”, “Stratified” and “Winter 2006” time periods

Figure 6-31 Average Total Phosphorus concentrations per mesocosm for “Winter 2005”, “Stratified” and “Winter 2006” time periods
The initial winter 2005 period clearly shows the effect of the substrate additions, particularly in M5 and M6 where the increases in nutrient concentrations were most pronounced.

In the summer stratified period, the effect of the additional dose to M4 was apparent. Across all measures, M4 had the highest average concentrations. The effect of the low application rate (0.2g/L) in M3 is less distinguishable during this time period, when compared to the control mesocosms M1 and M2. The high application rate mesocosms, M5 and M6 continued to have higher nutrient concentrations compared to the controls.

In the last period, winter 2006, concentrations in M4 remained high when compared to the other mesocosms. In general, TP and SRP concentrations in the other mesocosms were proportional to application rate, however NO₃ and TN concentrations were not. In M4, M5 and M6, the effect of anaoxic conditions in the
hypolimnion may have reduced NO$_3$ concentrations, as ammonia (NH$_3$) is preferentially produced by remineralisation. Lake water incursion into M6 may also have affected the concentrations of nutrients, especially in the epilimnion.

The thermal stratification cycle of the lake, will ultimately distribute nutrients from the substrate throughout the lake water. Overall, nutrient concentration increases measured in the water columns appeared to be related to treatment dose rate additions.

6.6.5.3 Nutrient Bioavailability

The ratios of NO$_3$ to TN, and SRP to TP indicates nutrient bioavailability. Both NO$_3$ and SRP are simple inorganic forms of the nutrients, that are readily taken up by phytoplankton (Barsanti et al. 2006). Bioavailability is a key indicator of how the addition of the substrate may stimulate primary production. Having established that nutrient concentrations can increase as a result of organic substrate additions, the benefits will be limited if the nutrients are in a form that cannot be easily utilised in primary production.

Speciation ratios are presented in Figure 6-33 and Figure 6-34 following the same temporal treatment of data ("Winter 2005", "Stratified" and Winter 2006") as used in the previous section.
Nitrogen bioavailability was good (~ 50% or more of nitrogen is in a bioavailable form) in the first two periods, with NO\textsubscript{x} : TN ratios ranging from approximately 0.5 to 0.65. Nitrogen bioavailability was highest in M5 and M6, the "high application rate" mesocosms. In the last period, winter 2006, NO\textsubscript{x} : TN ratios decreased in all mesocosms except M1. This may be due to available nitrogen being consumed in the epilimnion, and the anoxic conditions in the hypolimnion of M4, M5 and M6 resulting in the formation of ammonia rather than NO\textsubscript{x}. Ratios of NO\textsubscript{x} : TN in August 2006 following lake turnover (and reoxygenation of deep water) were: M1 = 0.72; M2 = 0.25; M3 = 0.45; M4 = 0.92; M5 = 0.8; M6 = 0.5.

Although no pattern is obvious in terms of application rate, the results show that a significant proportion of N is bioavailable, mostly in the surface layer where primary production occurs (see Table 6-4).
In the control mesocosms, M1 and M2, SRP concentrations were generally below the detectable limit (0.01 mg/L) and so M1 and M2 have been omitted from the graphs. For the treated mesocosms (M3 to M6), results were consistently above the detection limit and phosphorus bioavailability appeared to be high. On average, ~ 50-60% of total phosphorus was bioavailable for the entire experiment.

6.6.5.4 Nitrogen to Phosphorus Ratios

The ratio of nitrogen to phosphorus in water indicates which nutrient is likely to limit primary production of phytoplankton and macrophytes. Mesocosm experiments showed other factors, such as habitat, can also play an important role as filamentous algae colonised the surfaces of all of the mesocosms, including the untreated control mesocosms.
Of the substrates added, the biosolids had an approximate TN to TP ratio of 1.5 (South West Water Authority biosolids testing data, 2004) and greenwaste, as tested by McCullough et al. (2006) has an approximate ratio of 10:1 TN:TP.

In studies of Victorian western district lakes (De Deckker et al. 1988; Mitchell et al. 1987) total nitrogen was assessed as limiting below a TN:TP ratio of 10. Total phosphorus was considered limiting above a ratio of 17 (Forsberg et al. 1978). The ratio of TN to TP for the mesocosm experiment is presented in Figure 6-35.

![Figure 6-35 Mean Total Nitrogen (TN) to Total Phosphorus (TP) ratios per mesocosm for “Winter 2005”, “Stratified” and Winter 2006” time periods](image)

The average TN:TP ratio from the initial July 2005 (T = 0) sampling across all mesocosms was approximately 70, with a range from 31 to 134 (not shown). In all mesocosms therefore, phosphorus was the limiting nutrient, based on the criteria described above. The ratios decreased immediately, and over time post-treatment, suggesting that phosphorus became less limiting over time. As the biosolids have a very low TN:TP ratio, this could be due to additional phosphorous being added by remineralisation. A relatively low TN:TP ratio in M1 for the winter 2006 period
results from one high TP measurement in the epilimnion in August (0.15 mg/L). This result is an outlier compared to the other 12 lower measurements, and could be due either to analytical error, or contamination by bird excrement. M2 consistently showed TP as the limiting nutrient throughout the experiment. In general, by winter 2006, the addition of substrate had lowered the TN:TP ratio below the balanced range of 10 to 17, and toward N limitation.

Abundant phosphorus (i.e. nitrogen limitation) can lead to the dominance of blue green algae, which can meet nitrogen requirements by other means, such as fixation from the atmosphere (ANZECC 2000; Smith 1983). Blue green algae are not desirable, due to their potential toxicity. Although in studies of Lake Purrumbete (Mitchell et al. 1987), TN to TP ratios indicated nitrogen limitation, blue green algae was not identified as becoming dominant or problematic.

In this experiment, the results suggest that the addition of organic substrate with a relatively high phosphorus concentration has improved the TN : TP ratio in the water column from its initial phosphorus limited state. In any application of a biosolids-greenwaste substrate for bioremediation, a balancing of nutrient ratios could be achieved by further additions of nitrogen or phosphorus fertilisers if required.

