

***NITRATE REMOVAL USING COMPOST AND PINE BARK
AS A CARBON SOURCE***



**Submitted in fulfillment of the requirements for the degree of Master of Science in
Engineering in the Civil Engineering Programme, University of Kwa Zulu Natal,
Durban, South Africa.**

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Life is a journey, not a destination.
(Aerosmith, 1984)

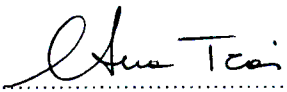
PREFACE

The work presented in this dissertation was carried out under the supervision of Dr. Cristina Trois of the School of Civil Engineering, Surveying and Construction, University of Kwa-Zulu Natal, Durban, South Africa. This dissertation has been compiled in accordance with *The Style Guide for Dissertations*, prepared by the Faculty of Engineering of the University of Kwa-Zulu Natal, Durban and represents work done by Giulia Pisano, unless otherwise stated in the text.

Date.....

.....

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Dr. Cristina Trois
Research supervisor

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ABSTRACT

Emissions resulting from waste degradation processes have a high polluting potential and are responsible for negative impacts on the environment. Landfill leachate is hazardous for the human health and the environment and requires treatment before being discharged, in order to comply with the South African legislation indicated in the Government Gazette, 1999. However, treated leachate may still contain high levels of nitrate that require an additional denitrification step. Biological denitrification occurs with the addition of a carbon source that increases the operational costs of the process, particularly if chemical compounds are employed (such as methanol). This research aims to explore the efficiency of denitrification using low cost carbon sources such as garden refuse compost and pine bark, easily available in South Africa and currently disposed of in general waste landfills. Denitrification processes in fixed bed reactors were simulated in laboratory scale leaching columns packed with immature compost and pine and irrigated with biologically treated leachate from the Mariannhill landfill site Sequencing Batch Reactor (SBR). The overall objective is to assess efficiency, kinetics and performance of the substrates using batch tests and columns tests. A secondary objective is to assess the feasibility of using organic waste compost and pine bark as by product of an integrated waste management system to denitrify landfill leachate.

INTRODUCTION

The discharge of landfill leachate is responsible for one of the highest impacts associated with the degradation of waste.

The technical-scientific community is striving towards the development of waste management strategies that minimize waste, improve reuse and recovery of materials and energy and aim at reducing harmful emissions to an acceptable level for humans and the environment (Stegmann, 1995; Driessen et al., 1995).

The environmental acceptability level of a landfill is related to quality and quantity of the emissions produced over a certain period of time which is generally set at 30 years of post-closure period. The permit requirements state that during this aftercare time, the landfill operating company is still responsible for control, management and treatment of the landfill emissions (DWAF, Minimum requirements, EU Landfill Directives).

Landfill leachate is usually treated in multi-reactor plants or Sequencing Batch Reactors (SBR) to reduce COD, solids and ammonia but treated leachates may still contain high nitrate concentrations that exceed the discharge limits. It is possible to denitrify these effluents by using a carbon source like methanol, ethanol, or natural matter like sugar, molasses and immature organic compost etc..

In the Ethekewini Municipality a suitable carbon source, in the form of compost from garden refuse, will be generated as part of a composting operation that falls under a ZERO WASTE operational strategy adopted by Durban Solid Waste in 2006. Currently, the three major eThekewini landfills receive an average of 3000 tons of garden refuse monthly, separately from the main waste stream. "Up to 1,5 million m³ of pine bark are produced as a by product of the paper and pulp industry in South Africa every year" (Trois and Polster, 2007). Most of this pine bark is disposed of in landfills. The Mariannhill Landfill site, in particular, will be soon operated in a close-loop mode, so that recyclables will be separated and recovered, putrescibles disposed, garden refuse and organic waste (including pine bark) diverted from the main stream and composted.

The focus of this project is to investigate the feasibility of using the organic compost that will be produced at the Mariannhill Landfill site and the pine bark to denitrify treated leachate from a Sequencing Batch Reactor also present at the site. This leachate, contains high levels of nitrates and is currently used as dust suppressant, hence does not require any further treatment. However, after closure of the landfill (expected in the

near future) the leachate will be discharged in the natural environment and it will then require specific attention. The employment of immature organic compost or the pine bark to denitrify the leachate will avoid excessive treatment costs, encouraging the development of a real integrated waste management strategy.

The main objectives of this research are:

- To assess the potential for nitrate removal (denitrification) of organic waste compost, in particular garden refuse compost and fresh pine bark.
- To study the kinetics and the efficiency of nitrate removal in relation to degree of maturity, biodegradability, size distribution and quality of organic substrates.
- To assess the applicability of using organic waste compost and pine bark as by product of an integrated waste management system to denitrify landfill leachate.

Pine bark, produced by Mondi paper and disposed at the Mariannhill Landfill site and local garden refuse compost were selected for this study. The effluent from the SBR at the Mariannhill landfill site was treated in pilot scale fixed beds reactors packed with the above mentioned organic substrates.

The solid substrates and the leachate were fully characterized to assess treatability, biodegradability and suitability for the treatment. Small scale anaerobic batch tests were performed to determine the removal kinetics of the two substrates in optimum conditions for microbial activity (surface contact, pH, temperature, oxygen content). The results of the batch tests constituted the foundations for the design of treatment trials in leaching columns operated as fixed bed reactors, again in optimum and controlled conditions.

The columns were monitored over a period of time to assess the efficiency of nitrate removal and the longevity in terms of performance of the substrates.

This dissertation is structured in six chapters:

The Literature review is presented in the chapters 1, 2 and 3, and it was designed to collect available information to understand the problems connected with landfill leachate production and nitrate contamination, the risk associated with human and environmental exposure to nitrogenous emissions from waste and treatment options.

The methodological approach followed in the research is shown in chapter 4. All the results of the present research are exposed and discussed in the chapter 5 while conclusions and recommendations are reported in chapter 6.

1 LANDFILL PROCESSES AND NITROGEN CYCLE IN LANDFILLS

1.1 Introduction

As the living standards and the growing population are increasing, both in South Africa and worldwide, large volumes of waste are generated posing a serious threat to the environment because of the liquid and gaseous emissions produced.

Since this work focuses primarily on denitrification of treated leachate, it is necessary to introduce landfill leachate, its compounds, in particular nitrogen compounds and their danger in relation to South African legislation.

1.2 Waste disposal management

Waste management involves collecting, transporting, processing and disposing of waste material (Figure 1.1). The two most common ways of disposal are incinerations and landfilling.

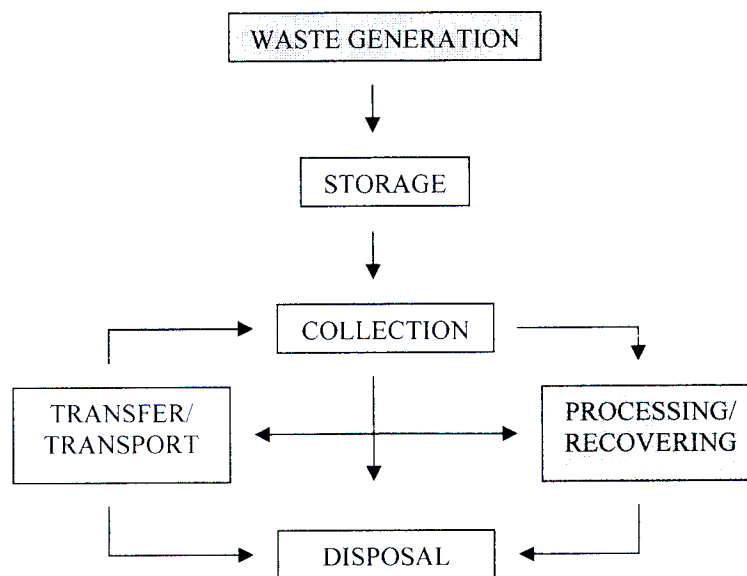


Figure 1.1 - Waste hierarchy

Landfilling is the most common practice in many countries as it is a relatively inexpensive method of disposing of waste materials, and in some cases the only available disposal option (Stegmann, 1995).

In South Africa, over 95% of all urban waste is disposed of in landfills (DWAF, 1998) and when using other forms of waste treatment, the landfill is required as a final disposal option for the residues (Peavy, 1985).

A sanitary landfill can be defined as “a scientific method of disposing of solid waste on land”, with the dual purpose of eliminating public and environmental hazards and minimizing nuisances without contamination of surface or groundwater resources (Lombard, 2005).

Incineration is the controlled thermal treatment of waste by burning and involves the stabilization of organic and/or hazardous compounds by means of thermal oxidation at high temperatures (750-1000°C); waste incineration prevents the production of methane or leachate and reduces the amount of waste to be landfilled by up to 90% of volume responding to the strong necessity of some countries of reducing waste volumes disposed in landfills.

This solution is adopted in some European countries like Germany or Sweden to eliminate most of the waste produced and the thermal energy is used for electricity generation during the combustion of the fractions with a high calorific value.

In South Africa, incineration is not employed for general waste disposal, but restricted to medical waste, because of the prohibitive costs associated with this high level technology.

1.3 Landfill processes and emissions

The landfill can be considered as a large reactor, an anaerobic digester in which waste and water (by rain, infiltration and waste humidity) are the inputs (Figure 1.2) and leachate and biogas are the products of biological and physical-chemical processes (Ghiani, 1997).

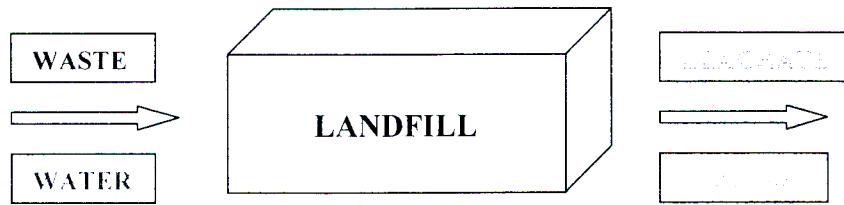


Figure 1.1 - Schematic view of inputs and outputs in landfill

Progressive decomposition of a significant proportion of putrescible organic substances in the waste is achieved by a population of micro organisms established within the waste body. (Gendebien et al., 1992).

The process of waste degradation produces harmful gases (biogas) that carry offensive odours and the moisture that percolates through the waste body becomes highly polluted (leachate).

Landfill leachate is a very complex, highly polluted liquid containing dissolved or suspended material leached out from the refuse: nutrients, large bacterial populations, pathogens, toxins and heavy metals (Peavy et al, 1985). Its nature depends on the type and composition of the waste, the moisture content of the waste body, on the state of waste biodegradation, and the landfill operational procedures (Andreottola et al., 1992). In most cases the landfill leachate may, through subsurface migration, contaminate groundwater resources and down stream surface waters (Christensen, 1989 and Robinson et al, 1992). Migrating leachate from a landfill site also has the potential to transport explosive landfill gas outside the boundaries of the site (Robinson et al, 1992). Landfill leachates may contain dissolved methane (CH_4) in concentrations of 10 to 15 mg/l, where a concentration of dissolved methane as low as 1.4 mg/l is capable of producing explosive atmospheres (Robinson, 2001).

1.4 Effects of waste deposition on leachate

Most of the available studies on the decomposition of waste in a landfill, both at full and laboratory scale, have identified 5 stages of biodegradation. The characteristics of the

leachate change noticeably during each one of the phases and before any type of treatment can be chosen, the variability and quality of the leachate must be evaluated and a basic understanding reached (Robinson and Gronow, 1998).

Research agrees on the following phases of degradation (Figure 1.3):

- I. Hydrolysis and aerobic degradation phase
- II. Acidogenic and acetogenic phase
- III. Anaerobic transitory phase
- IV. Methanogenic phase
- V. Final aerobic phase

1.4.1 Hydrolysis and aerobic degradation

During the first phase, polysaccharides are hydrolyzed into monosaccharides, fats into glycerin and fatty acids and proteins into amino acids. The hydrolysis is accelerated by the enzymatic catalysis of an established aerobic bacterial population (enzymatic hydrolysis).

The oxygen trapped in the waste body and in the upper part of the landfill, causes decomposition of the organic substances via *aerobic biological processes*.

During this phase several important biochemical reactions occur:

- From amino acids, as a result of an aerobic process, carbon dioxide, water, nitrates and sulphates are formed;
- Monosaccharides are transformed into carbon dioxide and water;
- Fatty acids and glycerin into simple catabolites (carbon dioxide and water) through the formation of volatile fatty acids (VFAs) and alkalis (Andreottola and Cannas, 1992);
- The enzymes degrade cellulose into glucose, which is consumed by bacteria thus transforming it into carbon dioxide and water.

Temperature of the fill may rise to around 60-70°C due to the exothermic nature of the biological oxidation reactions, it is a very fast reaction and biogas production is not marked because all of the oxygen is transformed into carbon dioxide (Ghiani, 1997).

No substantial leachate generation will occur (Andreottola and Cannas, 1992) but the COD (Chemical Oxygen Demand) is high with pH between 6 and 7.

1.4.2 Acidogenic and acetogenic phase

The first part of anaerobic degradation is the acid fermentation (Ghiani, 1997) and involves the biodegradation of complex organic materials, like glucose molecules, into simpler organics such as acetic acid (CH_3COOH), butyric acid ($\text{CH}_3\text{C}_2\text{H}_4\text{COOH}$), ethanol ($\text{CH}_3\text{CH}_2\text{OH}$), propionic acid ($\text{C}_2\text{H}_5\text{COOH}$) and pyruvic acid (CH_3COCOOH) or other simple organics and acids (Ehrig, 1989). In the second part, a large amount of volatile fatty acids such as acetic acid, hydrogen and carbon dioxide are produced as the result of the acetogenic bacterial activity.

The main characteristics of the leachate during this phase are as follows:

- Low pH (around 4-5) due to the high production of volatile fatty acids such as acetic acid (Qasim and Chiang, 1994) and the high partial pressure of CO_2
- High concentrations of volatile acids and inorganic ions such as Cl^- , SO_4^{2-} , Ca^{2+} , Mg^{2+} , and Na^+
- High BOD_5 (5-days Biochemical Oxygen Demand) values (commonly greater than 10,000 mg/l)
- High $\text{BOD}_5:\text{COD}$ ratios (commonly 0.7 and greater), which reflects the high proportion of readily biodegradable soluble organics in the fill (Andreottola and Cannas, 1994)
- Ammonia levels are between 500-1000 mg/l (Robinson, 1989).

During this phase the presence of biogas is evident and it is characterized by the reduction of nitrogen and oxygen and an increase in both carbon dioxide and hydrogen (Mulamoottil, 1998).

The transition to an anaerobic environment will cause sulphates to be reduced to sulphides, which may then induce the precipitation of various heavy metals contained in the leachate (Andreottola and Cannas 1992).

There are often only traces of methane released during this phase.

1.4.3 Anaerobic transitory phase

This is a transitory phase between the acetogenic conditions and the more stable methanogenic conditions. This phase can be rapid (in the range of months) but typically it takes several years (Robinson 1995a). This phase is characterized by the initial formation of methanogenic bacteria. These bacteria consume soluble organic compounds (VFA) and convert them into carbon dioxide (CO_2) and methane gas (CH_4).

The value of the pH increases because of the conversion of fatty acids.

In this more alkaline environment a decrease of calcium, iron, manganese and heavy metals in the leachate occurs (Andreottola and Cannas 1992).

Ammonia production reaches its peak during this phase and it will continue to be produced throughout the lifetime of the landfill.

Degradation of the ammonia does not occur in an anaerobic environment; therefore it will be removed only by flushing (Ghiani, 1997).

1.4.4 Methanogenic Phase

As a result of the dynamic equilibrium between acetogenic and methanogenic bacteria (Robinson 1995), and of neutral/alkaline values of pH reached (Ghiani, 1997), more methane is produced remaining stable between 50 and 60% in volume during this phase.

The volatile acids produced from the first stage of anaerobic decomposition along with other organic matter decrease because they are converted to methane and carbon dioxide (Qasim and Chiang 1994).

Leachate is characterized by a lower BOD/COD ratio, since by this phase the majority of organics have been degraded, and ammonia concentrations are steady (Andreottola and Cannas 1992).

This phase is the most biologically active and is of particular importance when examining the pollution potential of a landfill leachate because this phase lasts longer than the others (Robinson 1989) and the constituents in the leachate (referred in the literature as “stabilized”) remain fairly constant.

1.4.5 Final aerobic phase

When the decomposable substrate in the landfill has been degraded, there is a decrease in the population of bacteria and the production of volatile acids and methane.

This methane level is so low that air will diffuse from the atmosphere causing the redox potentials to rise and thus not allowing further methane production (Christensen and Kjeldsen 1989).

The COD concentrations in leachate, range around a few hundreds mg/l as a result of biorefractory organic matters (Ghiani, 1997). From this phase the values of BOD and BOD/COD ratios are relatively low (Robinson 1995a). As the matter in the landfill continues to be degraded, ammonia concentrations tend to remain high. Other inorganic materials such as iron, sodium potassium, sulphates, and chloride may continue to be found in leachate (Robinson 1995a; Robinson and Gronow 1995).

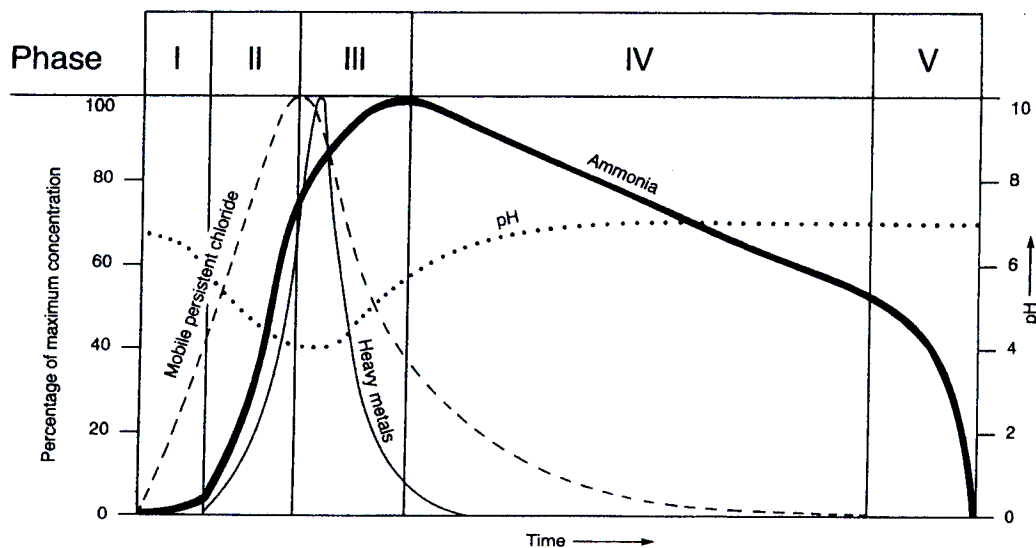


Figure 1.2 - Degradation phases in landfill (Qasim and Chiang, 1994)

As a result of the landfill processes, a typical landfill leachate composition is showed below (from Qasim and Chiang, 1994).

Table 1.1 - Typical landfill leachate composition (Qasim and Chiang, 1994).

Constituent	Unit	New Landfill (less than 2 years)		Mature landfill (greater than two years)
		Range	Typical	Range
BOD ₅ (5-day biochemical oxygen demand)	mg/l	2,000 – 30,000	10,000	100 – 200
TOC (total organic carbon)	mg/l	1,500 – 20,000	6,000	80 - 160
COD (chemical oxygen demand)	mg/l	3,000 – 60,000	18,000	100 – 500
Total suspended solids	mg/l	200 – 2,000	500	100 – 400
Organic nitrogen	mg/l	10 – 800	200	80 – 120
Ammonia nitrogen	mg/l	10 – 800	200	20 – 40
Nitrates	mg/l	5 – 40	25	5 – 10
Total phosphorus	mg/l	5 – 100	30	5 – 10
Ortho phosphorus	mg/l	4 – 80	20	4 – 8
Alkalinity as CaCO ₃	mg/l	1,000 – 10,000	3,000	200 – 1,000
pH	pH unit	4.5 – 7.5	6	6.6 – 7.5
Total hardness as CaCO ₃	mg/l	300 – 10,000	3,500	200 – 500
Calcium	mg/l	200 – 3,000	1,000	100 – 400
Magnesium	mg/l	50 – 1,500	250	50 – 200
Potassium	mg/l	200 – 1,000	300	50 – 400
Sodium	mg/l	200 – 2,500	500	100 – 200
Chloride	mg/l	200 – 3,000	500	100 – 400
Sulfate	mg/l	50 – 1,000	300	20 – 50
Total Iron	mg/l	50 – 1,200	60	20 – 200

In the table above, typical nitrogen and nitrate concentrations are highlighted.

The pollutants in leachate are highly variable over the course of the lifetime of the landfill and between different landfills. This makes choosing an appropriate treatment option difficult. A decision must be made by considering the following technical factors: landfill design, leachate quantity and quality, degree of treatment needed and ultimate disposal methods of effluent and residues. Also non-technical factors such as legal issues, regulatory constraints and public participation should play a role in determining the planning and design of the treatment system (Qasim and Chiang 1994).

1.5 Nitrogen cycle in nature

Nitrogen is essential for many biological processes and is crucial for life here on Earth. It is found in all amino acids, is incorporated into proteins, and is present in the bases that make up nucleic acids, such as DNA and RNA.

Air, which is 79% nitrogen gas (N_2), is the major reservoir of nitrogen.

The nitrogen cycle is the biogeochemical cycle that describes the transformations of nitrogen and nitrogen-containing compounds in nature (Figure 1.4).

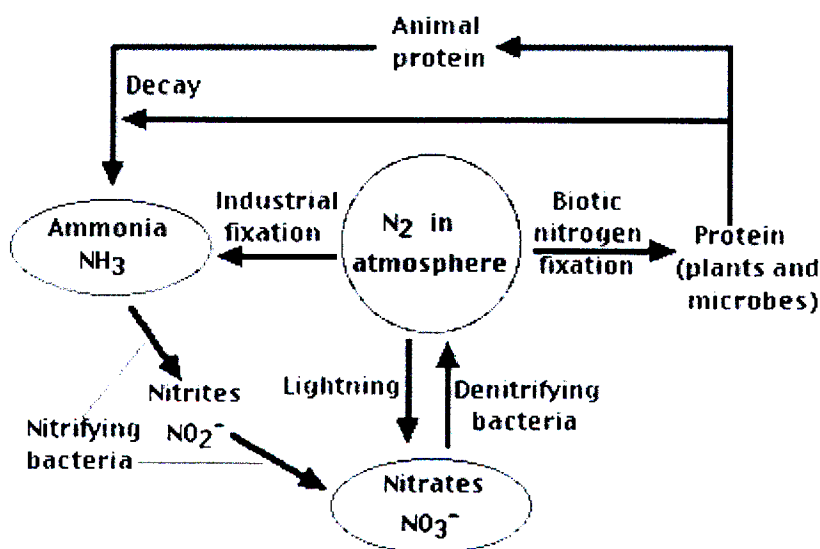


Figure 1.3 - Nitrogen cycle in nature (Ghafari, 2007)

Most organisms cannot use nitrogen in the form of complex compounds as proteins and nucleic acids; for example plants must secure their nitrogen in "fixed" form, i.e., incorporated in compounds such as:

- nitrate ions (NO_3^-)
- ammonia (NH_3)
- urea ($(\text{NH}_2)_2\text{CO}$)

Animals secure their nitrogen supply (and all others) from plants (or animals that have fed on plants).

Four processes participate in the cycle of nitrogen through the biosphere:

- nitrogen fixation
- decay
- nitrification
- denitrification

Microorganisms play major roles in all four of these (Ghafari, 2007).

1.5.1 Nitrogen Fixation

The nitrogen molecule (N_2) is inert and a substantial amount of energy is required to make it available for combination with other atoms.

Three processes are responsible for most of the nitrogen fixation in the biosphere:

- **atmospheric fixation** by lightning
- **biological fixation** by certain microbes (alone or in a symbiotic relationship with plants)
- **industrial fixation**

1.5.2 Decay

The proteins present in plants enter and pass through food webs. At each trophic level, their metabolism produces organic nitrogen compounds that return to the environment,

mainly as excretions. The final beneficiaries of these materials are microorganisms of decay. They break down organic molecules and dead organisms into **ammonia**.

1.5.3 Nitrification

Ammonia can be absorbed directly by plants, usually through their roots. However, most of the ammonia produced by plants decay is converted into **nitrites**. This is accomplished in two steps:

- Bacteria of the genus **Nitrosomonas** oxidize NH_3 to **nitrites** (NO_2^-).
- Bacteria of the genus **Nitrobacter** oxidize the nitrites to **nitrates** (NO_3^-).

These two groups of autotrophic bacteria are called **nitrifying bacteria**. Through their activities (which supply them with all their energy needs), nitrogen is made available to the plants roots.

Many soils also contain archaeal microbes, assigned to the Crenarchaeota, that convert ammonia to nitrites.

Many legumes, in addition to fixing atmospheric nitrogen, also perform nitrification — converting some of their organic nitrogen to nitrites and nitrates. These reach the soil when they shed their leaves.

1.5.4 Denitrification

The three processes above remove nitrogen from the atmosphere and pass it through ecosystems.

Denitrification reduces nitrates to nitrogen gas, thus replenishing the atmosphere through bacterial action.

Soil bacteria use nitrates as an alternative to oxygen as final electron acceptors in their respiration.

1.6 Effects of nitrogen and its compounds

Nitrogen, in all its oxidation states, has negative effects on the natural receptors like eutrophication and dissolved oxygen demand.

Ammonia is toxic for many aquatic species (especially fish) at very low concentrations, around 0.2 mg/l (Reed et al, 1995, Kadlec and Knight, 1996 and Hammer and Hammer, 2001).

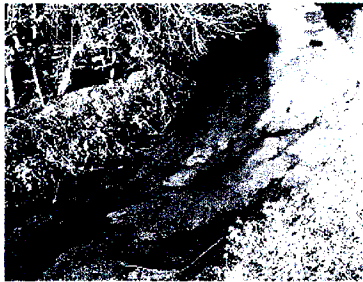


Figure 1.4 – Eutrophication in a water receptor.

Eutrophication (Figure 1.5) is the accelerated production of algae due to excessive nutrient inputs in natural water bodies.

The algae metabolize the nutrients (Nitrate nitrogen and phosphorous) and take energy from sunlight multiplying very quickly, covering the entire water surface, preventing the penetration of sunlight, which is essential for other aquatic species. In this process, oxygen is released as a waste product, however if sunlight is not available, the algae catabolize stored food for energy and use oxygen as an electron donor, thus creating an oxygen demand on the natural water.

In addition to accelerated growth, algae also decay quickly. The high numbers combining with the rapid decay leads to an accumulation on the bed of the aquatic system. The material formed is called 'necron mud', which continues to consume oxygen slowly as it decays. This further reduces the oxygen level and increases the sediment levels of the aquatic system, making it shallower. Animals that are adapted to the original aquatic system depth also begin to stress to a point where most of the aquatic species, except for algae, disappear from the natural water (Peavy et al, 1985 and <http://www.thegeographyportal.net>).

Other adverse effects on water quality due to algae include taste and odor problems (Peavy et al, 1985).

Presence of ammonia also causes a restriction in the use of drinking water and reduces the chlorination efficiency in the waste water treatment plants (Curreli, 2000).

Ammoniacal nitrogen ($\text{NH}_4\text{-N}$), nitrite nitrogen ($\text{NO}_2\text{-N}$) and nitrate nitrogen ($\text{NO}_3\text{-N}$) are the most polluting inorganic nitrogen forms.

Ammonium ion (NH_4^+ , ionized ammonia) and ammonia (NH_3 , unionized ammonia) are the two aqueous ammoniacal nitrogen forms that exist depending on temperature and pH (Figure 1.5)

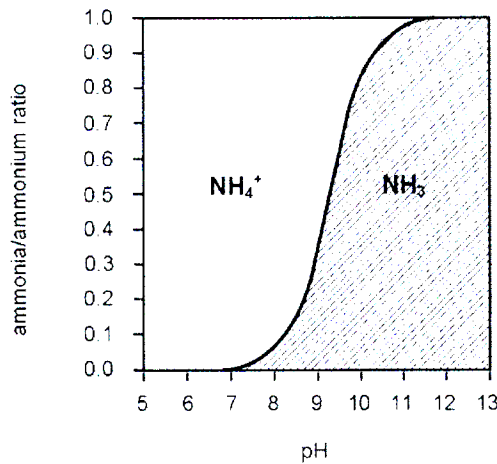
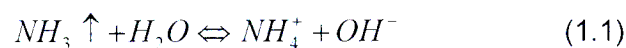


Figure 1.6 - Dependence of NH_4^+ and NH_3 from pH (Carucci, 2003).

The reaction is regulated by the equilibrium equation shown below (Peavy et al, 1985, Tchobanoglous and Burton, 1991, Kadlec and Knight, 1996 and Hammer and Hammer, 2001):



When the equilibrium is shifted to the left, ammonia becomes the most predominant species at high temperatures and basic pH ($\text{pH} > 7$). Conversely at low temperatures and acidic pH ($\text{pH} < 7$), ionized ammonia becomes the predominant species (Kadlec and Knight, 1996). Ammoniacal nitrogen in landfill leachates can range from 1 to 4000 mg/l (Andreottola and Cannas, 1992 and Department of the Environment (UK), 1995), which may be readily oxidized in natural waters, resulting in an oxygen demand on the natural water ($\pm 4.3 \text{ gO}_2/\text{gNH}_4\text{-N}$).

Under aerobic conditions, ammoniacal nitrogen is oxidized to nitrite and then to nitrate. Nitrite is not chemically stable and is usually found in trace levels in polluted waters, ranging from 0 to 25 mg/l in landfill leachates (Andreottola and Cannas, 1992 and Department of the Environment, 1995). Nitrate, however, is chemically stable and persists in polluted waters, ranging from 0.1 to 50 mg/l in landfill leachates (Andreottola and Cannas, 1992).

1.7 Nitrate contamination

In surface water and groundwater nitrate contamination is an increasingly important problem all over the world. Although nitrate is found in most of the natural waters at moderate concentrations, elevated levels in ground water mainly result from human and animal wastes, and excessive use of chemical fertilizers (Samatya et al., 2006). The other most common sources of nitrate are uncontrolled land discharges of municipal and industrial wastewaters, run off from septic tanks, processed food, dairy and meat products and decomposition of decaying organic matter buried into ground. Fertilizers and wastes are sources of nitrogen-containing compounds which are converted to nitrates in the soil. Nitrates are extremely soluble in water and can move easily through soil into the drinking water supply (Shrimali et al., 2001)

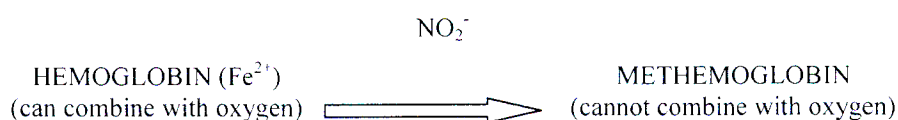
Nitrate is identified as one of the hazardous contaminants in potable water that, when digested is reduced to nitrosamines, that may cause gastric cancer (Glass and Silverstein, 1999; Galvez et al., 2003).

The effect of nitrate itself is described as *primary toxicity*, as its high intake causes abdominal pains, diarrhea, vomiting, hypertension, increased infant mortality, central nervous system birth defects, diabetes, spontaneous abortions, respiratory tract infections, and changes to the immune system (Lohumi et al., 2004).

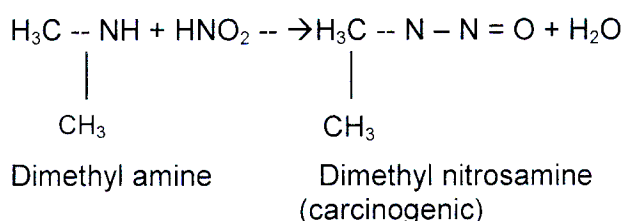
Secondary toxicity of nitrate is microbially reduced to the reactive nitrite ion by intestinal bacteria. Nitrate has been implicated in methemoglobinemia (the blue baby disease), especially to infants under six month of age (Kross et al., 1992; Mori, 1996).

