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Absorption of Nitrogen from a Liquid by Pine Bark

A thesis submitted in partial fulfilment of the requirement for the Degree of Masters of Applied Science at Lincoln University

by

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Lincoln University

1996
This study investigated the potential of *Pinus radiata* (pine) bark to absorb organic and ammoniacal-nitrogen, from an aqueous solution and from a dairy slurry, in the laboratory and in a field treatment of dairy slurry. Preliminary experiments indicated that the initial nitrogen concentration of the solution had a significant effect on the rate of nitrogen absorption. The range of physical and chemical characteristics in pine bark and dairy slurry was found to give some variation in the quantity of nitrogen absorbed over time. Some unexplained results were attributed to differences in pine bark characteristics between samples, and to undefined analytical errors which may have included microbial activity in stored samples. Phenyl mercuric acetate was found to have no significant effect on the analysis of ammoniacal nitrogen using the Flow Injection Analyser, and was hence used for the inhibition of microbial activity in subsequent samples during storage.

Empirical models were developed for predicting the amount of ammoniacal and organic nitrogen absorbed, from an aqueous solution of ammonium sulphate and from a dairy slurry, by pine bark over time as a function of pH and initial nitrogen concentration in the laboratory and in the field. The proportion of ammoniacal-nitrogen absorbed over 24 hours, at pH 6 to 8, ranged from 35% from an initial
concentration of 50 ppm-N, to 15% from an initial concentration of 200 ppm-N. After three weeks, approximately 75% of the ammoniacal-nitrogen was absorbed from a solution of 100 ppm-N ammonium sulphate at pH 6. About 50% of the total nitrogen in a dairy slurry was absorbed by pine bark over two weeks. The model developed for predicting the absorption of ammoniacal-nitrogen was tested and found not appropriate for describing the absorption of total nitrogen from a dairy slurry. A new empirical model was developed for the dairy slurry.

Initial pH of the solution was shown to have an effect on the mechanism of nitrogen absorption. At pH 6 there was no difference in the absorption of nitrogen by sterilised and non-sterilised bark, whereas at pH 8 there was a significant reduction in absorption of nitrogen by sterilised bark. This suggested that the absorption of nitrogen at pH 6 was largely chemical, whereas at pH 8 there was significant microbially mediated absorption.

Infra-red spectroscopy was used to deduce some possible functional groups involved in the absorption of various forms of nitrogen. A simple on-farm dairy effluent treatment system was designed and tested, with some success. In conclusion, pine bark was shown to have potential to remove significant quantities of organic and ammoniacal-nitrogen from a slurry, and in the process creating added value for the pine bark as a product which could be used in a horticultural context.
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CHAPTER 1. GENERAL INTRODUCTION

Pine bark is becoming a waste problem in New Zealand, with only limited use as a horticultural product. In areas of high timber production such as Nelson there is an over supply of pine bark chips from de-barked logs, which is increasing with time as the timber industry in New Zealand expands.

Chemical characteristics of pine bark have not been studied in detail, and the potential uses of pine bark have not been realised. This study was initiated to investigate the potential of pine bark to absorb organic and ammoniacal-nitrogen, which could make it suitable for use in many situations as a biofilter. Once saturated with nitrogen, it is proposed that the bark could be composted to form a stable product, useful in a horticultural context.

Major research objectives of the present study were to examine:

1. The quantity of nitrogen that pine bark is able to absorb from an aqueous solution,
2. Factors affecting the absorption of nitrogen from an aqueous solution by pine bark,
3. The mechanism of nitrogen absorption from an aqueous solution by pine bark, and
4. The potential of pine bark to act as a biofilter for high nitrogen aqueous solutions.
CHAPTER 2. LITERATURE REVIEW

2.1 Introduction
Although a native of North America, the radiata pine has proven to be tremendously successful in New Zealand, with an increasing domestic and export market being developed for the timber in various forms and degrees of processing. According to the New Zealand Ministry of Forestry (Barton et al., 1993), the output of roundwood in New Zealand (as at 31 March 1992) was approximately 14.4 million cubic meters. Barton et al (1993) estimated that New Zealand will have a sustainable output of timber in excess of 22 million cubic meters by the year 2003. With a growing trend for export of debarked clear logs (Steel, pers. comm.), and taking into account the conservative estimate that 10% of a pine tree is bark (Society of American Foresters, 1958), by the year 2003 there is going to be something in the order of 2.2 million cubic meters of pine bark per year.

2.2 Uses of pine bark
Pine bark has been defined as “the tissues of the stem branch and root outside the (vascular) cambium layer” (Society of American Foresters, 1958) which defines the practical separation point when stripping bark from a tree. Due to environmental and financial pressures, the industries involved in producing pine bark have had to develop some way of using it. Waste bark is being burnt in special furnaces to provide heat for industrial plants, a process which is limited by the high moisture content of fresh bark (Harris and Nash, 1973). Pine bark has been used in a number of studies, with varying degrees of success, as the basis for a growth media (Leda and Wright, 1991;
Alspach and Burrows, 1985; Gislerod, 1985; Starck and Okruszko, 1984), or it can be used for simple horticultural purposes such as mulching and beautification. A final option is to dump it in a landfill area, a costly and wasteful exercise.

In regions of low timber production, such as Canterbury, the available bark is becoming a sought-after horticultural commodity. By contrast, Nelson has an oversupply, with large quantities available but not utilised, because it is generally not financially viable to transport it any significant distance. Further research to realise the potential of pine bark is needed so as to change a potentially significant waste problem into a product worthy of transport to the site of demand.

2.3 Pine bark characteristics

The physical properties of pine bark in general (not specifically *Pinus radiata*) have been extensively studied (De Boodt and Verdonk, 1972; Bunt, 1974; Verdonk et al., 1974, 1985; Gislerod et al., 1985), however there are distinct interspecies differences in both the physical properties (Harris and Nash, 1973) and anatomy (Patel, 1973), and detailed study of the physical properties and anatomy of New Zealand grown *radiata* has not been reported in the literature.

Detailed study of the chemical characteristics of *Pinus radiata* bark, which are complex and variable, has not been reported either (Porter, 1973), and this lack of knowledge is a major barrier to the efficient use of this resource. Of the general chemical characteristics which are understood, four major aspects which affect the way pine bark can be used are:
• There is a high carbon to nitrogen ratio in fresh bark (Zucconi et al., 1985; Solbraa, 1986) leading to nitrogen immobilisation as the bark decays in a growth media or soil.

• Agents such as manganese, resins, turpentine and tannins have been found in bark and identified as phytotoxins. The general effects have been studied, although little detail has been established (Bunt, 1976; Starck and Okruszko, 1984; Smith, 1986).

• Raw pine bark has a high cation exchange capacity (Roberts and Smith, 1988) allowing it to absorb a wide variety of cations used by plants.

• The wide variation of physical and anatomical properties, and chemical content makes it difficult to predict harvesting volumes, and difficult to design reliable processes which utilise pine bark.

Some empirical observations on the chemical characteristics of pine bark have been made. Aged bark was found to have a lower carbon to nitrogen ratio than fresh bark (Cobb and Keever, 1984). Urea amendment has been shown to produce greater plant biomass than a limestone ammonium nitrate or calcium nitrate amendment when used as a pre-enrichment of bark for growing cabbage seedlings (Wright and Smith, 1989). These observations and others of a similar nature suggest that different nitrogen forms react with bark in different ways. Studies to provide an understanding of how nitrogen reacts with pine bark have not been conducted.

The types of chemicals which may be chemically extracted have been studied. Porter (1973) gives a brief summary, but there is little indication of where these chemicals occur in the bark structure, or of their reactivity and availability.
2.4 High nitrogen liquid wastes
Liquid wastes with a high nitrogen content, until recent times, have frequently been disposed of in waterways. These waterways have often been incapable of absorbing the high nitrogen content and high biological oxygen demand (BOD) inherent in these wastes, without detrimental effect on the biota. Recent changes in legislation and public perspective have placed a greater responsibility on the producers of such wastes to find alternative disposal methods.

2.5 Pine bark and nitrogen
High nitrogen liquid wastes contain organic nitrogen, ammonia, nitrates, and nitrites. The pH of the liquid has an effect on the form of these species, particularly ammoniacal-N. At low pH ammoniacal-N is present mostly in the form of ammonium (NH₄⁺), and at higher pH ammonia (NH₃) becomes more prominent (Haynes and Sherlock, 1986).

\[ \text{NH}_4^+ + \text{OH}^- \leftrightarrow \text{NH}_3 + \text{H}_2\text{O} \]

Anhydrous ammonia has been used as a fertiliser in the United States for some time (He et al., 1990), and as a nitrogen amendment for pine bark (Bollen, 1969; Lloyd, 1977; Aaron, 1982; Foster et al., 1983). Although ammonium is not generally reactive, microbes are capable of absorbing it in this form, whereas ammonia is more likely to be chemically fixed (He et al., 1990). Mortland (1958) suggests that “NH₃ will react with carboxyl, phenol, aldehyde, ketone and alcohol groups to form amines, amides, and imides of various kinds, which suggests the possibility of a variety of reactions with soil organic matter”.

Studies such as an unpublished report from the Forest Research Institute (Lloyd, 1977) and several others (Bollen, 1969; Solbraa, 1979a, b, c, d; Aaron, 1982; Foster et al., 1983; Guedes-de-Carvalho et al., 1991), indicate that pine bark can permanently absorb large quantities of ammonia. High numbers of cation exchange sites in soil have been shown to reduce the concentration of ammonium ions in the aqueous phase within the soil (Wild, 1988), and cation exchange sites in pine bark are likely to perform the same function. Functional groups, such as polyphenols (Bollen, 1969) and carboxyls (Wild, 1988) present in pine bark will react with ammonium ions. It has been suggested that the presence of tannins in the soil increase a plant's demand for ammonium and nitrate (Koch and Oelsner, 1916), and Lloyd (1977) suggested that ammonia absorbed by pine bark is permanently bonded to the flavenoid carbonyl groups in the tannins.

2.6 Pine bark as a filter

Pine bark is very porous, has low water retention in growth media (Odneal and Kaps, 1990), and shows strong non-coherence and resilience (Aaron, 1982), making it ideal as a filtering medium.

Pine bark has been used in several environmental studies to remove ammonia from the waste air stream of piggeries with high levels of efficiency (Van Langenhove et al., 1988; Zeisig, 1988; Van Asseldonk and Voermans, 1989; Van de Sande-Schellekens and Backus, 1993). Moisture was added in each case to provide an interface between the bark and the gases. When bark is used as a litter for animals, it shows evidence of absorption of water, ammonia, and other foul gases (S. Meyer, pers. comm.). The
ability of pine bark to absorb organic and ammoniacal-nitrogen from a liquid has not been studied.

2.7 Pine bark and compost

As an anthropogenic process, composting can be defined as "a complex aggregation of natural microbial and chemical degradations under controlled conditions, to concentrate and maximise these processes for the benefit of mankind" (Hoitink and Poole, 1980; Guedes-de-Carvalho et al., 1991). Although there is a degree of artificiality in a compost heap, as evidenced by the manufacture of heat by microbes to the point where some species are killed off (Kitto, 1988), enhanced composting of pine bark with nitrogen (and other nutrients) is readily accepted as a natural method of redressing the nitrogen imbalance in fresh pine bark destined for growth media.

When decomposing a substrate with a high C:N ratio, such as pine bark, microbes require a source of nitrogen (Starck and Oswiecimski, 1985). Microbial biomass provides a slow release of a more complete range of nutrients into the growth medium than can be provided in an inorganic fertiliser (Ostalski, 1976; Kitto, 1988).

Fresh pine bark shows strong evidence of phytotoxin pathogenicity, such as suppression of Phytophthora rot (Orlikowski and Wojdyla, 1988; Zajicek and Heilman, 1991; Boehm and Hoitink, 1992) and suppression of fungus gnat emergence (Lindquist et al., 1985). Although fresh bark shows natural phytotoxicity towards plants, composting is known to reduce this effect (Campbell et al., 1986), and tannins may be readily leached from pine bark (Starck et al., 1973). Well fermented
(composted) bark is noted as both *Rhizoctonia* and *Fusarium* suppressive (Pera and Calvet, 1989; Deulofeu and Aguila-Vila, 1984).