### 6.6.6 Metals

Analysing for a range of metals in the water column was completed, to monitor any changes in concentration due to additions of the biosolids-greenwaste substrate. Firstly there was potential for metals to be released from the biosolids and greenwaste, thereby increasing concentrations. Secondly, decreases in concentrations could result from precipitation by pH increases and reaction with hydrogen sulphide, or uptake by biogenic processes (Martin et al. 2003). From previous testing in 1990 by the Victorian Rural Water Commission, and initial
testing in this study, it was established that Wensleydale coal mine lake had very low metal concentrations (Table 3-3).

Sampling for metals was completed every second month during the experiment, however not all samples were analysed given the low concentrations detected. “Dissolved” (ie. filterable) metal analysis was completed for aluminium, copper, iron, manganese and zinc, for the months of July and September 2005 (after addition of organic substrate) and March 2006. “Total” metal analysis was completed for aluminium, arsenic, cadmium, chromium, cobalt, copper, iron, lead, manganese nickel, tin and zinc, for the months of July 2005, January 2006 and May 2006.

Total concentrations of cadmium, chromium, lead, and tin always had low concentrations below their detection limits (cadmium 0.0001 mg/L, other metals 0.001 mg/L), throughout the experiment. Arsenic was measured in 3 mesocosms on one occasion in May 2006 (0.002 mg/L in M4, and 0.001 mg/L in M5 and M6). Dissolved copper testing did not return a result above the detection limit of 0.01 mg/L for any mesocosms, at any stage throughout the experiment.

Of the remaining metal results, filterable concentrations for the initial July 2005 testing and a sample set near the conclusion of the experiment (March 2006) are detailed in Table 6-6. Total metals are compared for July 2005 and May 2006 in Table 6-7.
Table 6-6 Filterable metal concentration (mg/L), July 2005 and March 2006

<table>
<thead>
<tr>
<th>Metal</th>
<th>July-05</th>
<th>Mar-06</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Max</td>
</tr>
<tr>
<td>Al</td>
<td>0.21</td>
<td>0.25</td>
</tr>
<tr>
<td>Fe</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Mn</td>
<td>0.11</td>
<td>0.12</td>
</tr>
<tr>
<td>Zn</td>
<td>0.09</td>
<td>0.11</td>
</tr>
</tbody>
</table>

*E = epilimnion, H = hypolimnion

Table 6-6 shows that filterable aluminium and zinc concentrations reduced across all mesocosms. Concentrations of iron and manganese showed little change. As evident from the range of locations where maximum concentrations were measured, no patterns relating to substrate application rates were identified.

Table 6-7 Total metal concentrations (mg/L), July 2005 and May 2006

<table>
<thead>
<tr>
<th>Metal</th>
<th>July-05</th>
<th>May-06</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Max</td>
</tr>
<tr>
<td>Fe</td>
<td>0.2</td>
<td>0.23</td>
</tr>
<tr>
<td>Al</td>
<td>0.17</td>
<td>0.18</td>
</tr>
<tr>
<td>Cu</td>
<td>0.004</td>
<td>0.005</td>
</tr>
<tr>
<td>Mn</td>
<td>0.116</td>
<td>0.119</td>
</tr>
<tr>
<td>Zn</td>
<td>0.078</td>
<td>0.123</td>
</tr>
</tbody>
</table>

* E = epilimnion, H = hypolimnion

Table 6-7 shows that average and maximum concentrations of total metals reduced from July 2005 to May 2006. Both M4 (~ 4g/L substrate) and M1 (control) recorded the majority of the maximum concentrations in May 2006, again suggesting no direct correlation between substrate application rate and metal concentrations.
The metal analyses indicate that no increases in metal concentrations occurred in the water column as a result of substrate addition. Over time, general decreases in metal concentrations were measured across all mesocosms, suggesting that biogenic scavenging like that tested by Martin et al. (2003), or biosorption similar to that tested by Norton (2004) may be the most influential factor, rather than pH increase or reactions with hydrogen sulphide. In particular, M3 consistently had the lowest metal concentrations of all mesocosms. Speculatively this may be due to M3 not experiencing significant lake water incursion, in combination with the low rate of substrate addition. Importantly, the results indicate that biosolids and greenwaste are unlikely to increase metal concentrations.

6.6.7 Sulphate and Chloride

Sulphate and chloride concentrations were measured in each mesocosm every 3 months to monitor any changes due to organic substrate additions. Chloride concentrations in the upper and lower section of the mesocosms are presented in Figure 6-36 and Figure 6-37 respectively.

Figure 6-36 Chloride concentration in the upper section (epilimnion) of the mesocosms
Chloride concentrations remained relatively constant, indicating that organic sediment addition had no significant effect on chloride concentrations.

Sulphate concentrations in the upper (epilimnion) and lower (hypolimnion) are presented in Figure 6-38 and Figure 6-39 respectively.
Sulphate concentrations increased by approximately 30-40% across all mesocosms during the experiment period. Increases were initially seen in the deeper water samples, nearest the sediment. At the conclusion of the experiment, the concentrations ranged from 43 to 50 mg/L, and no direct relationship between application rate and sulphate concentration was evident. Sulphate increases in mesocosm M3-M6 may be at least partially attributed to substrate additions, given that biosolids have a total sulphur content of ~ 5 g/kg (personal communication SWWA), however increases in the control mesocosms cannot be due to this. The following possibilities were assessed as an explanation for the observed increases:

- Leaching from materials – the “Canvacon” material is derived primarily from polyethylene and is not a likely source of sulphate.
- Laboratory error – Deakin’s Water Quality Laboratory is NATA accredited and the error using method APHA 4500 Sulphate is +/- 1 mg/L. The observed changes in concentrations were an order of magnitude larger than this.
- Addition of biosolids – does not explain increases in controls, and no obvious trends were observed between application rate and sulphate concentrations. (Assuming the sulphur content of biosolids of 5 g/kg then at a dose rate of 2g/L of biosolids added in the ‘super’ dose to M4, the
amount of sulphur added equates to 10 mg/L. This figure is approaching the range of increases observed however, the 2g/L dose was only added to M4 and the calculation also assumes that all sulphur present in the biosolids was released into the water column.)

