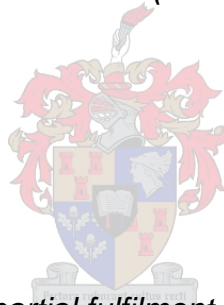# *IN SITU* DENITRIFICATION OF NITRATE RICH GROUNDWATER IN MARYDALE, NORTHERN CAPE

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Thesis submitted in partial fulfilment of the requirements of
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DECLARATION

I declare that this research is my own and that it was supervised by Prof. M. V. Fey, Dr G. Tredoux and Dr P. Engelbrecht. No part of this research has been submitted in the past or is being submitted, as a degree at another university.

Sumaya Clarke (Israel)

Signature　　　　　　　　　　　　　　*Date*

ABSTRACT

South Africa is a water scarce country and in certain regions the quantity of surface water is insufficient to provide communities with their domestic water needs. In many arid areas groundwater is often the sole source of water. This total dependence means that groundwater quality is of paramount importance. A high nitrate concentration in groundwater is a common cause of water being declared unfit for use and denitrification has been proposed as a potential remedy.

In groundwater of the Marydale district in the Northern Cape Province, nitrate levels are high enough to be of concern for domestic and livestock consumption. A review of the literature indicates that bacterial denitrification of groundwater can be achieved *in situ* by using a suitable energy substrate. The technology has been tested elsewhere in the world but more certainty is needed on whether it is a feasible option for local groundwater remediation using local, cost-effective energy substrates and exploiting bacterial populations present naturally in the regolith.

The objective of this study was to perform denitrification experiments by laboratory incubation using soil and groundwater samples collected in Marydale in order to determine; 1) The effectiveness of different carbon sources; 2) The effect of using soil sampled at different depths; 3) The effect of C:N ratio of the carbon substrate; and 4) The quality of resultant water.

Various experiments were set up using 10 g soil and 40 mL groundwater with different concentrations of carbon sources (sawdust, glucose, maize meal and methanol). All experiments were done under a nitrogen atmosphere to exclude oxygen and temperature was kept constant at 23 °C. Indicator parameters were selected based on literature review, and major cations and anions and some metals were analysed for initially and at selected times during each experiment to evaluate whether major ion chemistry was changing over time. Parameters analysed in supernatant solutions after varying periods of time to indicate progress of denitrification and reduction included nitrate, nitrite, sulfate, alkalinity, chloride, acetate, basic cations, ammonium, pH, electrical conductivity, dissolved organic carbon, heterotrophic plate count, iron and manganese.

The Marydale groundwater in some boreholes is of predominantly NaCl type and the nitrate concentration of 19-32 mg/L as N exceeds ideal limits for drinking water of 6mg/L as N . Two soil materials were sampled at different depths from a red sand overlying calcrete (Plooysburg form, Family Py1000).

The incubation experiments showed denitrification was complete within a period of between 1 and 6 weeks depending on the carbon substrate and C:N used. Higher rates of nitrate removal were achieved where greater C:N was used. Readily degradable carbon substrates e.g. glucose showed rapid denitrification, while sawdust, a slowly degradable substrate, effected slower denitrification, hence it was concluded that intermediately degradable carbon substrates e.g. wheat straw may prove more suitable. Use of shallower soil material containing initially higher nitrate levels resulted in better denitrification rates, however, both soil materials effected denitrification.. Heterotrophic plate counts increased with time, this presence and growth of heterotrophic bacteria confirmed that conditions were optimum for growth and denitrification and that inoculation with bacteria is not a requirement for *in situ* denitrification. Dissolved organic carbon (DOC) concentration could be directly correlated to the initial input of carbon substrate as soil and groundwater lacked organic material. Results showed that reaction products such as acetate and nitrite, and basic cation concentrations were elevated in the supernatant solution in preliminary experiments. This was interpreted to be attributed to incomplete oxidation of organic material and excess soluble and available carbon for reaction. Cation concentrations were interpreted to have resulted from a decrease in pH brought on by organic acids produced during denitrification. The method used showed specificity, as the only parameters affected by the denitrification experiment were DOC, alkalinity, nitrite, nitrate, and the heterotrophic plate count. The DOC and HPC did not comply with acceptable levels for drinking water. Removal of HPC by boiling or chlorinating is required to ensure that the resultant water composition is of potable quality.

For further research with slowly degradable carbon sources it is recommended that a C:N ratio of more than 12 should be employed, and monitoring should focus on soluble carbon nitrate, nitrite, and heterotrophic plate count.

The study confirmed that denitrification of this groundwater with a range of carbon sources is possible within a short period of anaerobic contact with local soil material. With sufficient knowledge of the characteristics of the soil and groundwater in the area, establishment of a working *in situ* denitrification plant is probably feasible.

UITTREKSEL

Water in Suid-Afrika is skaars en veral in gebiede waar oppervlakwater nie voldoende is om aan gemeenskappe water te voorsien nie. Grondwater is in hierdie gebiede die enigste bron van drinkwater. Dit is dus baie belangrik dat die grondwatergehalte van sodanige aard is dat dit met die minimum behandeling geskik is vir mens en dier. Dit is egter so dat hoë nitraat–vlakke in baie gevalle die algemene rede is waarom grondwater ongeskik verklaar word vir huishoudelike gebruik. As gevolg hiervan word *in-situ* denitrifikasie van grondwater voorgestel as 'n moontlike oplossing vir hierdie probleem. Die nitraatvlakke in die grondwater in Marydale in die Noord-Kaap is verhoog en word as 'n potensiële risiko gesien vir mens en dier. Bakteriologiese denitrifikasie is 'n natuurlike proses, maar is volgens die literatuuroorsig ook moontlik met *in-situ* behandeling met behulp van 'n geskikte koolstofbron. Alhoewel die tegnologie in ander lande getoets is, is verdere toetse nodig om te bepaal of dit plaaslik toegepas kan word met geskikte, goedkoop koolstofbronne en met behulp van natuurlike denitrifiserende bakterieë wat in die grond en grondwater voorkom.

Die doel van hierdie studie was dus om laboratorium denitrifikasie eksperimente uit te voer op grond- en grondwatermonsters wat in die Marydale in die Noord-Kaap versamel is om te bepaal:1) Hoe geskik verskillende koolstofbronne vir denitrifikasie is; 2) Wat die uitwerking op denitrifikasie wanneer gronde van verskillende dieptes gebruik word is; 3) Wat die mees geskikte koolstof: stikstof (C:N) verhouding is, en 4) of die produkwater aan die watergehalte-standaarde voldoen.

Verskeie eksperimente is opgestel met mengsels van 10 g grond in 40 ml grondwater met verskillende koolstofbronne (houtsaagsels, glukose, mieliemeel en metanol). Die eksperimente was onder 'n stikstofatmosfeer gedoen om suurstof uit te sluit en die temperatuur is konstant op 23 °C gehou. Inligting uit die literatuurstudie is gebruik om denitrifikasie aanwysers te kies. Katione (kalium, natrium, kalsium, magnesium, ammonia) anione (sulfaat, nitraat, nitriet en chloried), en metale (yster en mangaan) is aan die beginpunt van die eksperimente, sowel as op bepaalde tye, ontleed om enige moontlike veranderinge in die ioonchemie met tyd te evalueer. Monsters was op bepaalde tye gedurende die eksperimente geneem en ontleed vir die gekose aanwysers om die vordering van denitrifikasie te bepaal. Dit sluit nitraat, nitriet, sulfaat, alkaliniteit, chloried, asetaat,, ammonium, pH, elektriese geleiding, opgeloste organiese koolstof, heterotrofiese plaattelling, yster en mangaan in. Marydale se grondwater is hoofsaaklik 'n natrium-chloried tipe water. Die nitraatvlakke (as N) wissel tussen 19 en 32 mg/L (ongeveer 82 tot 133 mg/L

as NO_3^-) wat die ideale nitraatvlak vir drinkwater van 6 mg/L (as N) oorskry. Twee grondmonsters, (rooi sand bokant 'n kalkreetlaag: "Plooysburg form, Family Py1000") is by verskillende dieptes bemonster.

Die denitrifikasie-eksperimente het bewys dat totale denitrifikasie, afhanklik van die tipe koolstofbron en die C:N verhouding, binne 1 tot 6 weke kon plaasvind. Hoër reaksietempo's van nitraatvermindering en redusering was bereik waar groter C:N verhoudings gebruik was. Vinnigafbreekbare koolstofbronne (bv. glukose) het vinnige denitrifikasietempos bereik, terwyl stadige afbreekbare koolstofbronne (houtsaagsels) stadiger denitrifikasietempo gehad het. Die vlakker grond en hoë nitraatvlakke aan die begin van die eksperiment het gelei tot beter denitrifikasie reaksietempo's. Alle gronddieptes het egter gelei tot effektiewe denitrifikasie. Heterotrofiese bakteriese telling het met tyd vermeerder. Dit is 'n aanduiding dat omstandighede optimaal is vir groei en denitrifisering. Dit dui verder aan dat dit onnodig is om die grond met kunsmatige bakterieë aan te vul. Opgeloste organiese koolstofkonsentrasies kon direk gekorreleer word met die beginpunt koolstofkonsentrasie omdat die grond en grondwater 'n tekort aan organiese koolstof het. Resultate het gewys dat produkte soos nitriet, asetaat en die basiese ionekonsentrasie in die vloeistof met tyd in die voorlopige eksperimente verhoog. Konsentrasies van opgeloste organiese koolstof en die heterotrofiese bakteriese telling het die aanbevole konsentrasievlakke vir veilige drinkwater in die eindwater oorskry. Die voorkoms van asetaat en nitriet word vervaardig as gevolg van onvolledige oksidasie van organiese materiaal en 'n oorvloed van koolstof in die reaksie. Verhoogde ioonkonsentrasies is as gevolg van 'n daling in pH wat veroorsaak word deur organiese sure wat gedurende denitrifkasie gevorm word. Die metode bewys ook selektiwiteit, aangesien die enigste aanwysers wat beïnvloed was, opgeloste organiese koolstof, nitriet, nitraat en die heterotrofiese bakterieë telling was. Verwydering van die heterotrofiese bakterieë deur byvoorbeeld water te kook is nodig om die produkwater drinkbaar te maak sonder nagevolge.

Vir verdere navorsing met stadig afbreekbare koolstofbronne, soos houtsaagsels, word aanbeveel dat C:N verhoudings van > 12 gebruik word. Monitering moet oplosbare koolstof, nitraat, nitriet en heterotrofiese plaattelling insluit.

Hierdie studie het getoon dat die denitrifikasie van grondwater met 'n verskeidenheid koolstofbronne moontlik is oor 'n kort tydperk onder anaerobiese toestande in kontak met plaaslike gronde. Hierdie studie het ook getoon dat afbreekbare koolstofbronne wat teen 'n gemiddelde spoed afbreek, soos byvoorbeeld strooi dalk meer geskik is om te gebruik as

houtsaagsels wat baie stadig afbreek of glukose wat vinnig afbreek. Met genoeg inligting ten opsigte van die grond en grondwater van 'n gebied kan '*in-situ* denitrifikasie moontlik suksesvol bedryf word.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
CCl$_4$	Carbon-tetrachloride
DCE	dichloroethene
DOC	Dissolved Organic carbon
DWAF	Department of Water Affairs and Forestry
EC	Electrical conductivity
HCB	Hexachlorobenzene
HPC	Heterotrophic Plate count
ISBD	*In Situ* Biological Denitrification
ISRM	*In Situ* Redox Manipulation
ITRC	Interstate Technology Regulatory Cooperation Work Group
PRB	Permeable Reactive Barrier
TCE	trichloroethene
TDS	Total Dissolved Solids
WHO	World Health Organisation

CHAPTER 1: INTRODUCTION

Groundwater is a very important resource, more especially so in semi-arid regions where surface water quantities are too small to supply communities with water for drinking and other uses. Where evaporation rates exceed that of recharge or rainfall events, groundwater is often the sole source of water (Figure 1). This total dependence on the resource makes it of utmost importance that the water is of a good enough quality to be consumed by people and animals alike.
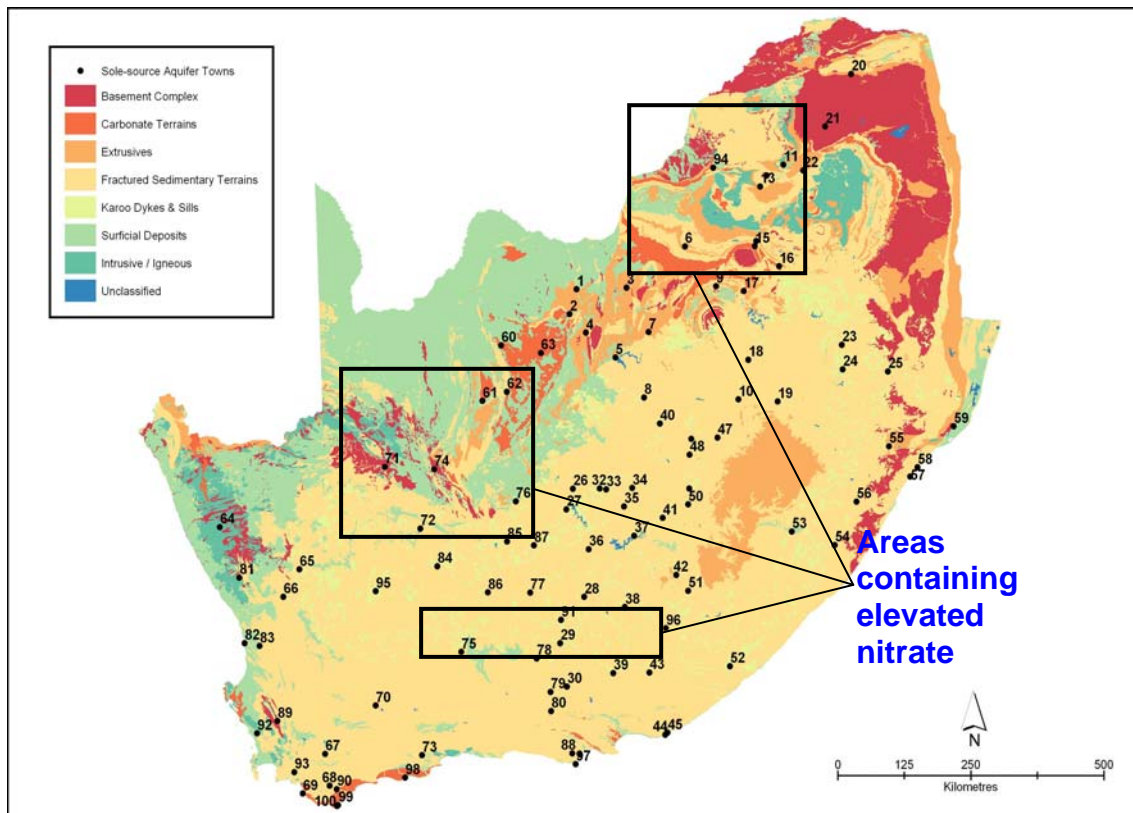
Figure 1:   Map of South Africa, the numbers on the map represent towns dependant on groundwater as a sole source of water used for drinking, washing, preparation of food, etc. The blocked areas represent sole source towns to which high nitrate concentrations in groundwater are a potential threat (after Tredoux *et al.*, 2004).

Certain chemical elements hamper the use of groundwater; among these are fluoride, nitrate, arsenic, iron and manganese to mention but a few. This study investigates the nature of nitrogen species in the subsurface. In nature, chemical and biological processes remove nitrate, and certain requirements exist for these processes to take place successfully. Where the required conditions do not exist, natural denitrification is not likely to occur.

In South Africa, the ideal drinking water according to DWAF (1996) ("blue", i.e. Class 0) has less than 6 mg/L nitrate (plus nitrite) as N while the "marginal" water quality ("yellow", i.e. Class II) has a maximum concentration of 20 mg/L. This is generally in agreement with the WHO guidelines. However, in many areas of the South Africa nitrate levels exceed the maximum concentration of 40 mg/L of "poor" water quality and levels of 100 mg/L or even greater than 200 mg/L are found in various places. Water with nitrate exceeding 40 mg/L, belongs to the category of "unacceptable" drinking water quality ("purple", i.e. Class IV).

Records of nitrate concentration have been documented for many areas by Tredoux *et al.* (2000), and the distributions of these levels were mapped to identify trends and severity of elevated nitrate concentrations.

High nitrate concentrations have been found to occur from sources ranging from agricultural fertilizing to anthropogenic pit latrines and explosives (Tredoux, 2004; and Heaton, 1984). Nitrate distribution stretches from the north-western parts of Southern Africa to Namibia and Botswana across the continent to the Northern Province of South Africa. Some point sources could be owed mainly to sources like pit latrines and other activities polluting primary aquifers by direct infiltration of polluted water.

This study aims to address groundwater dependence in areas that is often related to economic status. Rural areas, that are far from business centres often lack the funding for establishing large and complicated treatment plants. Treatment of nitrates with minimal costs and safe methods is a required technological endeavour in the more rural parts of Africa.

Literature documents the environmental conditions under which nitrogen undergoes various transformations in all spheres of the environment (sections 2.1 and 2.2). Denitrification is discussed with respect to the conditions that favour or hamper its occurrence or completion in Chapter 2.3. *In situ* denitrification technologies practised internationally has proven to be successful in many countries including New Zealand, Australia, Canada, Israel, Austria and the USA currently have either pilot or field scale operational sites (Tredoux *et al.*, 2004).

In situ denitrification methods include *in situ* redox manipulation, permeable reactive barriers, the Nitredox method, *in situ* biological denitrification with different site-specific configurations (Tredoux *et al.,* 2004).

Permeable Reactive Barriers (PRB) have been tested over a long period from bench scale to full-scale implementation plants (Blowes *et al.*, 2000; Schipper and Vojvodic-Vukovic, 2000; McRae *et al.*, 1999; Liang *et al.*, 2000; and Robertson and Cherry, 1995 & Robertson *et al.* 2000) in the USA, Canada, and New Zealand.

1.1 Problem Statement

Parts of Southern Africa are currently in a period of water scarcity, and more towns are opting to use groundwater. Many regions have become solely dependant on groundwater. Where elevated concentrations of e.g. nitrate, Fe, Mn etc. occur, it complicates the water shortage problem, as these waters are often not safe to use. *In situ* treatment is a robust and effective technique for removal of nitrate, iron, manganese etc.

1.2 Research objectives

- The main objective of the study is to perform laboratory studies using various carbon sources to evaluate their suitability and suitable carbon to nitrogen ratios for selected carbon sources;

- Secondary to this, to monitor indicator physical and chemical parameters during the laboratory experiments, which discern trends that occur during denitrification;

- To note all changes occurring during the experimental phase and to assess them in terms of drinking water standards set for South Africa by DWAF;

- To address the key questions that follow.

1.3 Key questions

The following key questions are addressed in this study:
1) How effective are the different carbon sources in denitrifying experiments?
2) Is there any distinct difference between reactions using soil of different depths?
3) What is the most suitable C:N ratios?
4) Does the resultant water comply with drinking water standards?

1.4   Work plan

- To examine literature for nitrogen species and the nitrogen cycle (Chapter 2);
- To research methods used for denitrification in the international arena (Chapter 3);
- To select a suitable site and do a site characterisation (Chapters 3.4 and 4);
- To do laboratory treatability and suitability studies to select suitable carbon sources and to examine different carbon to nitrogen ratios (Chapter 5);
- To present recommendations for field application (Chapter 6).

CHAPTER 2: NITRATE IN GROUNDWATER- A REVIEW ON CAUSES AND CONSEQUENCES

The supply of nitrogen to soils is an important factor in crop production. Inputs of nitrogen to the soil environment are often increased by fertilisation. The biogeochemical nitrogen cycle is a complex and important one. Organic nitrogen (e.g. proteins, nucleic acids), inorganic nitrogen (NH_4^+, NH_3), gaseous nitrogen (NO, N_2O, NO_2) and nitrate (NO_3^-), which is the most oxidised and mobile form of nitrogen all form part of this cycle and are either formed or consumed as part of this universal cycle in the processes of mineralization, nitrification, immobilisation, ammonification, assimilation and denitrification (Patrick, 1982). A brief discussion of these processes and their pathways follow. Figure 2 is a representation of the cycle as it occurs in nature.
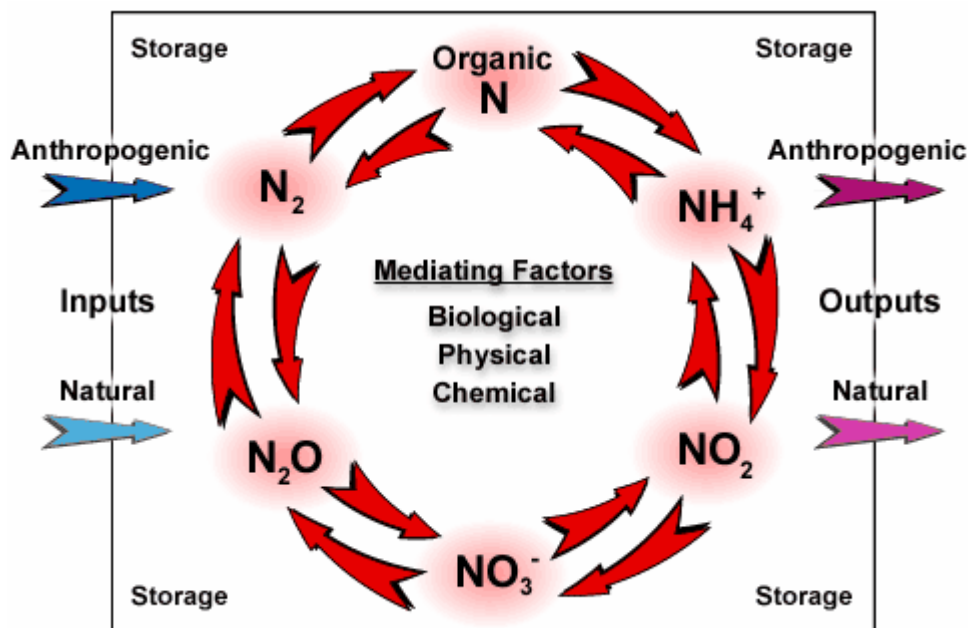
Figure 2:   The biogeochemical nitrogen cycle, after Deng *et al.* (1998)

Figure 2 shows all the pathways followed by nitrogen in the subsurface. Emphasis will be placed on denitrification and how other transformation reactions as well as environmental conditions (e.g. redox, organic matter, pH, etc.) affect the occurrence and successful completion of the denitrification reaction.

Nitrogen *mineralization* can be defined as the transformation of nitrogen from the organic state (proteins, nucleic acids etc.) into the inorganic forms of NH_4^+ or NH_3. Heterotrophic soil organisms that use nitrogenous organic substances as an energy source perform this process. N immobilization is the transformation of inorganic N compounds (NH_4^+, NH_3, NO_3^-, and NO_2^-) into the organic state. Soil organisms assimilate N compounds and transform them into part of their cells and tissue. The equation for the mineralization is as follows:
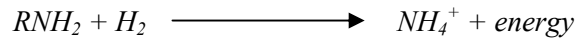
$$RNH_2 + H_2 \longrightarrow NH_4^+ + energy$$
Where R= organic matter

Equation 1

Under usual soil conditions, where microbial activity is limited by availability of C and energy, NH_4^+ is rapidly oxidised to NO_3^-, this is referred to as *nitrification*. This is however not the only means by which NO_3^- is introduced to soils, NO_2^- oxidation also contributes to the NO_3^- pool. Factors that limit nitrification in soils include substrate NH_4^+, O_2, CO_2, pH and temperature. Optimum conditions for the reaction vary in different soil environments. Conditions here refer especially to pH and temperature. Equations for NH_4^+ oxidation to NO_2^- and NO_2^- to NO_3^- (nitrification) follow:

$$2NH_4 + 3O_2 \longrightarrow 2NO_2^- + 2H_2O + 4H + energy$$

Equation 2

$$2NO_2^- + O_2 \longrightarrow 2NO_3^- + energy$$
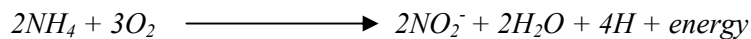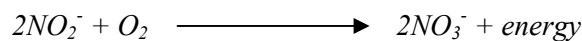
Equation 3

The products of nitrification i.e. NO_2^- and NO_3^- are both mobile, with NO_2^- being the more reactive of the two nitrogen species, it is also said to be highly toxic to microorganisms, (Schmidt, 1982). NO_3^- mobility poses a threat when it is leached from soils into groundwater and consumed by humans and animals. In Southern Africa particularly, many farmers have serious problems with cattle dying from nitrate poisoning. Areas in the northern parts of the country are the most affected by this phenomenon.

Nitrate removal or reduction from groundwater seems to be the most likely path to take. *Denitrification* is a natural process and an integral part of the nitrogen cycle that converts

NO_3^- to nitrogen gas with a few probable intermediates. Oxidation states of nitrogen species changes throughout the cycle, with NO_3^- being the most oxidized form, and NH_4^+ being the most reduced form of nitrogen.

Table 1 is a summary table of the transformations, chemical reaction and a brief description.

Table 1:   A summary of nitrogen transformation reactions adapted from Hauck and Tanji (1982)

Transformation	Chemical Reaction	Description
N- fixation	$0.5\ N_2 \longrightarrow R\text{-}NH_2$	Plants and some microorganisms use N_2 from the air and convert it to ON in a symbiotic relationship with microbes.
N- mineralization	$R\text{-}NH_2 + H_2O + H^+ \longrightarrow R\text{-}OH + NH_4^+$	Transformation of organic N to inorganic N (NH$_4$) as microorganisms decompose organic matter.
N-immobilization from nitrate from NH_4^+	$NO_3^- + 2e\text{-} \longrightarrow NO_2 + 6e\text{-} \longrightarrow NH_4^+$ $NH_4^+ + R\text{-}OH \longrightarrow R\text{-}NH_2 + H_2O + H^+$	Transformation of inorganic N into organic N as microorganisms incorporate N into their structures or humus during decomposition
NH$_3$ volitization first stage (in water) from water to air	$NH_3^+ \longrightarrow NH_3\ (aq) + H^+$ $NH_3\ (aq) \longrightarrow NH_3\ (air)$	Loss of ammonia from soil water to air
Nitrification By nitrosomonas By nitrobacter	$NH4^+ + 1.5\ O_2(aq) \longrightarrow NO_2^- + H_2O + 2H$ $NO_2^- + .5\ O_2(aq) \longrightarrow NO_3^-$	Transformation of ammonium to nitrite (NO$_2$) and nitrate (NO$_3$) by microorganisms.
Denitrification to N$_2$(g) to N$_2$O	$NO_3^- + 1.25\ [HCHO] \longrightarrow$ $0.5\ N_2 + 0.75\ H_2O + 1.25CO_2 + OH$ $NO_3^- + [HCHO] \longrightarrow$ $0.5\ N_2O + 0.5\ H_2O + CO_2 + OH$	Transformation of nitrate to nitrogen gases

Areas in Southern Africa that are adversely affected by high nitrate concentrations in groundwater, as a result of mobile nitrogen being leached and hence lost from the soil profile, include the Northern Cape, Limpopo Province, Namibia and Botswana. Processes

reducing concentrations of the NO_3^--N in groundwater or controlling the nitrification process needs to be well understood so that the problem of high nitrate levels in groundwater or loss of the soil N content can be alleviated. Considering all the above information, the process of denitrification and other transformations that affect it will be looked at more closely in the section that follows.

2.1 Nitrogen transformations and environmental conditions

Denitrification
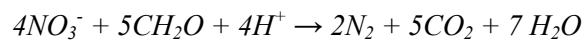
$4NO_3^- + 5CH_2O + 4H^+ \rightarrow 2N_2 + 5CO_2 + 7\,H_2O$	Equation 4

The reaction above is best described as biological denitrification as microbial communities in the soil environment facilitate it. Denitrification as explained in the previous section is a reductive sequence that nitrate/ nitrogen undergoes to form gaseous products. Conditions and parameters important for the occurrence of the reaction such as temperature, oxygen content, carbon content, pH, Eh, and other conditions will be discussed in more detail.

2.2 Conditions that affect denitrification

The soil type is defined by the chemical conditions prevailing at the time of formation as well as the prevailing physical-chemical conditions at any given time; this section will therefore discuss the soil chemical properties and their effects on denitrification.

2.2.1 Redox and O_2 content

It is known that the absence of O_2 or a reduced O_2 availability favours denitrification. According to McBride (1994), denitrification is favoured under moderately reducing conditions i.e. where $-4 < p\epsilon < 12$ (Figure 3 shows this range and relates it to the oxidation states of nitrogen in the soil environment). Redox potentials at which denitrification has been reported to be significant range from 350 to 650 mV (Firestone, 1982), and oxygen contents at which denitrification has been observed in the soil environment range from 4 to 17% oxygen. Oxygen entering a denitrification system affects the reaction metabolically as well as kinetically due to the inhibitory effect of oxygen on denitrification (Plòsz *et al.*, 2003). This inhibitory effect of oxygen on denitrification becomes larger with greater amounts of oxygen

entering the anoxic environment (Plòsz *et al.*, 2003). If a small amount of oxygen enters the system and reacts with organic matter present, its effects on denitrification will be negligible. When a larger amount of oxygen is present, microbes/bacteria would preferentially utilize this oxygen as an electron acceptor, thus inhibiting the denitrification reaction (Plòsz *et al.*, 2003). Figure 3 shows the redox range at which nitrogen species occur.
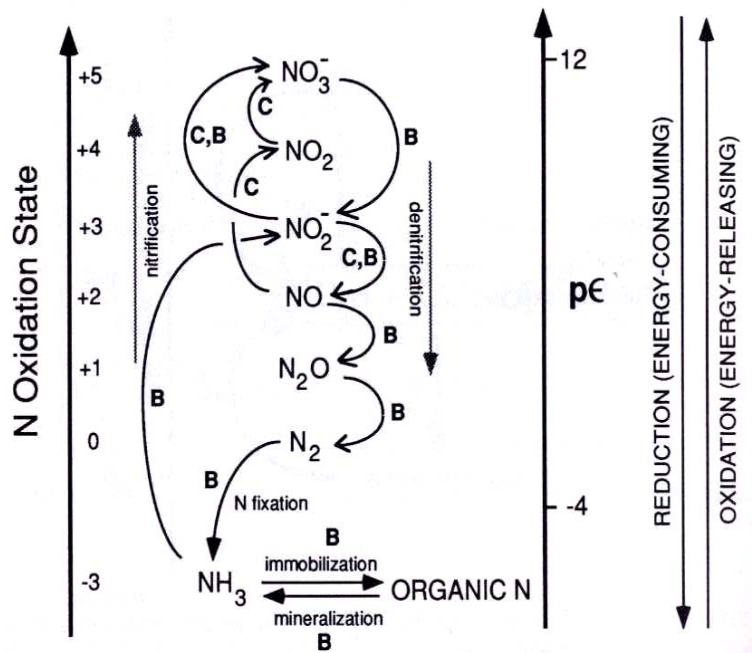
Figure 3: Redox range of nitrogen related to oxidation states of nitrogen species in the soil profile, McBride (1994).

2.2.2   Carbon to Nitrogen ratio

Denitrification rate is determined by the stoichiometric relationship between the organic carbon used and nitrate present in the soil environment. Carrera *et al*, (2003) found that the average COD/N ratio was calculated to be 3.7 ± 0.9 mg COD mg N^{-1}, they used the ratio between the difference between COD initially and at t=24hrs and the difference between N initial and at t=24. The carbon source utilized in this case was not pure, but rather a mixture of methanol, acetone and isopropilic alcohol. Carrera *et al.* (2003) and other workers used methanol as a carbon source and found COD:N ratios of 4.6 and 4.45 mg COD.mgN^{-1} in separate studies. Camberato (2001), says that where low C:N ratios exist (i.e. less than 15:1) the N content of the soil is relatively high and the microorganisms rapidly release nitrogen, in other words, mineralization is high. When the C:N ratio is high (i.e. 30:1), this

indicates low nitrogen content and slow mineralization. If the C:N ratio is very high, nitrogen is removed from the soil (immobilized), this occurs frequently where carbon compounds like sawdust, some composts, and different types of sludge are added to the soil, Camberato (2001).

2.2.3   Carbon availability/ soil organic matter (including humus)

Firestone (1982) says that it is well established that denitrification in soils is strongly dependant on the amount of carbon in the soil environment both as electron donors and a source of energy of cellular material. The presence of enough C also stimulates the consumption of O_2 hence directly enhancing the potential for denitrification. Paavolainen *et al.* (1999) showed that denitrification is in fact greater in humus layers than in mineral layers of soils. They also found that a low carbon availability lead to decreased denitrification enzyme activity and thus decreased denitrification. According to Griffiths *et al.* (1998) denitrification in forest soils was limited by the carbon content (they used glucose) rather than the NO_3^- availability, while in other soils both may limit it. Firestone (1982) mentioned that soil organic matter content and denitrification activity in soils can be closely correlated and that it is not merely the presence of organic matter that is important to the process, but rather the availability of the carbon. It has been reported by most workers, that soils with a high organic content is most likely to have a high denitrifying capacity as compared to soils with a lack of organic material. When organic soils become flooded, the presence of NO_3^- may limit the denitrification capacity as this leaches most of the nitrate from the soil. The effects of addition of carbon sources to soils on denitrification are dependant on the quality of the carbon source (Carrera *et al.*, 2003). Addition of readily degradable carbon substrates rich in N will enhance denitrification, while carbon sources not readily degradable may enhance immobilization by converting NO_3^- to NH_4^+. Carbon sources used by different workers include glucose, sucrose, ethanol, methanol, acetic acid, and lactic acid. It is not clear from the literature which of these is more effective in terms of rate of denitrification, but cost and availability of the solvent should also be taken into account in the selection of a suitable external carbon source (Carrera *et al.*, 2003).

2.2.4   Microbial communities

Denitrifying bacteria can grow in the absence of O_2 while reducing NO_3^- and NO_2^- to N_2, (Firestone, 1982). The general requirement for denitrification includes the presence of bacteria possessing the metabolic capacity, an energy source (organic carbon, reduced S

compounds, or molecular hydrogen) and terminal electron acceptors (N oxides). It is mentioned by Firestone (1982) that nitrifying organisms shift to denitrification when O_2 content is low. Firestone (1982) stated that the capacity to denitrify has been shown in about 23 genera of bacteria. Most denitrifying bacteria are chemotrophs (they use chemical energy and not light energy and organic carbon as a source of electrons and cellular C).

2.2.5   pH

The increase of pH in the humus layer of the soil leads to initiation of nitrification and increased leaching of nitrate from the soil (Paavolainen *et al.*, 1999). When pH is decreased to 5.3 or lower, the production of $NO_2^-+NO_3^-$ was inhibited. Denitrification rates are higher in humus than in mineral soil layers, Paavolainen *et al.* (1999), while an increase in pH was the leading cause for nitrification to occur in their study. Wild (1988) recorded a peak pH for denitrification of pH 7 to 8. He also mentions that denitrification occurs readily at neutral to calcareous pH's but less in acidic soils. Wild (1988) also mentions that pH is a major limiting factor for denitrification.

2.2.6   Temperature

Griffiths *et al.* (1998) measured denitrification at 25˚C. Carrera *et al.* (2003) although working with wastewater treatment reactors, used varied temperatures to measure the rate of denitrification at these temperatures. The temperature ranged from 6˚C to 25˚C. Rates of denitrification were lowest at 6±0.5˚C ($0.020±0.009mgN.mgVSS^{-1}.d^{-1}$) and highest at 25±0.5˚C ($0.28±0.03mgN.mgVSS^{-1}.d^{-1}$) (Carrera *et al.*, 2003). The rate of denitrification generally increased with an increase in the temperature at which the reactions were run. According to Wild (1988), nitrate loss can double with a temperature increase of 10˚C over a range from 10 to 35˚C. In the lower temperature ranges such as 0 to 5˚C denitrification rates are low but measurable, and more nitrous oxide than dinitrogen is produced. Wild also mentions that denitrification is typically favoured by warm wet soil conditions where little O_2 is present.

2.2.7   Depth at which denitrifying activity occurs

Soil characteristics that change with depth such as pH, redox conditions, temperature, porosity/ permeability, organic matter content and water table are important controllers of denitrification (Cosadndey *et al.*, 2003). Cases where denitrification decreases with depth are recorded in the literature. Depths varying from surface level up to 150cm deep are

discussed in the literature. The decrease is owed to microbial activity (150cm), the presence of carbon and anoxic microsites (at 60cm), the presence of populations capable of denitrifying (here a decrease in the rate of denitrification with depth was observed) (Firestone, 1982). It is also important to note that organic matter may accumulate at depth due to leaching or layer formation during soil forming or transforming processes. This carbon availability may support high denitrification rates.

2.2.8   Water content of soils

Paavolainen *et al.* (1999) used sprinkling filtration as a form of artificial groundwater recharge in southern Finland. This caused an increase in the pH of the humus layer of the soil from about 5 to 6.5. They found that high soil moisture favours denitrification. This can be explained by low oxygen content and reducing conditions. An increase in denitrifying enzyme activity often followed an increase in soil water content (Griffiths *et al.*, 1997). When soils are flooded, NO_3^- is mobilized and may limit the occurrence or rate of denitrification, (Firestone, 1982). Jacinthe *et al.* (2000) used water table management as a technique to stimulate denitrification. They increased the saturation of the upper part of the soil profile hence replacing O_2 with water in pores and generating an anaerobic environment. They encountered a problem with N_2O evolution during their experiments, which would eventually contribute to global warming. An interesting point raised by Jacinthe *et al.* (2000) was that a prolonged period of anoxic conditions (i. o. w. a high water table) would decrease the mole fraction of N_2O in the N gases emitted.

2.3   Factors affecting denitrification

2.3.1   Crop removal

Crop removal is seen as an activity that disturbs the natural equilibrium in the environment. It results in the release of gases and the addition of oxygen into the subsurface. This has a negative effect on denitrification as it disturbs the anaerobic state that is required for the reactions to proceed to completion. The introduction of oxygen promotes nitrification, (Henry *et al.* 1999). This leads to higher nitrate concentrations in the soil profile. The presence of nitrate would start denitrification, which will be extremely slow in the presence of oxygen. Once the oxygen is consumed, denitrification will be the dominant nitrogen transformation (Henry *et al.* 1999).

Removal of crops also implies the removal of organic matter. Organic matter is essential for denitrification to occur as it acts as an electron donor in this redox reaction (Henry *et al.* 1999). Another consequence of crop removal will be a decrease in the plant nitrogen uptake, which would probably result in a larger amount of leachable nitrate in the soil profile. Tilling of the soil will also mobilize the natural soil organic nitrogen in the form of nitrate (Heaton, 1985; and Conrad *et al.*, 1999).

2.3.2   Humus

Humus refers to a large amount of compounds that will not be discussed in much detail here [for detailed account of humic and fulvic acids approach (Stevenson, 1982). The humus content of the soil can be correlated to some extent to the cation exchange capacity and the buffer capacity, (Mc Bride, 1994). It is also a source of slowly degrading carbon. Humus is able to enhance the NH_4^+ mineralization if the conditions allow, this process being important to the nitrifying activity and the coupling of nitrifying and denitrifying activity (Nielsen and Revsbech, 1997). Humus can primarily be described as N-containing organic compounds, and thus provide a stored N-content to the soil, (Henry *et al.* 1999 and Mc Bride, 1994). This relates to a potential for mineralization as discussed above. Due to its ability to increase the water holding capacity of soils, it would increase the potential for maintaining anaerobic conditions and hence enhance the probability for denitrification, (Mc Bride, 1994).

2.3.3   Nitrification

Nitrification as explained above produces NO_3^- and NO_2^-. When other conditions such as pH, temperature and redox potential in the soil are optimum, the products of nitrification will favour the occurrence of denitrification (Schmidt, 1982, Henry *et al.* 1999 and Mc Bride, 1994). The process of nitrification feeds into denitrification in the nitrogen cycle as is illustrated by Figure 2 and Figure 4. Nitrification typically occurs at temperatures above freezing, pH 5.5 to 10 with an optimum pH of 7, the presence of more than 10% oxygen is also important for this reaction (Schmidt, 1982). Once the oxygen is removed or released from the system, some nitrifying bacteria transform to denitrifying bacteria, and nitrification is inhibited. The denitrification reaction will then proceed as predicted by the equations (Table 1). Figure 4 shows the nitrogen cycle with emphasis on denitrification.
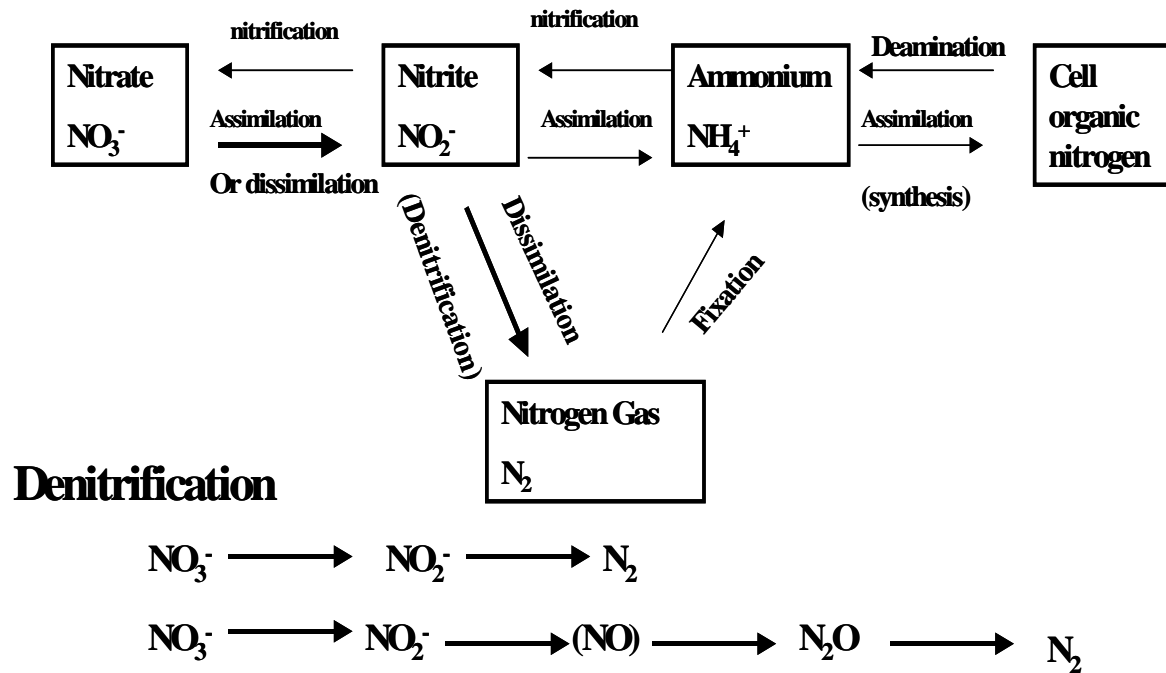
Figure 4: The Nitrogen cycle with emphasis on denitrification, modified after Henry *et al.* 1999.

2.3.4 Volatilization

Volatilization here refers primarily to ammonia volatilization. This contributes a loss of nitrogen. NH_3 gas formation or loss is closely linked with urea hydrolysis in soils (Camberato, 2001). Urea hydrolysis results in an increased pH and a shift in nitrogen species from ammonium to ammonia, which is released into the atmosphere as a gas. The buffer capacity of soils plays an integral role in controlling the pH increase and hence the extent of volatilization of ammonia gas.