Pine bark provides a good structural base for a compost or growth medium (Marfa et al., 1986; Milbocker, 1991), due to its porosity and low density, as well as the nutritional value (Golueke, 1982), suggesting that composting could be an effective treatment for pine bark which had been used to absorb nitrogen (in any form), resulting in a stable product for use in growth media.
CHAPTER 3. PRELIMINARY EXPERIMENTS

3.1 Preliminary Experiment I: Absorption of nitrogen from a solution by pine bark over time as affected by nitrogen concentration

3.1.1 Introduction

Although ammoniation of pine bark has been reported (Bollen, 1969; Lloyd, 1977; Aaron, 1982; Foster et al., 1983), absorption of nitrogen from a liquid by pine bark has not been studied in detail, and proven methodology was unavailable. Analytical methods for soil have been shown to be inappropriate for lightweight growth media, including bark based mixes, due to the very different chemical and physical characteristics (Bunt, 1986). The analytical methods routinely used by the Soil Science Department at Lincoln University were therefore used only as a starting point for developing the methodology for this study.

These preliminary experiments were necessary to focus on and attempt to characterise the period of time during which significant nitrogen absorption occurs, and on the effects of nitrogen concentration and shaking method on nitrogen absorption rate.

Keeping the main research objectives in mind (page 1), experiments were designed around the principle of approximating a batch filtration system, by shaking a bark sample in an imitation waste liquid. Continued development of the methodology played a substantial part in the study as further sources of error were identified.
The main aim of this experiment was to provide a broad indication of the rates of absorption of ammonium from a solution by pine bark over time, as affected by nitrogen concentration. Seven sampling intervals (1, 2, 3, 5, 7, 21, 24 hours) and five concentrations (0, 25, 50, 75, 100 ppm ammoniacal-N) were examined in a factorial design.

3.1.2 Materials and methods

3.1.2.1 Materials

The materials and laboratory equipment commonly used throughout this study are listed in Appendix A.

3.1.2.2 Reaction vessels

Corked 250 mL conical flasks were used as reaction vessels. Each flask contained 25 g of bark and 100 mL of ammonium sulphate solution at one of five concentrations (0, 25, 50, 75 and 100 ppm-N), buffered at pH 6 (Appendix B). A separate flask was used for each of the seven sampling intervals and five concentrations, making a total of 35 flasks.

The buffer pH of 6 was chosen for these first experiments to represent the pH of a dairy slurry as measured in a sample taken from the first sump on Darryl Petheram’s farm, Days Road, Springston, Canterbury.

3.1.2.3 Methods

A rotational shaker table was used to provide agitation to the reaction vessels at approximately 150 revolutions per minute (rpm).
Sampling involved removing the appropriate flask from the shaker and filtering the liquid with Whatman No. 4 filter paper into a 100 mL plastic sample bottle. The bottle was then stored at 4°C until analysis using the Flow Injection Analyser. It was assumed that the acidic nature of the solution (pH 6) would have prevented any volatilisation of ammonia during sampling and storage (Haynes and Sherlock, 1986). Results were expressed as concentration of nitrogen remaining in solution.

3.1.2.4 Statistical analysis

ANOVA for the data was calculated with Minitab software, with time and measured initial concentration as factors. The coefficient of variation (CV%) of all the samples was calculated as a measure of the overall variation present in the experiment.

The Flow Injection Analyser is known to have an inherent error of approximately ±5% (McCloy, pers. comm.), which was taken into account when interpreting data analysis.

3.1.3 Results and discussion

3.1.3.1 Absorption of nitrogen from solution

The data obtained (Figure 3.1) show that the concentration of nitrogen in the ammonium sulphate solution was reduced over time in each concentration treatment except the zero initial concentration, indicating that nitrogen was absorbed by the pine bark.
3.1.3.2 High overall variation

The high variation within concentration treatments, as evidenced by a CV = 17.5%, made it inappropriate to fit a predictive model to the data. It is possible that the high variation was caused in part by the use of a different bark sample for each sampling interval.

3.1.3.3 Effect of nitrogen concentration

Despite the variability found in the small bark samples, the results of the ANOVA (Table 3.1) showed that there was a significant difference between concentration treatments. The rate of nitrogen absorption was shown to be proportional to the initial concentration, as in many biological systems, allowing a reduction in the number of concentrations studied in the subsequent work.
Table 3.1. ANOVA for preliminary experiment I.

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<tr>
<th>Source</th>
<th>DF</th>
<th>Mean Sq</th>
<th>F ratio</th>
<th>P</th>
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<td>Hours</td>
<td>6</td>
<td>99.7</td>
<td>2.67</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Linear</td>
<td>1</td>
<td>187.6</td>
<td>5.02</td>
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<tr>
<td>Lack of fit</td>
<td>5</td>
<td>82.12</td>
<td>2.20</td>
<td>n.s.</td>
</tr>
<tr>
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<td>129.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Linear</td>
<td>1</td>
<td>19228.5</td>
<td>51.41</td>
<td>&lt;0.001</td>
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<tr>
<td>Lack of Fit</td>
<td>3</td>
<td>45.87</td>
<td>1.23</td>
<td>n.s.</td>
</tr>
<tr>
<td>Linear x linear Interaction</td>
<td>1</td>
<td>46.4</td>
<td>1.24</td>
<td>n.s.</td>
</tr>
<tr>
<td>Error</td>
<td>23</td>
<td>37.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n.s. = not significant

3.1.3.4 General observations

By the end of the first hour, most of the bark was well wetted and submerged in liquid, but remained floating near the top. The ends of one or two longer splinters of bark and wood remained protruding from the solution. By the end of the experiment some splinters remained partially above the level of the liquid, but the remaining bark was stratified relatively evenly from top to bottom of the flask.

Over the period of soaking, there was a slight dissolution of organic matter into the solution, giving it a yellowish-brown tinge, but this was not considered sufficient to affect the results of ammonium analysis (McCloy, pers. comm.).

3.2 Preliminary experiment II: Improving the precision of measurement of nitrogen absorption rate

3.2.1 Introduction

The main aim of this experiment was to reduce the variation found in Section 0.
3.2.2 Materials and methods

3.2.2.1 Reaction vessels

The bark sample size was increased to 50 g with 500 mL of liquid, and 600 mL plastic jars were used as reaction vessels. The reaction vessels were shaken briefly by hand so as to thoroughly wet the bark before being placed on the rotational shaker table.

3.2.2.2 Ammonium solutions

Three concentration treatments were used (0, 50 and 100 ppm-N) as suggested in Section 3.1.3.3, each with three replicates, all buffered at pH 6 (Appendix B). Time zero (initial) concentrations were measured on samples taken before the solution was added to the bark.

3.2.2.3 Sampling procedure

At each sampling interval the jars were removed from the shaker table and given a brief vigorous shake by hand to remove any concentration gradients. A 10 mL aliquot of each solution was removed by 15 mL disposable syringe and injected into a scintillation vial through Whatman No. 4 filter paper. Immediately following removal of the sample, each jar was re-capped and replaced on the shaker. The vials were stored at 4°C until analysis by the Flow Injection Analyser.

Although sampling by removal of solution reduced the ratio of liquid to bark by 2.4% it was assumed that the absorption rate in the reaction vessels was unaffected by sampling.
3.2.4 Statistical analysis

The data were analysed under a General Linear Model (GLM) with time as a covariate. The S.E.M. and CV% were calculated from the ANOVA of this model.

3.2.3 Results and discussion

3.2.3.1 Absorption of ammonium

The results obtained (Figure 3.2) showed a reduction in the variability within concentration treatments as compared to Section 0, and a reduction in nitrogen concentration over time, indicating that there was absorption of nitrogen by the pine bark over time. Approximately 25% of the initially available nitrogen was absorbed after 24 hours in each concentration treatment.

![Figure 3.2. Concentration of nitrogen remaining in solution over time with three initial nitrogen concentrations.](image)
There was a reduction in the experimental variation, as evidenced by a CV of 11.13%, which is a decrease of 35% from the previous experiment (Section 0). The only thing that was changed from the previous experiment is the use of repeated sampling on a bark sample, instead of multiple samples of bark over time, thus the reduction of error may be attributed largely to this change. The variation in the ability of individual bark samples to absorb nitrogen, which can now be seen to be significant in samples of this size, has not been documented in the literature.

The slopes of the fitted model are significantly different with (Table 3.2), and proportional to (Equation 1), nitrogen concentration. Because of the variation found in the previous experiment (Section 0) it was not appropriate to statistically compare the rate of nitrogen absorption found in Section 3.1.3.1 with the rate of absorption found in this experiment, but visual comparison showed no apparent difference in slopes.

**Equation 1. Preliminary model for prediction of the nitrogen concentration remaining in solution over time.**

\[ NRS = 0.563 + 0.8118IC - 0.0391T - (0.00718 \times IC \times T) \]

\[ R^2 = 75.6\% \]

*\(NRS = \) Nitrogen remaining in solution
*\(IC = \) Initial concentration
*\(T = \) Time

**Table 3.2. ANOVA for Preliminary Experiment II.**

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial concentration</td>
<td>2</td>
<td>62050</td>
<td>42952</td>
<td>21476</td>
<td>1015.18</td>
<td>0.000</td>
</tr>
<tr>
<td>Time</td>
<td>1</td>
<td>588</td>
<td>588</td>
<td>588</td>
<td>27.8</td>
<td>0.000</td>
</tr>
<tr>
<td>Initial concentration by Time</td>
<td>2</td>
<td>328</td>
<td>328</td>
<td>164</td>
<td>7.75</td>
<td>0.001</td>
</tr>
<tr>
<td>Error</td>
<td>57</td>
<td>1206</td>
<td>1206</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>64172</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
As expected the results confirmed that there was no measured activity where the initial nitrogen concentration in the solution was zero. This confirms that there was no significant soluble ammoniacal-nitrogen present in the bark.

3.2.3.2 Sources of variation

Concentrations of 81.2 and 42.1 ppm-N remaining in solution were predicted at time zero for measured initial concentrations of 100 and 50 ppm-N respectively. Four potential sources of variation needing further investigation were identified:

1. By shaking the reaction vessel to remove any concentration gradient before sampling, it is likely that a part of the air remaining in the pores of the bark would be dislodged, thus increasing the surface area of bark available for contact with the liquid. Because the shaking was by hand, and of no defined period or force, each bark sample would have had a different amount of air dislodged from its pore space.

2. Because of the large crystals and small quantities of solid ammonium sulphate, and because the liquid quantities were quantified by measuring cylinder, it is likely that there was variation in the initial concentration of the nitrogen solutions. This error would be reduced by making one large quantity of buffered ammonium sulphate solution in a volumetric flask, and then dividing it into sample volumes.

3. The variability between bark samples may cause different initial rates of water absorption. The wide variation in bark physical properties (Harris and Nash, 1973), including pore size and quantity, and the surface area available (dependent on the proportions of different particle sizes) would effect the speed at which the bark is wetted, giving the initial period of nitrogen absorption unavoidable fluctuations.
4. There may have been errors in the analytical method, such as inherent error in the Flow Injection Analyser, or loss of ammonia during storage of samples by microbial activity.

3.2.3.3 General observations

Some difficulty was experienced in expelling samples from the 10 mL syringes due to small bark particles becoming trapped in the nozzle of the syringe.

3.3 Preliminary experiment III: Characterisation of the variation in nitrogen concentration during the first hour

3.3.1 Introduction

The aim of this experiment was to characterise the variation in nitrogen concentration during the first hour of contact, as noted in Section 3.2.3.1.