- Algal growth – all mesocosms were observed to have a proliferation of filamentous algae during the experiment. Sulphur is present in freshwater algae at a ratio of approximately 1 S atom to 100 C atoms (Barsanti et al. 2006). During algal decomposition by microbial processes sulphur would be released as sulphate in aerobic conditions. The observation of black sediment on the floor of the control mesocosms (see Figure 6-14) supports an explanation based on increased sulphate concentrations from decay of algae.

- Bird excrement – this factor is very hard to quantify. A small number of random deposition events are unlikely to produce a similar general trend across the mesocosms, however over the long time period of the experiment (~ 1 year), the possibility that all mesocosms received a similar excrement loading becomes more plausible.

- Local environment – the lake water, which was not sampled, is also a possible source of additional sulphate concentrations. Section 3.3 on Wensleydale climate provides rainfall data over the duration of the experiment. For the period July 2005 to August 2006 only 527 mm was recorded compared to the annual average of 737 mm. The lower than average rainfall combined with ongoing spring and groundwater inflows could shift the relative proportions of inputs into the lake. An increased proportion of groundwater could increase sulphate concentrations given the pyritic material present in geological strata. Unfortunately testing of sulphate concentrations of spring or groundwater was not completed. It is possible that sulphate concentration did increase in the lake and that this effected the mesocosm sulphate concentrations through ingress (this could have affected some mesocosm more than others, i.e. the mesocosms facing the centre of the lake).
The interval between sampling (3 months) means that processes with time scales of days to weeks, would not be detected. Increased frequency of sulphate sampling may have given better evidence for the possible causes of the sulphate increases. Also more comprehensive spatial sampling of sulphate in the water column may also have provided better evidence. SRB in the sediment would reduce sulphate, however this effect would be localised to the anoxic sediment. Any sulphide released into the water column would be rapidly oxidised as it has a half life of 15 - 30 minutes (Millero et al. 1987). In the case of the high dose M4, where anoxic conditions extended up into the water column, sulphate reduction was measured in the water column. The sulphate concentration decreased by 7 mg/L between April and August, when all other mesocosms showed an increase.

The most likely explanations of the general observation of sulphate increases across all mesocosms is the proliferation of filamentous algae, and or the addition of bird excrement.

As significant changes in chloride concentrations were not measured, processes such as evaporation would appear to have had minimal effect on the sulphate levels in the water. From the increase in sulphate concentration it would appear that there was an adequate supply of sulphate in the system for SRB activity and that overall, the activity did not reduce sulphate concentrations to limiting conditions.
6.6.8 Biological and Ecological Observations

Limnological investigations at Wensleydale found biological diversity and productivity in general, to be very low, especially relative to other lakes in the western district. In addition to monitoring water quality in the bioremediation experiment, biological changes which might result from the additions of biosolids and greenwaste were also investigated. Biological monitoring included:

- measuring chlorophyll a concentrations, for an assessment of phytoplankton abundance
- identification of phytoplankton diversity
- identification and quantification of zooplankton populations

Chlorophyll a testing ceased after 7 months due to difficulties associated with obtaining a representative algae sample. Filamentous algae attached to the walls of the mesocosms were the most abundant. Chlorophyll a results based on Lund hose pipe samples varied, depending how many filamentous algae were captured in the sample. Algae identification was continued throughout the experiment to monitor changes in diversity.

6.6.8.1 Phytoplankton

In August 2005, 1 month after dosing, filamentous algae was observed to be colonising the ropes and the mesocosm material, both inside and out (Figure 6-40). Before being halted, chlorophyll a analyses ranged from 0 to 45 µg/L, for 7 months across all mesocosms.
Phytoplankton taxa identified in the mesocosms included *Cryptomonas*, *Cyclotella*, *Fragillaria*, *O-ocystis*, *Peridinium*, *Klebsormidium*, *Mougeotia*, *Oedogonium*, *Rhizoclonium*, and *Ulothrix*. Some temporal variation in individual mesocosm diversity was noted, but changes appeared to be random. Although nutrient concentrations increased in the mesocosms that received sediment, it would appear that the provision of an attachment surface was the most significant factor in increasing phytoplankton production in all mesocosms. Blue-green algae were not detected in any of the mesocosms during the experiment.

6.6.8.2 Zooplankton

In July 2005 only calanoid copepods were detected in all mesocosms in small numbers at an average count of 20 (standard deviation 14) per sample (Schindler trap ~ 24 L). In October 2005 calanoid copepods, cladocera and rotifers were
identified. These zooplankton remained as the dominant taxa identified throughout the experiment. Ostracods were found, but only in M5, and only on the last two sampling occasions.

No trends in zooplankton speciation or abundance could be linked to application rates. Colonisation of the mesocosms by phytoplankton appears to have been sufficient to increase zooplankton population by providing habitat and food. In October 2005, the average calanoid copepod count from all mesocosms was 490 and the average cladocera count was 325 per sample (24 L). For the remaining sampling occasions in January 2006, April 2006 and July 2006, the average calanoid copepods counts were 277, 338, and 360, and average cladocera counts were 202, 314 and 216, respectively.

No differences in population and diversity were apparent between the mesocosms. The results indicate that zooplankton abundance is most likely to have increased as a result of the installation of the mesocosms, and the subsequent provision of an attachment surface, rather than from the additions of biosolids and greenwaste.

6.6.8.3 Biological and Ecological Discussion

The installation of the mesocosm and supporting frame in the lake created a new “hard surface” habitat in the photic zone. The lake’s morphometric characteristics limit the extent of the littoral zone. Naturally occurring hard substrates are rare in the Wensleydale coal mine lake, with a single dead tree with branches protruding from the water being the only obvious example. At the conclusion of the experiment, phytoplankton and zooplankton had increased only marginally in diversity, but abundance had increased significantly, primarily as a result of the physical structure itself. Insects such as water boatmen, spiders, and birds were also noted to utilise the new habitat. These observations suggest that holistic rehabilitation of an acidic lake could be more involved than just supplying organic
rich sediment, but may include the placement of habitat such as logs and floating barge islands, to encourage ecological and biological development.