It can thus be said that soils with high organic matter, clays and humus will have high buffer capacity and therefore have minimized volatilization, while sandy soils with low buffer capacity will have a substantial amount of volatilization. Volatilization removes NH_4^+ and NH_3 from the soil; hence, the nitrification as well as the denitrification reactions of a particular soil would be decreased due to the volatilization of ammonia gas. Figure 5 explains ammonia volatilization in the soil environment.
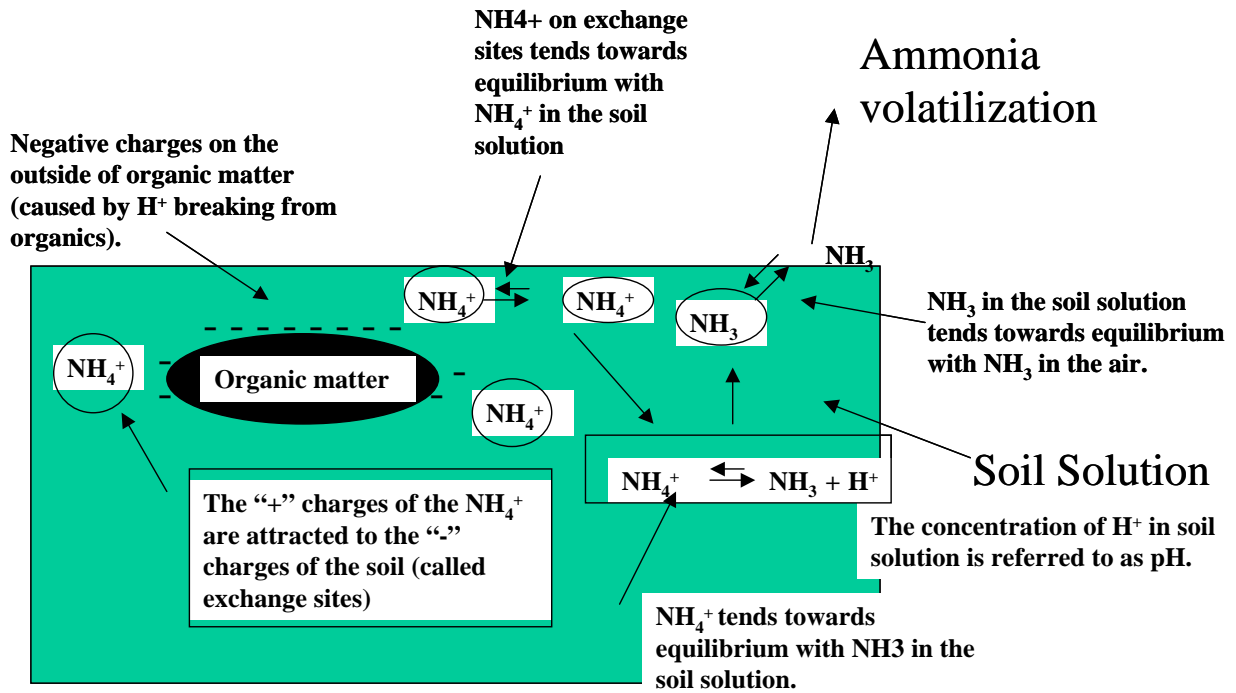
Figure 5:   Ammonium volatilization and the processes that leads to its occurrence, modified after Henry *et al.* 1999.

Buffer capacity and cation exchange capacity can be correlated refer 2.3.2. It is possible that ammonia will be adsorbed on cation exchange sites. This will decrease the amount of N loss in the soil environment (Camberato, 2001). Volatilization will occur more rapidly at higher temperatures, lower soil moisture, and higher air speeds.

2.3.5   Plant uptake and immobilization

Plant roots also provide carbon which serves as a source of energy for microbial populations capable of denitrification and acts as an electron donor for nitrate reduction when the O_2 availability is low (Jansson and Persson, 1982). Plant roots have a great effect on the soil O_2 content and availability for denitrification. Plant roots remove nitrate from the soil and contribute to anaerobic conditions in certain zones due to their respiration processes (consumption of O_2) (Jansson, and Persson, 1982). Figure 6 shows how immobilization occurs within the soil environment. Immobilization refers to the process during which mineral nitrogen (e.g. NH_4^+) is taken up by microorganisms and converted back to organic matter. Immobilization usually occurs in nutrient poor soils (e.g. high carbon content, lack of nutrients).

Immobilization and Plant uptake

NH$_4^+$

Organic matter (R-NH$_2$)

NO$_3^-$

The microorganisms and plants use NH$_4^+$ and NO$_3^-$ in synthesis of new biomass

Soil water
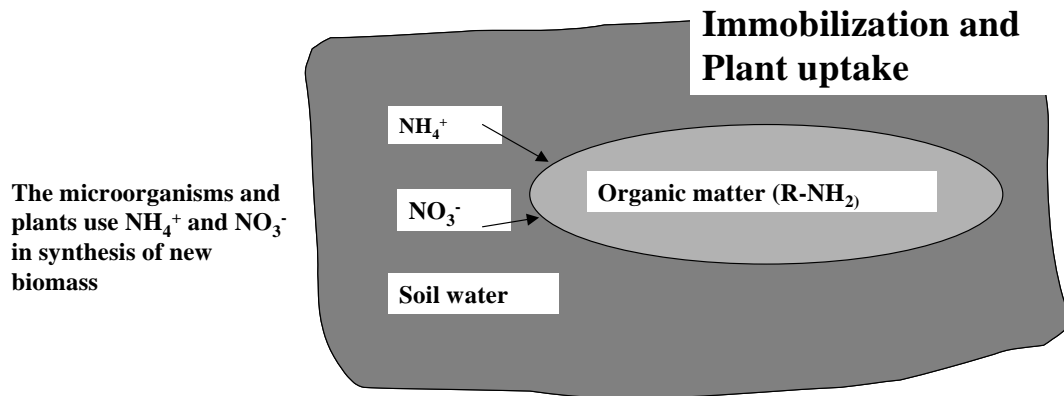
Figure 6:   Conceptualization of the processes of immobilization and plant up take of NH$_4^+$ and NO$_3^-$, modified after Henry *et al.* 1999.

Immobilization inhibits denitrification as it removes NH$_4^+$ and NO$_3^-$ from the soil profile.

2.3.6   Mineralization/ Ammonification

Mineralization and immobilization are predicted by considering the C:N. Large ratios (e.g. 30:1) favour immobilization, while smaller ratios (20:1) favour mineralization. Ratios between 20 and 30:1 favour both immobilization and mineralization.   Warm wet conditions and soil pH >5.5 are optimum conditions for mineralization/ammonification Camberato (2001). Figure 7 shows how nitrogen mineralization occurs in the soil environment. It occurs by the breakdown of organic compounds to release N compounds. The resultant of the organic molecule breakdown (oxidation) is CO$_2$, H$_2$O, and minerals.

Microorganisms break the organic bonds to obtain energy. This is decomposition.

Nitrogen mineralization

Organic matter (R-NH$_2$)

NH$_4^+$

Soil water
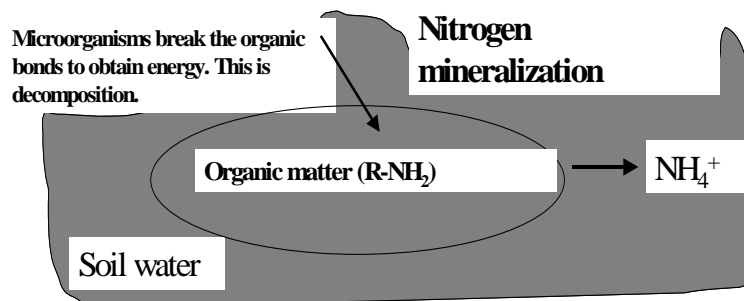
Figure 7:   Conceptual nitrogen mineralization processes as it occurs in the soil profile providing a supply of NH$_4^+$, modified after Henry *et al.* 1999.

NH_4^+ is the first nitrogen species to be available in the soil profile.

2.3.7 Leaching

Leaching can be defined as the downward movement of nitrogen with water percolation through the soil profile. Most soils have little anion exchange capacity; this allows anions such as nitrate to percolate, often passing the root zone and into the groundwater, McBride (1994). Cations such as ammonium are retained and remain on exchange sites. Soils with limited cation exchange capacity do allow the leaching of ammonium to occur, Camberato (2001). Leaching of NO_3^- into groundwater or other water bodies result in a net loss of nitrogen to soil. This causes significant health problems in human and ecological environments. So much so that denitrification has to be simulated to reduce the nitrate levels in certain groundwater supply boreholes. Figure 8 shows nitrification and nitrate leaching as it would occur in the soil environment.

The microorganism Nitrosomonas oxidizes the NH_4^+ to NO_2^-, in order to get energy

The microorganism Nitrobacter oxidizes the NO_2^- to NO_3^-, in order to get energy

NH_4^+ → NO_2^- → NO_3^-

Nitrification and nitrate leaching

H^+

Soil solution

This first step releases H^+ which acidifies the soil

As NO_3^- is negatively charged, it moves freely through the soil with excess rain
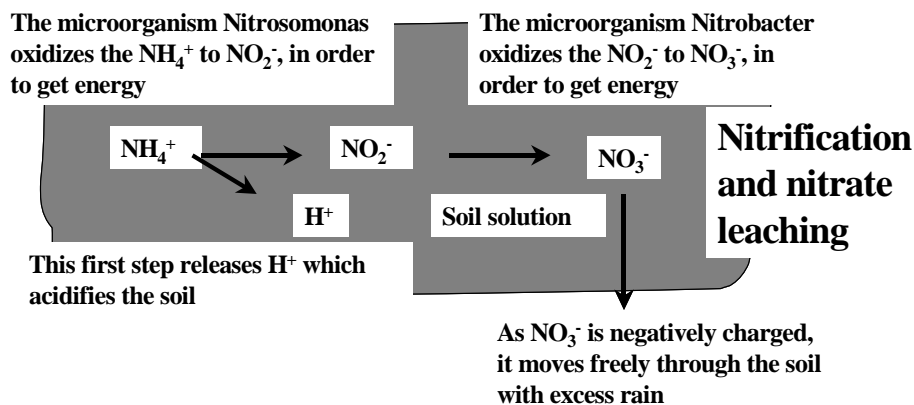
Figure 8:   Processes and pathways for nitrification and leaching of NO_3^- from the soil profile, modified after Henry *et al.* 1999.

Leaching is most likely to occur under the following conditions (Dodds and Fey, 1995):
- High rates of N loading;
- Low ratios of C:N, increasing the availability of N for mineralization;
- Well aerated soils-this encourages nitrification;
- Low levels of plant uptake- (little or no vegetation);
- High levels of precipitation or irrigation;
- High vertical permeability;
- A shallow unconfined water table.

Any combination of the above could result in nitrates leaching to groundwater.

2.4   Nitrate and health

Nitrate concentration in groundwater is of concern due to potential effects on human health as well as effects on livestock, crops, and industrial processes at high concentrations.

2.4.1   Human health

A condition called methaemoglobinaemia also known as "blue baby syndrome" results from the ingestion of high concentrations of nitrate in its inorganic form. Indigenous bacteria in the small intestine of individuals with low stomach acidity chemically reduce the nitrate to nitrite, a more reactive form of nitrogen. The nitrite is then absorbed through the walls of the small intestine into the blood stream where it combines with haemoglobin to form methaemoglobin that blocks the oxygen carrying capability of the blood (ITRCWG, 2000). This ultimately leads to death by asphyxiation. The body does not possess the ability to convert methaemoglobin back to effective haemoglobin. Infants as well as children and adults suffering from maladies or treatments that lower the levels of stomach acid, are vulnerable to methaemoglobinaemia (ITRCWG, 2000). Methaemoglobinaemia has been reported in a few states in the US. Cases of this disease are not reported frequently as it is not a routine test for infants. Cyanosis, an illness of oxygen starvation, is called methaemoglobinaemia when nitrogen compounds are the cause (Canter, 1997).

Other suspected conditions that could be linked to high nitrate concentrations include spontaneous abortions in females consuming excess nitrate and stomach cancer (ITRCWG, 2000).

As a result of the AIDS epidemic, mothers are forced to bottle feed infants; this places them in danger of exposure to high nitrate water consumption (Colvin, 1999). Nitrosamines are harmful to humans of any age (Stadler *et al.*, 2004).

2.4.2 Animal health effects

Nitrate concentrations affect livestock similarly to what it affects humans. Above 300mg/L, nitrate poisoning may result in the death of livestock consuming water. At lower concentrations, other adverse effects occur in animals, these include:

- Increased incidence of still born calves;
- Abortions;
- Retained placenta;
- Cystic ovaries;
- Lower milk production;
- Reduced weight gains, and
- Vitamin A deficiency.

Recommended levels of nitrate for stock watering (livestock and poultry) in the US is below 100mg/L (ITRCWG, 2000 and Innovative Technology, 2000). Symptoms of nitrate-nitrite poisoning in livestock include cyanosis in and above the non-pigmented areas (mouth and eyes), shortness of breath, rapid heartbeat, staggered gait, frequent urination, and collapse (Canter, 1997). In severe cases, convulsions, coma, and death may result within hours (Canter, 1997).

2.4.3 Environmental health

Nitrogen and phosphorus are the two most important nutrients limiting primary productivity. Excessive inputs of nitrogen and phosphorus to soils and in the resultant run-off to rivers and lakes increase the rate of eutrophication in lakes and other surface water bodies (ITRCWG, 2000). The effects of nutrient loading on water quality and productivity of surface water bodies is of great concern as it may serve as a drinking water source for communities. Heavy rainfall events often cause accumulated nitrate in the soil profile to be flushed down to the groundwater table. This causes a loss of nitrate and hence smaller amounts available to plants.

Certain plant species are believed to have died off due to irrigation by run-off of water with elevated nitrate concentrations.

2.5   Microbial geochemistry of denitrification

Nitrate reduction is an anaerobic process in which a reduced substrate (e.g. CH_2O, H_2S or H_2) is oxidized at the expense of nitrate (Krumbein, 1983). Genera capable of denitrification (table 2) include (Canter, 1997):

Table 2:   Genera of bacteria capable of effecting denitrification modified from Firestone (1982) and Kumbrein (1983)

Genus	Hydrogen Donor	Important characteristic of species
Alcaligenes spp.	Cl⁻compounds	Commonly isolated from soils
Agrobacterium		Some species are plant pathogens
Azospirillum		Capable of N_2 fixation, commonly associated with grasses
Bacillus	Cl⁻compounds	Thermophilic denitrifiers reported
Flavobacterium		Denitrification species recently isolated
Halobacterium		Requires high salt concentrations for growth
Hyphomicrobium		Grows on one-carbon substrates
Paracoccus denitrificans	Hydrogen	Capable of both heterotrophic and lithotrophic growth
Propionibacterium		Fermentors capable of denitrifying
Pseudomonas spp.	Cl⁻compounds	Commonly isolated from soils
Rhizobium		Capable of N_2 fixation in symbiosis of legumes
Rhodopseudomonas		Photosynthetic bacteria
Thiobacillus	Reduced S compounds	Generally grow as chemoautotrophs
Achromobacter spp.	Cl⁻compounds	
Thiobacillus thioparus	Reduced S compounds	
Thiomicrospira denitrificans	Reduced S compounds	
Thiosphera pantotropha	Reduced S compounds	
Pseudomonas pseudoflava	Hydrogen	

When oxygen is available, these organisms are able to oxidize carbohydrate substrates to CO_2 and H_2O as follows (Canter, 1997):

$$C_6H_{12}O_6 + 6\,O \longrightarrow 6\,CO_2 + 6\,H_2O$$
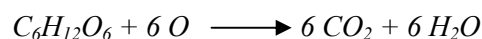
Under oxygen free conditions, some microorganisms oxidize a carbohydrate substrate to CO_2 and H_2O using nitrate instead of oxygen as an electron acceptor and converting nitrate to N_2 gas as follows (Canter, 1997):
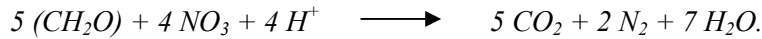
$$5 \ (CH_2O) + 4 \ NO_3 + 4 \ H^+ \longrightarrow 5 \ CO_2 + 2 \ N_2 + 7 \ H_2O. \qquad \text{Equation 5}$$

Other Processes

Certain denitrifying genera are capable of oxidizing H_2S and S^0 to sulfate, Krumbein (1983), one such denitrifying bacterium is *Thiobacillus denitrificans*. According to Sorensen *et al.* (1979), active denitrification has been demonstrated in sediments/ layers where sulfate reducing bacteria were producing H_2S.

Krumbein (1983) mentions that the rate of sulfate reduction is controlled by:
(a)   The concentration of organic matter and
(b)   its degradability

In other words, substrates that are not readily degradable will not affect sulfate reduction due to the bacteria's inability to consume or oxidize the substrate.

Reduction of SO_4^{2-} by sulfur reducing bacteria produces acetate as a by-product. Possible mechanisms are shown in many published text, here a starting organic material is lactate:
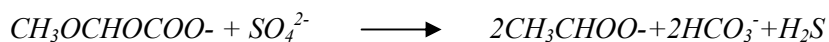
$$CH_3OCHOCOO\text{-} + SO_4^{2-} \longrightarrow 2CH_3CHOO\text{-}+2HCO_3^-+H_2S \qquad \text{Equation 6}$$

Acetate is produced due to incomplete oxidation of the organic material (Krumbein, 1983). The bacteria mentioned as affecting such reactions are designated desulfovibrio (Krumbein, 1983). It is also mentioned that this process introduces HCO_3^- to solution 2 mol for every mol of sulfate reduced. So, how do we deal with the phenomenon of elevated nitrate concentrations in nature with a minimal altering of ecosystems? The chapter that follows addresses some methods used to treat elevated nitrate as used in countries like USA, Canada, Australia and New Zealand.

CHAPTER 3: IN-SITU DENITRIFICATION FOR NITRATE REMOVAL FROM SOIL AND WATER – A REVIEW

3.1   Introduction

In Situ denitrification refers to the denitrification process occurring while soil or groundwater is still within the sub-surface. Methods of *in situ* treatment used internationally have been identified as part of this study. Several *in situ* groundwater treatment methods have been developed, this includes:

- *In Situ* redox manipulation (3.2.1);
- Permeable reactive barriers (3.2.2);
- *In Situ* biological denitrification (3.2.3), and
- The nitredox method (3.2.4).

The applicability of each of these depends on the particular contaminant characteristics, the aquifer matrix properties and its chemical composition. This chapter will give an overview of these methods and their operational mechanisms where available.

3.2   Permeable reactive barrier (PRB) methods

This entails the placement of a permeable physical or chemical "barrier" in the flow-path of the groundwater. The configuration of the barrier varies depending on the type of pollution source and the aquifer properties. The chemically reactive part of the barrier also varies depending on the actual contaminant being treated. Two types of barriers are discussed. In the first type, i.e. *In Situ* Redox Manipulation (ISRM), the aquifer material is chemically modified to serve as a chemical redox barrier. The second type, i.e. Permeable Reactive Barriers (PRB), involves the construction of a physical barrier consisting of chemically reactive material.

3.2.1 In Situ Redox Manipulation

In Situ Redox Manipulation (ISRM) is a process that is based on chemical manipulation of natural redox processes to change the mobility or form of contaminants (Innovative Technology, 2000). In the case of nitrate, removal by ISRM involves chemical denitrification by reduced metal species. The method is based on creating a reactive barrier, in the most permeable part of the subsurface in order to achieve optimum treatment capacity.

Literature documents the presence of iron in the aquifer, which can be reduced from its oxidized state in the aquifer sediments to serve as a long-term reducing agent, as a requirement for the successful implementation of the method.

3.2.1.1 Operating principle

By injection of chemical reagents, ISRM creates a permeable treatment zone in the subsurface down gradient of the contaminant source. The type of reagent is selected according to its ability to alter the oxidation/reduction state of the aquifer materials and groundwater to such an extent that it will allow the destruction or immobilization of specific contaminants.

Sodium dithionite (along with pH buffers) is injected into the aquifer through a well and allowed to react with the aquifer material for approximately 18 hours to create a treatment zone. Water containing the reaction by-products and any remaining reagent is abstracted, tested for hazardous constituents, and disposed (Mallinckrodt Baker Inc., 2001). Placement of the redox treatment zone is designed in such a way that contaminated groundwater flows naturally through the zone allowing the contaminants to react with the reduced iron in the sediment.

The sulfoxyl radical is a strong and highly reactive reducing agent. The reducing agent reacts as follows with the naturally occurring iron in the sediments:
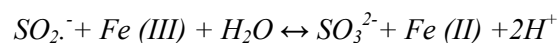
$$SO_2^{\cdot-} + Fe\ (III) + H_2O \leftrightarrow SO_3^{2-} + Fe\ (II) + 2H^+ \qquad \text{Equation 7}$$

The reduced Fe (II) then acts as a reducing agent for various contaminants in the groundwater. In the case of nitrate removal, denitrification occurs by the reaction of nitrate with the ferrous iron.
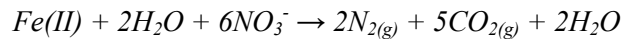
$Fe(II) + 2H_2O + 6NO_3^- \rightarrow 2N_{2(g)} + 5CO_{2(g)} + 2H_2O$	Equation 8

3.2.1.2 Site specific conditions that favour application of the method

ISRM method can be successfully applied where sand or sand and gravel aquifers have adequate hydraulic conductivity dimensions to allow injection and significant migration of dithionate solution before it reacts to form sulfate, thiosulfate, and sulphide (Innovative Technology, 2000). Low permeability aquifers and fractured rock aquifers are not suitable for this method. The method can be applied to sites where groundwater contamination by redox sensitive metals, such as chromium, uranium, and technetium, inorganic ions, radio nuclides or chlorinated hydrocarbons are dispersed over large areas and are deeper than 10 metres below the surface (Innovative Technology, 2000).

The following aquifer characteristics are essential for the successful application of ISRM:

- High permeability, porous primary aquifers;
- Appreciable natural iron (hydr)oxides, preferably iron-coated sands;
- A simple, well characterised subsurface flow system;
- Impermeable bedrock;
- Injection borehole(s) between contaminant source and abstraction borehole.

3.2.2 Permeable Reactive Barriers (PRB)

Permeable Reactive Barriers (PRB) are also referred to as "passive treatment walls" and "*in situ* reactive barriers" and have been tested over a long period from bench scale to full-scale implementation plants (Blowes *et al.*, 2000; Robertson and Cherry, 1995 & Robertson *et al.* 2000; Schipper, and Vojvodic-Vukovic, 2000 & 2001). Laboratory studies show that the methods can be used for the treatment of many inorganic contaminants including arsenic, cadmium, chromium, copper, mercury, iron, manganese, molybdenum, nickel, lead, selenium, technetium, uranium, vanadium, nitrate, phosphate, and sulfate. Cadmium, chromium, copper, iron, nickel, lead, nitrate, phosphate, and sulfate have also been treated in field studies (Blowes *et al.*, 2000). O'Hannesin (1998) lists the various types of contaminants (Table 3) that can be treated and the reactive materials that have been used to treat these contaminants.

Table 3:   Contaminants and reactive materials used for treatment (modified from O' Hannesin, 1998)

Contaminant	Reactive Material
Halogenated Organics (CCl$_4$, HCB, DCE, TCE, etc.)	Fe^0, bimetallic materials, Al, Fe, Zn, Mg, Sn.
Metals	Fe^0, organic carbon
Acid mine drainage	Organic carbon
Gasoline/petrol derivatives	Oxygen releasing compounds
Nitrate	Organic carbon or mixed organics + bacteria
Phosphorus	Metal oxides, limestone
Cr(VI), Cr(III)	Fe(II) in aquifer material, Fe(0)

Permeable Reactive Barrier technology has been studied extensively and over 100 references testify to its usefulness in ridding groundwater from the above listed contaminants. Its application stretches over the USA, Canada, Austria, Australia, New Zealand and Russia with operational and test sites at industries, water works, municipal well fields, homes and wastewater treatment plants. In certain countries the Lasagna technology for *in situ* soil remediation of nitrate is used (Ho *et al.*, 1999).

3.2.2.1   Operating principle

Permeable reactive barriers are constructed in the path of a migrating plume of contaminated groundwater. The porous treatment wall is constructed below the water table, perpendicular to the groundwater flow (Schipper and Vojvodic-Vukovic, 2000). They are typically designed as a continuous trench, filled with permeable, reactive material or a funnel-and-gate configuration, which includes impermeable sections, directing the groundwater flow through the permeable "gates".

PRB systems can be applied for the removal of anions, cations, organic compounds and inorganic compounds and utilise various processes such as reduction and precipitation, adsorption and precipitation, and biologically mediated reduction and precipitation (Blowes *et al.*, 2000). Various processes have been employed in different configurations and the system design is generally both site and contaminant specific.

Treatment of nitrate is achieved by adding a slowly degrading carbon source, such as sawdust or woodchips, to the matrix of the permeable wall, instead of zero-valent iron. The carbon acts as an electron donor, promoting an anaerobic environment and providing an

energy source for denitrifying bacteria (Schipper and Vojvodic-Vukovic, 2000). Biological denitrification is the main mechanism of nitrate removal in these systems.

Schipper and Vojvodic-Vukovic (2000) used the following equation to calculate nitrate removal rates using a denitrification wall:

$$N \, removal = \frac{q \: x \: A \: x \: \Delta \left[NO_3 - N\right]}{Soil \: volume}$$

<div align="right">Equation 9</div>

The numerator is the mass of nitrate removed in the wall, where q = groundwater flow rate (m day^{-1}); A = cross sectional area conducting groundwater (1 m^2 x the porosity); and Δ [NO$_3$ - N] (g.m^{-3}) = the difference between the nitrate concentration entering the denitrification wall and that arriving at the borehole. The denominator is the volume of the matrix in the wall that the nitrate passes through (Schipper and Vojvodic-Vukovic, 2000).

3.2.2.2   Site specific conditions that favour application of the method:

This method could be applicable where:
- A shallow water table is present, preferably a primary aquifer;
- Bedrock should be at about 10meters deep;
- Aquifer parameters such as (t) Transmissivity, (s) storativity, (q) discharge and (k) hydraulic conductivity should be well understood or known;
- Soil and groundwater chemistry should be known prior to wall emplacement.

Denitrification walls could be constructed as a PRB between on-site sanitation and water supply boreholes in rural and peri-urban settings.

3.2.3   In Situ Biological Denitrification (ISBD)

This treatment method is a viable option when the rate of contaminant biodegradation is faster than the rate of contaminant migration. Rates are dependant on the type of contaminant, the microbial community, and the subsurface hydrogeochemical conditions. Treatment techniques are focussed on optimising the conditions that support natural denitrifying microorganisms.

Several techniques for nitrate removal rely on natural microbiological reactions, which convert nitrate into other forms of nitrogen, particularly nitrogenous gases. For ISBD, substrates containing organic carbon are added as an energy source to enhance the activity of the microorganisms.

3.2.3.1   Operating principle

The principle behind the technique is similar to the addition of carbon sources in denitrification walls, except that the high molecular weight carbon sources in the wall have a slow-release action, whereas those used for ISBD are readily available, low molecular weight compounds. The reagents such as ethanol, methanol, acetic acid, glucose or sucrose are usually injected in liquid form into wellpoints or boreholes in the affected area (ITRCWG, 2000; Bates and Spalding, 1998, Deng *et al.*, 1998; and Nuttall *et al.*, 2001). If there is no resident population of suitable microbial communities, microorganisms may also be artificially introduced.

The influx of oxygen into the system inhibits denitrification, and depletion of natural oxygen may cause delays in the reaction of microbes, which then become insufficiently fast to contain contaminants (ITRCWG, 2000). Lack of a sufficiently large size microbial population may also limit the clean-up rate. The size of a microbial population is, in turn, affected by the environmental conditions prevailing in the area. Often conditions of slightly high or low pH, organic carbon availability or the ambient temperature can enhance or inhibit growth, depending on the optimum conditions for growth of a specific microbial population (Nuttall, 2001). Salinity is also an important control on microbial activity. Section 2.5 lists all the genera of bacteria that may effect denitrification under varying conditions.

The denitrification system operates on the same general principles as other *in situ* treatment systems i.e. an organic substrate is injected into the aquifer to introduce the carbon source (electron donor), which is required for denitrification. Phosphate is also injected with the carbon source to stimulate microbial growth. Anoxic conditions develop at the injection well, creating a natural bioreactor where nitrate is reduced to nitrogen gas.

3.2.3.2   Site specific conditions that favour application of the method:

The effectiveness of ISBD is governed by:

- The presence or availability of chemical species such as carbon, oxygen containing species (e.g. nitrate) and the environmental conditions, which affect microbial activity;

- If bacterial regeneration is inhibited, the denitrification rate will decrease and eventually stop as bacteria die off;

- Case studies show successful application of the method in coastal aquifers, alluvial and aeolian settings

Since there are many configurations of this method, one could apply it in a range of hydrogeological settings.

3.2.4   The Nitredox method

The Nitredox method is a modified configuration of the biological denitrification technique, designed for *in situ* treatment of nitrate (Braester and Martinell, 1988). It involves injection of an organic substrate to enhance denitrification, but also includes an additional phase of injection with aerated water once the nitrogen is removed.

The largest full-scale *in situ* denitrification plant uses the Nitredox principle. This plant is located at Bisamberg, Vienna (Austria) and has been operating successfully for more than a decade (Jechlinger *et al.*, 1991). It uses ethanol as the carbon substrate and the process is regulated to ensure that the raw water nitrate, which exceeds 15 mg/L, is reduced to approximately 9 mg/L in the product water.

3.2.4.1   Operating principle

The system consists of one pumping borehole located at the centre of two concentric circles of injection boreholes. Glucose or ethanol is injected into the boreholes in the outer ring to form a reduction zone where nitrate is reduced. At the inner ring, which acts as an oxidation zone, aerated water is injected and iron and manganese oxides are precipitated. The groundwater recovered from the central production borehole is partly free of nitrate and free of iron or manganese by-products (Braester and Martinell, 1988).

The injection boreholes are operated on a cyclic rotation such that on each circle only one borehole is injecting at a time. During injection, the two boreholes on either side of the injection borehole are pumped. The water pumped from the inner circle is sent through a degassing system to remove the nitrogen gas created by denitrification, so that the build up of gas in the subsurface does not decrease the effective permeability of the aquifer.

Monitoring boreholes are positioned between the reduction and oxidation rings and between the oxidation ring and the production borehole to monitor redox potential and changes in chemistry as the process continues. Microorganisms may also be injected if there is no suitable resident community of denitrifying bacteria

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3.2.4.2   Site specific conditions that favour application of the method:

Since the method is one of injection of a carbon substrate:
- High permeability and porosity would be favourable in the aquifer;
- Sand or gravel would make for suitable aquifer material;
- An adequate amount of resident denitrifying bacteria present.

3.3   Operational in situ denitrification plants worldwide

Tables 4 and 5 provide an overview of operational biological denitrification sites known at this stage and their experiences. These include ISBD and Nitredox methods in various configurations. The PRB systems require little or no maintenance. These methods have the potential for significant cost savings on expenses such as training and salaries for operators who would otherwise be required on site all the time.

In terms of permeable reactive barriers; factors that may affect the performance of the wall, or denitrification/nitrification rates include competing biological reactions such as dissimilatory nitrate reduction to ammonium and nitrogen immobilisation (Schipper and Vojvodic-Vukovic, 2000). Before constructing a denitrification wall, both the soil and the groundwater should be sampled. Determination of groundwater flow rates in the aquifer is also an important part of the process that precedes emplacement of the wall. In simple, saturated flow systems, flow rates can be determined using Darcy's Law and measurements of hydraulic gradients, porosity and saturated conductivity at the site (Schipper and Vojvodic-Vukovic, 2000).

The longevity of a wall may be grossly overestimated using only the denitrification reaction, since various other reactions can also remove organic carbon. For example, organic carbon is consumed or decreased by sulfate reduction and excess DOC leaching, as well as reaction with oxygen that enters the system.

Table 4: Operational site information for *in situ* nitrate treatment methods used internationally (after Robertson & Cherry, 1995; Schipper & Vojvodic-Vukovic, 2000; Blowes *et al.*, 2000).

Treatment method	Nitrate concentration (initial)	Aquifer type	Carbon substrate	% NO_3^- removed
PRB, Canada	5-57mg/L	Primary	Sawdust/woodchips	58-91
PRB, New Zealand	5-15 mg/L	Unconfined, sandy	Sawdust	95+
Electrokinetics/ Fe-wall, USA	Controlled amounts	Primary/ Secondary	None: Abiotic	84-87
NitrEl system, Canada	Up to 1000 mg/L (as N)	Primary/ unsaturated zone	None: electrochemical electrodes	levels down to 0.1 mg/L

Table 5: Summary of pilot and field-scale *in situ* denitrification plant published information (modified from Cartmell *et al.*, 2000).

Reference, treatment & location	Aquifer type	Carbon substrate	Operational details			NO_3^- -N mg/l	% NO_3^- reduced	Miscellaneous operational and other details
			Injection regime	Injection boreholes	Abstraction rate			
Jechlinger *et al.* (1991), Nitredox, Bisamberg, Austria	sand & gravel	ethanol or methanol	cyclic pumping	16 boreholes at 18m radii from abstraction well	5 ML/day	23	75	The full-scale plant at Bisamberg has been successfully in operation for more than a decade.
Khan & Spalding (1998), daisy wheel ISBD, Nebraska, US	sand & gravel	ethanol	continuous & pulse (C&P)	8 boreholes at 12m depth and 12m radii, rate = 0.065 ML/day	6 ML/day	40	35=C 90-100=P	This operation has proved successful at pilot scale. The continuous (C) regime gave higher denitrification efficiency than the pulse (P) regime but it also led to complete biofouling after 10 days. This could perhaps have been prevented by recirculation of treated water through the system to dilute the high nitrate water. This system also used an inner oxidation ring at 6 m radius, (presumably to oxidise any residual nitrite).
Hamon & Fustec (1991), daisy wheel ISBD, Carbonne, France	shallow alluvial aquifer	ethanol	continuous & pulse (C&P)	15 boreholes at 25m radii	0.7 ML/day	23	70 for both C & P	This was a successful field demonstration, lowering local NO_3^- concentration to below the EC limit. Increasing the no. of boreholes increased homogeneity of the clean up. Clogging was limited by pulsing (P) carbon supply (1 hour on/1 hour off), or, when pumping was continuous (C), using limiting carbon concentration. Recirculation of some of treated water also ameliorated clogging risks.
Mercado *et al.* (1988), daisy wheel ISBD, Shivat Zion, Israel	Hetero-genous	sucrose	pulse	2 operational boreholes at 100m depth, 15-25m radii, rate = 0.048-0.1 ML/day	1.2-1.4 ML/day	14	10	Clogging was experienced in one of the wells, rendering it inoperable. However, local clogging of substrate injection wells could be reduced by intermittent substrate injection. Aquifer denitrification efficiency depended on hydrodynamic dispersion and local hydrogeological conditions. The authors recommended the use of more injection wells.
Janda *et al.* (1988), Vsetaty, Central Bohemia	fine gravel & sand	ethanol with 5 % methanol	continuous – with and without recirculation	4 boreholes at 17m depth and 12-15m radii	0.5 ML/day	25-27	20-30 no recirc. 30-50 with recirc.	Initially, this method was operated as a trial without the recirculation of groundwater. Under this regime, inadequate mixing was demonstrated with very high carbon concentration in some parts, causing breakthrough, whilst in other parts, groundwater was flowing through the system without any exposure to carbon at all. The change in operation to include the recirculation of some of the treated

Reference, treatment & location	Aquifer type	Carbon substrate	Operational details			NO$_3^-$ -N mg/l	% NO$_3^-$ reduced	Miscellaneous operational and other details
			Injection regime	Injection boreholes	Abstraction rate			
								water through the aquifer improved overall efficiency. More boreholes were also recommended to further improve efficiency.
Kruithof *et al.* (1985), horizontal doublet design ISBD, Van Heek, Netherlands	phreatic	methanol	continuous & pulse (C&P)	min of 3 boreholes at 10-25m radii	-	19	30=C 50=P	This method was found to remove nitrate, but gave rise to an accumulation in nitrite. Also, clogging of aquifer was reported. Intermittent methanol dosing did not ameliorate clogging in this instance.
Chevron *et al.* ISBD, line of injection boreholes, Calais, France	chalk	ethanol	-	clusters of 3 boreholes, 3m apart	-	226-565	80	Natural *in situ* denitrification was evident prior to remediation in this instance, but was limited by carbon. The pulse injection regime used depended on the fissuring of the chalk. Denitrification was achieved in long time operation (450 days). Improvements to the system could have reduced this time period. Rates of denitrification were improved when trace metals were supplied in conjunction with the carbon substrate.

3.4 Permeable Reactive Barrier Design - A review

Contaminants, conditions and Reactants for design of PRBs

3.4.1 Desirable characteristics of reactive media

Reactive media used at any particular site, should be compatible to the subsurface environment at that site. To keep costs of PRBs down the following are important considerations should be made for the material used in the barrier (EPA, 1995):

a)   It should persist over long periods of time;

b)   It should not be readily soluble or depleted by reactivity;

c)   It should be readily available at a medium to low cost;

d)   It should minimize the constraints on groundwater flow;

e)   It should preferably be unimodal in grain size;

f)   It should be safe for handling by workers;

g)   It should cause no adverse chemical reactions or by-products when reacting with constituents in the contaminant plume;

h)   It should not act as a contaminant itself.

3.4.2 Treatability of contaminants

A large amount of contaminants are treatable using *in situ* treatment technologies. Table 6 lists contaminants treatable using *in situ* treatment technologies.

Table 6: Contaminants treatable by reactive materials in Permeable reactive barriers (PRBs), Powell *et al.* (1998) and O'Hannesin (1998).

Organic compounds		Inorganic compounds	
Methanes	Tetrachloromethane Trichloromethane Dichloromethane	Trace metals	Chromium Nickel Lead Uranium Technetium Iron Manganese Selenium Copper Cobalt Cadmium Zinc
Ethanes	Hexachloroethane 1,1,1-trichloroethane 1,1,2-trichloroethane 1,1-dichloroethane	Anion	Sulfate Nitrate Phosphate Arsenic
Ethenes	Tetrachloroethene Trichloroethene Cis-1,2-dichloroethene Trans-1,2-dichloroethene 1,1-dichloroethene vinyl chloride		
Propanes	1,2,3-trichloropropane 1,2-dichloropropane		
Aromatics	Benzene Toluene ethylbenzene		
Other	Hexachlorobutadiene 1,2-dibromoethane Freon 113 N-nitrosodimethylamine		

There are some contaminants that cannot be treated using *in situ* technologies, and some contaminants that have not been tested for treatability.

Permeable reactive barriers are designed to provide adequate residence time in the treatment zone for degradation of parent compounds as well as breakdown products that are generated, (Powell *et al.*, 1998; Blowes *et al.*, 2000; Robertson and Cherry, 1995 & Robertson *et al.* 2000; Schipper and Vojvodic-Vukovic, 2000 & 2001). In the case of "cocktails" the barrier design is determined by the least reactive contaminant (Powell *et al.*, 1998).

3.4.3 Carbon source and concentration

Substrates including glucose, sucrose, methanol, ethanol, acetic acid and other carbon compounds have been assessed in various studies as organic substrates for *in situ* denitrification (Mercado *et al.*, 1988). Hydrogen and reduced sulfur were also assessed as inorganic substrates (Cartmell, 1999). With reference to elevated nitrate concentrations, it is important to be certain that the stoichiometric requirement of carbon to allow for de-oxygenation and denitrification is present. Any excess carbon will lead to undesirable carbon compounds and by-products being produced in the potable water.

3.4.4 Conditions for denitrification

The main controlling factors of denitrification (Tredoux *et al.*, 2004 and Korom, 1992), when N oxides are present include:

a) pH (optimum range 7-8);

b) temperature (denitrification is possible from 10ºC, optimal denitrification occurs at 60-70ºC);

c) presence of bacteria possessing appropriate metabolic capacity;

d) presence of organic carbon as an electron donor;

e) dissolved oxygen concentration.

Nutrients which are often necessary for microbial growth include:

- Major elements: H, O, P,and S;
- Minor elements: K, Na, Mg, Ca, and Fe;
- Trace elements: Mn, Zn, Cu, Co, and Mo.

Most aquifers contain adequate quantities of these elements (Cartmell *et al.*, 1999).

3.5 *Remediation feasibility, laboratory treatability and PRB design studies*

PRBs installations have been designed and implemented based on the results of laboratory incubation and column studies used to test reactive materials and the kinetics of contaminant removal. The data obtained from these studies are used in combination with site specific information such as:

a) groundwater velocity

b) contaminant type

c)   contaminant concentration

d)   and the total mass-flux of the contaminant requiring treatment

Laboratory incubation tests and column experiments will be briefly discussed in context of PRB design.

3.5.1 Laboratory treatability studies

The need for these studies is dependant on:

a)   contaminants present

b)   contaminant concentration

c)   and geochemical conditions at the site

Contaminants for which behaviour in the subsurface is well documented may not require treatability studies, but rather use available databases to design barriers. Where mixtures of contaminants occur, and geochemical conditions are different to sites previously tested, or where reactive mixtures or sequential zones of reactive materials are proposed, treatability tests proves highly instructive toward design of barriers (Powell *et al.*, 1998; Innovative Technology, 2000; Blowes *et al.*, 2000; Robertson and Cherry, 1995 & Robertson *et al.* 2000; Schipper and Vojvodic-Vukovic, 2000 & 2001).

These studies can be used to compare reactivity and longevity of reactive materials under uniform controlled conditions, (Schipper and Vojvodic-Vukovic, 2000). The half-life of contaminants, important knowledge during barrier design, can be estimated. Treatability studies should be conducted using groundwater and aquifer material from the site.

3.5.2 Incubation/ batch studies

Incubation/batch treatability studies are suitable for rapid comparison or screening of reactive materials. Results obtained for various reactive materials gives an indication of relative rates that may be useful for selecting appropriate reactive materials for subsequent testing or field application. Incubation tests are usually faster, cheaper and simpler to set up than column tests.

The limitations of incubation tests include the following:

a)   mass transport and diffusion effects are not taken into account

b)   the use of very low ratios of reactive material to solution relative to column tests and actual field implementation

3.5.3   Column studies- methodologies, and data interpretation

Column tests are more useful for determining contaminant removal rates under conditions that more closely compare with operating conditions anticipated in the field e.g. flow velocity. Rates determined in column tests are the basis on which design parameters used to determine residence time required for the contaminant in the reactive material. Using the residence time and the flow rate, the treatment zone thickness can be determined. Information concerning potential mineral precipitation in the reactive material caused by changing pH and Eh conditions can be detected by measuring the major ion concentrations of the influent /effluent water during column tests (Powell *et al.*, 1998).

Column tests have the following advantages over incubation tests:

1.   more realistic field performance rates

2.   a better opportunity to examine products of reactions

3.   and it can provide useful information with respect to log term performance

Methodologies

Column size used varies and is typically 10-100cm long, and 2.5 to 3.8cm inside diameter, with sampling ports at the influent and effluent points as well as along the flow path. The sampling ports should be designed so as to enable sampling along the central axis. Groundwater from the site should be used and a laboratory flow rate that should closely approximate field flow velocity should be used. Concentrations of the major ion as well as alkalinity should be measured to predict the potential for mineral precipitation (Powell *et al.*, 1998).

Data Interpretation in columns

Contaminant concentrations are plotted as a function of distance along the column. The flow rate is used to determine the residence time at each sampling position (relevant to the influent). Kinetics models are used to calculate the degradation or disappearance rate constants for each contaminant in the influent groundwater (Powell *et al.*, 1998).