3.3.2 Materials and Methods

3.3.2.1 Nitrogen concentration

Having confirmed in Section 3.2.3.1 that there was a linear relationship between initial nitrogen concentration and the rate of nitrogen absorption, a single concentration was used to characterise the response over the first hour. To this end a solution containing 100 ppm-N ammonium sulphate and buffered at pH 6 (Appendix A) was used with nine replicates. Bark sample sizes and reaction vessels remained the same as in Section 3.2.2.1.

3.3.2.2 Sampling method

In order to make the sampling interval more accurate, starting times for each vessel were staggered at 1.5 minute intervals. The sampling method was similar to that in
Section 3.2.2.3, except that only one sample was taken at a time due to the staggered start, and that a Terumo 25 mL syringe with a wider nozzle was used.

3.3.2.3 Statistical Analysis

ANOVA for the data was calculated with Minitab software, from which the S.E.M. and the CV% were calculated.

3.3.3 Results and discussion

3.3.3.1 Nitrogen concentrations

Results obtained (Figure 3.3) show high overall variation, with no absorption of nitrogen. There was no significant effect of time on nitrogen absorption (Table 3.3), indicating that there was no absorption of nitrogen during the 45 minutes examined. Clearly the first hour of exposure of pine bark to the ammoniacal-nitrogen solution is characterised by variation in nitrogen levels. The experimental error (CV = 13.08%) was similar to that found in the previous experiment, indicating that the method used to make up the ammonium sulphate solutions did not contribute to the observed error.

![Figure 3.3. Concentration of nitrogen remaining in solution during the first hour.](image-url)
Table 3.3. ANOVA for the absorption of nitrogen over the first hour.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>2</td>
<td>288.86</td>
<td>144.43</td>
<td>2.03</td>
<td>0.174</td>
</tr>
<tr>
<td>Time</td>
<td>6</td>
<td>86.71</td>
<td>14.45</td>
<td>0.2</td>
<td>0.969</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>853.86</td>
<td>71.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>1229.43</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As with the previous experiment (Section 3.2.3.1), the initial nitrogen concentration as measured by the Flow Injection Analyser is lower than expected. These values were once again obtained without exposure of the solution to the pine bark, so the only explanations for this variation remaining from Section 3.2.3.2 are;

1. There may have been errors in the analytical method, such as inherent error in the Flow Injection Analyser, or loss of ammonia in samples by microbial activity during storage.

2. The high variation between bark samples.

3.4 Preliminary experiment IV: Comparison of three different shaking methods

3.4.1 Introduction

The main aim of this experiment was to examine the absorption of nitrogen from a liquid by pine bark using three different methods of shaking.

3.4.2 Materials and Methods

The three shaking methods studied were rotational, reciprocal and end-over-end shaking. An aqueous solution of nitrogen at 100 ppm-N and a pH of 6 (Appendix A) was used under conditions as described previously (Section Error! Reference source not found.) with the shaking method as the only variable. Each shaking method involved 9 replications.
3.4.2.1 Statistical Analysis

Sequential regressions in Minitab were used to fit a linear model to each method. Significant differences between the linear models were determined by comparing the error terms from the ANOVA on the linear models.

3.4.3 Results and discussion

3.4.3.1 Comparison of shaking methods

The data (Figure 3.4) clearly showed different rates of nitrogen absorption by the three methods, with the end-over-end shaker showing less nitrogen absorption than the reciprocal method, which showed less nitrogen absorption than the rotational shaking method. The error bars represent the S.E.M. of the experiment (3.39), and the labelled lines represent the fitted model (Equation 2), for which the coefficients and their significance are summarised in Table 3.4.

![Figure 3.4. Concentration of nitrogen remaining in solution during shaking by three different methods.](image-url)
Equation 2. General model for prediction of the nitrogen concentration remaining in solution over time using different shaking methods.

\[ NRS = a + bT \]

Where:
- \( NRS \) = Nitrogen remaining in solution
- \( T \) = Time

(see Table 3.4 for other constants)

\[ R^2 = 89.9\% \]

<table>
<thead>
<tr>
<th>Shaking method</th>
<th>a</th>
<th>S.E.M.</th>
<th>p-value</th>
<th>b</th>
<th>S.E.M.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reciprocal</td>
<td>82.9</td>
<td>2.2</td>
<td>&lt;0.0001</td>
<td>-0.048</td>
<td>0.023</td>
<td>&lt;0.07</td>
</tr>
<tr>
<td>Rotational</td>
<td>95.3</td>
<td>2.2</td>
<td>&lt;0.0001</td>
<td>-0.140</td>
<td>0.023</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>End-over-end</td>
<td>94.4</td>
<td>3.3</td>
<td>&lt;0.0001</td>
<td>0.003</td>
<td>0.029</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s. = not significant

The end-over-end shaking treatment showed no significant absorption of nitrogen over time (Table 3.4). The reciprocal and rotational shaking treatments showed linear trends, although the fit of the model to the reciprocal shaking treatment was not quite significant at 5%. Because the rotational shaking treatment showed the most nitrogen absorption, and because the logistics with the reciprocal and end-over-end methods were complicated with increased turbidity inhibiting the filtering process, the rotational shaker was chosen as the method for all subsequent work.
3.5 Preliminary experiment V: Inhibition of microbial growth in ammoniacal-N samples by phenyl mercuric acetate

3.5.1 Introduction

Phenyl mercuric acetate (PMA) at a concentration of 50 ppm has been shown to inhibit growth of bacteria and fungi in aqueous mineral-nitrogen solutions (Bremner, 1965). The main aim of this experiment was to determine the degree of interference of PMA in the analysis for ammonium nitrogen by the Flow Injection Analyser.

3.5.2 Materials and Methods

3.5.2.1 Phenyl mercuric acetate solution

A solution of PMA was made by dissolving 1 g of PMA in 1 L of nano-pure water (Appendix A), which was nearly a saturated solution. Dilution of a 20 mL sample with 1 mL of this PMA solution would result in a concentration of 50 ppm PMA in the sample (Bremner, 1965).

3.5.2.2 Ammonium solution

Twelve samples, each containing 20 mL of ammonium sulphate solution, were made up at 100 ppm-N in a phosphate buffer at pH 6.

3.5.2.3 Addition of phenyl mercuric acetate

Each of six 20 mL sample was diluted with 1 mL of PMA solution, achieving a PMA concentration of 50 ppm. Samples were refrigerated at approximately 4°C for a period of two weeks before analysis.
3.5.2.4 Analysis

All samples were diluted and analysed for NH$_4$-N by Flow Injection Analyser. An unpaired t-test was used to look for significant differences.

3.5.3 Results and discussion

The data (Table 3.5) showed no significant difference between PMA treated and untreated samples. It was therefore concluded that PMA has no effect on analysis for NH$_4$-N by the Flow Injection Analyser.

It was not proven that the presence of PMA inhibited microbial growth, or that any of the variation present in previous work was due to microbial activity in the stored samples.

<table>
<thead>
<tr>
<th>Mean without PMA</th>
<th>23.917 ppm-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean with PMA</td>
<td>24.183 ppm-N</td>
</tr>
<tr>
<td>Pooled S.E.M.</td>
<td>0.161</td>
</tr>
</tbody>
</table>

There was no significant difference between the treatments.

3.6 Conclusions of preliminary experiments

The results of these preliminary experiments showed that:

1. The initial quantity of nitrogen present in the liquid produced a significant effect on nitrogen absorption by pine bark, as in many biological systems.

2. The most important period of absorption appears to be within the first 24 hours.
3. Fluctuations in measured nitrogen concentrations during initial exposure of pine bark to a liquid containing nitrogen were unavoidable, and caused by either analytical error or variation in the bark samples.

4. Rotational shaking was the most appropriate method available to maximise nitrogen absorption from a liquid.

5. The presence of phenyl mercuric acetate (PMA) at a concentration of 50 ppm did not significantly affect the ammonium analysis by Flow Injection Analyser. It was assumed that PMA was suitable for the inhibition of microbial growth in samples during storage.
CHAPTER 4. WASTE CHARACTERISATIONS

4.1 Introduction
In order to provide a reference point for comparison of other situations to this research it was essential that the bark and the slurries used in the present study were characterised.

4.2 Materials

4.2.1 The bark
All the bark used in the present laboratory studies came from the one source, the Hornby Garden Market in Shands Road, Christchurch in March 1995. As far as it was possible to determine, the bark was all of the same type (*Pinus radiata*). The market receives fresh bark supplies daily, and screens the bark to a maximum chip size of 500mm on site. The bark was spread on a large plastic tray to air dry at room temperature for at least two weeks before use.

The following measurements (Section Error! Reference source not found.) were taken on 50 g samples and adequately describe the general characteristics of the bark supplied. The variation inherent in pine bark has been described in Section 2.3.

4.2.2 The slurries
Five 600 mL samples of dairy slurry were taken from the outlet pipe of the first holding tank on the farm of Darryl Petheram, after an evening milking.
4.3 Materials and Methods

4.3.1 Bark particle size distribution and wood content

Three 50 g bark samples were sieved into a range of particle sizes. The fractions were then visually separated into wood and bark fractions (with the exception of the < 1.4 mm fraction, due to the visual similarity) and weighed.

4.3.2 Bulk density

Bulk density of a soil sample is usually calculated using a 1 L measuring cylinder (Joiner and Conover, 1965; Goh and Haynes, 1977), but the large particle size of the bark chips meant that a larger volume was necessary. A 2 L measuring cylinder was filled with air-dry bark, tapping firmly during addition to compact the mix, and the weight of bark recorded.

Oven dry bark was not used due to the fact that the particles are crisped by the drying process, which would effect the compaction characteristics. The moisture content of the air dry bark used (14.0%) was calculated in Section 4.3.5.

4.3.3 Total pore space

Because pine bark chips have no cohesion, it was not appropriate to use the water tension table method as for a soil core. The sample of bark used in Section 4.3.2 was soaked in water for 48 hours and weighed. The bark in the cylinder was then drained overnight and reweighed, representing the free draining pore space. The cylinder and contents were then dried at 105°C for 24 hours and reweighed, giving the total pore space (Cameron, pers. comm.).
4.3.4 Total carbon and nitrogen content

Six 50 g air-dry samples of bark were oven dried, finely ground, subsampled six times and analysed for total carbon and nitrogen on a Leco Elemental Analyser furnace (Appendix A).

4.3.5 Moisture content

Six 50 g samples of air-dried bark were weighed out and dried for 24 hours in an oven at 105°C, before reweighing. The difference in weight was taken as the moisture content of the air-dried sample.

4.3.6 Total water holding capacity

Six 100 g samples of air-dried bark were accurately weighed and placed in tins, which had holes drilled in the bottom and Whatman No. 4 filter paper placed over the holes. The tins were then placed in a deep tray, and water was added. To keep the bark below the surface a 1 L beaker, which fitted neatly inside the tin, was placed on top of each sample. Water was added to leave all the bark below water level, and the samples were left to soak for 24 hours.

The tins were then removed gently from the water bath and placed on a rack to drain for one hour, after which they were weighed and placed in an oven at 105°C for 24 hours, and re-weighed. The total water holding capacity was taken as the quantity of water lost during oven drying.

4.3.7 Slurry pH

The dairy samples were analysed immediately with a pH meter (Appendix A).
4.3.8 Total nitrogen and ammonium content of the dairy slurry

Four 20 mL sub-samples were taken from each sample of slurry, two for total nitrogen analysis by the Kjeltech autoanalyser (Appendix A), and two for ammoniacal-nitrogen analysis by Flow Injection Analyser (Appendix A).