Methemoglobin (MetHb) is formed when nitrite (for our purposes, formed from the endogenous bacterial conversion of nitrate from drinking water) oxidizes the ferrous iron in hemoglobin (Hb) to the ferric form. MetHb cannot bind oxygen, and the condition of

methemoglobinemia is characterized by cyanosis, stupor, and cerebral anoxia. Symptoms include an unusual bluish gray or brownish gray skin color, irritability, and excessive crying in children with moderate MetHb levels and drowsiness and ethargy at higher levels (Samatya et al., 2006)



In *tertiary toxicity*, the reaction between nitrite and secondary or tertiary amine in acidic medium can result in the formation of N-nitroso compounds, some of which are known to be carcinogenic, teratogenic, and mutagenic (Mikuska et al., 2003).



A diet, adequate in vitamin C, partially, protects against the adverse effects of nitrate–nitrite. Methaemoglobinaemia in infants can only be mitigated by blood transfusion (Schoemann et al., 2003).

To protect consumers from the adverse effects associated with the high nitrate intake, nitrate consumption should be limited and standards have been established (Elmidaoui et al., 2002). According to TSE 266, WHO, EC standards, drinking water must contains no more than 50 mg/L of nitrate and EPA established a maximum contaminant level of 45 mg/L.

European Community recommends levels of 25 mg-NO₃⁻/L (Lohumi et al., 2004).

Also, the United States Environmental Protection Agency (EPA) and WHO have set the maximum contaminant level (MCL) to 10 mg NO₃⁻/L in drinking water (Cast and Flora,

1998). The same concern in Europe set MCL to 12 mg NO₃⁻/L in drinking water (Glass and Silverstein, 1999).

1.8 Water quality standards and South African legislation

Over the years, as scientific knowledge has grown, water quality standards have evolved, internationally and locally.

Effluent water quality standards are set in order to protect the receiving water body and usually take into account its beneficial use, for example more stringent standards will be enforced for receiving waters that are used as drinking water resources and for recreational purposes. In setting effluent discharge limits two aspects need to be satisfied: the first being 'Best Available Technology Economically Achievable' (BATEA) and the second being the state and use of the receiving water body (Hammer and Hammer, 2001). This leads to water quality based standards and technology-based standards. Technology based standards do not guarantee that a treated wastewater will not pollute a receiving water body as they do not take into account the state of the receiving water body. Water quality based standards apply to the waters receiving wastewater discharges (Hammer and Hammer, 2001).

The Uniform Effluent Standards (UES) has been in place for over twenty years in South Africa and is aimed to regulate the input of effluents into the receiving water body, and takes into account Best Available Technology Not Entailing Excessive Costs (BATNEEC) (Pulles et al, 1996). This approach has its drawbacks as it ignores the possibility of existing high background concentrations in the river system and its ability to assimilate pollutants (Pulles et al, 1996).

Although the application of the UES has led to the decrease in the rate of pollution, deterioration has still continued (Pulles et al, 1996).

In 1990 the Department of Water Affairs and Forestry (DWAF) realized that a more advanced approach would be necessary and included the following principals (Pulles et al, 1996):

- "The desired quality of a water resource is determined by its present and/or intended uses. This quality should be stated as a list of water quality objectives."

- “It is accepted that the water environment has a certain, usually quantifiable, capacity to assimilate pollutants without detriment to predetermined quality objectives.”
- “The assimilative capacity of a water body is part of the water resource and, as such, must be managed judiciously and shared in an equitable manner amongst all water users for the disposal of their wastes.”
- “For those pollutants which pose the greatest threat to the environment, because of their toxicity, extent of bio-accumulation and persistence, a precautionary approach aimed at minimizing or preventing inputs to the water environment should be adopted.”

The Government Gazette No. 20526 8 October 1999 indicates the actual requirements for discharge of waste or water containing waste into a water resource through a pipe, canal, sewer or other conduit. In this document, rivers are classified according to use and either excluded from or included in the general authorization for discharge. Those that are included are further subdivided into rivers accepting effluents that have complied with the special limit values or the general limit values. It also gives an indication of the monitoring requirements for domestic wastewater discharges.

The General and Special limit values are presented in Table 1.3.

Table 1.2 - Discharge limit value applicable to effluents into a water resource (Government Gazette, 1999)

Parameter	General limit	Special limit
Faecal Coliforms (per 100 ml)	1000	0
COD (mg/l)	75*	30*
pH	5.5 to 9.5	5.5 to 7.5
Ammoniacal Nitrogen (mg/l)	3	2
Nitrate/Nitrite nitrogen (mg/l)	15	1.5
Free Chlorine (mg/l)	0.25	0
Suspended Solids (mg/l)	25	10
Electrical Conductivity (mS/m)	70 mS/m above intake to a max. of 150 mS/m	50 mS/m above background receiving water to a max. of 100 mS/m

Ortho-Phosphate (mg/l)	10	1 (median) and 2.5 (max.)
Fluoride (mg/l)	1	1
Soap, oil or grease (mg/l)	2.5	0
Dissolved Arsenic (mg/l)	0.02	0.01
Dissolved Cadmium (mg/l)	0.005	0.001
Parameter	General limit	Special limit
Dissolved Chromium (mg/l)	0.05	0.02
Dissolved Copper (mg/l)	0.01	0.002
Dissolved Cyanide (mg/l)	0.02	0.01
Dissolved Iron (mg/l)	0.3	0.3
Dissolved Lead (mg/l)	0.01	0.006
Dissolved Manganese (mg/l)	0.1	0.1
Dissolved Selenium (mg/l)	0.02	0.02
Dissolved Zinc (mg/l)	0.1	0.04
Mercury and its compounds (mg/l)	0.005	0.001
Boron (mg/l)	1	0.5

*After the removal of algae

As explained in this section, nitrogen and its compounds in landfill leachate are hazardous for environment and human health. Ammonia levels in raw landfill leachate are higher than the legislation limits and nitrates are in excess in treated leachate. Leachate from Marianhill landfill, our case study, can reach 2400 mg/l of nitrate.

In order to achieve the legislation limits additional treatments of nitrification and denitrification are required before discharging.

The next chapter will focus on principles and kinetics of denitrification and available technology.

2 PRINCIPLE AND KINETICS OF DENITRIFICATION - AVAILABLE TECHNOLOGY

2.1 Introduction

Nitrogen management requires knowledge of nitrification and denitrification processes that involve complex reactions influenced by many factors. This chapter focuses on kinetic aspects of nitrification and denitrification and presents an overview of available technologies.

2.2 Nitrification

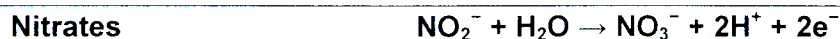
Nitrification is the biological oxidation of ammonia (NH₄-N) with oxygen into nitrite (NO₂-N) followed by the oxidation of nitrites into nitrates (NO₃-N).

The nitrifying bacteria derive energy from the oxidation of ammonia and/or nitrite and carbon dioxide is used as a carbon source for synthesis of new cells (Vymazal 2006).

As mentioned in chapter 1, the oxidation of ammonia into nitrite, and the subsequent oxidation to nitrate are performed by two different nitrifying bacteria (Curreli, 2000), the first step is performed by bacteria of (amongst others) the genus *Nitrosomonas* and *Nitrosococcus*.



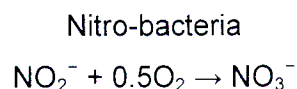
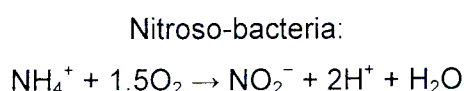
The second step (oxidation of nitrite into nitrate) is mainly due to bacteria of the genus *Nitrobacter*. All organisms are autotrophs.



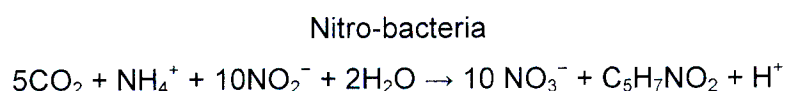
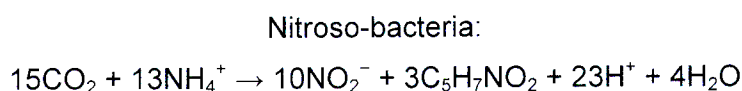
In most environments both organisms are found together, yielding nitrate as the final product.

Together with ammonification, nitrification forms a mineralisation process which refers to the complete decomposition of organic material, with the release of available nitrogen compounds. This replenishes the nitrogen cycle (Metcalf & Eddy 2004).

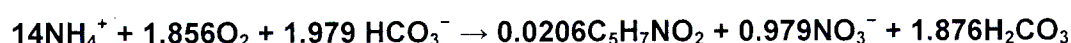
The oxidation reactions can be expressed as follows (Curreli, 2000):



The synthesis reactions can be expressed as follows:



Total nitrification reaction from ammonia to nitrate:



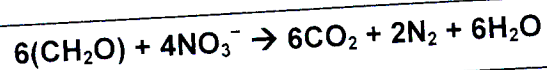
Nitrification is influenced by temperature, pH, alkalinity of the water, inorganic carbon sources, moisture, microbial population, concentrations of ammonium-N and dissolved oxygen (Vymazal, 1995). The optimal temperature for nitrification in pure cultures ranges from 25 to 35 °C and in soils from 30 to 40 °C. The minimum temperatures for growth of Nitrosomonas and Nitrobacter are 5 and 4 °C, respectively (Cooper et al., 1996). Strachan et al. (2000) reported that optimum pH values may vary from 6.6 to 8.0, however, acclimatized systems can be operated to nitrify at a much lower pH value (Cooper et al., 1996). Approximately 4.3 mg O₂ per mg of ammoniacal nitrogen oxidized

to form nitrate nitrogen. In the conversion process, a large amount of alkalinity is consumed, approximately 8.64 mg HCO_3^- – per mg of ammoniacal nitrogen oxidized (Cooper et al., 1996).

2.3 Denitrification

Denitrification is most commonly defined as the process in which nitrate is converted into dinitrogen gas via intermediates nitrite, nitric oxide and nitrous oxide (Haandler et al., 1981; Platzer 1999).

From a biochemical viewpoint, denitrification is a bacterial process in which nitrogen oxides (in ionic and gaseous forms) serve as terminal electron acceptors for respiratory electron transport. Electrons are carried from an electron-donating substrate (usually, but not exclusively, organic compounds) through several carrier systems to a more oxidized N form. The resultant free energy is conserved in ATP, following phosphorylation, and is used by the denitrifying organisms to support respiration. Denitrification is illustrated by the following equation (Henze et al., 1995):



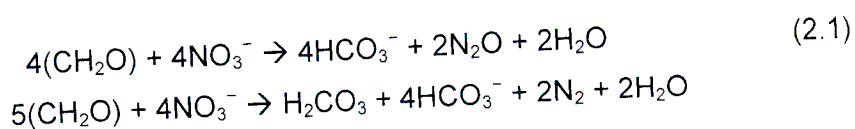
This reaction is irreversible, and occurs in the presence of available organic substrate only under anaerobic or anoxic conditions ($E_h = +350$ to $+100$ mV), where nitrogen is used as an electron acceptor in place of oxygen.

The main player in the denitrification reaction is the **Nitrate Reductase**, an enzyme produced in the absence of oxygen that ensures electron transport from the reducing substance to the oxidizing one.

In the presence of oxygen this enzyme is not produced and the aerobic process is advantaged because bacteria prefer to use free oxygen.

Evidence is available in literature that nitrate reduction can occur in the presence of oxygen. Hence, in waterlogged soils nitrate reduction may also start before the oxygen is depleted (Robertson and Kuenen, 1984).

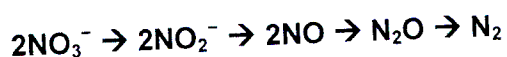
Gaseous N production during denitrification can also be depicted by the following equations (Henze et al., 1995):



Diverse organisms are capable of denitrification such as organotrophs, lithotrophs, phototrophs, and diazotrophs (Paul and Clark, 1996). Most denitrifying bacteria are chemoheterotrophs. They obtain energy solely through chemical reactions and use organic compounds as electron donors and as a source of cellular carbon (Henze et al., 1995). The genera *Bacillus*, *Micrococcus* and *Pseudomonas* are probably the most important in soils; *Pseudomonas*, *Aeromonas* and *Vibrio* in the aquatic environment (Grant and Long, 1981). When oxygen is available, these organisms oxidize a carbohydrate substrate to CO_2 and H_2O (Chow et al., 1988). Aerobic respiration using oxygen as an electron acceptor or anaerobic respiration using nitrogen for this purpose is accomplished by the denitrifiers with the same series of electron transport system. This facility to function both as an aerobe and as an anaerobe is important in the practice because it enables denitrification to proceed at a significant rate soon after the onset of anoxic conditions (redox potential of about 300 mV) without change in microbial population (Henze et al., 1995).

Because denitrification is carried out almost exclusively by facultative anaerobic heterotrophs that substitute oxidized N forms for O_2 as electron acceptors in respiratory processes, and because these processes follow aerobic biochemical routes, it is often referred to an anaerobic process, but it essentially occurs under anoxic conditions (Henze et al., 1995).

The actual non-stoichiometric sequence of biochemical changes from nitrate to elemental gaseous nitrogen is:



2.4 Kinetic aspects of denitrification

Using the Monod's kinetic approach (1942), the denitrification rate is expressed as follow:

$$\frac{d(N - NO_3)}{dt} = -\frac{\mu_{ndf}}{Y_d} \cdot \frac{(N - NO_3)}{K_{df} + (N - NO_3)} \cdot \frac{(C)}{K_{cl} + (C)} \cdot X \quad (2.2)$$

Where (N-NO₃) is the nitric nitrogen concentration (mg/l), (C) is the carbon substrate concentration (mg/l), Y_d is the yield coefficient for denitrification, K_{df} and K_{cl} are the saturation constant respectively for denitrification and carbon substrate.

In the literature the data concerning K_{df} indicate a very low value, around 0.1 mg (N-NO₃)/l then the denitrification kinetic can be considered of zero order in comparison with azotized substrate.

Using methanol as a carbon source, K_{df} value is around 0.2-0.3 mg(N-NO₃)/(mg*d) at 20°C; lower values using organic matter present in urban sewage (Carucci, 2003).

Referred to methanol the constant K_{cl} vary from 29-50 mg CH₃OH/l to 0.1mg/l (Carucci, 2003); it is common to consider a zero order kinetic for the denitrification also in relation to the carbon substrate obviously taking into account the COD/TKN (Total Kjeldahl Nitrogen) ratio necessary to ensure no nitrates in the effluent.

In integrated systems with pre-denitrification the minimum COD/TKN ratio to ensure a complete denitrification in the first anoxic reactor is 11-15 (Water Pollution Control Federation Manual, 1983)

Environmental factors known to influence denitrification rates include the absence of O₂, redox potential, soil moisture, temperature, pH value, presence of denitrifiers, soil type, organic matter, nitrate concentration and the presence of overlying water (Carucci et al. 1999).

K_{df} value can be around 0.2 – 0.3 mgN-NO₃/(mg_{vss}*d) at 20°C using methanol as a carbon source, inferior values (0.10 mgN-NO₃/(mg_{vss}*d) at 20°C) using organic substance present in urban sewage (Carucci 2003).

The difference in the kinetics (methanol and urban sewage) can be in part attributed to the different process schemes in which these substances are used.

Urban sewage is used only with integrated schemes and the kinetic is inferior in comparison with methanol (used in the separated schemes) in which all the heterotrophic biomass can use nitrates as an electron acceptor (Water Pollution Control Federation manual, 1983).

The specific rate of denitrification is expressed as follow:

$$(v_D) = \frac{(v'_D)_{20} (NO_3^- - N)}{[k_N + (NO_3^- - N)]} \cdot \frac{c \theta^{(T-20)}}{(k_c + c)} \quad (2.3)$$

$(v'_D)_{20}$	specific denitrification rate without limiting factors
$(NO_3^- - N), c$	concentration of nitrates and organic biodegradable matter
k_N, k_C	semisaturation constants of nitrates and substrate

The constants k_N and k_C are limited and do not constitute limiting factors, so $v_D = v'_D$ and they can change only in relation to the available of the carbon source (Curreli, 2000).

The estimation of denitrification rates varies widely in the literature between 0.003 and 1.02 g N m⁻² d⁻¹ (Carucci et al., 1999).

Studies on N conversion under low-oxygen and anaerobic conditions have shown that ammonia can be converted to dinitrogen by processes other than conventional nitrification of ammonia to nitrate followed by denitrification of nitrate to dinitrogen gas (Strachan et al., 2000). Under low-oxygen conditions, the production of nitrite from ammonia is favored over the production of nitrate. The nitrite can then be denitrified to nitrous oxide and/or dinitrogen without being converted to nitrate. This process has been termed “partial nitrification–denitrification” (Carucci 2003).

2.4.1 Dissolved Oxygen (DO)

The presence of oxygen inhibits **Nitrate reductase**, the enzyme that ensures electron transport in the denitrification process; moreover free energy variation is higher in the aerobic process than in the reaction with nitrate. It is evident that in the simultaneous presence of oxygen and nitrate, the aerobic process will be favored.

Nevertheless, in many experiments, the denitrification may occur in presence of DO (Carucci, 2003).

This aspect is important in the design of treatment processes where the removal efficiency is higher because of the denitrification occur in aerobic conditions.

2.4.2 Temperature

Denitrification is also strongly temperature dependent. From 5 to 25 °C, the Arrhenius equation ($k = Ae^{-E_a/RT}$) describes temperature influence in experiments with suspended biomass.

The k_{dT} variation law with the temperature $T(^{\circ}\text{C})$ can be expressed with the formula:

$$k_{dT}(T) = k_{dT(20)}\theta^{(T-20)} \quad (2.4)$$

in which $k_{dT(20)}$ is the absolute value of kinetic constant at 20°C and θ the temperature coefficient. (Carucci, 2003).

Rates of denitrification increase to a maximum of 60 to 75 °C and then decline rapidly (Paul and Clark, 1996). Denitrification proceeds at very slow rates, at temperatures below 5 °C (Van Oostrom and Russell, 1994) where relatively large mole fractions of N_2O and NO are reported and ceases at 3 °C. Molecular N_2 is the major product at higher temperatures (Paul and Clark 1996).

2.4.3 pH

Paul and Clark (1996) reported that the optimum pH range for denitrification lies between 6 and 8.

Denitrification is slow but may still remain significant below pH 5 and is negligible or absent below pH 4.

The denitrification process produces alkalinity: in the industrial discharge with high nitrate concentration is necessary to add acid while in urban sewage alkalinity production is balanced by acidity production in nitrification process (Carucci, 2003).

2.4.4 Inhibiting substances

Little information is available about denitrification inhibitors. For some type of bacteria the sensitivity of nitrate reductase to cyanide is known. Nitro reductase enzymes of the same species are inhibited by bivalent copper and some mercury compounds (Carucci, 2003).

Substances directly involved in the nitrogen removal process, like ammonia and methanol do not have adverse effects on the reaction.

Nitrite can have an inhibiting effect in concentrations higher than 20mgN/l (Beccari, 1979), no nitrate effects have been noted up to 2.2gN/l; for higher concentrations, selective effects on bacteria species have been studied (Focht, 1977).

2.5 Available technology employed for denitrification

Denitrification is considered as a major removal mechanism for nitrogen in most types of reactors. The concentrations of nitrate, however, are usually very low in wastewater (the exception is drainage water from the agriculture and some industrial wastewaters) and, therefore, denitrification must be coupled with nitrification. Different requirements for the presence of oxygen for nitrification and denitrification are the major obstacle in many treatments.

The conventional processes of water treatment method including coagulation, filtration, and disinfection which are applied to potable water are not efficient for the complete removal of nitrate ions. Therefore, to remove nitrate ion, a supplementary method is necessary.

Nitrate removal from water or wastewater can be achieved using two main groups of treatment processes: physicochemical and biological.

2.5.1 Abiotic nitrate removal methods

The most common conventional treatment processes used to remove nitrate are reverse osmosis (RO), ion exchange (IE), electro-dialysis (ED) and activated carbon adsorption in conjunction with pH adjustment (Islam and Suidan, 1998; Ergas and Reuss, 2001; Shrimali and Singh, 2001; Feleke and Sakakibara, 2002; Prosnansky et al., 2002).

Also, there is a wide interest towards some new methods such as metallic iron-aided abiotic nitrate reduction (Huang et al., 1998; Choe et al., 2000, 2004; Devlin et al., 2000; Hu et al., 2001; Huang and Zhang, 2002, 2004; Huang et al., 2003; Chi et al., 2004; Ginner et al., 2004).

Thermally activated hydrotalcite based upon a Zn/Al hydrotalcite with carbonate in the interlayer has been used to remove nitrate anions from an aqueous solution resulting in the reformation of a hydrotalcite with a mixture of nitrate and carbonate in the interlayer (Frost et al., 2006). Nitrate removal using natural clays modified by acid thermoactivation has been performed by Duran et al. (2007).

In particular, the effects of organic and metal ion concentration on the simultaneous biological removal of NH_4^+ and NO_3^- under anaerobic condition were studied by Kim et al. (2006).

Abiotic nitrate reduction has been performed using modified noble metals, with Pd based compounds as catalysts (Maia et al., 2007; Gasparovicova et al., 2006).

Nitrate reduction by zero valent iron has been investigated and reported in recent publications (Choe et al., 2000; Alowitz and Scherer, 2002; Westerhoff, 2003; Su and Puls, 2004; Zhang and Huang, 2005; Ruangchainikom et al., 2006a,b).

There are some disadvantages with these conventional methods that limit their employment, due to costly operations and long term maintenance and dispose of the bioproduct, as brine etc.

For instance, the IE process removes nitrate and sulfate simultaneously but subsequently wastewater is produced from the resin regeneration process (Shrimali and Singh, 2001).

RO, although is able to separate and concentrate the compounds contained in water without any change in their molecular structures, it is of limited application due to high costs and production of concentrated waste brine that may pose a disposal problem (Ergas and Reuss, 2001; Shrimali and Singh, 2001).

Even for the new methods of metallic iron-aided abiotic nitrate reduction, although complete or partially nitrate removal are reported, formation of ammonia along with nitrogen gas as end-products is an undesired consequence of this method (Biswas and Bose, 2005).

2.5.2 Biological denitrification methods

In contrast to abiotic methods, which fail to treat nitrate and are able to separate or remove it from the stream and result in some problematic by-products, biological methods provide a robust treatment option. Although aerobic denitrification has been reported (Joo et al., 2005; Robertson and Kuenen, 1984; Zart and Eberhard, 1998; Chiu et al., 2007), most of the reported denitrification processes are performed by facultative anaerobes in the absence of oxygen (Rijn et al., 2006).

Biological denitrification is a mechanism by which denitrifying bacteria use nitrate as terminal electron acceptor in their respiratory process in the absence of oxygen. Denitrifying bacteria, in this method, reduce inorganic nitrogen compounds such as nitrite and nitrate into harmless elemental nitrogen gas (Prosnansky et al., 2002) so that no further treatment is imposed. Denitrifying bacteria are ubiquitous in nature (Gamble et al., 1977; Szekeres et al., 2001) and numerous researchers cultivated them using mix cultures taken from wastewater treatment plants as seeds. Moreover, microbial removal of nitrate may be the most economical strategy for the reclamation of nitrates polluted waters and wastewaters (Soares, 2000). As a result, there has been large interest towards microbial removal of nitrate as the most environmentally friendly and cost-effective method, although biological denitrification is usually slow particularly for industrial wastewaters containing high concentrations of nitrate (Foglar et al., 2005). The Inorganic Contaminants Research Committee of USA (Islam and Suidan, 1998) reported that different denitrification strategies, such as the use of granular activated carbon (GAC), packed beds, rotating biological contactors and soil-aquifer denitrification systems have been undertaken by various researchers. Combination of biological and other abiotic methods, such as membrane biofilm reactors (MBR) are being studied (Terada et al., 2003).

2.6 Heterotrophic versus autotrophic denitrification

Review of numerous studies on biological denitrification of nitrate contaminating, aquaculture wastewaters and industrial wastewater confirmed the potential of this method and its flexibility.

Biological denitrification is normally conducted by facultative anaerobes which require essential food and energy sources which are organic or inorganic (Cast and Flora, 1998; Rijn et al., 2006). This fact classifies denitrifiers into two main groups of heterotrophs and autotrophs. The denitrifiers can be fed on organic compounds (heterotrophs) or inorganic (autotrophs) substrates displaying different performances.

Review of heterotrophic and autotrophic denitrification with different food and energy sources concluded that autotrophic denitrifiers are more effective in denitrification (Ghafari et al., 2007).

Autotrophs utilize carbon dioxide and hydrogen as the source of carbon substrate and electron donors, respectively. The application of this method has also been studied in bio-electro reactors (BERs) (Ghafari et al., 2007).

2.7 Processes in biological reactors

The systems used for the biological nitrogen removal can be grouped into two categories: **separate systems** and **single-sludge systems**; the system A/B shown in figure 2.1 present intermediate characteristics.

The following process schemes are referred to plant using suspended biomass reactors; these are the most commonly utilized because the suspended biomass is easily moved from one zone to another in the reactor or from one reactor to another, allowing for higher efficiencies.

Most of the considerations below can be extended also to the biofilm reactors.

2.7.1 Separate system

Separate systems had been developed primarily in the USA: the anaerobic phase came after the aerobic one and the nitrifying bacteria are separate from denitrificans biomass.

In the *two step system* (Figure 2.1 A) the influent comes from the primary sedimentation and enters in the first reactor where the carbon aerobic removal and the nitrification take

place; the denitrification occur in a second reactor, separated from the first and in anoxic conditions where an external carbon source like methanol is added.

The outgoing mix from the denitrification reactor is then re-aerated to afford the exceeded methanol removal and the gaseous Nitrogen stripping in order to avoid the sludge rising in the sedimentator.

The *three step system* (Figure 2.1 B) comprehends three separate reactors for the three main processes.

The three step system, compared to the two step system, optimizes each treatment step; it is also less sensitive to the operative parameters fluctuation and, partially protects the nitrificans bacteria from toxic substances that can be biologically removed or absorbed in the first reactor.

Nevertheless the plant and maintenance cost are higher and the sludge production is elevated: the removal of the only organic substances need a value of sludge age lower than the one adopted in the reactor also performing nitrification.

Further it has been found that it is not easy to maintain stable working conditions in the nitrification reactor when most of the organic substances have been oxidized: the reason seems to be the low value of the nitrificans bacteria growth yield and their good attitude to flocculation resulting in a loss of biomass in the effluent (Carucci, 2003).

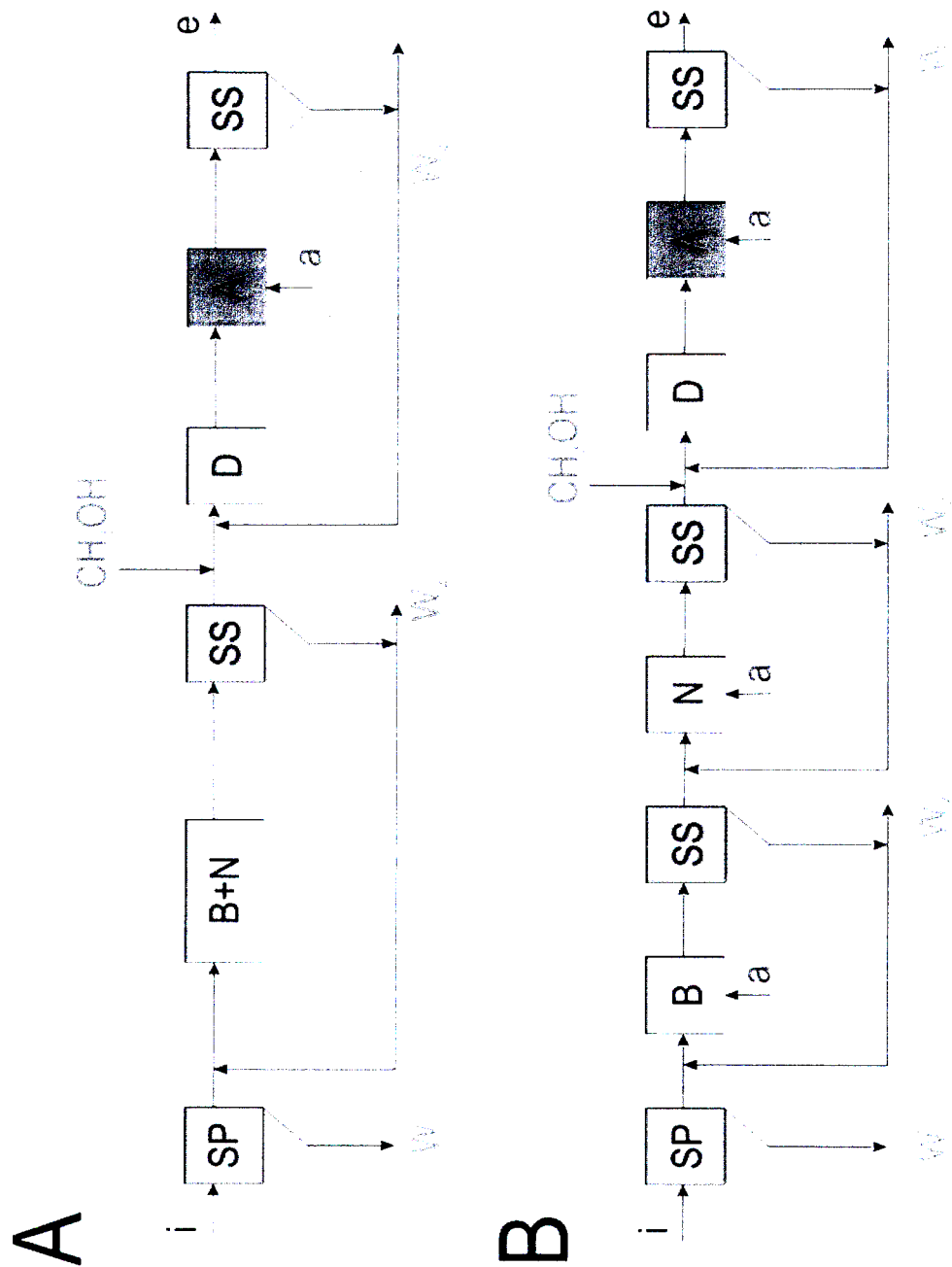


Figure 2.1 – Separate systems schemes for organic carbon and nitrogen removal: two stages system (A) and three stages system (B). SP: primary sedimentation; B: organic carbon oxidation; N: nitrification; D: denitrification; SS: secondary sedimentation; A: re-aeration; i: influent; e: final effluent; a: air; W1: primary sludge; W2: biologic sludge (IRSA-CNR, 2003).

2.7.2 Single-sludge systems

In the single-sludge system, nitrification and denitrification are performed by the same biomass.

The Wuhrmann process (1957) is the first example of single-sludge system.

This process is performed in two reactors in series (Figure 2.2 A). The first reactor works in aerobic conditions oxygenating and nitrifying organic substances.

The second reactor is anoxic and works in endogenous mode respiration: the matter from cellular lyses is used as energy source and carbon source (endogen carbon) for denitrification.

The effluent from the anoxic reactor flows in a sedimentator; the sludge taken from the bottom of the settling tank is recirculated in the aerobic reactor.

In the Wuhrmann process the denitrification rate is very slow, then the volume of the anoxic reactor is large and the nitrification efficiency is limited. The organic nitrogen and the ammonia released with cellular lyses in the anoxic reactor concur to increase the concentration of total nitrogen in the effluent.

In order to eliminate inconveniences caused by using endogenous carbon for denitrification, single-sludge system were developed, where biodegradable organic substances present in the effluent (internal carbon) are used as a carbon source for denitrification.

A sufficient amount of internal carbon is necessary to achieve optimum denitrification reaction (at least 8kg of BOD₅ for kg of N-NO₃ reduced); therefore in these systems, primary sedimentation, in which the BOD₅ removal fraction is higher than the nitrogen removal fraction, is absent.