For VOCs and chromate, the first order model is used:

$$C = C_0{}^{e^-}kt$$	Equation 10

Where:

C = contaminant concentration in solution at time t

C_0 = initial contaminant concentration of the influent solution

k = the first order rate and

t = time

To calculate the half-life of a particular species/compound, C/C0=0.5 which by rearranging the equation (11):

$$ln(C/C_0) = -kt.$$ and $\qquad t_{1/2} = 0.693/k$	Equation 11

For organic compounds, the first order rate kinetic model should determine the degradation rates and conversion factors of the parent materials and breakdown products/ intermediates (Powell *et al.*, 1998).

Inorganic compounds rely on precipitation and adsorption of a chemical constituent; hence it is important that laboratory incubation and column data should be combined with geochemical modelling to assess the stability of potential precipitates, adsorbates, and to assess the potential utility of reactive mixtures for remediation of inorganic compounds. Comprehensive water analyses and characterisation during incubation experiments is thus important to enable one to do speciation calculations using a programme like PHREEQC (Appelo and Postma, 1998).

3.6   Residence time determination in PRBs- inorganic constituents

Reaction rates vary widely and depend on site specific characteristics of the aquifer, the groundwater and the reactive material. Laboratory incubation tests using site groundwater and aquifer material, or pilot scale studies should be used to estimate reaction rates representative of field conditions.

Reaction rates can be incorporated into reactive solute modelling to estimate the reaction rates in a barrier. The results of column tests would give a more reliable estimate of the reaction rate than incubation tests; however the limitations of the duration of the test as well as some secondary product formation may exist/ occur.

Biologically mediated systems might be more susceptible to variations in nutrient concentrations. Field pilot scale barriers are warranted until the limiting factors of biologically mediated systems are better understood. Potential variability of the constructed barrier should also be taken into account when field reaction rates and residence time determinations are done.

3.7   Conclusions

Currently operational sites show that denitrification in the field is possible and successful with some sites experiencing some initial problems. It is however important to know that each site or geological setting is unique and that proper site characterisation including:

- Chemical characterisation of water, soil and rocks;
- Lithological make up as well as structural geology, and
- Groundwater flow characteristics and hydraulic properties.

Parameters that stand out as being important for successful denitrification experiments include:

- C:N ratio and oxidisability of the organic matter;
- the presence or availability of chemical species such as carbon, oxygen containing species (e.g. nitrate);
- If bacterial regeneration is inhibited, the denitrification rate will decrease and eventually stop as bacteria die off.

It is important to know the history of a particular site e.g. was there a waste site at the location previously, were there any previous pollution plumes, etc.

Incubation experiments are described in the literature as testing the different parameters to achieve optimum quantities or concentrations of variables so that field-testing can be done with more confidence and a better understanding of the processes involved. Chapter 4 shows information gathered at the site, and Chapter 5 describes laboratory studies as part of this study to evaluate various carbon sources for denitrification.

CHAPTER 4: SITE CHARACTERISATION

4.1   Introduction

Before attempting any remediation, one needs to do a site characterization in order to understand certain parameters of the site. Geology, lithology, hydrogeology, chemistry of soil and groundwater, previous contamination and any other related data need to be collected for the site as background information before any remediation can even be planned. This information will provide insight as to what methods could best be applied at a specific location in terms of construction and monitoring and actual chemical outcomes based on the known chemistry. The site selected for this study had to have the following characteristics:

- A rural town solely dependant on groundwater for all water uses;
- An area with groundwater of inferior quality and elevated nitrate concentrations (i.e. exceeding drinking water limit of 10 mg/L as N) in groundwater at most of its boreholes;
- A shallow primary aquifer of about 10m deep

Based on the above information and previous sampling Marydale was selected as a suitable study area. This chapter discusses the study area with respect to location; geology, groundwater quality, and hydrogeology (4.2, 4.2.1, and 4.2.2), soil and groundwater data collected during a sampling run (section 4.3) and describes the current status of soil and groundwater in the study area with respect to chemistry and suitability for denitrification experiments.

4.2   The study area

Marydale is situated in the Karoo area of the Northern Cape Province, between the towns of Prieska and Groblershoop, with the nearest large town being Upington approximately 180 km north west of Marydale (Figure 9). Figure 9 shows the topography and locality within South Africa is indicated.
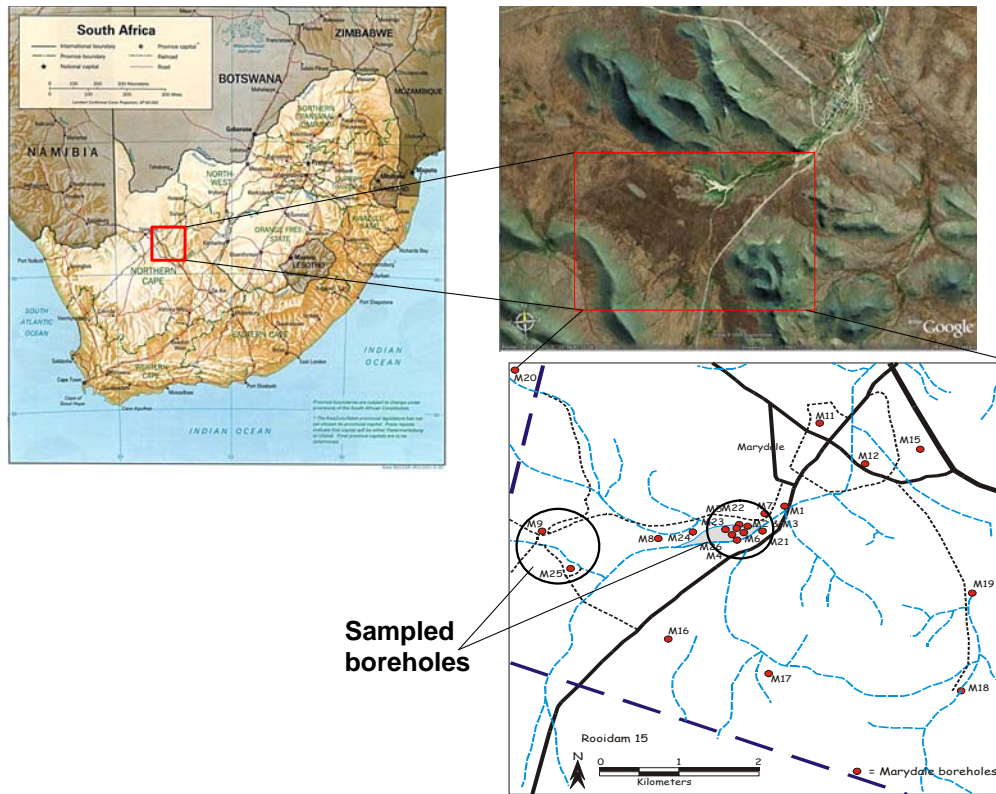
Figure 9:   The study area, Marydale, Northern Cape, South Africa. Left: location in South Africa. Upper right: topography (extracted from Google Earth, 2006). Bottom right: distribution of boreholes in the area (Tredoux *et al.*, 2004)

Marydale is a small town with 2039 inhabitants that is solely dependent on groundwater (Department of Water Affairs and Forestry, 2003). Most of their boreholes were drilled in a riverbed and adjacent flood plain with some boreholes located several kilometres away on neighbouring farms.

4.2.1   Geology

Marydale is situated on the contact between the Kaapvaal craton and the Namaqua metamorphic belt. The contact passes through the town in a northwest southeast direction. The dominant rock types in the southwest are metamorphic rocks of the Namaqua metamorphic belt, primarily quartzite, schists and porphyritic granite. Northeast of the contact there are primarily gneissic granites of the Kaapvaal craton. Quaternary aeolian sands of the Kalahari group occur in the lower lying areas between the ridges formed by more resistant rocks (Figure 9).

The brakbosch fault passes through the area in a north-south direction, although the surficial position of the fault is not known (Hofmann, 1997).

4.2.2 Hydrogeology of the area

VSA Geoconsultants and Ninham Shand performed a hydrocensus in 1997. Aquifer types, water levels, aquifer thickness, aquifer parameters, flow direction, aquifer material, groundwater quality, and fitness for use were considered in their study. According to their findings, a primary and a secondary aquifer are present in the Marydale area. It was concluded that the primary aquifer yields water of a better quality than that of the secondary aquifer. The flow pattern within the secondary aquifer is not well understood, however, average yields are lower than in the primary aquifer. In summary, the primary aquifer has a higher yield and better quality than the secondary aquifer.

The primary aquifer occurs in the low lying areas between ridges, and is composed mainly of aeolian sedimentary deposits (sandstone and silt) up to 12 m thick with bedrock composed of either quartzitic gneiss or granite. Sand grains are sub rounded and grain size varies from coarse grained (with some cobble sized grains) at depth to medium and fine grained at the surface. A large percentage of fine-grained matrix material is present.

Water levels measured in the area vary from about 5 m to approximately 25 m, with 10 m and shallower ascribed to the primary aquifer, with deeper levels relating to the secondary aquifer. Transmissivity calculated from pumping test data by Hofmann (1997) shows a variation from 50 m^2/day to 610 m^2/day. This is indicative of the heterogeneity within the aquifer; it may also relate to the contrast between the yields of the primary and secondary aquifers.

4.2.3 Groundwater quality at Marydale

Inorganic chemistry data (Appendix B, groundwater sample analyses) from borehole water in Marydale confirms that groundwater is primarily of sodium chloride type water, "very hard", salty water. Hydrochemical data for groundwater samples obtained from Marydale boreholes indicated that it was dominated by sodium chloride. Apart from the salinity the water also had a relatively high calcium concentration which indicated that the water was hard. Nitrate and fluoride exceeded the maximum allowable concentrations for drinking water. As a result, Hoffmann (1997) recommended that the feasibility of a denitrification plant be tested. The

health risk to babies (in the case of nitrate) and dental health of the community (in the case of fluoride) are factors that have to be noted.

4.3 Groundwater and soil characterisation

4.3.1 Introduction

Soil type is defined by the chemical conditions prevailing at the time of formation as well as the prevailing physical-chemical conditions at any given time.
Controlling factors and requirements of denitrification in soils and groundwater includes, (2.2 and 2.3):

- the presence of bacteria possessing the metabolic capacity;
- an energy source (organic carbon, reduced S compounds, or molecular hydrogen);
- terminal electron acceptors (N oxides);
- A peak pH for denitrification of 7 to 8;
- the absence of O_2 or a reduced O_2 availability;
- Stoichiometric relationship between the organic carbon used and nitrate present in the soil environment;
- availability of the carbon;
- temperatures ranging from 6ºC-35ºC, with greater rates of denitrification at higher temperatures, and
- water table depth.

4.3.2 Sampling

Samples were collected in Marydale, Northern Cape to characterize soil and groundwater and to evaluate conditions present prior to denitrification experiments. Ten soil samples were collected on the basis of colour and texture changes with depth along a profile dug in the study area in Marydale, Northern Cape. Boreholes sampled included MAR 9, MAR 10, and MAR 23. Samples collected in the study area were analysed at the CSIR (microbiology and chemistry), and BEMLAB (soil chemistry and grain size distribution); solutions were analysed to provide baseline data and to properly characterize the site conditions in terms of chemistry (methods are discussed in Appendix A).

4.4 Water and soil baseline data

4.4.1 Water samples

Water samples were collected from boreholes in the town of Marydale according to methods outlined in (Weaver, 1992). These were analysed by the CSIR accredited laboratories (methods documented in Appendix A).

Major anions and cations in solution show the relationships between ions within the groundwater environment and the magnitude of concentration of ions and compare these to the minimum requirements for safe drinking water. A piper diagram was generated to characterize the water type in the Marydale area (Figure 10).
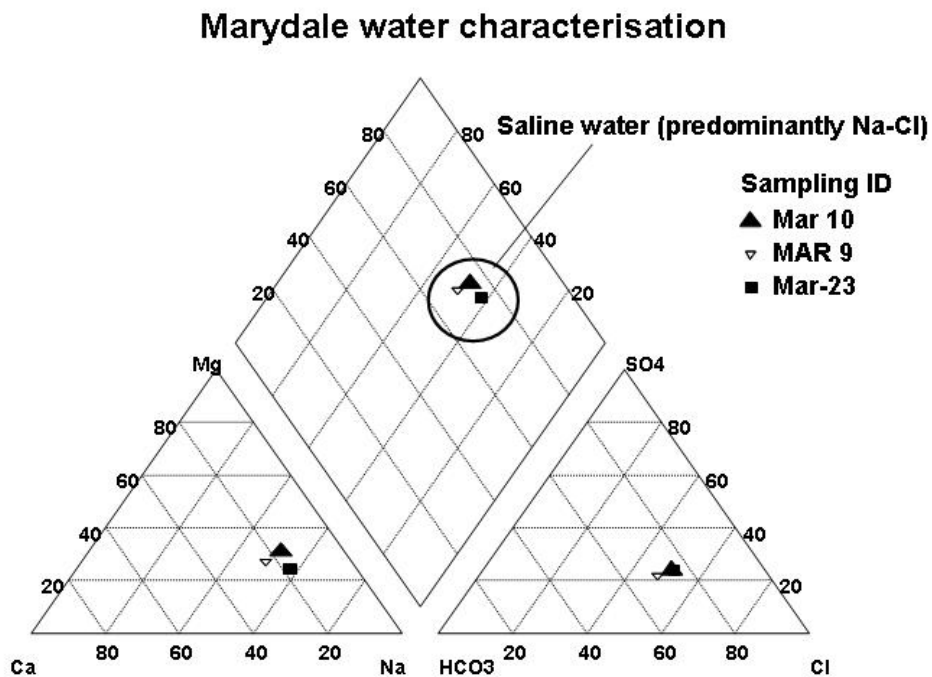
Figure 10:   Results of groundwater sample analyses displayed using a piper diagram to characterise the water composition of Marydale's groundwater.

Marydale has predominantly sodium-chloride rich waters. Total dissolved solids values calculated from EC measurements of water samples range from 885.5mg/L for borehole MAR 23 to 1245mg/L for MAR 10. All samples may be characterised as "very hard waters" with hardness ranging from 371 to 535 mg/L (measured as $CaCO_3$). Electrical conductivity, alkalinity and pH were measured and hold some evidence for hardness and total dissolved solids. Groundwater data was also recorded in Appendix B.

Dissolved organic carbon was measured in the groundwater as an indication of the amount of organic carbon in water that may aid denitrification. The results (Table 7) show that the dissolved organic carbon is generally < 1 mg/L and only reached 1 mg/L in MAR 10, hence dissolved organic carbon level in the selected borehole was below detection limits. This indicates that carbon present is either in another phase, or not present at all. The total alkalinity of the groundwater was measured as $CaCO_3$ for each borehole (Table 7). Water from borehole MAR 23 was selected for use during a 28-day laboratory denitrification experiment. Borehole MAR 23 was selected based on its location and probable use as a site for field-testing at a later stage. All the boreholes in the Marydale area have nitrate concentrations above that of the target < 6 mg/L as N.

Table 7:   Summary data for boreholes sampled in Marydale, borehole 23 (shaded) was selected as the groundwater source for incubation experiments

Parameter	Borehole MAR 9	Borehole MAR 10	Borehole MAR 23
Na (mg/L)	169	270	214
K (mg/L)	20	31	21
Ca (mg/L)	68	77	58
Mg (mg/L)	49	85	49
Cl (mg/L)	185	346	212
SO_4^{2-} (mg/L)	114	222	133
Alkalinity as $CaCO_3$ (mg/L)	289	301	313
NO_3^- + NO_2^- as N (mg/L)	23	32	19
EC (mS.m)	148	224	161
pH	7.7	8.2	7.8
DOC (mg/L)	<1	1	<1

The maximum allowable nitrate concentration of 20mg/L as N is exceeded in boreholes 9 and 10. The pH of borehole 23 is within the range of the optimum pH for denitrification.

4.4.2 Soil samples

A soil profile was sampled in Marydale. Ten samples were collected along the vertical profile represented by Figure 11. Similar soil types are grouped together and significant colour and texture changes were used as criteria for distinguishing different soil types. The detailed data obtained from the soil analyses of all ten samples are presented in Figure 11 data also in Appendix B.
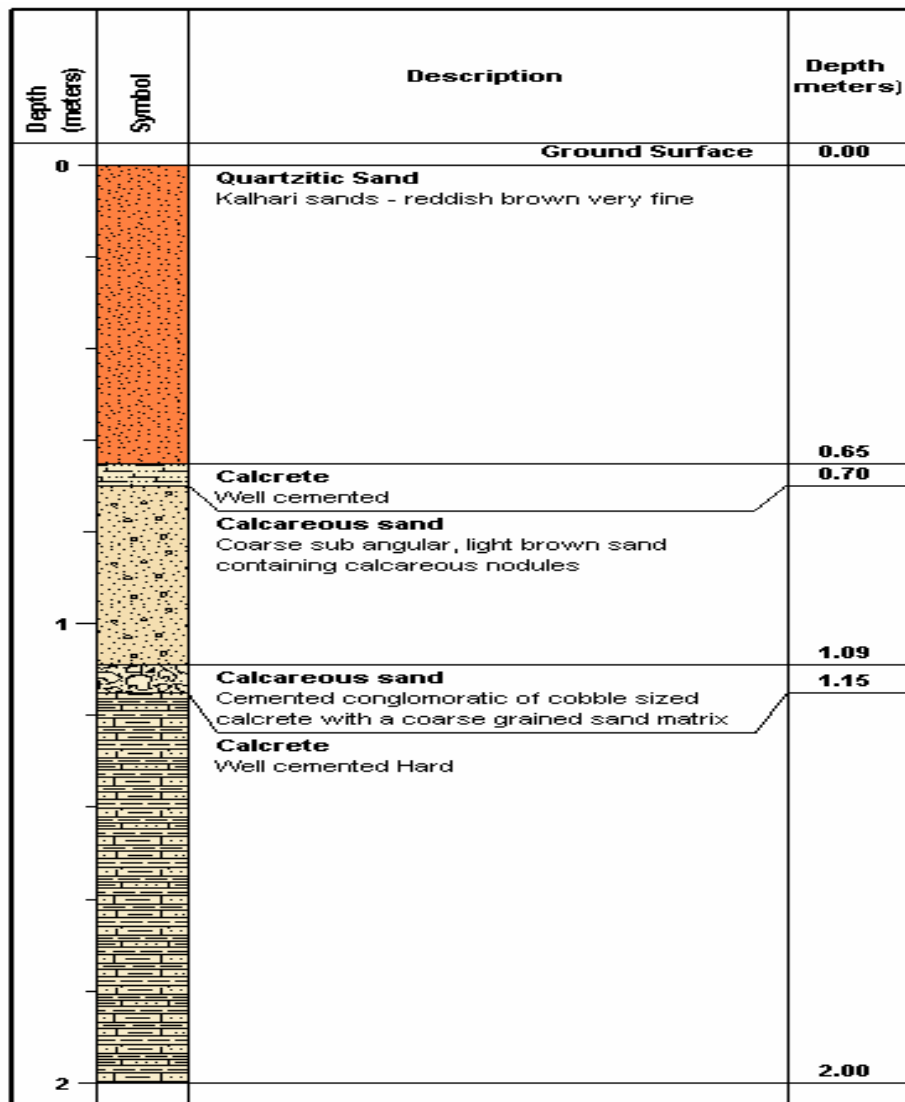
Figure 11:   Soil profile description from field observations, generated in Winlog by the CSIR.
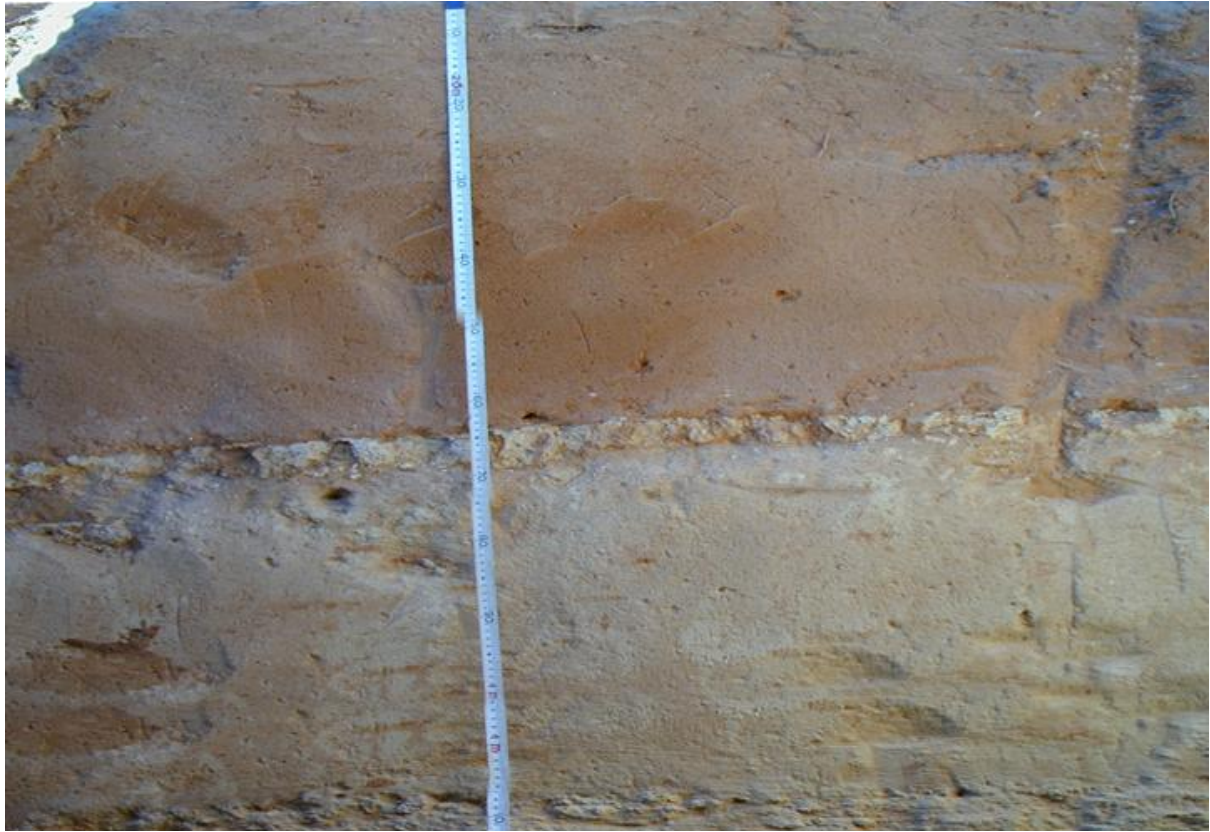
Figure 12:   Photograph taken of the vertical profile, (pictures taken by Gideon Tredoux).

Considering Figure 11 as well as photographic evidence in Figure 12, soils can be classified according to the South African system (SCWG, 1991). The sampled profile contained an orthic A horizon, a red apedal B horizon, and a hardpan carbonate horizon. The profile sampled can thus be classified as belonging to the Plooysburg soil form, and more specifically to the Brakkies soil family (Py1000).

Moisture % was determined in the laboratory for each soil depth (Figure 13).
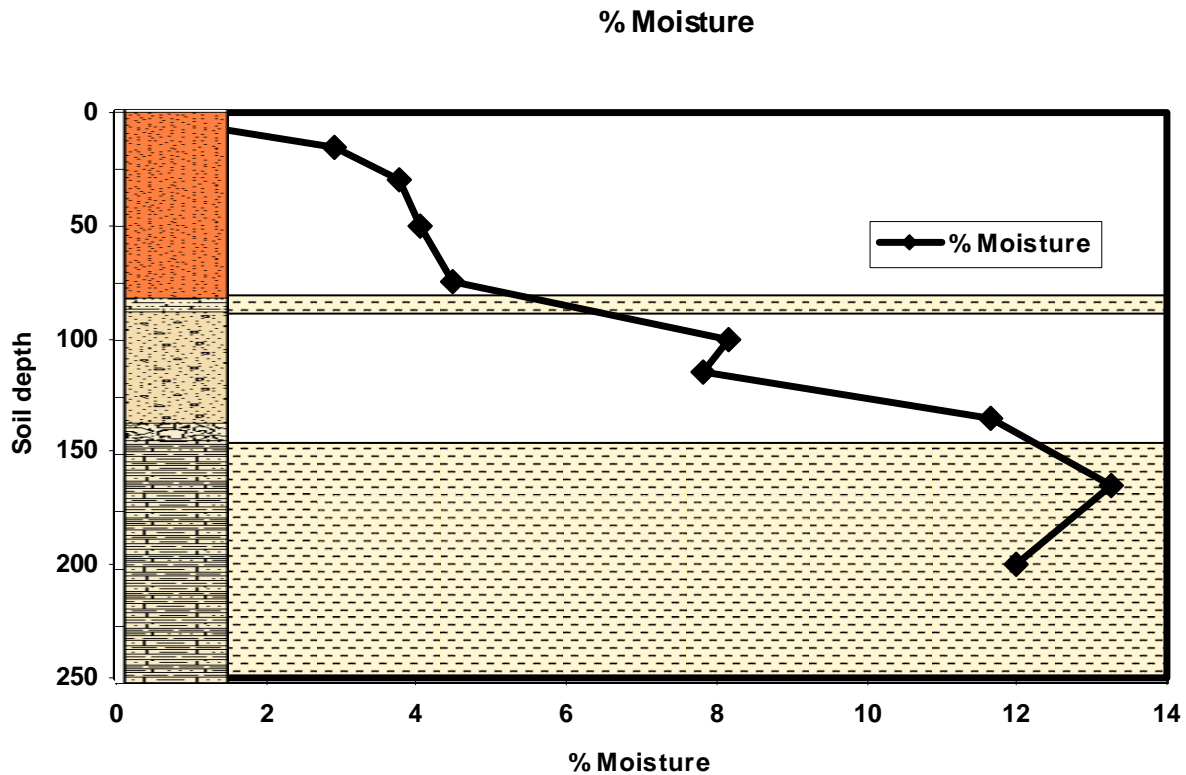
% Moisture

Figure 13:   Soil moisture percentage as measured in the laboratory by weight difference between wet (fresh samples from the field) and oven dried samples. The yellow and black layers represent calcrete rich layers present along the profile. The log on the left is a representation of the soil profile with respect to depth of layers as well as colour and texture of soil types.

Analyses show that soil moisture increases with depth. The rate of increase in moisture is greater from 75cm. This coincides with the presence of a thin calcrete layer at 65-70cm. A decrease in moisture occurs below 150cm, which correlates with the position of a second hard calcrete layer in the profile. The presence of a hard calcrete layers along the profile could act as inhibitors of evaporation and evapo-transpiration for the deeper soil layers.

Samples were then allowed to air dry for 24 hours. Dried and sieved (2mm) samples were analysed for total sulfur, nitrogen and carbon (Figures 14 and 15). The percentage nitrogen and carbon graph shows that there is a maximum of ten times more carbon than nitrogen in the soil. This compares favourably with data found in most literature. The measurement here however is as a total carbon and nitrogen and does not give an indication of what proportion is organic and inorganic. Figure 14 shows the ratio between total carbon and nitrogen at each soil depth.

Soil Depth (cm)

C:N along profile
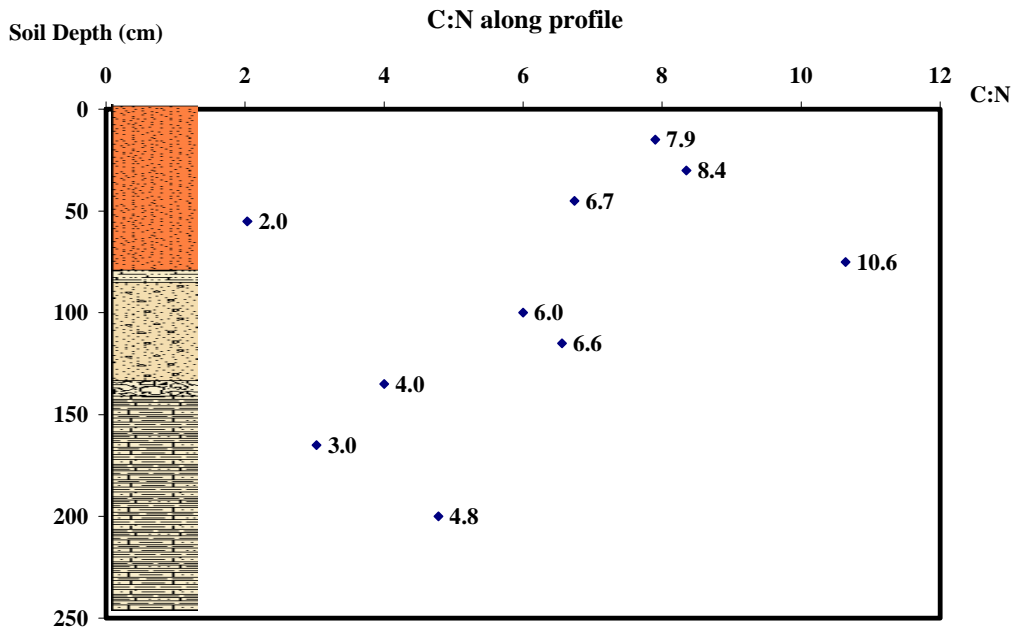
Figure 14: Carbon to nitrogen ratio in soil samples collected along a profile at Marydale, Northern Cape from surface to 200 cm depth. The log on the left is a representation of the soil profile with respect to depth of layers as well as colour and texture of soil types. The yellow and black layers represent calcrete rich layers present in the profile.

The total sulfur in the soil showed a dual peak of up to 240 mg/kg at 50 cm and 100 cm. The chemical form of sulfur was not a certainty and SO_4^{2-} was further examined to evaluate whether all the sulfur was in fact present as SO_4^{2-} or whether there were other potential sources of sulfur. Calcareous sulfate containing nodules occurred at most soil depths sampled; this could also contribute to the SO_4^{2-} concentration. Figure 15 shows the total sulfur in the soil profile.
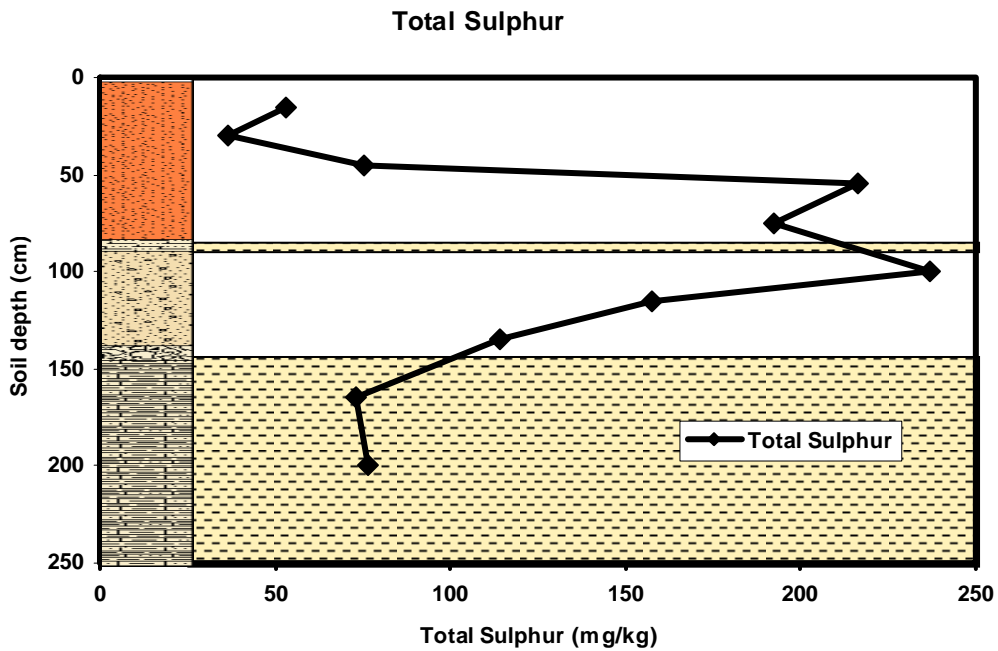
Figure 15:   Total sulfur concentration along a soil depth profile at Marydale, Northern Cape. Depth on the y-axis and sulfur concentration on the x. The log on the left is a representation of the soil profile with respect to depth of layers as well as colour and texture of soil types. The yellow and black layers represent calcrete rich layers present along the profile.

The highest sulfur concentration occurred in the depth interval from 45cm to 135cm. The high levels of sulfur at 50 and 100 cm may be due to dissolving of sulfur containing minerals present in the profile. It indeed possible that gypsum may be present at 50 and 100cm in the profile, however, this did not part of the investigation.

Saturated pastes were prepared and analysed for pH, EC, and alkalinity (Figures 16 and 17).

pH of Saturated paste
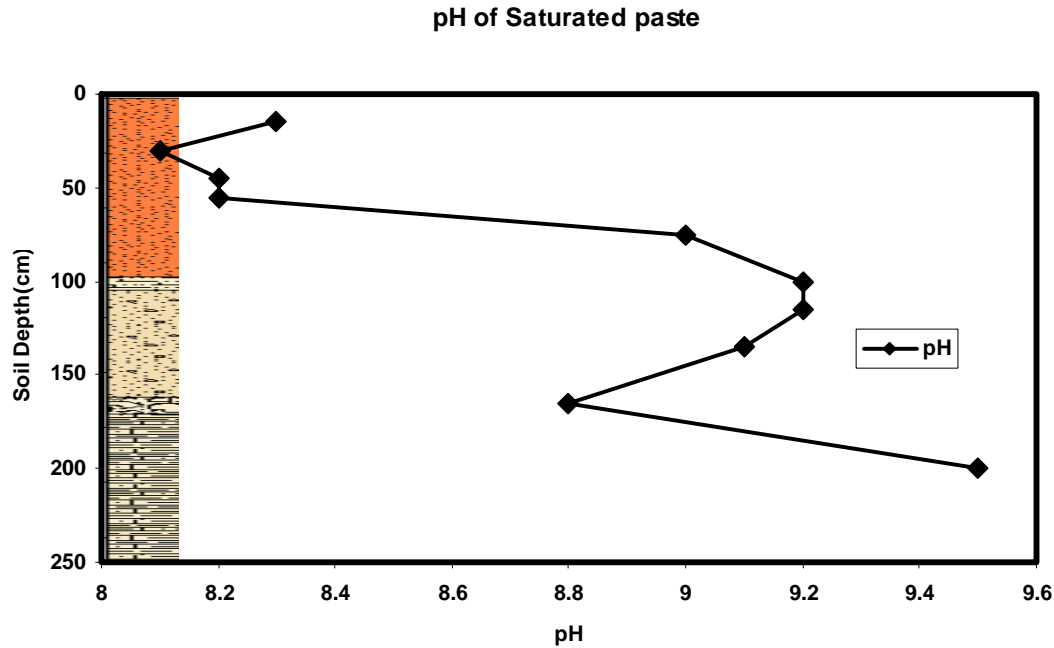
Figure 16: Saturated paste pH as measured prior to filtration. The yellow and black layers represent calcrete rich layers present along the profile. The log on the left is a representation of the soil profile with respect to depth of layers as well as colour and texture of soil types.

The pH values measured in the saturated pastes show that the optimum pH for denitrification to take place (i.e. 7-8) is exceeded in all the soil samples collected. Samples that have pH values exceeding 8.5 can be expected to have high soluble or exchangeable Na^+, and low solubility of micronutrients metal cations. The pH seems to be a function of the presence or content of calcium carbonate or calcrete in the profile.

Electrical conductivity (EC) and alkalinity of the saturated pastes for the ten soil samples are presented graphically in Figure 17.

EC and alkalinity of saturated paste
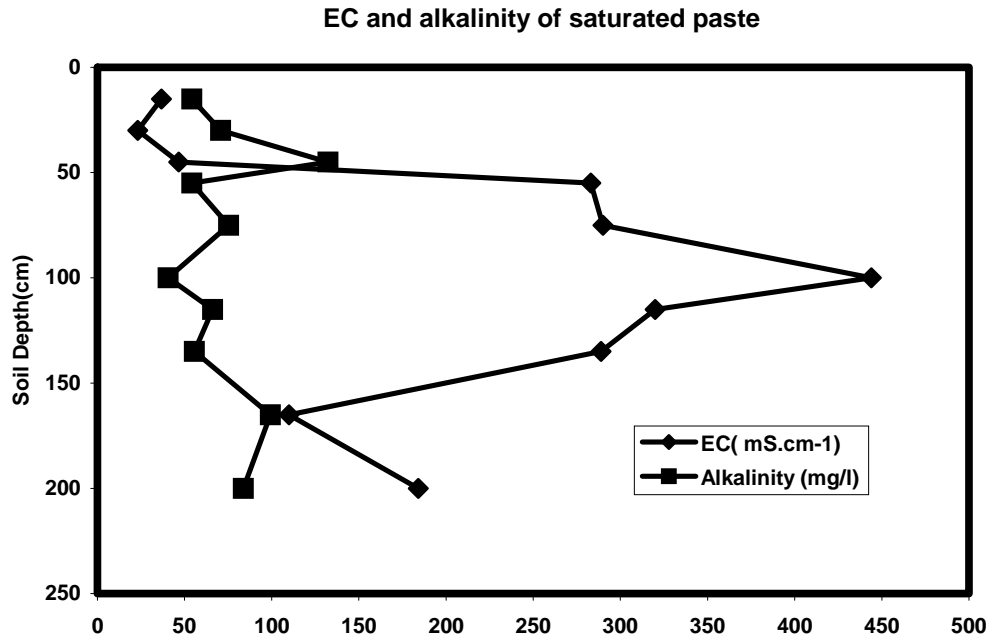
Figure 17:   EC and alkalinity of soils at varying depths along a profile dug in Marydale, Northern Cape. With depth on the y-axis and concentration on the x-axis.

A dual peak in electrical conductivity occurs at soil depths 55cm and 145cm. This indicates a zone of elevated salinity at these depths. Figures for pH, EC and saturated paste cations classify the soils of depth greater than 55cm as saline sodic soils. The filtrate was analysed for major cations and anions (Figures 18 and 19).

Saturated paste extracts- cations
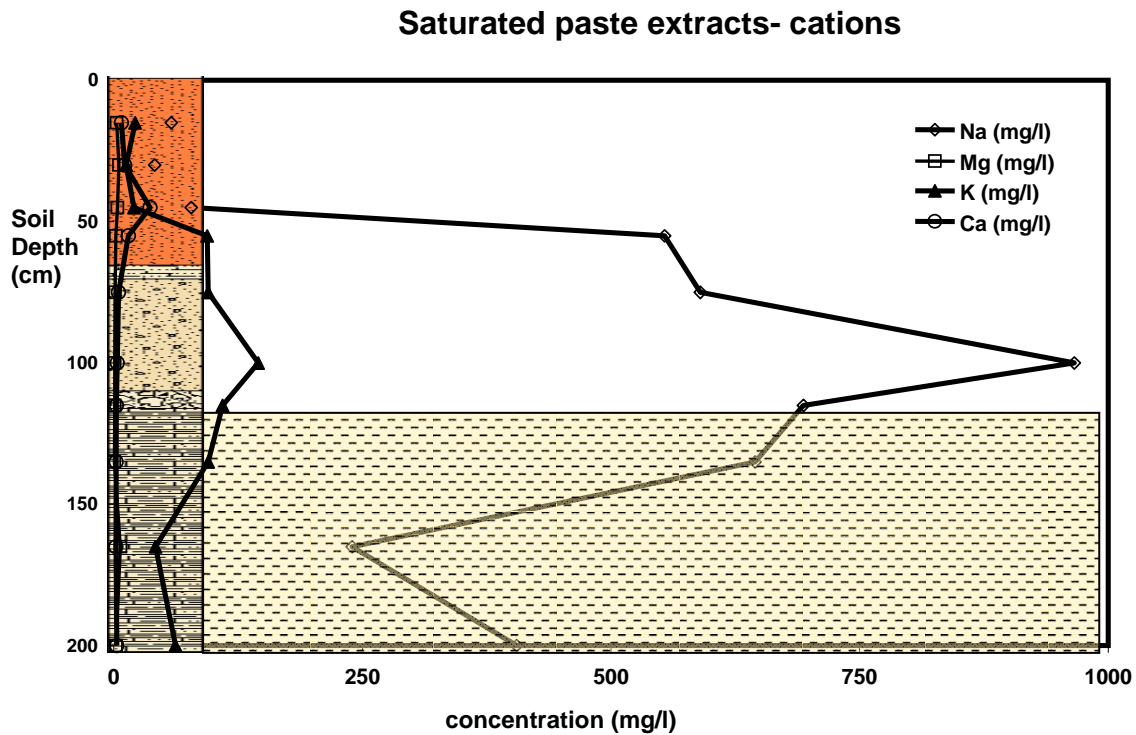
Figure 18: Soluble cations versus depth as measured from a saturated paste extract prepared from samples collected along the profile dug in the study area. The yellow and black layers represent calcrete rich layers present along the profile. The log on the left is a representation of the soil profile with respect to depth of layers as well as colour and texture of soil types.

Sodium and potassium are the major cations in solution for the saturated paste extract. This can be expected with the pH being greater than 9.

Chloride and sulfate are the dominant anions in the saturated paste extract. These ions contribute to the EC values seen in Figure 17. Ammonium extracts were prepared and analysed for the exchangeable cations in solution Figure 20.
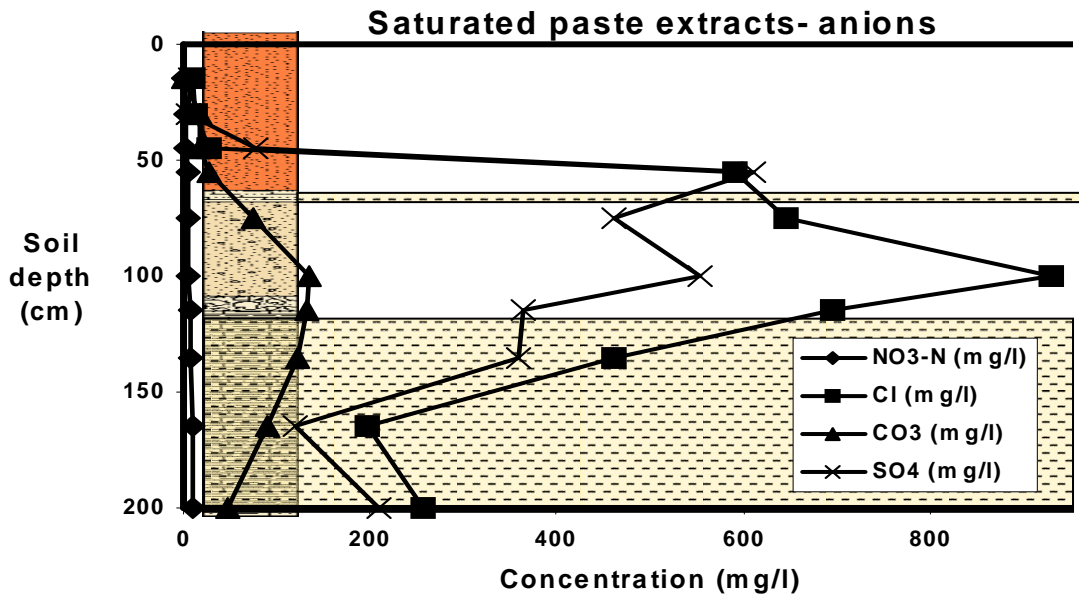
Figure 19: Soluble anions versus depth along the profile as measured from a saturated paste extract. The yellow and black layers represent calcrete rich layers present along the profile. The log on the left is a representation of the soil profile with respect to depth of layers as well as colour and texture of soil types.

Calcium was the dominant exchangeable cation, followed by sodium and potassium. This could be due to the fact that ammonium in the ammonium acetate extract solution displaces all cationic species from the soil matrix during mixing, and the presence of a calcrete layer would warrant calcium-rich minerals in the profile.