4.4 Results and discussion

The bark and slurry characteristics are summarised in Table 4.1.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Characteristic</th>
<th>Measured Value</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bark</td>
<td>Free draining pore space</td>
<td>554.6 cm³ L⁻¹</td>
<td>-</td>
</tr>
<tr>
<td>Bark</td>
<td>Total pore space</td>
<td>730.1 cm³ L⁻¹</td>
<td>-</td>
</tr>
<tr>
<td>Bark</td>
<td>Carbon content</td>
<td>51.2%</td>
<td>2.8</td>
</tr>
<tr>
<td>Bark</td>
<td>Nitrogen content</td>
<td>0.2%</td>
<td>0.0</td>
</tr>
<tr>
<td>Bark</td>
<td>C:N ratio</td>
<td>232.5</td>
<td>-</td>
</tr>
<tr>
<td>Bark</td>
<td>Moisture content</td>
<td>14.0%</td>
<td>0.3</td>
</tr>
<tr>
<td>Bark</td>
<td>Total water holding capacity</td>
<td>108.1%</td>
<td>0.8</td>
</tr>
<tr>
<td>Slurry</td>
<td>pH</td>
<td>6.7</td>
<td>0.1ᵃ</td>
</tr>
<tr>
<td>Slurry</td>
<td>Ammonium content</td>
<td>71.5 ppm-N</td>
<td>3.6ᵃ</td>
</tr>
<tr>
<td>Slurry</td>
<td>Total nitrogen content</td>
<td>278.1 ppm-N</td>
<td>15.0ᵃ</td>
</tr>
</tbody>
</table>

ᵃ = Standard deviation.

4.4.1 Pine bark particle size distribution and wood content

The particle size distribution of wood and bark fractions is shown in Figure 4.1. A wide range of particle sizes is apparent, although the distribution is skewed towards the larger particle sizes. The smaller particle sizes are present in sufficient quantity to offer a large surface area and therefore must not be discounted. It can be seen that the wood constitutes only a small percentage of the sample (11%).
Because of the difficulty in picking up the finer particles to get a representative sample of bark, it was decided to separate the supply of bark into coarse (>5.6 mm) and fine fractions by sieving. All samples in subsequent work were taken by adding separate quantities of the two fractions in a ratio of 17.9:1, as shown in Figure 4.1.

4.4.2 Pore space of the bark

The pore space measurements do not have an S.E.M. because only one sample was examined.

4.4.3 Carbon and nitrogen content of the bark

The carbon content of the bark (51.2%) was similar to that found by Bollen (1969) of 53.97%, although the nitrogen content (0.2%) is approximately twice the content found by Bollen (0.11%). This may be due to differing ages of the pine bark samples for these two studies. The carbon to nitrogen ratios of the individual samples varied,
but with no consistent pattern (Figure 4.2), reflecting the wide variation inherent in Pine bark (Harris and Nash, 1973).

![Figure 4.2. Relationship between carbon and nitrogen contents of pine bark.](image)

### 4.4.4 Moisture content and water holding capacity of the bark

The moisture content (14.0%) had low variability, showing consistency. The water holding capacity of 108.1% was similar to that found by Bunt (1974).

### 4.4.5 pH, total nitrogen and ammonium content of the slurry

The pH of the dairy slurry was 6.7, which was very different to the pH of a sample taken on the neighbouring Lincoln University Dairy Unit, which had a pH of 7.5 (Muller, pers. comm.). The total nitrogen and ammonium contents of this slurry (278.1 and 71.5 ppm-N respectively) were also very different from the sample taken by Muller (448.8 and 33.3 ppm-N respectively). The most likely explanation for this is the difficulty in obtaining consistent samples due to the wide variation in slurry quality over the period of milking. The samples in this study were all taken at the
same time and from the same point in the discharge programme. A different farm and discharge program may be expected to yield different results.

The age of the slurry has also been shown to affect the forms of nitrogen present in a dairy slurry (Haynes and Williams, 1993). Fresh slurry contains a high proportion of urea, which over time is converted to ammoniacal-nitrogen, and then to mineral-nitrogen. It is possible that the slurry examined in the present study was of a different age to the slurry examined by Muller.
CHAPTER 5. NITROGEN ABSORPTION AND EXTRACTION

5.1 Development of an ammonium extraction technique

5.1.1 Introduction

When pine bark absorbs organic and mineral nitrogen from an aqueous solution, a range of different bonds are formed between the bark and the nitrogen, from covalent bonding with functional groups and molecules in the bark structure (Lloyd, 1977), to loosely associated aqueous nitrogen within the pore spaces of the bark. Thus it is possible to separate the less firmly bonded (or "readily available") nitrogen from the "firmly bonded" nitrogen.

Measurement of the nitrogen remaining in solution before and after exposure to a bark sample allows the quantity of nitrogen absorbed to be calculated, and analysis of the bark by the Leco Elemental Analyser (Appendix A) will give the total nitrogen content. The quantity of nitrogen which is readily available to plants is quite different to the total nitrogen present in a growth medium. Although pine bark chips are widely used in growth media and composts in commercial nurseries, there is no test currently available for estimating the quantity of readily available nitrogen in the pine bark.
The aim of this experiment was to develop an extraction technique for estimating the “readily available” and the “firmly bonded” fractions of absorbed nitrogen in pine bark.

5.1.2 Materials and Methods

5.1.2.1 Absorption of aqueous nitrogen

To provide a consistent quantity of absorbed nitrogen available for extraction, 50 g pine bark was shaken in 500 mL of buffered (pH 6) ammonium sulphate (100 ppm-N) for 24 hours (Appendix B). The quantity of nitrogen remaining in the solution was then determined, and the difference between this amount and the initial quantity of nitrogen in the solution was used as an estimate of the quantity of nitrogen absorbed by the pine bark.

5.1.2.2 Constant temperature

To keep the temperature constant around the reaction vessels during the absorption of nitrogen, a cabinet was designed and built by Rob McPherson of the Lincoln University Soil Science Department (Figure 5.1). The cabinet consisted of a carpet lined cupboard (dimensions: 80 cm wide, 70 cm high, 50 cm deep), with a fan unit in the top left side of the roof attached to a thermostat to maintain an upper limit on the internal temperature range. A second fan was left constantly running to circulate the air inside the cabinet, and was placed next to a light bulb connected to a second thermostat to control the minimum internal temperature.
The internal and external temperatures of the cabinet were monitored with a TinyTalk data monitor during four absorption periods to establish the range of temperatures encountered inside and outside the cabinet.

5.1.2.3 Separation of bark and liquid

Sequential extraction requires that the solid (pine bark) be separated from the extractant ready for subsequent extractions. Because of the wide range of particle sizes in bark, no single method of separation was appropriate. The very fine bark particles blocked filter paper very quickly, even with the use of a Buckner funnel under vacuum, thus suggesting that centrifugation was necessary as an alternative. The larger bark particles had to be removed first, and several methods of sieving were tested, including various combinations of metal and cloth mesh, until the sieve lid (Figure 5.2) was developed. This method allowed rapid attachment of the sieve for ease of use, and prevented spillage around the sides of the sieve.
Three sieve lids were made, due to the fact that only three bark samples could be dealt with at one time because of limitations in the availability of centrifuge space. Three spare 1200 mL reaction vessel lids had a circular hole (63 mm diameter) cut in the top and a piece of 2 mm stainless steel mesh glued in place with Araldite. Use of the sieve lid involved screwing the sieve lid onto a reaction vessel which was then upturned into an 800 mL beaker. The size of the beaker was such that the reaction vessel remained suspended near the top of the beaker.

Once the larger particles of bark were separated from the extractant, the fine particles had to be removed. It was found that centrifuge tubes of greater than 250 mL allowed too much internal circulation of the extractant after spinning, which stirred up the sediment. Speeds of less than 8,000 rpm failed to compact the sediment, while at speeds greater than 13,000 rpm, the sediment tended to form flakes which floated freely and disintegrated when the tube had stopped spinning. A combination of a 250 mL high speed centrifuge tube spun at 10,000 rpm for six minutes gave maximum
sedimentation with a minimum of wasted time. The supernatant could then be filtered through Whatman No. 4 filter paper in a Buckner funnel under vacuum.

5.1.2.4 Extractants

Two extractants were chosen to separate the two fractions of absorbed nitrogen:

1. Water extraction, as a measure of the "readily available" nitrogen, and

2. Potassium chloride (2 M) extraction, as a measure of the total extractable nitrogen

The difference between total extractable nitrogen and "readily available" nitrogen was taken as the estimate of the "firmly bonded" fraction.

Factors examined which had the potential to affect the extraction of nitrogen included:

(a) the number of extractions required to achieve an acceptable result.

(b) the appropriate duration of extraction.

(c) the appropriate volume of extractant for a 50 g bark sample.

5.1.2.5 Experimental design

The experiment was a completely randomised design, treatment combinations including one of two volumes of extractant (250 or 500 mL), one of three durations of extraction (30, 60 or 90 minutes), and one of four combinations of extractions (1 or 2 water extractions followed by 1 or 2 KCl extractions). Each treatment combination had three replicates (Table 5.1).

5.1.2.6 Extraction technique

The technique described in Section 5.1.2.3 was used to separate the bark from the initial liquid and from the extractant after each extraction. The volume of liquid recovered was measured, and two 20 mL samples were taken and treated with PMA
Table 5.1. Combinations of extractant volume and type, and duration of extraction.

<table>
<thead>
<tr>
<th>Extraction combination</th>
<th>Volume of extractant (mL)</th>
<th>Number of water extractions</th>
<th>Number of KCl extractions</th>
<th>Duration of extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>250</td>
<td>1</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>1</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>2</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>2</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>1</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>250</td>
<td>1</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>250</td>
<td>2</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>8</td>
<td>250</td>
<td>2</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>9</td>
<td>250</td>
<td>1</td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td>250</td>
<td>1</td>
<td>2</td>
<td>90</td>
</tr>
<tr>
<td>11</td>
<td>250</td>
<td>2</td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>12</td>
<td>250</td>
<td>2</td>
<td>2</td>
<td>90</td>
</tr>
<tr>
<td>13</td>
<td>500</td>
<td>1</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>14</td>
<td>500</td>
<td>2</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>15</td>
<td>500</td>
<td>2</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>16</td>
<td>500</td>
<td>1</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>17</td>
<td>500</td>
<td>1</td>
<td>2</td>
<td>60</td>
</tr>
</tbody>
</table>

(Section 3.5), to inhibit microbial growth, and stored at 4°C until analysis by the Flow Injection Analyser. The quantity of nitrogen recovered could then be calculated. When subsequent extractions were required, the following steps were followed.

The 800 mL beaker, the sieve lid, the Buckner funnel and filter paper, and the centrifuge tube(s) used in separation of the bark from the liquid were washed into the reaction vessel with the next extractant (Table 5.1). The quantity of this extractant used for washing was determined by difference of weight in the reaction vessel, and the volume required to make the volume to 250 or 500 mL (Table 5.1) calculated and added ready for the next extraction.

Extraction of absorbed nitrogen involved shaking the reaction vessels on an end-over-end shaker in a constant temperature room at 16°C for the appropriate duration (Table
5.1. Sampling and preparation of the bark for subsequent extractions was as described at the beginning of this Section.

5.1.2.7 Statistical analysis

Results were expressed as a quantity of nitrogen recovered, and as a percentage recovery of the nitrogen absorbed, both corrected to the equivalent of a 50 g bark sample.

Because an initial water extraction was included in each combination, it was used as a covariate to stabilise the variation in the subsequent extractions in a General Linear Model (GLM) of ANOVA with Minitab software. To further stabilise the variance, the square root of the data from the 2nd, 3rd and 4th extractions was used to account for the higher variation found with higher nitrogen recoveries. The covariate means were then examined for significant differences to determine which treatments had a significant effect.

5.1.3 Results and discussion

5.1.3.1 Constant temperature chamber

The results of temperature monitoring over the four absorption periods were:

Internal temperature: 26.8 ºC (S.D. 1.3 ºC)

External temperature: 18.4 ºC (S.D. 1.3 ºC)

These show that the temperatures were kept fairly constant during the extractions.
5.1.3.2 Nitrogen recovered

Results for the percentage recovery of the different extraction combinations (Figure 5.3) showed high overall variability, but no systematic variation was apparent. This indicates that there was no real difference between the extraction combinations.

Covariate adjusted means are presented in Table 5.2. The data for the 2nd, 3rd and 4th extractions were separated by extraction type before looking for significant differences. There was no pattern to these significantly different means, indicating that although there were statistical differences between combinations, there was no real difference between the extraction combinations examined.