6.7 Field Experiment Discussion

Of the many potential microbial processes that can contribute to water quality remediation, enhancing sulphate reducing bacteria (SRB) is the favoured process in bioremediation of AMD, as sulphate is generally always in abundance (Wendt-Potthof et al. 1998). Other remediation processes such as photosynthesis, which was particularly evident from changes in M2 and M3 epilimnion water quality, were observed in this experiment. The sulphate reduction process was targeted here through additions of biosolids and greenwaste, to form an anaerobic sediment substrate. Formally, 3 treatment application rates were tested - 0.2 g/L, 2 g/L and 4 g/L. However by default, a fourth rate that could be called “enhanced natural” was observed in the control mesocosms as sediment accumulated from the increased productivity observed on the mesocosms themselves, relative to the lake. This observation highlights an ongoing challenge to lake remediation, regarding how a lake's productivity can be factored into the calculations for determining a sediment application rate. This experiment, similar to those conducted by Brugram et al. (1995), Koschorreck et al. (2002), Totsche et al. (2003), and Lewis et al. (2003), has its limitations. As discussed in section 6.2, the experiment scale and time frame differs by orders of magnitude relative to lake succession, and results from this experiment can only be extrapolated with relative care, and with limitations clearly acknowledged.
6.8 Field Experiment Findings and Key Points

The field scale mesocosm experiment has demonstrated that:

- the pH of acid mine water can be raised by using additions of biosolids and greenwaste to stimulate a bioremediation process. The pH increases, specifically in the hypolimnion appear to be proportional to the rates of substrate added. Algae photosynthesis was also responsible for pH variation in the epilimnion.

- thermal stratification does not inhibit bioremediation, and more likely, improves the process by aiding the establishment of an anoxic sediment environment

- anoxia was established in all mesocosms. The length of water column which became anoxic was proportional to the dose rate - in the 4g/L application rate, anoxia extended throughout the hypolimnion, thereby providing the environment favoured by the sulphate reducing bacteria responsible for the target bioremediation process

- alkalinity was increased using additions of biosolids and greenwaste to stimulate a bioremediation process

- nutrients released by the substrates and sediment became available in the water column

- after 1 year, the remediation process was still ongoing

- some unforeseen external factors affected results, including potential leaching of sulphate from the mesocosm structure, filamentous algae proliferation with the provision of a “hard surface” substrate, and birds perching on the structure and potentially adding nutrients and biota

- concentrations of metals were not detected at problematic levels

The mesocosm experiment indicated that the acidity of Wensleydale coal mine lake water could be improved by additions of biosolids and greenwaste to form an organic rich sediment, and thereby stimulating a bioremediation process.
Importantly, the experiment demonstrated the compatibility of the bioremediation process with the annual thermal stratification patterns of deep lakes in western Victoria. SRB bioremediation could be viable, both environmentally and economically at Anglesea, if this is determined to be a desirable and acceptable outcome for the community.
7 Creating Lake Anglesea

This chapter applies findings from the limnological study of the Wensleydale coal mine lake, and from the laboratory and field phases of bioremediation experiments, to the future scenario where a lake may be created in the void created by Alcoa’s Anglesea coal mine. The poor ecological state of Wensleydale and other examples such as German mines described by Charles (1998), provide evidence that active management will be required to ensure the development of a healthy lake at Anglesea.

Active management will not only require the application of acid water bioremediation processes, but will also involve other aspects involved in lake transformation, such as planning, design and logistics. The latter are as much a part of a holistic bioremediation and rehabilitation plan as acidic water bioremediation is. These factors will be essential if government agencies’ visions that “…the health of the environment will be improving, and all enterprises will use resources in a way that maintains ecological processes…” are to be met (Corangamite Catchment Management Authority 2003).

An analysis into management options should take into consideration the local and regional environment, and how a future lake may function. As McCullough (2006a) outlines, “…the potential for pit lakes to provide benefit to companies, communities and the environment is frequently unrecognised” (p.1). In our dry continent, and with climate modelling suggesting that even drier times may lay ahead, benefits to local water management may be substantial, if the creation of a future lake at Anglesea is well-planned and well-executed.

The analysis discussed in this chapter is organised within a logical remediation framework, starting with considerations regarding the future lake’s morphological characteristics. Issues surrounding the logistics of applying an organic substrate
for the creation of an organic sediment are modelled and discussed, and the chapter concludes with a discussion on habitat creation for flora and fauna.

Because the actual size of the mine void cannot be accurately defined until mining ceases, a number of assumptions have been made regarding the life of the mine, and the volume of coal mined in this time, in order to give a final lake volume. For the purposes of this chapter the void’s volume has been assumed to be 50,000 ML, based on the fact that approximately 1 million m$^3$ of coal are mined every year, and assuming the power station and mine will close in 2019 after 50 years of operation. This volume equates to a lake of ~1 km$^2$ surface area, which is ~50m deep.

### 7.1 Morphology – shaping a mine to become a lake

At Anglesea the mining process and hence mine void shape is determined by a number of factors including geology and coal-overburden ratio, mining efficiency and economy, boundaries of the mine lease, and overburden stability. Historically this has led to the development of a void with steep-sided walls and a relatively flat floor. It is the steep-walled feature of the mine void that will produce the characteristics of very high relative depth and limited shallow or littoral zones in the future lake. The littoral zones of a lake are considered to be the most productive and hence should be maximised in designing for lake morphology (Wetzel 2001). Additionally, wetlands are a well-recognised water treatment method (Acid Drainage Technology Initiative 1998; CLAIRE 2002; Lloyd et al. 2003; Ziemkiewicz et al. 2003), therefore incorporation of wetlands around the lake’s perimeter may treat acid runoff or seepage prior to entering the lake’s main water body.
The transition from mine void to lake may need to involve the following engineering and earth moving aspects:

- mine wall stability control works to prevent slumping, particularly once the geological formations becomes saturated with water
- wave action and shore line erosion management, either by installation of shoreline beaching, or reducing shore angles to dissipate wave energy
- construction of an outlet structure, such as a reservoir outlet tower with variable water depth extraction points
- construction of a flood spillway

These works do not address the ecological need to maximise littoral and wetland zones however. The construction of significant littoral areas (0-5m water depth), fringing wetland and riparian areas to promote remediation could be incorporated into the engineering and earthmoving works. This could be achieved by either adding more fill to certain areas of the void or, if possible, by further excavation into the surrounding area. The creation of littoral areas would also help address wave and bank erosion management, and could be an economically attractive control measure compared to other options such as rock beaching.