There are three types of single-sludge systems employing this approach:

- Recirculation systems
- Simultaneous nitrification-denitrification system
- Alternating flow systems

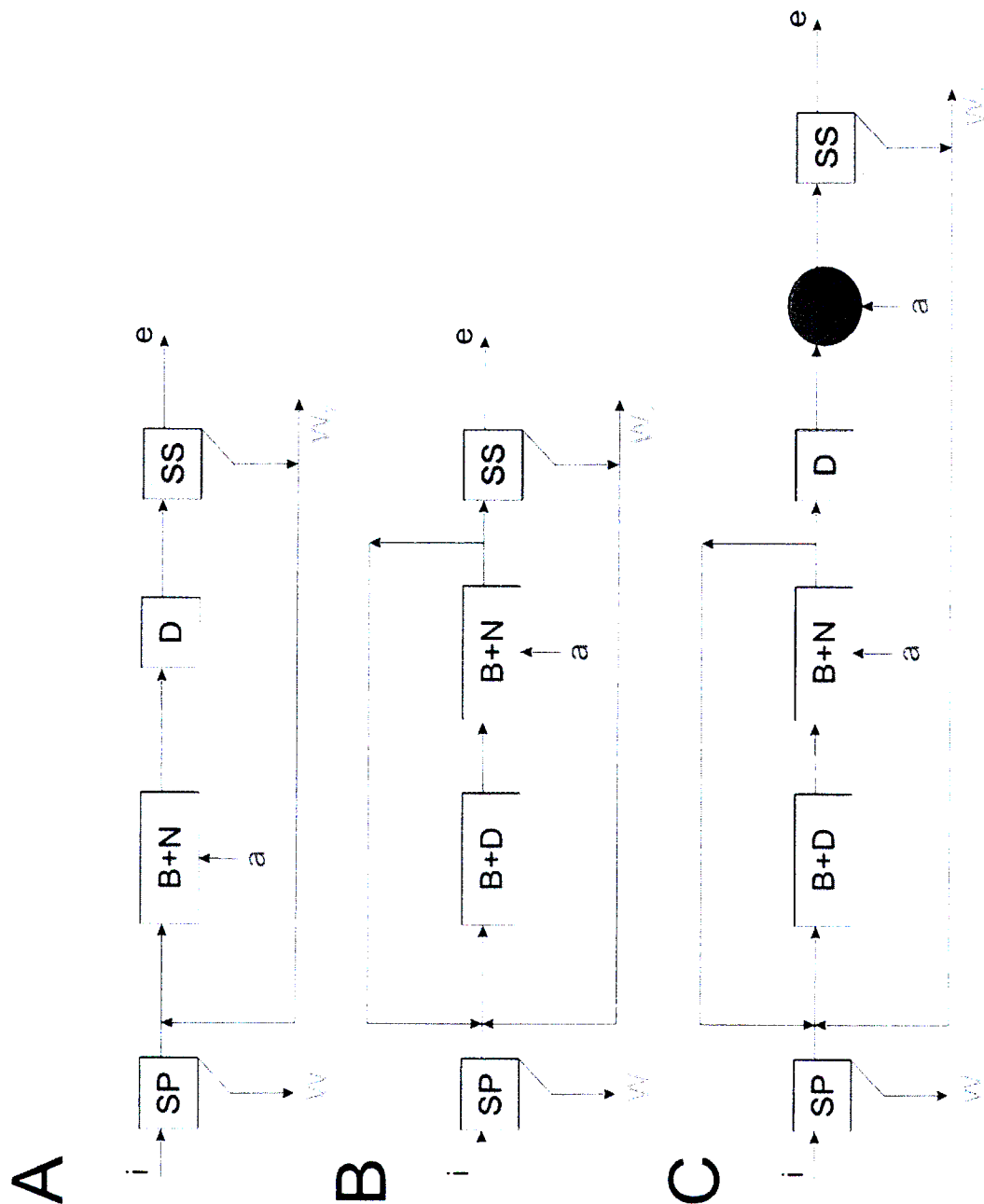


Figure 2.2 – Single sludge systems schemes for organic carbon and nitrogen removal: Wuhrmann system (A); Ludzack-Ettinger modified system (B); Bardenpho system (C); i: influent; e: final effluent; a: air; W1: primary sludge; W2: biologic sludge; SP: primary sedimentation; B: organic carbon oxidation; N: nitrification; D: denitrification; SS: secondary sedimentation; A: re-aeration; (IRSA-CNR, 2003).

2.7.3 Recirculation systems

In the **Ludzack-Ettinger modified** process (1962) (Figure 2.2 B), the influent in the first reactor is kept in anoxic conditions and the denitrification process is achieved using internal carbon as an energy source; the second reactor is aerated to perform the nitrification reaction; the bottom flow is recirculated in the anoxic reactor; another nitrate flow coming into the anoxic reactor is provided by a second recirculation current from the aerobic reactor.

This process represents a significant improvement compared with the Wuhrmann's procedure because it employs internal carbon instead of endogenous carbon, allowing for higher denitrification rates; Nevertheless this process does not achieve a complete nitrate removal because a fraction of the nitrates from aerobic reactor is not recirculated in the anoxic reactor and reside in the final effluent.

The **Bardenpho process** (Barnard 1974, 1975) (Figure 2.2 C) developed in South Africa, combines the Ludzac-Ettinger modified process with the Wuhrmann process in order to avoid the incomplete denitrification.

In this process, a second anoxic reactor is needed in which the endogenous carbon is employed for the removal of residual carbon from an aerobic reactor; sometimes methanol is necessary to avoid the negative effects of low temperature, daily fluctuation of the BOD_5/TKN ratio that may lead to incomplete denitrification.

The re-aeration stage, before the sedimentation, performs nitrogen stripping and nitrification.

2.7.4 Simultaneous nitrification-denitrification systems

Simultaneous nitrification-denitrification systems have been developed in Austria (Matsche', 1972) and the experience has shown that Through a proper oxygen concentration management it is possible to obtain, in the whole reactive zone, a sequence of well aerated zones, where the nitrification occur and poorly aerated zones in which denitrification takes place. Normally the reactors utilized in those systems have an elliptic channel shape in which the mix liquor-sludge flows (Carousell process); the aeration system also provides energy for the mix circulation.

The apparatus is simple in that the recirculation of the nitrates flow is not necessary and the operation is flexible: in fact, changing the aerators depth or the air flow in the reactor is easily to modify the extension of the anoxic and aerobic zones.

This type of system is also very stable in relation to overloading and possible toxic substances.

Moreover, it is not easy to dimensioning it because the aerating zone and the anoxic zones are not strictly characterized, not only from a geometric point of view but also from the kinetic aspect.

2.7.5 Alternating flow systems

Alternating flow systems have been developed in Denmark (Carucci, 2003); they are based on the principle of changing the amount of air flow to obtain alternately aerobic and anoxic condition in every element of the reactor.

A process of n cycles of alternating aeration during the permanence of liquor is equal to a process of simultaneous nitrification-denitrification characterized by n aerobic zones and n anoxic zones.

In the alternating flow systems for the nitrogen removal the Bio-Denitro process (Figure 2.3) is the most well-known; normally the working cycle involves four steps:

Phase A: The effluent flow is sanded to the reaction tank 1 (RT1) where the anoxic condition is ensured by a very low turbulence just enough to keep the biomass suspended; the nitrates produced in the preview phases have been reduced biologically using organic matter in the influent as a carbon source for denitrification.

In the reaction tank 2 (RT2), receiving the stream from RT1, high aeration is maintained to achieve biological oxidation of ammonia still present in the effluent from RT1 (in anoxic condition). From the RT2 the flow feeds a sedimentator that provide a clarified effluent and sludge flow recirculated at the head of the plant.

Phase B: Each tank is kept in aerobic condition; the stream from RT2 still feed the sedimentator; the difference with the preview phase is that the influent is sent to RT2 and not RT1 that stay momentarily isolated. The goal of this phase, relatively short, is to oxidize the ammonia in the RT1 before the connection with the sedimentator and so with the exit of the plant.

Phase C: This phase is equivalent to the A phase, the only difference is the inversion of the tanks: in the RT1, linked with the sedimentator, the nitrification reaction occurs while in the RT2, fed with the influent, the denitrification reaction takes place.

Phase D: This phase is equivalent to the B phase (both tanks in aerobic conditions), but this time the RT2 is isolated while RT1 is fed with the influent and connected with the final sedimentator.

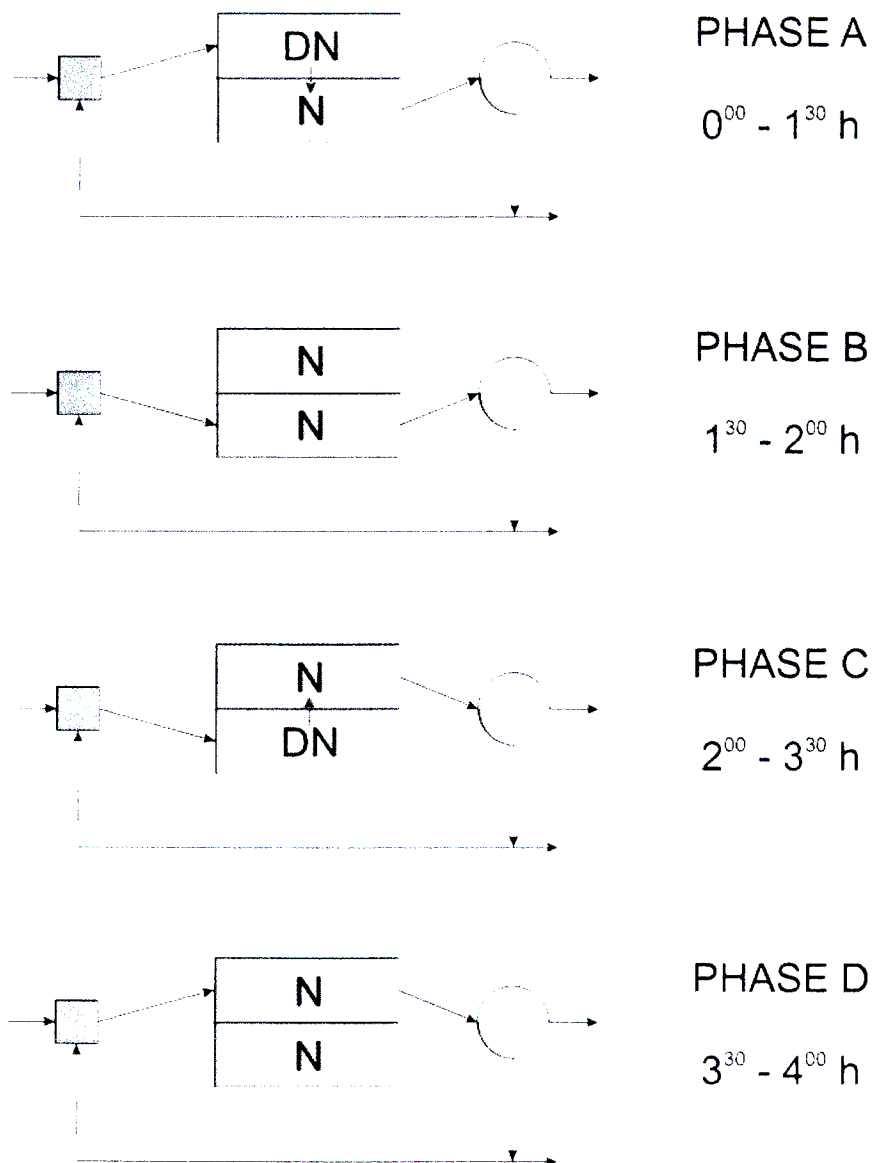


Figure 2.3 - Bio Denitro process scheme (DN: denitrification; N: nitrification).

2.8 Natural Systems - wetlands

Constructed wetlands are engineered systems that have been designed and constructed to utilize the natural processes involving wetland vegetation, soils, and their associated microbiology to assist in treating wastewater. They are designed to take advantage of many of the processes that occur in natural wetlands, but do so within a more controlled environment (Hammer and Bastian, 1989). Synonymous terms to “constructed” include “man-made”, “engineered” or “artificial” wetlands.

The basic classification is based on the type of macrophytic growth; further classification is usually based on the water flow regime (Figure 2.4) and they are classified in: constructed wetlands with free-floating plants (FFP), free water surface CWs with emergent plants (FWS) and sub-surface CWs with horizontal (HSSF or HF) and vertical (VSSF or VF) flows (Vyzimal, 2006).

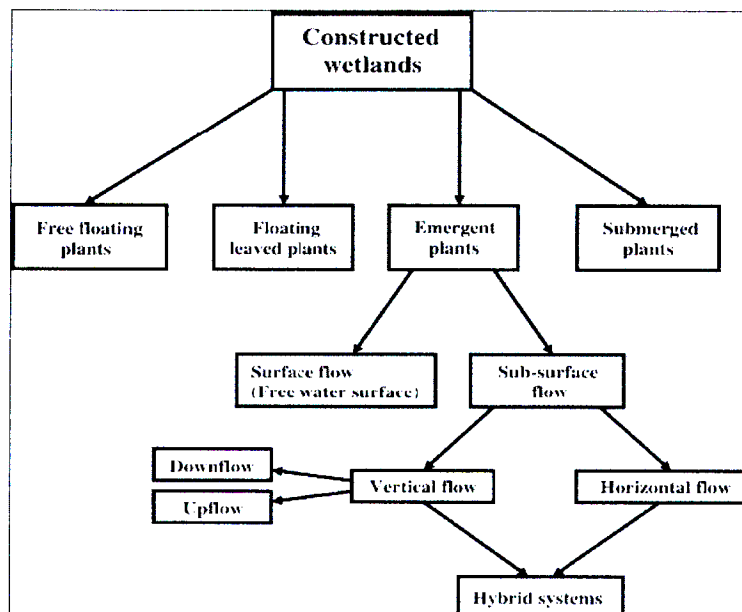


Figure 2.4 - Classification of constructed wetlands for wastewater treatment (Vymazal, 2001).

The most important forms of inorganic nitrogen in constructed wetlands are ammonia (NH_3^+), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrous oxide (N_2O) and dissolved elemental nitrogen or dinitrogen gas (N_2). Nitrogen may also be in many organic forms: urea, amino acids, amines, purines and pyrimidines (Kadlec and Knight, 1996). The organic nitrogen which is associated with suspended solids may be removed by the

same mechanisms previously discussed for suspended solids through physical separation followed by ammonification of the settled sediment (EPA, 2000). Ion exchange of ammonium within the medium's matrix may play a short-term role in nitrogen removal, until the medium's ion exchange capacity has been depleted (Reed et al, 1995 and EPA, 2000). The quantity of the relative species of aqueous ammonia in the water column is pH and temperature dependant and for a typical wetland system under average environmental conditions of 25°C and a pH of 7, un-ionised ammonia is only 0.6% of the total ammonia present; at a pH of 9.5 and a temperature of 30°C, the percentage of un-ionised ammonia increases to 72%. The volatility of un-ionised ammonia results in ammonia losses from the wetlands under high temperature and pH conditions, which may occur in CW during active photosynthesis (Kadlec and Knight, 1996 and EPA, 2000). The biologically mediated transformations of the nitrogen species are the most important mechanisms of nitrogen removal (Figure 2.5) (Reed et al, 1995).

If the influent content of organic nitrogen is high, the first microbial reaction will be ammonification. Ammonification will also take place during the break down of internally generated organic nitrogen. During this reaction organically combined nitrogen is transformed to ammoniacal nitrogen (EPA, 2000), thus adding to the influents ammoniacal nitrogen concentration. Approximately 4.3g of dissolved oxygen and 7.14g of alkalinity, as CaCO₃, are required to nitrify 1g of ammoniacal nitrogen. As discussed, due to the oxygen limitations in both VSB and FW CW, nitrification of high concentrations of ammoniacal nitrogen is limited and the use of hybrid systems is more appropriate (Van Oostrom and Russel, 1994; Reed et al, 1995; Kadlec and Knight, 1996; Cooper, 1999; Cooper et al, 1999).

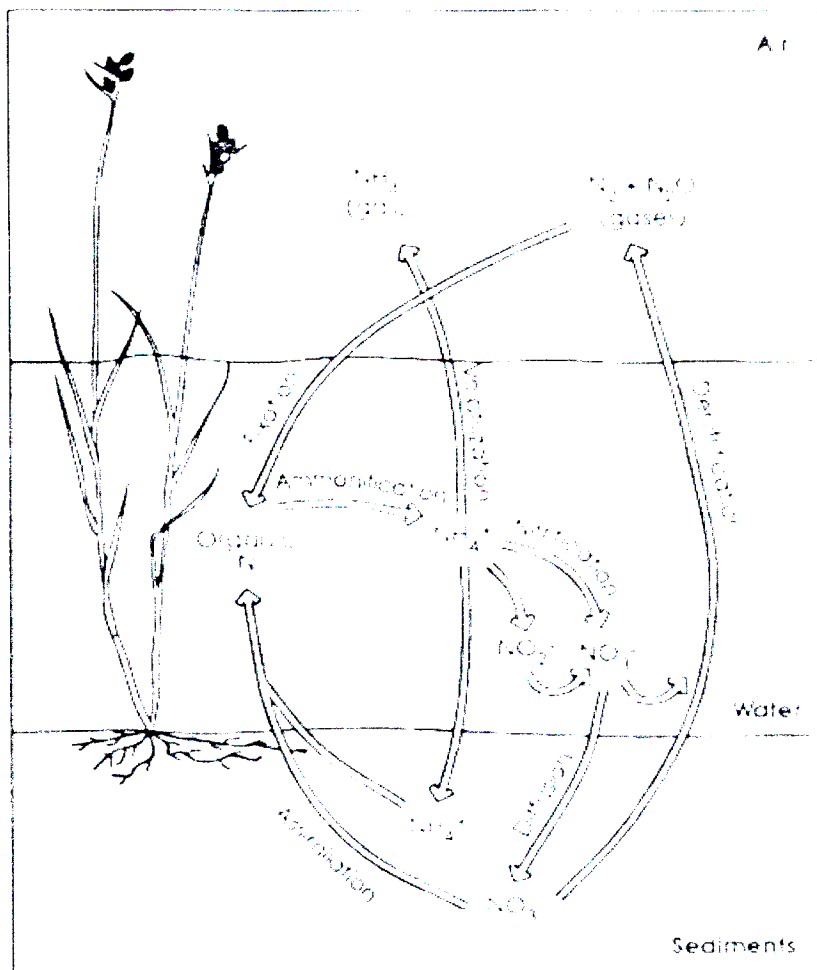


Figure 2.5 - Constructed wetland biological nitrogen cycle, from Kadlec and Knight (1996).

Once the ammoniacal nitrogen has been nitrified, either due to pre-treatment or through sequential constructed wetland cells, and it is in the form of nitrate nitrogen, the final biological step in the removal process is denitrification. Denitrification takes place under anoxic conditions where nitrate is the electron acceptor and carbon is the electron donor; nitrate nitrogen is converted to N_2 and N_2O gasses that readily exit the wetland (Reed et al, 1995, Kadlec and Knight, 1996 and EPA, 2000). The nitrogen gas formed through denitrification and present in the water column through atmospheric re-aeration may, however, be converted back to organic nitrogen through nitrogen fixation, mediated by specific bacteria and blue-green algae under aerobic and anaerobic conditions (EPA, 2000).

Plants may also have a significant potential to remove nitrogen from wastewaters. The removal of nitrogen by plant uptake must, however, be regarded as temporary removal as most of the nutrients taken up are returned back to the system once the plants die and decompose (Wetzel, 1993). Algae may also remove considerable amounts of nitrogen, but release all nutrients after death, since algae contain less structural refractory material (Rogers et al, 1985).

As shown in this chapter, biologic denitrification needs a carbon source; an overview of typical carbon sources is provided in the next chapter, with particular focus on organic material.

3 COMPARISON AMONG CARBON-SOURCES

3.1 Introduction

Denitrification occurs mainly under anoxic conditions in the presence of nitrate as an electron acceptor. Heterotrophic denitrifying bacteria utilize organic matter as a carbon and energy source (Tiedje, 1982). However, effluents from secondary treatment plants normally contain very low concentrations of easily degradable organic matter. Hence, carbon and energy from external sources are commonly supplied to the reactor to enable the biological denitrification process to proceed satisfactorily.

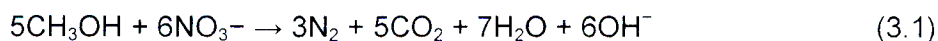
Nitrate can be converted into ammonium via assimilative reduction or into gaseous nitrogen byproducts, forming nitrite as an intermediate product in dissimilative reduction (Madigan et al., 1997).

3.2 Carbon sources and relative kinetics

Substantial research has been done on the design of bioreactors for denitrification (McCleaf and Schroeder, 1995; Reising and Schroeder, 1996; Shanableh et al., 1997). Most designs require a supplemental carbon source such as sucrose (Sison et al., 1995), methanol [CH_3OH] (dos Santos et al., 2004), ethanol [$\text{C}_2\text{H}_5\text{OH}$] or acetic acid [$\text{C}_2\text{H}_4\text{O}_2$] (Constantin and Fick, 1997), or methane [CH_4] (Thalasso et al., 1997) to be effective. They also require a high level of management for on site or off side treatment of subsurface drainage water. Solid carbon sources would appear to be more appropriated to field application.

Some examples of stoichiometric reactions for different electron donors are shown below:

Methanol:



Acetate:

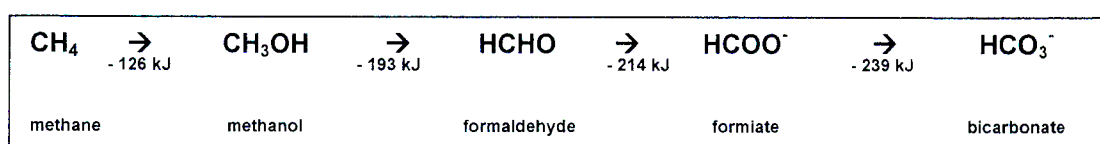


(Metcalf & Eddy, 2004)

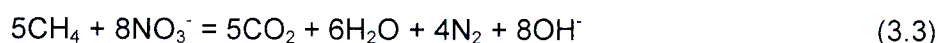
Full-scale waste water treatment plants have commonly used methanol as external carbon source (Liessens et al., 1993; Louzeiro et al., 2002). Nevertheless, the need for additional

exogenous electron donor sources increases operational costs, possibly representing a drawback for the use of innovative anaerobic process-based technologies.

The existence of microorganism consortia that can use methane to produce electron donors for denitrification under certain environmental conditions has been demonstrated (Houbron et al., 1999; Costa et al., 2000). According to Thalasso et al. (1997), denitrification using methane as the sole carbon source would proceed if a consortium of methanotrophic and denitrifying bacteria could be developed and maintained inside the reactor. In the presence of oxygen, such organisms are able to convert methane into intermediate compounds (as shown in the sequence below, Madigan et al., 1997), utilized by denitrifying bacteria as electron donors for reducing nitrate to nitrogen gas.



The denitrification reaction using methane as a carbon source can be written as follows (Modin et al., 2007):



Potentially inexpensive, methane can be generated on site due to the anaerobic digestion of sludge in wastewater treatment plants and degradation of organic waste in landfills.

Denitrification rates depend both on the carbon and energy sources used and on the carbon to nitrogen (C/N) ratio. Low C/N ratios can cause nitrite to accumulate (Bandpi and Elliot, 1998), while the dissimilative reduction to ammonium can occur at high C/N ratios (Gylsberg et al., 1998), damaging the denitrification process.

Callado and Foresti (2001) obtained very efficient denitrification rates at C/N ratios of 0.9 to 1.7.

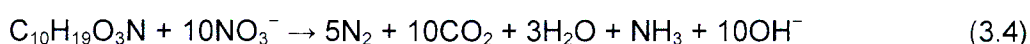
It is well known that kinetic parameters are useful tools for a comparative process analysis. Some studies have presented the denitrification kinetics as following a single zero-order reaction (Shieh and Mulcahy, 1986; Ros, 1995). Nevertheless, the process can be better represented if a sequence of reactions in series is applied. This approach can lead to a better understanding of the process development and control, thus permitting some

operating variables to be monitored to avoid an intermediate product accumulation or a poor performance.

Referring to the equation 3.1, (page 44), using methanol as a carbon source, a value of 0.2-0.3 mgN-NO₃/mg_{V_S}*d at 20°C for the constant k_{dt} has been reported; using organic substances present in the waste water (C₁₀H₁₉O₃N - U.S. EPA, 1993) lower values are obtained: 0.1 mgN-NO₃/mg_{V_S}*d (Carucci, 2003).

The denitrification reaction can be expressed as follows:

Wastewater:



Moreover, the supposed difference in the kinetic behavior of methanol and C₁₀H₁₉O₃N can be, partially, linked with the different process schemes.

C₁₀H₁₉O₃N as a carbon source is employed as organic matter only in the single sludge system (see paragraph 2.6.2) and the denitrification reaction shows a lower kinetic related to CH₃OH used in a separate system because not all heterotroph mass can utilize nitrate as an electron acceptor (Water Pollution Confederation Manual, 1983).

In the following table a comparison between Methanol and Urban Waste Water is shown.

Table 3.1- Comparison between Methanol and Urban Waste Water (Carucci, 2003).

	Specific consumption:	Growth yield
Methanol	2.5 – 3 mg CH ₃ OH/mg N-NO ₃ 2.9 mg COD/mg N-NO ₃	0.23 mg VSS/mg COD
Urban waste water	8.6 mg COD/mg N-NO ₃	0.42 mg VSS/mg COD
Aerobic process	-	0.4 – 0.5 VSS/mg COD

Another option that can be considered is the use of Molasses. Molasse is a by product of the sugar production cycle with high sugar content (48–50%). This by-product is a cheap carbon source used for various industrial fermentations (Miranda et al., 1996; Najafpour and Shan, 2003). Some researchers have used molasses as an external carbon source for denitrification (Boaventura and Rodrigues, 1997; Ten Have et al., 1994). However, the major components in molasses, polysaccharides, have carbon chains that are too long to be used quickly by denitrifying bacteria and need to be hydrolyzed to reduce sugars such as sucrose, glucose and fructose (Najafpour and Shan, 2003).

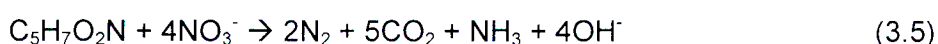
To increase the effect of molasses as a carbon source in denitrification, molasses can be thermohydrolyzed under acidic conditions (Quan et al., 2005).

Quan et al., 2005 compared the effectiveness of hydrolyzed molasses for the treatment of artificial sewage in a SBR with the commonly used carbon source, methanol.

The denitrification rate was 2.9–3.6 mg N/g VSS h with hydrolyzed molasses, in which the percentage of readily biodegradable substrate was 47.5%.

During the 14 days (28 cycles) of operation, the SBR using hydrolyzed molasses as a carbon source showed $91.6 \pm 1.6\%$ nitrogen removal, which was higher than that using methanol ($85.3 \pm 2.0\%$). These results show that hydrolyzed molasses can be an economical and effective external carbon source for the nitrogen removal process (Quan et al., 2005).

The denitrification reaction involving endogenous carbon can be expressed as follows:



When $\text{C}_5\text{H}_7\text{O}_2\text{N}$ is the cellular mass from sludge endogenous respiration

Figure 3.1 reports the variation of k_{dT} with the temperature in $[\text{mgN-NO}_3^* \text{ mg}^{-1} \text{ VSS}^* \text{d}^{-1}]$ when the carbon source perform an endogenous denitrification (U.S.EPA, 1975).

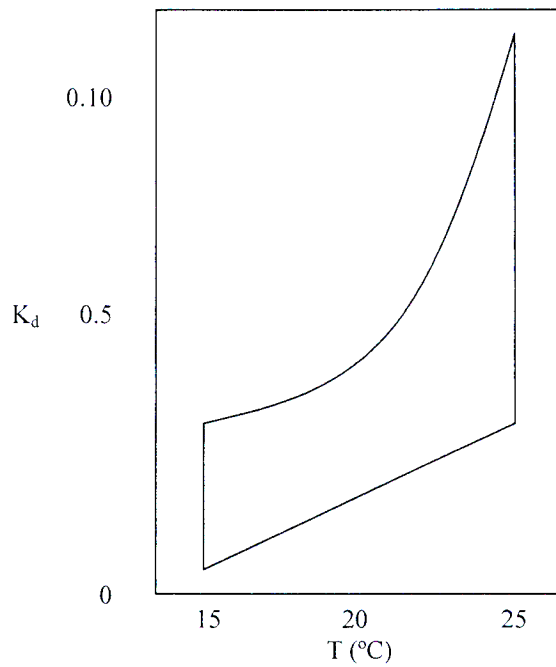


Figure 3.1 - Denitrification kinetic constant values with temperature using endogenous carbon as a carbon source (U.S.EPA, 1975).

Apart from the carbon sources previously described, a list of industrial waste waters has been used as an organic counterpart in the denitrification process. Table 3.2 reports the main substances tested and the relating denitrification constants.

Table 3.2- Denitrification rate at 20° C for industrial organic substrates (Monteith et al., 1980).

Effluents	Denitrification rate [mg N-NO ₃ * mg _{VSS} ⁻¹ * d ⁻¹]	Specific consumption carbon substrate	
		[mg COD/mg N-NO ₃]	[mg TOC/mg N-NO ₃]
Petrol industry	0.33	2.2	0.8
Alimentary industry	0.16 – 0.26	2.6 – 5.7	-
Distillery	0.12 – 0.21	5.0 – 10.2	1.4 – 2.3
Brewery	0.16 – 0.2	5.3 – 6.7	1.4 – 2.5
Amid industry	0.16	3.3	-
Organic chemistry	0.11 – 0.14	3.9 – 6.0	1.4 – 1.7
Paper mill	0.13 – 0.14	2.5 – 3.9	0.8
Fuel industry	0.14	6.2	1.8
Cider extract	0.11	5.7	2.6
Isopropanol	0.1	3.6	1.8
Milk serum	0.1	9.7	0.9
Destrosium	0.1	8.2	2.6
Formaldehyde	0.05	1.4	-

Volokita et al. (1996) used shredded newspaper as a C source in laboratory columns to obtain N removal rates ranging from 0.056 to 0.875 mg N g⁻¹ newspaper d⁻¹. Blowes et al. (1994) used a fixed-bed bioreactor filled with a compost mixture of sand, tree bark, wood chips, and leaves to treat drainage water from a farm field. Over a year, a 200-L bioreactor was able to remove nearly all NO₃ from a 10 to 60 L d⁻¹ discharge of field drainage water containing 3 to 6 mg L⁻¹ NO₃-N. Neither study differentiated between assimilation and denitrification as the NO₃ loss mechanism.

Robertson and Cherry (1995) demonstrated the NO₃ removal potential of a bioreactor constructed in situ. They filled a 0.6-m-wide trench that extended 0.75 m below a shallow water table with sand containing 20% (v/v) coarse sawdust and measured the concentration of NO₃ in ground water before and after flowing through the mixture. Very high NO₃-N concentrations (57–62 mg L⁻¹) were reduced to 2–25 mg L⁻¹ in the ground water passing through the bioreactor. They attributed the removal of NO₃ to heterotrophic denitrification,

with the sawdust serving as C source, and estimated that this denitrification wall would have an effective lifetime of 20 to 200 years. However, they offered only indirect evidence that denitrification was the primary removal mechanism, citing only reduced O_2 and SO_4 concentrations in the water. In a similar study with a constructed denitrification wall, Schipper and Vojvodic-Vukovic (1998) found NO_3-N concentrations to be reduced from between 5 and 16 $mg\ L^{-1}$ to $<2\ mg\ L^{-1}$ in shallow ground water passing through a wall. They attributed the NO_3 removal to denitrification and reported that denitrifying enzyme activity reached a plateau of $906\ ng\ g^{-1}\ h^{-1}$ after 6 months of operation.

3.3 Pine bark and compost

The present study is focused on denitrification of treated leachate using available pine bark and compost as a carbon sources.

The incoming garden refuse at the Bisasar Road Landfill Site can be separated into two main groups: pine bark and local garden refuse including mainly tree and bush trimmings as well as grass clippings.