### Table 5.2. Covariate adjusted means for different extraction combinations.

<table>
<thead>
<tr>
<th>Extraction combination</th>
<th>Extraction number</th>
<th>1st H₂O</th>
<th>1st KCl</th>
<th>2nd H₂O</th>
<th>2nd KCl</th>
<th>1st KCl</th>
<th>2nd KCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2145.5&lt;sup&gt;ab+&lt;/sup&gt;</td>
<td>79.97&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>45.84&lt;sup&gt;m&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2047.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.87&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>9.83&lt;sup&gt;h&lt;/sup&gt;</td>
<td>69.14&lt;sup&gt;kl&lt;/sup&gt;</td>
<td>73.9&lt;sup&gt;i&lt;/sup&gt;</td>
<td>40.26&lt;sup&gt;o&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2686.7&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>9.08&lt;sup&gt;h&lt;/sup&gt;</td>
<td>40.1&lt;sup&gt;m&lt;/sup&gt;</td>
<td>70.16&lt;sup&gt;kl&lt;/sup&gt;</td>
<td>64.52&lt;sup&gt;jk&lt;/sup&gt;</td>
<td>35.76&lt;sup&gt;no&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2243.7&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>80.02&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>5.24&lt;sup&gt;h&lt;/sup&gt;</td>
<td>64.33&lt;sup&gt;jk&lt;/sup&gt;</td>
<td>61.09&lt;sup&gt;ij&lt;/sup&gt;</td>
<td>32.37&lt;sup&gt;n&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>5</td>
<td>2206.4&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>75.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.33&lt;sup&gt;i&lt;/sup&gt;</td>
<td>74.66&lt;sup&gt;jl&lt;/sup&gt;</td>
<td>73.01&lt;sup&gt;i&lt;/sup&gt;</td>
<td>34.07&lt;sup&gt;n&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>6</td>
<td>2310.8&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>79.88&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>17.33&lt;sup&gt;i&lt;/sup&gt;</td>
<td>64.52&lt;sup&gt;jk&lt;/sup&gt;</td>
<td>61.09&lt;sup&gt;ij&lt;/sup&gt;</td>
<td>32.37&lt;sup&gt;n&lt;/sup&gt;</td>
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</tr>
<tr>
<td>7</td>
<td>3166.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.08&lt;sup&gt;h&lt;/sup&gt;</td>
<td>40.1&lt;sup&gt;m&lt;/sup&gt;</td>
<td>70.16&lt;sup&gt;kl&lt;/sup&gt;</td>
<td>64.52&lt;sup&gt;jk&lt;/sup&gt;</td>
<td>35.76&lt;sup&gt;no&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>8</td>
<td>1974.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.55&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>42.85&lt;sup&gt;m&lt;/sup&gt;</td>
<td>64.33&lt;sup&gt;jk&lt;/sup&gt;</td>
<td>61.09&lt;sup&gt;ij&lt;/sup&gt;</td>
<td>32.37&lt;sup&gt;n&lt;/sup&gt;</td>
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<td>80.55&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>42.85&lt;sup&gt;m&lt;/sup&gt;</td>
<td>64.33&lt;sup&gt;jk&lt;/sup&gt;</td>
<td>61.09&lt;sup&gt;ij&lt;/sup&gt;</td>
<td>32.37&lt;sup&gt;n&lt;/sup&gt;</td>
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</tr>
<tr>
<td>10</td>
<td>2349.6&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>6.56&lt;sup&gt;h&lt;/sup&gt;</td>
<td>20.18&lt;sup&gt;i&lt;/sup&gt;</td>
<td>74.66&lt;sup&gt;jl&lt;/sup&gt;</td>
<td>73.01&lt;sup&gt;i&lt;/sup&gt;</td>
<td>34.07&lt;sup&gt;n&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2054.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.24&lt;sup&gt;g&lt;/sup&gt;</td>
<td>7.49&lt;sup&gt;h&lt;/sup&gt;</td>
<td>73.01&lt;sup&gt;i&lt;/sup&gt;</td>
<td>34.07&lt;sup&gt;n&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2792.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.58&lt;sup&gt;h&lt;/sup&gt;</td>
<td>6.58&lt;sup&gt;h&lt;/sup&gt;</td>
<td>73.01&lt;sup&gt;i&lt;/sup&gt;</td>
<td>34.07&lt;sup&gt;n&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>2654.4&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>85.18&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>25.15</td>
<td>5.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1957.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.99&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>5.9</td>
<td>5.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>2499.4&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>81.99&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>5.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>16</td>
<td>3539.1&lt;sup&gt;d&lt;/sup&gt;</td>
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<td></td>
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<td>3595.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.9</td>
<td>5.9</td>
<td>5.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Data shown for extractions two, three and four is the square root of the raw data
<sup>2</sup>Means in each column with no significant difference share the same superscript

LSD 588 5.9 5.9 5.9 5.9 5.6
NB: see Table 5.1 for details of extraction combinations.

Figure 5-3. The percentage of nitrogen recovered using different combinations of H₂O and KCl extractant... volumes of 250 mL and 500 mL. (Each bar represents the mean of three replicates.)
Only a limited number of extraction combinations were undertaken with 500 mL of extractant, as there was no significant difference from the 250 mL ratio, and the logistics were considerably complicated by using 500 mL of extractant.

5.1.3.3 Choosing an efficient method

Factors considered in choosing the most efficient method of extraction were:

1. The time required for the extraction process. This was important due to the large numbers of extractions required later in this study, and

2. A complete extraction of nitrogen was unnecessary, because not all of the extractable nitrogen is readily available to plants.

5.1.3.4 The chosen combination

Because the second water extraction did not extract an appreciable quantity of nitrogen (1.6% of the total extracted), it was considered unnecessary. There were some significant differences in effects of the time treatment, but they were not systematic, and were not considered to contribute a real difference to the recovery rate, so the shortest duration (30 minutes) was chosen. The quantity of extractant made a significant difference in the 60 minute treatment, but the difference was not considered sufficient to warrant the extra work, thus the smaller volume (250 mL) was chosen. The chosen combination was therefore one water extraction, followed by two KCl extractions, with 250 mL of extractant, and an extraction duration of 30 minutes.
5.2 Model for the absorption of nitrogen by pine bark

5.2.1 Introduction

The aim of this experiment was to develop a predictive model capable of calculating the quantity of nitrogen, in the form of ammonium or ammonia that pine bark chips are capable of absorbing from solution, with variations in temperature, initial nitrogen concentration, and pH. The results from the earlier Sections were used for scaling of the factors examined with the aim of representing the range of conditions present in the field.

5.2.2 Materials and Methods

5.2.2.1 Factors examined

As suggested in Section 3.1.3.1., the time period covered was 24 hours, with six sampling intervals (0, 1, 2, 4, 6, and 24 hours). As found in Section 4.4.5 the nitrogen content and pH of a dairy slurry varies widely, so three initial concentrations of ammonium sulphate solutions (50, 100, and 200 ppm-N) were used with three buffer systems (pH 6, 7 and 8) and a control with nano-pure water (Appendix A) instead of buffer. Each combination of treatments had nine reaction vessels, each containing 50g bark and 500 mL solution, with three concentrations and one pH per block (ie. three replicates per concentration, all at the one pH).

5.2.2.2 The sampling method

The sampling method was adapted from Section 3.3.2.2. The appropriate vessel was taken from the shaker and a 12 mL aliquot removed with a Terumo 25 mL syringe. The vessel was replaced on the shaker, and the sample filtered with Whatman No. 4
filter paper into a scintillation vial containing one drop of concentrated sulphuric acid, to bring the pH below 6 and prevent volatilisation of ammonium (Haynes and Sherlock, 1986). For samples taken after 24 hours shaking, the turbidity was such that filtering was extremely slow, and the 12 mL aliquots were spun in centrifuge tubes for six minutes at 10,000 rpm before filtering the supernatant as above.

Each sample was diluted to bring the concentration range within 0-50 ppm-N (Section 3.3), then treated with phenyl mercuric acetate for inhibition of microbial growth (Section 3.5.2.1) and stored at 4°C before analysis by the Flow Injection Analyser. Results were expressed as the concentration of nitrogen remaining in solution, corrected to the equivalent concentration of nitrogen remaining if the bark sample had been exactly 50 g, using the relationship described in Equation 3.

**Equation 3. Relationship between measured absorption and corrected absorption.**

\[
CA = MA \times \frac{50}{SW}
\]

Where

- CA = Corrected absorption
- MA = Measured absorption
- SW = Bark sample weight

5.2.2.3 Extraction of available nitrogen

The bark from the reaction vessels with an initial nitrogen content of 100 ppm was separated from the liquids after 24 hours and analysed for "readily available" and "firmly bonded" fractions of nitrogen (Section 5.1).
Because of the high variability observed in the results for the initial concentrations of the nitrogen solutions, it was not possible to accurately measure the initial quantity of nitrogen available for absorption. The variation was assumed to be analytical error, and the initial quantity of nitrogen available for absorption was taken to be 50,000 μg (the quantity available in 500 mL of 100 ppm-N solution).

The volume of liquid remaining after 24 hours of absorption was taken as 452 mL (500 mL minus 4 x 12 mL aliquots), meaning that the liquid in the pore space of the bark was included as liquid remaining. The nitrogen concentration of the liquid and this volume were used to calculate the quantity of nitrogen absorbed and corrected to the equivalent of a 50 g sample of bark.

The recovery of nitrogen was calculated as a quantity in micrograms at each extraction, corrected to the equivalent of a 50 g bark sample. This quantity was then expressed as a percentage of the quantity absorbed.

5.2.3 Results and discussion

5.2.3.1 Nitrogen absorption

The results (Figure 5.4) show a clear effect of nitrogen concentration treatments, and of pH treatments. The order in which the pH treatments appear on the graph (Figure 5.4) suggests a systematic difference between them.
Figure 5.4. Nitrogen remaining in solution after shaking pine bark in nitrogen solutions with different pH and initial nitrogen concentrations.
The 50 ppm-N concentration treatment shows a slightly lower rate of absorption (Figure 5.4), but a high proportion (35%) of the available nitrogen was absorbed. The rates of absorption for the 100 and 200 ppm-N concentration treatments were very similar, so that although the quantity of nitrogen absorbed was similar, the percentage absorbed in the 100 ppm-N treatment (28%) was twice that in the 200 ppm-N treatment (14%).

Data for the quantities of nitrogen remaining in solution were analysed by Genstat to fit a predictive model. The general model is given in Equation 4, with the constants given in Table 5.3.

**Equation 4. General model for prediction of the nitrogen concentration remaining in solution over time from buffered ammonium sulphate solution with different initial nitrogen concentrations.**

\[
NRS = -34.8 - a + 1.37IC - bIC - 0.00142IC^2 - dT - 0.00399(IC \times T)
\]

where:

- \(NRS\) = Nitrogen remaining in solution
- \(IC\) = Initial concentration of nitrogen
- \(T\) = Time
- \(a, b, d\) = constants (Table 5.3)

**5.2.3.2 The effects and interactions of time and concentration on nitrogen absorption**

<table>
<thead>
<tr>
<th>Buffer</th>
<th>a</th>
<th>S.E.M.</th>
<th>b</th>
<th>S.E.M.</th>
<th>d</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 6</td>
<td>1.13</td>
<td>3.51</td>
<td>0.0735</td>
<td>0.025</td>
<td>-0.0025</td>
<td>0.19</td>
</tr>
<tr>
<td>pH 7</td>
<td>1.14</td>
<td>3.51</td>
<td>-0.150</td>
<td>0.025</td>
<td>0.017</td>
<td>0.19</td>
</tr>
<tr>
<td>pH 8</td>
<td>-55.07</td>
<td>3.51</td>
<td>0.0552</td>
<td>0.025</td>
<td>0.0097</td>
<td>0.19</td>
</tr>
<tr>
<td>Unbuffered</td>
<td>0</td>
<td>*</td>
<td>0</td>
<td>*</td>
<td>0.00363</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Results obtained (Table 5.4) show that there was a significant effect of time and of concentration within each pH treatment, showing that there was significant absorption of nitrogen over time, and confirming that the initial nitrogen concentrations were significantly different.