### 7.2 Hydrology – creating a lake in the Anglesea Catchment

At Anglesea, it is likely that the mine void would fill with water without human intervention. As will be discussed, recharge of groundwater in the upper Eastern View Formation (EVF) aquifer and direct rainfall and runoff, primarily from Salt Creek, could fill the void in less than 10 years.

The task of filling the void becomes more complex however, if various alternatives to a “natural fill” are considered. These engineered alternatives may be more attractive for a number of reasons, and range from altering the local
hydrological system (for example, by connecting Salt Creek and/or Marshy Creek into the lake), to using recycled water or treated sewage that is currently discharged to the sea. These alternative scenarios to fill the mine void from various water sources (each with their own water quality) have implications for the remediation process including:

- filling time frame
- initial lake water quality
- acidity generation and transport
- bioremediation substrate application
- overall remediation and rehabilitation time frame

Furthermore, the alternative hydrological scenarios have broader implications for long term water management in the region. For example if recycled water was piped into the system, then in the longer term, the lake could act as a reservoir and the recycled water could be reused. Analysis of this option is beyond the scope of this study, but may be worthy of consideration in future planning phases.

Lessman et al (2003) suggest that to minimise acid generation and transportation, a mine void should be filled as quickly as possible with water that is as alkaline as possible. As a result of rapid filling, acid transportation will theoretically be away from the void and into the regional aquifer, assuming the hydraulic head in the lake is greater than that of the regional water table. This approach also assumes that the regional aquifer is able to receive acid flows, and that mitigation of acidity will occur through dilution.

For the various fill scenarios that seem plausible for Anglesea, a filling time frame has been calculated using a basic water balance model. Water balance models carry a degree of uncertainty depending on the model's complexity, the assumptions that are made, and estimations of data to be inputted. The main uncertainties in this application relate to the complex hydrogeology of the area (including the cone of groundwater depression around the mine and power
station), the limited amount of hydrologic data on stream flows, and the unpredictable climate (currently the region is in a record drought). Despite these uncertainties, the model estimates the time frames for filling the lake for a number of scenarios, allowing comparisons to be made.

A schematic water balance model for the Anglesea void depicting the various water inputs and outputs is shown in Figure 7-1.

Figure 7-1 Schematic hydrological model used for mine void filling of a future Anglesea Lake
The characteristics of each component are:

"Inputs":

- **"Mine Lake"** - a deficit of water is required to fill the mine lake (void) and the cone of groundwater depression in the aquifer surrounding the mine. The deficit has been assumed to be 50,000 ML.

- **"Rain and run-off"** is the direct precipitation that falls on the surface area of the mine void and its catchment. Lake surface area of 1 km\(^2\) has been estimated and an annual precipitation of 600mm used in calculations. As the void catchment area and run-off yield is uncertain, a conservative approach, assuming zero runoff, has been taken.

- **"Groundwater"** is groundwater recharge to the Upper EVF aquifer. This has been calculated assuming that the Upper EVF has an area of 45 km\(^2\), and that recharge is 15% of the 600mm annual rainfall (Sinclair Knight Merz 1994)

- **"Salt Creek"** is Salt Creek’s discharge. Based on stream flow data from 1975 to 1982, an average of 2920 ML/year has been assumed.

- **"Marshy Creek"** is Marshy Creek’s discharge. Flow data was limited on Marshy Creek, however given its similar catchment area to Salt Creek a figure of 3000 ML/year has been assumed.

- **"Recycled water"** is the volume of recycled water discharged from the Anglesea and Black Rock sewerage treatment plants. Anglesea discharges 230 ML/year, and Black Rock discharges 16,500 ML/year

- **"Flood water"** is the potential flow from either or both Salt and Marshy creeks in a flood event. A flood event describes an abnormally high flow in the creeks. A volume of 5000 ML per flood event has been assumed, based on estimated flood volumes measured in the adjacent Painkalac Catchment, which has a similar catchment area to Salt or Marshy Creeks (Pope 2006).
"Outputs":

- "Evaporation" is the water loss due to evaporation. This has been estimated at 1000mm/year from Bureau of Meteorology regional weather maps.

- "Groundwater" is the loss of water from the lake to groundwater systems. As the mine void is the hydraulic low point in the Upper EVF it is assumed that there will be no flow from the lake while it is filling.

- "Anglesea River" is the water released from the lake to provide the base flow to the estuary that has been supplied by the mine for approximately 30 years. A figure of 2 ML/day has been adopted, based on historical discharge volumes.

A summary of the water balance components and their assigned annual water volumes for the water balance calculation is listed in Table 7-1, along with an estimated potential volume range for each component.
Table 7-1 Water balance components, their assigned annual water volumes and potential range in volume.