3.3.1 Pine bark material

Bark is a non technical term that refers to that tissue outside of the vascular cambium. When debarking, the vascular cambium is also removed, so the term “bark” is everything on the exterior of the last ring of the secondary xylem (Trois and Polster, 2007).

Origin and formation of pine bark

Pines can be divided into two main groups:

- APLOXYLON – with branches and young stems smooth and without fissures
- DIPLOXYLON – with branches and young stems rough and fissured

The species used in southern Africa belong to the latter group, including *Pinus patula*, *Pinus elliottii*, *Pinus taeda*, *Pinus radiata* and *Pinus pinaster*.

The first three are grown in the summer rainfall region, the last four in the constant rainfall region of the southern cape, while the last two grow mainly in the Western Province. Over 1.5 million m^3 of bark, mainly from *Pinus patula*, *P. elliottii* and *P. taeda* are produced in South Africa (Smith, 1992). Almost all the available pine bark comes from debarkers, such as ring and drum debarkers that remove only a little wood from logs and generally yield bark

with a wood content of less than 10 percent. Rosserhaed debarkers are used at sawmills for large diameter logs, remove a considerable amount of wood in addition to bark. The size of the chunks bark depends on the debarking machine and on the pine species. For ring debarkers the pieces should not be as bigger as 10cm in length because of the distance between each cut (Smith, 1992).

Chemical composition of pine bark

The chemical composition of pine bark is important and should be considered when using as input material for an aerobic biological degradation process. The content of chemical compounds varies with species and age of the trees. A representative chemical composition of wood waste is presented in $C_{295}H_{420}O_{186}N$ (Maggs, 1985).

Organic constituents

- Cellulose

Cellulose is a linear polymer of repeating glucose units, linked by β (1 – 4) glycosidic bonds. The degree of polymerization of D – glucose can be up to 15.000, with an average of 8.000. The Cellulose content of bark differs with different species and the younger the trees and branches the higher the percentage is found in the bark. The inner bark also contains more cellulose than the outer (Maggs, 1994).

During composting the cellulose is most readily broken down by microbes and so it is the cellulose content that determines the nitrogen requirement. The cellulose content of pine bark requires only 0,2% N and is far lower than most hardwood barks, requiring 2% N used for composting (Hoitink et al., 1993).

- Lignin

Lignin is an aromatic polymer of phenylpropane units, based upon p-coumaryl, coniferyl and sinapyl alcohols. Free radical copolymerization of these alcohols produces a heterogeneous, cross linked polymer. More than 10 linkage types occur, the dominant type being the β -aryl-ether (β – 0 – 4) linkage. During polymerization, secondary reactions lead to cross linking between lignin and hemicelluloses. The degree of binding of lignin to the other constituents of lignocelluloses is one of the major factors affecting the ability of micro organisms and their enzymes to biodegrade organic materials (Hoitink et al., 1993).

Most barks contain slightly more lignin than cellulose. The lignin contained in bark is resistant to enzymatic degradation and only a little loss of lignin occurs during the composting process (Smith et al., 1990).

Because of the high lignin content, bark is a stable medium that does not lose its structure and physical properties during the composting process.

- ***Tannins***

Tannins give fresh pine bark its characteristic odour, and inhibit seedlings and plant growing in the bark. The bark may contain different levels of tannins in relation to the pine species, climatic conditions, soil type and the age of the tree.

Tannins are water soluble and denatured by heat. The time, required to drop the level below the non toxic concentration of 2% ranges in literature from 30 days (Still, 1974) up to 12 weeks (Van Schoor et al., 1980).

Elemental composition of pine bark

The elemental composition of bark also differs according to the tree species, age, ambient conditions (soil type, climate, etc.) and even seasons.

- ***General***

With the exception of Nitrogen and Phosphorus, the concentrations of all nutrients are sufficient to cover the needs of the micro organisms (Solbraa, 1986). With decreasing maximum bark particle size increases the availability of Phosphorus (Aaron, J. R. 1972):

- ***Nitrogen and C/N – ratio***

The C/N – ratio in pine bark is very high. In Literature values differ between 723:1 (Willson, G. B. 1989), 580:1, (Schliemann, G. K. G. E. 1974), 480:1 (Lamb, 1982) and 300:1 before composting and 150:1 after composting (Gartner, 1979).

According to the previous section pine bark contains large quantities of lignin, cellulose and tannins. All of them contain dominantly carbon causing the high C/N – ratio. Due to the fact that these constituents cannot be readily broken down during composting, the active C/N – ratio will approach that of other compost input materials. Furthermore pine bark has less cellulose and other available carbon sources than hardwood bark and generally requires less nitrogen for composting (Maggs, 1984).

Micro – organisms in pine bark

According to Maggs (1984) the number of mesophiles and thermophiles bacteria is relatively constant and shows no detectable seasonality. There are greater numbers of bacteria than fungi present in the bark and there is furthermore a predominance of mesophiles over thermophiles micro organisms.

Researchs, described in (Maggs, 1984), about the ageing of bark prior to composting showed that microbial numbers do not vary significantly, but a quality change in the composition occurs. The starter microflora appears to deteriorate with prolonged time. Bark aged for longer periods (3 – 5 month) showed relatively smaller increases in the number of thermophiles than no aged, or aged bark for a short period (0 – 1 month). This may be caused by a predominance of facultative anaerobic bacteria, as the heaps are bad ventilated during ageing.

Physical properties of pine bark

- ***General***

An analysis of wood – waste, published in (Schliemann, 1974): showed the following physically properties of pine bark:

-	dry density (bulk)	[kg/m ³]	180 – 250
-	electrical resistance	[ohm]	780
-	Ignition loss	[% of dry mass]	96

- ***Decomposition***

Allison (1975) reports in about the decomposition of bark when incubated in soil for 60 days; therefore nitrogen was added to prevent nutrient rate limitations. The results of these studies are presented in Table 3.3:

Table 3.3 - Decomposition of pine barks - incubated in soil for 60 days, with addition of nitrogen (Allison, 1975)

Species	% of bark carbon released as CO ₂
White pine	3
Loblolly pine	3,5
Slash pine	5,5
Longleaf pine	9,3
Ponderosa pine	11,1
Western white pine	13,8
Lodgepole pine	23,2
Sugar pine	3,8
Shortleaf Pine	4,1
Average for all investigated pine species	8,6

There appears to be rather high difference in the decomposition of various pine species in the soil. Many of the pine barks exhibited relatively low decomposition after 60 days in soil. The decomposition for all pine barks averaged around 8,6%.

3.3.2 Compost

As presented in paragraph 3.2, when designing bioreactors for nitrate removal, a supplemental carbon source is usually required for supporting the denitrification processes. Several biodegradable carbonaceous compounds, such as glucose, methanol, ethanol, propionate, or acetic acid are used. These products, however, may not be suitable for applications in developing countries because of their high cost. Moreover, because of their high solubility, they could be easily transported in the surface or ground water. Other solid materials, such as tree bark (Blowes et al., 1994), wood chips and corncobs (Doheny et al., 2000), newspaper (Vолоkita et al., 1996), and sawdust (Robertson and Cherry, 1995) have been proposed the carbon.

Because these solid carbon materials were not as biodegradable as the more water-soluble carbon products, their use in denitrification may require a relatively long time for the biomass to accumulate and to yield significant efficiencies.

Therefore, an alternative solid organic material that could sustain abundant initial microbial activity could be a more desirable carbonaceous source for the denitrification purpose.

Compost is made by the microbial transformation of organic matter and can sustain a large population of microorganisms.

The high microbial activity in compost could readily utilize the carbon source for denitrification. Immature yard-waste compost has larger carbon content when compared with mature compost (Trois and Polster, 2007). Moreover, the immature compost would contain greater percentage of liable carbon source than the matured compost (Haug, 1993).

Composts, because of their high organic matter content, make a valuable soil amendment and are used to provide nutrients for plants. When mixed into the soil, compost promotes proper balance between air and water in the resulting mixture, helps reduce soil erosion, and serves as a slow-release fertilizer.

Composting involves the aerobic biological decomposition of organic materials to produce a stable humus-like product (see Figure 3.2). Biodegradation is a natural, ongoing biological process that is a common occurrence in both human-made and natural environments. To derive the highest benefit from this natural, but typically slow, decomposition, it is necessary to control the environmental conditions during the composting process.

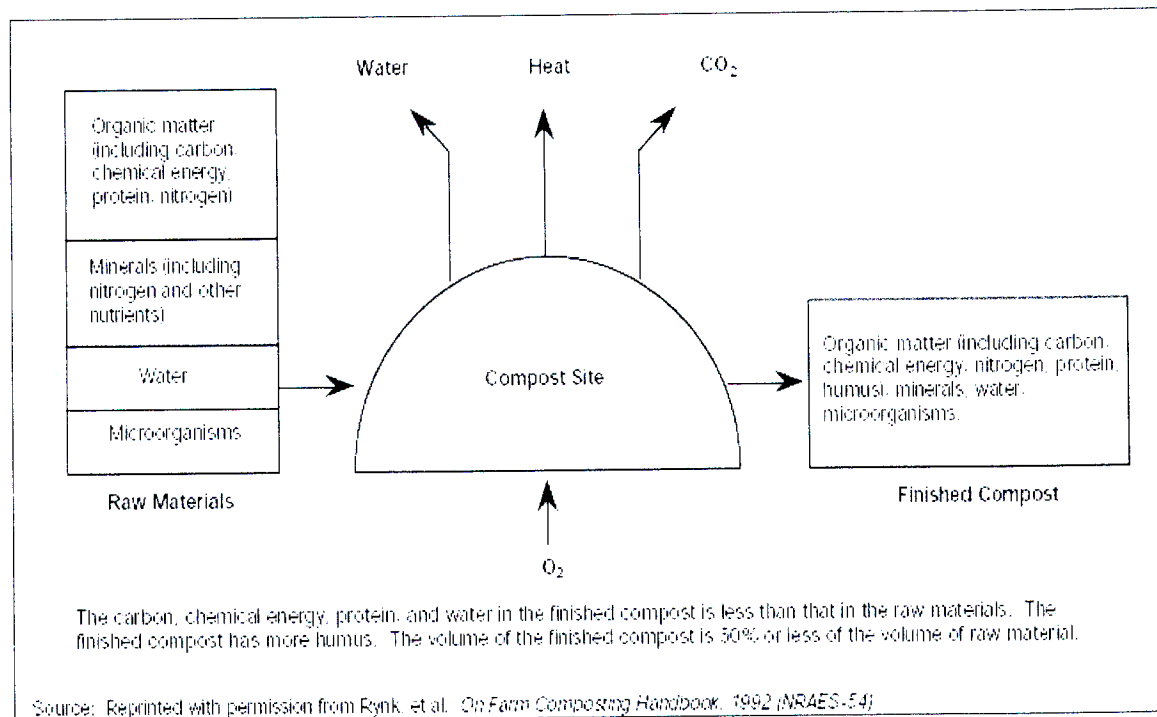


Figure 3.2 - Schematics of the composting process

The main targets of composting operations are:

- ✓ Stabilizing organic biodegradable substance reducing the volume at the same time
- ✓ Destroying pathogens and others dangerous organisms through high temperature (60 – 70°C in the thermophilic stage)
- ✓ Retaining nutrients (N, P, K)
- ✓ Obtaining a good manure

Municipal solid wastes and yard trimmings contain up to 70 percent by weight of organic compounds.

In addition, certain industrial by-products from the food processing, agricultural, and paper industries, are mostly constituted of organic materials. Composting organics, therefore, can significantly reduce the waste stream. Diverting such materials from the waste stream frees up landfill space.

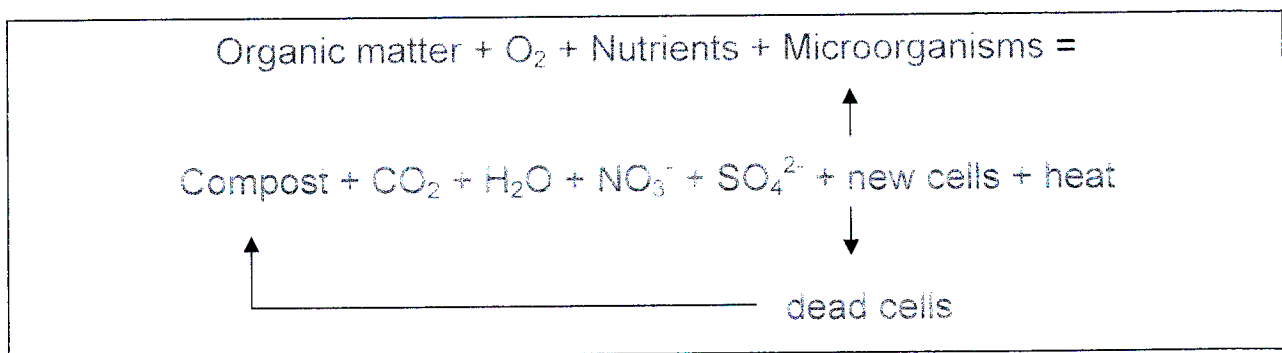
Composting is nowadays increasing for several factors, including increased landfill tipping fees, shortage of landfill capacity, and increasingly restrictive measures imposed

by regulatory agencies. In addition, composting is indirectly encouraged by states with recycling mandates that include it as an acceptable strategy for achieving mandated goals. Consequently, the number of existing or planned composting programs and facilities has increased significantly in recent years.

Composting may also offer an attractive economic advantage for low income communities. In some cases, the benefits of reducing disposal needs through composting may be adequate in fact it will be ultimately used for other purposes such a landfill cover.

Transformations in the composting process and influent parameters

The aerobic transformation process of organic matter, during composting, can be described as follows:



Where main components are proteins, aminoacids, fats, carbohydrates, cellulose, lignin, ashes and mainly cellulose are lignin and ashes.

New cells produced in the process are part of the active biomass involved in the transformation of organic matter while dead cells became part of the compost.

The chemical environment is largely determined by the composition of material to be composted. In addition, several modifications can be made during the composting process to create an ideal chemical environment for rapid decomposition of organic materials. Several factors determine the chemical environment for composting, especially:

- a) the presence of an adequate food/energy source: carbon to nitrogen ratio (C/N)
- b) a balanced amount of nutrients,
- c) the correct amount of water,

- d) adequate oxygen,
- e) appropriate pH,
- f) absence of toxic constituents that could inhibit microbial activity.

Carbon/Nitrogen ratio (C/N) (U.S.EPA, 1998).

The C/N ratio is considered critical in determining the rate of decomposition. The ratio must be established on the basis of *available* carbon rather than *total* carbon. In general, an initial C/N of 25 - 50 is considered ideal to obtain good compost. Higher ratios tend to retard the process of decomposition because of the excessive CO₂ production and the relative slowing down of bacterial activity, while ratios below 25 may result in nitrogen loss as NH₃ stripping and odor problems.

Typically, C/N ratios for yard trimmings range from 20 to 80, wood chips 400 to 700, manure 15 to 20, and municipal solid wastes 40 to 100.

To lower the C/N ratios, nitrogen-rich materials such as yard trimmings, animal manures, or biosolids are often added. Adding partially decomposed or composted materials (with a lower C/N ratio) as inoculums may also lower the ratio. Attempts to supplement the nitrogen by using commercial fertilizers often create additional problems by modifying salt concentrations in the compost pile, which in turn impedes microbial activity.

As temperatures in the compost pile rise and the C/N ratio falls below 25, the nitrogen in the fertilizer is lost in gaseous form (ammonia) to the atmosphere creating odors.

As the composting process proceeds and carbon is lost to the atmosphere, this C/N ratio is reduced (Figure 3.3). Mature compost should have C/N ratios around 15 to 20 (Trois and Polster, 2007).

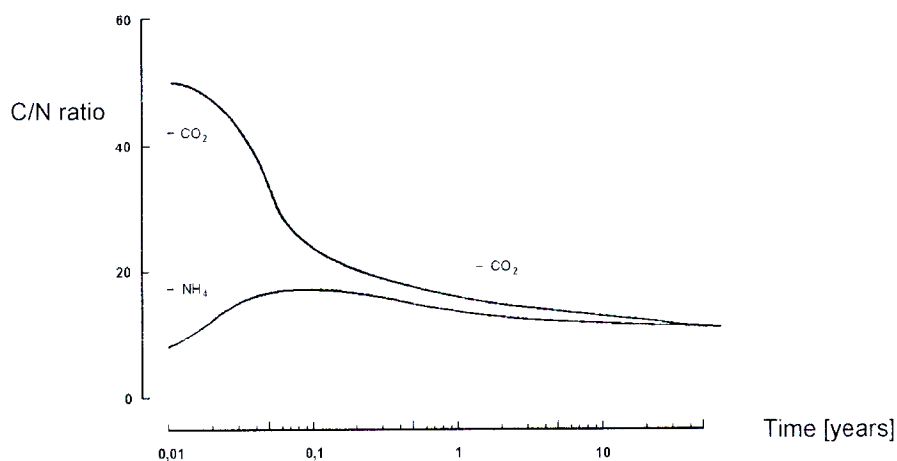


Figure 3.3 - Schematic view of the C/N evolution in two materials rich in carbon and nitrogen (US EPA, 1998).

Moisture

A moisture content of 50 to 60 percent of total weight is considered ideal in the composting process; in fact the excess lead to free flow of water and movement caused by gravity, potential liquid management problems and potential water pollution and odor problems (Trois and Polster, 2007).

Excess moisture also impedes oxygen transfer to the microbial cells and can increase the possibility of anaerobic conditions developing and may lead to rotting and obnoxious odors.

Microbial processes contribute to increase the moisture content during decomposition.

While moisture is being added, however, it is also being lost through evaporation. In such cases, adding moisture may be necessary to keep the composting process performing at its peak.

Evaporation from compost piles can be minimized by controlling the size of piles.

Oxygen (U.S.EPA, 1998).

Composting is an *aerobic* process and the compost pile should have enough void space to allow free air movement so that oxygen from the atmosphere can enter the pile and the carbon dioxide and other gases emitted can be exhausted to the atmosphere. In some composting operations, air may be mechanically forced into the piles to maintain adequate oxygen levels. In other situations, the pile is turned frequently to expose the microbes to the atmosphere and also to create more air spaces.

A 10 to 15 percent oxygen concentration is considered adequate, although a concentration as low as 5 percent may be sufficient for leaves. While higher concentrations of oxygen will not negatively affect the composting process, they may indicate that an excessive amount of air is circulating, which can cause for example, cooling of the pile, excess in evaporation, slowing the composting rate down. Excess aeration is also an added expense that increases production costs.

The air needed for the composting process depends on the amount of biodegradable organic substances. The oxygen consumption average is around $0.5 \text{ m}^3/\text{kg}\cdot\text{d}$.

Temperature (U.S.EPA, 1998).

All microorganisms have an optimum temperature range. For composting this range is between 32° and 60° C (mesophilic or termophilic). Although composting can occur at a

range of temperatures, the optimum temperature range for thermophilic microorganisms is preferred, for two reasons:

- promote rapid composting
- destroy pathogens and weed seeds (Table 3.4).

Temperatures above 65° C are not ideal for composting.

Pathogen destruction is achieved when compost is at a temperature of greater than 55° C for at least three days. At these temperatures, weed seeds are also destroyed. After the pathogen destruction is complete, temperatures may be lowered and maintained at slightly lower levels (51° to 55° C).

Table 3.4 Temperature and exposition time required for pathogens and parasites destruction (U.S.EPA 1998).

<u>Microorganism</u>	<u>Time/temp.</u>	
Salmonella typhosa	30'/60 °C	(stasis after 46°C)
Salmonella sp.	20'/60 °C	(60'/56°C)
Shigella sp.	60'/55 °C	
Escherichia Coli	20'/60 °C	(60'/55°C)
Entamoeba histolytica cysts	few	min/45 °C
Taenia saginata	few	min/55 °C
Trichinella spiralis	immediate/60	°C
Brucella abortus or suis	3'/62 °C	(60'/55 °C)
Micrococcus pyrogenes	10'/50 °C	
Streptococcus pyrogenes	10'/54 °C	
Mycobacterium tuberculosis	20'/66 °C	
Corynebacterium diptheriae	45'/55 °C	
Necator americanus	50'/45 °C	
Ascaris lumbricoides eggs	60'/50 °C	

pH

The compost pH, like the temperature, changes during the process (Figure 3.4), in general a pH between 6 and 8 is considered optimal (Trois and Polster, 2007).

pH affects the amount of nutrients available to the microorganisms, the solubility of heavy metals, and the overall metabolic activity of the microorganisms. The pH can be adjusted upward by addition of lime or downward with sulfur, but such additions are normally not necessary.

The composting process itself produces carbon dioxide, which, when combined with water, produces carbonic acid. The carbonic acid could lower the pH of the compost. As the composting process progresses, the final pH varies depending on the specific type of feedstocks used and operating conditions. Wide swings in pH are unusual. Because organic materials are naturally well-buffered with respect to pH changes, down swings in pH during composting usually do not occur (U.S.EPA, 1995).

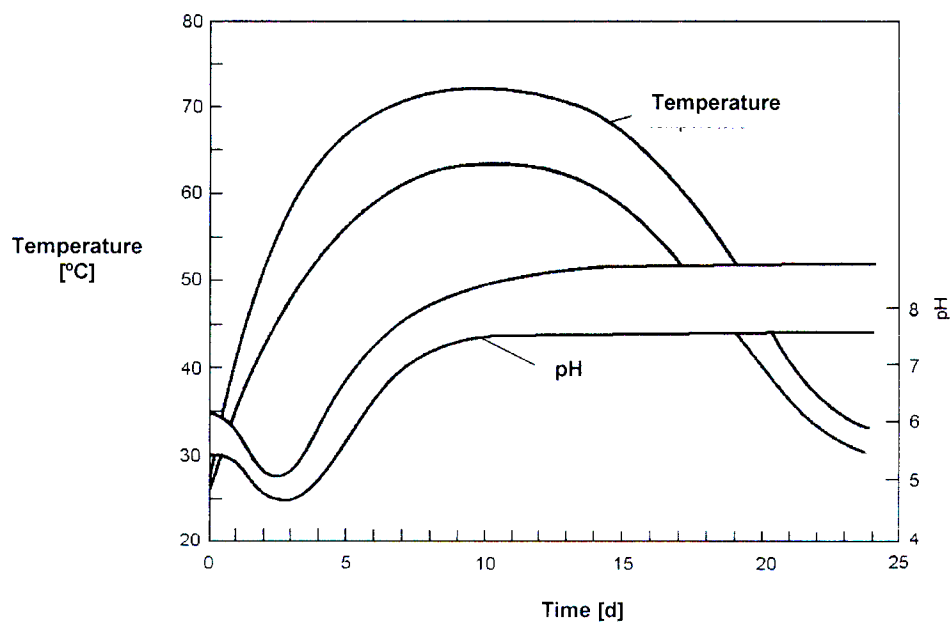


Figure 3.4 – Temperature and pH variation in a natural fermentation composting process (Curreli, 2000).

4 MATERIALS AND METHODS

4.1 Introduction

This experimentation was developed in order to study the potential of organic substrates such as immature compost from garden refuse and pine bark for nitrate removal (denitrification). The investigation focused on the study of the kinetics of nitrate removal in relation to degree of maturity, biodegradability, size distribution and quality of the organic substrates.

The goal is to assess the suitability of organic waste compost and pine bark as by products of an integrated waste management system in denitrifying treated landfill leachate.

Leachate collected from the Mariannhill Landfill Leachate Treatment Plant in Durban was treated in leaching columns operated as static beds packed with garden refuse compost and pine bark.

The first step of the research was to characterize both substrates and the leachate sample using conventional tests performed on the solid material and on eluates. Preliminary anaerobic batch tests were then designed to investigate the optimal removal efficiency and process kinetics using different nitrate concentrations. A series of batch tests were then conducted in larger leaching columns to assess the process kinetics and the longevity of the substrates.

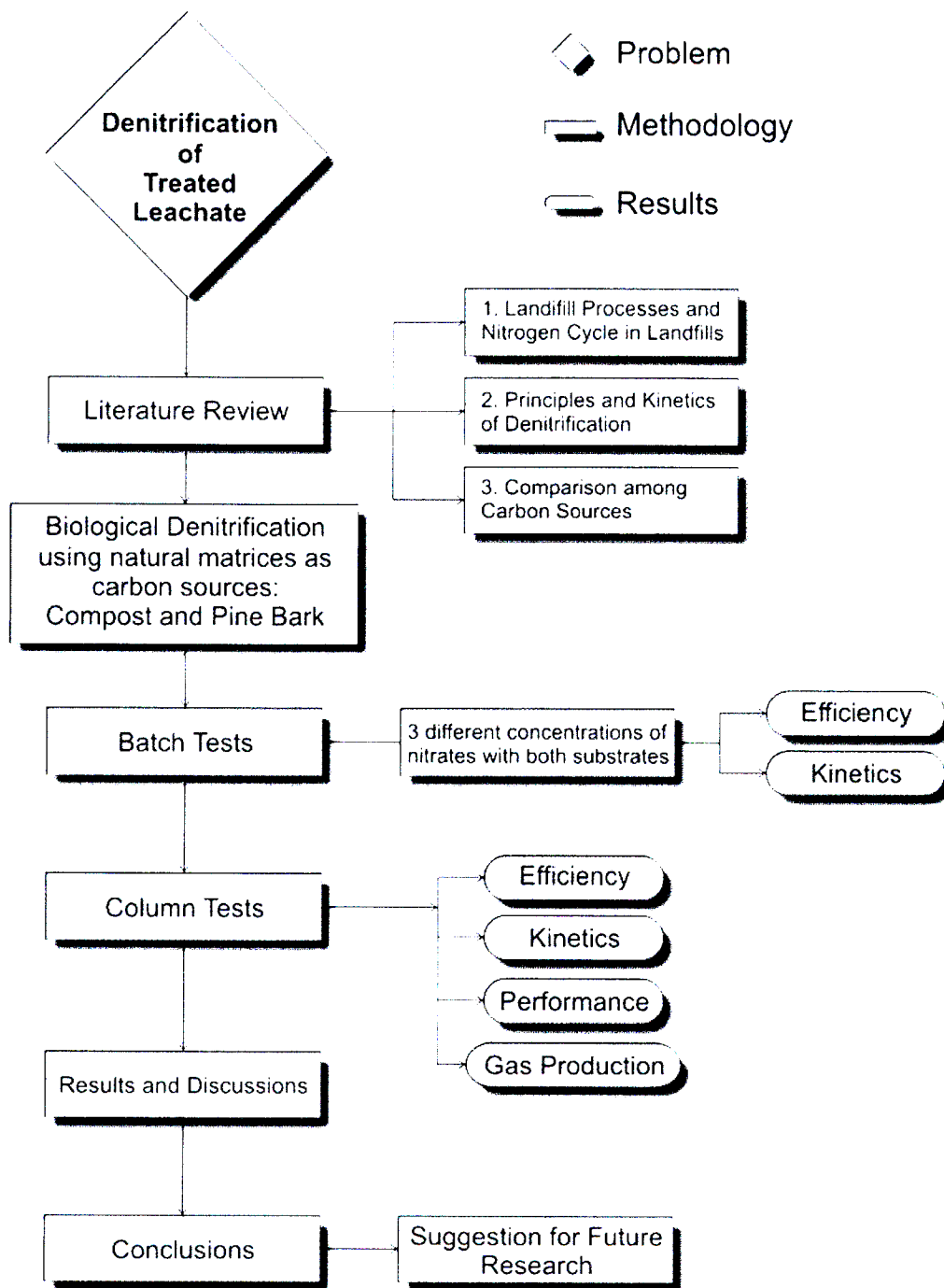


Figure 4.1 Research framework.

4.2 Materials

Large quantities of pine bark are produced daily by Mondi paper and disposed of in local landfills. The pine bark used for this investigation was collected at the Mariannhill Landfill site in Durban.



Figure 4.2 - Fresh pine bark

The compost employed in this study was sourced from a small composting operation at the Sundown Retirement Village in Durban where garden refuse is treated in turned open windrows for approximately 4 to 6 months and sieved to a 40-50mm particle size. The compost used in this research was collected at the end of the 4th month cycle.



Figure 4.3 - Garden refuse compost

The leachate sample was collected from a Sequencing Batch Reactor (SBR) at the Mariannhill Landfill site that is designed to treat up to 50 cubic meters of influent from the landfill, daily.

The plant also comprises of a 280 square meter lined reed bed, which provides a polishing treatment for the removal of residual BOD, COD and solids. The treated effluent from the SBR is fed into a balance tank, which is level controlled to supply a

portion of the effluent to a standpoint for the site water tanker for dust suppression and a portion to the reed bed. The effluent from the reed bed is used for irrigation of the vegetated areas within the conservancy area. The leachate used for this study was collected from the balance tank before entering in the reed bed.

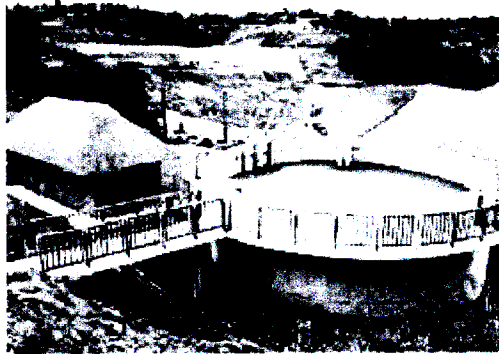


Figure 4.4 - Mariannhill Landfill SBR

4.3 Quality Characterization

An extensive quality characterization of the substrates and leachates used was conducted through the use of standard analytical methods; as described by standard test procedures published by ASTM (Clesceri et al, 1989).

Most of the analyses were conducted in the Environmental Engineering laboratory at the University of KwaZulu-Natal, Durban; the analysis of Total Carbon and Total Nitrogen on the solid matter was performed at the BermLab laboratory in Somerset West (Cape Town); Nitrates analysis, TKN and TOC on the liquid samples were analyzed at the BN Kirk laboratory in Durban, South Africa.

4.4 Sampling

In order to obtain a representative sample the solid substrates were quartered according to the following standard method:

the solid matter was mixed and turned to ensure a good homogeneity. The pile was divided in 4 parts with 2 perpendicular diametral lines, then 2 opposite quarters were chosen and the others 2 removed (Figure 4.5, A). The material in the 2 quarters was spreaded in the adjacent quarters and the circular mass was divided in 4 parts with 2 orthogonal diameters at 45° from the previouses (B). The material in the 2 opposite

quarters was removed as in the previous case. The mass was mixed again and arranged in a circle with the same thickness and diameter 0.7 of the initial one. In this mass the previous operations were repeated obtaining, in this way, the last quartering, to the representative sample for the tests.

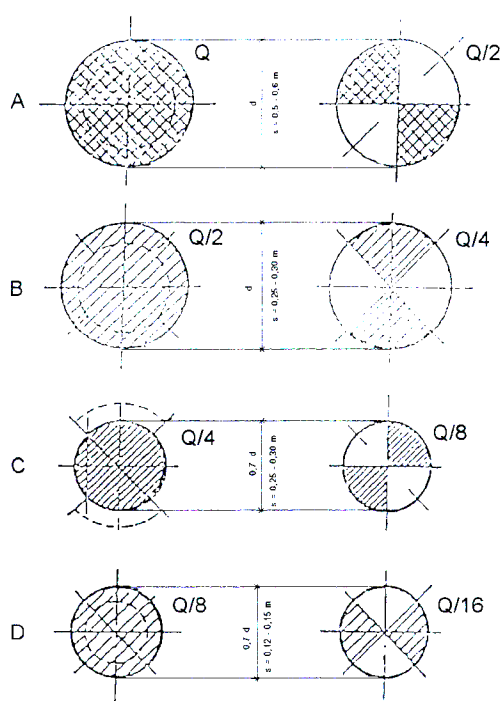


Figure 4.5 – Schematic view of quartering method



Figure 4.6 - Quartering procedure

4.5 pH

(Procedure – Modified Metodi Analitici IPLA – 1984 “Analisi chimica del suolo” - Gazzetta Ufficiale - 1999)

The measuring of pH was carried out using a Labotec Orion 410A pH meter. For the solid matter pH was measured on a slurry of 10g of solid matter (previously dried and sieved at 2mm), mixed with 100g of distilled water for 30min and then left to settle for 15min. For the eluates and the leachates, pH was determined dipping the probe in a known volume of liquid.