**Table 5.4. The significance's of effects and interactions of concentration and time for each pH treatment.**

<table>
<thead>
<tr>
<th></th>
<th>pH 6</th>
<th>pH 7</th>
<th>pH 8</th>
<th>No Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time</td>
<td>0.028</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Concentration by Time</td>
<td>0.937</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.322</td>
</tr>
</tbody>
</table>

A significant interaction between time and concentration is shown in the pH 7 and 8 treatments, suggesting that the rate of absorption changes with initial concentration in the two higher pH treatments. This interaction was not present in the pH 6 and unbuffered treatments, suggesting that there may be a different mechanism of absorption for these pH treatments.

The mean coefficient of variation (CV) for the pH 7 and pH 8 treatments was 8.0%, as compared to 17.9% for the pH 6 and unbuffered treatments. It is possible that variations in pH during absorption, due to the lack of a stabilising buffer system, may explain the difference in the unbuffered treatment. It is more likely that this observation supports the previous suggestion that there was a different absorption mechanism for the pH 6 and unbuffered treatments.
5.2.3.3 Comparison of pH treatments

Genstat was used to compare the effects of pH treatments. The means of all the replicates in each pH treatment (including all the concentration treatments) were taken to show the overall effect of pH, and are shown in Figure 5.5.

![Figure 5.5. Comparison of pH treatments.](image)

Each pH treatment showed a significant linear response, but with a different slope. Once again, the pH 7 and pH 8 treatments belong to a separate group to that of the pH 6 and unbuffered treatments.

Results also show that the four pH treatments tend towards a common point at 24 hours absorption, suggesting that over a longer time period there may be little difference between treatments. It is possible that the bark was overcoming the pH buffering effect, although this was not measured.
5.2.3.4 Extraction of absorbed nitrogen

The results show (Table 5.5) a very similar recovery of absorbed nitrogen over all pH treatments except pH 6. At pH 6, 50% less nitrogen was extracted by the water extraction (Table 5.5). This suggests that at pH 6 the nitrogen was more firmly adsorbed to the bark, again suggesting a different absorption mechanism from the other pH treatments.

Table 5.5. The percentage recovery by different extractions of nitrogen absorbed at different pH.

<table>
<thead>
<tr>
<th>Extraction</th>
<th>pH6</th>
<th>S.E.M.</th>
<th>pH7</th>
<th>S.E.M.</th>
<th>pH8</th>
<th>S.E.M.</th>
<th>Unbuffered</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>8.7</td>
<td>2.3</td>
<td>17.6</td>
<td>2.3</td>
<td>15.9</td>
<td>2.3</td>
<td>16.4</td>
<td>1.8</td>
</tr>
<tr>
<td>KCl 1</td>
<td>26.0</td>
<td>2.3</td>
<td>27.9</td>
<td>2.3</td>
<td>32.5</td>
<td>2.3</td>
<td>30.8</td>
<td>1.8</td>
</tr>
<tr>
<td>KCl 2</td>
<td>8.1</td>
<td>2.3</td>
<td>7.6</td>
<td>2.3</td>
<td>8.71</td>
<td>2.3</td>
<td>9.4</td>
<td>1.8</td>
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<td>Total</td>
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<td>4.0</td>
<td>57.1</td>
<td>4.0</td>
<td>56.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

5.3 Long term absorption of nitrogen

5.3.1 Introduction

It was indicated earlier (Section 3.6) that the greatest quantity of ammonium sulphate absorbed by pine bark in a buffer system at pH 6 occurred over the first 24 hours. This estimation was used in the design of all the subsequent experiments. It is not likely that this length of time would be sufficient in a working waste treatment system. The aim of this experiment was to estimate the ability of the model developed in Section 5.2 to predict the absorption of nitrogen over a period of time greater than 24 hours.
5.3.2 Materials and methods

The method used in Section 5.2 was used with sampling over a period of three weeks. A solution of ammonium sulphate at 100 ppm-N and buffered at pH 6 was used with six replicates, each containing 50 g of bark and 500 mL of nitrogen solution. After 478 hours, the extraction of "readily available" and "firmly bonded" nitrogen (Section 5.1) was conducted on three of the six replicates.

5.3.3 Results and discussion

5.3.3.1 Nitrogen remaining in solution

Results obtained (Figure 5.6) show that about 75% of the nitrogen in solution had been absorbed by the pine bark after 478 hours; and that the absorption was continuing. This demonstrates that a pine bark batch filter has the potential to absorb a high proportion of the ammonium present in a waste stream.

![Figure 5.6. Concentration of nitrogen remaining in solution over three weeks.](image-url)
5.3.3.2 Absorption model

A linear model was fitted to the data using Minitab software (Equation 5). There was no significant deviation from a linear model. The predicted initial concentration (72.7 ppm-N) was lower than the measured initial concentration (100 ppm-N). This could be due to the model not explaining all the variation and/or errors in the measurement of the nitrogen concentration.

**Equation 5. General model for prediction of the nitrogen concentration remaining in solution over three weeks.**

\[ NRS = 72.7 - 0.107T \]

\[ R^2 = 75.6\% \]

where:

\( NRS \) = Nitrogen remaining in solution

\( T \) = Time

**Equation 6. Simplified model from Equation 4 for prediction of the nitrogen concentration remaining in solution over time from a solution of 100 ppm-N ammonium sulphate buffered at pH 6.**

\[ NRS = 79.52 - 0.397T \]

\[ R^2 = 95.3 \]

where:

\( NRS \) = Nitrogen remaining in solution

\( T \) = Time

The short term model for a buffer of pH 6 and an initial concentration of 100 ppm-N (Section 5.2.3.1) can be simplified to Equation 6 by entering the constants for pH 6 and an initial nitrogen concentration of 100 ppm-N. This shows a similar predicted initial nitrogen concentration, but a steeper gradient of absorption. This suggests the possibility that further investigation may show long term absorption of nitrogen to have a curvilinear component in the model.
5.3.3.3 Forms of nitrogen recovered

A total of only 0.47% of the absorbed nitrogen was recovered as "readily available" nitrogen, and 1.35% as total extractable nitrogen (Table 5.6). This suggests that the majority of the nitrogen absorbed was firmly bonded to the pine bark.

Table 5.6. Percentage recovery of absorbed nitrogen after long term absorption.

<table>
<thead>
<tr>
<th>Form of nitrogen</th>
<th>Percentage recovered</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Readily available</td>
<td>0.47</td>
<td>0.075</td>
</tr>
<tr>
<td>Firmly bonded</td>
<td>0.88</td>
<td>0.075</td>
</tr>
<tr>
<td>Total extractable</td>
<td>1.35</td>
<td>0.075</td>
</tr>
</tbody>
</table>
CHAPTER 6. THE NATURE OF NITROGEN
ABSORPTION BY PINE BARK

6.1 Biological and chemical absorption of nitrogen

6.1.1 Introduction

It has not been established in the literature whether the absorption of ammonia or ammonium by pine bark is chemical or biological. The differing response to pH shown in the previous work suggested that there may be different mechanisms of nitrogen absorption operating in the bark at different pH. Comparison of the absorptive capacities of sterilised pine bark and those of raw pine bark at different pH should give an indication of the proportion of biologically to chemically absorbed nitrogen.

Gamma-irradiation and autoclaving, two common methods of sterilisation, are expected to have different effects on the structure of the bark, particularly due to the heat involved with autoclaving.

6.1.2 Materials and Methods

The absorption method as described in Section 5.2 was used, with nine replicates for each sterilisation and pH treatment. Sterility was maintained as necessary by using the laminar flow cupboard (in the Animal Sciences Department of Lincoln University) which filters airborne organisms from a constant airflow. The modifications are as described below.
6.1.2.1 Autoclaved bark

To withstand the temperatures and pressures involved in autoclaving, IL centrifuge tubes were used as reaction vessels. A 50 g sample of pine bark was placed in each of the nine reaction vessels, which were loosely capped and autoclaved at 120°C and 103 kN/m² for 40 minutes, then left to cool overnight. There was no moisture absorbed by the bark during this process as sample weights did not change.

6.1.2.2 Gamma irradiated bark

Eighteen 50 g samples of pine bark were sealed into plastic bags, and sent to Mallinckrodt Veterinary Limited in Upper Hutt, where they were irradiated with a minimum of 2.5 Mrads. This dose is commonly accepted as sufficient to kill all microbes of medical significance. Upon return, the bark was transferred in sterile conditions into 1200 mL plastic jars which had been sterilised with ethanol.

6.1.2.3 Nitrogen solutions and bark

Ammonium sulphate solution (100 ppm-N) buffered at pH 6 or 8 was prepared (Appendix B), and 500 mL added to each of the bark samples in sterile conditions. The reaction vessels were then placed on a rotational shaker at 150 rpm in the constant temperature cupboard (Section 5.1.2.2).

6.1.2.4 Sampling

Sampling involved opening the reaction vessel in sterile conditions and using a syringe, which had been sterilised with ethanol, to take a 12 mL aliquot of solution. The vessels were replaced on the shaker, and the fluid samples were then treated as in Section 5.2.
6.1.3 Results and discussion

When compared to nitrogen absorption curves of untreated bark from previous work at the appropriate pH (Figure 5.5), the present results show (Figure 6.1 and Figure 6.2) that sterilisation treatment did not reduce the amount of nitrogen absorbed from solution by the pine bark at pH 6, whereas at pH 8 both sterilisation methods reduced the nitrogen absorption. There was no significant difference between the two sterilisation methods.

Because sterilisation of the pine bark has no significant effect on the quantity of nitrogen absorbed at pH 6, it can be concluded that the absorption of nitrogen by pine bark at this pH was mostly a chemical process.

At pH 8, there was a significant proportion of absorption which was apparently microbially mediated, although it is possible that some of the nitrogen absorption may be chemically mediated.

![Figure 6.1. Concentration of nitrogen remaining in solution with sterilised and unsterilised bark at pH 6.](image-url)
Thus at pH 6 a different mechanism of nitrogen absorption occurred compared to that at pH 8 (and presumably at pH 7). These results are supported by the observations made in Sections 5.2.3.3 and 5.2.3.4.

The results are not consistent with the findings of He et al. (1990) who suggest that ammonia in soil is likely to be chemically fixed, and that ammonium is able to be absorbed by soil microbes. Haynes and Sherlock (1986) have stated that ammoniacal-nitrogen is present mostly in the form of ammonium at pH 6, with ammonia becoming increasingly important at pH 8.

6.2 The probable location of nitrogen absorption in pine bark as indicated by infra-red spectroscopic analysis

6.2.1 Introduction

Lloyd (1977) detailed a simple method of analysis to indicate the locations within pine bark which have changed after exposure to ammonia gas. Changes in the solid phase
infra-red spectrum, using a KBr disk, were assumed to indicate the location of attachment for the nitrogen, although it is difficult to prove this. Oven drying the bark at 105 °C after absorption of ammonia did not affect the results of the infra red spectrum, suggesting strong attachment. The changes noted by Lloyd (1977) were in the 1740 cm⁻¹ wavelength, which was taken to suggest a reaction with the carbonyl groups of the bark, resulting in possibly ammonium salt or imine formation.

The aim of this experiment was to use the infra-red technique of Lloyd (1977) to provide information about the mechanism of absorption of nitrogen by pine bark.

6.2.2 Materials and methods

Coarse (< 30 mm) and finely ground (< 4 mm) bark samples, with the wood removed, were exposed for 24 hours to solutions of ammonium sulphate or glycine at pH 6 or 8, or to gaseous ammonia (Table 6.1).