Exchangeable cations
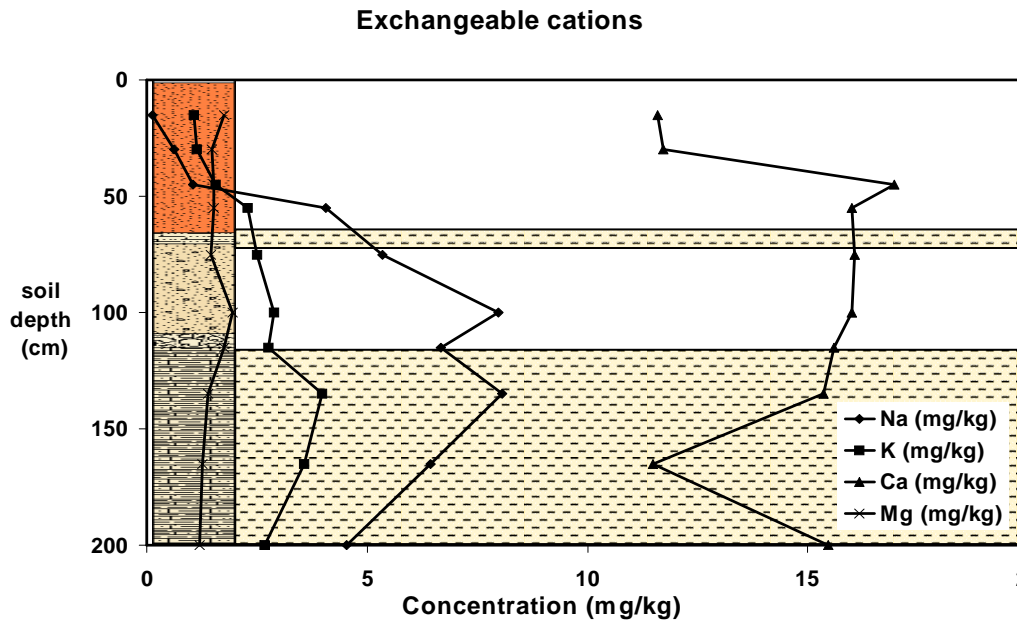
Figure 20:   Ammonium acetate extract to determine the exchangeable cations. Curves from left are Magnesium, potassium, sodium and calcium. The yellow and black layers represent calcrete rich layers present along the profile. The log on the left is a representation of the soil profile with respect to depth of layers as well as colour and texture of soil types.

Here, Ca appears to be the dominant cation. The soil samples were also characterized in terms of grain size distribution in the fine earth fraction (Table 8).

Table 8:   Grain size distribution of soil samples collected along a profile dug in Marydale, Northern Cape, South Africa, for particle size <2mm. % Clay, silt and sand were measured in order to classify the soil texture at each depth

Sample Depth (cm)	%Clay	%Silt	%Sand	Classification
15	2.6	3	94.4	Sa
30	3.2	1.8	95	Sa
45	3	2.2	94.8	Sa
55	2.2	1	96.8	Sa
75	1.8	3.2	95	Sa
100	3.2	5.6	91.4	Sa
115	2.4	3	94.6	Sa
135	0.4	2.6	97	Sa
165	0.4	2.2	97.4	Sa
200	0.4	2	97.6	Sa

The lower section of the profile was characterised by calcareous nodules and a hard calcareous semi cemented layer. The above table represents the portion of grains below 2mm in diameter. All the samples analysed were classified as sands with very low percentages of clay and silt present. This is an indication of high porosity and permeability for soils in the study area.

Heterotrophic plate count was analysed in all soil samples to assess the extent to which heterotrophic bacteria are present at different depths of soil (Figure 21).

It was expected that the surface soil layer would have the highest amount of microbiological activity. Figure 21 shows that microbial activity generally decreases with depth for this soil profile.

It also shows that the presence of heterotrophic bacteria at all depths of soil would warrant using any depth for denitrification experiments when other conditions such as temperature, carbon availability etc. are also present or favourable.

Heterotrophic plate count of soil of varying depths
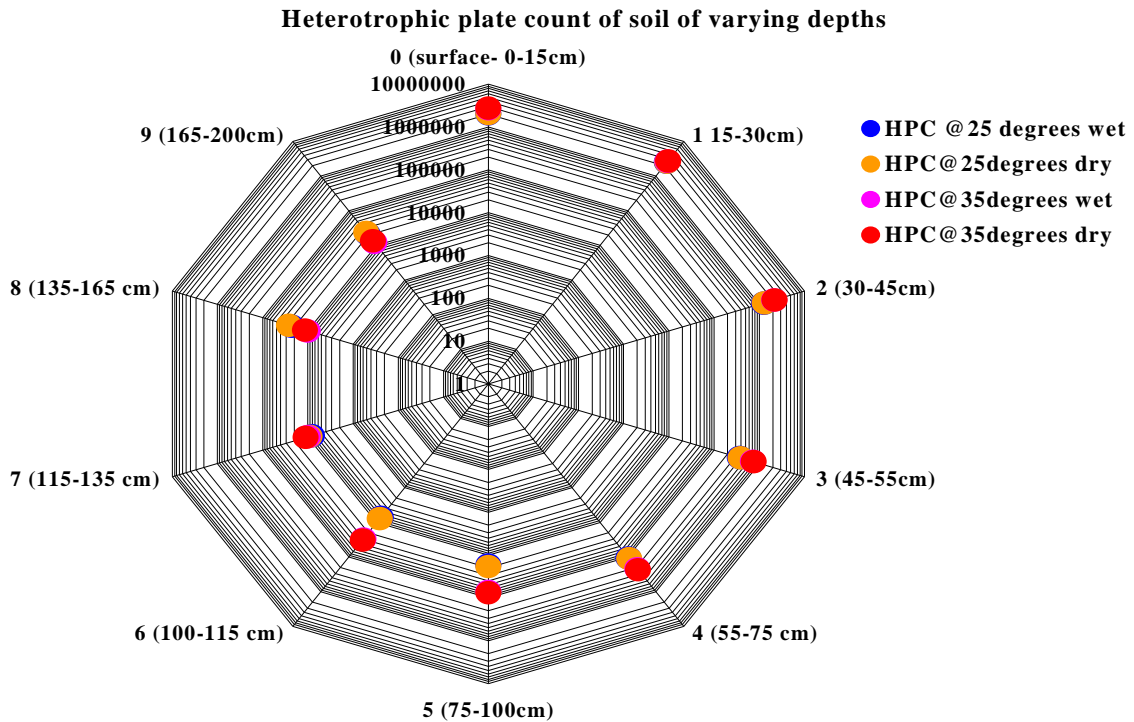
Figure 21: Heterotrophic plate count of each different soil depth along the profile, samples were selected on the basis of texture and colour changes with depth along the profile.

4.5 Ground water quality and DWAF guidelines

The results show that groundwater in the Marydale area has a high salt content, reflected in the TDS and EC values. The water here is likely to have a salty taste and may affect certain users who are on low salt intake diets or suffer from diarrhoea, and individuals with congestive heart failure or with kidney disease who follow salt restricted diets (Department of Water Affairs and Forestry, 1998). Some risk is posed to infants under the age of one from both the high salt load, as well as the nitrate concentration that might lead to methaemoglobinaemia.

The groundwater in this region classifies as "very hard". This condition would lead to impaired lathering of soap while washing and considerable scaling of pipes. This hardness is mainly due to the concentrations of divalent cations Ca^{2+} and Mg^{2+}.

Marydale waters are NaCl rich "seawater" like in composition (Figure 10). Possible sources of sulfate include oxidation of S^0, dissolution or weathering of sulfur/sulfate containing rocks and minerals which may be present within the calcrete layer in the form of gypsum or other sulfate containing minerals.

Marydale's nitrate concentrations range of 18.6-30 mg/L as N, which poses a threat to infants in the area. The guidelines classify 0-6mg/L as N as the target water quality range, any concentrations above this poses some threat of methaemoglobinaemia to infants, and at concentrations >20mg/L as N mucous membrane irritation can occur in adults. Much higher concentrations have adverse effect on cattle and other livestock. Some high nitrate concentrations can be owed partly to run-off of effluent and infiltration into the shallow alluvial aquifer in the study area. The presence of KNO_3 in areas that may be hydraulically connected may also be a cause of naturally high nitrate concentrations.

Other constituents of groundwater analysed for (i.e. Na^+, Cl^-, K^+, Ca^{2+}, HCO_3^-, Mg^{2+}) are all within the target values set by DWAF for domestic use, although they contribute to the scaling effects, impaired lathering of soap, hardness and salty/ bitter taste of the groundwater. The results of water analyses compare favourably to that found by Hoffman (1997).

4.6   Soil chemical characteristics

Soil pH according to literature is considered as the master variable in describing soil chemical characteristics. Soil samples analysed had pH values greater than 8 and even some greater than 9. This is indicative of samples having high soluble or exchangeable Na^+, and all samples with pH above 7 are likely to have low solubility of micronutrients metal cations. The high Na^+ solubility is confirmed by the results of the saturated paste extract, which has Na^+ as the major ion and K^+ closely following as the second most abundant cation.

Na^+ and Cl^- are the major constituents of the soil samples. This is confirmed by Figures 18 and 19, and is also reflected in the EC (Figure 17). The plots for EC and Na^+ have similar trends with depth. The soils from 55cm to 200cm can be described as saline sodic soils based on the pH and EC data for these soil depths.

Soil moisture shows an increase in moisture with depth, with the increase in moisture being greater for depths beyond 55 cm. This may be due to the presence of a semi consolidated calcrete layer at this depth possibly acting as a barrier to evaporation and evapotranspiration.

The total Sulfur and sulfate curves have a similar trend with depth along the profile. Two possible explanations,

1) All the sulfur detected is as sulfate in solution, or
2) sulfur is introduced and passes through the profile by dissolution of sulfate containing minerals within the calcareous layer at 55 cm.

Ammonium acetate extracts data varies from the saturated paste extracts in that calcium is the dominant exchangeable cation in solution, followed by sodium and potassium. This can be explained by the fact that ammonium in the ammonium acetate extract solution replaces all adsorbed cations as well as cations that form part of the soil matrix. Large amounts of calcium would be available from the calcrete particles within the soil profile. The abundance of calcrete nodules can be correlated with the calcium concentration with depth.

Grain size distribution was used to classify soil samples. All samples were classified as sand with more than 90% sand sized particles and very low clay and silt percentages. This relates to a high porosity and permeability in the study area. Heterotrophic plate count served as a good indicator of microbiological activity at all depths of soil as it considers a wide range of possible microbial species which includes denitrifiers and it is cost effective and reliable as a method.

4.7 Sample selection criteria for laboratory denitrification experiment

Samples were carefully selected based on locality, chemistry and suitability for denitrification. The water from borehole 23 was selected from the boreholes sampled as

- pH of the water sample falls within the favourable range for denitrification
- The dissolved organic carbon was below detection limits;

The addition of a carbon source would provide a carbon substrate for denitrification. One 25L sample was collected from this borehole for the laboratory denitrification experiment.

It was decided that two soil depths would be selected for denitrification experiments to compare the reactivity of the different soil depths as well as the influence of different soil chemical make up on denitrification. These were the 75-100cm and 165-200cm samples. The selection was based on the contrast in chemistry between the two depths. The 75-100cm sample falls within the zone of high salinity and displays elevated sulfate, sodium, and chloride. Samples of the 165-200cm soil depth did not show the elevated sulfate levels. Both soil samples had pH greater than 9, which is greater than the favourable pH for denitrification. Total nitrogen for the selected samples was 0.032% for both soils, while the total carbon was 0.192% and 0.153% respectively.

4.8 Conclusions

This chapter set out to characterize the current status of soil and groundwater chemistry for the study area and to compare this with the requirements for denitrification as well as soil fertility and health.

Water chemistry data shows that
- Marydale has predominantly sodium-chloride rich waters;
- "very hard waters" with hardness ranging from 371 to 535 mg/L;
- Marydale's nitrate concentrations range of 18.6-30 mg/L as N;
- The water is likely to have a salty taste and may affect certain users who are on low salt intake diets or suffer from diarrhoea, and individuals with congestive heart failure or with kidney disease who follow salt restricted diets.

Soil data shows that
- soil moisture increases with depth;
- The presence of a hard calcrete layers along the profile could act as an inhibitors of evaporation and evapo-transpiration for the deeper soil layers;
- The high levels of sulfur at the two depths of soil may be owed to dissolution of sulfur containing minerals in the calcareous layer present in the profile.

In general it was concluded that
- Marydale's groundwater is of inferior quality both chemically and aesthetically;

- This is based on the fact that result show that the water chemistry is dominated by NaCl and nitrate concentrations are above that of the acceptable levels for human consumption and close to the maximum allowable levels (20mg/L as N);

- pH measured in the water sample is within the range of optimum pH levels for denitrification;

- The soil can be described as a saline sodic soil with pH greater than 8 in all samples, hence not within the optimum range for denitrification and even greater than 9 in certain of the samples;

- Elevated sulfate levels may be due to the presence of sulfur/sulfate containing calcrete layer within the soil profile;

- Soil moisture along the profile may also be influenced by the presence of the semi consolidated calcrete layer;

- Predominantly sandy soils are located in the study area, this relates to high porosity and permeability within the soil profile.

CHAPTER 5: LABORATORY EVALUATION OF CARBON SUBSTRATES FOR DENITRIFICATION

5.1 Introduction

This study investigates the nature of nitrate occurrence in the subsurface and attempts to carry out denitrification using soil and groundwater of elevated nitrate concentration, to attain concentrations that are acceptable compared to drinking water standards. Nitrate presents adverse effects on the health of humans at concentrations above the maximum allowable concentration in drinking water. In South Africa the maximum allowable concentration is 20 mg/L as N, while the WHO guidelines refer to maximum allowable concentration of 10 mg/L as N (WHO, 1998).

Treatment of nitrates with minimal costs and safe methods is considered a required technological endeavour in the more rural parts of Africa.

Denitrification is a natural process occurring under certain favourable conditions and forms an integral part of the nitrogen cycle that converts NO_3^- to nitrogen gas with a few probable intermediates.

$$4NO_3^- + 5CH_2O + 4H^+ \longrightarrow 2N_2 + 5CO_2 + 7\,H_2O$$
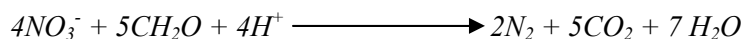
Equation 12
denitrification

The above reaction is best described as biological denitrification as microbial communities in the soil environment facilitate it. Conditions and parameters affecting of the reaction include temperature, oxygen content, carbon content, pH, Eh, carbon availability, soil moisture or water content, the presence of appropriate microbial communities, the carbon to nitrogen ratio, as well as other reactions taking part in the nitrogen cycle.

In situ denitrification refers to the reduction process occurring in the soil/ aquifer or groundwater within the sub-surface. The focus of this study is laboratory treatability studies for assessing denitrification in a field setting. Design of denitrification systems is primarily

dependant on the contaminant concentration and the geochemical conditions at the site. Chapter 4 discusses the soil and groundwater chemistry of the study area in detail.

The C:N ratio and oxidisability of the organic matter have been found to be important parameters in denitrification (Dodds and Fey, 1995). Readily oxidisable, carbon-rich substrates such as sucrose or glucose promote rapid denitrification, while straw, grass and wood chips are less effective over the short term, but may provide a slowly degradable form of carbon for long-term applications (Dodds and Fey, 1995 and Robertson and Cherry, 1995). The effectiveness of denitrification is governed by the presence or availability of chemical species such as carbon, oxygen containing species (e.g. nitrate and sulfate) and suitable environmental conditions, which affect microbial activity. If bacterial regeneration is inhibited, the denitrification rate will decrease and eventually stop as bacteria die off. This chapter addresses some of the key questions raised in Chapter 1 section 1.3.

Laboratory experiments were performed to evaluate the difference between the reactivity of soils from two different depths, as well as the performance of different carbon sources during denitrification over time.

The objectives of the experiments were:
1. To establish which of four carbon sources were more suitable as a carbon source for denitrifying a soil and groundwater mixture
2. To consider various carbon to nitrogen ratios of selected carbon sources
3. To consider a single carbon source for further experimentation
4. To establish what the effects of lengthening the incubation time and increasing the carbon concentration is on denitrification

5.2   Materials and methods

Laboratory experiments were performed to evaluate the difference between the reactivity of soils from two different depths, as well as the performance of different carbon sources during denitrification over time. Initial experiments (1 & 2) were run over 30 days with sampling and analyses at day 1, day 7, day 14 and day 30. Table 9 contains details of the sample make up as well as times of sampling for experiment 1.

A third set of experiments had sampling at t=0, 1/8, 1/4, 1/2, 1, 2, 4, 7, 10, 14, and 28 days. The fourth experiment had sampling and analyses performed at t=0, 3, 7, 10, 14, 21, 29, 43 days. Tables 11, 12 and 13 show the sample make up and sampling times of subsequent experiments.

Cation concentrations were measured using an atomic absorption spectrometer, while anions were measured using ion chromatography. EC and pH were measured for every sample. Samples were frozen prior to analyses to slow down or stop any microbial activity while awaiting analyses. Two depths of soil were used, as a contrast in chemistry between the soils was evident from initial soil analyses. Soil is likely to form part of the barrier when constructed in a field situation thus it was used in this experiment as the aquifer or matrix material. The soil is also the main source of denitrifying bacteria. Measurement of EC, pH, C, N, were taken prior to the incubation experiment.

Table 9:   List of substrates (organic compounds) used and times at which samples were removed from nitrogen atmosphere for analyses during initial treatability studies (experiment 1).

Soil depth	Carbon substrate	Time	Water composition
50cm	Untreated (reference) Glucose Methanol Maize meal Sawdust	sub samples removed from incubator at 4 intervals; at t=1 day, t= 7days, t= 14 days, and t=30 days	Groundwater from MAR 25 in Marydale was used to ensure that a known nitrate level was present at t=0
110cm	Untreated (reference) Glucose Methanol Maize meal Sawdust	sub samples removed from incubator at 4 intervals; at t=1 day, t= 7days, t= 14 days, and t=30 days	Groundwater from MAR 25 in Marydale was used to ensure that a known nitrate level was present at t=0

Parameters selected as possible indicator parameters for the denitrification experiment included nitrate, nitrite, sulfate, alkalinity, pH, electrical conductivity, dissolved organic carbon, ammonia, potassium, heterotrophic plate count, iron and manganese. Full chemical analyses included all major cations and anions. Samples were analysed by the chemical and microbiology laboratories at the CSIR. Sawdust samples were analysed by BEMLAB.

10g of soil were weighed into 50ml bottles. 40ml groundwater was added to this. Carbon sources were added as a 2% by weight of the soil, therefore 0.4g of solid carbon sources (i.e. glucose, maize meal and sawdust) and 0.4ml of methanol for initial treatability studies (Table 9). These were perceived as being an excess of carbon substrate, and were used

purely to evaluate which of the carbon sources would denitrify the soil and groundwater in the shortest time and with the least by-products.

Results in Table 10 show sawdust contained 0.25% nitrogen and 53.25% carbon. These percentages were incorporated into an equation to find the mass of sawdust to use for specific C:N ratios. Major ions as well as trace metals were analysed as these could contribute to the reactivity of the sawdust.

Table 10:   Composition of sawdust used for the laboratory denitrification experiments

	Lab. No.	Carbon	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
		%							mg/kg				
Sawdust	20022	53.25	0.25	0.03	0.08	0.10	0.04	124	20	58	1	10	5

Trace metals such as Fe, Mn, Cu and Zn act as enhancers for denitrification (Labbé *et al.*, 2003 & Lee, 1996).

Table 11:   Experimental setup for incubation experiments performed under a N_2 (g) atmosphere. Selected carbon sources were sawdust and glucose used in varying C:N ratios; 1.1m deep soil (10g), groundwater (40mL) and the carbon sources (different C:N ratios) was used.

Soil Depth	Water Composition	Treatment	Time
110 cm	Groundwater samples from MAR 25 in Marydale was used to ensure that a known nitrate level is present at t=0	Glucose (C:N) 75:1 50:1 25:1	Each sample had 4 sub samples removed and analysed at t=1 day, t= 7days, t= 14 days, and t=30 days
110 cm	Groundwater samples from MAR 25 in Marydale was used to ensure that a known nitrate level is present at t=0	Sawdust (g/kg soil); C:N 5; 12.6:1 10; 24:1 15; 34:1	Each sample had 4 sub samples removed and analysed at t=1 day, t= 7days, t= 14 days, and t=30 days

* C:N ratio is glucose C: dissolved NO_3-N in groundwater

All samples were placed in an incubator set at 23ºC, the temperature for the groundwater measured in the field, with nitrogen gas flowing through the system at a slow constant rate. All samples were sealed so that the nitrogen gas could not enter the containers. The nitrogen atmosphere was maintained to prevent any oxygen from entering the system.

Previous experiments had only 4 sampling times, which gave some idea of the concentration trends of nitrate and other parameters, but the initial part of the experiments were lacking

some data that was thought to be invaluable to the study. Table 12 shows a further experiment had more sampling events than the previous ones including a greater amount of sampling events during the first 24 hours of the experiment.

Table 12:   Experimental set up for incubation experiments using sawdust, groundwater, and soil mixtures. Identical carbon source (sawdust), two depths of soil (75-100cm, and 165-200cm) and three C:N ratios were used. Samples were incubated at 23 degrees Celsius under a N_2 (g) atmosphere.

Soil Depth	Water Composition	Treatment	C:N	Time
75-100cm	Water from Borehole 23 in Marydale	Sawdust 5g/kg of soil 10g/kg of soil 15g/kg of soil	12.6:1 24:1 34:1	Sampling times included t= 0, 3, 6, 12, 24, 48, 96, 268, 240, 336, and 672 hours.
165-200cm	Water from Borehole 23 in Marydale	Sawdust 5g/kg of soil 10g/kg of soil 15g/kg of soil	12.6:1 24:1 34:1	Sampling times included t= 0, 3, 6, 12, 24, 48, 96, 268, 240, 336, and 672 hours.

The following experiment (Table 13) includes further investigation based on the outcomes of experiment 3 (Table 12), evaluating higher C:N ratios of sawdust and a longer incubation period. Fine grained homogeneous sawdust particles (1000 µm) versus heterogeneous particles of sawdust were also evaluated.

The duration of the experiment was 43 days as opposed to the previous experiments running over a maximum of 30 days. Statistical evaluation of the data follows from the replication of identical sampling times and sample make up. One sample out of the four prepared had a uniform and finer grain size of sawdust (1000 µm), while the other three contained heterogeneous sawdust grain size mixtures of identical mass.

Table 13: Experimental setup used for incubation experiments investigating denitrification over a 43 day period. Soil depth (75-100cm) from the study area, groundwater from the study area, and sawdust at two different C:N ratios were used.

Soil Depth	Water Composition	Treatment	C:N	Time
75-100cm	Water from Borehole 23 in Marydale	Sawdust 15g/kg of soil 25g/kg of soil	34:1 50:1	Sampling times included t= 0, 3, 7, 10, 14, 21, 29, and 43 days. 4 replicates were prepared and analysed.

Full chemical analyses were done on days 0, 10 and 43. Only indicator parameters were analysed for at days 3, 7, 14, 21, and 29.

5.3   Results

The materials and methods describes the experimental design and analyses and parameters that were monitored over time, experiment 1 will be referred to as Carbon substrate effects on denitrification, Experiment 2 will be referred to as effect of C:N, experiment 3 will be referred to as sawdust as a carbon source and experiment 4 will be referred to as further investigation on sawdust in terms C:N and incubation time in the sections that follow.

5.3.1   Carbon substrate effects on denitrification

Figures 22-25 show the concentrations of these ions over a thirty-day period as analysed at days 1, 7, 14 and 30. Figure 22 shows the results of nitrate concentration over the thirty days of incubation. Data collected during this experiment also occurs in Appendix C.
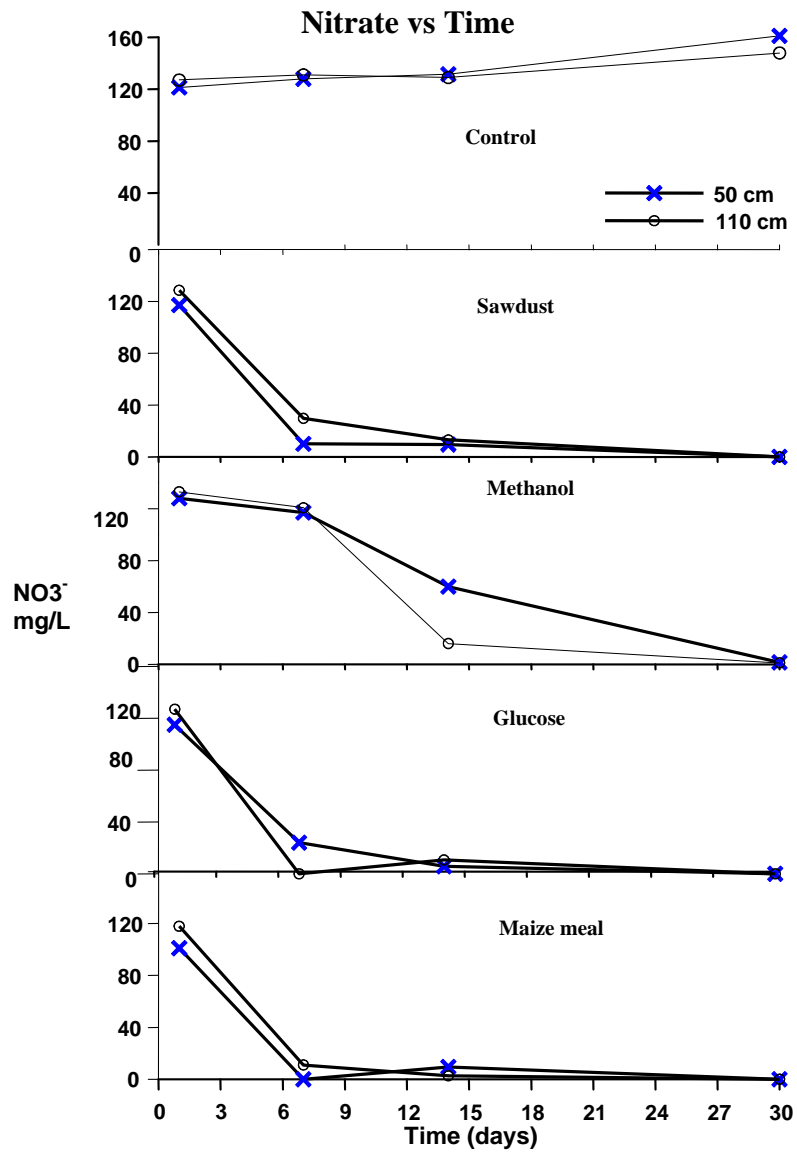
Figure 22:   Nitrate concentration as a function of time in relation to carbon substrate and soil source.

Figure 23 shows the changes in sulfate concentration during the thirty day incubation period.
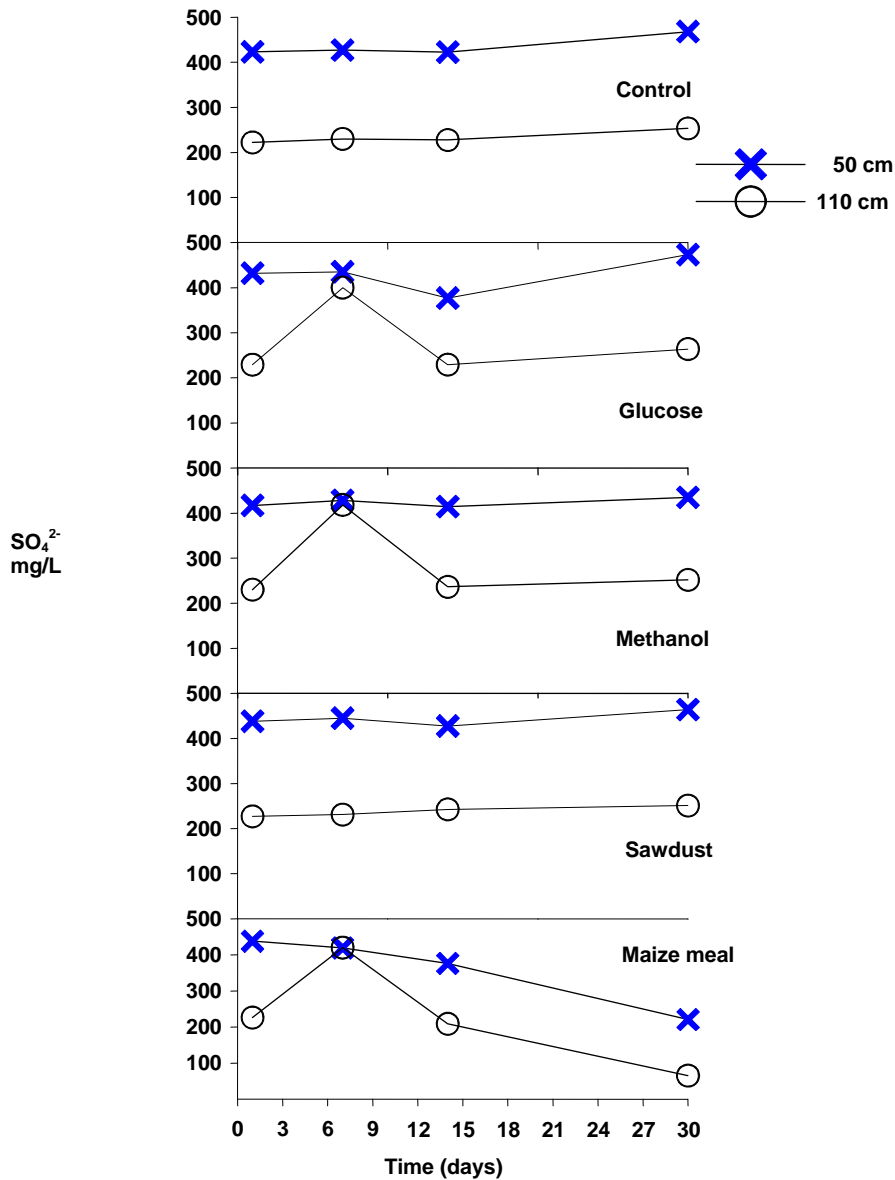
Figure 23: SO_4^{2-} concentration as a function of time in relation to carbon substrate and soil source.

Figure 24 shows the formation of acetate using the various substrates. Methanol and sawdust treated samples have much the same trend as the untreated sample. The glucose and maize meal treated samples show the greatest changes in the acetate concentration.
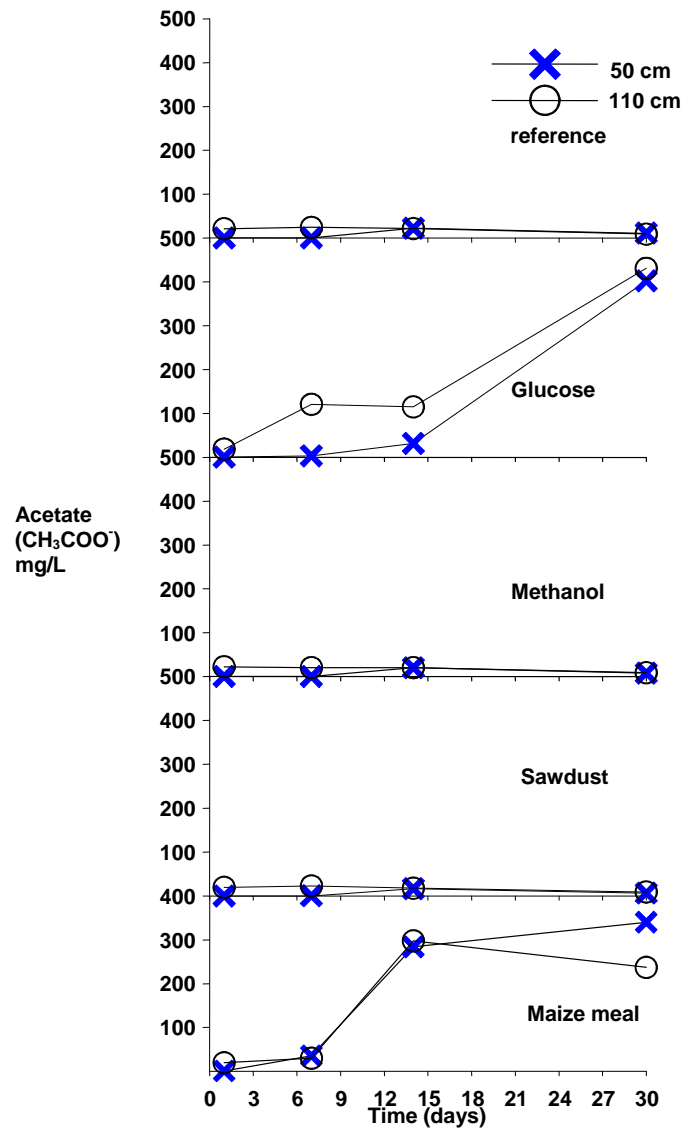
Figure 24:   Acetate concentration as a function of time in relation to carbon substrate and soil source.

Methanol contains only one carbon in its structure and hence cannot form acetate. Figure 25 shows the behaviour of the electrical conductivity during the incubation experiment.
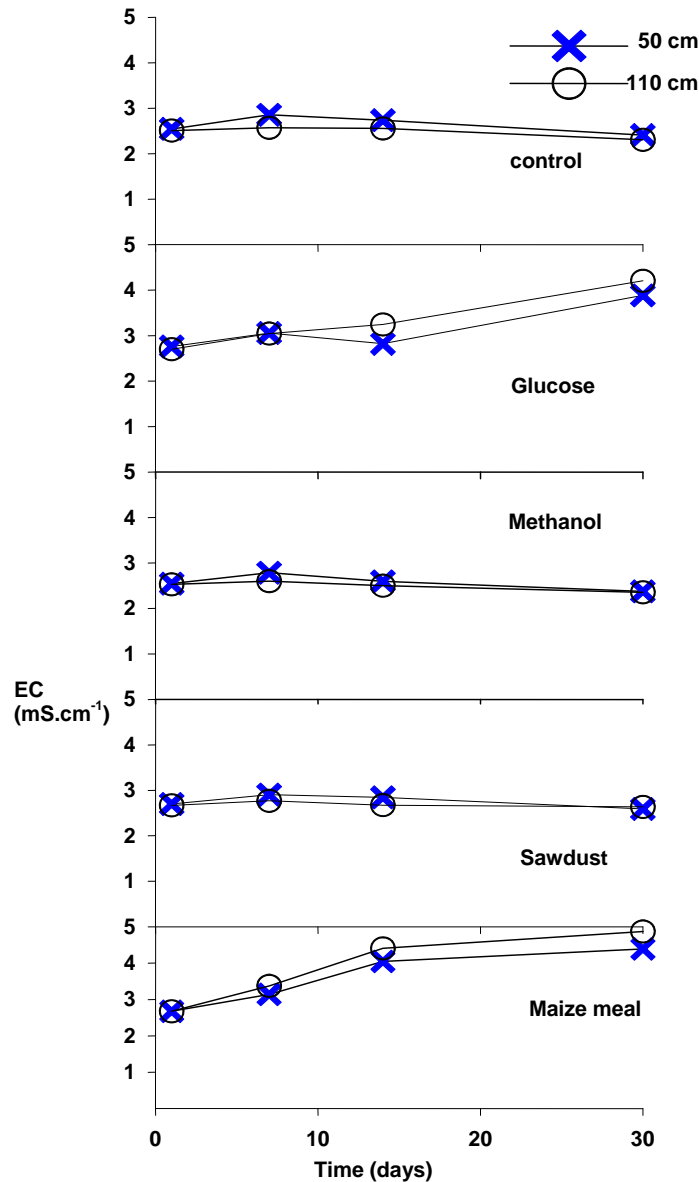
Figure 25:   EC as a function of time in relation to carbon substrate and soil source.

Sawdust and methanol show small changes in EC for both soil depths. EC graphs for the glucose treated soils show an overall increasing trend for both soil depths, with a slight decrease between days 7 and 14 for the 0.5m samples (Figure 25). Maize meal treated samples show an overall increasing trend in the EC values; with a steeper gradient between days 7 and 14 (Figure 25).

Figure 26 shows glucose treated 1.1m-soil depth sample for the ease of comparing all the measured parameters over the 30-day period.
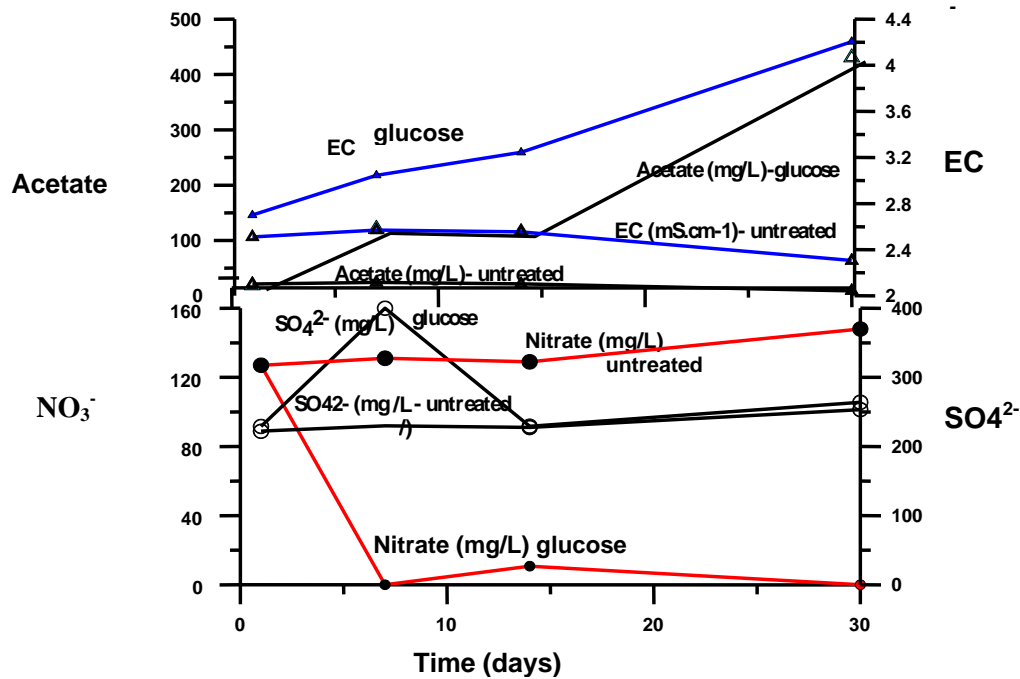
Figure 26:   The above figure displays results typical of adding an excess of readily available carbon source to groundwater with elevated nitrate concentration and 1,1m deep soil sample for denitrification compared to an untreated sample of the same composition.

Days 1-7: Denitrification is the dominant process. An increase in the sulfate, EC and acetate concentrations occurs here.

Days 7-14: Total removal of nitrate at day 7 occurs for this treatment (an excess of glucose in 1.1m soil). Nitrate concentration shows a small increase, while a decrease in the sulfate concentration occurs. A gradual increase in the EC occurs, while the acetate concentration remains the same.

Days 14-30: Remaining nitrate is removed from the sample, while the sulfate concentration levels out to a similar concentration range as the initial concentration in the sample. A large increase in the acetate concentration occurs, while EC also shows a steady increase here.

Samples that do not conform to this behaviour include:

- ▪ Sawdust in that no significant increase in sulfate concentration occurred (Figure 23);

- Methanol and sawdust had background levels of acetate, and
- Maize meal samples showed a considerable reduction in sulfate from day 14 to 30.

5.3.2 Effect of C:N ratios

Glucose and sawdust was selected as the most suitable carbon sources based on the results from the first experiment. Data collected during this experiment also occurs in Appendix C. Table 14 shows the % nitrate removed during the experimental work.

Table 14: Percentage of nitrate removed over the thirty day incubation period for glucose and sawdust treated soils using a mixture of 1.1m deep soils (10g), groundwater (40mL) and the carbon sources (different C:N ratios).

Treatment	Nitrate removed (percentage of initial concentration)			
	Day 1	Day 7	Day 14	Day 30
Glucose (C:N)	-	-	-	-
25:1	24.9	83.5	100	100
50:1	14.8	87.4	100	100
75:1	22.7	89.5	100	100
Sawdust (g/kg soil)	-	-	-	-
5; 12.6:1	0.46	54.8	59.7	92.7
10; 24:1	13.2	72.2	100	100
15; 34:1	15.8	84.5	100	100

Sulfate and acetate concentrations were plotted to compare the outcomes of this experiment with that of experiment 1 (5.2.1, 5.2.2, and 5.2.3). To have a greater comparison between sawdust and glucose as well as a better understanding of ions contributing to the increase in electrical conductivity seen in the first experiment, parameters analysed and plotted here include Ca, Mg, K, EC and pH.

The maximum acetate concentration detected for glucose treated samples is approximately 60mg/L (Figure 27). The 50:1 samples shows a constantly increasing acetate concentration with time, while 25:1 as well as the 75:1 samples shows an increase in acetate concentration between days 7 and 14 only. The 75:1 glucose ratio shows a levelling out of the curve as of day 14, while 25:1 shows a decrease in the acetate concentration for the same period.
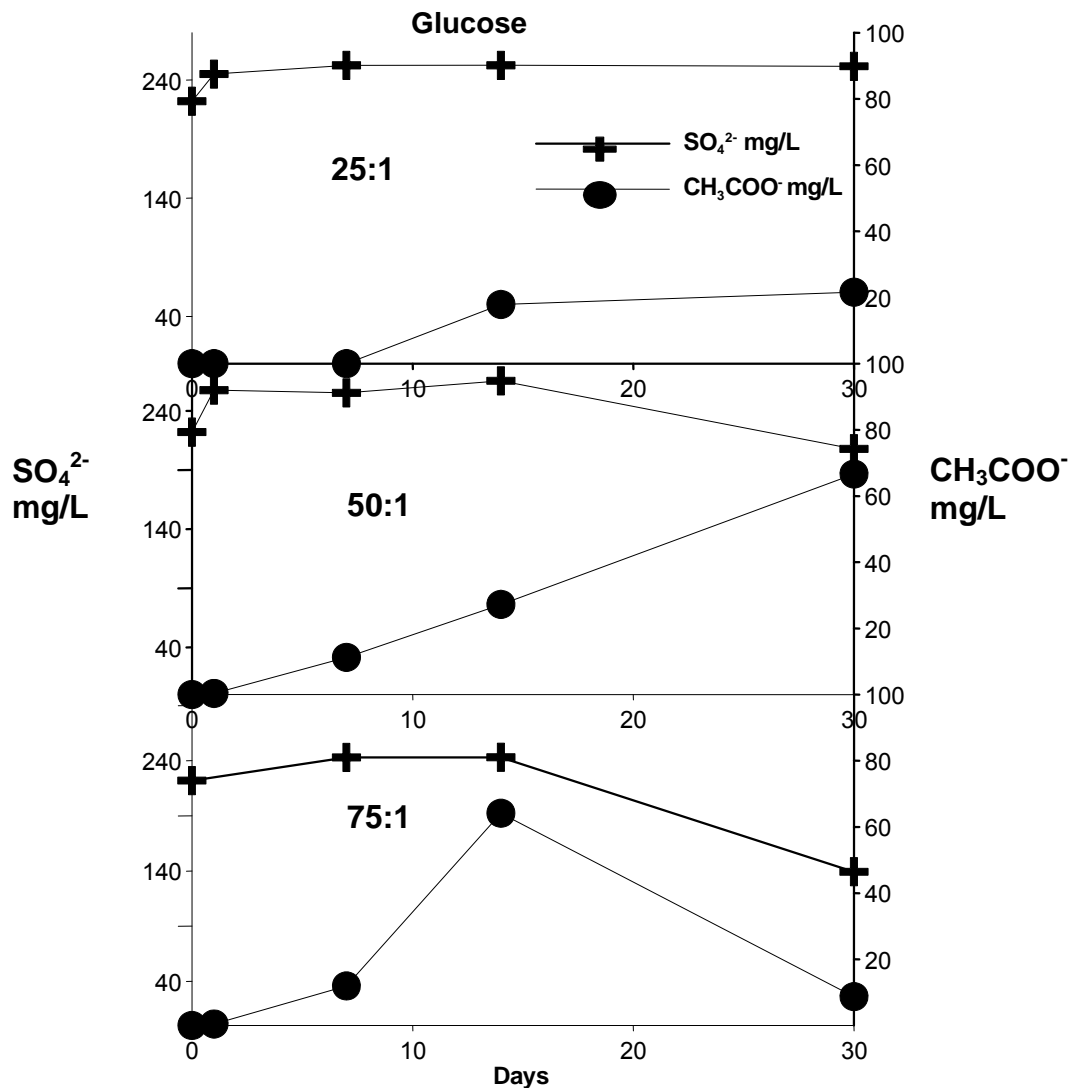
Figure 27: Sulfate and acetate concentration with time for glucose treated samples of varying C:N ratios recorded during a 30-day incubation experiment using 10g 1.1m deep soil, 40mL groundwater, and the respective C:N ratios. The top graph represents the 25:1 C:N, the middle represents the 50:1 C:N, and the bottom represents the 75:1 C:N for glucose. Where C is from carbon in glucose and N is from nitrate concentration in groundwater and soil.