4.6 Moisture content

Moisture content is defined as the ratio of the volume of water to the total volume of a porous media (Bedient et al., 1999). Moisture content was measured with the following procedure:

100-200g of a solid sample are weighed at natural moisture content and then desiccated at 105 °C for 24 hours. After cooling down in a desiccator, the sample is weighed again and the amount of water contained in its structure is determined as in equation 4.1:

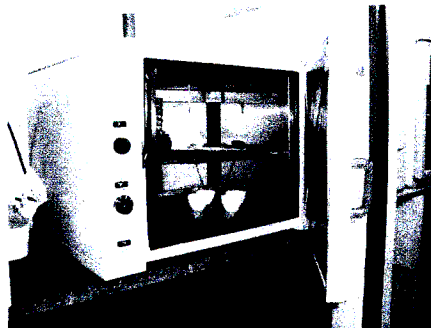


Figure 4.7 - Oven

$$W_{tot} = \frac{W_w - W_d}{W_w} \quad (4.1)$$

W_w = wet sample weight;

W_d = dried sample weight.

4.7 Field capacity

Field Capacity (FC) is defined as the maximum percentage of moisture in the pores of solid material held against gravity (Das, 1998).

With the evaluation of FC is possible to quantify the retained water (adsorbed + pellicular) and the capillary water inside the sample.

The representatively of this sample is ensured by the quartering procedure as in paragraph 4.4.

A supersaturated sample of substrate is weighed and placed on a plastic grid inside a funnel. The known amount of water used to supersaturate the sample is left to percolate through it by gravity for 24 hrs. The funnel and the sample are covered by with cling wrap to avoid water losses by evaporation. After 24hrs the sample is then dried in an oven for other 24hrs and weighed again (Figure 4.8).

The FC is determined by the formula 4.2:

$$FC = \frac{W_h - W_d}{W_d} \cdot 100 \quad (4.2)$$

W_h = Weight of the wet sample

W_d = Weight of the dry sample

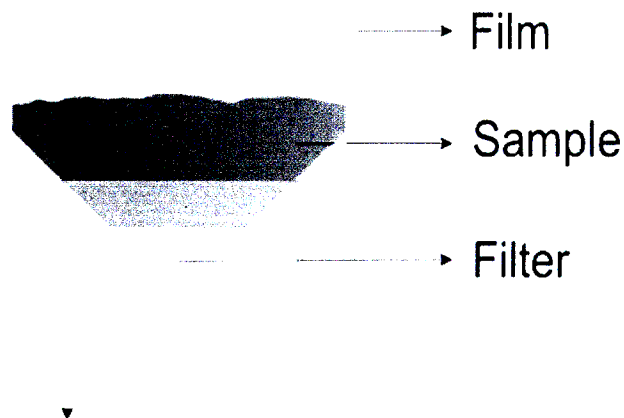


Figure 4.8 – Schematic of the apparatus used for field capacity test.

4.8 Density

Density is defined as the ratio between the weight of a particular material and its volume. To determine the substrates' density 20 samples of known volumes (from 10 ml to 500ml) were weighed and an average density calculated.

ρ_d (density) = total weight / volume

The obtained value was then compared with typical ranges found in literature to confirm its representativity.

4.9 Porosity

Porosity (n) of a solid porous medium is defined as the ratio of the volume of voids to the total volume (Das, 1998), or:

$$n = \frac{V_v}{V} \quad (4.3)$$

To evaluate the porosity, an indirect method was used, based on the determination of the following parameters:

- Density;
- Volume of the solid.

The porosity was evaluated with the following expression (4.4):

$$n = 1 - \frac{\rho_d}{\rho_{sd}} \quad (4.4)$$

where:

- ρ_d (density) = total weight / volume
- ρ_{sd} (dry density) = dry weight / dry volume

A more complex procedure, as explained below, was used to determine the volume of the solid: a known amount of the substrates (M_s) was weighed and placed in a flask. The flask was then inserted in a desiccator connected with a light vacuum pump used to eliminate the air from the voids of the porous medium. After 24hrs of vacuum deoxygenated water was poured inside the flask very slowly to avoid any excessive air ingress in the soil matrix (Figure 4.9).

In this way, it was possible to assume that all the voids were filled with water and the substrate was fully saturated. In this condition, water was added to a predetermined level and the flask with the sample weighed again.

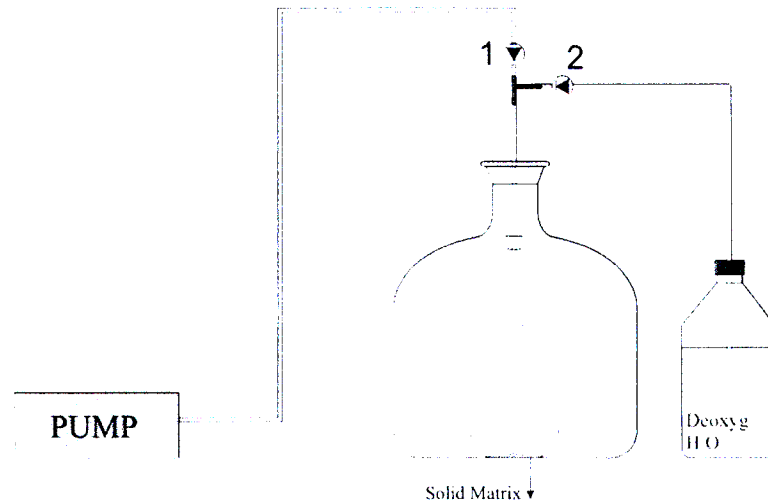


Figure 4.9 - Schematic view of the porosity test

The computation procedure to calculate the density is shown below.

Water weight: $M_w = M_{(\text{water+sample})} - M_s$

Water volume: $V_w = M_w / \rho_w$ with $\rho_w = 1 \text{ kg/l}$

Solid sample volume: $V_s = V_{\text{tot}} - V_w$

Dry density:
$$\rho_{sd} = \frac{M_s}{V_s}$$

4.10 Respirometric Test (RI₇)

In order to evaluate the biodegradability of the substrates, the Respirometric Index at 7 days (RI₇) was determined using a respirometric system type OxiTop®. This system provides an indirect reading in mgO₂/g_{dry mass} of the amount of oxygen consumed by an indigenous biomass present in the sample to degrade the available organic substances.

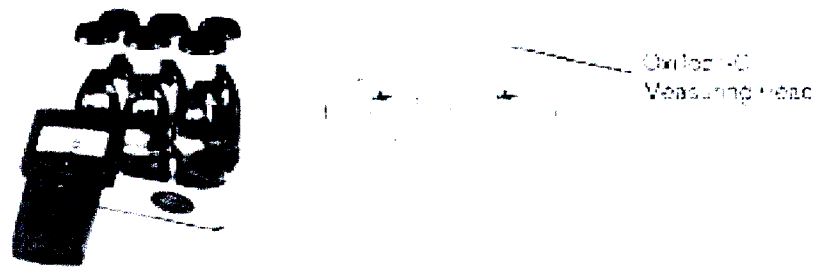


Figure 4.10 - OxiTop System: OC 110 Controller and schematic view Measuring Heads.

To evaluate the RI7 the following procedure was followed:

20g of solid material and distilled water in a liquid to solid ratio as presented in Table 4.1 are incubated in an airtight 1500ml vessel for 7 days at 20°C. The Oxitop bottles are equipped with a pressure sensor lid that records the gas pressured developed during the biodegradation process of the organic matter.

The L/S ratio was calculated in relation to the density and porosity test explained in the previous paragraphs.

Table 4.1– Characteristics of the samples.

	Pine Bark	Compost
Dry matter [g]	20	20
Water from moisture content [g]	25.5	17.7
Added water [g]	94.5	36.3
L/S ratio	6	2.7

As biodegradation progresses, oxygen is consumed and carbon dioxide produced. This is then absorbed by 10 drops of potassium hydroxide added in the head of the vessel together with Allythiourea (ATH) used to avoid nitrification.

There is a direct proportionality between the negative pressure measured by the pressure sensor and the amount of carbon dioxide absorbed by the potassium hydroxide which is, hence, equal to the amount of oxygen consumed in the biodegradation process. After 7 days the pressure difference can be measured by equation 4.5.

$$\Delta p = \frac{BOD_7}{\frac{M(O_2)}{R \cdot T} \cdot \left[\frac{V_t - V_l}{V_l} + \alpha \frac{T_m}{T_0} \right]} \quad (4.5)$$

Where:

BOD_7 = results from the tests in mg/l

M = Molecular weight (3200mg/mol)

T_0 = Reference temperature = 273.15K (STP)

T_m = 20°C = 293.15K (STP) –Testing Temperature

V_t = Volume of the vessel (ml)

V_l = Volume of the sample (ml)

R = Gas constant - 83.144 mbar/mol.k

α = Bunsen absorption coefficient (0.03103)

The standard atmospheric gas composition in the vessel at starting conditions is assumed as in Table 4.2.

Table 4.2 - Standard atmospheric gas composition

O ₂	N ₂	CO ₂
21%	78%	1%

Nitrogen (P_{pN}) and oxygen (P_{pO}) partial pressures are measured as follows:

$$P_{pN} = 1 \text{ atm} \cdot 0.78$$

$$P_{pO} = 1 \text{ atm} \cdot 0.21$$

Ignoring a small amount of CO₂, the number of the total moles is calculated as a sum of oxygen and nitrogen moles. Considering P_f the final pressure measured, the total number of moles (n_f) is calculated through the Perfect Gas Law $PV = nRT$ as in the following equation.

$$n_f = \frac{P_f V}{RT} \quad (4.6)$$

Since Nitrogen is inert and the number of its moles (n_N) doesn't change during the reaction, the final oxygen moles (n_{of}) are calculated as follows:

$$n_{of} = n_f - n_N \quad (4.7)$$

Finally, the mass of oxygen consumed in relation to the mass of the dry material is as in the equation below expressed in [$\text{mg}_O/\text{g}_{\text{dry mass}}$]

$$g_o = n_{of} \cdot MW \quad (4.8)$$

MW = (molecular weight)

4.11 Total Carbon, Total Nitrogen and C/N ratio

The Total carbon and total nitrogen analysis were carried out in order to evaluate the compost's C/N ratio. This index enables to estimate the compost quality in relation to quality standards and to measure the amount of carbon available for denitrification in the substrate matrix.

The analysis of total Carbon and total Nitrogen in the solid samples was conducted by the BemLab in Somerset West (Cape Town) using a CHN analyzer.

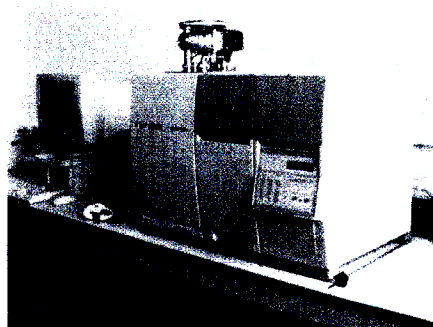


Figure 4.11 - CHN analyzer

4.12 Biogas production

Denitrification occurs with production of N_2 gas and CO_2 that were analyzed using two systems: a respirometric method using a respirometer model Oxitop-WTW and a liquid displacement method. The potential of both the substrates and the leachate for methane

generation in an anaerobic environment was assessed by testing the organic material in contact with leachate and with distilled water.

4.12.1 Respirometric method - Oxitop

The Oxitop test is performed using a representative known amount of substrate mixed with leachate at L/S ratios as presented in Table 4.1. The mixture is placed in a 1500ml airtight vessel in an incubator at 20°C for almost 50 days. As seen in Figure 4.12 the anaerobic bottles are equipped with two silicone septa that avoid gas diffusion, but allow suppressing CO₂ during the test. The carbon dioxide produced during degradation of the sample is suppressed with KOH and a pressure reading gauge in the lid of the respirometer measures the amount of methane produced as positive absolute pressure.

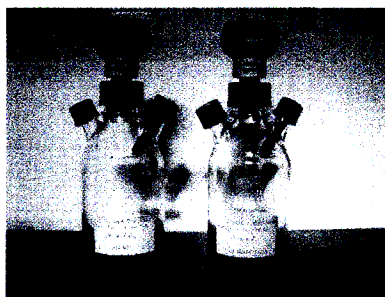


Figure 4.12 - Samples in vessels

The sample composition and the liquid to solid ratios adopted for the oxitop method are presented in Table 4.3.

Table 4.3- Samples' composition and liquid to solid ratios

	Pine Bark	Compost
Solid matter [g]	24.6	47.7
Moisture content [g]	31.4	42.3
Leachate [g]	116.5	86.5
L/S ratio	6	2.7

Assuming a standard atmospheric gas composition in the vessel as in table 4.2, the total volume of gas produced in the vessel is determined as in paragraph 4.10 in according to the general equation:

$$V_{gas} = \frac{n_f RT}{P_{atm}} \quad (4.9)$$

4.12.2 Liquid displacement method

This method was employed to evaluate the biogas production of the two substrates (compost and pine bark) in contact with distilled water in anaerobic conditions.

The system, based on the liquid displacement principle (Figure 4.14), is constituted of a 2 liters glass bottle used as a reservoir and of a 1.2 liters graduated glass burette with two taps: one is connected to the sample bottle (reactor vessel) and the other to the gas analyzer for quality readings.

The burette is filled with a liquid solution of sodium chloride (NaCl), sulphuric acid (H₂SO₄) and a colorant solution in order to avoid the absorption of any gas into the liquid. The test is performed in a thermostatically controlled room at 25-30°C and the reactors are insulated to avoid temperature variations during biodegradation.

Figure 4.14 illustrates the general layout of the liquid displacement method.

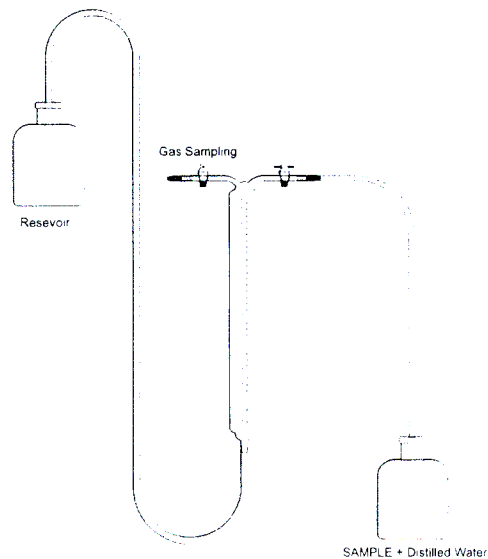


Figure 4.13 - Schematic layout of the liquid displacement method.

As biogas is produced, the liquid in the burette is displaced. The volume of biogas is equivalent to the volume of solution displaced and measured in the graduated burette.

A gas analyzer is used (GA 2000) to determine the percentage by volume in air of methane (CH_4), carbon dioxide (CO_2) and oxygen (O_2) and to level the liquid displaced in the burette with that in the reservoir (at atmospheric pressure) after each measurement.

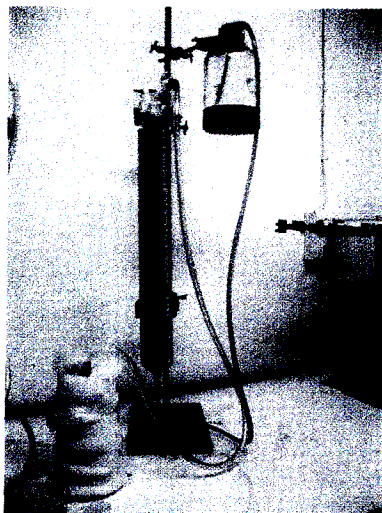


Figure 4.14 - Liquid displacement method set up



Figure 4.15 - Gas analyzer

4.13 Eluate tests

Eluate tests were conducted to assess amount and nature of the compounds released by the substrates when in contact with water (Collivignanelli, 1992).

The main factors that affect performance and accuracy of eluate tests are:

- the physical state of the solid material;
- leaching solution used (distilled water or acid solutions);
- contact time between liquid and solid;
- contact mode (static or dynamic) between the leaching solution and the solid;
- liquid to solid ratio (L/S);
- operation temperature;
- solid-liquid separation mode.

Representative quantities of pine bark and compost were mixed to distilled water in a liquid to solid ratio of 10:1 and shaken for 24 hours (20g of solid in 2litre of water).

The eluate formed was centrifuged twice at 6000 rpm for 20 minute in a Hettich EBA 12 centrifuge (Figure 4.17) and filtered with a 0.45µm filter paper (Figure 4.18).

The filtered eluate was analyzed for the following parameters: COD, BOD₅, NH₃, NO₃, pH, Conductivity, Total Solids, Volatile Solids, TKN and TOC.

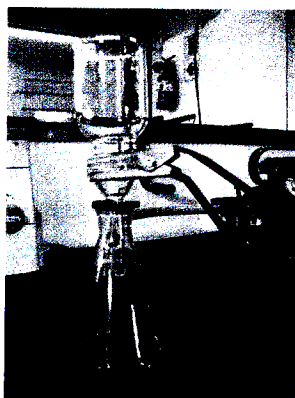


Figure 4.16 - Filtration 0.45µm



Figure 4.17 - Centrifuge

4.14 pH

pH was measured using a Orion 410A pH meter and following the same procedure explained in paragraph 4.5.

4.15 Total Solids (TS) and Volatile Solids (VS)

(Standard methods nº 2540 D,E, Clesceri et al., 1989)

The measurement of Total Solids (TS) represents quantity of the total solid residue remaining after a sample was desiccated in an oven at 105°C for 24 hours and is calculated as follows:

$$TS[g/l] = W_d \cdot \frac{1000}{V_s} \quad (4.10)$$

Where:

W_d = dry weight

V_s = sample volume

Volatile Solids are measured placing the residue of the Total Solids test in a furnace (Figure 4.19) at 550°C for 4 hours. The non-volatile fixed residue after incineration is then weighed and calculated using equation below:

$$FS[g/l] = W_{FS} \cdot \frac{1000}{V_s} \quad (4.11)$$

Therefore, the VS are determined as in the following equation:

$$VS[g/l] = TS - FS \quad (4.12)$$

where:

W_{TS} = mass of the total solids

W_{FS} = mass of the fixed residue remaining after incineration (ashes)

V_s = volume of the sample

FS = concentration of non-volatile fixed solids (mg/l),

1000 = multiple to convert the concentrations in g/l.

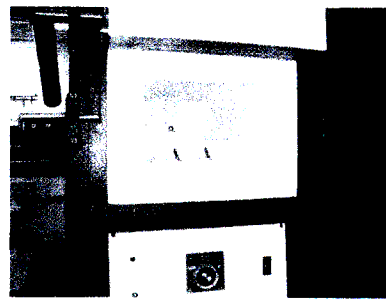


Figure 4.18 - Furnace

4.16 Conductivity

Conductivity is a numerical expression of the ability of an aqueous solution to carry an electric current (Standard method, Clesceri et al., 1989). The conductivity of a solution gives an indication of the amount of dissolved ions and total dissolved solids. Conductivity tests were carried out using a Corning conductivity meter (Figure 4.20).

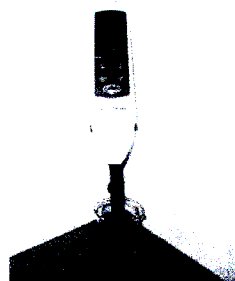


Figure 4.19 - Conductivity meter

4.17 Chemical Oxygen Demand (COD)

The COD test procedure used follows the ASTM standard method n° 5220 and 1990 (Standard method, Clesceri et al., 1989), using the closed reflux method, which was performed in a COD digester Hach (Figure 4.21). The Chemical Oxygen Demand (COD) is a measurement of the amount of oxygen that is required for the chemical oxidation of the organic matter contained in a sample. A sample of leachate is added to a solution of potassium dichromate ($K_2Cr_2O_7$), a strong oxidant, and sulphuric acid (H_2SO_4).

The samples are digested for two hours at $180^\circ C$ and then let to cool; the dichromate remaining in each sample mixture is detected in a spectrophotometer set at 600nm.

The consumption of the oxidant is expressed in terms of oxygen equivalent according to the following equation:

$$COD[mg_{O_2}/l] = \frac{(A - B) \cdot a}{V} \quad (4.13)$$

where:

A = absorbance of the sample

B = absorbance of the blank sample

a = conversion coefficient (6189)

V = volume of the sample (ml)

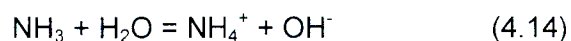


Figure 4.20 - COD digester

4.18 Ammonia

The ammonia test procedure used follows the ASTM standard method number B, D 4500 (Clesceri et al, 1989).

Ammonia nitrogen exists in aqueous form (NH_3) and as an ion of ammonia (NH_4^+) depending on the pH of the solution in connection with the following equilibrium reaction:



At pH 7 the ammonium ion (NH_4^+) exists in solution while at pH 12 the solution contains NH_3 as a dissolved gas.

The sample is modulated to pH=12 to permit ammonia absorption by the boric acid solution and, then distilled; the concentration of ammonia (NH_3) is then determined by titration of the distillate with a standard 0.02N H_2SO_4 titrant.

The apparatus and an ammonia sample are showed in Figure 4.22.

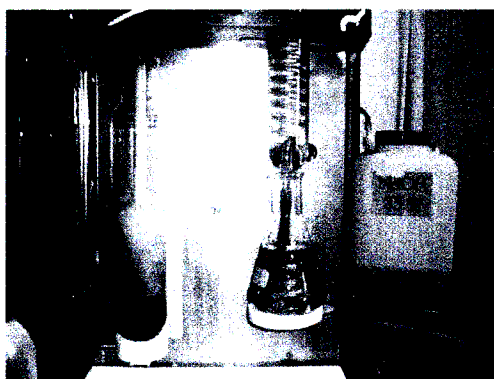


Figure 4.21 - Ammonia distiller apparatus

4.19 Nitrates

The analysis of the nitrates was carried out by the BN Kirk certified laboratory in Durban in according with the standard method n° 4500 – B, (Clesceri et al., 1989). Most of the results were crossed checked with sticks based on a colorimetric method (Nitrate Test stick Merckoquant).

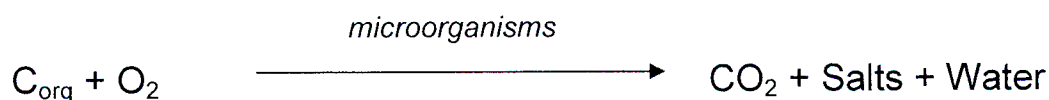
4.20 BOD

The Biochemical Oxygen Demand is defined as the quantity of oxygen consumed in the aerobic degradation of organic substances by an established micro flora; this is directly proportional to the amount of biodegradable matter contained in the sample.

BOD is thus a substantial feature in determining the effect of discharged effluents on the oxygen content of a water-course or on the oxygen demand of an effluent treatment plant. BOD levels are stated in mg/l of oxygen and are usually measured over a period of 5 days (BOD₅) in order to avoid the influence of nitrification on the measurement.

Measurement Principle

Microorganisms (bacteria, fungus, archaea and protozoa) feed on the organic compounds contained in a waste water sample, by using oxygen to biochemically oxidize them to carbon dioxide (CO₂), inorganic salts (mineralization) and water as expressed by the non stoichiometric reaction:



The barometric method used for the BOD-determination is based on the fact that all the oxygen consumed is converted to carbon dioxide, this is then removed from the air space by the use of potassium hydroxide KOH (HÜTTER, 1984). Therefore, in the closed system BOD-flask/BOD-sensor, a drop in pressure occurs, which is proportional to the amount of oxygen consumed.

The BOD₅ measurement was carried out using a Sensomat scientific System with BSB/BOD Sensomat and Sensor-IR (figure 4.23)



Figure 4.22 - Sensomat scientific System with BSB/BOD Sensomat and Sensor-IR

The respirometric measurement is a measurement of pressure difference: if oxygen is consumed in a closed vessel at a constant temperature, a negative pressure develops. If

a gas is released, an overpressure develops. The sensor-IR measures and stores this pressure for the entire duration of a measurement once started.

The Sensomat collects the pressure values from the sensor-IR and processes them giving a BOD value in mg/l.

The sample preparation including pH adjustment, homogenization and volume of the sample, inhibiting of nitrification, sample sealing and tempering are explained in Sensomat Scientific manual.

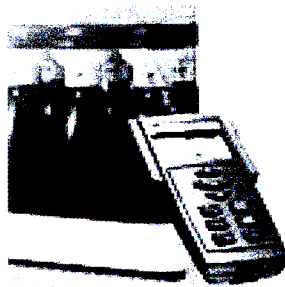


Figure 4.23 - Connection IR head - remote (BSB-BOD Sensomat).

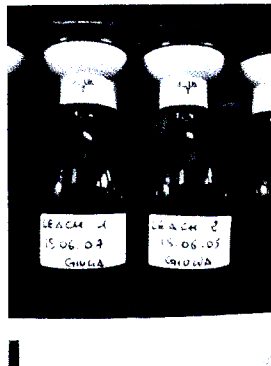


Figure 4.24 - Bottle in the thermostatically-controlled cabinet.

4.21 Total Kjeldahl Nitrogen (TKN) and Total Organic Carbon (TOC)

The analysis of TKN and TOC was conducted by BN Kirk Certified Laboratory – Durban - South Africa, according with Standard methods n° 4500-N_{org} B and C.

4.22 Batch Tests

A series of static batch tests was designed to evaluate the optimal kinetic constants for the denitrification of the leachate sample using compost and pine bark as a carbon source, in relation to different nitrate concentrations.

Known amounts of substrate were mixed with leachate from the Mariannhill Landfill with a $L/S = 2.7$ for the compost and $L/S=6$ for the pine bark. The L/S ratios were determined in relation to porosity, density and natural moisture content of the substrates, as previously presented in paragraph 4.12, Table 4.3.

Three nitrate concentrations were selected for the batch tests: $\text{NO}_3\text{-N} = 1100 \text{ mg/l}$; 700 mg/l and 350 mg/l . The tests were conducted in double in 1500ml anaerobic bottles equipped with two airtight silicone septa (figure 4.29) that allow for continuous sampling avoiding air ingress (figure 4.28). The bottles are filled for $\frac{3}{4}$ of their capacity and placed in a shaker to ensure full liquid to solid contact (Labcon shaker at 250 rpm, figure 4.27). The experiment was conducted in a thermostatically controlled room at 25°C , checked once a week, through the use of an MT digital thermometer.



Figure 4.25 - MT digital thermometer

The size of the pine bark had to be reduced to 2-3 cm and the leachate had to be deoxygenated with a light vacuum to accelerate the onset of anaerobic conditions.

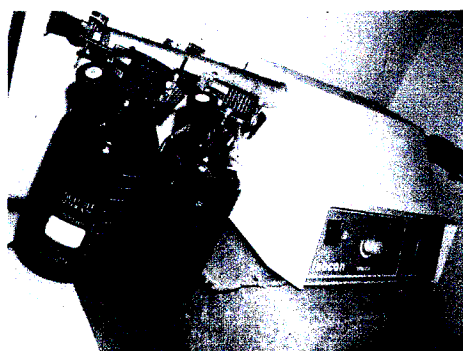


Figure 4.26 - Labcon shaker

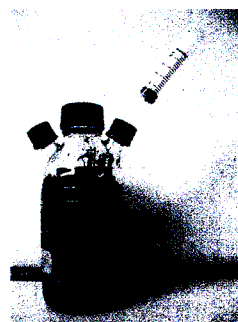


Figure 4.27 - Batch sampling

Sampling was conducted every hour for the first day and once a day thereafter with a precision syringe connected with a 0.45 μ m filter (figure 4.3). The nitrates and nitrites concentrations in the solutions extracted during the batch tests were analyzed using Nitrate Test Sticks type Merckoquant (figure 4.31) that employs a colorimetric method. This method was selected because it requires a little amount of solution so avoiding large variations in the L/S ratios and it is reasonably accurate (error within 15%).

A full characterization of the solid substrates and the denitrified leachate after the batch tests were also conducted focusing on residual organics and nutrients, in particular COD, pH, TOC, TKN, C/N.

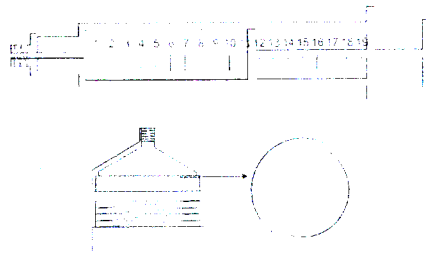


Figure 4.28 – Siring 0.45 μ m filter and schematic view.

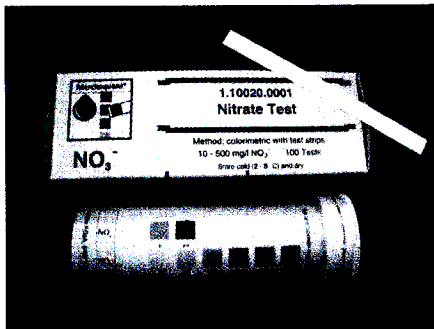


Figure 4.29 - Nitrate Test stick
Merckoquant

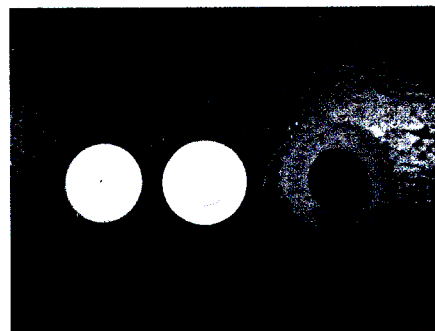


Figure 4.30 - Rubber septum

4.23 Column tests

Leaching columns were used to simulate the denitrification process in a natural filter packed with compost and pine bark. These tests aimed at determining the process kinetics and the longevity of the carbon sources in a larger scale than the one used considered for the batch tests. The leachate containing a NO_3 concentration of 600mg/l was selected for this experiment.

Columns setup

The columns have the following characteristics: transparent PVC cylindrical body, 1m length, 160mm in diameter and 20 liters capacity.

PVC was chosen for its high resistance to chemical and mechanical damage and because it is chemically inert to most compounds. Transparent PVC also allowed a good visual control of the process.

A typical leaching column is showed in Figure 4.32.

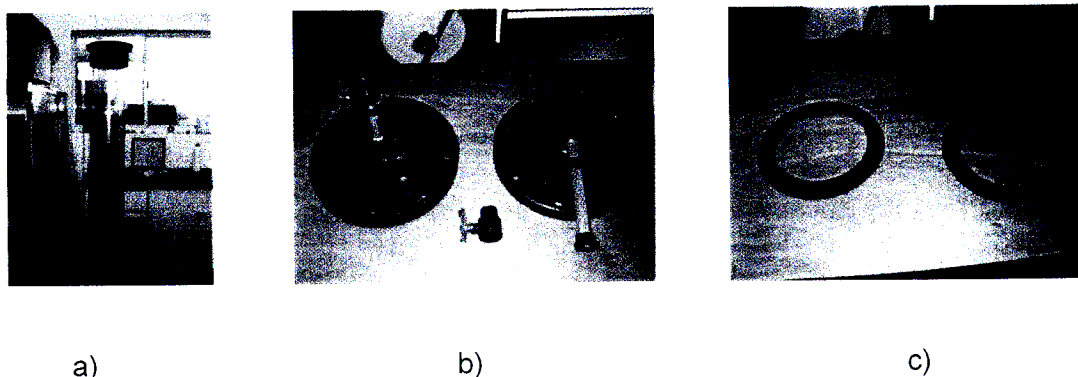


Figure 4.31 (a,b,c) - Leaching column; Upper and lower flanges; Rubber rings

The upper and lower part of each column is closed with two pairs of 25 mm thick flanges and 280 mm diameter screwed together (Figure 4.32 b). The hermetic fit is assured by a rubber gasket (Figure 4.32 c), with a thickness of 20 mm, laid between the flanges.

The upper flange has two orifices; the first is connected to a tap to allow the column to be filled with leachate while the second is connected to a plastic pipe for biogas measurements. The lower flange has only one orifice connected to a pipe and a tap for the collection of leachate.