<table>
<thead>
<tr>
<th>Water Balance Component</th>
<th>Description</th>
<th>Volume (ML/year)</th>
<th>Range (ML/Year)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inputs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rain (R)</td>
<td>600mm over the area of the mine, ~1 km²</td>
<td>600</td>
<td>400 - 800</td>
<td>Based on current rainfall</td>
</tr>
<tr>
<td>Groundwater Recharge</td>
<td>15% annual 600mm rainfall over 45 km², which in time flows to the void – lake,</td>
<td>4050</td>
<td>2700 - 5400</td>
<td>SKM (1994)</td>
</tr>
<tr>
<td>(GWR) Upper EVF</td>
<td>hydraulic low point</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt Creek (SC)</td>
<td>Salt Creek from 1975-1982 data, 8 ML/day (~ 2920 ML/year)</td>
<td>2920</td>
<td>?</td>
<td>Historical monitoring data</td>
</tr>
<tr>
<td>Marshy Creek (MC)</td>
<td>unknown - assume equivalent to Salt Creek</td>
<td>3000</td>
<td>?</td>
<td>Estimate</td>
</tr>
<tr>
<td>Recycled Water – Anglesea STP (RA)</td>
<td>Recycled water discharged from sewerage treatment plant</td>
<td>230</td>
<td>200 – 250</td>
<td>Barwon Water Website</td>
</tr>
<tr>
<td>Recycled Water – Black Rock STP (RB)</td>
<td>Recycled water discharged from sewerage treatment plant</td>
<td>16500</td>
<td>10,000 – 17000</td>
<td>Barwon Water Website</td>
</tr>
<tr>
<td>Flood Diversion (FD)</td>
<td>Diverting flood waters into the mine void from either Salt Creek, Marshy Creek, or both</td>
<td>5000</td>
<td>1000 – 10000</td>
<td>Pope (2006)*</td>
</tr>
<tr>
<td><strong>Outputs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaporation (E)</td>
<td>1000mm annually over 1 km²</td>
<td>1000</td>
<td></td>
<td>Estimated from BOM¹</td>
</tr>
<tr>
<td>Groundwater Discharge (GWD)</td>
<td>Assume no GWD as the void is hydraulic low point in the aquifer</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anglesea River</td>
<td>2 ML/day, based on historical discharges from power plant</td>
<td>730</td>
<td></td>
<td>Pope (2006)</td>
</tr>
<tr>
<td>Environmental Flow (Ang)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*from flood data for the adjacent Painkalac Catchment (Pope 2006)

¹ BOM = Bureau of Meteorology
The fill scenarios, ranging from natural to engineered, and their water balance calculations are presented in Table 7-2.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Annual Balance (ML)</th>
<th>Approx Fill Time (Years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated Lake, = GWR + R – E</td>
<td>3650</td>
<td>13.7</td>
</tr>
<tr>
<td>Isolated Lake less Anglesea River Enviro Flow = GWR + R – E – Ang</td>
<td>2920</td>
<td>17.1</td>
</tr>
<tr>
<td>Lake with Salt Ck connected = GWR + SC + R – E</td>
<td>6570</td>
<td>7.6</td>
</tr>
<tr>
<td>Lake with Salt Ck connected and diverted flood water inputs = GWR + SC + FD + R - E (assumes flood diversion every year)</td>
<td>11570</td>
<td>4.3</td>
</tr>
<tr>
<td>Lake with Salt and Marshy Ck connected less Anglesea Enviro Flow = GWR + SC + MC + R - E – Ang</td>
<td>8840</td>
<td>5.7</td>
</tr>
<tr>
<td>Isolated Lake with recycled Anglesea STP water input = GWR + R + RA – E</td>
<td>3880</td>
<td>12.9</td>
</tr>
<tr>
<td>Isolated Lake with recycled Anglesea STP less Anglesea River Enviro Flow = GWR + R + RA - E – Ang</td>
<td>3150</td>
<td>15.9</td>
</tr>
<tr>
<td>Isolated Lake with recycled Anglesea and Black Rock STP inputs less Anglesea River Enviro Flow = GWR + R + RA - E – Ang</td>
<td>19650</td>
<td>2.5</td>
</tr>
<tr>
<td>Lake with Salt Creek connected and with recycled Anglesea STP inputs less Anglesea River Enviro Flow = GWR + R + RA - E – Ang</td>
<td>6070</td>
<td>8.2</td>
</tr>
</tbody>
</table>
The aim of the water balance calculations was to estimate filling times for various plausible mine void filling scenarios. The water balance calculations show that filling time frames range from 2.5 years for the piped recycled water scenario, up to approximately 17 years for the natural fill which also provides an environmental flow to the Anglesea River. One possible option that was not modelled was the addition of seawater into the lake, which has been done at the Island Copper Mine in Canada (Wilton et al. 1998). This has not been evaluated in this study, as the protection of groundwater quality and the greater attraction of creating a potential freshwater supply are likely to rule this option out.

Of the modelled scenarios, the piping of recycled water from Anglesea and Black Rock Sewage Treatment Plants (STP), approximately 30 km, into the lake seems very promising, considering:

- the uncertainty of climate and rainfall
- the long time frame for a natural fill to occur, when compared to filling with recycled water
- the potential benefit that a quick fill may have on acidity management

Black Rock STP provides the most significant, controllable, and reliable input of water into the system and therefore would be best able to deliver some certainty to the filling process, regardless of the other possible inputs.

A final decision regarding the method of filling the mine void, and its ultimate use, will involve regional water resource and environmental managers, as well as other stakeholders such as tourism operators, fishing organisations, and the Anglesea community at large.
7.3 Bioremediation - with a constructed organic sediment

The bioremediation experiments have established that biosolids and greenwaste are suitable materials with which to construct an organic rich sediment, and to stimulate SRB activity. The experiments also defined a probable range of application rates to achieve the desired improvement in water quality. This section will discuss the issues, and practical and logistical considerations that may arise regarding the sourcing and application of organic sediment materials to a lake environment.

7.3.1 Sourcing Organic Sediment Materials

The size and scale of an Anglesea Coal Mine bioremediation project means that it will require large quantities of organic materials and therefore, ideally, they should be available locally and also be attractive economically. Biosolids and greenwaste are by far the most preferred materials based on these criteria. Production of both materials is ongoing, and because the materials are still often seen as waste products with little value, they are potentially obtainable at low or zero cost.