Glucose treated samples, 25:1 C:N shows a stabilizing of the sulfate concentration at approximately 250mg/L, while higher C:N shows a decrease in the sulfate concentration up to day 30. Results of experiment 1 (section 5.2.2) (Figure 23) show that similar increase in sulfate concentration for glucose treated samples occurs for the first day in the 1.1m samples. Small reductions in the sulfate concentration are evident in the glucose samples, but not to the extent to which it was noted in the maize meal and glucose treated samples in experiment 1 (section 5.2.2). Figure 28 shows the sulfate and acetate concentrations over the 30 day period for sawdust treated samples.
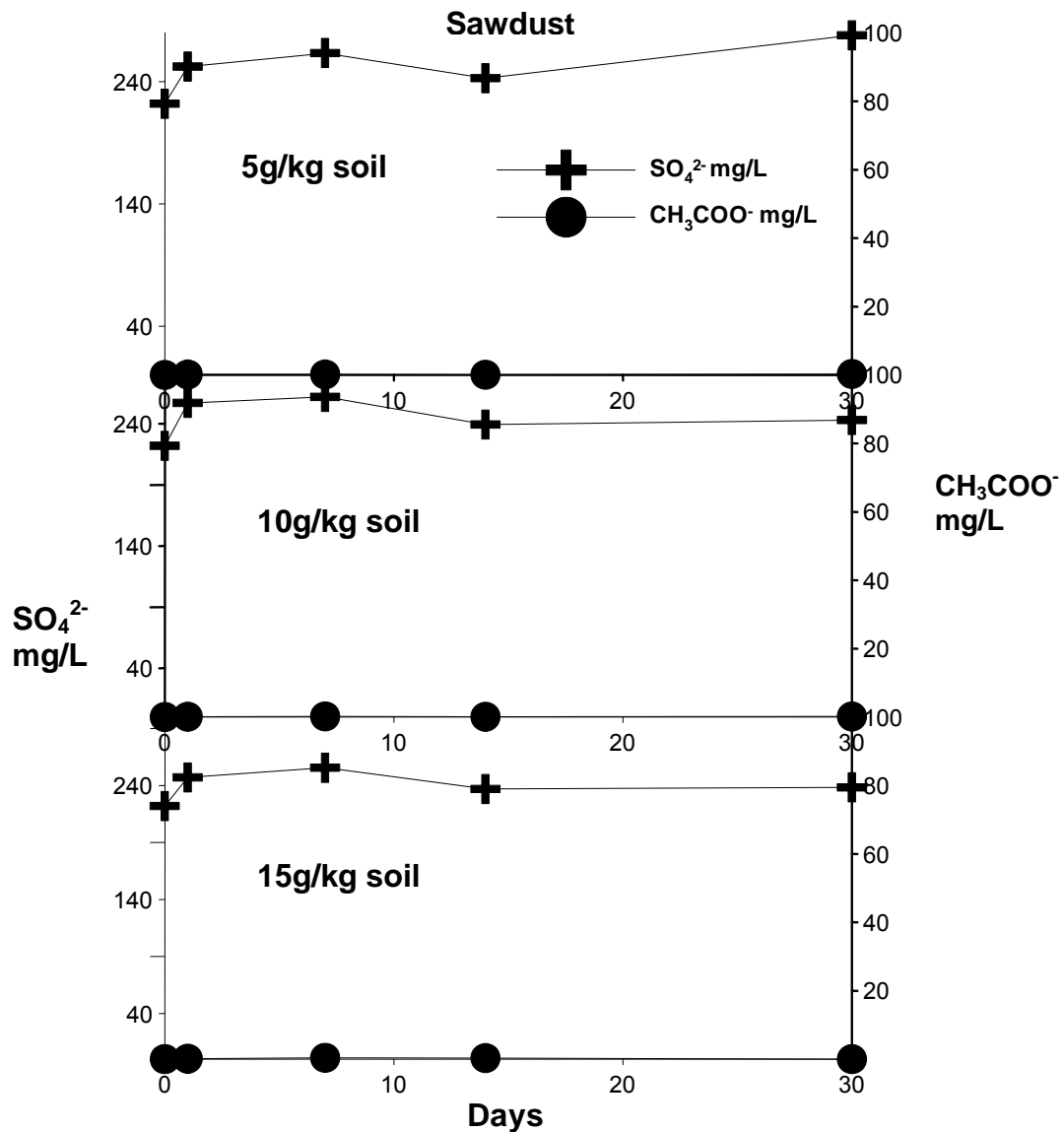
Figure 28: Sulfate and acetate behaviour with time for sawdust treated samples of varying C:N ratios recorded during a 30-day incubation experiment using 10g 1.1m deep soil, 40mL groundwater, and the respective C:N ratios incubation took place under a nitrogen atmosphere. The top graph represents 5g/kg of sawdust, the middle represents 10g/kg, and the bottom represents 15g/kg of sawdust, which is equivalent to the ratios of glucose used.

Changes in acetate concentrations for sawdust treated samples are almost negligible when compared to those produced in glucose treated samples and can be compared to background concentrations (Figures 27 and 28), sulfate concentrations are similar up to day 14 for glucose and sawdust treated samples. The solution pH change over the 30-day incubation period (Figure 29) was tested to evaluate whether the pH range remained within the acceptable pH for domestic water uses, as well as to get a better understanding and comparison of the pH value for the various treatments.
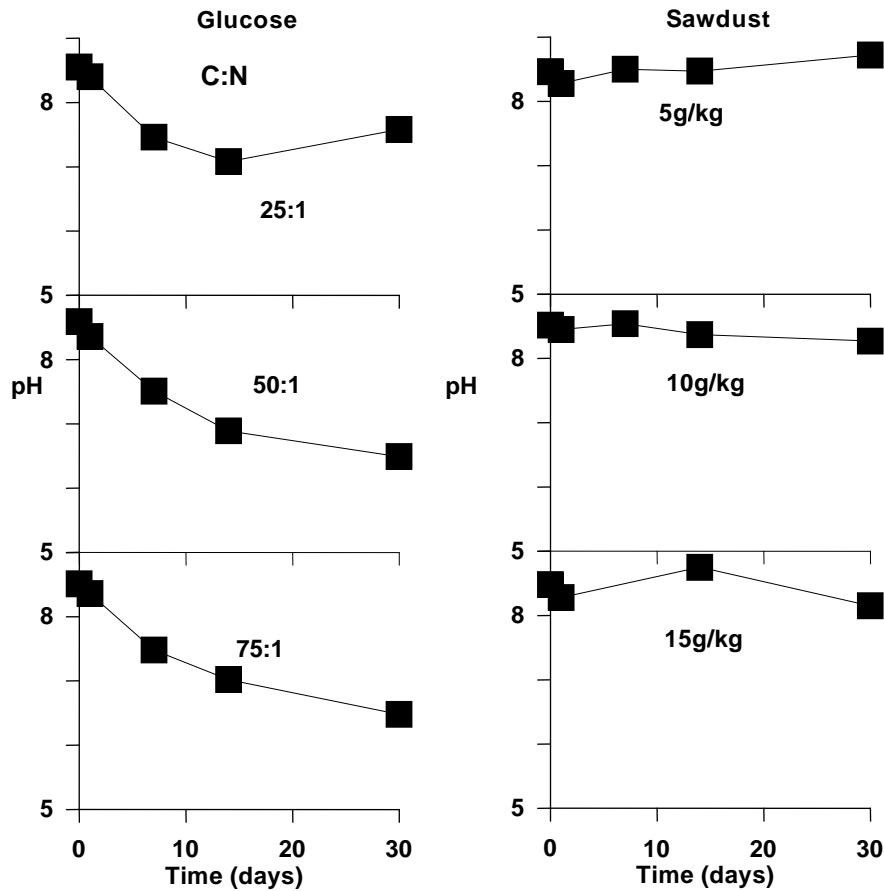
Figure 29: pH readings recorded with time for glucose and sawdust treated samples at varying C:N ratios. Mixtures contained varying C:N ratios, groundwater from the study area (40mL), and soil from the study area (10g) and were incubated under a nitrogen atmosphere.

The results show that for both glucose and sawdust treated samples of all C:N the pH remains within the target guideline value of 6-9 set by (Department of Water Affairs and Forestry, 1993 & 1996). The initial pH of the groundwater and soil mixture is just above 8 as can be seen in Figure 29. Most treatments affect an initial decrease in pH at day 1. Glucose treatment shows a steady decrease in the pH with time to below 7, while sawdust treatments show a stable pH range above 8. It is known that a decrease in pH causes many ions to be released into solution or replaced on exchange sites of soils. Hence, major cations have been plotted to evaluate the effects of pH on ion dissolution and the extent of dissolution. The 25:1 glucose treated sample shows an increase in pH after day 14, while the 50:1 and 75:1 ratios shows a continued decrease in pH. Electrical conductivity was also plotted, as a change in pH would affect dissolution of ions and an increase in EC is also to be expected for samples displaying a pH decrease. Figure 30 shows the EC for all treatments done in experiment 2. Examination of this figure reveals that pH and EC has an inverse relationship.
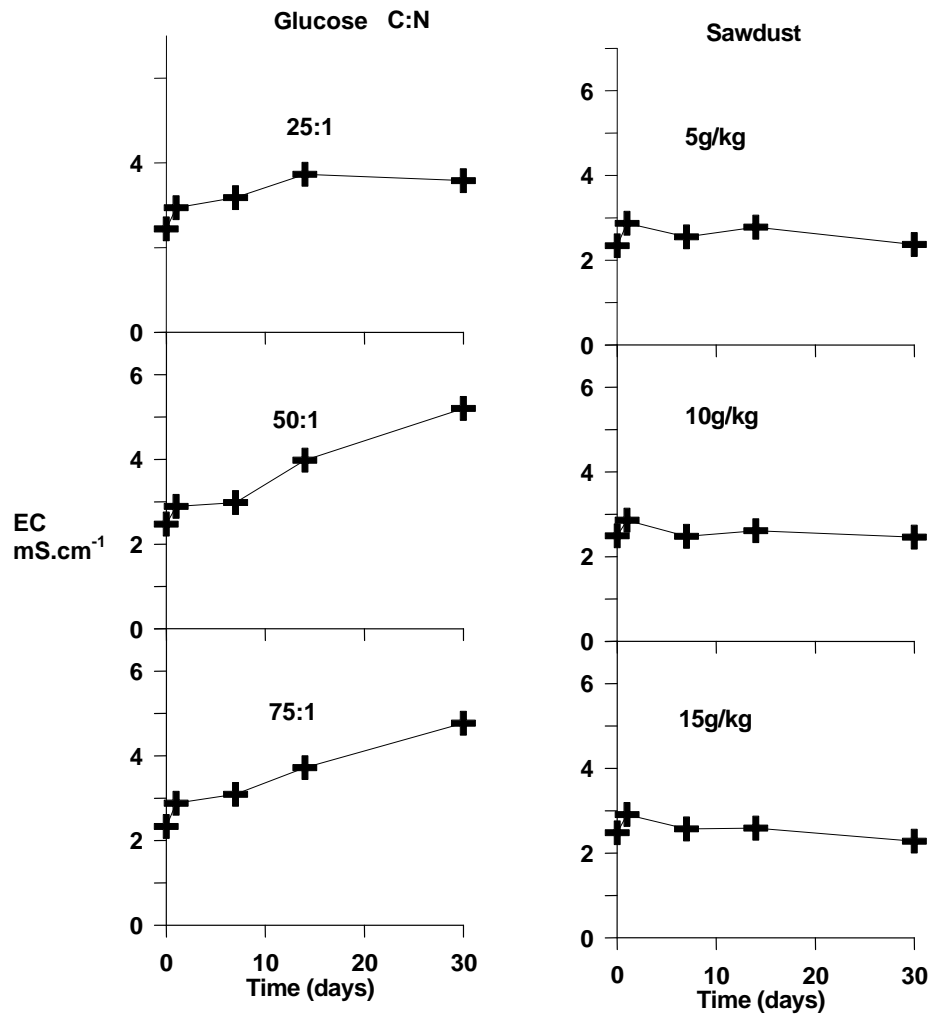
Figure 30: Electrical conductivity recorded for a 30-day incubation experiment using glucose and sawdust in different C:N ratios for denitrification. Mixtures contained varying C:N ratios, groundwater from the study area (40mL), and soil from the study area (10g) and were incubated under a nitrogen atmosphere.

This can be explained by the expected dissolution of ions into solution with changes in pH. Figures 31-33 shows the behaviour of Ca, Mg, and K respectively to assess the extent to which the pH change affected ion dissolution. Due to the change in EC, cations were considered to see which the major contributors to the high EC were.
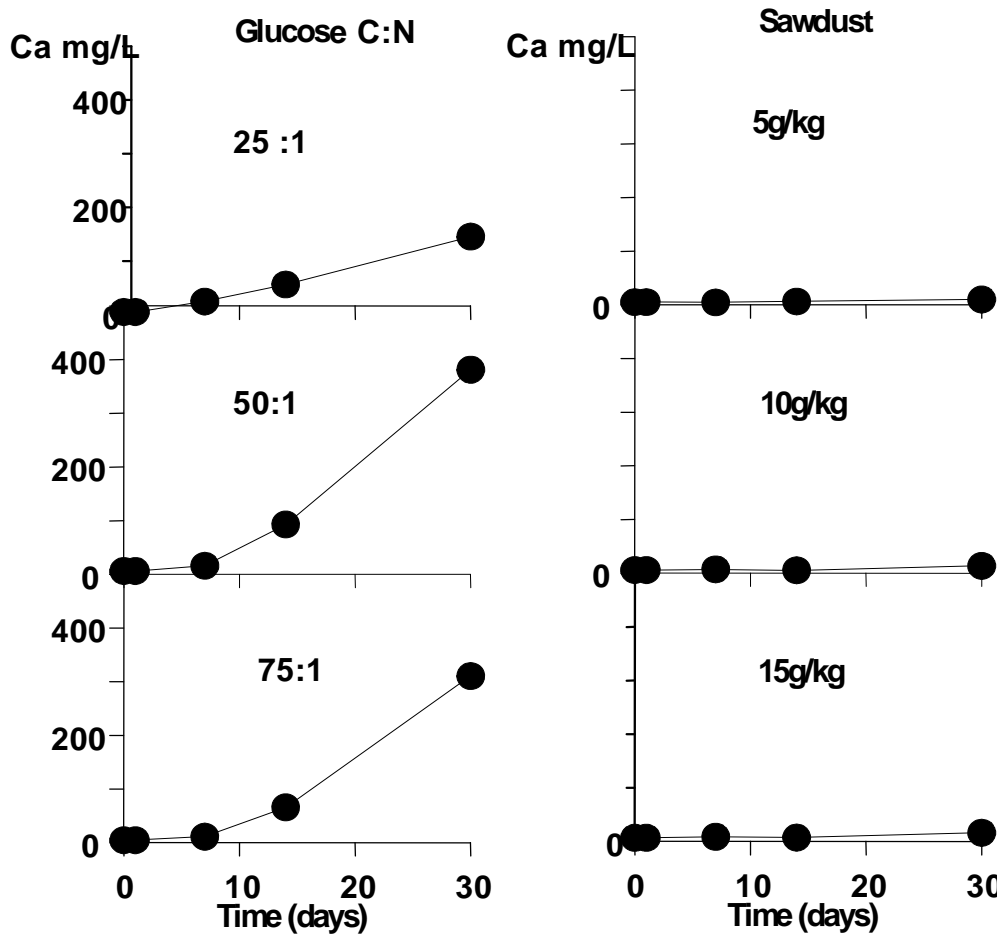
Figure 31:   Calcium concentration with time for a 30 day incubation experiment for glucose and sawdust treated samples of different C:N ratios. Mixtures contained varying C:N ratios, groundwater from the study area (40mL), and soil from the study area (10g) and were incubated under a nitrogen atmosphere.

EC and calcium curves show the same trend, and have an inverse relationship with the pH curve for each treatment. Calcium is thus a major contributing ion to the high EC. Here, the 50:1 and 75:1 glucose treatments show a huge increase in the calcium concentration, which may contribute to problems of $CaCO_3$ hardness and problems of scaling. The sawdust treatments show no marked increase in the calcium concentration.
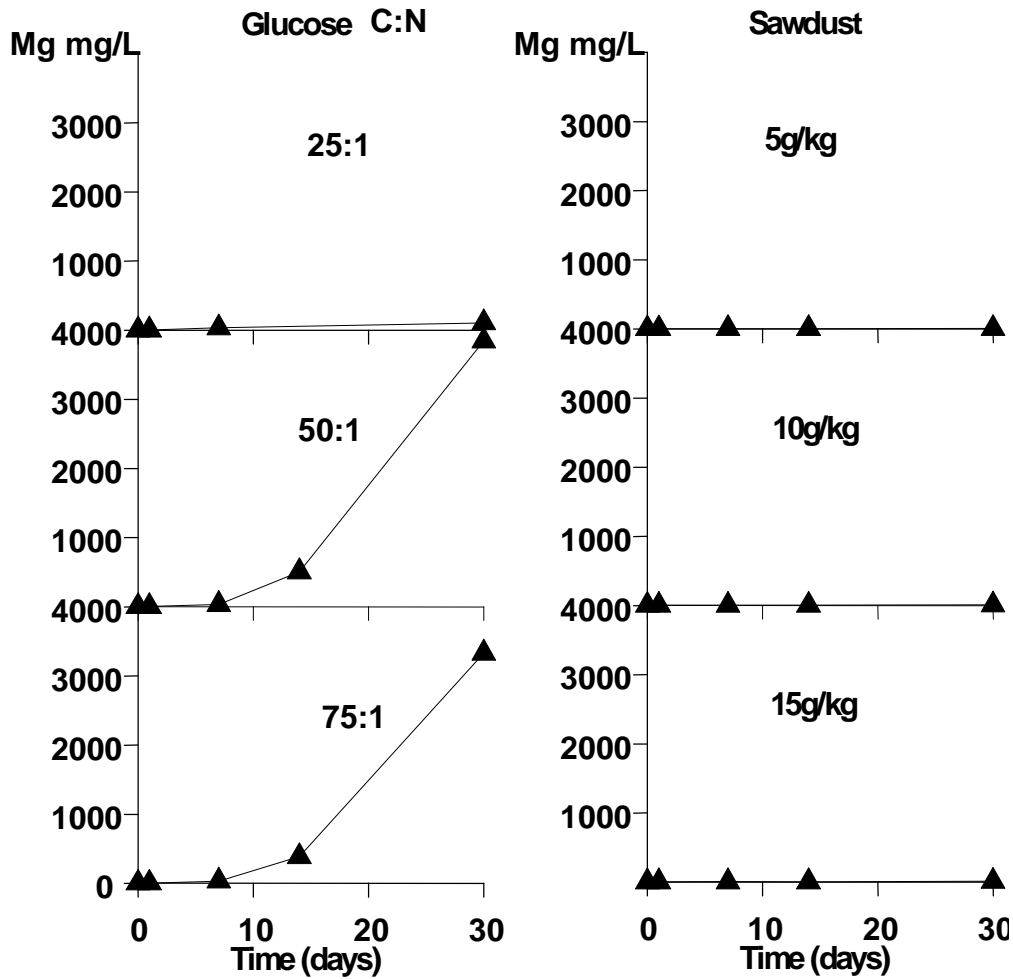
Figure 32: Magnesium concentration with time for a 30-day incubation experiment for glucose and sawdust treated samples at different C:N ratios. Mixtures contained varying C:N ratios, groundwater from the study area (40mL), and soil from the study area (10g) and were incubated under a nitrogen atmosphere.

Magnesium behaves similar to the calcium for all treatments. These excess concentrations of calcium and magnesium would contribute to alkalinity, hardness and scaling. Excess magnesium and sulfate concentrations may result in diahhroea. Potassium concentrations show different behaviour (Figure 33).
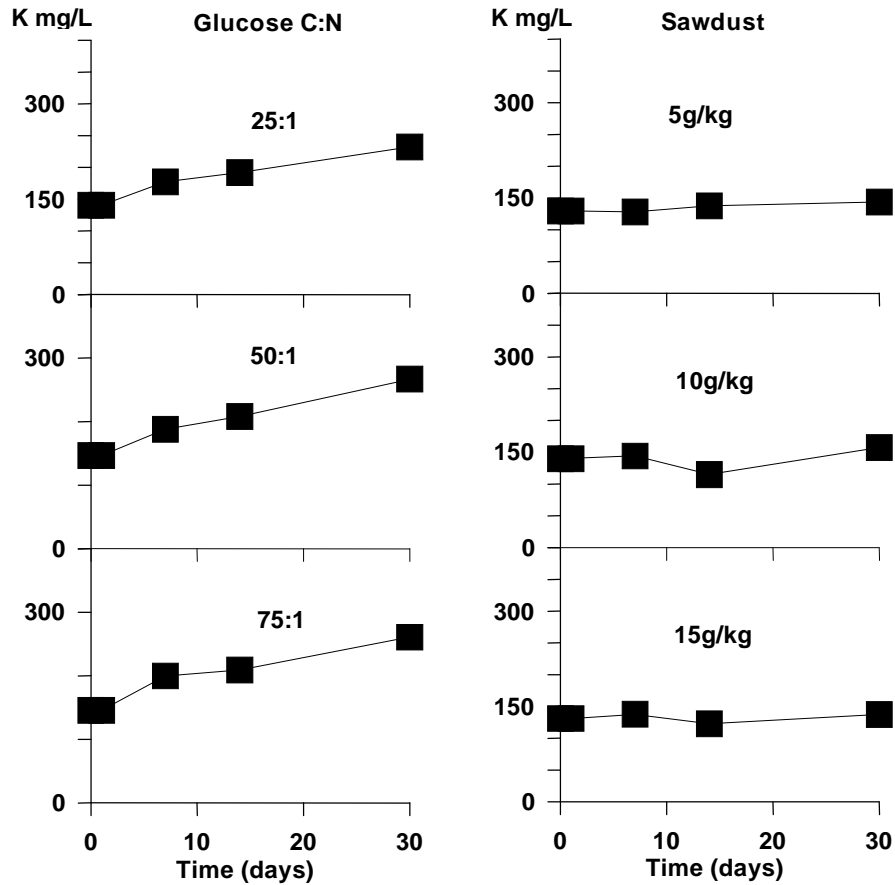
Figure 33: Potassium concentration with time for glucose and sawdust at different C:N treated samples in a 30-day incubation experiment. Mixtures contained varying C:N ratios, groundwater from the study area (40mL), and soil from the study area (10g) and were incubated under a nitrogen atmosphere.

Potassium has an initial solution concentration of about 140mg/L. Glucose samples show an increase in the concentration by about 100mg/L for all C:N ratios, while sawdust treated samples shows an increase of only 10 mg/L over the thirty day incubation period.

5.3.3 Sawdust as a substrate

Mixing of soil and groundwater analysed and discussed in Chapter 4 resulted in the following solution chemistry (table 15). Data collected during this experiment also occurs in Appendix C.

Table 15:   Indicator parameters after mixing of various amounts of sawdust, groundwater (40mL) and soil (10g) for laboratory testing of sawdust as a carbon source for denitrification

Treatment	untreated	12.6:1	24:1	34:1	untreated	12.6:1	24:1	34:1
Soil Depth		75-100				165-200		
Ammonia as N mg/L	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Alkalinity as $CaCO_3$ mg/L	322	345	346	345	328	328	342	328
Nitrate + nitrite as N mg/L	23.29	25.00	24.76	24.51	20.85	21.10	21.34	20.61
NO_3- N mg/L	23.29	25.00	24.76	24.51	20.85	21.10	21.34	20.61
NO_3 mg/L	103	111	110	109	92	93	95	91
Nitrite as N mg/L	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
D OC mg/L	2.6	10.7	13.4	17.2	3.2	7.7	18.3	27.8
Conductivity mS/m (25°C)	200	215	218	215	180	180	190	180
pH (Lab) (20°C)	8.4	8.4	8.4	8.3	8.2	8.2	8.4	8.0
HPC	2115	2165	2040	2620	5850	4600	4650	6250

Note that there is no ammonia and nitrite present at the start of the experiment. Nitrate concentrations are above the maximum allowable by Department of Water Affairs and Forestry drinking water standards (1996) and the WHO.

The pH is above that of the optimum range for denitrification described in the literature [see 2.2.5 and 3.4.4(a)], but considerably less than what it was for the soils. Dissolved organic carbon is directly proportional to the amount of sawdust added. This confirms that sawdust is the main contributor to the organic carbon present in the mixture.

Heterotrophic plate count is higher in the 165-200cm-soil depth mixture. Sodium, potassium, sulfate, nitrate, chloride, TDS and electrical conductivity is greater in the shallower soil while calcium and magnesium concentrations are greater in the deeper soil.

Nitrate-N, nitrite-N, DOC, and alkalinity levels were plotted for each treatment and for both soil depths for the 30-day incubation for which laboratory testing took place in Figures 34 and 35.
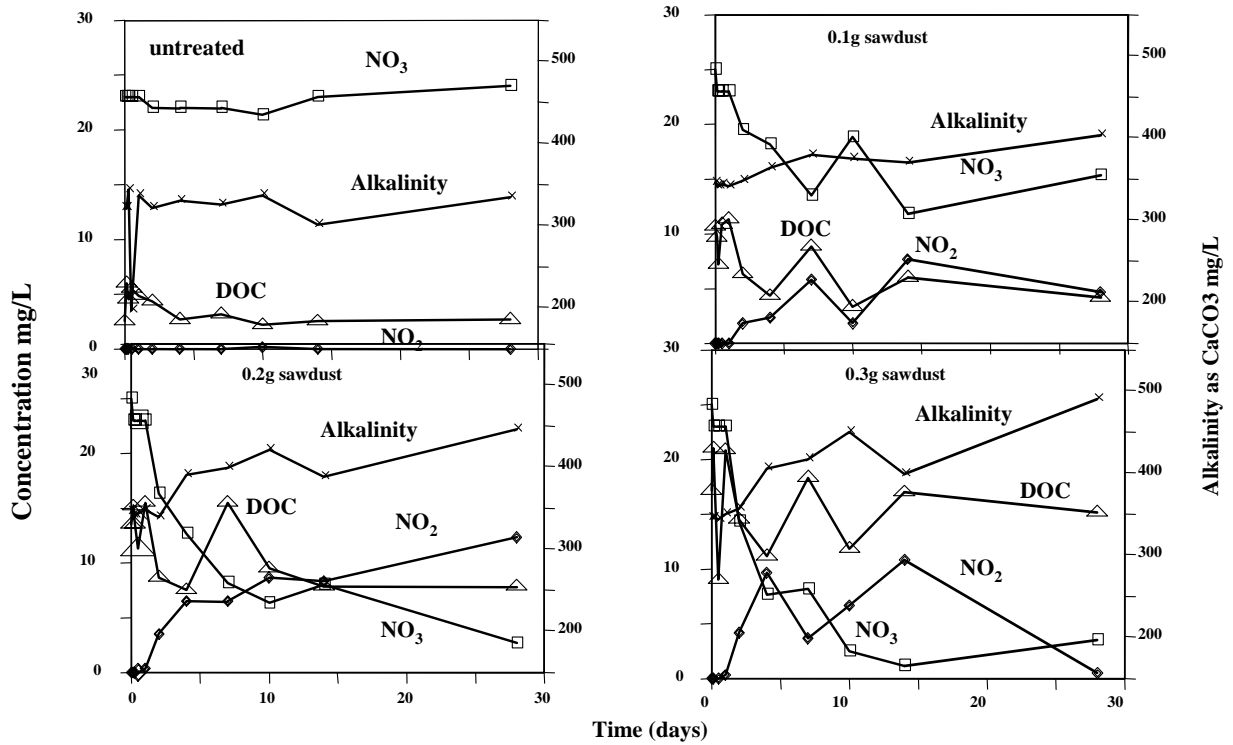
Figure 34: Concentration of nitrate-N, nitrite-N, dissolved organic carbon (DOC), and alkalinity as a function of incubation time using 40mL groundwater 10g soil (75-100cm layer) and various C:N ratios.

The general trend in the data is that the nitrate concentration decreases with time. The aim of the experiment was to reduce the nitrate concentration to within guideline values of below 20 mg/L as N for acceptable drinking water. All the treatments reduce the nitrate concentration to below the maximum allowable within the period of laboratory testing. Results for soil and groundwater mixtures for the 75-100cm deep soil show two of the treatments where nitrate concentration to within the acceptable level.

A similar graph was plotted for the 165-200cm soil depth in Figure 35 to evaluate whether there was any difference between the results.
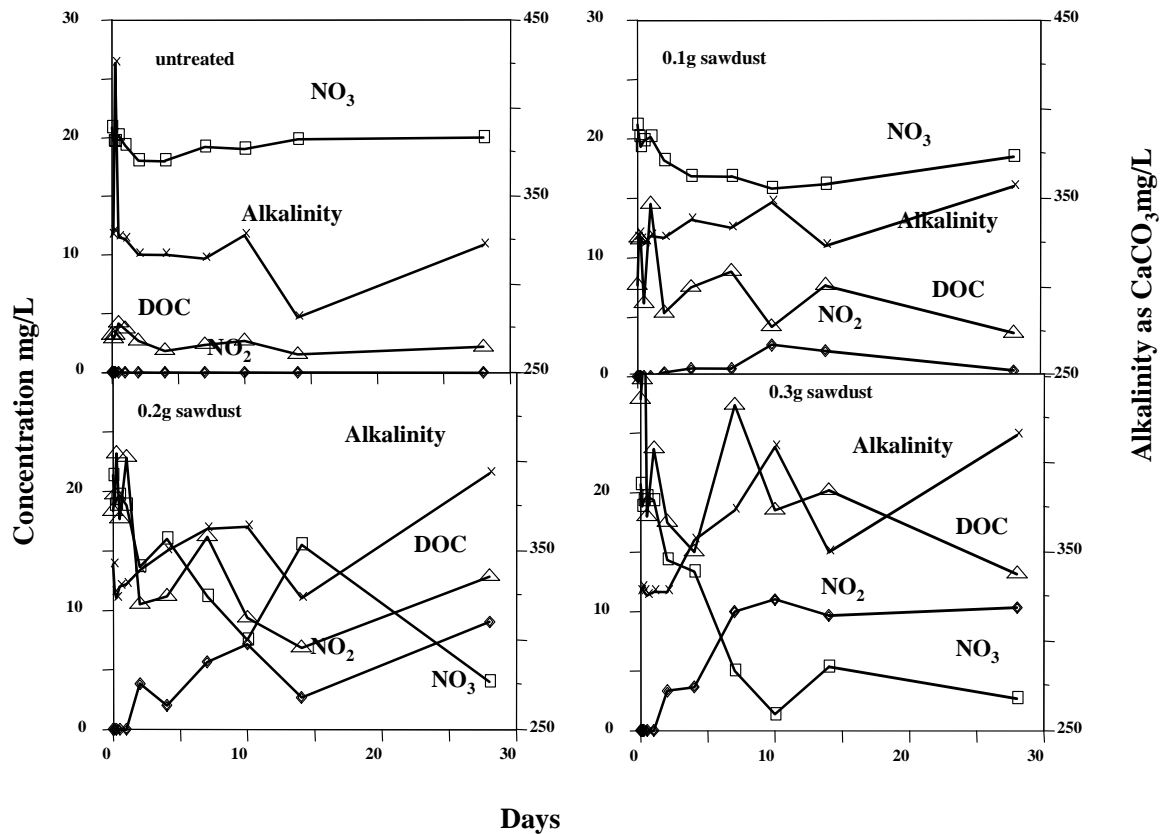
Figure 35:   Concentration of nitrate-N, nitrite-N, dissolved organic carbon (DOC), and alkalinity as a function of incubation time using 40mL groundwater 10g soil (165-200cm layer) and various C:N ratios. Samples were incubated under a nitrogen atmosphere.

The 165-200cm deep soil and groundwater mixture shows that the 24:1 (0.2g sawdust) and 34:1 (0.3g sawdust) C:N ratios reach a nitrate concentration within the acceptable levels for drinking water. The 75-100cm depth of soil and groundwater mixture seems to be more effective than the 165-200cm in reducing the nitrate concentration.

The 24:1 and 34:1 treatments resulted in a nitrate concentration within the acceptable levels for both soil depths. Dissolved organic carbon shows the amount of carbon available in the dissolved phase to take part in the denitrification reaction at any given time during the experiment. Nitrate and nitrite were plotted to see to what extent denitrification proceeded.

Although denitrification occurred and nitrate concentration reached levels within that of acceptable levels, it was found that for most treatments, nitrite was being produced. It increased as the nitrate concentration decreased. This was an indication of an incomplete reaction, and that nitrate was being reduced to nitrite. The levels of nitrite produced within the maximum allowable range of concentration for nitrite-N.

The 75-100cm soil treated samples showed a slightly different result (Figure 34). Although the graph shows an increase in the nitrate concentration for the last sample, it also shows that the nitrite was totally removed from the system. This production of nitrite and incomplete denitrification reaction could be owed to the limited availability of carbon. The dissolved organic carbon shows that availability of carbon fluctuates with time. The denitrification may have been limited by carbon as sawdust is a slowly releasing carbon substrate.

Since nitrite was produced in all treatments i.e. 12.6:1, 24:1, and 34:1 for both soil depths, nitrite vs. nitrate was plotted to see what the relationship between the two parameters was. The trend line in the figure represents the time series over the 30 day experiment. The optimum reaction would have no nitrite being produced and denitrification proceeding to produce nitrogen gas. In experiment 1 (5.2.2) nitrite was produced only at one time during the sawdust treated soil and groundwater mixture and was later not detected in the system again. This gave an indication that the nitrite being an intermediate of denitrification, was introduced, but later reacted to also form nitrogen gas (figure 36).
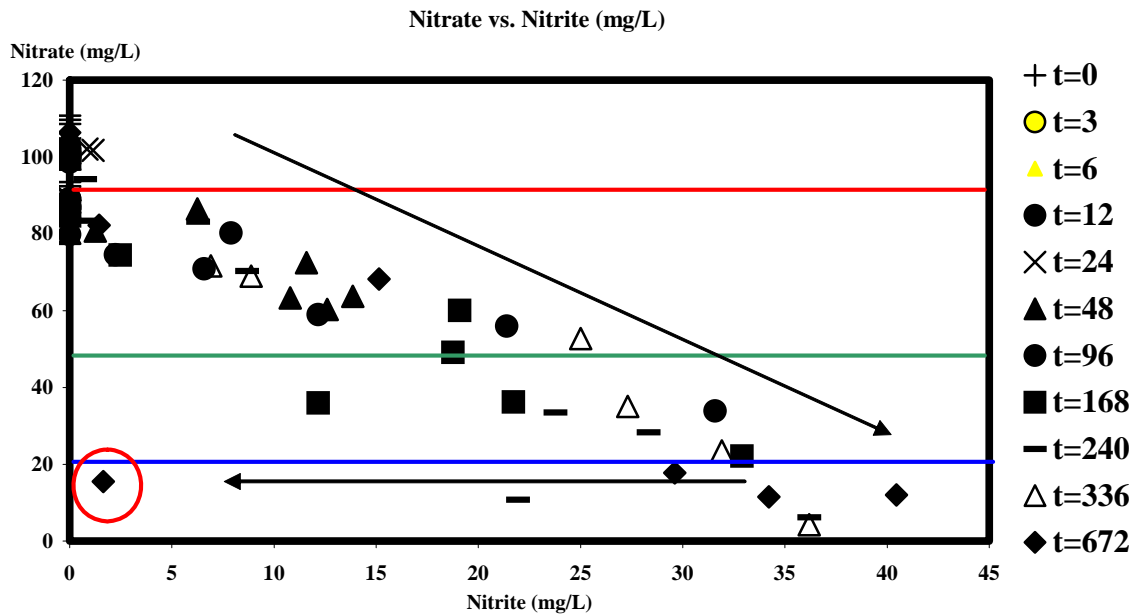
Figure 36: Nitrate against nitrite with data for each time allocated a different symbol according to the legend. This relationship persisted between nitrite and nitrate in most treatments. The bottom line represents the recommended nitrate levels in drinking water, while the middle horizontal line represents the maximum allowable by the WHO, and the top horizontal line represents the maximum allowable in South Africa. The arrows represent the time scale.

It is evident from the graph that nitrite increases or is produced while nitrate is reduced during the reaction. As nitrite is an intermediate of denitrification, one would expect it to come into solution where the reaction is incomplete, but to be removed from solution as the denitrification proceeds further.

The points in the upper left area of the graph represent the starting point of the experiment as well as the untreated samples' results. Only one treatment in the 75-100cm soil shows the reaction proceeding further and removing nitrite from the system. The circled point on the graph represents the ideal outcome of the experiment. With most of the nitrate removed i.e. nitrate levels within the acceptable range for drinking water, and nitrite levels approaching zero.

The experiment that follows shows that a longer incubation period and higher C:N ratios lead to total denitrification with both nitrate and nitrite removed (5.5.2 and 5.5.3).

The heterotrophic plate count was measured throughout the experiment to evaluate how quickly the denitrifying bacteria establish their colonies and whether there is a noticeable die-off in the curve. Figure 37 and Figure 38 represents the heterotrophic plate count for each treatment for every sampling time, samples that were analysed twice (that of t=48) are also included to highlight the importance of analysing samples within 24 hours of removal from incubator.

In general, biological growth occurs in phases, and one can expect any such curve to show a lag phase initially, a phase of exponential or logarithmic growth, a stationary phase and a die-off phase.

At t=0 all treatments show more or less similar number of colonies. A lag phase is present from t=0 to t=3. Major growth occurs between t=6 and t=24. After t=24, growth slows down and most of the treatments maintain similar numbers of colonies.
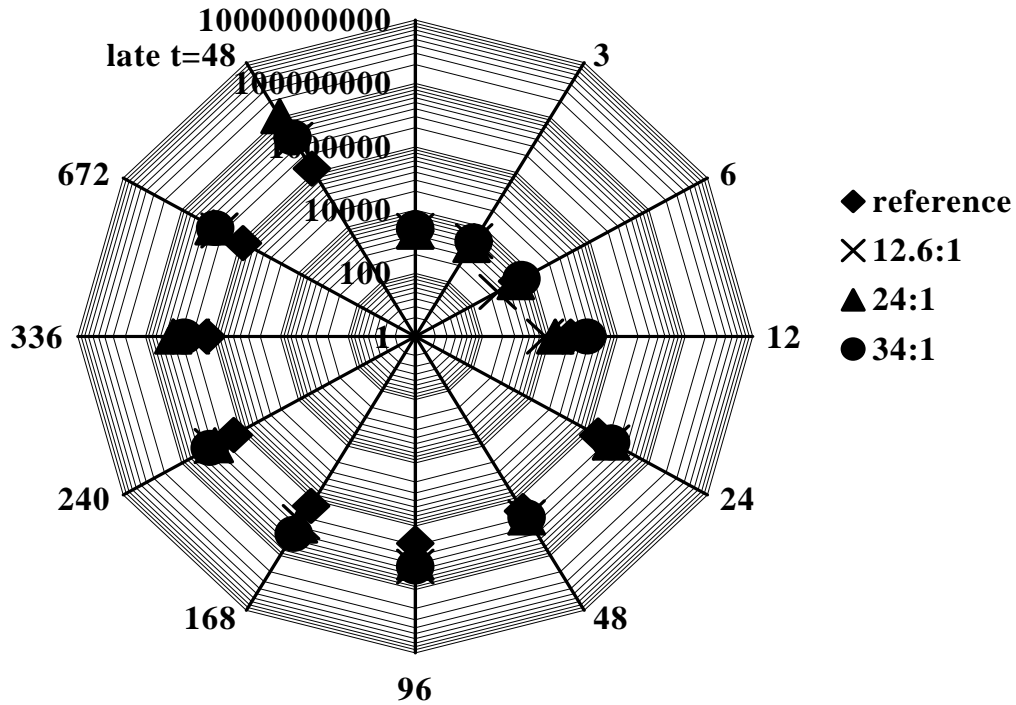
Figure 37:   Heterotrophic plate count data for 30 day denitrification incubation experiments of 75-100cm soil, 40mL groundwater and sawdust at various C:N ratios. Samples that were analysed later than the day of removal were placed in a refrigerator in order to slow down microbial growth.

The untreated and 0.1g sawdust treated in the 165-200cm soil do not conform. Instead it shows growth between t=6 and t=12 and then between t=24 and t=48. After t=48 most of the points occur within a band of values.
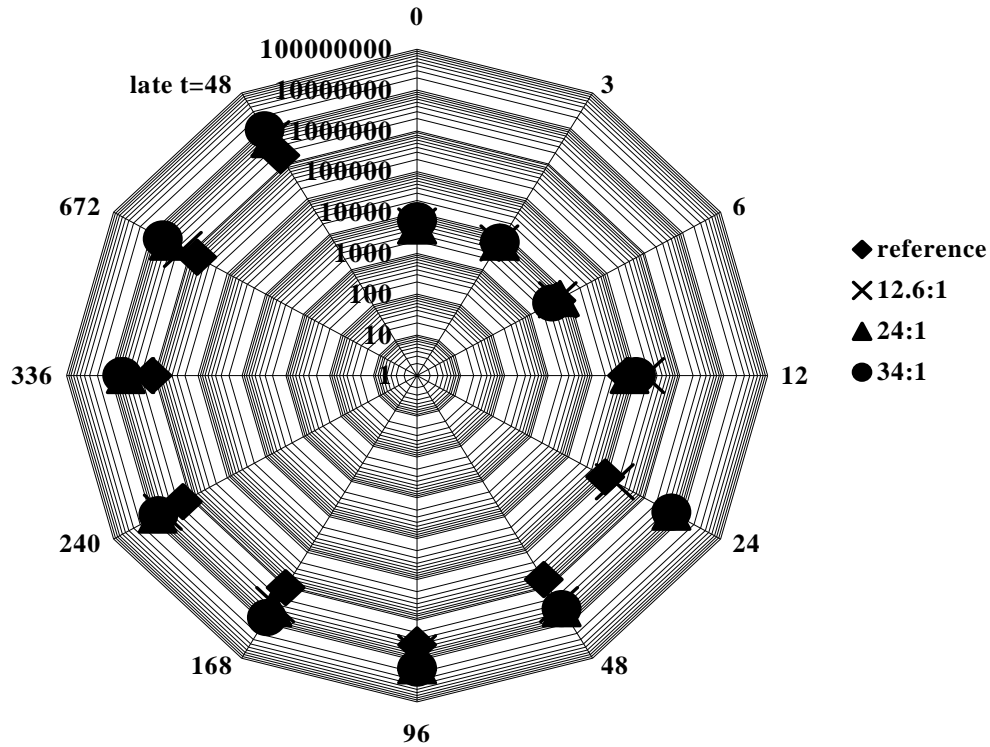
Figure 38:   Heterotrophic plate count for 165-200cm soils for 30 day denitrification incubation experiment using 40mL groundwater, 10g soil and sawdust at various C:N ratios. Samples that were analysed later than the day of removal were placed in a refrigerator in order to slow down microbial growth.