To allow for the leachate extraction and to avoid obstruction of the system by particulate matter, a drainage layer composed of glass marbles of 25 mm diameter was placed at the base of each column.

Filling of the columns

The two columns have been packed with:

- COLUMN 1: Fresh pine bark;
- COLUMN 2: Garden refuse compost

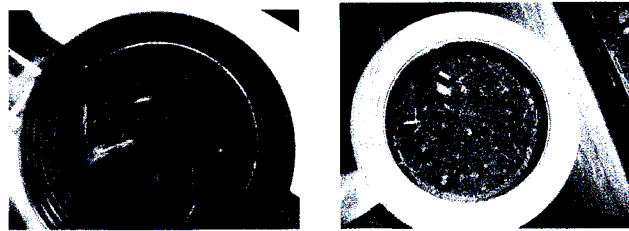


Figure 4.32- Column filling

The substrates were then filled with the treated leachate using the same liquid to solid ratio (L/S) calculated for the batch tests (as in Table 4.3).

Table 4.4 - Starting conditions for each column

COLUMN INPUT	Pine Bark [Kg]	Compost [Kg]
Total Input Mass	5.24	9.8
Moisture Input	2.9	4.6
Dry Mass	2.3	5.2
Added Leachate	11.7	7.7
Total Moisture	14.6	12.3
L/S Ratio	6.3	2.4

Total input mass = Moisture Input + Dry mass

Total moisture = Moisture Input + Added leachate

L/S ratio = Total moisture / Dry mass

The leaching tests were designed to determine the maximum contact time necessary to reduce the nitrates concentration in the leachate within the discharge limits (to zero in certain instances). In order to test the longevity of the substrates, at the end of each treatability trial the column was drained, a global sample collected and a new batch of leachate was then poured back into the column. The sampling from the columns was designed on the basis of the results of the batch test for the same substrate-leachate combination. The effluents, sampled generally once a week for the first week and once a day thereafter, were analysed for: NO_x , NH_3 , pH, DO, Eh, conductivity, COD.

4.24 Dissolve Oxygen (DO)

The analysis of Dissolved Oxygen (DO) was performed in order to assess the evolution of aerobic, anoxic or anaerobic conditions during the column trials.

The methods that are most commonly used to measure DO can be sorted into three major groups: colorimetric, titrimetric, and polarographic (see below). Since oxygen can easily diffuse into water during handling (and alter the natural concentration of dissolved oxygen) samples must be collected and processed without contact with air.

The measurement was carried out using a DO/OUR Meter (figure 4.34) with a Dissolved Oxygen Probe YSI Model 5239 (figure 4.35).



Figure 4.33 - DO/OUR Meter

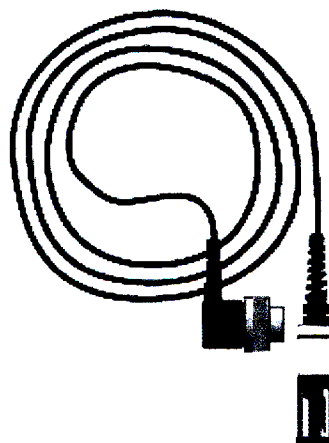


Figure 4.34 - Dissolved Oxygen Probe
YSI Model 5239

The 5239 probe is a Clark type polarographic (Volta-metric) dissolved oxygen sensor. The sensor is made of a silver anode and a gold cathode (see Figure 4.36), and is separated from the measured medium by a semi-permeable Teflon membrane. A temperature sensing element is mounted next to the oxygen sensor vertically, providing temperature readings for the DO system.

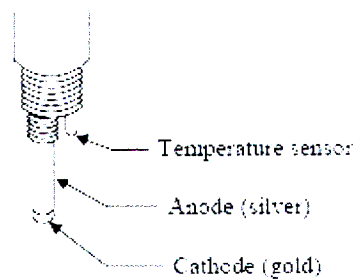
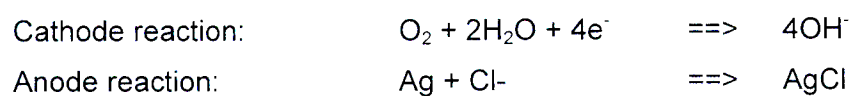


Figure 4.35 - DO probe

The membrane selectively allows oxygen to permeate into the sensor, but prevents most interfering molecules and fouling materials. Upon permeating through the membrane, oxygen is reduced at the gold cathode. The current resulting from this reduction is diffusion-limited and is proportional to the partial pressure of oxygen in the sample. The counter reaction is the oxidation of silver at the anode/reference electrode which completes the overall electrolytic reaction in the chloride medium (KCl electrolyte) behind the membrane. These reactions, at the cathode and the anode, are as follows:



The oxygen reduction current is sampled and processed, by the meter, and displayed as either %-air saturation or mg/L. While the parameter of %-air (partial pressure) is independent of temperature and salinity, mg/L (solubility of oxygen) is a function of temperature and salinity. For instance, the same %-air reading (same partial pressure) would give a higher mg/L reading at a lower temperature than at a higher temperature. Also the higher the salinity the lower the solubility for the same %-air reading at the same temperature.

4.25 Redox potential (Eh)

Redox potential is a measure of a systems' capacity to oxidize a material; it is an intensity parameter of an overall redox reaction potential in the system (similar in concept to pH).

In the literature, redox potential is generally reported as Eh, which is the potential generated between a platinum electrode and a standard hydrogen electrode when placed into a liquid sample, where hydrogen is considered the reference electrode. The redox potential is, therefore, a measure (in volts) of the affinity of a substance for electrons —electro-negativity — compared with hydrogen (which is set at 0).

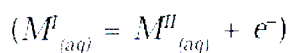
Substances more strongly electronegative than (i.e., capable of oxidizing) hydrogen have **positive** redox potentials. Substances less electronegative than (i.e., capable of reducing) hydrogen have **negative** redox potentials.

The following are examples of Eh for different reactions and conditions (expressed in order of decreasing Eh):

Aerobic Organic Degradation	+250mV and higher
Oxygen - Nitrogen	+250mV to 100mV
Iron - Mn (IV)	+100mV to 0mV
Sulfate	+0mV to -200mV
Methane - Hydrogen	-200mV and lower

Water in contact with air will have an Eh in the range of 350mv to 500mv. Microbially mediated redox processes may decrease the redox potential to values as low as -300mV (Orion Aplus manual).

A rigorous quantitative interpretation of a measurement of Eh requires interactive access to an aqueous speciation code. It is important to exercise caution when interpreting a measured Eh using the Nernst equation for a simple half-cell reaction as:



That enables to determine Eh as:

$$Eh = E^o + 2.303RT/nF \log (a^{II}_{M(aq)} / a^I_{M(aq)})$$

where:

R = gas constant;

T = temperature, in degrees Kelvin;

n = number of electrons in the half-cell reaction;

F = Faraday constant; and

$a_{M(aq)}^I$ and $a_{M(aq)}^{II}$ = thermodynamic activities of the free ions $M_{(aq)}^I$ and $M_{(aq)}^{II}$

4.26 Biogas measurement system

The columns were equipped with a biogas measurement system based on the liquid displacement method as explained in paragraph 4.12, figure 4.14.

This system is connected to the top flange of the column as in Figure 4.37.

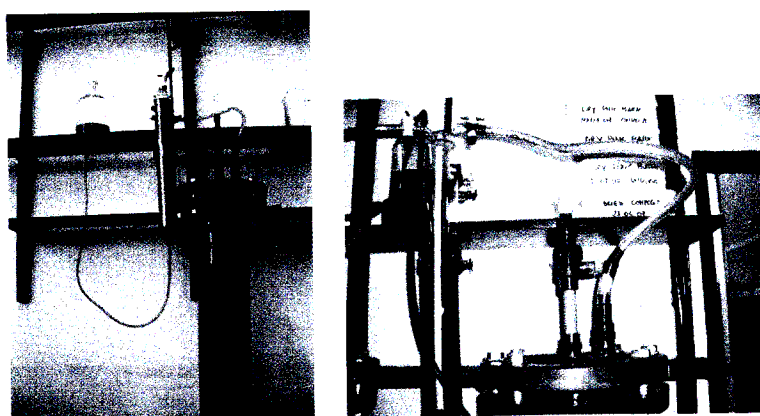


Figure 4.36 - Biogas system in the column

Biogas production and concentration were conducted in a quasi-regular frequency during the column trials; CH_4 , O_2 and CO_2 were measured using a gas analyzer type GA2000.

4.27 Accuracy and Repeatability Testing

All the analyses listed before were carried out in triplicate. The results presented in Chapter 5 are an average of the measured values; the raw data are presented in the Appendices. For each set of data, media, standard deviation and coefficient of variation (variance) were calculated and an example of these calculations is reported below in Table 4.5.

Table 4.5 - Repeatability checks on Ammonia testing

Sample	Repeats			Ave	Result	Std Dev	Var
	1	2	3				
					[mg/l]		[%]
PB eluate	0.2	0.25	0.24	0.23	4.14	0.026	0.000
COMPOST eluate	0.45	0.41	0.43	0.430	7.74	0.02	0.000
LEACHATE	0.5	0.42	0.41	0.443	7.98	0.049	0.002
PB leachate	0.5	0.6	0.6	0.567	10.20	0.057	0.003
COMPOST leachate	0.89	0.9	0.87	0.887	31.93	0.0152	0.000

AVE = average, STd DEV = standard deviation, VAR = coefficient of variation (%)

The average is expressed as follows:

$$\bar{C} = \frac{\sum_{i=1}^n C_i}{n}$$

Where C_i is the single observation and n is the total number of observations.

The standard deviation of the data from the multiple analyses of a single sample was calculated using the following equation (Robertson et al, 1995).

$$SD = \sqrt{\frac{\sum_{i=1}^n (C_i - \bar{C})^2}{n - 1}}$$

where

S_M = standard deviation for multiple analyses of a single sample,

C_i = concentration of sample i .

The precision tests conducted on the triplicate samples showed a high level of precision.

5 RESULTS

5.1 Introduction

The main objective of this investigation was to assess the suitability of garden refuse compost and pine bark as carbon sources for the denitrification of high strength landfill leachates. The effluent from a SBR at the Mariannhill landfill site in Durban was selected for treatment trials in columns. Two leaching columns were set up and operated in batch, as static beds packed with compost and pine bark mixed with leachate in L/S ratios determined on the basis of the substrates' physical properties.

Both solid substrates and leachates used were fully characterized before and after the treatment trials. Small-scale shaken batch tests in anaerobic vessels (1500ml capacity) were designed to determine the kinetics of nitrate removal in optimal conditions for different nitrates concentrations. The longevity of the substrates and the efficiency of nitrate removal were then measured in leaching columns operated as fixed bed plug-flow reactors for 32 weeks. Biogas production and nature was monitored throughout the columns' trials. Key parameters such as NH_3 , COD, pH, Conductivity and Eh were regularly measured in the effluents from the columns together with NO_3 concentrations.

The results of the characterization campaign, batch tests and column tests are presented in this chapter.

5.2 Quality characterization

5.2.1 Substrates

The results of the characterization on the solid matter and on the eluates are shown below.

Table 5.1 - Characterization of the solid substrates.

	pH	moisture content	TS	VS	porosity	density	RI 7	tot C	tot N	C/N
		[%]	[%]	[%]	[%]	[g/cm ³]	[mgO ₂ /gTS]	[%]	[%]	
Pine Bark	5	56%	44%	95	85	0.167	2.8	38.84	0.62	62.65
Compost	7.8	47%	53%	30	82	0.3	1.6	14.01	1.24	11.30

Table 5.2 - Characterization of the eluates.

	COD	BOD	pH	Cond.	NH₃	NO_x	TS	VS	TKN	TOC
	[mg/l]	[mg/l]		[μS/cm]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]
Pine Bark	1415.90	386	5.67	559.3	3.22	7.51	2	0.3	9	410
Compost	881.46	152	6.28	1815	7.74	73.39	11.09	3.75	22	415

From Tables 5.1 and 5.2, it is evident that the pine bark is slightly acidic and this can negatively influence the denitrification process since the optimal pH for biologic denitrification is between 6 and 8 (Trois et al., 2007). The higher carbon content, in the form of COD, BOD and Total carbon in the pine bark rather than in the compost is justified by the fact that the former hasn't undergone a stabilization process. The C/N ratio for the pine bark falls within the expected range as in literature, while the low value for the compost suggests a type V mature substrate (DIN 4187). The low RI7 values for the compost suggest that the nature of the organic carbon is slowly biodegradable.

5.2.2 Leachate

Tree different types of leachate were collected from the SBR at the Mariannhill Landfill site. These samples display slightly different characteristics as reported in Table 5.3. Never the less, these differences allowed for comparisons between the different batch tests. The results of the quality characterization of the leachate used for batch tests and column trials are presented in the following table.

Table 5.3 - Input leachate characterization

LEACHATE	NO₃	COD	BOD	pH	Cond.	NH₃	TS	VS	TKN	TOC
	[mg/l]	[mg/l]	[mg/l]		[mS/cm]	[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mg/l]
Input test 1	1100	494.00	4	7.9	9.9	7.98	7.6	1.1	25	205
Input test 2 and 3	700	282.93	15.3	7.73	6.39	10.15	4.8	1.17	11	120
Input column tests	600	546.28	15.6	7.93	9.85	6.63	7.4	1.12	20	245

Table 5.3 shows that as a result of the SBR treatment the leachate is characterized by low levels of ammonia, and residual/humic organics (low BOD, COD and TOC values). The high nitrates levels are also a result of the aerobic treatment.

5.2.3 Biogas Production Tests

In order to establish the capacity of the substrate to produce biogas an anaerobic test using the liquid-displacement method with the organic substrates in distilled water has been set up. The results are presented below.

Table 5.4 - Biogas test with distilled water

Biogas test	solid sample	distilled water	duration	gas production	specific production
	[g]	[g]	[d]	[ml]	[ml/gdm]
Pine bark	114	54	30	4.9	0.1
Compost	226	95	30	1.2	0.01

Both substrates display a small reactivity in anaerobic conditions with distilled water. The compost's reactivity is negligible and this may be justified by the low organic content in its matrix and low BOD in the eluate as presented in Tables 5.1 and 5.2.

5.3 Results - Batch test

Batch tests have been carried out with the following purposes:

- To investigate the kinetics of the denitrification process associated with the two substrates (pine bark and compost) for different nitrates loadings.
- To perform a screening in order to select the most significant results as a base for the column tests.

The three loadings chosen and the duration of the tests are shown in Table 5.5 below.

Table 5.35 - Nitrate loadings and duration of the batch tests

	substrate	name	nitrate concentration [mg/l]	duration [h]
Batch test nº1	pine bark	BT1 PB	1100	129.5
	compost	BT1 CO		450
Batch test nº2	pine bark	BT2 PB	700	85
	compost	BT2 CO		160
Batch test nº3	pine bark	BT3 PB	350	54.5
	compost	BT3 CO		121.5

At the end of each test, the eluates were analysed for pH, conductivity, ammonia and COD, while the residual solid matter tested for total carbon and total nitrogen.

5.3.1 Batch tests - Pine bark

The following table presents the results of the quality characterization for the influent leachate and the solid substrate in the batch tests with pine bark.

Table 5.6 - Characteristics of leachate and solid substrates used in the batch tests with pine bark.

	COD	pH	NH₄	NO₃	TOC	TKN	
	[mg/l]	-	[mg/l]	[mg/l]	[mg/l]	[mg/l]	
Influent leachate	499	7.9	7.98	1100	205	25	

BT1 PB	COD	pH	NH₄	NO₃	tot C	tot N	C/N
	[mg/l]		[mg/l]	[mg/l]	[%]	[%]	
Output leachate	5200	7.62	10.88	0	-	-	-
Output substrate	-	-	-	-	19.2	0.35	54.86

BT2 PB	COD	pH	NH₃	NO₃	tot C	tot N	C/N
	[mg/l]		[mg/l]	[mg/l]	[%]	[%]	
Output leachate	3352	6.25	8.82	0	-	-	-
Output substrate	-	-	-	-	21.91	0.54	40.57

BT3 PB	COD	pH	NH₃	NO₃	tot C	tot N	C/N
	[mg/l]		[mg/l]	[mg/l]	[%]	[%]	
Output leachate	1898	5	6.75	0		-	-
Output substrate	-	-	-	-	35.87	0.58	61.84

Figures 5.1 and 5.2 illustrate the evolution of the nitrate concentration with time during batch tests 1, 2 with pine bark and high nitrate concentrations.

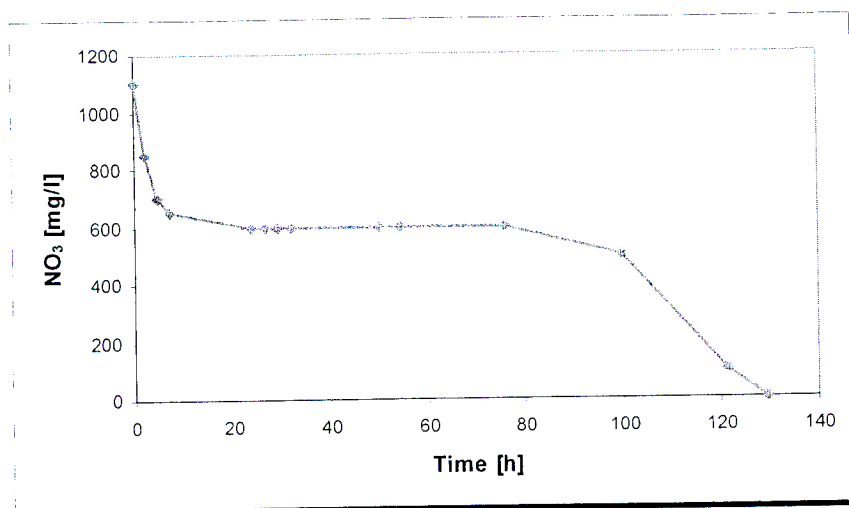


Figure 5.1 - Evolution of the Nitrate concentration in BT1 PB.

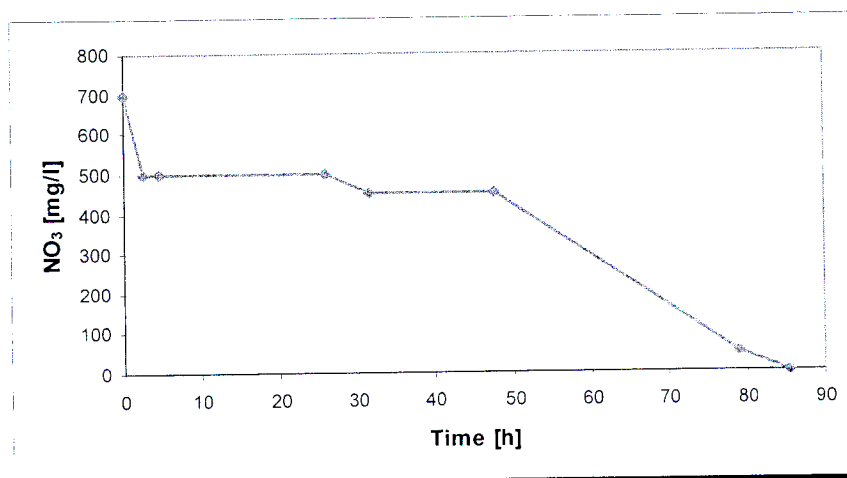


Figure 5.2 - Evolution of the Nitrate concentration in BT2 PB

A typical plateau of 50-70 hours is necessary for the system to reach regime, particularly for high initial nitrate concentrations. This trend is not evident for lower concentrations, although a short and early plateau can be detected in the first 5-10 hours of treatment. To allow for a more in depth understanding of the process, the kinetic models fit on the measured values were presented in discrete sections characterized by a specific kinetic constant.

Batch Test 1 - pine bark (BT1 PB)

Figure 5.3 (a, b and c) presents the kinetic model suggested for batch test 1 with pine bark split in three main sections.

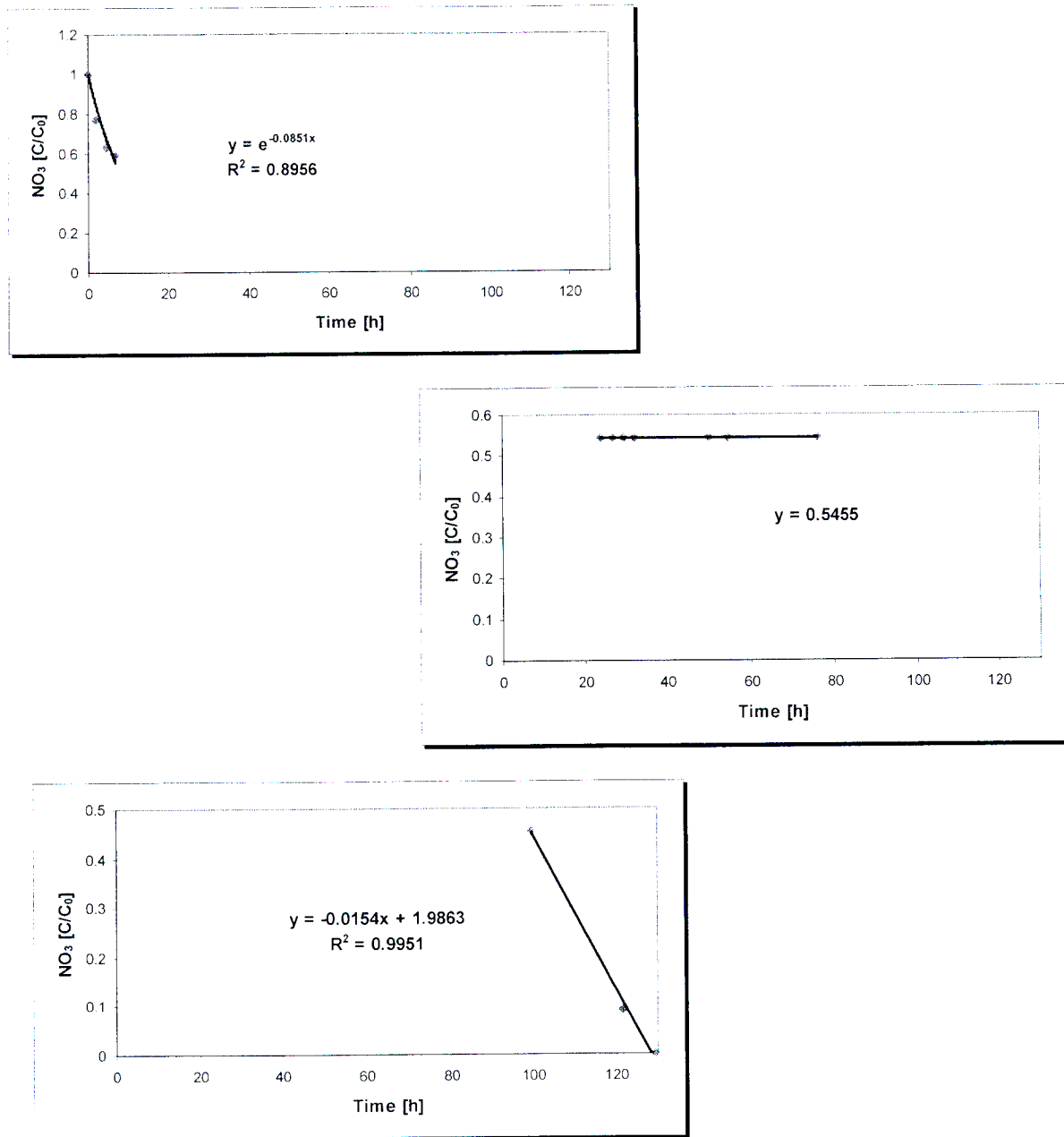


Figure 5.3 - Breakdown of the nitrate removal trend observed in batch test 1 with pine bark.

Batch Test 2 - pine bark (BT2 PB)

Figure 5.4 (a, b and c) presents the kinetic model suggested for batch test 2 with pine bark split in three main sections.

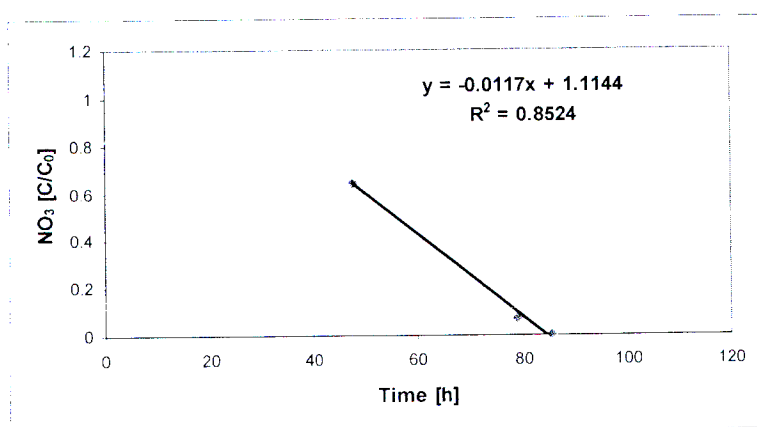
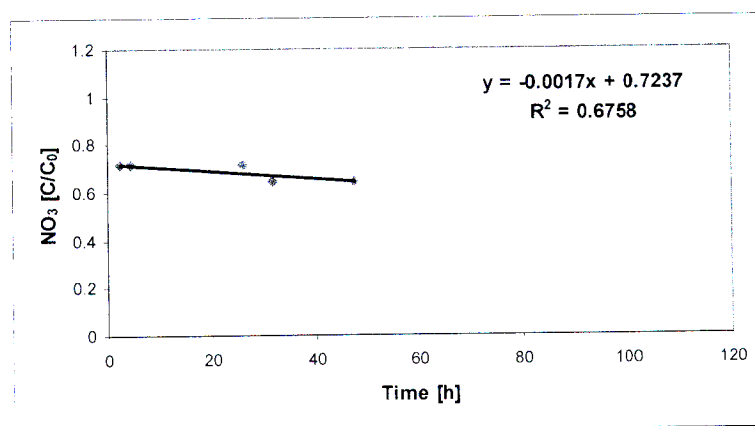
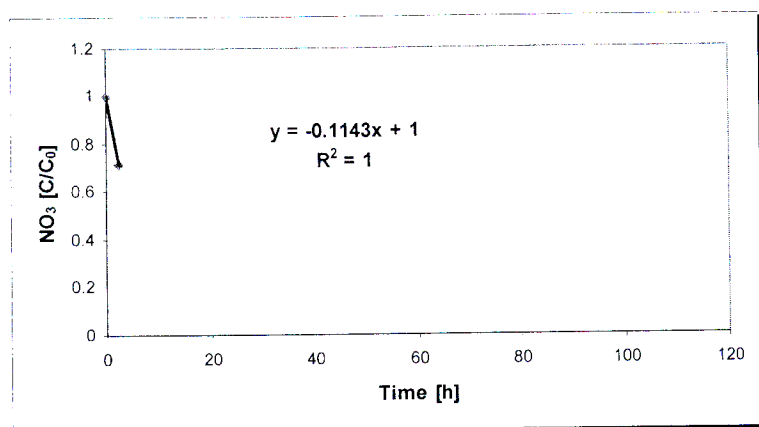


Figure 5.4 - Breakdown of the nitrate removal trend observed in batch test 2 with pine bark.

From the figures above, it is evident that the nitrate removal rate is initially fast following a zero order kinetic model, to gently slowing down towards the end of the experiment. An average of 45.5% and 28.6% nitrate removal efficiencies, respectively for BT1 and BT2, are achieved during this phase. The initial removal rate is described by a zero order model with $R^2 = 0.84$ in BT1 and $R^2=1$ in BT2. The transition between the fast removal rate in the upper part of the graph and the lower part of the model occurs with an intermediate plateau that generally lasts an average of 40 hours. In BT1 this plateau is described by a straight line with $R^2 = 1$ while in BT2 the kinetic model is also zero order one with $R^2 = 0.67$. In the last part of the experiment the removal efficiencies are 54.5% in BT1 and 64.4% in BT2, with a rate of degradation comparable to a zero order kinetic model with $R^2 = 0.995$ and $R^2 = 0.85$, respectively.

The above considerations are summarized in Table 5.7.

Table 5.7 - Kinetic considerations for BT1 and BT2.

	Stage 1			Stage 2 (plateau)			Stage 3		
	<i>time</i>	<i>degradation</i>	R^2	<i>time</i>	<i>degradation</i>	R^2	<i>time</i>	<i>degradation</i>	R^2
	[h]	%		[h]	%		[h]	%	
BT1 PB	24	45.5	0.84	52	0	1	53.5	54.5	0.995
BT2 PB	2.5	28.6	1	45	7	0.67	58	64.4	0.85

The Table and figures above clearly show that for high nitrate concentrations the response of the system is rapid in the first 10 hours when the high concentration of readily biodegradable carbon (rbCOD) available in the fresh pine bark is consumed.

The following plateau may be due to the presence of slowly biodegradable carbon (sbCOD) that, coupled with the inhibitory effect of low pH (typical of pine bark), retard the denitrification process.

After 50-70 hours the system reaches a regime and the efficiency of nitrate removal increases rapidly. This may be attributed to two main reasons: in accordance with the denitrification process described in chapter 3, OH^- ions are produced, providing alkalinity and buffering the low pH to optimal levels for bacterial growth. The large molecules of sbCOD still available at the end of stage 1 are now broken down and ready to be used by the denitrifying microorganisms.

Batch Test 3 - pine bark (BT3 PB)

The evolution of the nitrate concentration with time during batch test 3 (BT3) with pine bark is shown in Figure 5.5 below.

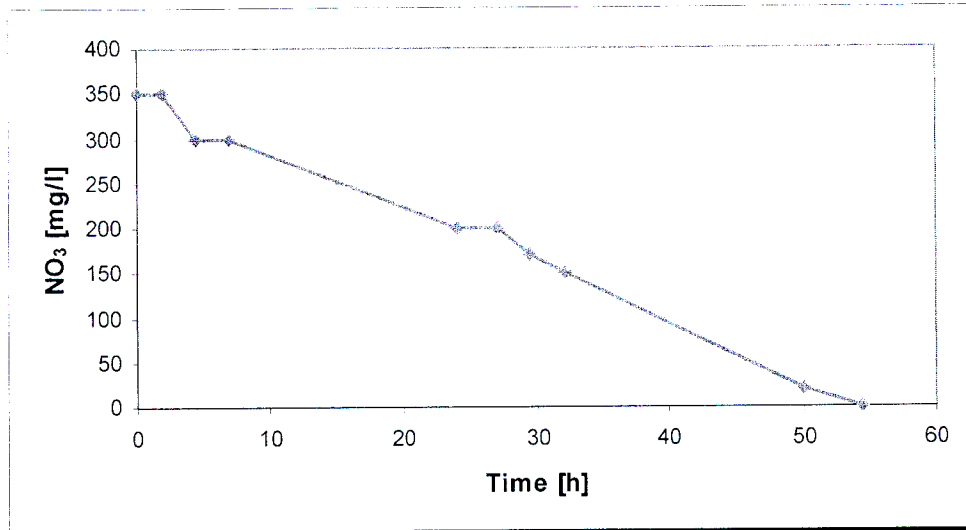


Figure 5.5 - Evolution of the Nitrate concentration in BT3 PB.

In Figure 5.6 the measured nitrate concentrations are normalized to the initial concentration (C_0) and described by a linear model $R^2 = 99\%$.