Samples of treated and untreated bark were freeze dried and ground to a fine powder with the Tecator mill (Appendix A). The solid phase infra-red spectrophotometer (Appendix A) at the Wool Research Organisation of New Zealand, Lincoln, was used to measure the changes in reflectance spectra of samples in a finely ground KBr disk. Reflectance spectrophotometry provides the same information as absorption spectrophotometry (Worth, pers. comm.). An increase in absorbance indicates a decrease in reflectance.
Table 6.1. Treatment combinations and results of infra red analysis.

<table>
<thead>
<tr>
<th>Conc. (ppm-N)</th>
<th>Nitrogen source</th>
<th>Bark size</th>
<th>Sterilisation</th>
<th>pH 6$^+$</th>
<th>pH 6$^+$</th>
<th>pH 8$^+$</th>
<th>pH 8$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1740 cm$^{-1}$</td>
<td>1610 cm$^{-1}$</td>
<td>1740 cm$^{-1}$</td>
<td>1610 cm$^{-1}$</td>
</tr>
<tr>
<td>100</td>
<td>(NH$_4$)$_2$SO$_4$</td>
<td>coarse</td>
<td>x</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>500</td>
<td>(NH$_4$)$_2$SO$_4$</td>
<td>coarse</td>
<td>x</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1000</td>
<td>(NH$_4$)$_2$SO$_4$</td>
<td>coarse</td>
<td>x</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1000</td>
<td>(NH$_4$)$_2$SO$_4$</td>
<td>fine</td>
<td>gamma</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1000</td>
<td>(NH$_4$)$_2$SO$_4$</td>
<td>fine</td>
<td>gamma</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>glycine</td>
<td>coarse</td>
<td>x</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1000</td>
<td>glycine</td>
<td>coarse</td>
<td>x</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>glycine</td>
<td>fine</td>
<td>x</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1000</td>
<td>glycine</td>
<td>fine</td>
<td>x</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1000</td>
<td>glycine</td>
<td>coarse</td>
<td>gamma</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>1000</td>
<td>glycine</td>
<td>fine</td>
<td>gamma</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>gas</td>
<td>NH$_3$(g)</td>
<td>coarse</td>
<td>x</td>
<td>2</td>
<td>2</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>gas</td>
<td>NH$_3$(g)</td>
<td>fine</td>
<td>x</td>
<td>2</td>
<td>2</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>gas</td>
<td>NH$_3$(g)</td>
<td>coarse</td>
<td>gamma</td>
<td>2</td>
<td>2</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>gas</td>
<td>NH$_3$(g)</td>
<td>fine</td>
<td>gamma</td>
<td>2</td>
<td>2</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

*Degree of increase in reflectance was visually graded as: (1) different or (2) substantially different*

6.2.3 Results and discussion

The results of the various treatments are summarised in Table 6.1. It can be seen that for all the treatments there was an increase in the reflectance at 1740 and 1610 cm$^{-1}$ peaks. The increase in reflectance for the gaseous treatments was more substantial than for the other treatments.

The results confirm those of Lloyd (1977) which showed a reduction of absorption (ie. increase in reflectance) in pine bark at 1740 cm$^{-1}$ after exposure to gaseous ammonia due to probable reaction with carbonyl groups. The present results indicate that this change also occurs but to a lesser extent in pine bark which has been exposed to ammonium sulphate or glycine solutions. Furthermore, an enhancement of infra-red reflection occurred at 1610 cm$^{-1}$ in all treatments in the present study. This was not
reported by Lloyd (1977), thus groups other than carbonyl groups may be involved in the absorption of nitrogen by pine bark.

It can be seen that in all the infra-red spectra presented here (Figure 6.3, Figure 6.4, and Figure 6.5) there was a substantial increase in reflectance at the 1740 and 1610 cm\(^{-1}\) peaks, with no real difference between the treatments. On comparing the spectra in Figure 6.4 and Figure 6.5 it is obvious that there was no substantial difference between the coarse and fine bark treatments. This method of analysis does not support the suggestion (Section 2.3) that different forms of nitrogen react in different ways with pine bark.

Figure 6.3. Infra-red reflectance spectra for coarse untreated bark, and for coarse sterilised and unsterilised bark treated with ammonia gas.
Figure 6.4. Infra-red reflectance spectra for coarse untreated bark, and for coarse bark treated with 1000 ppm-N ammonium sulphate at pH 6 and pH 8.

Figure 6.5. Infra-red reflectance spectra for coarse untreated bark, and for sterilised and unsterilised fine bark treated with 1000 ppm-N ammonium sulphate at pH 6.
The present infra-red results suggest that there was chemical activity which changed the infra-red reflectance characteristics of the pine bark at 1740 and 1610 cm\(^{-1}\) in all treatments studied. Table 6.2 contains some potential sources of resonance in these regions. The wide variety of possibilities makes it impractical in this experiment to accurately determine what was happening.

### Table 6.2. Possible assignment of changes in infra-red spectra in the regions of 1740 and 1610 cm\(^{-1}\), based on Bellamy (1958) and Stevenson and Goh (1971).

<table>
<thead>
<tr>
<th>1610 cm(^{-1})</th>
<th>1740 cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>• C=C</td>
<td>• 5-membered ring ketones</td>
</tr>
<tr>
<td>• C=N (conjugated or cyclic)</td>
<td>• saturated aldehydes</td>
</tr>
<tr>
<td>• N=N</td>
<td>• α-halogen acids</td>
</tr>
<tr>
<td>• CONH(_2)</td>
<td>• saturated esters 6- and 7-ring lactones</td>
</tr>
<tr>
<td>• NH(_3)</td>
<td>• αβ-unsaturated 5-ring lactones</td>
</tr>
<tr>
<td>• NH</td>
<td>• anhydrides</td>
</tr>
<tr>
<td>• O-NO(_2)</td>
<td>• Y-lactams</td>
</tr>
<tr>
<td></td>
<td>• β-lactams</td>
</tr>
</tbody>
</table>

The most likely source of infra-red resonance at 1740 and 1610 cm\(^{-1}\) is from carbonyl groups (Stevenson and Goh, 1971; Kemp and Vellaccio, 1980) which show intense resonance within the 1800 - 1600 cm\(^{-1}\) region. In particular, a simple aldehyde shows resonance at 1740 - 1720 cm\(^{-1}\), and imines and amides show resonance in the 1690 - 1640 cm\(^{-1}\) region (Kemp and Vellaccio, 1980), which may account for the change at 1610 cm\(^{-1}\) encountered in this experiment, although the imine band is usually weak.

The two characteristic absorptions found with most ammonium salts are in the regions 3200 cm\(^{-1}\) and 1430-1390 cm\(^{-1}\) (Bellamy, 1958). The spectra recorded in the present study showed no substantial change in these regions: Thus it can be concluded that the ammonium salt content of the bark did not change substantially with treatment.
It is possible that $^{13}$C-NMR spectroscopy would give a more accurate spectrum, from which more definite conclusions could be drawn (Stevenson, 1982). It is likely that the analysis of a greater number of samples would eliminate some of the noise encountered in this experiment, and further define the changes taking place. Future work in this area holds much potential, with the possibility of further defining the chemical activity occurring with the absorption of ammonium from a liquid by pine bark, and achieving more efficient absorption of nitrogen from a better understanding of the process.
CHAPTER 7. NITROGEN ABSORPTION FROM DAIRY SLURRY

7.1 Absorption under laboratory conditions

7.1.1 Introduction

A model has been developed to estimate the quantity of nitrogen, in the form of ammonium sulphate, which may be absorbed from an aqueous solution by pine bark. The aim of this experiment was to test the applicability of this model, under laboratory conditions, to total nitrogen in a dairy slurry.

7.1.2 Materials and Methods

The method of Section 5.2 was used, with nine replicates of dairy slurry taken from the second sump at Darryl Petheram's farm, shortly after milking was complete.

A linear model was fitted to the data using Minitab software.

7.1.3 Results

The results are graphically shown in Figure 7.1, and the model is presented in Equation 7. A total of 28% of the available nitrogen was absorbed over the 24 hour period. The predicted initial total nitrogen content of the slurry was thus 117.5 ppm-N, which is less than half the content (278.1 ppm-N) measured in the slurry in Section 4.4.5. This is evidence of the high variation inherent in the flow of slurry from a dairy shed.
Equation 7. General model for prediction of the nitrogen concentration remaining in solution over time from a dairy slurry over 24 hours.

\[ NRS = 117.5 - 1.36T \]

where:
\( NRS \) = Concentration of nitrogen remaining in solution
\( T \) = Time

\( R^2 = 89.4\% \)

The rate of absorption of nitrogen from solution (-1.36 ppm, hour\(^{-1}\)) was approximately four times the rate of nitrogen absorption predicted in Equation 6 (-0.397 ppm, hour\(^{-1}\)). The source of this difference is not apparent, but the model developed in Section 5.2.3.1 is clearly not appropriate for prediction of total nitrogen absorption from a slurry by pine bark. With hindsight the absorption of ammoniacal-nitrogen should also have been measured, which would have allowed a more appropriate comparison with Equation 6.

![Figure 7.1. Concentration of nitrogen remaining in a dairy slurry over time in laboratory conditions.](image-url)
7.2 A pilot waste treatment program

7.2.1 Introduction

The previous work in this study has provided the necessary data for design of a pilot treatment system for dairy slurry. The aim of this experiment was to test the knowledge acquired in the earlier stages of this study in a practical waste disposal situation.

Darryl Petheram, a dairy farmer in the Lincoln area, made his facilities available for this trial. He had recently installed an expensive system of disposal for his slurry, involving collecting the slurry first in a holding tank, then transferring it to a sump from which it was irrigated directly on to the land of his farm (Figure 7.2). The pumps were controlled with electrical contacts to switch on and off automatically. The presence of sumps and pumps specifically designed to cope with dairy slurry made it a simple task to collect samples for analysis and for the trial.

7.2.2 Materials and Methods

To form a batch filter, a large trailer with sides, having a capacity of 9.5 m$^3$, was lined with plastic and 5 m$^3$ of bark added. The second pump in the farm irrigation system was used to add approximately 4000 litres of slurry, slightly more than the estimated average production at a single milking on this farm (3500 litres).

The ratio of bark to slurry (4 m$^3$ of liquid to 5 m$^3$ of bark) was slightly higher than that used in the lab situation (50 grams of bark to 500 mL of liquid). This quantity of liquid filled the free air space around the bark, with a little extra liquid (17%) to allow for absorption of water into the pore space of the bark. The greatest possible quantity
of bark was therefore in contact with the liquid, to compensate for no agitation of the filter.

Samples of liquid were taken from three parts of the trailer, described as the front (approximately 700 mm from the front), middle and back (approximately 700 mm from the back). Sampling involved using a sampling probe (Figure 7.3), consisting of a plastic scoop on a long pole, and pushing this through the upper layer of bark into the slurry. Because the pressure of the bark and liquid on the top of the scoop closed it slightly, the scoop was still filling by the time it reached the middle of the slurry. A
sub-sample was then taken from the scoop, labelled and frozen until it could be analysed for total nitrogen content, using a Kjeldahl digestion auto-analyser (Appendix A) in the Animal Science Department of Lincoln University.

After eight days of soaking, a simple recirculation system was set up. The grain chute in the tailgate of the trailer was partially opened, allowing a stream of liquid to fall through a series of five sieves ranging from 50 mm to 1 mm and into a tank. A twelve volt bilge pump was used to pump the collected liquid through a pipe into the top front of the trailer. Samples of liquid were taken as the liquid re-entered the trailer and analysed for total nitrogen content.

After 24 hours the liquid was allowed to drain away. Samples of bark were then taken at approximately the same points as liquid samples were taken before recirculation started (front, middle and back), at approximate depths of 20 and 40 cm. These were
dried and analysed for total carbon and nitrogen by the furnace elemental analyser (Appendix A).