This shows that initial and final heterotrophic plate count data are similar for all treatments. The line late t=48 represents data recorded for samples that were analysed twice. This represents the data collected from samples t=48 when the samples were accidentally analysed a few days later. This results in an inconsistency between the two results. This is indicative of the sensitivity of the method of analyses to time and storage conditions. Establishment and growth of heterotrophic bacteria is an indication that the conditions in the system are indeed suitable for their growth and for denitrification to be facilitated by microbial colonies present.

To compare the results of the initial samples, the final water chemistry is tabulated in Table 16. These are compared to water quality guidelines for domestic use (drinking).

The bold values in the table represent the water quality guideline values for the marginal class. Ideal water quality was not used in this case as the initial water quality of the samples was already outside of the ideal class for some parameters.

Table 16: Chemistry data for samples analysed after the 30 day laboratory denitrification experiment, the 4 treatments on the left represent the 75-100cm soil source depth, while the next 4 represent the 165-200cm soil source depth

Treatment	Untreated	12.6:1	24:1	34:1	Untreated	12.6:1	24:1	34:1	Water Quality *Guidelines
K mg/L	86	91	90	89	72	72	72	74	**50-100**
Na mg/L	392	405	400	403	301	321	309	313	**200-400**
Ca mg/L	13	19	25	24	23	25	29	33	**150-300**
Mg mg/L	12	18	22	22	20	22	27	28	**100-200**
N mg/L	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	**0-1**
SO_4 mg/L	150	158	172	191	120	145	142	147	**400-600**
Cl mg/L	304	306	300	302	248	244	235	237	**200-600**
Alkalinity mg/L	335	403	445	491	322	357	393	415	
$NO_2^- + NO_3^-$ as N mg/L	24.0	20.0	15.0	4.0	20.0	19.0	13.0	13.0	**10-20**
NO_3 as N mg/L	24.0	15.4	2.7	3.5	20.0	18.6	4.0	2.6	**10-20**
NO_2 as N mg/L	<0.1	4.6	12.3	0.5	<0.1	0.44	9.0	10.4	**10-20**
Si mg/L	20	28	29	29	35	35	44	45	
DOC mg/L	2.6	4.1	7.9	15.1	2.2	3.6	12.8	13.1	**10-20**
EC mS/m (25°C)	211	217	220	221	168	183	180	185	**150-370**
pH (Lab) (20°C)	8.4	8.1	8	8.2	8.3	8	7.9	7.9	**<4 & >10**
TD S (Calc) mg/L	1350	1389	1408	1414	1075	1171	1152	1184	
HPC (cpm)	800000	5000000	8000000	7000000	620000	1500000	3500000	5000000	**>1000**

marginal water quality range: shows some effects on health of sensitive individuals, above this range water can be dangerous to consume.

Results of samples analysed after 30 days of incubation at 23ºC (the groundwater temperature measured in the field) are tabulated above. These have been compared to drinking water standards set by the Department of Water Affairs and Forestry, 1993 & 1996 (see last column in table). EC, chloride and sodium exceed that of target values for irrigation, while sodium and TDS exceed target values for stock watering. Nitrate levels have decreased to within the target range for the 24:1 and 34:1 nitrogen to carbon ratios. Nitrite levels in certain of the treatments have increased to within the maximum allowable range for some treatments. Only the 34:1 treatment using the 75-100cm-soil depth shows the nitrite levels approaching zero. Dissolved organic carbon is also within the range of marginal quality. The heterotrophic plate count is indicative of poor quality water.

5.3.4   Further investigation on sawdust in terms of C:N and incubation time

Data collected during this experiment also occurs in Appendix C. Overlay plots including NO_3^-, NO_2, Alkalinity, and DOC were prepared in JMP6 to have an overview of chemical species behaviour over time Figures 39 and 40.
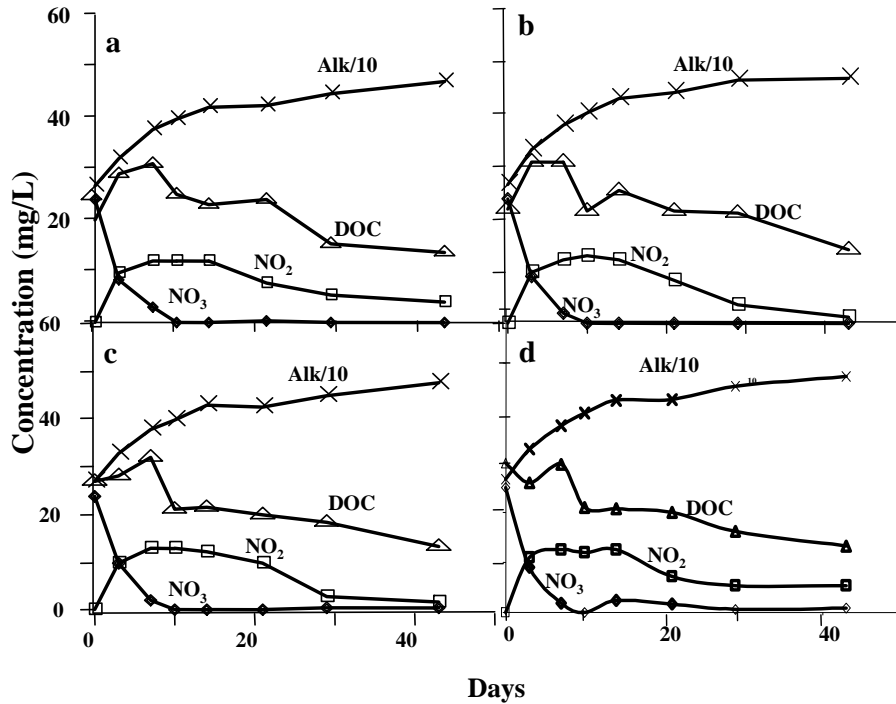
Figure 39:   Concentration of nitrate-N, nitrite-N, dissolved organic carbon (DOC), and alkalinity/10 as a function of incubation time using 40mL groundwater, with 10g soil (75-100cm layer) and 0.3g sawdust (a,b, and c: replicated experiments, d: same but with fine fraction of sawdust).

The denitrification and nitrite production with time was evaluated. The behaviour of all indicator parameters were evaluated over the 43 day incubation period. The 0.5g treated soil and groundwater mixture (Figure 40) shows similar results.
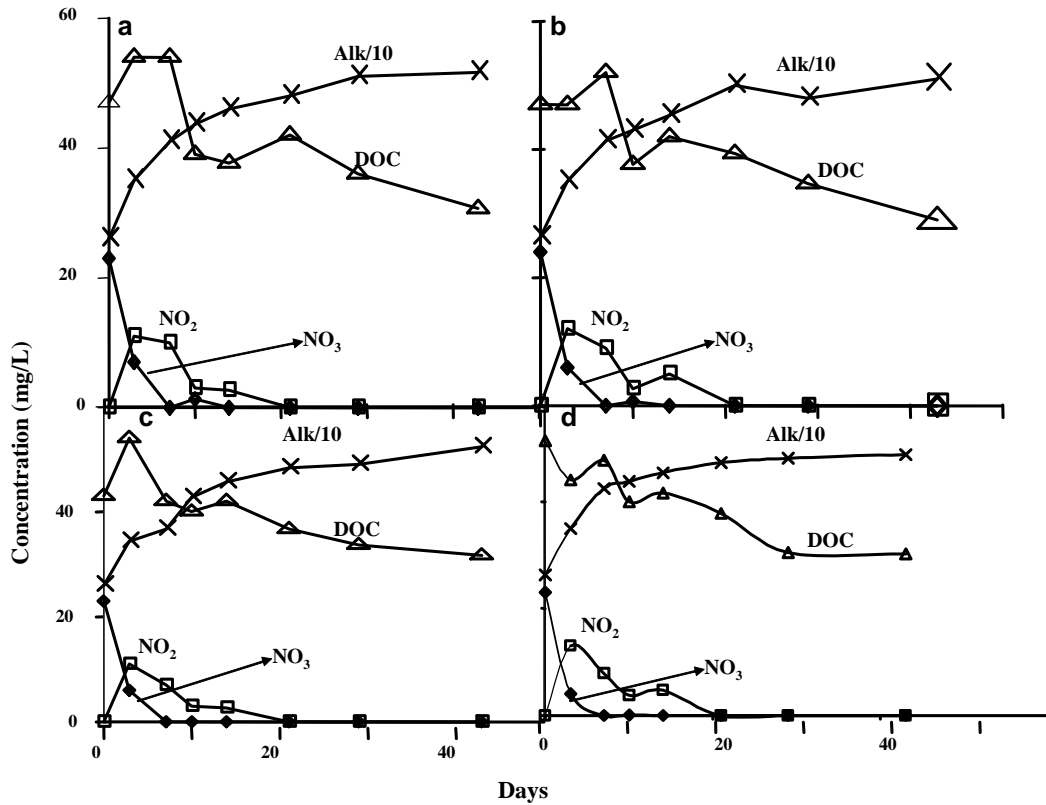
Figure 40: Concentration of nitrate-N, nitrite-N, dissolved organic carbon (DOC), and alkalinity/10 as a function of incubation time using 40mL groundwater, with 10g soil (75-100cm layer) and 0.5 g sawdust (a,b, and c: replicated experiments, d: same but with fine fraction of sawdust).

Figure 40 shows that the dissolved organic carbon (DOC) is generally higher for the 0.5 g than in the 0.3g sawdust treatment. Nitrate is completely removed by day 7, and nitrite is completely removed from day 21. All parameters show the same trend as in the 0.3g treatment, but the reaction rate is faster.

Statistical evaluation in the form of variability plots, box and whisker plots and correlation matrices were prepared (Appendix D). Full major cation and anion analyses were performed on initial samples, after ten days and after 43 days to evaluate the change in water chemistry and to compare parameter concentrations to that of the marginal water quality range for drinking water standards (Table 17) above which chemical species may induce hazards to human health.

Table 17: Water chemistry of samples at various stages of the denitrification experiment in comparison with target water quality (DWAF 1996). Water sample GW-Bh M23 was mixed with 75-100cm deep soil to yield the chemistry at day 0 for the 0.3 and 0.5g samples. The sequence in the table follows from initial water chemistry (on the left) to final water chemistry and water quality guidelines (on the right).

	GW- Bh M23 day 0	Average 0.3g day 0	Average 0.5g day 0	Average 0.3g day 10	Average 0.5g day 10	Average 0.3g day 43	Average 0.5g day 43	Domestic Use Maximum Allowable (Marginal) WQ Range
K	22	68.8	70.0	91.9	91.8	89.1	88.7	**50-100**
Na	215	375.0	368.8	408.3	409.6	399.4	399.4	**200-400**
Ca	57	8.1	8.5	17.4	18.0	22.1	23.7	**150-300**
Mg	50	13.5	13.5	15.5	15.7	21.4	25.5	**100-200**
NH_4 as N	ND	ND	ND	ND	ND	ND	ND	**0-1**
SO_4	138	164.3	169.3	201.7	204.6	210.0	207.0	**400-600**
Cl	229	290.0	289.8	304.5	305.7	286.3	289.3	**200-600**
Alkalinity	318	266.5	261.3	398.5	432.0	472.6	509.1	
NO_3+NO_2	18.6	24.3	23.3	12.5	3.6	3.5	0.0	**10-20**
NO_3	NA	24.3	23.3	0.0	0.5	0.4	0.0	**10-20**
NO_2	NA	0.0	0.0	12.5	3.2	3.2	0.0	**10-20**
Fe	0.09	0.1	0.1	NA	NA	NA	NA	**1.0-2.0**
DOC	<1.0	24.6	47.0	22.2	39.2	13.6	30.5	**10-20**
EC mS.cm^{-1}	173	203.3	202.3	217.8	217.3	225.8	227.3	**150-370**
pH	7.9	8.7	8.5	8.1	8.0	8.1	8.0	**<4 & >10**

Default unit mg/L, NA= not analysed, ND= not detected

Marginal in the above table refers to water quality within a range that may have some effects on health of sensitive individuals. Shaded parameters are above the guideline range and would occur in the poor or dangerous category of water quality.

Results of incubation over a longer period with larger C:N in Table 17 shows that DOC is the only parameter that is above the range of marginal water quality. Data from a previous experiment (Table 16) showed similar results and included the heterotrophic plate count, which was not analysed for during this experiment. Most parameters have more elevated concentrations after mixing groundwater, soil and sawdust. Major differences between the two C:N ratios are the amount of available carbon in the form of DOC, which affects the denitrification reaction rate. This and microbiological activity has bearing on the extent of production of alkalinity. The results confirm that the method is specific.

5.4 Discussion

5.4.1 Carbon substrate effects on denitrification

Denitrification occurred successfully in all samples by the end of the 30-day incubation period. Glucose treated samples had 79% of nitrate reduced by the seventh day of incubation in 0.5m soil, and 100% nitrate reduced in the 1.1m soil. Methanol treated samples had very little nitrate reduction up to day 7, only 9% nitrate reduction occurred in the 0.5m samples, while 88% of nitrate was removed in the 1.1m methanol treated sample by day 7 of incubation. The untreated samples showed a slight increase in the nitrate concentration, this could be owed to the presence of some oxygen in the soil being utilized for nitrification, or possible nitrogen fixation followed by subsequent mineralization and nitrification (Robertson *et al.*, 2003). The rate at which reactions occur is indicative of the presence of resident microbial communities and the necessary enzymes to catalyse them.

The initial slow reaction in the methanol treated sample could be owed to methanol's toxicity to certain denitrifying bacteria, and once a microbial community was established, this after day 7 denitrification proceeded at a greater rate. We can therefore say that it took 7 days for optimum conditions for denitrification to be established in the methanol treated samples. This compares favourably with research done by (Dodds and Fey, 1995), where no decline in nitrate concentration occurred over their ten-day incubation period of methanol treated samples and Nyberg *et al.* (1996) who said that the start-up of their experiment using methanol as a carbon source resulted in a lag adaptation period before full effect of the carbon source added was reached. They owed it to methanol's ability to destroy aerobic heterotrophs such that anaerobiosis could not occur and inhibition of methanol utilizing anaerobic populations. The results of this work differ in that after day 7 denitrification occurred.

Sawdust treatment was observed to have effected denitrification as of day 1, by day seven 97% of nitrate had been denitrified in the 0.5m soil, while 78% denitrification occurred in the 1.1m soil. This is followed by a slow rate up to day 30 where results show that no nitrate was detected in all sawdust treated samples. The initial high rate of denitrification may be due to the soluble organic constituents of the sawdust (i.e. tannic acids etc.) initially being utilized in the denitrification reaction, Robertson *et al.* (2003), followed by the less readily available organic carbon portion during the period from day 7 to day30. Schipper and Vojvodić-

Vuković (2000) and Beauchamp *et al.* (1989) contrasted readily available carbon sources such as ethanol and other soluble carbon substrates with sawdust and concluded that the effectiveness of soluble carbon compounds are likely to be short lived in comparison to particulate substrates such as sawdust.

Maize meal and glucose treated samples also undergo total nitrate reduction after day 7, followed again by a slow reaction rate. A slight increase in the nitrate concentration in the glucose (0.5m) samples and the maize meal (1.1m) samples can be owed to the denitrification reaction being nitrate limited; Robertson *et al.* (2003) and Schipper and Vojvodić-Vuković (2000) in their studies also concluded that the reactions were nitrate limited rather than carbon limited. This could be expected as excess carbon source was used in the experimental to ensure that the reaction proceeds and carbon availability or release from sawdust was slow.

Nitrite was detected only on day 7 in the glucose (0.5m), methanol (1.1m), sawdust (0.5 and 1.1m), and maize meal (1.1m) samples. This shows incomplete denitrification at this stage of the experiment. A further increase in NO_3^- concentration after day 7 for the above samples may be due to the occurrence of nitrification in these samples.

Day 7 also seems to signify the end of the dominance of denitrifying activity. The concentration of sulfate in solution during the denitrification activity was increased from day 1 to day7 in all the 1.1m deep samples. It appears that a starting point for sulfate reduction or a limiting sulfate concentration might play a role in this experiment. An increase in sulfate concentration occurs between days 1 and 7 which correlates exactly with the period during which denitrification is dominant. This can be explained by the possible presence of resident denitrifying bacteria capable of oxidising sulphides to sulfate (e.g. *Thiobacillus denitrificans*) (Krumbein, 1983). The sulfate concentration reaches a maximum when nitrate concentration reaches its minimum; this enables sulfate-reducing bacteria to become dominant. Days 7 to 14 in Figure 23 show a marked decrease in the sulfate concentration for methanol, glucose and maize meal treated samples in the 1.1m soil/groundwater solution. The methanol 0.5m samples also do not show any signs of sulfur oxidation or reduction, this could be owed to the fact that the rate of denitrification in these samples is slower than that of the 1.1m samples and other treatments, and the fact that the conditions in this sample are still optimum for a gradual denitrification reaction. Methanol treated samples containing soil of a 1.1m depth does not seem to conform entirely to the idea that denitrifying bacteria capable

of oxidizing SO_4^{2-} are present, as a small decrease in the NO_3^- concentration occurs within the first 7 days. Other samples show similar rates of denitrification and sulfur oxidation. The production of H_2S from sulfate reduction during these treatments may prove harmful or toxic to aerobic organisms in a field situation (Krumbein, 1983). The occurrence of sulfate reduction after total denitrification is consistent with experiments done by Robertson *et al.* (2003) working with wood based filters. According to Robertson *et al.* (2003) the BOD also increases because of sulfate reduction; this parameter was not measured during this experiment.

Furthermore, at the onset of sulfate reduction, an unusually large peak registering as fluoride was detected using ion chromatography. This appeared unusual as no known source of fluoride occurred in the soil or groundwater. A sample of acetate was then run as the peak retention was similar to that of fluoride, and possible interference could have occurred. Results showed that it was indeed acetate and not fluoride. Acetate was then measured at every sampling period. Figure 24 shows the acetate concentration with time for the different treatments. The acetate levels of methanol and sawdust treated samples were similar to background levels and hence negligible as a product of any processes. Glucose and maize meal treated samples showed up to 430mg/L in the 1.1m glucose samples, 402mg/L in the 0.5m glucose samples, 340mg/L in the 0.5m maize meal samples, 297mg/L on day 14 in the 1.1m maize meal samples and 236mg/L in the 1.1m maize meal sample on day 30. This increase in acetate concentration may be a result of sulfate reducing bacteria reacting with residual carbon after denitrification is complete to reduce SO_4^{2-} and produce acetate as a by-product. A possible mechanism is shown here, an example of a starting organic material is lactate:

$$CH_3OCHOCOO- + SO_4^{2-} \longrightarrow 2CH_3CHOO-+2HCO_3^-+H_2S \qquad \text{Equation 13}$$

Acetate is produced due to incomplete oxidation of the organic material, (Krumbein, 1983). Methanol and sawdust react differently because methanol is already a single carbon containing compound, and sawdust is a slowly degradable carbon substrate, hence a slow reaction. The reference sample is untreated and hence one would not expect the formation of acetate as reagents in the reaction vessel are not sufficient to form acetate. The bacteria mentioned as affecting such reactions are designated desulfovibrio (Krumbein, 1983). It is also mentioned that this process introduces HCO_3^- to solution.

Electrical conductivity measurements were taken with each sampling episode and the following became evident:

- The processes of nitrate reduction and sulfur oxidation increased the EC;
- Acetate production also had an increasing effect on EC;
- Samples in which sulfate reduction did not occur experienced a stable EC i.e. methanol and sawdust treated.

The first two processes mentioned introduced a large amount of ions into solution, SO_4^{2-} and intermediates to denitrification. Acetate being a negatively charged ion also contributed to the EC of the solution. Methanol and sawdust treated samples, which show a steady decrease in EC values may be due to a decrease of ions in solution and an increase of gaseous phase e.g. N_2 (g), H_2S (g), CO_2 (g).

Two carbon sources were selected for further laboratory experiments based on the results of experiment 1. These were glucose and sawdust. Maize meal was ruled out as a suitable source due to sulfate and acetate production as well as sulfate reduction, which were not desired. The chemical make up of maize meal is complex and a variety of side reactions may result. Compounds like vitamins and iron have to be accounted for. Methanol was ruled out due to its initial delay in reaction and probable toxicity to certain naturally available microbes/bacterial strains capable of denitrification. Sawdust was selected as it showed little or no sign of side reactions except for production of some nitrite at day 7. Acetate produced for the sawdust treatment was comparable to that of the untreated samples. Glucose was selected on the grounds that the denitrification started almost immediately, and although there was a considerable amount of acetate produced, it is believed to be related to the amount of glucose used and the C:N in the sample.

5.4.2 Effect of C:N ratios

Glucose and sawdust were used in this experiment at different ratios of C:N to evaluate the difference or significance in the results at different C:N. Chemical parameters measured include pH, EC, Na^+, Ca^{2+}, K^+, Mg^{2+}, SO_4^{2-}, NO_3^-, CH_3COO^-, NO_2^-, Cl^- at intervals during a 30-day experiment. Samples were run in a nitrogen atmosphere to try to keep any oxygen from entering the sample containers. Special care was taken to properly seal all containers before placing them under a nitrogen atmosphere.

Analyses of nitrate concentration with time showed that there was not a clearly different rate of removal in the glucose samples, it was noted that analysing samples between days 1 and 7 might have revealed a different or more presentable result. The typical sequence of reduction processes in flooded soil as observed in silty clay amended with rice straw and incubated in suspension without oxygen is demonstrated by Turner and Patrick (1968) and shows that nitrate is already removed by 2.5 days. The depletion of nitrate begins shortly before oxygen disappears.

Although not plotted, the production of nitrite did occur during the experiment. Glucose treatment only produced nitrite on the first day, after which it was again consumed or converted to other species of nitrogen. The sawdust samples produced nitrite at a later stage of the experiment. Nitrite is detected up to day 7 in the samples where greater amounts of sawdust are used. The 5g/kg sawdust treatment has detectable concentrations of nitrite up to day 30. This may be linked to the availability of carbon and the ability of resident bacteria to facilitate the complete denitrification, (Schipper and Vojvodić-Vuković, 2000 & 2001; Beauchamp *et al.*, 1989; Robertson and Cherry, 1995; and Robertson *et al.*, 2000 & 2003). As nitrite is less stable than nitrate, it is most likely to react very quickly after it is formed, unless there is insufficient available carbon to provide the energy required for the reaction. This is demonstrated in the experiment by nitrite only being detected at one stage of the experiment.

Sulfate and acetate were analysed for, as it proved undesirable by-products of using an excess amount of carbon source, side reactions occurring or by-products of microbial activity. The results showed that acetate levels in the glucose treated samples were an order of magnitude less than that for the glucose treatments in experiment 1 (5.2.2 and 5.2.3). Sulfate concentrations have a similar initial increasing trend as in experiment 1 for all glucose treatments; however, after day 7 there is a slight difference in the reactions in the three treatments. The 25:1 glucose ratio shows a larger decrease in the sulfate concentration than the other C:N ratios, as of day 14 to day 30 the sulfate concentration is halved. The initial oxidation of S in the soil or in solution causes a release of H^+ ions, which would relate to a decrease in pH, while the reduction of sulfate to H_2S would lead to the production of bicarbonate (Mc Bride, 1994). This relates to a pH buffering effect. This is evident if one compares the pH diagrams for these three glucose treatments. The 25:1 ratio shows an increase in pH after day 14, while the 50:1 and 75:1 show a steady decrease in pH.

Acetate and sulfate concentrations for sawdust treated samples are almost negligible when compared to those produced in glucose treated samples and can be compared to background concentrations.

Measurements of pH showed that for the sawdust treated samples pH remained between 8 and 9 throughout the experiment, while for glucose treated samples, the 50:1 and 75:1 ratios of glucose treatment showed a constant decrease in pH from the initial pH above 8, down to just above 6. The 25:1 glucose treatment only showed a decrease in pH up to the 14th day after which an increase in pH occurs.

Many naturally occurring processes contribute to the pH in the soil and groundwater environments. Some of the processes that increase the acidity of the solution include (Firestone, 1982;Mc Bride, 1994; Drever, 1997; and Canter, 1997):

- Nitrification- generates acidity;
- CO_2 released during denitrification and other reactions- generates carbonic acid induced by soil biological activity;
- Acidification may be due to metabolic activity of roots, microorganisms, production of organic acids from break down of organic compounds. Metabolic processes generate CO_2, soluble organic acids, and acidic soil residues, all of which behave like weak acids, displacing base cations from exchange sites. These mechanisms are only important and in operation when pH exceeds 5;
- Oxidation of sulphide particles- releases H+ into solution, acidification is immediate and extreme when anaerobic sulphide bearing soils are drained.

The above processes lead to the base cations being replaced on exchange sites and increases or decreases in pH and thus release of base cations into solution.

Some of the processes that act as buffering processes include (Firestone, 1982; Mc Bride, 1994; Drever, 1997; and Canter, 1997):

- Reduction of SO_4^{2-}, releases alkalinity (HCO_3^-) into solution. This could serve as a buffer of solution pH;
- Biological denitrification which consumes acidity.

Electrical conductivity has an inverse relationship with pH. This is owed to the release of ions into solution as the pH changes.

Cations analysed showed that, with a decrease in pH, Ca and Mg concentrations increased by orders of magnitude, while potassium did not show the same dramatic increase. This could be owed to the fact that, higher electrolyte concentrations favour K^+ and Na^+ adsorption on exchange sites (Mc Bride, 1994).

5.4.3   Sawdust as a substrate

Different C:N ratios of sawdust were used as a substrate for denitrification. Soil and groundwater from the study area (Chapter 4 for groundwater and soil characterization) was mixed with the sawdust as determined by calculation of the required amounts to ensure that the C:N includes all carbon and nitrogen present in the soil, groundwater and sawdust. Initial sample analyses (Table 15) show that mixing soils and groundwater with sawdust result in pH values of above the optimum range for denitrification of 7-8 specified in the literature. Heterotrophic plate count is higher in the deeper soil initially. The nitrate concentration is greater in the mixture containing the shallower soil depth. The initial analyses reveal a direct proportionality between the dissolved organic carbon in solution and the ratio of C:N. Potassium, sodium, sulfate, chloride, EC, nitrate and TDS concentrations all exceed that of the target ranges set out by DWAF (1993 & 1996) but fall within the range of marginal quality for the initial samples. This differs from the original water sample in that the concentrations are elevated; however of the parameters mentioned above, only sulfate was within the target range for the water sample. Most literature reviewed are from countries outside of South Africa, and water quality guidelines used in literature were mostly WHO, where comparisons were made. In most cases, South Africa's guideline values exceed that of the World health organisation's.

Most of the cations and anions have higher concentrations in the mixture due to dissolution of ions from the soil. The resultant pH is between 8 and 8.5, while the soil pH was 9.2 and 9.5 respectively and the water pH was 7,8. The target DOC set by (DWAF, 1998) of 5 mg C/L is also exceeded.

Sawdust was selected on the grounds that in previous experiments sulfate reduction or production did not occur and no acetate was produced (5.2.2 and 5.3.2). Nitrite production

was only detected once during each treatment and was later removed form the system (5.4.2). The results of sawdust analyses show that it contained more than 50% carbon and a small percentage of nitrogen. The presence of trace metals is believed to enhance denitrification (Table 10). Sawdust is a slowly degrading carbon source and would most likely initially release the soluble organic constituents of the sawdust (i.e. tannic acids etc.), followed by the less readily available organic carbon portion,a similar statement is made by Robertson and Cherry (1995) and Robertson *et al.* (2000 & 2003).

Overlay plots of nitrate, nitrite, alkalinity/10 and DOC (Figures 34 and 35) for each soil depth shows that the denitrification occurs at a greater rate in the 75-100cm soils than in the 165-200cm soils. This could be linked to the initial nitrate concentration in the 75-100cm-soil being greater than that in the 165-200cm-soil, groundwater and sawdust mixtures. Here one should consider Le Chatelier's principle that if more reactants are present, the forward reaction will be favoured. If one considers that the amount of sawdust was identical for identical treatments in the two soil depth mixtures, then the nitrate concentration would be the only contributing factor in the denitrification reaction that was different. This is in agreement with findings by Greben and Tjatji (2004) and Mohn *et al.* (2000) who both said that higher initial nitrate levels or input yields a higher denitrification rate if all other variables are similar. Schipper and Vojvodić-Vuković, 2000 & 2001; Beauchamp *et al.*, 1989; Robertson and Cherry, 1995; and Robertson *et al.*, 2000 & 2003, also mention that dewnitrification rates are higher where nitrate inputs are higher, and that micro-organisms can rapidly respond to changes in nitrate inputs.

The nitrate concentration is reduced to concentration lower than the maximum allowable for South Africa in all treatments. The 24:1 and 34:1 treatments in both soils effect denitrification to concentrations below or within the acceptable nitrate concentration of 6mg/L as N. It is evident in both figures that DOC is consumed and produced slowly during the experiment. This can be explained by the fact that sawdust is slowly degradable and that not all the organic carbon in sawdust is available at once, this compares favourably with many authors (Schipper and Vojvodić-Vuković, 2000 & 2001; Beauchamp *et al.*, 1989; Robertson and Cherry, 1995; and Robertson *et al.*, 2000 & 2003; Bates and Spalding, 1998). Nitrate concentration is reduced for all treatments (compare Table 15 and Table 16), while nitrite production during the experiment is an indication of incomplete denitrification Hunter (2003) attributed the production of nitrite during denitrification not only to carbon limitation, but also to phosphate limitation. This can be attributed to the limited available carbon during the

reaction, bare in mind that the DOC is directly proportional to the C:N ratio, so smaller C:N ratios would have less carbon available at any time.

The aim of the experiment was to reduce nitrate concentrations to a minimum or to acceptable levels while minimizing the amount of side reaction and by-products present in solution. This is achieved in the 34:1 treatment for the 75-100cm soil and groundwater mixture (Figure 35). All other treatments however result in the production of nitrite throughout the experiment. The main reason that this is the case is that the denitrification reaction was carbon limited, this is contrary to most authors (e.g. Schipper and Vojvodić-Vuković, 2000 & 2001; Beauchamp *et al.*, 1989; Robertson and Cherry, 1995; and Robertson *et al.*, 2000 & 2003)., who state that the reaction is nitrate limited rather than carbon limited, but the contradictory result may be linked to the short time span of the experiment as well as the different scales on which the studies were done. Dodds and Fey (1995) had similar incomplete and delayed denitrification results for their 10 day experiments. The denitrification did not proceed to completion within the duration of this laboratory experiment, however, the fact that one of the treatments showed nitrite being removed from the system suggests that had more carbon been available in other treatments, or had the experiment run for a longer period, the denitrification may have been complete. One could thus say that the ratios 12.6:1, 24:1, and 34:1 are over-estimations of the actual carbon nitrogen ratios as the sawdust does not release its 53% carbon at once, and that the ratio is not constant during the reaction due to the periodic release of organic carbon from sawdust.

Heterotrophic plate count was plotted to discern the different parts of the growth curve for denitrification (Figures 37 and 38), they show that t=0 to t=6 represents the stationary phase, while t=12 and t=24 represents logarithmic growth. Growth seems to slow down and start die-off after t=96. Two treatments do not conform to this i.e. the untreated and 12.6:1 samples for the 165-200cm soil, for these samples t=12 and t=24 represent equal number of colonies, while exponential growth occurs between t=24 and 48hours. The heterotrophic plate count for the untreated and 12.6:1 samples of the 165-200cm soils is less than that for other treatments. This can be correlated with the results for the denitrification (Figure 35) where the denitrification occurs at a slower rate in the 165-200cm soils than for identical treatments in the 75-100cm soil (Figure 34). These treatments show maximum amount of growth at t=48 and a decline in the number of colonies later than t=48, which signifies the start of die-off. The 34:1 treatments show the greatest amount of colonies established and also the highest rate of denitrification (Figure 34, Figure 35, Figure 37, and Figure 38). The

fact that the micro-organisms that effect denitrification is present in the study area, indicates that one wouldn't have to add appropriate species to effect the reaction. Many authors (Schipper and Vojvodić-Vuković, 2000 & 2001; and Robertson *et al.*, 2003)   have measured either denitrifying enzyme activities or microbial biomass as an indicator of microbial activity in their studies, their work shows that there was no downward trend in the microbial biomass measured during the experiment (Schipper and Vojvodić-Vuković, 2001). According to the authors, this is an indication that carbon availability did not limit the size of the microbial population.

Final water quality (Table 16) contains the chemistry for samples after a 30 day laboratory denitrification experiment. This was compared to the initial chemistry of the groundwater, soil and sawdust mixture as well as the DWAF guidelines for domestic water use. Why are we considering the final water quality? If field scale denitrification should materialize in South Africa, one would like to know that the product water would be potable. If it is not, then the technology is of no use to people who may need it.

How do the results compare to drinking water standards? Certain parameters are elevated due to dissolution of certain ions from the soil namely sodium, potassium, chloride, electrical conductivity, and total dissolved solids. These parameters were above the acceptable levels in the original groundwater sample, but became even higher after mixing the soil, groundwater and sawdust. In terms of DWAF drinking water standards, those parameters that exceed the target water quality ranges as mentioned above, do not all have adverse health effects. The only parameters that present some risk of effect on health include:

- Dissolved organic carbon, depending on its composition;
- Sodium, in infants and people with Na-restricted diets;
- And potassium, in infants and people suffering from renal disease.

Aesthetic effects include a noticeably salty taste in the water, which is contributed to by all anions, and cations in solution, which is evident from the high EC values in Tables 15 and 16.

Even though the target water quality range is exceeded, the water chemistry after the 30 day laboratory experiments is similar to the original water quality except that the nitrate concentration (which presented the most adverse effects for the study area) has been lowered to within the acceptable levels for drinking water for two of the three treatments

used. The production of nitrite is seen to be a negative result for this experiment; however the main reason for this is the carbon availability which is related to an over-estimation of the actual C:N as sawdust is a slowly degradable carbon source and would hence release carbon slowly.

The amount of heterotrophic colonies per millilitre exceed all the classes set by DWAF and would hence make the water unfit for drinking as the possibility of the presence of pathogenic micro-organism, bacteria, viruses or parasites cannot be ruled out. So, even though bacteria was not added to the system, the amount generated by adding carbon sources is way beyond the maximum allowable for drinking water. Interestingly, the data (Figures 37 and 38) indicates that a die-off process had started after about 96 hours of the laboratory scale experiment.

When water use by agriculture (livestock watering) is considered the parameters that exceed the target range values include only the total dissolved solids that may have some effect on livestock production, but no significant adverse effects. In terms of irrigation, chloride, electrical conductivity and total dissolved solids exceed the target water quality ranges. Nitrogen (which includes nitrate, nitrite and ammonia) also exceeds the target water quality range, but are within marginal quality range for drinking water. Mainly sensitive crops are likely to be affected during agricultural practices.

5.4.4   Further investigation on sawdust in terms of C:N and incubation time

The previous experiment revealed that C:N ratio calculations may have yielded over estimations of available C at any time t due to slow release from sawdust. Higher C:N ratios were used over a 43 day period to evaluate its effects and the extent of the denitrification reaction. Established facts in the study included:
1) DOC is a function of C:N ratio,
2) Nitrite is produced as a product of incomplete denitrification
3) The reactions may be carbon limited.

With all this in mind, denitrification using the 34:1 C:N (0.3g sawdust or 30g/kg soil) was studied over a 43 day period, as well as a higher 54:1 C:N (0.5g sawdust or 50g/kg soil) ratio. Samples were prepared in triplicate to determine the reproducibility of the experiment and the statistical significance of the results.   Results (Figures 39 and 40) show that both 0.3g and 0.5g treatments remove all nitrate from the system by the tenth day. This is a

function of the carbon availability in the system. Nitrite is produced as in previous experiments (5.2.2, 5.3.2, and 5.4.2), but is removed/reduced with time, and totally removed from the 0.5g treatment by the 21st day. This shows that the greater availability of carbon speeds up the rate of the reaction. The longer incubation period allowed for the completion of the denitrification reaction.

Data for the 0.3g treatment with 75-100cm soil layer of this experiment was compared to that of the previous experiment (Figure 34, Figure 35, Figure 39, and Figure 40, and Appendix D). It shows that the data obtained during this experiment is comparable with that for the 0.3g treatment (75-100cm soil layer) data in the previous experiment.

Four samples were prepared, a triplicate set for analyses as well as a fourth sample of identical make-up but with a homogeneous finer fraction of sawdust for each treatment, in order to compare whether grain-size has an effect on the denitrification. Results show that the only difference between the three replicates and the fourth sample is the initially higher available carbon as DOC (mg/L). The triplicate data shows good reproducibility for all parameters analysed (Appendix D). Standard deviation values are low except for DOC which may be due to its slow release and varying rate of release within different samples (Appendix D). DOC shows no real discernable trend (Appendix D), this compares favourably with the work done by Schipper and Vojvodić-Vuković, 2001; Beauchamp *et al.*, 1989; Benner *et al.*, 1999; Robertson and Cherry, 1995; and Robertson *et al.*, 2000 who mention a continued slow release of available organic carbon from sawdust to support microbial populations. All of these studies done over years showed that, less than 10% of total organic carbon had been lost during their experiments in the field situation. Box and whisker plots give an indication of the mean, standard error and spread of the data at specific points in time (Appendix D). Significant changes in concentrations of parameters can also be observed in these figures for each parameter.

Correlation matrices for both treatments (Appendix D) show that pH has a positive correlation with nitrate, while EC and alkalinity have a negative correlation with nitrate. Alkalinity and EC have negative correlations with pH and a positive correlation with each other. Results show a negative correlation between alkalinity and DOC. There is no correlation between nitrite and any of the other measured parameters except DOC for the 0.5 g sawdust treated samples where a positive correlation value of 0.67 between nitrite and

DOC exist. This relationship may be as a result of the greater reaction rate and available carbon at any point in time in the 0.5g treatment as compared to the 0.3g treatments.

Water chemistry is determined initially (the groundwater sample) and at three points during the experiment i.e. days 0, shortly after mixing groundwater, soil and sawdust, day 10 (after ten days of incubation) and day 43 (the last day of the experiment) (Table 17).

Mixing of soil, groundwater and sawdust initially causes an increase in the potassium in solution as well as a decrease in the calcium and magnesium levels. This can be explained in theory by the fact that calcium and magnesium in solution in the 2^+ state having a higher positive charge (groundwater sample in Table 17) would replace potassium on exchange sites in the 1+ state in soil and hence be available in solution, while the calcium and magnesium would be attached to the negatively charged soil surfaces, (Mc Bride, 1994). Table 15 shows that the soil pH of above 9 and the water pH of 7.9 together with buffering effects of the total base cations (Alkalinity) results in solution pH values of above 8 which is higher than the optimum for denitrification as documented in literature references. Anion concentrations increase as these are unlikely to be attached to soil surfaces, and it would thus represent an addition of ions from soil, groundwater and sawdust. The increase in EC is as a result of the increase in ions in solution. Alkalinity decreases as a result of buffering pH and adsorption of calcium and magnesium onto exchange sites.

The reaction is specific in the sense that reduction of nitrate is the dominant process occurring. The increase in certain ion concentrations is as a result of the decrease in pH induced by biological activity and CO_2 release by processes. The calcium and magnesium concentrations increase eventually due to a natural buffering effect within the system. Nitrite is produced as an intermediate of the denitrification reaction, in other words, after ten days the denitrification reaction is incomplete. The cause of the resultant decrease in pH between days 0 and 10 can be due to one or a combination of mechanisms (5.3.3, page 85).

The samples analysed after 43 days, shows a decrease in potassium, sodium, and chloride, while calcium, magnesium and sulfate show an increase in their concentrations. Alkalinity increases throughout the experiment as nitrate and nitrite decreases, this can be explained by the fact that denitrification produces alkalinity. DOC is consumed by microbiological denitrification and hence decreases. EC increases due to more ions in solution. The pH seems to stabilize at about 8 in both treatments. The sulfate concentration increases

throughout the experiment. This is ascribed to dissolution of sulfur containing minerals e.g. gypsum in the soil, which may be linked to gypsum ($CaSO_4$) within a calcareous layer in the vertical profile. The total sulfur in the soil layer used for this experiment is the highest in that part of the profile. The decrease in sodium and potassium concentrations is negligible between days 10 and 43, and so is the change in magnesium and calcium.

The final water quality was compared to DWAF (1993 & 1996) guidelines for domestic use and effects on health of people. Calcium, magnesium, nitrate, nitrite, and nitrate + nitrite are within the target water quality range for both treatments. These were all elevated initially with calcium and magnesium about double the target range and the nitrate and nitrate + nitrite above the maximum allowable according to the guidelines for domestic use. Potassium, sodium, chloride and electrical conductivity are within the marginal water quality range, this relates to have health effects only on sensitive users of the resource. DOC is the only parameter analysed during this experiment that is within the dangerous range, however, the effects on human health is dependent on the actual organic compound making up the dissolved organic carbon.

5.5   Conclusions

5.5.1   Carbon substrate effects on denitrification

Four carbon sources were assessed for suitability using excess amounts of carbon. The following conclusions were drawn based on the results of the experiment:

- Denitrification occurred successfully in all samples by the end of the 30-day incubation period;
- The rate at which reactions occur is indicative of the presence of resident microbial communities and the necessary enzymes to catalyse them;
- The initial slow reaction in the methanol treated sample could be owed to methanol's toxicity to certain denitrifying bacteria;
- Acetate production in certain of the treatments was as a result of incomplete oxidation of organic material;
- Sulfate was produced due to dissolution of salts or oxidation of S(-2) to S(6) in the soil;
- Sawdust and glucose were the most suitable of the 4 carbon sources;
- The main differences between the carbon sources was the production of by-products and side reactions.

5.5.2 Effect of C:N on denitrification

The C:N ratio for glucose and sawdust as the C:N ratio or excess C used in experiment 1 was considered to have been the main reason for the acetate production and sulfate reduction or dissolution. Conclusions drawn from the results of this experiment are that:

- C:N ratios used all showed positive results with respect to denitrification;
- The results showed that acetate levels in the glucose treated samples were an order of magnitude less than that for the glucose treatments in experiment 1;
- The decrease in pH for glucose treatment may be due to CO_2 released from denitrification as well as to CO_2, soluble organic acids, and acidic soil residues, all of which behave like weak acids, displacing base cations from exchange sites; generated by metabolic processes;
- Sawdust does not display the same pH decrease or dissolution of cations due to its slow degradation.

5.5.3 Sawdust as a substrate

Different carbon to nitrogen ratios of sawdust were used during laboratory experiments and parameters including pH, EC, DOC, nitrate, nitrite, ammonia, sodium, potassium, calcium, magnesium, sulfate, iron, manganese, total dissolved solids, alkalinity, chloride, silica, and hardness were measured during a 672-hour (28 days) experiment. The following conclusions were drawn from the results:

- NO_3^- concentration is reduced to below the acceptable limit in best cases;
- NO_2^- being present and not fully reduced is an indication of incomplete denitrification DOC is directly proportional to the carbon to nitrogen ratios;
- Ratios calculated may have been over estimated as it is based on total carbon and nitrogen present that took into account the 53% carbon measured for sawdust;
- Low availability of C in sawdust as sawdust is a slowly degrading source of carbon;
- For this experiment, the 75-100cm soil displayed greater denitrification rates than the 165-200cm due to higher initial nitrate concentration.
- The presence and growth of heterotrophic bacteria confirms that conditions are optimal for growth and denitrification, and that injection or addition of bacteria is not a requirement for *in situ* denitrification;
- The method showed some specificity, as the only parameters affected by the denitrification experiment are DOC, nitrite, nitrate, and the heterotrophic plate count;

- The best case scenario is represented by the 34:1 C:N using 75-100cm soil, and groundwater as nitrate and nitrite levels approach zero, however, DOC and heterotrophic plate count do not comply with acceptable levels for drinking water.