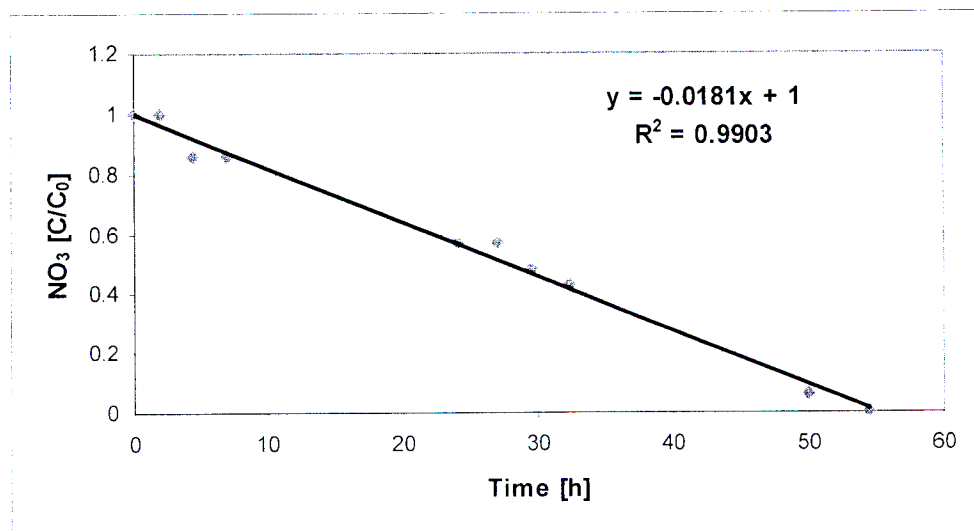


Figure 5.6 - Evolution of C/C_0 with time and best fit model in BT3 PB.

For lower concentrations the response of the system is rapid because the initial concentration is modest and the total degradation is reached in only 54 hours.

In this case the influence of the low pH or the rbCOD is minimized and is not possible to distinguish a clear plateau.

Considering five time intervals (4.5, 29.5, 54.5, 105.5 and 129.5 hours) during the batch tests and recording the percentages of nitrate removal, a clear dependence of the system's behavior with the initial nitrate concentration is noted.

After 4.5 hours the percentage of nitrate removed is 36% in BT1, 29% in BT2 and 14% in BT3, and therefore it can be concluded that the higher the initial concentration the more rapid the degradation in the first part of the test (5-10 hours).

After 29.5 hours the conditions are totally different: BT1 and BT2 display a plateau associated with minimum removal efficiency: 9% in BT1 and 4% in BT2; BT3, on the other hand, doesn't show the plateau and the degradation efficiency increases of 37% in the third interval. After 54.5 hours BT3 has achieved 100% overall removal efficiency, while BT2 has just entered the third stage with an overall removal at the end of this phase equal to 32%; complete removal is achieved after 92 hours. At the third stage, BT1 is still in the plateau and displays 0% removal, but rapidly increases of 54.5% at the end of the fourth interval, reaching 100% efficiency only after 129.5 hours.

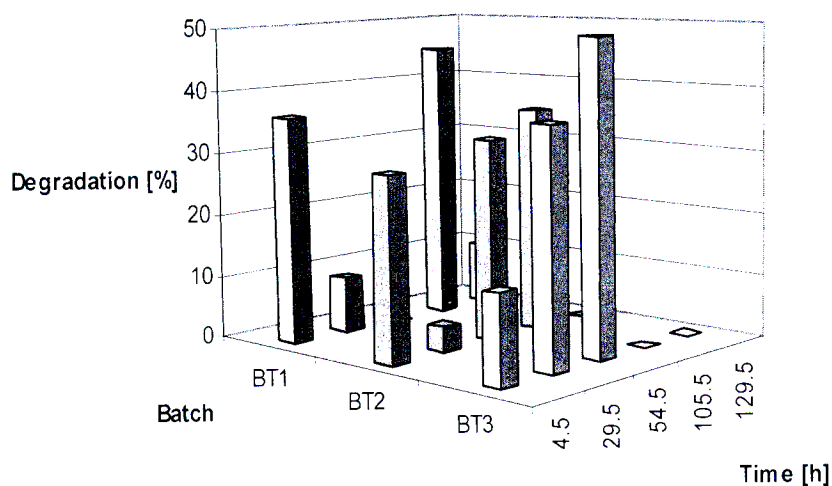


Figure 5.7 - Percentage of Nitrate removal at five intervals (4.5, 29.5, 54.5, 92 and 129.5 hours) in the batch tests with pine bark.

It is interesting to note that, although the denitrification process is strongly dependent on the initial nitrate concentration, their relationship is not linear but logarithmic as expressed by equations 5.1 and 5.2 and presented in Figure 5.8.

$$y = 36.949 \cdot e^{0.0012x} \quad \text{and} \quad R^2 = 99.7\% \quad (5.1 \text{ and } 5.2)$$

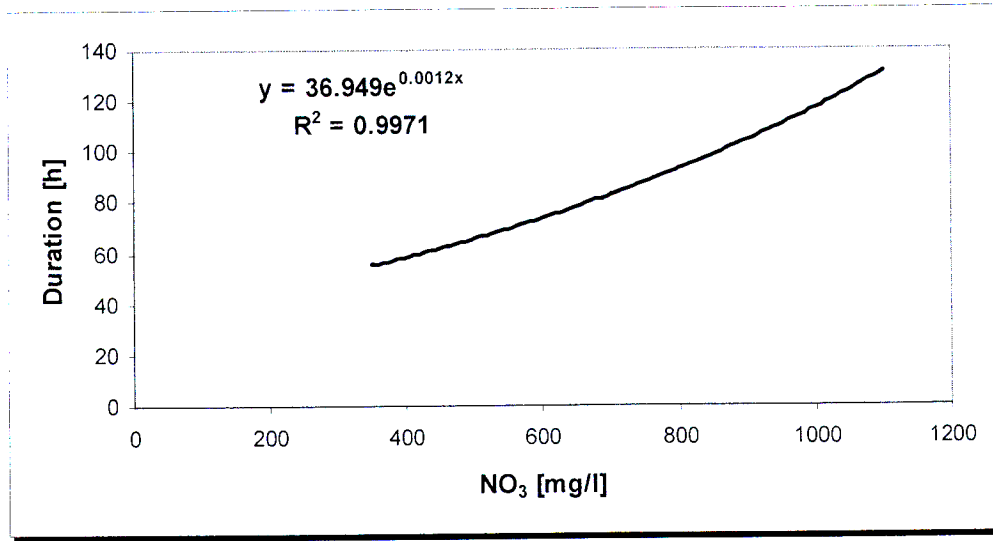
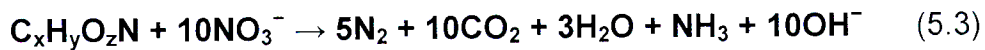


Figure 5.8 - Dependence between the initial nitrate concentration and duration of the batch tests with pine bark.

The denitrification reaction shown below (5.3) suggests that the process occurs with production of ammonia, nitrogen gas, carbon dioxide, water and alkalinity.



The alkalinity produced has the capacity to buffer the initial low pH, typical of fresh pine bark. The higher the initial nitrate concentration, though, the longer the transitional period for the system to buffer the acidity and enters into regime.

Another consequence of denitrification is a net total carbon reduction in the solid matter, as it is consumed by the denitrifying microorganisms and transformed into CO_2 or leached out as soluble COD. The total nitrogen in the substrate also decreases during the denitrification process causing an increase in ammonia in the leachate and a production of nitrogen gas.

5.3.2 Batch tests – Compost

Table 5.8 presents the results of the quality characterization for the influent leachate and the solid substrate in the batch tests with compost.

Table 5.8 - Characteristics of the influent leachate and solid substrates used in the batch tests with compost

	COD	pH	NH ₃	NO ₃	TOC	TKN	
	[mg/l]		[mg/l]	[mg/l]	[mg/l]	[mg/l]	
Influent leachate	499	7.9	7.98	1100	205	25	

BT1 CO	COD	pH	NH ₃	NO ₃	tot C	tot N	C/N
	[mg/l]		[mg/l]	[mg/l]	[%]	[%]	
Output leachate	2114	7.95	61.57	0	-	-	-
Output substrate	-	-	-	-	6.6	0.78	8.46

BT2 CO	COD	pH	NH ₃	NO ₃	tot C	tot N	C/N
	[mg/l]		[mg/l]	[mg/l]	[%]	[%]	
Output leachate	1145	8.2	44	0	-	-	-
Output substrate	-	-	-	-	11.77	1.19	9.89

BT3 CO	COD	pH	NH ₃	NO ₃	tot C	tot N	C/N
	[mg/l]		[mg/l]	[mg/l]	[%]	[%]	
Output leachate	1119	7.5	27.37	0	-	-	-
Output substrate	-	-	-	-	12.62	1.21	10.43

It is interesting to note the high concentrations of ammonia and COD in the output leachate and the low carbon-to-nitrogen ratio in the solid substrate at the end of the batch tests with compost. The slightly basic pH does not constitute an obstacle for the onset of denitrification.

The evolution with time of the nitrate concentration in the batch tests with compost is shown in Figures 5.9, 5.10 and 5.11.

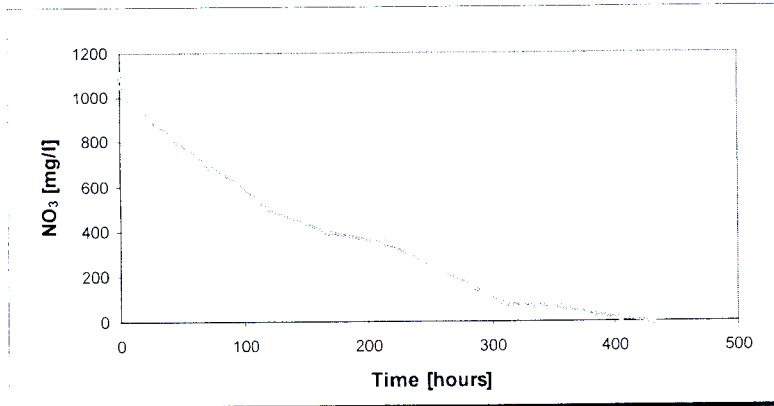


Figure 5.9 - Evolution of the Nitrate concentration in BT1 CO.

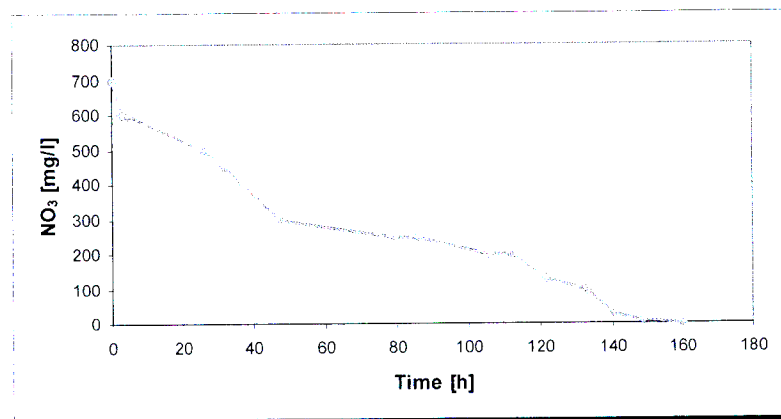


Figure 5.10 - Evolution of the Nitrate concentration in BT2 CO.

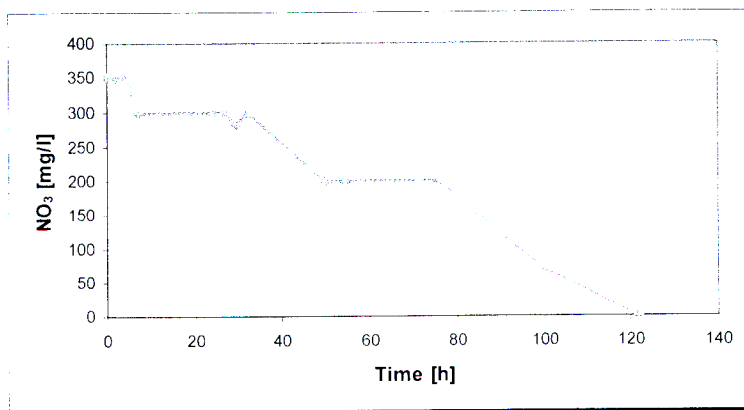


Figure 5.11 - Evolution of the Nitrate concentration in BT3 CO.

An optimum environment for microbial degradation is established immediately at the onset of the batch tests, showing a regular trend with a direct dependence between initial nitrate concentration and the time required to reduce the nitrates to zero. Two short plateaus are detected during the batch tests at 350mg/l initial nitrate concentration.

Figures 5.12, 5.13 and 5.14 present the kinetic model suggested for the batch tests with compost. Note that the nitrate concentrations have been normalized to the initial concentrations C_0 .

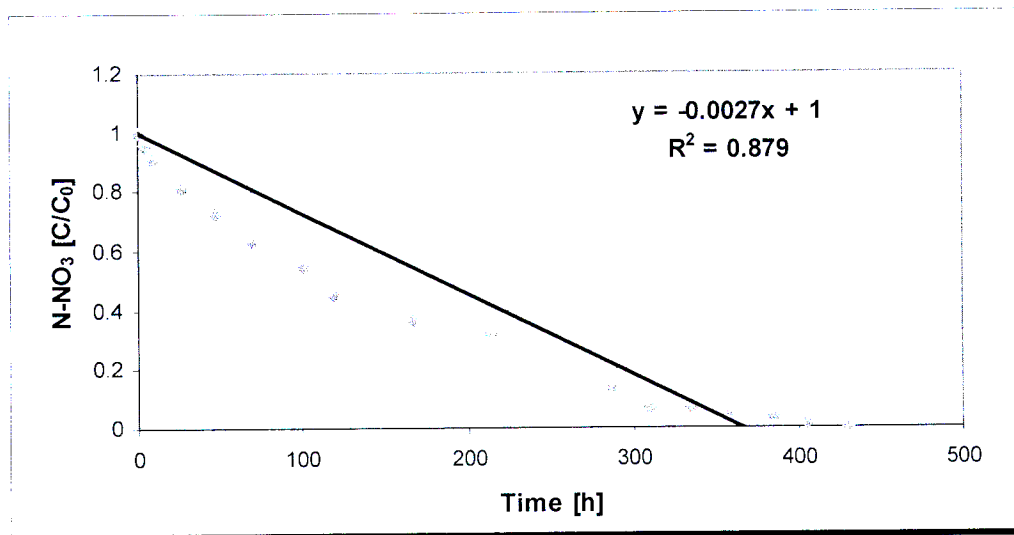


Figure 5.12 - Evolution of C/C_0 with time and best fit model for BT1 CO.

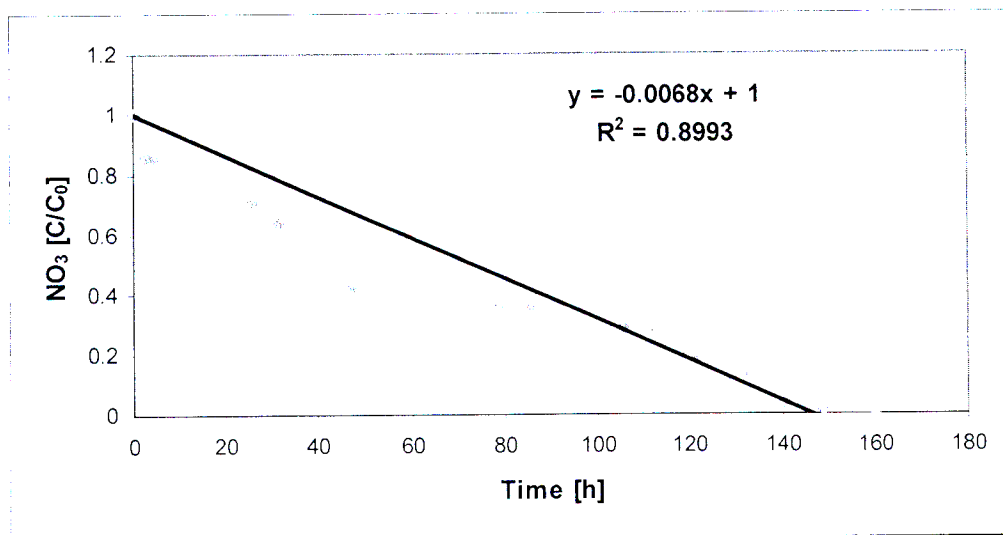


Figure 5.13 - Evolution of C/C_0 with time and best fit model for BT2 CO.

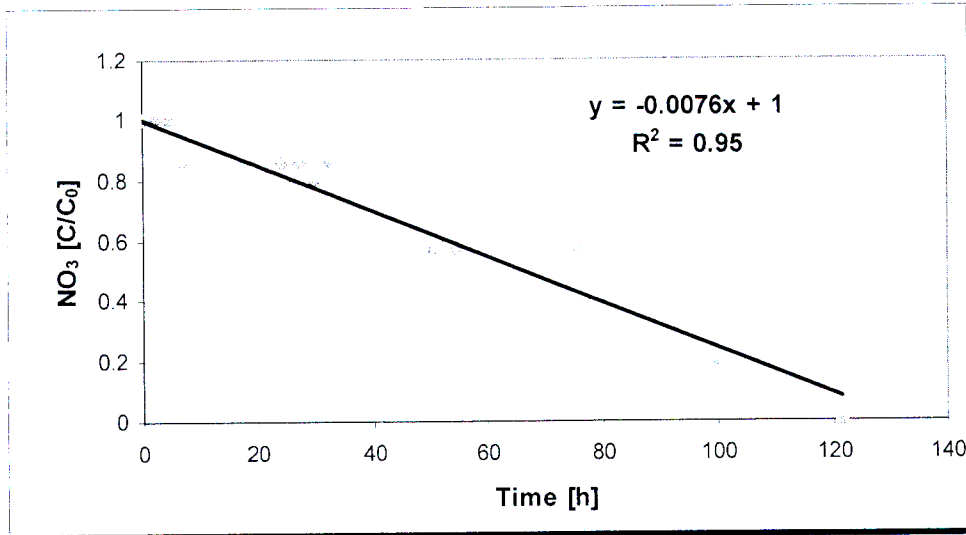


Figure 5.14 - Evolution of C/Co with time and best fit model for BT3 CO

The denitrification process is described by a zero order kinetic model type $c = at + c_0$ with the following R^2 values:

- BT1 CO, $R^2 = 88\%$
- BT2 CO, $R^2 = 90\%$
- BT3 CO, $R^2 = 95\%$

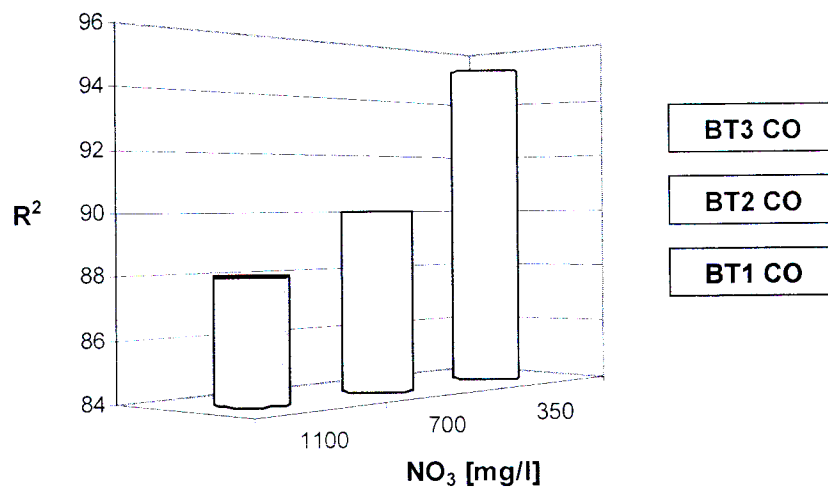


Figure 5.15 - Relationship between R^2 and NO₃ initial concentration in the batch tests with compost.

Figure 5.16 shows a direct relationship between the R^2 values of the proposed kinetic models and the initial nitrate concentration in the leachate used for the batch tests with compost, suggesting that concentrations as high as 1000 mg/l do not negatively affect the process which follows a linear evolution trend.

As observed in the batch tests with pine bark, there is a strong dependence between the initial nitrate concentration and the duration of the test. This dependence is described by equations 5.4 and 5.5 and presented in Figure 5.16. Occurrence and duration of the plateaus in the third batch test are considered negligible in the overall degradation process.

$$y = 58.133 \cdot e^{0.0018x} \quad \text{and} \quad R^2 = 92.1\% \quad (5.4 \text{ and } 5.5)$$

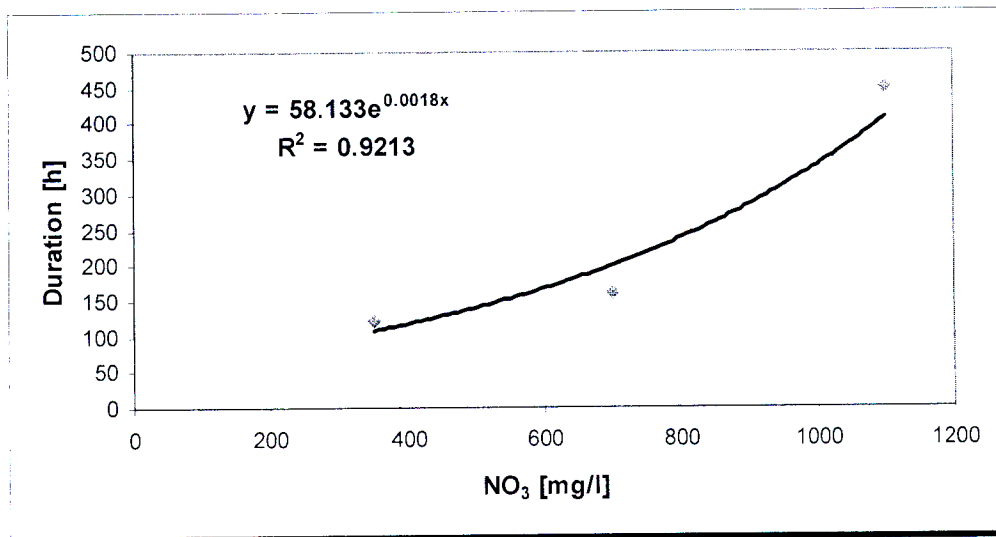


Figure 5.16 - Dependence between the initial nitrate concentration and duration of the batch tests with pine bark.

Compost is an aerobically treated substrate, the rbCOD has been consumed during the composting process and the available carbon is in the form of sbCOD. The neutral pH provides optimal conditions for denitrification. The high nitrogen concentration in the compost reflects in high ammonia production in the output that does not inhibit the process development. The organic content in the leachate increases during the test as it is leached out of the organic-rich compost. As in the pine bark batch tests, the total carbon decreases during the batch tests with compost as a consequence of the

microbial activity and the total nitrogen is reduced according with the denitrification reaction with consequent production of ammonia and nitrogen gas.

5.4 Results – Columns (leachate)

The column tests were designed to investigate the substrates' performance and potential for gas production during the denitrification process at a larger scale than the one used for the preliminary batch tests.

As explained in chapter 4, two columns, filled with pine bark (column 1) and compost (column 2), were used for this experiment and operated as fixed bed reactors, irrigated with leachate ($C_0=600\text{mgNO}_3/\text{l}$) in a plug-flow mode.

5.4.1 Nitrates

The evolution of the nitrate concentration in the leachate from the columns with time is presented below in relation to the specific solid substrates.

Pine bark

Column 1 was filled 6 times in 126 days (18 weeks) and the results of the evolution of the nitrate concentration with time are presented in Figure 5.17.

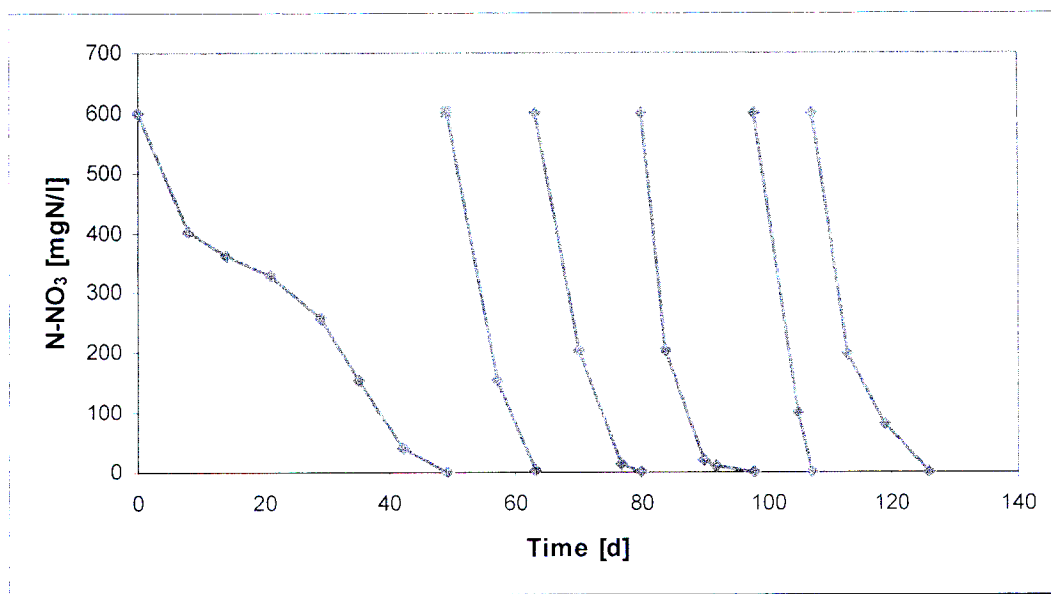


Figure 5.17 - Evolution of the nitrate concentration in the column test with pine bark.

Figure 5.18. reports the evolution with time of the nitrate concentration normalized to the initial concentration (C/C_0) and the kinetic model used to describe the process during the first filling.

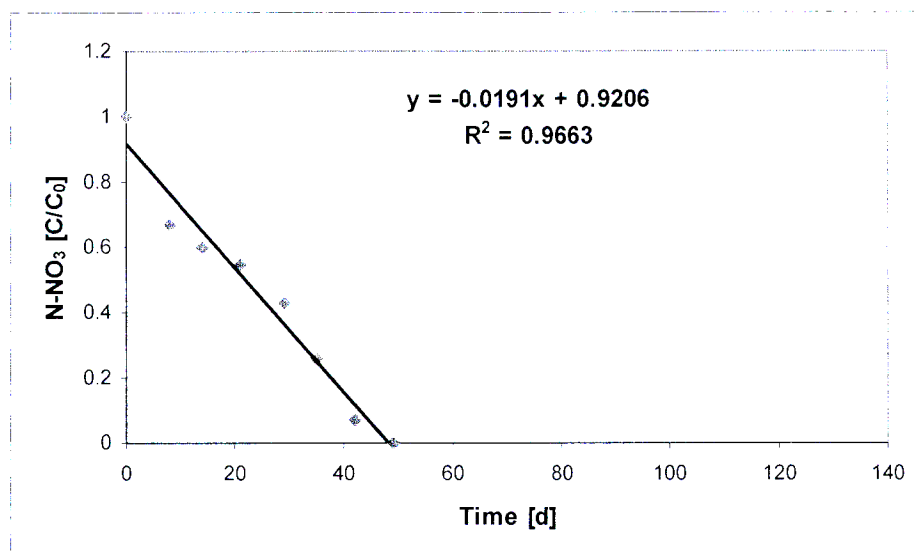


Figure 5.18 - Evolution of C/C_0 in the column test with pine bark during the first filling.

The trend observed in the batch tests with high nitrate concentrations is evident again in the columns. The system requires a certain amount of time to reach regime and buffer the inhibitory effect of the low pH and of the high CO_2 production occurring due to the high organic content in the substrate. The following fillings display a more rapid linear trend. Except for the final part of the fourth filling, the degradation seems to follow a zero order kinetic model. Focusing on the first filling a comparison with the batch test 2 (BT2 PB) is presented in Figures 5.19.

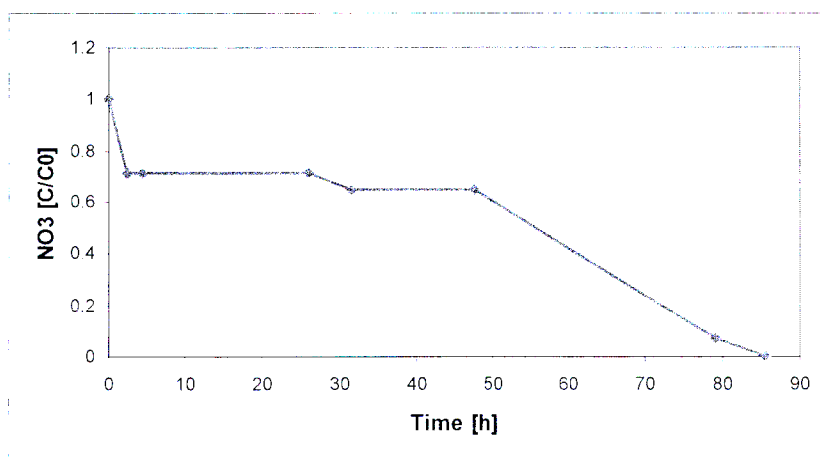


Figure 5.19 - Evolution of C/C_0 and best fit model for BT2 PB.

The variation of the nitrate concentration with time at both scales is comparable, showing a tendency of the system to reach regime in the first 10 days of the process. The large differences in scale, though, make a direct comparison between the tests difficult. None the less, it is possible to note that the pine bark requires about 50 days in columns to acclimatize and completely degrade the nitrates.

A breakdown analysis of Figure 5.19 above is presented in Figures 5.20 (a, b and c).

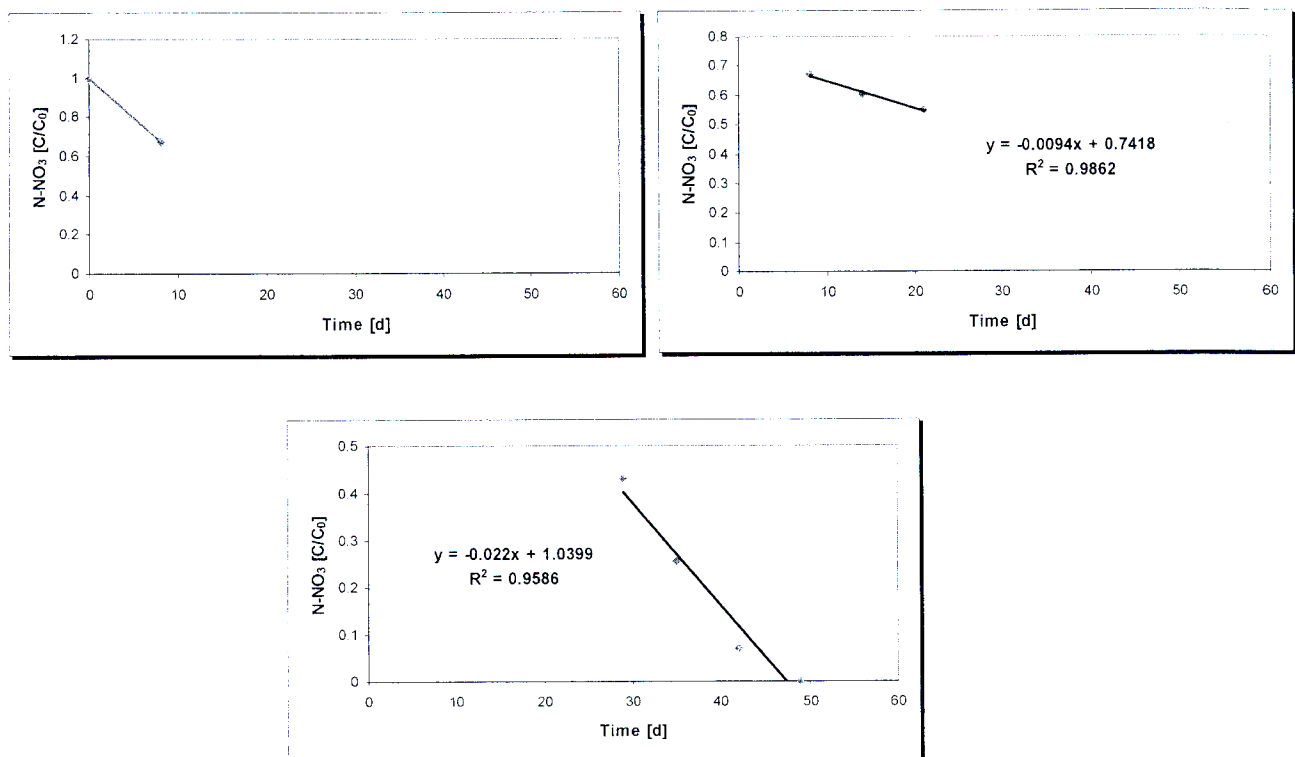


Figure 5.20 (a, b and c) - Detailed kinetic analysis of the C/C_0 evolution during the first filling of the column test with pine bark

The first part of the curve (Figure 5.20a) shows a rapid response of the system as the readily biodegradable carbon (rbCOD) is consumed; a slight deflection is also evident as compared to the plateau identified in BT2 PB (Figure 5.20b), possibly also due to the difficulty in degrading the slowly biodegradable carbon (sbCOD) and buffering the low pH. The last part of the curve shows a rapid response of the system passed the acclimatization time (Figure 5.20c).