Anglesea, although a relatively small town, is located ~30 km from Geelong, Victoria’s second largest city with a population of approximately 200,000. Annual quantities of biosolids and greenwaste produced in the region have been gathered from Barwon Water, and Barwon Regional Waste Management Group (Table 7-3).
Table 7-3 Quantities of Biosolids and Greenwaste produced in the Anglesea Region

<table>
<thead>
<tr>
<th>Greenwaste</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>o Greenwaste collected from the kerbside in Geelong in 2005</td>
</tr>
<tr>
<td></td>
<td>~ 28,576 tonnes (wet weight).</td>
</tr>
<tr>
<td></td>
<td>o Greenwaste production from Greater Geelong, Surf Coast Shire,</td>
</tr>
<tr>
<td></td>
<td>Colac Otway Shire and Borough of Queenscliff, ~</td>
</tr>
<tr>
<td></td>
<td>38,600 tonnes/year.</td>
</tr>
<tr>
<td></td>
<td>(Source: Personal communication, Barwon Region Waste Management Group)</td>
</tr>
<tr>
<td>Biosolids</td>
<td>o Biosolids produced by Barwon Water ~ 7000 dry</td>
</tr>
<tr>
<td></td>
<td>tonnes/year (Source: Barwon Water Website)</td>
</tr>
</tbody>
</table>

Given the large quantities produced annually, bioremediation using these materials would appear feasible. Lake application rates will determine how many years’ production would be required. When rehabilitation plans are developed, a key initial step will be to confirm the supply of these materials if they are to be used.

7.3.2 Bioremediation Model – Organic Sediment

A conceptual model of the bioremediation process, based on acidity generation has been developed for Anglesea (Figure 7-2). The pH and alkalinity of the lake will be strongly influenced by water flows (and their respective water qualities) in the system. The estuary acidification and associated fish kill of October 2000 demonstrates that substantial acid flows do occur from the Anglesea River catchment. Algae and littoral zones are recognised as significant components of the bioremediation process, and they are discussed in further detail in section 7.4.
Information from the current study that has been applied into this model includes:

- Wensleydale coal mine lake appears to have remained at pH ~5 for over 15 years, based on testing in December 1989 (Rural Water Commission of Victoria 1990). Acidic springs on site (~ 100m from the Wensleydale lake) supply water of pH 3. Rain and associated runoff can input significant fresh water volumes to the lake, but this appears to only temporarily increase surface water pH (in February 2005, for example, pH rose to 6.5 at the surface following a storm). Therefore it is reasonable to conclude that currently Wensleydale is at a steady state, with pH ~5.

- The “Application Rate” bench experiments showed that pH increased from 3.3 to 5.5 after 28 days, at a dose rate of 2g/L (90:10 biosolids:greenwaste ratio). When additional organics were added in the form of sugar, a further pH rise (to a pH of 7.8 in one vessel) occurred after 270 days, as a result of SRB activity. A total dose of 3g/L (60:40 biosolids:greenwaste ratio) was applied.

- Results from the field experiment showed increases in alkalinity, and pH (5 to 5.8 from a dose of 2 g/L (50:50 biosolids greenwaste ratio)) after 1 month. After 1 year, an overall pH rise to approximately 6.3 was achieved, however the pH started at 5, which is higher than is likely at Anglesea,
where it is expected that the water will have an initial pH ~ 3, based on measurements of its current acid mine water.

- Based on the current study, an application of 2 g/L will neutralise some acidity immediately on application, by straight chemical reaction. Further acidity neutralisation, pH increase and alkalinity generation will occur from SRB activity in the sediment environment. Thermal stratification will assist the process, mixing the more neutral alkaline water that will develop adjacent to the sediment, when turnover occurs annually.

### 7.3.3 Sediment Application Strategy

To address the uncertainty associated with models, and how a bioremediation process will work at full scale at Anglesea, a staged remediation strategy tied to water volume and water quality could be adopted.

As it is likely to take a minimum of two to four years to fill the mine void (Table 7-2), and the water may come from a range of sources varying in quality, a key question arises regarding when, and at what rate, to apply the organic substrate (which will in turn become the lake’s organic-rich sediment). Three basic options exist for application, each with their own advantages and disadvantage. These options include:

- applying the substrate before the mine void is filled
- applying it after it is filled
- applying it in stages during filling.

As originally suggested by Tuttle et al. (1969), application of the substrate prior to filling the mine void should mitigate acid generation immediately, by reducing oxygen supply to the pyritic materials, and by stimulating SRB activity. This approach carries some risks however, if the application turns out to be an “overdose”. Potential problems associated with an overdose include eutrophication of water, excessive odour generation (hydrogen sulphide gas), and
groundwater pollution. The highest dose (~4 g/L) used in the field experiments did create anoxic conditions in the entire hypolimnion, indicating that overdosing is indeed a legitimate risk.

Application of the substrate after the mine void has filled with water reduces the risks associated with the pre-filling approach. A post-filling application could be tailored to the actual water quality and environment that eventuates. This advantage would be especially relevant if recycled water made up a significant portion of the water used to fill the void. Another benefit may also include an improvement to practical application logistics, with the potential to use a floating barge system for the delivery of the sediment. The drawback of this approach is in not having the treatment in place from the onset, and significant volumes of acid water may accumulate in the void until remediation is successful.

A compromise strategy is a staged application approach. This would entail a substrate application that is proportional to the volume and quality of water accumulated in the lake over the course of a year. For example, if the annual water input into the system is expected to be 5000 ML, and the majority of net water gain is assumed to occur in winter and spring, then 50% of the estimated organic sediment application would be dosed prior to this, in autumn. At the end of spring, an assessment would be made on the basis of actual data, water volume and quality, and the remaining substrate application would be determined. This approach incorporates the benefits of both pre- and post-filling applications, while at the same time being conservative, and reducing the risks associated with both approaches.

7.3.4 Application Practicalities and Logistics

The practicalities and logistics of the bioremediation substrate application will be partially dependant on strategy. Issues for consideration include the following:
• The shape of the mine void may determine access to its various parts, including its floor and walls, by different vehicles of substrate delivery. Dump trucks, for example, may be most appropriate for pre-filling application of substrate to the truck-accessible areas such as the mine floor, roads and future wetland areas. Substrate application to steeper areas may be most effective via a floating barge after the lake has been filled.