5.5.4 Further investigation of sawdust in terms of C:N and incubation time

The following conclusions were drawn for this experiment:
- Issues raised in the previous experiment were addressed e.g. nitrite removed from the system, more carbon made available for reaction etc.;
- All nitrate and nitrite can be totally removed from groundwater within 21 days at C:N ≥34:1;
- The rate of reaction is dependent on the available carbon at a given point in time, i.o.w. higher carbon to nitrogen ratios effects greater rate of denitrification;
- No foreign bacteria are required to effect denitrification, indigenous species in soil are capable of denitrifying;
- The method showed reproducibility, small standard deviation and standard errors;
- In terms of potability, the water quality was improved in most parameters;
- DOC in the final product water may need further attention depending on the constituent organic compounds.

CHAPTER 6: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

In South Africa, high nitrate concentrations in groundwater is the single most important reason for groundwater sources being declared unfit for drinking, i.e. nitrate N exceeding 10 mg/L (Marais, 1999). Although no statistics are available it is known from recorded incidences that infant methaemoglobinaemia occurs in southern Africa.

In South Africa, the ideal drinking water according to Department of Water Affairs and Forestry (1996) and DWAF and the Department of Health (1998),("blue" i.e. Class 0) has less than 6 mg/L nitrate (plus nitrite) as N while the "good" water quality ("green" i.e. Class I) has a maximum concentration of 10mg/L and the "marginal" water quality ("yellow" i.e. Class II) has a maximum concentration of 20 mg/L. This is generally in agreement with the WHO guidelines. However, in many areas of the South Africa nitrate levels exceed the maximum concentration of 40 mg/L of "poor"("red"-Class III) water quality and levels of 100 mg/L or even greater than 200 mg/L are found in various places. Water with nitrate exceeding 40 mg/L, belongs to the category of "unacceptable" drinking water quality ("purple", i.e. Class IV). Such levels are an order of magnitude higher than for example in Western Europe where water with nitrate N exceeding 5.5 mg/L will be denitrified. The incidence of methaemoglobinaemia and the occurrence of high nitrate levels in groundwater in Namibia and South Africa have triggered epidemiological studies for investigating the effects of the sub-lethal levels of methaemoglobin on children (Tredoux, *et al.*, 2005).

In approximately 280 towns, some of which have evaporation rates that exceed that of recharge or rainfall events, groundwater is the sole source of water. This total dependence on the resource increases the need to have groundwater that is of a good enough quality to be consumed by people and animals alike.

Denitrification is part of the biogeochemical nitrogen cycle that proceeds as follows with the help of bacteria and their enzymes:

$$5\ (CH_2O) + 6\ NO_3 + 4\ H^+ \longrightarrow 5\ CO_2 + 3\ N_2 + 7\ H_2O + 6\ OH^-$$
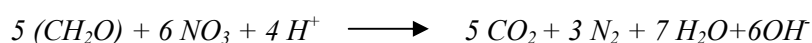
Equation 14

However, in nature, the reagents in the forward reaction are not always present in sufficient amounts to allow the reaction to proceed at the required rate. Incubation experiments were performed to investigate the amount of carbon source required for the forward reaction to proceed at an acceptable rate to yield nitrate concentration within the ideal drinking water ("blue", i.e. Class 0) category.

This study addresses groundwater dependence in rural areas and lack of remediation technologies that is often related to economic status. Rural areas, that are far from business centers often lack the funding for establishing large and complicated treatment plants. Treatment of nitrates with minimal costs and safe methods is a required technological endeavour in the more rural parts of Africa.

The following key questions are addressed here:
- Which carbon sources prove to be successful in promoting/facilitating denitrification?
- What are the main differences when using certain carbon sources?
- Is there any distinct difference between reactions when soils of different depths are involved?
- What is the most suitable C:N ratio?
- Does the resultant water comply with drinking water standards?

Currently operational sites show that denitrification in the field is possible and successful with a number of sites experiencing some initial problems. However, it is important to realize that each site or geological setting is unique and that proper site characterisation is important and includes:
- Chemical characterisation of water, soil and rock at the site;
- Delineating the aquifer in terms of lithological make up as well as structural geology, and
- Evaluating the groundwater flow characteristics and hydraulic properties of the aquifer.

Parameters that stand out as being important for successful denitrification experiments include C:N ratio and assimilability of the organic matter, the presence or availability of chemical species such as carbon, oxygen-containing species (e.g. nitrate). If bacterial regeneration is inhibited, the denitrification rate will decrease and eventually stop as bacteria die off.

Among other properties that may be unique to a specific site, it is important to know the history of a particular site e.g. was there a waste site at the location previously, were there any previous pollution plumes, etc.

Site characterization is important in any type of remediation. The site selected for this study had the following characteristics:

- A rural town;
- A town solely dependent on groundwater for all water uses (also called sole source);
- An area with elevated nitrate concentrations in their groundwater and or soils at most of its boreholes;
- A shallow primary aquifer of about 10m deep;

Marydale was selected as a suitable study area based on the above information and previous sampling in the area. Samples were selected in the study area to characterize soil and water and to evaluate conditions present prior to denitrification experiments. Ten soil samples were taken on the basis of colour and texture changes with depth along a profile dug in the study area in Marydale, Northern Cape.

It was concluded that Marydale's groundwater is of inferior quality both chemically and aesthetically by applying of the Department of Water Affairs and Forestry (1993, 1996 and 1998) guidelines. This is based on the fact that result show that the water chemistry is dominated by NaCl and nitrate concentrations are above that of the acceptable levels for South Africa and close to or exceeding the maximum allowable levels (20mg/L as N). pH measured in the water sample is within the range of optimum pH levels for denitrification.

The soil can be described as a saline sodic soil with pH greater than 8 in all samples, hence not within the optimum range for denitrification and even greater than 9 in certain of the samples. Elevated sulfate levels may be due to the presence of sulfur/sulfate containing calcrete layer within the soil profile. Soil moisture along the profile may also be influenced by the presence of the semi consolidated calcrete layer. Soils in the study area are predominantly sandy with high porosity and permeability within the soil profile.

Laboratory evaluation of carbon sources for denitrification presented several scenarios to consider. Laboratory experiments were undertaken to establish:

- Suitability of carbon sources;

- Main differences between different carbon sources;
- Whether outcomes would differ when soil from different depths were used;
- Suitable C:N ratios;
- Whether the resultant water quality complies with drinking water guidelines set by the DWAF.

General conclusions drawn from the laboratory incubation experiments include the following:

- Sawdust and glucose were the most suitable of the 4 carbon sources used in initial treatability evaluation;
- The production of by-products and side reactions were the main drawbacks of the other carbon sources; i.e. maize meal and methanol.
- The C:N ratio influences the extent to which denitrification, by-products and side reactions occur.
- NO_3^- is totally removed from the system in best cases;
- NO_2^- being present and not fully reduced is an indication of incomplete denitrification as well as possible toxicity to some heterotrophic bacteria, although best cases remove all nitrite from the system by the end of 43 day experiments
- Available DOC is directly proportional to the quantity of carbon source;
- Ratios calculated for sawdust may have been over estimated as it is based on total carbon and nitrogen present that took into account the full 53% carbon in sawdust;
- Low availability of C in sawdust presented some limitations to denitrification;
- Initial concentrations of nitrate and organic carbon in soils and water contribute to the rate at which nitrate is reduced in a particular soil and groundwater mixture;
- The presence and growth of indigenous heterotrophic bacteria confirms that conditions are optimal for growth and denitrification, and that injection or addition of foreign bacteria is not a requirement for *in situ* denitrification;
- The method showed some specificity, as the only parameters affected by the denitrification experiment are DOC, nitrite, nitrate, and the heterotrophic plate count;
- DOC and heterotrophic plate count do not comply with acceptable levels for drinking water, however removal of HPC can be effected by boiling or chlorination; however, operational conditions under in situ denitrification are expected to be different and the treatment, if any, determined on site.
- In agreement with Firestone (1982), Dodds and Fey (2004), and Greben *et al.* (2004) the soil organic matter and denitrification activity can be correlated. The availability or assimilability of carbon and not just the presence of carbon is important;

- Denitrification occurred successfully in all samples by the end of the incubation period, and the product water is fit for drinking with some conventional treatment required to reduce heterotrophic plate count.

With respect to the laboratory incubation experiments that formed part of this study, a few recommendations can be made:

- Readily degradable carbon sources e.g. glucose should be used at a carbon to nitrogen ratio equal to or less than 25:1 to avoid undesirable side reactions or by-products;

- When using sawdust (woodchips) as a carbon source, one has to monitor the nitrite levels in water as an indicator of incomplete denitrification;

- Recommended parameters to monitor during further studies include nitrate, nitrite, ammonia, dissolved organic carbon or total organic carbon, heterotrophic plate count, pH and EC, as these are the affected parameters and indicators of change within the system conditions;

- Bacteria able to facilitate denitrification are naturally present in the subsurface for the study area. It is recommended that the number of heterotrophic colonies be determined as part of site characterisation and if possible throughout experimentation if laboratory as well as field scale studies are performed;

- Field parameters in the study area such as hydraulic conductivity, direction of groundwater flow, rate of groundwater flow should be assessed before any field test studies can be conducted;

- Full environmental impact assessments have to be done prior to implementation of any *in situ* denitrification method according to the National Water Act (1998)and National Environmental Management Act (1998);

- Some of the methods assessed may produce waste products which have to be pumped out and disposed of, the incubation experiments show that certain chemical species may be produced as side reactions or as by-products if an excess of carbon is used. The challenge would be to find the optimum C:N ratio to avoid by-products formation and side reactions. The impacts of changes in EC, pH and ion concentrations need to be considered. The concentration of carbon sources used needs to be monitored throughout implementation in order to detect the rate of consumption along a particular flow path, also to avoid excessive addition of carbon to the aquifer as this may have some negative effects as demonstrated in the incubation experiments.

It is recommended that further field investigations and further site characterisation proceed for the study area. A field test study would prove invaluable to the town of Marydale and Southern Africa. The knowledge gap that exists in Southern Africa with respect to remediation of groundwater and soils that contain elevated concentrations of nitrate needs to be addressed and closed so that it may be applied and secure more sustainable use of the precious water resources.

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APPENDIX A:

METHODS OF ANALYSES

AND DETECTION LIMITS

Saturated soil paste and extract preparation

By Hand: A 250g air dry soil sample is placed in a suitable container and moistened with de-ionised water while mixing with a spatula. Consolidate the mixture from time to time by tapping the container on the work bench. Test for the properties of a saturated paste and add more de-ionised water if necessary. Allow to stand for at least an hour and test whether it still has saturation properties. If left overnight cover the container. Special care should be taken to ensure that water does not collect and that the paste does not dry out too much. Add more de-ionised water if required. If too much water was added, repeat the procedure. Note the total volume of water added (w).

Properties of
a saturated paste: In a saturated soil paste all the pores are filled with water
    It has the following characteristics:

  ✓ The surface is shiny;
  ✓ The paste flows slightly when the container is tilted;
  ✓ Free water does not collect when a small trench is drawn on the surface and
  ✓ It does not cling to the spatula (with the exception of clayey soil)

By capillary
 saturation: Based on the method of Longenecker and Lyerly (1964), sample holders are prepared from whatman no 50 filter paper, 180mm diameter. A 250g air dry soil sample is transferred to each filter paper holder, which is then placed on sand (about 40mm thick) in a plastic container with de-ionised water. The level of water is controlled to saturate the bottom 10mm of sand. The sample is allowed to absorb water for 24 hours. The sample is then emptied into a plastic dish and carefully mixed to ensure even distribution of soluble salts. Before extraction of moisture, determine mass of soil and absorbed moisture. Soils high in sodium or clay content do not saturate satisfactorily with this method and the hand method   should be used.

Preparation of saturation extract:
  • Filter the soil by suction through Whatman no. 50 paper on a Buchner or Richards funnel
  • Collect the filtrate in a test tube placed under the funnel in the suction flask
  • Repeat filtration if the solution is not clear
  • Store filtrate in a plastic bottle with a drop of toluene added as a bacteriostat

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Determination of nitrogen

This was done using a nitrogen analyser

Scope

This work instruction details the use of a nitrogen analyser for the measurement of nitrogen in plant materials and soil.

The measurement scope of the method, for a maximum sample weight of 250 mg, using a Leco FP528 nitrogen analyser, is:
- LLD – 0.04 $\%^m/_m$
- LQC – *0.053 $\%^m/_m$*
- Estimate of uncertainty of measurement – *3.8 $\%^m/_m$*

The evaluation data for this method has been recorded in the "*Method evaluation data*" file.

Safety precautions

This work instruction requires the use of a nitrogen analyser. Before operating the instrument, laboratory personnel must familiarise themselves with the safety procedures, as provided in the manufacturer's manual. If the information is not available, or not understood, consult the Technical Manager (or other suitably qualified senior staff member) before continuing. Laboratory personnel must also ensure that they are familiar with any hazards and safe handling practices associated with the reagents used in the nitrogen analyser.

Apparatus

- Nitrogen analyser
- Analytical balance (4 decimal place)

Reagents and consumables

- Appropriate reference materials with certified nitrogen content
- Tin foil cups
- Oxygen, UHP (99.999%)
- Helium, Instrument grade (99.999%)

Instrument start up and performance checks

- Refer to the instrument log book (in the instrument software) to ensure that the maintenance checks have been performed in accordance with the manufacturer's recommendations.
- Run the instrument checks as defined manufacturer's manual.
- Run blanks until the relative standard deviation of three successive blanks is less than 25%. Do not proceed until the instrument blank is stable.
- "Zero calibrate" the instrument.
- Run a suitable reference material. Proceed with analysis if the standards are within the defined tolerances.

Analysis of samples	Complete the steps in the following table to analyse samples.

Step	Requirement
1	Weigh 0.1500 g $\pm$ 0.05 g sample in a tarred tin foil cup and record sample weight. **Note:** The weigh may be increased to a maximum of 0.25 g for low levels of nitrogen. The weight can be reduced for samples with high nitrogen, *or if the sample size is too small.*
2	Run an appropriate secondary reference material every 20 samples. Record the result and manage the data in accordance with the procedure defined in BWI/G03 (see below).
3	Nitrogen concentrations are calculated by the instrument software. Check the results in the instrument database and, if accepted, download them to the BemLIMS database.

Verifying the analytical data	**Evaluation**: Evaluate the analytical data obtained from the nitrogen analyser in the following way. • Plot the concentrations for the nitrogen in the reference material on the control charts and evaluate the results by the method defined in BWI/G03. **Action:** Take the following action, as appropriate. • Continue with the processing of the data, if the results are within the defined limits (refer next section). • Notify the Technical Manager, or appointee, if incorrect results are suspected. (Note: Suspect analytical data is to be handled in accordance with processes defined in document 3.02.01). Do not continue to download the results.

Processing analytical data	Export the data to the file server once it has been checked and no incorrect results found. Check that the results have been expressed with the correct units (for example, mg/kg or %$^m/_m$), once the data has been downloaded. The Director, and Technical and Quality Managers, are authorised to access the database to make amendments. For example, converting units.

References	DONALD A HORNECK and ROBERT O MILLER., 1998. Determination Total Nitrogen in Plant Tissue, Y.P. Kalra (Ed) Handbook of reference methods for plant analysis, pp 81 - 83. CRC Press, Boca Raton. The documents referred in this work instruction are: • "Management of analytical data", Quality manual 2.05.04 • "Managing nonconformity", Quality manual 3.02.01 • "Statistical treatment of analytical data", Work instruction BWI/G03

Preparation of solutions

This method was used for sawdust analyses for the determination of analytes using ICP.

Scope

This method details the preparation of solutions for the determination of the concentrations of major (Ca, K, Mg, P) and minor / trace analytes (B, Cu, Fe, Mn, Na, Zn) by ICP-OES. The measurement scope of the method is given in the following tables.

Analyte	LLD (mg/*kg*)	LQC (mg/*kg*)	U of M	Cal. Range (mg/L)
Major analytes				
Ca	N/A	N/A	*2.9%*	0 - *600*
K	N/A	N/A	*7.2%*	0 - 600
Mg	N/A	N/A	*1.6%*	0 - 300
P	N/A	N/A	*2.3%*	0 - 150
Minor / trace analytes				
B	*0.007*	*0.024*	*3.0%*	0 - 2.0
Cu	*0.010*	*0.032*	*14.0%*	0 - 0.4
Fe	*0.007*	*0.024*	*9.8%*	0 - 4.0
Mn	*0.002*	*0.006*	*4.7%*	0 - 2.0
Na	*0.034*	*0.112*	*11.1%*	0 - *20*
Zn	*0.006*	*0.018*	*7.5%*	0 - 2.0

Abbreviations:

◊ LLD = Lower limit of detection
◊ LQC = Lowest quantifiable concentration
◊ U of M = Uncertainty of measurement
◊ Cal. Range = Calibration range for which the above data is valid
◊ N/A = Not applicable; required for minor / trace analytes only

The evaluation data for this method has been recorded in the "*Method evaluation data*" file.

Safety precautions

Before commencing analysis, laboratory personnel must familiarise themselves with the safe handling practices and emergency procedures, as provided in the appropriate material safety data sheets. If the safety data is not available, or not understood, consult the Technical Manager (or other suitably qualified senior staff member) before handling the chemicals.

Apparatus

♦ Volumetric flasks, 250 ml, 500 ml and 1 L capacity
♦ Measuring cylinder, 500 ml capacity
♦ Pipettes, 5.0 ml and 25.0 ml capacity
♦ Displacement pipettes
♦ Analytical balance (4 decimal place)

Reagents and consumables

• Standard ampoules (1000 mg/L) for B, Cu, Fe, Mn, and Zn, Merck Titrisol, or equivalent
• Reagents dried overnight at 100 °C $\pm$ 5 °C:-

- Calcium carbonate (99.5% min.)
- Diammonium hydrogen phosphate (98.5% min.)
- Potassium chloride (99% min.)
- Sodium chloride (99% min.)
- Magnesium metal ribbon (99% min.)
- Hydrochloric acid (32%)
- Hydrochloric acid solution, approximately 5M
- Deionised (or distilled) water

Preparation of reagent solutions

Hydrochloric acid solution, *(1:1 by volume)*

Transfer 500 ml hydrochloric acid into a 1 L volumetric flask containing approximately 300 ml water. Mix well.

Dilute to volume with water.

Preparation of calibration stock solutions

Use calibrated glassware (class 'A' or 'AS') and displacement pipettes for the preparation of all calibration solutions. *Standard ampoules, diluted to appropriate volumes, may be used in place of chemicals for the preparation of calibration standards .for the major analytes.*

Stock solution for minor / trace analytes (B, Cu, Mn, Zn)
Prepare individual stock solutions (1000 mg/L) for each of the minor / trace analytes by following the steps below.
1. Transfer, carefully, the contents of an ampoule to a 1 l volumetric flask.
2. Rinse the ampoule well, adding the rinsings to the flask.
3. Dilute to volume with water and mix thoroughly.

Stock solution for Fe
1. Transfer, carefully, the contents of an ampoule to a 500 ml volumetric flask.
2. Rinse the ampoule well, adding the rinsings to the flask.
3. Dilute to volume with water and mix thoroughly.
    This solution contains 2000 mg/L Fe.

Preparation of combined calibration solutions

Combined stock solution for all analytes
1. Weigh, accurately to within 0.0002 g, 14.3018 g potassium chloride, 0.6355 g sodium chloride, 18.7296 g calcium carbonate, 7.9941 g diammonium hydrogen phosphate and 3.7500 g magnesium ribbon, and transfer to a 500 ml volumetric flask.
2. Add approximately 100 ml water followed, slowly, by sufficient hydrochloric acid to dissolve the solids. Mix well.
3. Once the solids have dissolved, add 25.0 ml of each of the Fe (*2000mg/l*), B, Mn and Zn stock solutions (1000mg/l) and 5.0 ml Cu to the flask. Mix well.
4. Dilute to volume with water. Mix well.

This solution contains:

◊ 15000 mg/L K and Ca	◊ 100 mg/L of Fe
◊ 7500 mg/L of Mg	◊ 50 mg/L B, Mn, and Zn
◊ 3750 mg/L of P	◊ 10 mg/L Cu
◊ 500 mg/L of Na	

Working solutions for all analytes

1. Standard 1 is a reagent blank prepared by diluting 20 ml 5M hydrochloric acid solution to 250 ml with water.
2. Prepare three mixed working standards by pipetting the volumes, given in the table, into 250 ml volumetric flasks.

	Volume of stock solution (ml)		
Analyte	**Flask 1**	**Flask 2**	**Flask 3**
All analytes	2.5	5.0	10.0

3. Add 20 ml hydrochloric acid solution (5M) and dilute to volume with water. Mix thoroughly. These solutions contain the following concentrations:

	Concentration (mg/L)		
Analyte	**Flask 1**	**Flask 2**	**Flask 3**
Ca / K	150	300	600
Mg	75	150	300
P	37.5	75	150
Na	*5.0*	*10*	*20*
Fe	1.0	2.0	4.0
B / Mn / Zn	0.5	1.0	2.0
Cu	0.1	0.2	0.4

Preparation of sample solutions

Refer to document BWI/L02 for details of the method for the preparation of solutions of leaves for analysis, using ICP-OES.

ICP programme

Select the ICP programme "Leaves" to set up the analytical conditions. For the operating criteria in this programme refer to file "ICP programme parameters" in the ICP Programme file.

Follow the instructions in BWI/G04 to calibrate the ICP-OES and analyse samples.

Verification of the analysis

Verify the validity of the analytical data by following the steps given below.

1. Prepare a secondary reference material for leaves, along with the samples.
2. Run the solutions and evaluate the data by following the instructions detailed in document BWI/G04.

Calculations　　Calculate the concentration of the analytes using the following formula.

$$\text{Macro analyte concentrations} = \frac{a * 50}{10000 * m} \ \%$$

$$\text{Micro / trace analyte concentration} = \frac{a * 50}{m} \ \text{mg/kg}$$

Where a = the measured analyte concentration (mg/L) in the extract.
　　　 m = sample mass

Processing data　　Process the analytical data by following the method detailed in document BWI/G04.

References　　♦　ISAAC, R.A. & JOHNSON, W.C., 1998. Elemental determination by Inductively Coupled Plasma, Y.P. Kalra (Ed) Handbook of reference methods for plant analysis, pp 165-170. CRC Press, Boca Raton.

The documents referred in this work instruction are:
♦　"Determinations of analyte concentrations using an ICP-OES", Work instruction BWI/G04
♦　"Leaves – Preparation of samples for analysis using ICP-OES and a nitrogen analyser", Work instruction BWI/L02

Heterotrophic Plate Count (Pour Plate)

Method as used by the CSIR accredited microbiology laboratory in Stellenboch

Introduction and scope

This method quantifies viable bacteria in potable water, non-potable water and waste water. It is widely used in routine laboratories. The results of HPC represent those bacteria that are to form visible colonies in nutrient media under specified culture conditions. Heterotrophic plate count on its own is used to test the efficacy of water treatment processes. The heterotrophic plate count can be used for clear water i.e. borehole water or other potable water. It can also be used for heavily polluted turbid effluent water, where it will be necessary to make tenfold dilution.

Principle

A fresh water sample (analysed within 6 hours of sampling or a maximum of 24hrs) mixed with a nutrient agar, tryptone glucose yeast agar, is incubated for 48hours at 35ºC. The number of colonies that developed on the culture plate, represent the number of bacteria that grow within 48 hours at 35ºC for that particular sample. This number is only a portion of the total number of bacteria in the sample. The other bacteria may need different temperatures for longer periods to develop or may only grow in the absence of oxygen.

Reagents

Medium

Plate count agar

Ingredients:
	Agar	15g
	Tryptone	5g
	Yeast extract	2.5g
	Dextrose	1g
	Reagent grade water	1L

Dissolve agar, tryptone, yeast extract and dextrose in 100ml of the water. Stand for 5 minutes. Add 900ml boiling water and put the flask on a Bunsen burner until the agar dissolves and the medium becomes clear. Adjust the pH to 7,2 ± 0,2 at 25ºC. Pour 120ml quantities into suitable bottles and sterilize by autoclaving at 121ºC for 15 minutes. Each bottle is enough to pour six plates.

Store at 4ºC.

Apparatus and Laboratory supplies

- ❖ Incubator. Temperature 35ºC ± 0,5ºC.
- ❖ Waterbath. Temperature range 44ºC - 48ºC
- ❖ Sterile pipettes. 10ml and 1ml volume
- ❖ Colony counter with Quebec grid.
- ❖ 45ml sterile saline (0,85%) in medical flat for 10 fold dilutions

Interferences

Water with a high turbidity and suspended solids may not mix well with the agar and colonies may be over crowded at the site of inoculation.

Sampling and sample preparation

Samples should be analysed within 6 hours of collection. Refrigerate all samples if not analysed immediately. Sample from remote areas should be stored in a cooler bag at not more than 10ºC and should be analysed within 24 hours after sampling. Results from samples older than 24 hours are doubtful. Make sure that all samples are clearly marked.

Analytical Procedure

Setting up

Melt enough solid agar medium for the number of tests to be carried out. This can be done by placing the agar medium in the autoclave and heating it to 100ºC. Once the autoclave reaches the lowest pressure (appr. 10lb/m^2), switch the autoclave off. Do not resterilize the plating medium.

Maintain the melted medium in a water-bath between 44 ºC and 48 ºC until used. Discard medium not used within 8 hours.

Test Procedure

Dilutions should be selected so that the total number of colonies on a plate will be between 30 and 300. For most potable water, plates suitable for counting will be obtained by plating 1ml and 0.11 ml undiluted sample and 0.1 ml of a 10^{-1} dilution.
A sterile pipette should be used and start pipetting from the highest dilution. Should the pipette become contaminated before the transfers are completed, replace with a sterile pipette. Use decimal dilutions in preparing sample volumes of less than 0.1 ml inoculum of original sample. Prepare at least two replicate plates foe each sample dilution used. Limit the number of samples to be plated in any one series so that no more than 20 minutes elapse between dilution of the first sample and pouring of the last plate in the series. Dilutions should therefore be made immediately before the plating of the sample. Do not first dilute all the samples before the plating is started. Pour at least 18-20 ml liquefied medium maintained at 44-48ºC into each dish by gently lifting the cover just high enough to pour. This volume will just cover the surface of the petri dish. Start mixing the agar immediately after pouring one bottle of medium. Rotate the dish first in one direction and then in the opposite direction. Whilst keeping the plates on the surface of the bench, gently shake the plates by rapidly sliding them from side to side over the bench surface taking care not to spill the medium. Rotate the plates once more in opposite directions. The mixing should not take longer than 20 seconds. Leave the plates to solidify for about 10 minutes before stacking them. Invert the plates and place them in an incubator at 35ºC±0.5ºC for 48 hours.

Include negative control samples when doing only one sample. When more than one sample is tested use one bottle of agar to pour into one plate each of six different samples instead of using one bottle for each sample. When testing less than six samples at a time, use one bottle agar for only one of the duplicate sets of the different sample volumes of each sample and another bottle of agar for the remaining duplicate sample volume. In the event that one bottle of the culture medium was contaminated, the rest of the culture plates may still be used for counting.

Positive control samples should be done in parallel with total and/or faecal coliforms after new incubationes of culture media were prepared, using a pure culture of E. coli or Klebsiella.

Counting and Calculating of results

Count the colonies on those plates that contain between 30 and 300 colonies. Use the colony counter for manual counting. If the total number of colonies is less than 30, disregard the rule above of only counting plates that contain between 30 and 300 colonies.

If the number of colonies per plate exceeds 300, do not report the result as too numerous to count. If there are fewer than 10 colonies per centimetre, count colonies in 10 squares (of the colony counter),

having representative colony distribution. If possible, select five consecutive squares horizontally across the plate and five consecutive squares vertically. Multiply the sum of the number of colonies in the 10 representative square centimetres by 9 to compute estimated colonies per plate when the plate area is 90cm². When there are more than 10 colonies/ cm², count 5 representative squares, take the average count per square centimetre, and multiply by 18 to estimate the number of colonies per plate. Report as estimated colony-forming units per millimetre.

Expression of results

Results should be reported as the total number of colonies per millilitre.

Literature references

APHA, AWWA and WPCF, (1992) *Standard Methods for Examination of Water and Waste Water* (18th ed.) Greenberg, A. E., Clesceri, L., S., Eaton, A. D. (eds). APHA, Washington DC

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Van Vliet, H. R. *et al.* March (1988) *Analytical Methods Manual*, Hydrological Research institute, Department of Water Affairs Branch: Scientific Services. Pretoria, S. A.

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APPENDIX B:

SOIL AND GROUNDWATER

DATA TABLES

Data presented here are the result of field sample analyses for groundwater and soil samples collected in Marydale, Northern Cape.

Groundwater sample analyses

	Marydale				
	Mar-09	**Mar-10**	**BG M23**	**M25**	**Municipal**
Potassium as K mg/L	34	28	22	19	20
Sodium as Na mg/L	200	257	215	191	205
Calcium as Ca mg/L	68	73	57	72	63
Magnesium as Mg mg/L	58	86	50	61	54
Ammonia as N mg/L	<0.1	<0.1	<0.1	<0.1	<0.1
sulfate (SO_4^{2-}) mg/L ***	129.73	222.47	136.52	139.1	139.77
Chloride mg/L ***	255.69	424.2	245.47	277.5	267.35
Alkalinity as $CaCO_3$ mg/L	289	354	318	281	306
nitrate (NO_3^-) mg/L ***	83.29	116.98	68.52	91.97	77.92
Ortho phosphate as P mg/L	<0.1	<0.1	<0.1	<0.1	<0.1
Iron as Fe mg/L	0.15	<0.05	0.09	<0.05	<0.05
Manganese as Mn mg/L	<0.05	<0.05	<0.05	<0.05	<0.05
Silica as SI mg/L	31	32	33	33	33
Dissolved Organic Carbon mg/L	<1.0	1.4	<1.0	<1.0	<1.0
Conductivity mS/m (25°C)	176	234	173	180	175
pH (Lab) (25°C)	7.8	8.4	7.9	8.0	7.9
Saturation pH (pHs) (20°C)	7.3	7.2	7.4	7.3	7.3
Hardness as $CaCO_3$ mg/L	409	538	350	433	380
Sodium absorption ratio (SAR)	4.3	4.8	5.0	4.0	4.6

Water used in Exp 3 and 4 [ ]   Water used for Exp 1 and 2 [ ]

1: 5 Soil analyses

Ions	Soil					
	1	2	3	4	5	6
depth	**0.50**	**0.80**	**1.00**	**1.05**	**1.10**	**1.15**
Calcium (Ca) mmolc/L	0.39	0.35	0.27	0.45	0.26	0.11
Magnesium (Mg) mmolc/L	0.29	0.88	1.13	1.74	1.47	0.85
Sodium (Na) mmolc/L	0.97	1.43	1.33	1.39	1.41	1.15
Potassium (K) mmolc/L	0.60	0.75	1.26	0.76	0.75	0.64
depth	**0.50**	**0.80**	**1.00**	**1.05**	**1.10**	**1.15**
Fluoride (F) mmolc/L	0.00	0.00	0.00	0.01	0.01	0.01
Chloride (Cl) mmolc/L	1.62	2.71	2.18	1.84	1.71	0.43
nitrate (NO_3^-)mmolc/L	0.12	0.12	0.10	0.08	0.08	0.06
sulfate (SO_4^{2-}) mmolc/L	2.12	1.01	0.76	0.62	0.63	0.14

Soil Analyses

Sat. Paste Extracts (mg/l)

Depth	Soil depth (cm)	Sample	Na	K	Ca	Mg	SO$_4$	Cl	CO$_3$	NH$_4^-$ N	NO$_3$	NO$_3^-$N
0-15 cm	15	# 0	57.9	21.6	7.74	2.87	6.39	9.61	0	0	11.7	2.63
15-30 cm	30	# 1	41.2	12.4	10.9	5.24	5.93	11.36	17.75	0	4.03	0.91
30-45 cm	45	# 2	77.9	20.3	36.5	3.79	76.6	26.22	16.29	0	11.3	2.56
45- 55cm	55	# 3	553.9	94.1	14.9	2.06	612	590	26.62	0	174.1	39.3
55- 75cm	75	# 4	589.9	95.0	4.78	1.25	461.4	646	73.95	0	120.9	27.3
75- 100cm	100	# 5	966.1	145.4	3.53	1.08	553	930	134.6	0	203.3	45.9
100-115cm	115	# 6	693.6	109.3	2.67	0.99	365	695	131.63	0	127.6	28.8
115-135cm	135	# 7	644.9	95.1	2.59	0.59	359	462	121.28	0	103.7	23.4
135-165cm	165	# 8	239.7	42.0	2.30	6.67	120	198	90.22	0.63	24.8	5.59
165-200cm	200	# 9	405.2	62.0	3.16	2.32	210	257	47.33	0	55.8	12.6

Exchangeable cations (mg/kg)

Depth	Soil depth (cm)	Sample	Na	K	Ca	Mg
0-15 cm	15	# 0	0.14	1.07	11.6	1.76
15-30 cm	30	# 1	0.63	1.13	11.7	1.48
30-45 cm	45	# 2	1.04	1.57	16.9	1.52
45- 55cm	55	# 3	4.06	2.29	15.9	1.53
55- 75cm	75	# 4	5.36	2.50	16.1	1.46
75- 100cm	100	# 5	7.99	2.89	16.0	1.96
100-115cm	115	# 6	6.68	2.77	15.6	1.76
115-135cm	135	# 7	8.06	3.98	15.4	1.39
135-165cm	165	# 8	6.45	3.58	11.5	1.27
165-200cm	200	# 9	4.53	2.67	15.5	1.20

Grain size and classification

Depth	Soil depth (cm)	Sample	%clay	%silt	%sand	classification
0-15 cm	15	# 0	2.6	3	94.4	Sa
15-30 cm	30	# 1	3.2	1.8	95	Sa
30-45 cm	45	# 2	3	2.2	94.8	Sa
45- 55cm	55	# 3	2.2	1	96.8	Sa
55- 75cm	75	# 4	1.8	3.2	95	Sa
75- 100cm	100	# 5	3.2	5.6	91.4	Sa
100-115cm	115	# 6	2.4	3	94.6	Sa
115-135cm	135	# 7	0.4	2.6	97	Sa
135-165cm	165	# 8	0.4	2.2	97.4	Sa
165-200cm	200	# 9	0.4	2	97.6	Sa

Soil Chemical parameters

Depth	Soil depth (cm)	Sample	EC	HCO$_3^-$	pH	S (mg/kg)	% C	% N
0-15 cm	15	# 0	36.8	54.1	8.3	52.9	0.403	0.051
15-30 cm	30	# 1	23.2	70.7	8.1	36.7	0.334	0.040
30-45 cm	45	# 2	46.7	132.3	8.2	75.5	0.283	0.042
45- 55cm	55	# 3	283	54.1	8.2	216.5	0.252	0.124
55- 75cm	75	# 4	290	75.2	9	192.5	0.266	0.025
75- 100cm	100	# 5	444	40.6	9.2	236.6	0.192	0.032
100-115cm	115	# 6	320	66.2	9.2	157.5	0.223	0.034
115-135cm	135	# 7	289	55.6	9.1	114.2	0.168	0.042
135-165cm	165	# 8	110	99.2	8.8	73.3	0.194	0.064
165-200cm	200	# 9	184	83.7	9.5	76.3	0.153	0.032

Heterotrophic plate count

Depth	Soil depth (cm)	Sample	HPC @ 25°C[#]	HPC @ 35°C[#]	% Moisture	HPC @ 25°C*	HPC @ 35°C*
0-15 cm	15	# 0	2030000	2660000	0.99	2050000	2740000
15-30 cm	30	# 1	2470000	2660000	2.91	2540000	2740000
30-45 cm	45	# 2	1260000	2110000	3.77	1310000	2190000
45- 55cm	55	# 3	370000	720000	4.06	386000	750000
55- 75cm	75	# 4	105000	220000	4.47	110000	230000
75- 100cm	100	# 5	17000	70000	8.15	18500	76200
100-115cm	115	# 6	7100	30000	7.83	7700	32000
115-135cm	135	# 7	8100	10000	11.66	9150	11000
135-165cm	165	# 8	22300	10000	13.26	26000	11500
165-200cm	200	# 9	20700	12000	11.99	23500	13500

*Where # refers to 1gram of wet mass, and * refers to 1 gram of dry mass.*

Groundwater Data

*Groundwater data (*default unit mg/L)

LAB NUMBER	SAMPLE ID	SAMPLE DATE	K	Na	Ca	Mg	SO4	Cl	Alkalinity as $CaCO_3$	$NO_3^+ NO_2$ as N mg/L	DOC
2457	**Mar 9**	08-Jun	20	169	68	49	114	185	289	23	<1
2458	**Mar 10**	08-Jun	31	270	77	85	222	346	301	32	1.0
2459	**Mar-23**	09-Jun	21	214	58	49	133	212	313	19	<1

LAB NUMBER	SAMPLE ID	SAMPLE DATE	EC mS/m (25°C)	pH (Lab) (25°C)	% Difference	CATIONS meq/L	ANIONS meq/L
2457	**Mar 9**	08-Jun	148	7.7	2.08	15.33	15.01
2458	**Mar 10**	08-Jun	224	8.2	3.08	23.39	22.69
2459	**Mar-23**	09-Jun	161	7.8	2.42	16.77	16.37

APPENDIX C:

DATA FROM BENCH SCALE

DENITRIFICATION TESTS

Carbon substrate effect on denitrification, soil depth 0.5m

Day	Treatment	Acetate (mg/l)	Cl (mg/l)	NO_3 (mg/l)	% NO_3 removed	SO_4 (mg/l)	EC (mS.cm^{-1})	Br (mg/l)	NO_2 (mg/l)
0				121.3	0.00				
1		0.21	436	121.3	0.00	423	2.545	3	0
7	nil C source	0.22	439	128	-5.52	427	2.855	4	0
14		22.27	386.04	131.47	-8.38	422.65	2.735	16.04	0
30		10.94	530.37	161.09	-32.80	467.99	2.41	3.07	0
0				121.3	0.00				
1		0.88	458	115	5.19	432	2.76	4	0
7	Excess Glucose	3.42	414	24	80.21	435	3.055	1	12
14		31.85	361.79	5.79	95.23	376.91	2.82	16.15	0
30		402.23	511.55	0	100.00	473.37	3.885	2.06	0
0				121.3	0.00				
1		0.42	450	128	-5.52	417	2.545	4	0
7	Methanol	0.26	458	117	3.54	428	2.785	4	0
14		20.38	383.22	59.86	50.65	414.48	2.595	14.75	0
30		7.96	521.77	1.37	98.87	434.99	2.375	2.65	0
0				121.3	0.00				
1		0.10	456	117	3.54	438	2.7	3.3	0
7	Sawdust	0.33	454	10	91.76	445	2.905	6	35
14		16.66	393.17	9.53	92.14	427.59	2.845	18.33	0
30		6.45	534.11	0	100.00	464.07	2.59	2.67	0
0				121.3	0.00				
1		1.07	413	101	16.74	438	2.675	1.2	0
7	Mielie Meal	35.44	434	0	100.00	419	3.14	2	0
14		283.67	399.92	9.51	92.16	376.19	4.045	14.01	0
30		340.18	474.63	0	100.00	220.36	4.39		56.75

Carbon substrate effects on denitrification: Soil Sample Depth 1.1m

Day	Treatment	Acetate (mg/l)	Cl (mg/l)	NO$_3$ (mg/l)	% NO$_3$ removed	SO$_4$ (mg/l)	EC (mS.cm^{-1})	Br (mg/l)	NO$_2$ (mg/l)
0				127	0				
1		21.19	403	127	0.00	222	2.51	15	0
7	nil C source	24.14	398	131	-3.15	230	2.57	14	0
14		21.37	380.03	128.96	-1.54	227.44	2.555	16.01	0
30		8.88	535.23	147.94	-16.49	253.32	2.305	1.95	0
0				127	0.00				
1		18.82	403	127	0.00	229	2.7	15	0
7	Glucose	120.81	392	0	100.00	400	3.045	14	0
14		114.91	393.27	10.75	91.54	229.25	3.245	15.63	0
30		430.85	606.15	0	100.00	263.83	4.205	0	0
0				127	0.00				
1		22.60	404	133	-4.72	230	2.53	16	0
7	Methanol	20.54	376	120.7	4.96	418	2.6	17	42
14		20.48	395.73	16	87.40	236.37	2.5	14.25	0
30		8.93	537.35	1.06	99.17	251.79	2.355	0	0
0				127	0.00				
1		19.56	396	128.5	-1.18	227	2.67	13	0
7	Sawdust	22.81	390	29.7	76.61	231	2.77	16	46
14		17.63	399.71	13.08	89.70	242.59	2.675	11.03	0
30		9.06	540.42	0	100.00	251.02	2.635	3.31	0
0				127	0.00				
1		19.57	395	118	7.09	226	2.675	15	0
7	Mielie Meal	29.98	386	11	91.34	420	3.375	15	38
14		297.58	405.47	2.6	97.95	208.67	4.405	6.73	0
30		236.95	487.44	0	100.00	65.08	4.87	1.44	0

Effect of C:N ratio

Day	Treatment	Time	Acetate (mg/l)	Cl (mg/l)	NO_3 (mg/l)	% NO_3 removed	SO_4 (mg/l)	Ca (mg/l)	Mg (mg/l)	Na (mg/l)	K (mg/l)	pH	EC (mS.cm^{-1})	NO_2 (mg/l)
0		0	0	403	127	0	222					8.55	2.44	0
1		1	0.43	431	95.3	24.9	254	4.7	4.6	582	141	8.4	2.94	13.5
7	1:25 gluc	7	11.9	432	21	83.5	243	24.4	33.8	612	177	7.46	3.18	4
14		14	64.1	492	0	100	243	56.1		710	192	7.08	3.73	0
30		30	8.72	464	0	100	139	145	106.9	705	232	7.58	3.58	0
0		0	0	403	127	0	222					8.59	2.47	0
1		1	0.33	435	108	14.7	257.4	6.1	3.7	620	146.6	8.36	2.89	7.1
7	1:50 gluc	7	11.2	414	16	87.3	255.2	15.8	33	678	187.9	7.51	2.98	0
14		14	27.2	434	0	100	265.4	92.9	505	811	208.0	6.89	3.98	0
30		30	66.6	476	0	100	207.8	381	3846	828	266.9	6.49	5.20	0
0		0	0	403	127	0	222					8.51	2.33	0
1		1	0	431.1	98.2	22.7	245	4.64	3.9	644.2	145.6	8.36	2.88	20.4
7	1:75 gluc	7	0	424.2	13.3	89.4	252	11.1	31.6	705.8	199.8	7.48	3.09	0
14		14	17.9	429.7	0	100	252	65.7	385.3	649.1	209.3	7.02	3.72	0
30		30	21.6	436.1	0	100	251	310.3	3330.2	893.3	260.9	6.48	4.77	0
0		0	0	403	127		222					8.46	2.34	0
1		1	0.13	426.7	126.4	0.46	252	5.19	3.86	520.9	129.8	8.28	2.87	0
7	0.1g saw	7	0.14	442.5	57.3	54.83	263	4.52	4.44	549.4	128	8.50	2.55	42.6
14		14	0.06	438.8	51.2	59.65	243	6.21	4.08	565.2	137.8	8.47	2.78	15.2
30		30	0.27	501.2	9.23	92.73	278	9.85	5.48	711.6	144	8.72	2.37	20.5
0		0	0	403	127	0	222					8.52	2.49	0
1		1	0.08	432.3	110.2	13.22	257	5.60	4.07	513.7	140.1	8.45	2.86	14.9
7	0.2g saw	7	0.17	437.1	35.2	72.24	262	6.99	4.71	677.4	144.1	8.54	2.48	38.0
14		14	0.07	439.4	0	100	239	5.20	3.91	606.4	115	8.37	2.61	0
30		30	0.15	471.7	0	100	243	13.4	9.45	679.9	157.5	8.27	2.46	0
0		0	0	403	127	0	222					8.48	2.48	0
1		1	0.11	410	107	15.7	247	6.79	4.41	575.7	131.1	8.28	2.91	18
7	0.3g saw	7	0.38	436.7	19.62	84.5	256	8.65	5.17	660.5	137.6	8.45	2.57	18.2
14		14	0.30	409.2	0	100	237	7.25	4.46	642.9	122.6	8.75	2.59	0
30		30	0	472.2	0	100.00	238	16.2	11.97	784.2	137.5	8.15	2.28	0