7.2.3 Results and discussion

The concentration of nitrogen remaining in the slurry over time is shown in Figure 7.4. Where there are three points per time (48, 72, 168, and 192 hours) this represents sampling points at the front, middle and back of the trailer, with the highest concentration representing the front of the trailer, and the lowest at the back. The most likely reason for this difference was that the slurry was pumped into the trailer from a high pressure hose, and the slurry landed at the front of the trailer and flowed back. It is likely that a proportion of the nitrogen was trapped, probably in the solids, at the front of the trailer. The difference between sampling points reduced with time as the nitrogen levels in the trailer equilibrated.

The data showed a significant deviation from a linear model. A curvilinear model, which is different from the models in the previous work, is presented in Equation 8.

![Figure 7.4. Concentration of nitrogen remaining in the dairy slurry over time.](image-url)
Equation 8. Curvilinear model for prediction of the total nitrogen concentration remaining in solution over time from a dairy slurry.

\[ NRS = 318 - 1.748T + 0.00571T^2 \]

\[ R^2 = 64.4\% \]

where:
- \( NRS \) = Concentration of nitrogen remaining in solution
- \( T \) = Time

The linear model (Equation 9), although accounting for less of the variation in the experiment, showed a very similar absorption rate (-0.445) to the prediction model (-0.397) found in Section 5.2.3.1 (Equation 6).

Equation 9. Linear model for prediction of total nitrogen concentration remaining in solution over time from a dairy slurry.

\[ NRS = 318 - 0.445T \]

\[ R^2 = 51.0\% \]

where:
- \( NRS \) = Concentration of nitrogen remaining in solution
- \( T \) = Time

For short term prediction of total nitrogen absorption from a slurry by pine bark the linear model developed in Section 5.2.3.1 is appropriate. For longer term absorption the above model (Equation 8) is more appropriate, but further work is necessary to confirm these results, and to account for variations in initial nitrogen concentration and pH.

Clearly further work is necessary to reconcile the differences between the models for prediction of absorption of ammonia from solution, and of total nitrogen from a dairy slurry.
CHAPTER 8. CONCLUSIONS.

This study has shown that pine bark has the ability to absorb 50-75% of the nitrogen present in a solution of organic or ammoniacal-nitrogen over a period of three weeks.

As in many biological systems, the preliminary experiments showed that the initial concentration of ammoniacal-nitrogen in solution had a significant effect on the rate of nitrogen absorption. Variations in the rate of absorption of ammoniacal-nitrogen from solution were attributed to a combination of variability between bark samples, and undefined analytical errors such as the inherent variation in the Flow Injection Analyser and/or loss of ammoniacal-nitrogen during sample storage by microbial activity. It was assumed that the presence of phenyl mercuric acetate in samples from subsequent work suppressed microbial activity during storage, but further study of this aspect would seem appropriate.

Measurement of the chemical and physical characterisation of the pine bark and slurry highlighted the wide variation found in each of these products. The variability of the physical characteristics of pine bark, and of the pH and forms of nitrogen present in a dairy slurry, must be taken into account when designing a practical application of this work.

Empirical models were developed to predict the amount of ammoniacal-nitrogen that pine bark was able to absorb from an aqueous solution over 24 hours and over three weeks, with factors of initial nitrogen concentration and initial pH as variables. An
indication of the proportion of readily extractable ammoniacal-nitrogen was
developed in terms of "readily available" and "firmly bonded" nitrogen. These
proportions changed with pH, indicating that there were different mechanisms of
absorption at pH 6 and pH 8. Although the mechanism and rate of absorption appears
to change with pH, the amount of nitrogen absorbed over more than 24 hours appears
to be largely unaffected by pH. After three weeks the proportion of readily extractable
nitrogen at pH 6 was reduced to almost zero, indicating that the nitrogen becomes
more firmly bound to the bark over this period of time than over the initial 24 hours.

Some insight into the mechanism of absorption was developed through the use of
sterilised and non-sterilised bark. It was shown that at pH 6 the absorption of
ammoniacal-nitrogen was essentially chemical, and at pH 8 the absorption appeared to
be largely microbially mediated. This finding supported the earlier evidence that there
appeared to be differing mechanisms of nitrogen absorption at different pHs, but did
not support the findings of He et al. (1990) regarding absorption of ammoniacal-
nitrogen in soil. Infra-red spectroscopy was used in an attempt to deduce the possible
functional groups involved in nitrogen absorption. No firm conclusions were drawn,
although it was found that unidentified functional groups, as well as carbonyl groups,
were involved in the absorption of nitrogen by pine bark.

The ability of pine bark to absorb total (organic and mineral) nitrogen from a dairy
slurry was examined under both laboratory and field conditions. In hindsight it would
have been more appropriate to have measured the absorption of ammoniacal-nitrogen
from the slurry as well as the total nitrogen, because the earlier models developed for
absorption of ammoniacal-nitrogen did not compare well with the models developed for absorption of total nitrogen from a dairy slurry. It was shown that over 24 hours the rate of total nitrogen absorption is about 4 times the rate of ammoniacal-nitrogen absorption, but that over two weeks, the average rate of absorption of total nitrogen was similar to the rate of ammoniacal-nitrogen absorption determined previously.

Finally, it is concluded that there is potential for pine bark to be used in a dairy slurry treatment system, not only to remove nitrogen from the slurry in a waste treatment process, but to create added value for pine bark by generating a product which may be utilised in a horticultural context.
CHAPTER 9. REFERENCES


CHAPTER 10. ACKNOWLEDGMENTS

I would like to thank a great many people for their assistance and motivation in helping me to achieve this result.

Two important people are my father, David Musgrave, and Stewart Meyers. It was my father's inspection of Stewart's composting operation in Nelson, for the Bio-Gro certification label, which started this study. During this inspection there were several factors which did not fit in with what David had learned over the years. Upon questioning, Stewart put forward a number of unique ideas, which were very cleverly put together, and strayed from the acknowledged 'way of things', posing several questions which my father and I could not answer.

I began a Masters of Science at Canterbury, then transferred to Lincoln University, where Prof. Kuan Goh and Dr. Rob Sherlock agreed to supervise this project. The battle began. Theirs was the task of guiding me through the approaching mine field!

Because bark is such a unique material, the usual methods of analysis for plant material had to be severely modified, and in this endeavour, I was ably assisted by the technicians in the Soil Science Department of Lincoln University, namely Rob MacPherson, Roger Cresswell, Leanne Hassal, and Maureen McCloy. Keith Cameron's advice on measurement of pore space was invaluable, particularly due to the fact that I was a microbiologist, rather than a soil scientist!
During the design phase, it was the strongly practical nature of Dr Rob Sherlock which saved me from many a deep pit, and my eternal thanks go to him. Once the design was complete, the grind began, and this would never have been completed without the constant drive of Prof. Kuan Goh. His dedication to his work is legendary, and his experience in writing scientific literature helped mould the screeds of data into a meaningful thesis. Thank you Kuan.

As with life, there are always a host of people on the periphery of a project without whose support, suggestions, and occasional abuse, the motivation to continue would fade. A special thanks to my musical friends for their warmth and relaxing distractions. They are too many to thank individually, but to those who still care, thank you!
APPENDIX A. CHEMICALS AND EQUIPMENT USED

**Chemicals:**
- Potassium Dihydrogen Phospnate; KH₂PO₄ (anhydrous), Lab Reagent Quality
- Sodium Hydroxide; NaOH (anhydrous), Lab Reagent Quality
- Ammonium Sulphate; (NH₄)₂SO₄ (anhydrous), Lab Reagent Quality
- Phenyl Mercuric Acetate; C₈H₆HgO₂, Lab Reagent Quality
- Concentrated Sulphuric Acid; H₂SO₄, Lab Reagent Quality
- Buffer tablets at pH 4, pH 7 and pH 9.2 (for pH meter calibration)
- Nano-pure (de-ionised) water

**Glass and plastic-ware:**
- 300, 600 and 1200 mL white plastic jars (wide neck) with red lids
- 250 mL conical flasks
- 100 mL white plastic bottles with red lids
- 25 mL scintillation vials
- 20 cm diameter glass funnels
- Measuring cylinders; 50, 100, 500 and 1000 mL
- 2 L Volumetric Flask
- 3 L beaker

**General equipment:**
- Mortar and pestle
- Spatula

**Electronic equipment:**
- Tecator Kjeltec Auto 1035/38 Sampler System (Kjeldahl Auto-Analyser)
- Tecator FIAstar 5010 Analyzer with a 5027 sampler and 5032 controller
- Leco CNS-2000 Elemental Analyzer furnace V 4.03
- Tecator Cyclotec electric grinder
- Orion Research model 701A/digital ionalyzer with Ag/AgCl 91 series pH electrode
- Electronic timer
- Electronic scales
- Chiltern Scientific rotational shaker table
- End-over-end shaker
- Reciprocal shaker
APPENDIX B. BUFFERS AND AMMONIUM SOLUTIONS

The CRC Handbook of Chemistry (Weast D.R.C. 1974) was used to obtain appropriate quantities of 0.1 M Potassium Dihydrogen Phosphate (PDP) and 0.1 M Sodium Hydroxide solution to make buffers of pH 6, 7, and 8, as summarised in Table 11.1.

Table 11.1. Buffer component volumes (mL) required to achieve a particular pH.

<table>
<thead>
<tr>
<th>pH</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>PDP</td>
<td>NaOH</td>
<td>PDP</td>
</tr>
<tr>
<td>1L</td>
<td>899</td>
<td>100</td>
<td>632</td>
</tr>
<tr>
<td>2L</td>
<td>1798</td>
<td>201</td>
<td>1264</td>
</tr>
<tr>
<td>2.5L</td>
<td>2248</td>
<td>251</td>
<td>1580</td>
</tr>
<tr>
<td>3L</td>
<td>2697</td>
<td>302</td>
<td>1896</td>
</tr>
<tr>
<td>4L</td>
<td>3597</td>
<td>402</td>
<td>2528</td>
</tr>
<tr>
<td>5L</td>
<td>4496</td>
<td>503</td>
<td>3160</td>
</tr>
</tbody>
</table>

Stock solutions were made as follows and maintained in half gallon Winchesters:

0.1M potassium dihydrogen phosphate

The appropriate weight of PDP was weighed into a beaker then quantitatively washed into a 2L volumetric flask using nano-pure (NP) water. More NP water was added to the mark, the solution was mixed thoroughly and transferred to a Winchester bottle (2.5 L) for storage.

0.1M sodium hydroxide

The appropriate weight of NaOH was weighed into a beaker and quantitatively washed into a 1L volumetric flask using NP water. More NP water was added
to the mark, the solution was mixed thoroughly, and transferred to a reagent bottle (1 L) for storage.

Solutions of ammonium sulphate for the first two preliminary experiments were made with the following procedure:

Table 11.2 was used to determine the required quantities of ammonium sulphate, which were weighed into beakers. An appropriate quantity of buffer solution was added by measuring cylinder and mixed thoroughly until the ammonium sulphate solid was completely dissolved.

<table>
<thead>
<tr>
<th>ppm-N</th>
<th>in 100mL</th>
<th>in 1L</th>
<th>in 2L</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.012</td>
<td>0.118</td>
<td>0.236</td>
</tr>
<tr>
<td>50</td>
<td>0.024</td>
<td>0.236</td>
<td>0.471</td>
</tr>
<tr>
<td>75</td>
<td>0.035</td>
<td>0.354</td>
<td>0.707</td>
</tr>
<tr>
<td>100</td>
<td>0.047</td>
<td>0.471</td>
<td>0.943</td>
</tr>
</tbody>
</table>

Solutions of ammonium sulphate used in Section 3.3 and subsequent work were made with the following procedure:

The exact quantity of finely ground ammonium sulphate required (4.714, 9.429, or 18.858 g for 50, 100, or 200 ppm-N) was weighed out to +/- 0.0005 g. and quantitatively washed into a 1 L volumetric flask with nano-pure water. The flask was then filled to the mark and the solution distributed into ten labelled 100 mL volumetric flasks for storage. When diluted to 2 L with water or buffer solution, each of these flasks gave a solution of the appropriate concentration (50, 100, or 200 ppm-N)

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