• To ensure even distribution, tracking of substrate application may be completed by GPS plotting of the areas being dosed.

• Further processing of biosolids or greenwaste may be required, depending on government regulations and requirements (to date, no known application of biosolids or greenwaste has been done to a lake in Victoria). Stock piling of these materials may be required.

• Quantities of the biosolids and greenwaste, by volumes or by weight, will need to be measured

• Quantitative mixing of biosolids and greenwaste substrate components will be necessary to a specified ratio.

### 7.3.5 Substrate Application Rate

A range of substrate application rates that would result in water quality improvement in a future Anglesea lake was determined by this study. A staged application strategy has been suggested. Importantly, this strategy provides flexibility in the process, and acknowledges uncertainties associated with bioremediation management. However, determination of an initial target application rate is essential for planning of the process. The application rate experiments and field experiments showed that a suitable dosage rate is in the range of 0.2 g/L to 4 g/L, at a 1:1 ratio of biosolids to greenwaste. Recognising that remediation may also be assisted by other processes, such as increasing littoral zones, an application of 2 g/L is recommended as an initial target rate. At 2 g/L, approximately 100,000 tonnes of organic material would be required. This is approximately 2 years of the region’s greenwaste production, and 7 years of
Barwon Water's biosolids production. The ratio of the component organics in the mixture may be varied from 1:1 to suit supply, if tests demonstrated it was suitable to do so.

7.4 Revegetation and Ecological Establishment

Aquatic revegetation of wetland and littoral areas is an important element of a lake rehabilitation process, and should be given the same attention that terrestrial revegetation is given at mine sites.

During the Wensleydale field experiments some unforeseen, but positive ecological enhancements developed on and around the mesocosms and their floating support frame. Filamentous algae colonised the new mesocosm surfaces as a substrate to grow from, and consequently, zooplankton increased in diversity and abundance in the mesocosm environment. A population of aquatic insects such as water boatmen was established, and the structure also provided a perch for bird life, such as ducks and egrets.

Given these observations, consideration should be given not only to revegetation of wetland and littoral areas but also to the creation of other physical environments as habitat. Based on observations made at Wensleydale, colonisation of any new habitat can occur rapidly.

A number of topics for further investigation regarding aquatic revegetation and habitat creation may include:

- identification of appropriate indigenous aquatic and wetland plants
- techniques for propagation of identified plants
- practical revegetation techniques for the mine lake environment
- placement of large woody debris in the littoral zone
- creation of islands

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• creation of floating islands or structures
• creation of an artificial reef (with potential ultimately for recreational diving also)

Other options such as recreational fish restocking, could also be investigated.
8 Conclusions and Recommendations

The processes in the Anglesea Catchment which led to the fish kill event in 2000, and which subsequently instigated the current PhD project have been described. Additionally a water balance model has been developed to aid in the prediction of acid generation and transport to the Anglesea River estuary, specifically large or severe episodes. Based on this study, the following conclusions can be made:

- Acid generation and transportation is a function of the catchment’s geology and hydrological cycle
- Alcoa’s coal mine and power station operations had little effect on the fish kill event of 2000
- Acid generation and transportation is an ongoing process in the catchment, and the scale of the acid flush into the estuary determines its severity and therefore the likelihood of future fish kills occurring.

The current study has investigated bioremediation of an acidic mine lake using a constructed organic sediment, as well as other potential measures to promote optimal rehabilitation. Findings from the current study have demonstrated that:

- water quality improvements, including reduced acidity, and increased alkalinity and nutrient concentrations, can indeed result from the application of a biosolids and greenwaste substrate mixture, to form a rich organic sediment.
- substantial amounts, in the order of 5 years’ worth, of regional production of biosolids and greenwaste could be used to promote bioremediation via a constructed organic sediment
- if the mine void is filled using only natural water regimes in the Anglesea catchment, the time that this takes will be weather and climate dependant, but could range from ~ 4 to 17 years
The project’s limnological investigation of the Wensleydale coal mine lake, in combination with the laboratory and field experiments on acidic water bioremediation, have led to the development of a potential future transformation process of the Anglesea coalmine, from mine void to healthy mine lake. In addition to the primary bioremediation strategy, many other variables associated with transforming a mine void into a lake have been described and discussed, including the lake’s final morphology, and the creation of adequate littoral zones, wetlands, and riparian areas.

Perhaps the most vital recommendations regarding future directions for acidic lake bioremediation in general, and rehabilitation of the Anglesea Coal Mine in particular, are that a flexible and adaptive approach to the transition process be maintained, and that a closure plan outlining all facets of the process, from legal aspects to community consultation, be developed. Building from the current research project, the closure plan should include, at a minimum:

- identification of knowledge and confidence gaps, where further data collection, work or research is warranted or needed, including:
  - hydrological data regarding stream flows for both Salt and Marshy Creeks, Upper EVF water table heights, Anglesea Catchment specific evaporation rates and other data such as vegetation types and transpiration rates.
  - hydrological modelling of filling scenarios developed from the additional data collected, allowing for more informed and confident decisions to be made on the transformation from mine void to lake
  - littoral zone and aquatic revegetation’s influence on rehabilitation, including field trials at Wensleydale coal mine lake
  - bioremediation with a constructed sediment at a larger field scale trial, such as the entire Wensleydale coal mine lake, using local biosolids and mulched greenwaste from the Geelong and Anglesea areas
- modelling whether a lake could effectively buffer severe acid flush events from the catchment, preventing a fish kill in the estuary
- establishing realistic lead times for tasks such as the activities outlined above, substrate stockpiling and processing, and aquatic vegetation propagation.

As is often the case, this project has raised as many questions as it has answered, and many questions regarding the transition from mine void to healthy lake still exist. The current project is a first step in what will no doubt be a long journey. It is hoped that with an ongoing commitment to rigorous research, planning and community consultation, the scientific community, and the Anglesea community at large can rise to the social, economic and environmental challenges of creating a healthy Lake Anglesea, for all to use and enjoy, for generations to come.
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