Sawdust as a substrate using 75-100cm soil source

Treatment	Time (hrs)	K	Na	Ca	Mg	NH₄ as N	SO₄	Cl	Alkalinity	NO₂-N	NO₃-N	Si	DOC	EC	pH	HPC
	0	74	372	14	13	<0.1	150	285	322	<0.1	23	23.4	2.6	200	8.4	2115
	3	73		-	-	<0.1	150	261	322	<0.1	23		6.0	210	8.4	2960
	6	69		-	-	<0.1	170	297	344	<0.1	23		4.5	210	8.4	3020
	12	81		-	-	<0.1	167	300	196	<0.1	23		5.4	215	8.5	40000
	24	78		-	-	<0.1	152	290	337	<0.1	23		4.9	210	8.4	1800000
untreated	48	70				0.13	169		322	<0.1	22.00		4.4	205	8.4	2400000
	96	66				<0.1	173		331	<0.1	22.00		2.7	210	8.5	3500000
	168	70				<0.1	167		326	<0.1	22.00		3.1	209	8.4	1500000
	240	84	372	14	13	<0.1	178	290	337	0.2	21.3	18	2.2	215	8.4	1650000
	336	74				<0.1	173		302	<0.1	23.00		2.4	196	8.4	1500000
	672	86	392	13	12	<0.1	150	304	335	<0.1	24.00	20	2.6	211	8.4	
	0	76	394	18	16	<0.1	156	303	345	<0.1	25	24.2	10.7	215	8.4	2165
	3	76		-	-	<0.1	155	283	342	<0.1	23		9.7	215	8.4	3010
	6	78		-	-	<0.1	177	303	342	<0.1	23		7.1	215	8.4	650
	12	78		-	-	<0.1	160	297	343	<0.1	23		10.9	215	8.4	7000
	24	78		-	-	<0.1	152	271	340	<0.1	23		11.4	210	8.3	5000000
0.1g sawdust	48	70				<0.1	169		348	1.9	19.5		6.3	211	8.2	4000000
	96	71				<0.1	175		363	2.4	18.1		4.3	214	8.3	19000000
	168	74				<0.1	181		379	5.8	13.6		8.8	215	8.3	6000000
	240	84	372	16	15	<0.1	181	293	374	1.9	18.8	16	3.4	225	8.2	8400000
	336	76				<0.1	179		371	7.6	11.9		6.0	203	8.2	4500000
	672	91	405	19	18	<0.1	158	306	403	4.6	15.4	28	4.1	217	8.1	

Default unit mg/L, EC mS/m (25º C), pH (Lab) (20º C)

Sawdust as a substrate continued

Treatment	Time (hrs)	K	Na	Ca	Mg	NH$_4$ as N	SO$_4$	Cl	Alkalinity	NO$_3$ + NO$_2$ as N	NO$_2$ as N	Fe	Mn	Si	DOC	EC	pH	HPC
	0	79	409	16	15	<0.1	165	310	346	25	<0.1	<0.05	<0.05	26.6	13.4	218	8.4	2040
	3	73	0	-	-	<0.1	157	292	340	23	<0.1	<0.05	<0.05	0.0	15.1	215	8.4	2185
	6	78	0	-	-	<0.1	177	297	342	23	<0.1	<0.05	<0.05	0.0	13.5	210	8.4	2735
	12	78	0	-	-	<0.1	157	297	344	23	<0.1	<0.05	<0.05	0.0	11.3	215	8.4	14000
	24	80	0	-	-	<0.1	152	307	348	23	0.35	<0.05	<0.05	0.0	15.5	215	8.1	5000000
0.2g sawdust	48	67				<0.1	158		339	20	3.5				8.6	205	8.2	4000000
	96	70				<0.1	180		391	19	6.5				7.6	216	8.2	14000000
	168	74				<0.1	183		400	15	6.6				16	211	8.1	8000000
	240	84	372	22	19	<0.1	188	293	421	15	8.6	<0.05	<0.05	23.27	9.56	225	8.1	7200000
	336	80				<0.1	181		388	16	8.3				7.8	201	8.2	15000000
	672	90	400	25	22	<0.1	172	300	445	15.00	12.3			29	7.9	220	8	8000000
	0	76	398	18	17	<0.1	149	313	345	25	<0.1	<0.05	<0.05	27.3	17.2	215	8.3	2620
	3	78	0	-	-	<0.1	158	292	347	23	<0.1	<0.05	<0.05	0.0	21.0	215	8.3	2930
	6									-	-	-	-	-	-	-	-	4360
	12	78	0	-	-	<0.1	152	297	343	23	<0.1	<0.05	<0.05	0.0	9.1	215	8.3	125000
	24	79	0	-	-	<0.1	153	310	350	23	0.26	<0.05	<0.05	0.0	20.9	215	8.2	5000000
0.3g sawdust	48	65				<0.1	167		357	19	4.2				15	210	8	4000000
	96	68				<0.1	191		406	17	9.6				11	215	8.1	19000000
	168	77				<0.1	183		418	12	3.7				18	214	8.2	16000000
	240	84	372	22	19	<0.1	190	297	450	9.1	6.7	<0.05	<0.05	24	12	220	8.3	11000000
	336	77				<0.1	185	399	399	12	11				17	199	8.2	7000000
	672	89	403	24	22	<0.1	191	302	491	4.00	0.5			29	15	221	8.2	7000000

Default unit mg/L, EC mS/m (25º C), pH (Lab) (20º C)

Sawdust as a substrate results using 165-200cm soil source

Treatment	Time (hrs)	K	Na	Ca	Mg	NH$_4$ as N	SO$_4$	Cl	Alkalinity	NO$_3$ +NO$_2$ as N	NO$_2$ as N	Fe	Mn	Si	DOC	EC mS/m (25°C)	pH (20°C)	TDS	HPC
	0	59	308	26	21	<0.1	114	238	328	21	<0.1	<0.05	<0.05	30.5	3.2	180	8.2	1152	5850
	3	60	0	-	-	<0.1	130	235	330	20	<0.1	<0.05	<0.05		2.8	185	8.3	1184	5500
	6	62	0	-	-	<0.1	134	232	426	20	<0.1	<0.05	<0.05		3.3	180	8.2	1152	4700
	12	62	0	-	-	<0.1	121	232	327	20	<0.1	<0.05	<0.05		4.1	180	8.3	1152	62000
	24	64	0	-	-	<0.1	123	232	326	19	<0.1	0.06	<0.05		3.8	180	8.2	1152	90000
untreated	48	53				<0.1	126		317	18	<0.1				2.6	175	8.3	1120	600000
	96	51				<0.1	130		317	18	<0.1				1.8	178	8.3	1139	4000000
	168	59				<0.1	128		315	19	<0.1				2.4	175	8.4	1120	1000000
	240	67	300	22	18	<0.1	139	237	328	19	0.2	<0.05	<0.05	29	2.6	185	8.3	1184	1500000
	336	60				<0.1	187	282	282	20	<0.1				1.6	161	8.4	1030	1100000
	672	72	301	23	20	<0.1	120	248	322	20	<0.1			35	2.2	168	8.3	1075	620000
	0	60	308	25	21	<0.1	131	238	328	21	<0.1	<0.05	<0.05	30.5	7.7	180	8.2	1152	4600
	3	62	0	-	-	<0.1	130	230	330	20	<0.1	<0.05	<0.05		11.7	180	8.2	1152	5650
	6	62	0	-	-	<0.1	133	232	327	19	<0.1	<0.05	<0.05		11.5	180	8.1	1152	5100
	12	62	0	-	-	<0.1	117	232	325	20	<0.1	<0.05	<0.05		6.2	175	8.2	1120	160000
	24	63	0	-	-	<0.1	121	232	329	20	<0.1	<0.05	<0.05		14.5	180	8.0	1152	160000
0.1g sawdust	48	55				<0.1	124		328	19	0.38				5.3	179	8.2	1146	3000000
	96	53				<0.1	130		338	18	0.68				7.4	180	8.1	1152	6300000
	168	57				<0.1	129		334	18	0.75				8.8	175	8.0	1120	3000000
	240	65	297	22	21	<0.1	138	230	348	19	2.6	<0.05	<0.05	34	4.2	185	8.1	1184	5000000
	336	60				<0.1	150		324	18	2.1				7.7	164	8.3	1050	2700000
	672	72	321	25	22	<0.1	145	244	357	19	0.44			35	3.6	183	8	1171	1500000

Default unit mg/L, EC mS/m (25º C), pH (Lab) (20º C)

Sawdust as a substrate results continued

Treatment	Time (hrs)	K	Na	Ca	Mg	NH$_4$ as N	SO$_4$	Cl	Alkalinity	NO$_3$ +NO$_2$ as N	NO$_2$ as N	Fe	Mn	Si	DOC	EC mS/m (25°C)	pH (20°C)	TDS	HPC
	0	68	366	13	12	<0.1	146	256	342	21	<0.1	<0.05	<0.05	28.9	18.3	190	8.4	1216	4650
	3	62	-	-	-	<0.1	130	227	327	19	<0.1	<0.05	<0.05	-	19.6	180	8.1	1152	6000
	6	62	-	-	-	<0.1	133	232	323	19	<0.1	<0.05	<0.05	-	23.1	180	8.1	1152	5850
	12	62	-	-	-	<0.1	127	232	330	20	<0.1	<0.05	<0.05	-	17.7	180	8.1	1152	70000
	24	64	-	-	-	<0.1	121	235	331	19	<0.1	<0.05	<0.05	-	22.8	180	7.9	1152	5000000
0.2g sawdust	48	60	-	-	-	<0.1	127		339	17	3.8	-	-	-	11	180	8.0	1152	4000000
	96	54	-	-	-	<0.1	133		350	18	2.0	-	-	-	11	180	8.1	1152	13000000
	168	61	-	-	-	<0.1	138		362	17	5.7	-	-		16	179	8.1	1146	4000000
	240	65	283	25	21	<0.1	141	223	364	15	7.2	<0.05	<0.05	32	9.4	180	8.2	1152	6900000
	336	61				<0.1	150		324	18	2.7				6.8	164	8.2	1050	4500000
	672	72	309	29	27	<0.1	142	235	393	13.00	9.0			44	13	180	7.9	1152	3500000
	0	61	316	25	21	<0.1	136	238	328	21	<0.1	<0.05	<0.05	30.8	27.8	180	8.0	1152	6250
	3	69	-	-	-	<0.1	165	230	330	19	<0.1	<0.05	<0.05	0.0	29.5	180	8.3	1152	6000
	6	63	-	-	-	<0.1	133	232	328	19	<0.1	<0.05	<0.05	0.0	36.3	180	8.1	1152	3605
	12	62	-	-	-	<0.1	129	235	326	20	<0.1	<0.05	<0.05	0.0	18.0	180	8.2	1152	100000
	24	102	-	-	-	<0.1	122	265	328	19	<0.1	<0.05	<0.05	0.0	23.6	210	8.2	1344	5000000
0.3g sawdust	48	56	-	-	-	<0.1	131	-	328	18	3.3	-	-	-	17	175	8.0	1120	4000000
	96	55	-	-	-	<0.1	130	-	357	17	3.7	-	-	-	15	180	8.1	1152	15000000
	168	65	-	-	-	<0.1	152	-	374	15	10	-	-	-	27	180	8.1	1152	7000000
	240	69	303	32	23	<0.1	161	233	409	12	11	<0.05	<0.05	36	18	185	8.2	1184	6500000
	336	64				<0.1	161		350	15	9.7				20	164	8.1	1050	5400000
	672	74	313	33	28	<0.1	147	237	415	13.00	10.4			45	13	185	7.9	1184	5000000

Default unit mg/L, EC mS/m (25º C), pH (Lab) (20º C)

Further Investigation of Sawdust as a Substrate

Date	Treat-ment	Time (hrs)	Time (days)	K	Na	Ca	Mg	NH₄⁺ as N	SO₄	Cl	Alk as CaCO₃	NO₃ + NO₂ as N	NO₃ as N	NO₂ as N	Fe	DOC	EC	pH
20/03/2006	0.3gA	0	0	68	369	8.1	13	<0.1	162	286	267	24.0	24.0	0.0	<0.05	20	200	8.7
23/03/2006	0.3gA	72	3								321	18.0	8.3	9.7		29	210	8.3
27/03/2006	0.3gA	168	7								378	14.5	2.4	12.1		31	215	8.0
30/03/2006	0.3gA	240	10	91	403	18	16	<0.1	202	298	395	12	0.0	12		25	212	8.2
03/04/2006	0.3gA	336	14								420	12	0.0	12		23	221	8.2
10/04/2006	0.3gA	504	21								424	7.9	0.3	7.6	24	24	214	8.2
18/04/2006	0.3gA	696	29								446	5.3	0.0	5.3	15	15	217	8.0
02/05/2006	0.3gA	1032	43	87.6	393.3	27.9	23.1		222.7	290	471.5	4.1	0	4.1		14	228	8.0
20/03/2006	0.3gB	0	0	69	376	8.1	13	<0.1	162	290	264	24.0	24.0	0.0	0.18	22	203	8.7
23/03/2006	0.3gB	72	3								330	19.0	9.2	9.8		31	215	8.2
27/03/2006	0.3gB	168	7								378	14.2	2.1	12.1		31	220	8.0
30/03/2006	0.3gB	240	10	94	417	18	15	<0.1	202	307	403	13	0.0	13		22	221	8.1
03/04/2006	0.3gB	336	14								429	12	0.0	12		26	222	8.2
10/04/2006	0.3gB	504	21								443	8.1	-0.03	8.1	22	22	211	8.1
18/04/2006	0.3gB	696	29								464	3.6	0.1	3.5		21	224	8.1
02/05/2006	0.3gB	1032	43	89.2	403.7	19.6	20.0		210.9	288	469.5	1.4	0	1.4		14.1	225.0	8.1
20/03/2006	0.3gC	0	0	69	376	8.1	14	<0.1	168	292	268	24.0	24.0	0.0	0.07	27	205	8.7
23/03/2006	0.3gC	72	3								327	20.0	10.0	10.0		28	215	8.2
27/03/2006	0.3gC	168	7								378	15.0	2.1	12.9		12	220	8.2
30/03/2006	0.3gC	240	10	92	410	17	15	<0.1	202	309	396	13	0.0	13		21	219	8.1
03/04/2006	0.3gC	336	14								427	12	0.0	12		22	221	8.2
10/04/2006	0.3gC	504	21								426	10	0.04	10	20	20	214	8.1
18/04/2006	0.3gC	696	29								450	3.4	0.47	2.9		18	217	8.1
02/05/2006	0.3gC	1032	43	88.4	393.3	20.8	21.7		203.1	280	476	2.3	0.5	1.8		13.2	224.0	8.1
20/03/2006	0.3gD	0	0	69	379	8.2	14	<0.1	165	292	267	25.0	25.0	0.0	0.11	30	205	8.6
23/03/2006	0.3gD	72	3								327	20.0	9.0	11.0		26	215	8.2
27/03/2006	0.3gD	168	7								375	14.4	1.9	12.6		30	221	8.2
30/03/2006	0.3gD	240	10	91	403	17	16	<0.1	202	305	400	12	0.0	12		21	219	8.1
03/04/2006	0.3gD	336	14								426	15	2.4	13		21	225	8.2
10/04/2006	0.3gD	504	21								427	8.9	1.74	7.1	20	20	215	8.1
18/04/2006	0.3gD	696	29								453	5.8	0.5	5.3		16	221	8.1
02/05/2005	0.3gD	1032	43	90.9	407.4	20.2	21.0		203.1	287.0	473.5	6.3	0.9	5.4		13.2	226.0	8.0

Default unit mg/L, EC mS/m (25º C), pH (Lab) (20º C)

Date	Treatment	Hours	Days	K	Na	Ca	Mg	NH_4 as N	SO_4	Cl	Alkalinity	NO_3+NO_2 as N	NO_3 as N	NO_2 as N	Fe	DOC	EC	pH
20/03/2006	0.5gA	0	0	71	373	8.5	14	<0.1	166	296	259	23.0	23.0	0.0	0.07	47	200	8.5
23/03/2006	0.5gA	72	3								351	18.0	7.0	11.0		54	220	8.1
27/03/2006	0.5gA	168	7								409	10.0	0.0	10.0		54	223	8.0
30/03/2006	0.5gA	240	10	93	417	18	16	<0.1	207	307	435	4.2	1.2	3.0		39	221	8.1
03/04/2006	0.5gA	336	14								461	2.7	0.0	2.7		38	218	8.3
10/04/2006	0.5gA	504	21								479	0.0	0.0	0.0	42	42	215	8.2
18/04/2006	0.5gA	696	29								509	0	0.0	0.0		35.87	225	8.1
02/05/2006	0.5gA	1032	43	88.0	396.7	25.4	26.9		199.2	289.0	515.5	0.0	0.0	0.0		30.7	226.0	7.9
20/03/2006	0.5gB	0	0	71	376	8.7	13	<0.1	175	289	264	24.0	24.0	0.0	0.08	47	205	8.5
23/03/2006	0.5gB	72	3								349	18.0	6.0	12.0		47	220	8.2
27/03/2006	0.5gB	168	7								412	8.9	0.0	8.9		52	225	8.1
30/03/2006	0.5gB	240	10	92	408	18	16	<0.1	203	305	431	3.2	0.5	2.7		38	216	8.0
03/04/2006	0.5gB	336	14								454	4.9	0.0	4.9		42	221	8.3
10/04/2006	0.5gB	504	21								499	0.0	0.0	0.0		39	218	8.2
18/04/2006	0.5gB	696	29								478.5	0	0.0	0.0		34.78	222	8.1
02/05/2006	0.5gB	1032	43	87.6	393.3	23.8	26.2		203.1	283.0	510.5	0.0	0.0	0.0		29.2	225.0	7.9
20/03/2006	0.5gC	0	0	69	363	8.7	14	<0.1	168	281	259	23.0	23.0	0.0	0.08	43	200	8.5
23/03/2006	0.5gC	72	3								344	17.0	6.0	11.0		54	220	8.0
27/03/2006	0.5gC	168	7								365	6.9	0.0	6.9		42	220	8.0
30/03/2006	0.5gC	240	10	91	407	17	16	<0.1	203	307	425	3.1	0.0	3.1		40	214	8.0
03/04/2006	0.5gC	336	14								456	2.7	0.0	2.7		42	216	8.3
10/04/2006	0.5gC	504	21								484	0.0	0.0	0.0	37	37	214	8.2
18/04/2006	0.5gC	696	29								489.5	0	0	0.0		33.7	224	8.1
02/05/2006	0.5gC	1032	43	89.2	400.0	23.8	25.2		210.9	291.0	523.0	0.0	0.0	0.0		31.8	230.0	8.0
20/03/2006	0.5gD	0	0	69	363	8.1	13	<0.1	168	293	263	23.0	23.0	0.0	0.08	51	204	8.5
23/03/2006	0.5gD	72	3								350	17.0	4.0	13.0		44	220	8.0
27/03/2006	0.5gD	168	7								424	7.8	0.0	7.8		48	225	8.0
30/03/2006	0.5gD	240	10	92	407	19	16	<0.1	205	305	437	3.9	0.1	3.8		40	218	7.9
03/04/2006	0.5gD	336	14								454	4.8	0.0	4.8		42	220	8.2
10/04/2006	0.5gD	504	21								473	0.0	0.0	0.0		38	211	8.2
18/04/2006	0.5gD	696	29								480.5	0.0	0.0	0.0		30.43	225	8.1
02/05/2006	0.5gD	1032	43	90.0	407.4	21.7	23.6		214.8	294.0	487.5	0.0	0.0	0.0		30.2	228.0	8.0

Default unit mg/L, EC mS/m (25º C), pH (Lab) (20º C)

APPENDIX D:

CALCULATIONS AND DATA

EVALUATION

Table 18:   Ratio determining calculations for further investigation of sawdust as a substrate, page 100

	Total N	Total C		Total N	Total C
Soil					
75-100cm	0.032%	0.192%	Water		
165-200cm	0.032%	0.153%	mg/50ml	0.95	0
Water (mg/L)	19	0	mg/80ml	1.52	0
Sawdust	0.25%	53.25%			

In 1g soil	mg/g	OC (mg/g)	Sawdust		mg/g
75-100cm in 10g	3.2	0	2	5	1065
165-200cm in 10g	3.2	0	1	2.5	532.5
75-100cm in 20g	6.4	0	0.5	1.25	266.25
165-200cm in 20g	6.4	0	0.3	0.75	159.75
			0.2	0.5	106.5
			0.076	0.19	40.47

For 75-100cm (using 20g of soil)

Sawdust (g/kg) of soil	Sawdust (g)	C	N	C:N	C:N	moles C	moles N	Ratio C:N	Ratio N:C
50	1	532.5	10.42	51:1	1:51	44.4	0.74	60:1	1:60
25	0.5	266.25	9.17	29:1	1: 29	22.2	0.65	34:1	1:34
15	0.3	159.75	8.67	18.4:1	1:18	13.3	0.61	22:1	1:22
10	0.2	106.5	8.42	12.6:1	1:12.6	8.8	0.60	15:1	1:15
3.8	0.076	40.47	8.11	5:1	1:5	3.37	0.57	5.9:1	1:5.9

For 165-200cm (using 10g soil)

Sawdust (g/kg) of soil	Sawdust (g)	C	N	C:N	C:N	moles C	moles N	Ratio C:N	Ratio N:C
50	1	532.5	7.22	73:1	1:73	44.37	0.51	86:1	1:86
25	0.5	266.25	5.97	44.6:1	1: 44.6	22.18	0.42	52:1	1:52
15	0.3	159.75	5.47	29:1	1:29	13.3	0.39	34:1	1:34
10	0.2	106.5	5.22	20:1	1:20	8.8	0.37	24:1	1:24
5	0.1	53.25	4.91	10.8:1	1:10.8	4.43	0.35	12.6:1	1:12.6

Comparison of Data from Experiment 3 and 4

A comparison between these two experiments was considered to evaluate whether the data showed similar trends to assist in understanding the reproducibility of the method. Samples with identical make-up i.e. 0.3g sawdust treated samples with soil of the 75-100cm depth and 40mL groundwater used in experiment 3 and 4 were compared. Nitrate-N, Nitrite-N, and alkalinity was plotted vs. time (Figure 41-43) as well as nitrate vs. Alkalinity (Figure 43).
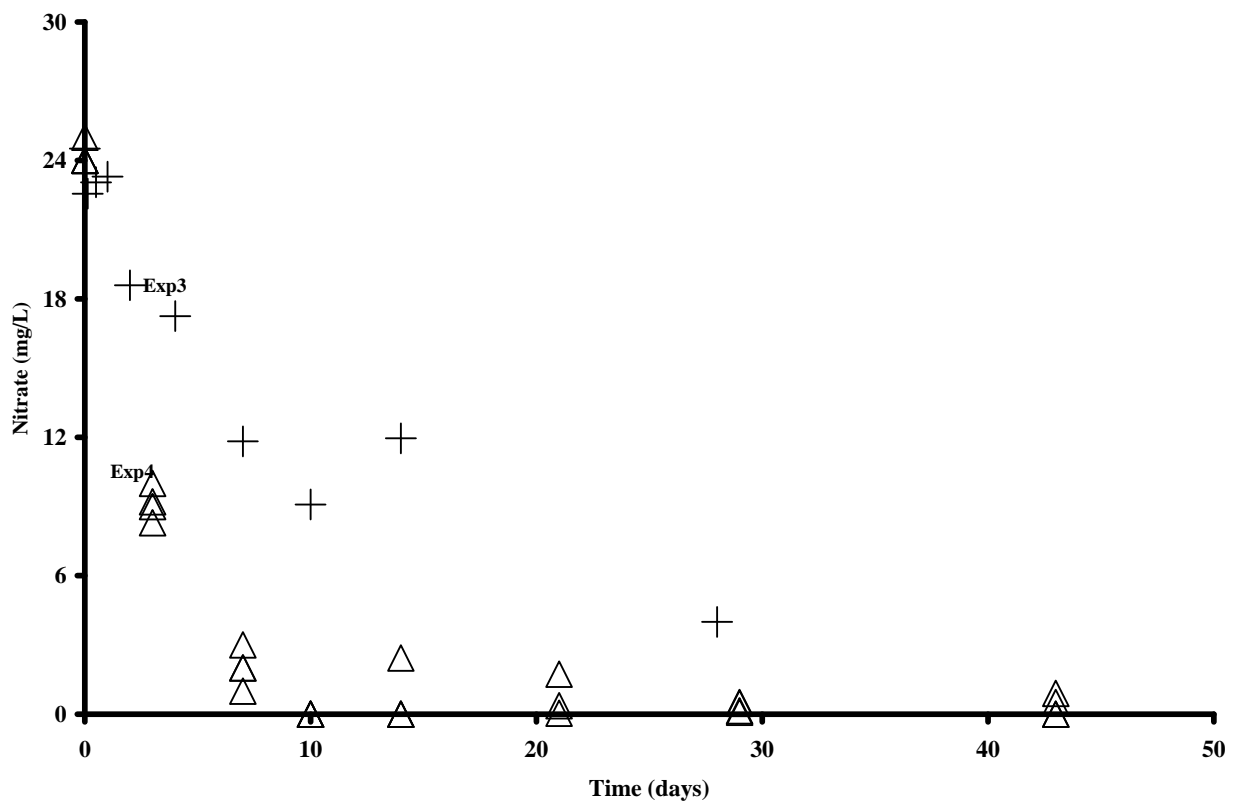

Figure 41:   Nitrate-N for the duration of incubation experiments. The + sign represents 0.3g sawdust treatment containing 40mL groundwater, 10g soil (75-100cm layer) incubated for 30 days (experiment 3), the triangle represents 0.3g sawdust, 40mL groundwater, 10g soil (75-100cm layer) incubated for 43 days and done in triplicate.
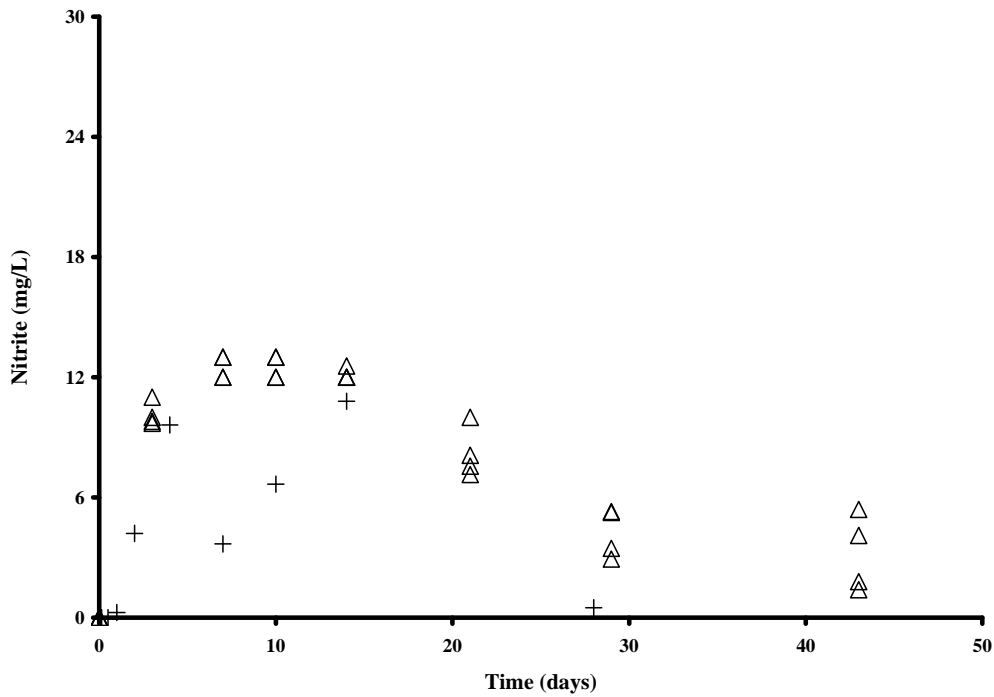
Figure 42: Nitrite-N for the duration of incubation. + represents 0.3g sawdust, 40mL groundwater, 10g soil(75-100cm layer) and 30 days incubation in experiment 3, while the triangle represents 0.3g sawdust, 40mL groundwater, 10g soil (75-100cm layer) and 43 days incubation in experiment 4 done in triplicate.
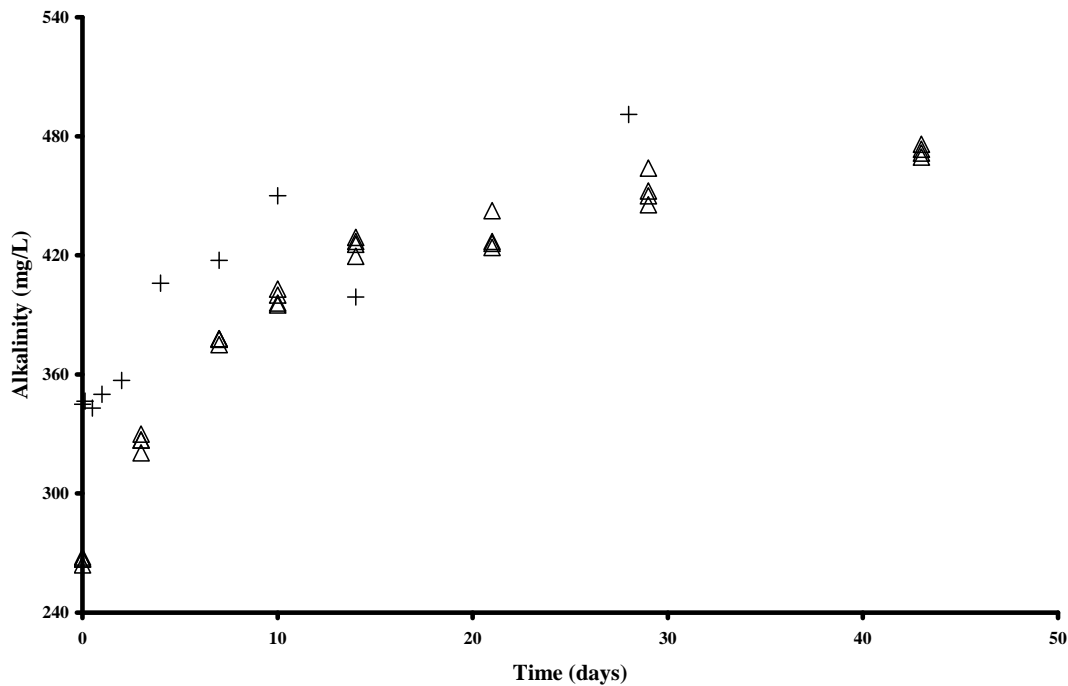

Figure 43: Alkalinity as $CaCO_3$ mg/L for the duration of incubation. The + sign represents 0.3g sawdust treatment containing 40mL groundwater, 10g soil (75-100cm layer) incubated for 30 days (experiment 3), the triangle represents 0.3g sawdust, 40mL groundwater, 10g soil (75-100cm layer) incubated for 43 days and done in triplicate.
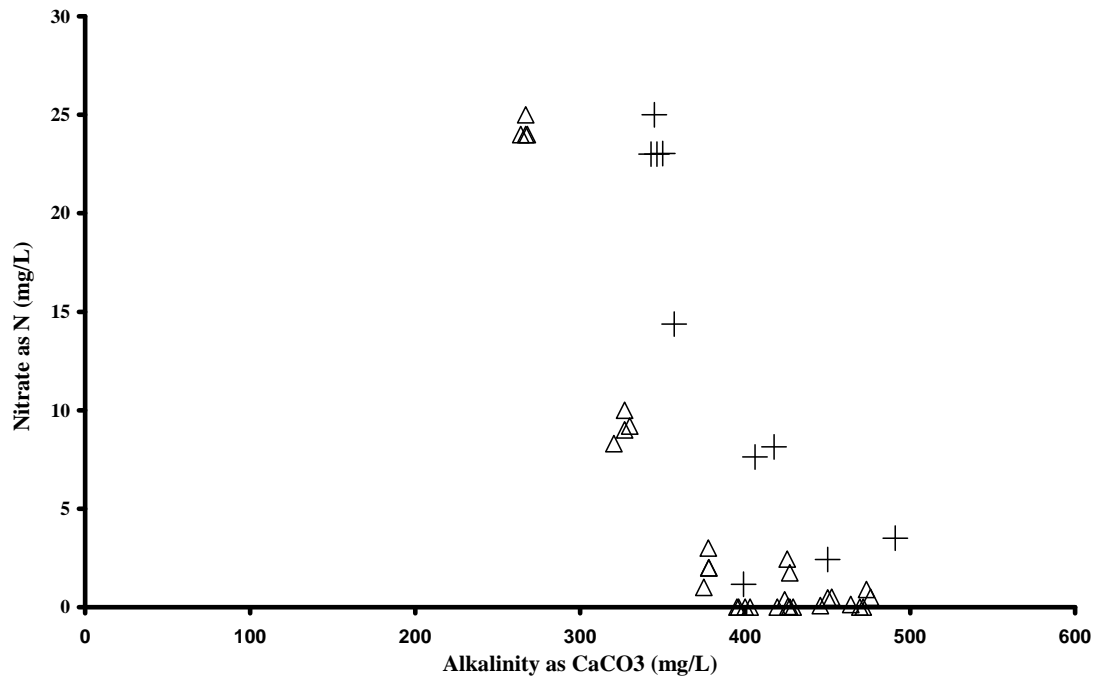
Figure 44:   Nitrate-N vs. Alkalinity for the duration of incubation experiments. The + sign represents 0.3g sawdust treatment containing 40mL groundwater, 10g soil (75-100cm layer) incubated for 30 days (experiment 3), the triangle represents 0.3g sawdust, 40mL groundwater, 10g soil (75-100cm layer) incubated for 43 days and done in triplicate.

Statistical Evaluation of data from Experiment 4

Samples were prepared and analysed in triplicate to get an overview of the spread of the data within a sampling time and to evaluate the repeatability of the experiment. Variability plots and box and whisker plots were prepared in JMP6 to show the mean and spread of the data for the two treatments used during this experiment Figures.
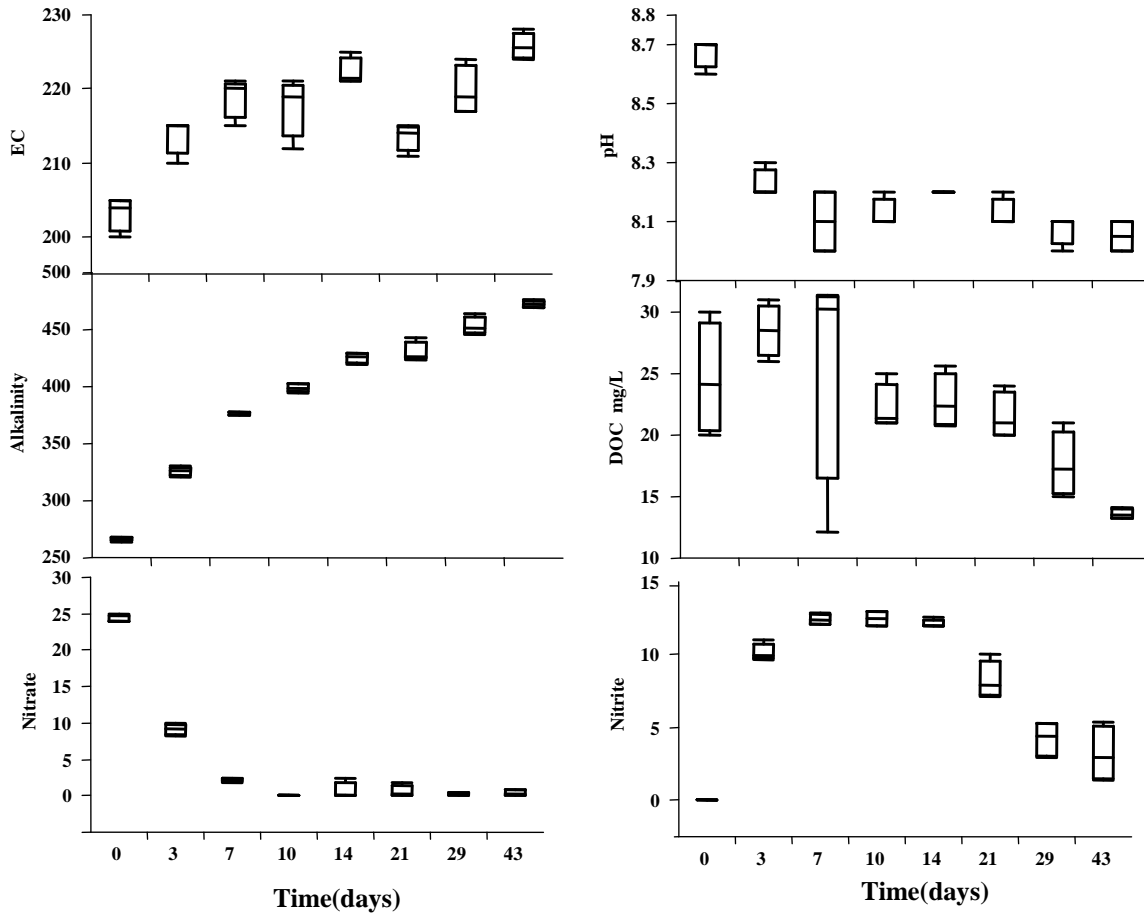
Figure 45: Box and whisker plots for indicator parameters analysed in triplicate for 43 day incubation experiments using 0.3g sawdust treated samples.
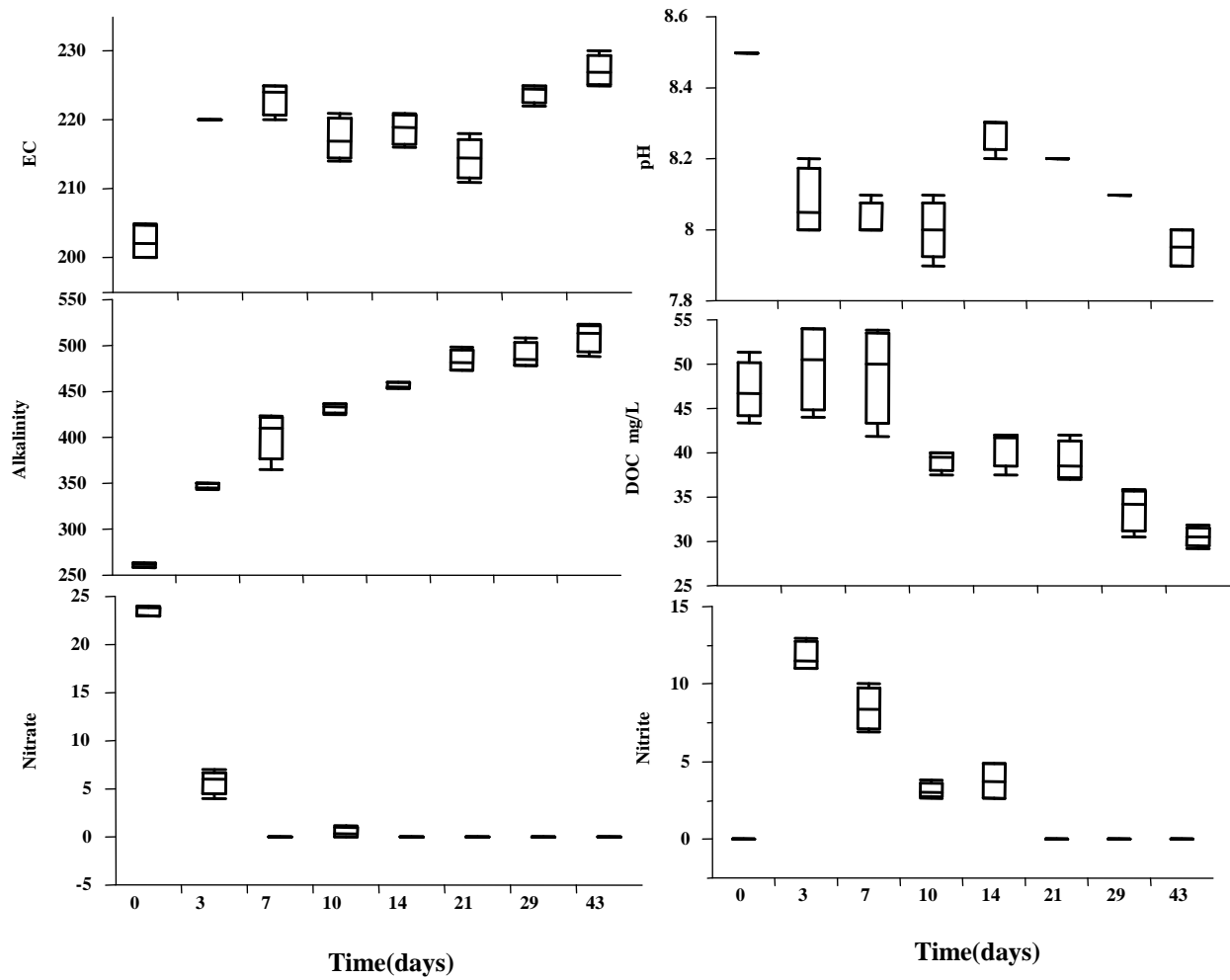
Figure 46:   Box and whisker plots for 43 day incubation experiments of 0.5g sawdust treated samples of soil and groundwater.

Correlation matrices were also prepared using JMP6 for each treatment to evaluate the degree of correlation between parameters analysed during the experiment Tables 19 and 20.

Table 19:   Correlation matrix of parameters during incubation denitrification experiment using 0.3 g sawdust (25 g/kg of soil), 10 g soil, 40 mL groundwater, incubated over a period of 43 days.

	Nitrate	pH	EC	Alkalinity	Nitrite	DOC
Nitrate	1.00					
pH	0.92	1.00				
EC	-0.80	-0.79	1.00			
Alkalinity	-0.89	-0.82	0.81	1.00		
Nitrite	-0.55	-0.48	0.35	0.15	1.00	
DOC	0.34	0.27	-0.42	-0.60	0.32	1.00

Table 20: Correlation matrix of parameters during incubation denitrification experiment using 0.5 g sawdust (50 g/kg of soil), 10 g soil, 40 mL groundwater, incubated over a period of 43 days

	Nitrate	pH	EC	Alkalinity	Nitrite	DOC
Nitrate	1.00					
pH	0.75	1.00				
EC	-0.80	-0.79	1.00			
Alkalinity	-0.87	-0.57	0.72	1.00		
Nitrite	-0.12	-0.28	0.24	-0.33	1.00	
DOC	0.43	0.31	-0.36	-0.72	0.67	1.00

END