From the analysis of Figure 5.19, it is evident that the buffering of the low pH occurs during the first filling and does not influence the rest of the test.

Figure 5.21 below shows the removal efficiency expressed in $\text{mg NO}_3^-/\text{l} \cdot \Delta t/\text{kg TS}$, where Δt is the time interval and TS is the total solids in the pine bark.

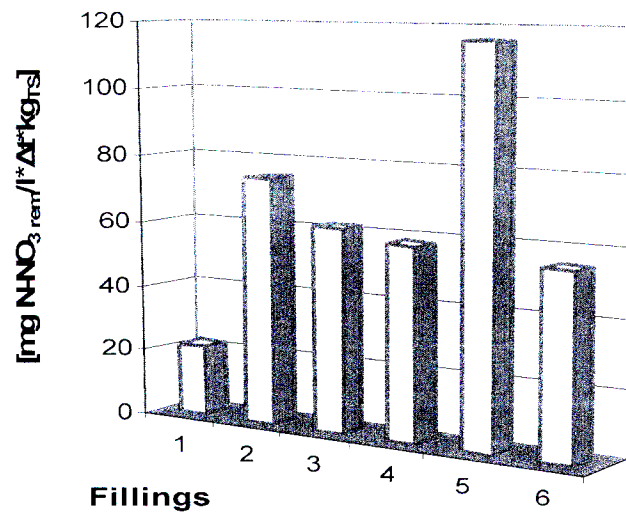


Figure 5.21 - Removal efficiency of pine bark in column test

The pine bark removed an overall concentration of nitrates equal to 3600 in 18 weeks without losing its capacity.

Compost

Column 2 was filled 3 times in 126 days (18 weeks) and the results of the evolution of the nitrate concentration with time are presented in Figure 5.22.

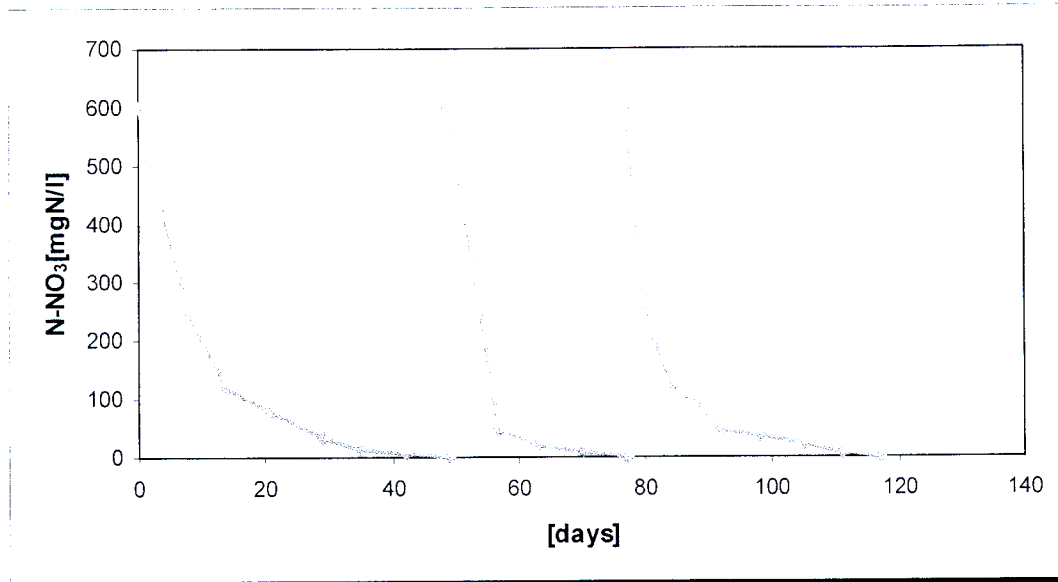


Figure 5.22 - Evolution of the nitrate concentration in the column tests with compost.

From the figure above, it is evident that the compost's performance is lower than the pine bark, although it is not affected by inhibitory factors such as a low pH. The system's response is certainly related to the absence of rbCOD and the difficulty to breakdown slowly degradable matter which requires high energy efforts.

Figure 5.23 reports the evolution with time of the nitrate concentration normalized to the initial concentration (C/C_0) and the kinetic model used to describe the process during the first filling.

It is evident that the degradation process in column 2 is described by a first order kinetic model.

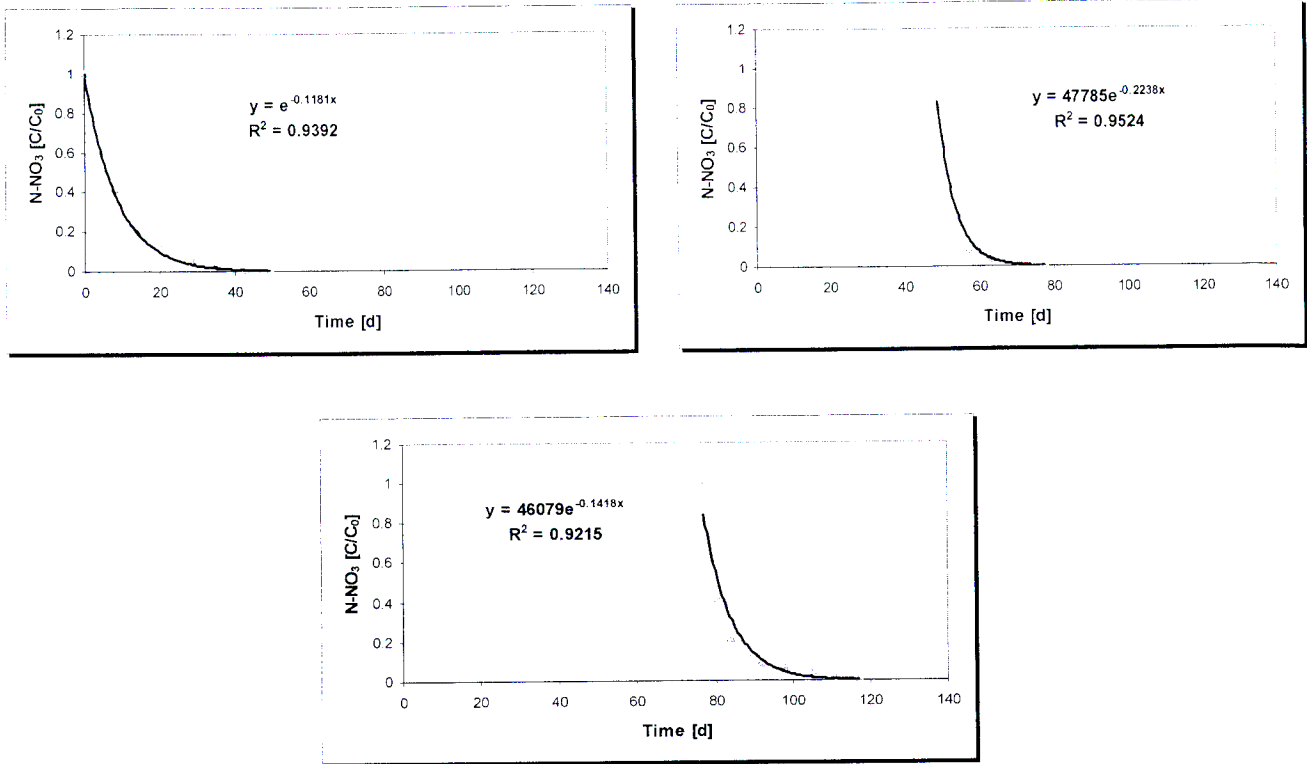


Figure 5.23 (a, b and c) - Detailed kinetic analysis of C/C_0 during the first filling of the column test with compost

Focusing on the first filling a comparison with the batch test 2 (BT2 CO) is presented in Figures 5.24.

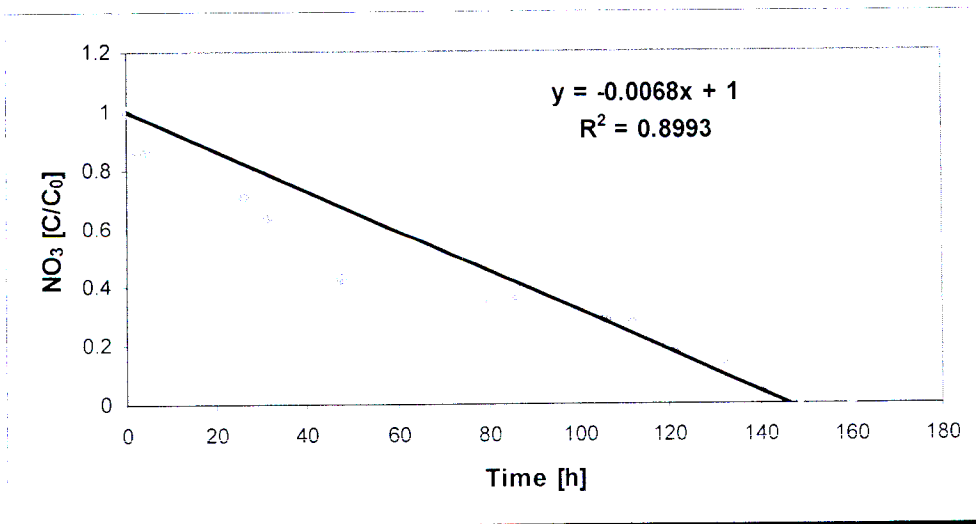


Figure 5.24 - Evolution of C/C_0 and best fit model for BT2 CO.

The trends displayed by the compost in the batch tests and the columns are comparable, but not described by similar kinetic models. It is remarkable that both substrates require similar acclimatization periods to reach regime (approx. 40 days), although the absence of inhibitory effects in the compost.

Figure 5.25 below shows the removal efficiency expressed in $\text{mgNO}_3/\text{l}/\Delta t/\text{kgTS}$ for the column tests with compost.

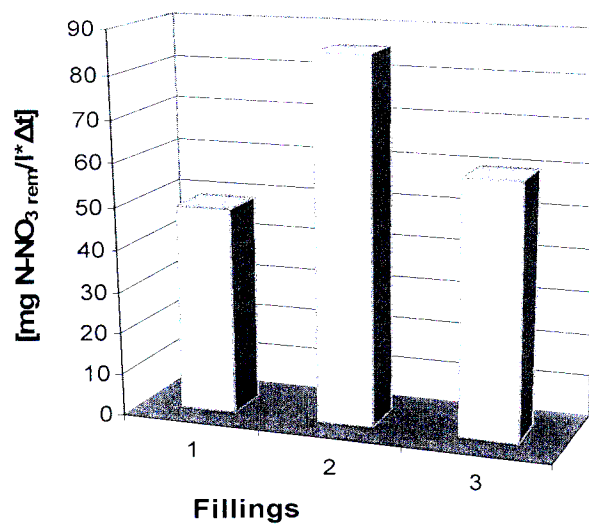
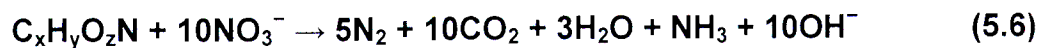


Figure 5.25 - Removal Efficiency expressed in $\text{mgNO}_3/\text{l}/\Delta t/\text{kgTS}$ for the column tests with compost

The compost removed 1800 mg/l of nitrates in 18 weeks without losing its denitrification capacity.

5.4.2 Ammonia

The denitrification reaction (5.6) occurs with an increment of ammonia.



The figures 5.26 and 5.27 below report the evolution of the ammonia concentrations with time and the cumulate ammonia production during the columns tests.

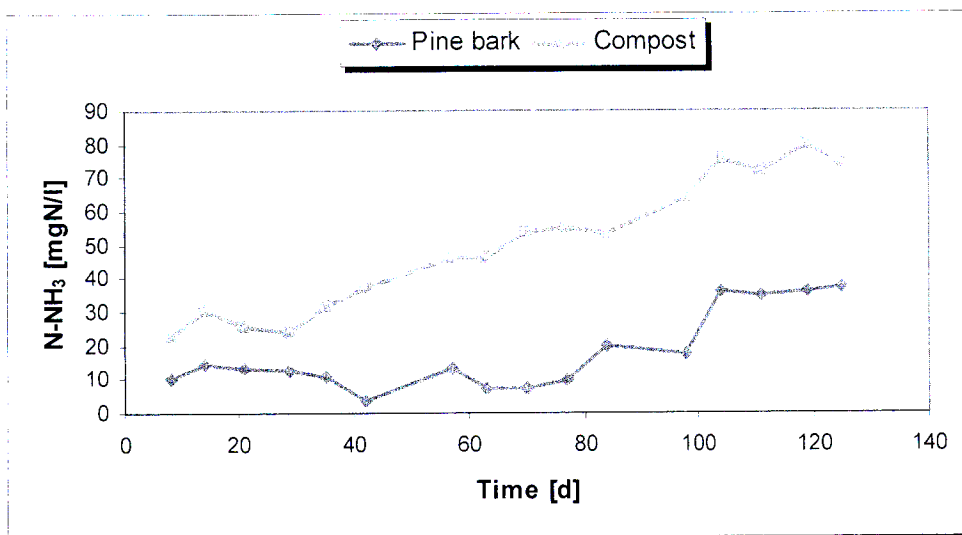


Figure 5.26 - Evolution of the nitrate concentration during the column tests.

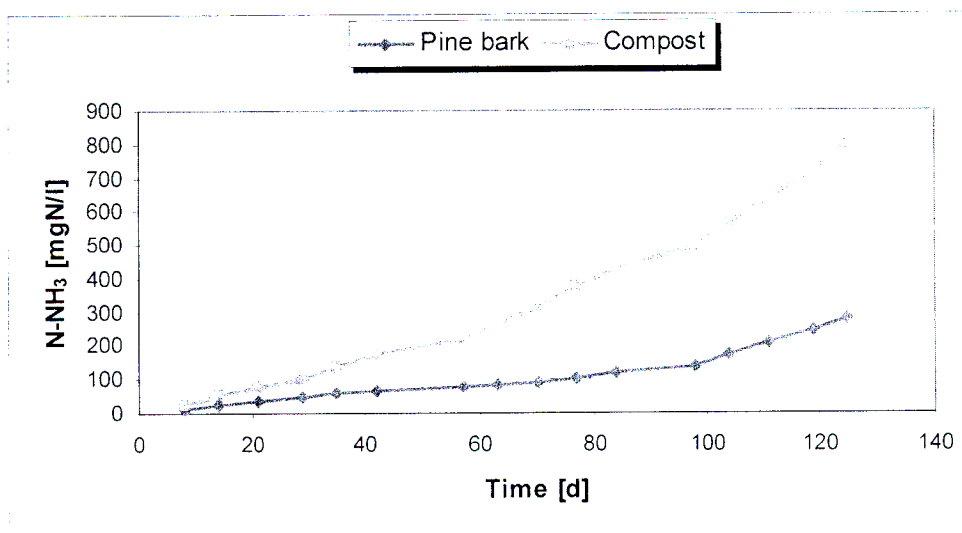


Figure 5.27 - Cumulate production of ammonia during the column tests.

The ammonia production is higher in the compost possibly due to the higher nitrogen content in the substrates' matter (Chapter 5, Page 87, Table 5.1). The production of ammonia has a long term inhibitory effect on the microflora established in the substrates.

5.4.3 pH

The pH variation during the column tests is shown below.

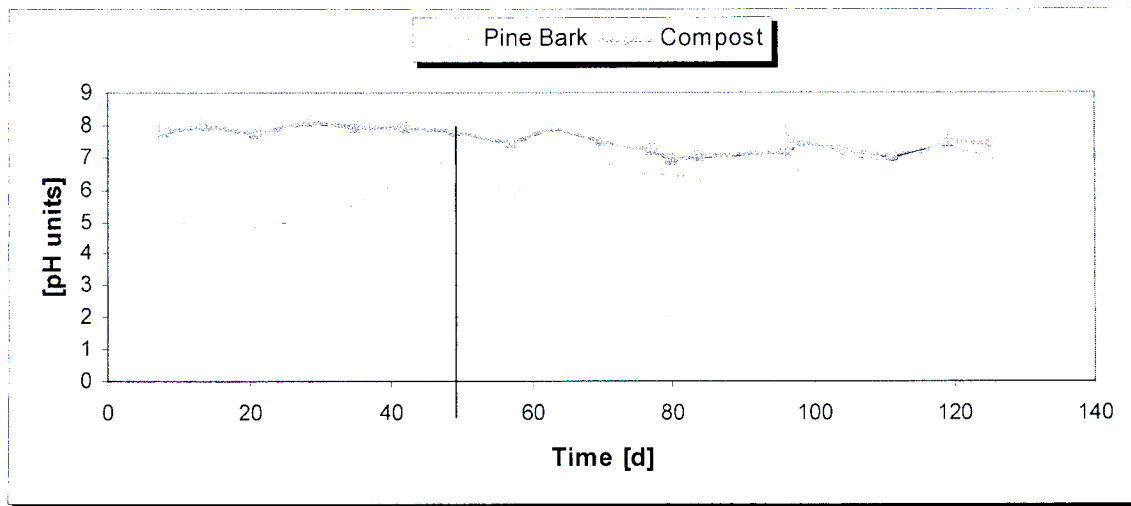


Figure 5.28 - pH during the column tests.

Focusing on the pine bark (Figure 5.28) it is evident that the first 40 days of treatment are critical to buffer the low pH, to reach neutrality and establish a regime.

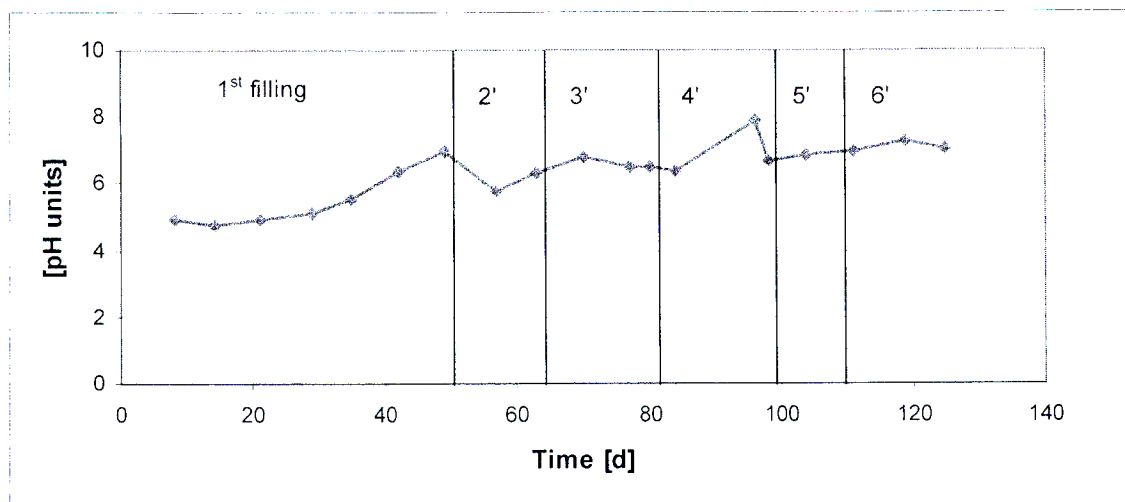


Figure 5.29 - pH variation during the column tests with pine bark.

A correlation between the evolution of the nitrates concentrations and the pH in the pine bark is presented below. The effect of the low pH is clear in the first filling.

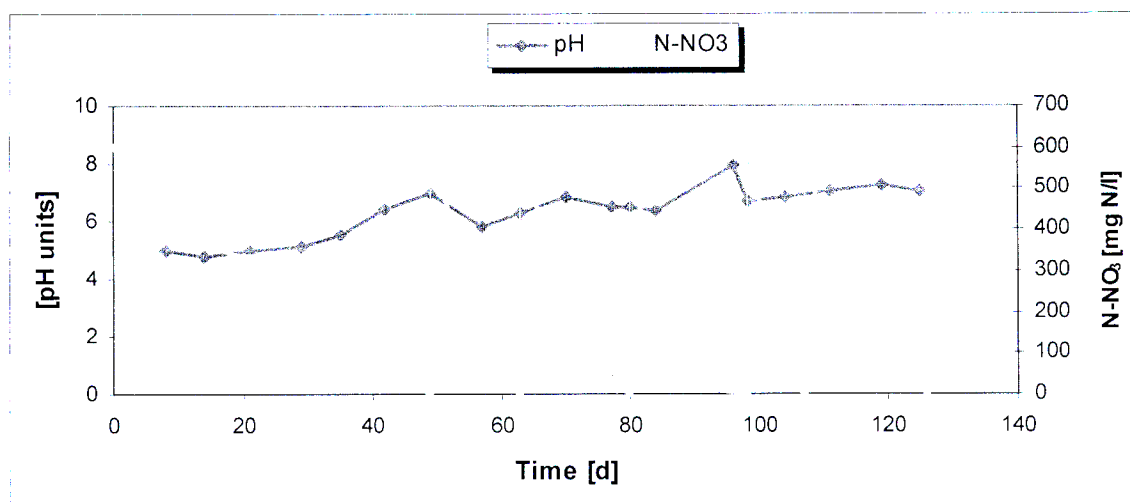


Figure 5.30 - pH and nitrate concentrations during the column tests with pine bark.

The pH in the compost is constantly around neutrality during the tests as confirmed by the quality characterization of the solid mater.

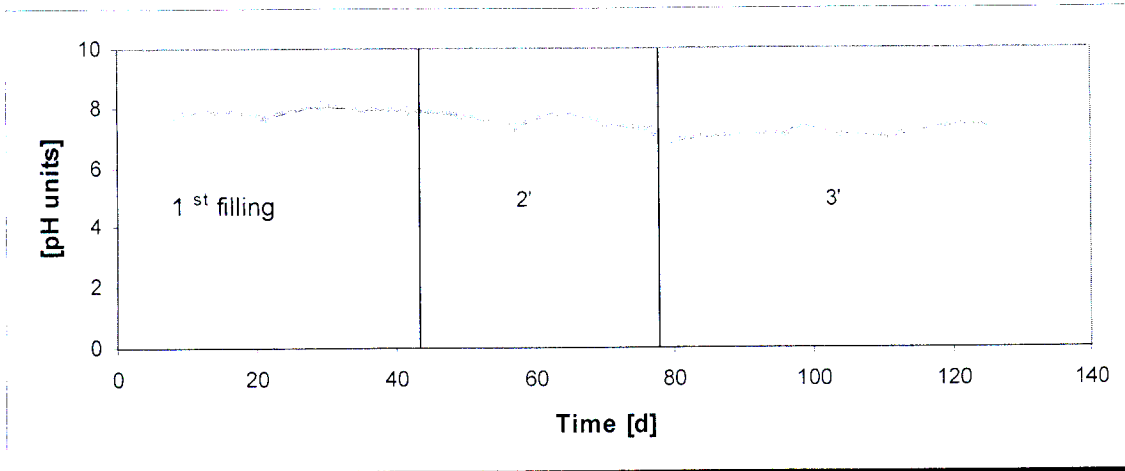


Figure 5.31 - pH variation during the column studies.

There is no interdependence between the variation of the nitrate concentration and the pH in the system as presented in Figure 5.32.

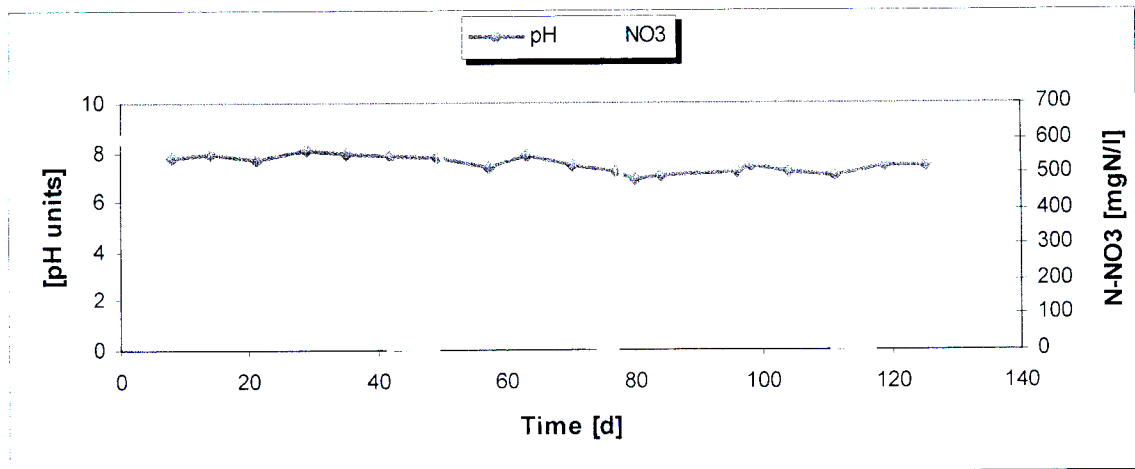


Figure 5.32 - pH and nitrate concentration rate during the column tests with compost.

5.4.4 Eh

The Eh measurement allowed assessing the oxidative state of the system. The results are presented in Figures 5.33 and 5.34.

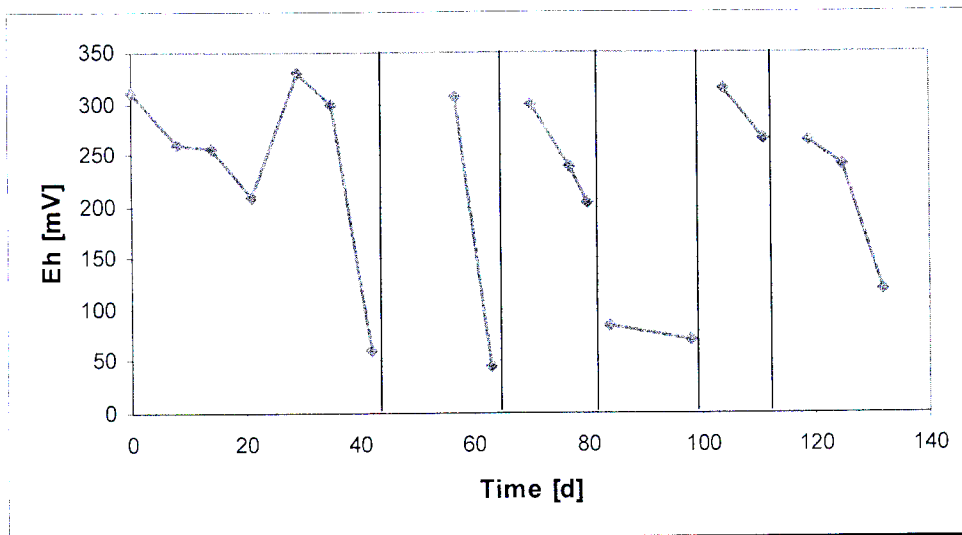


Figure 5.33 - Eh during the column test with pine bark.

During most of the trials, the leachate is in anoxic conditions ($E_h < 400\text{mV}$) favouring the denitrification process. Anoxic conditions are reached towards the end of each campaign after 40 days for the acclimatization period during the first filling and more rapidly (10 days) thereafter.

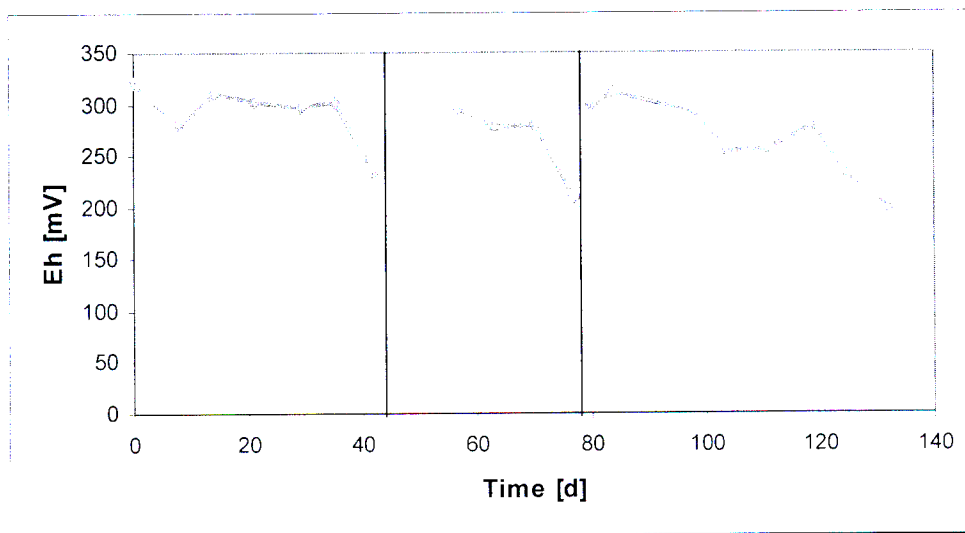


Figure 5.34 - Eh during the column tests with compost.

The establishment of anoxic conditions is not evident in the compost trials as confirmed by the dissolved oxygen concentrations presented in Figure 5.35.

This difference between the two substrates may be due to the higher reactivity of the pine bark and the larger readily degradable component of the C/N that induces a rapid consumption of oxygen and nitrates by the denitrifying bacteria after the first 40 days of acclimatization. The higher contact surface of the compost and lower porosity allow for a more efficient transport and diffusion of oxygen in the system, inducing the denitrifiers to operate in a semi-aerobic environment as facultative anaerobes. This reflects in a low initial performance.

5.4.5 DO

The DO measurements were carried to ensure the establishment of optimal conditions for denitrification. The results of the column tests are presented in Figures.....

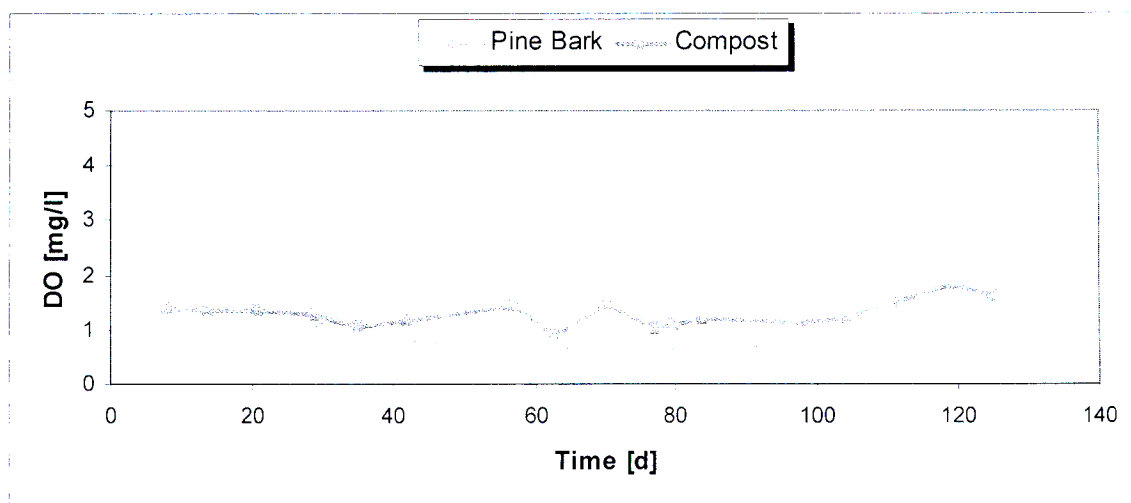


Figure 5.35 - DO during the column tests.

As noted before in Figure 5.35, the dissolved oxygen concentration is very low throughout the trials with pine bark, while semi-aerobic conditions are present in the column filled with compost. However, the positive DO concentrations in the compost may retard the onset of denitrification but do not constitute a real inhibitory effect for the denitrifiers that operate as facultative anaerobes.

5.4.6 COD

One of the problem linked with biological denitrification using organic matter as carbon source is the increase in COD in the treated leachate.

The following figures show the COD production during the column studies.

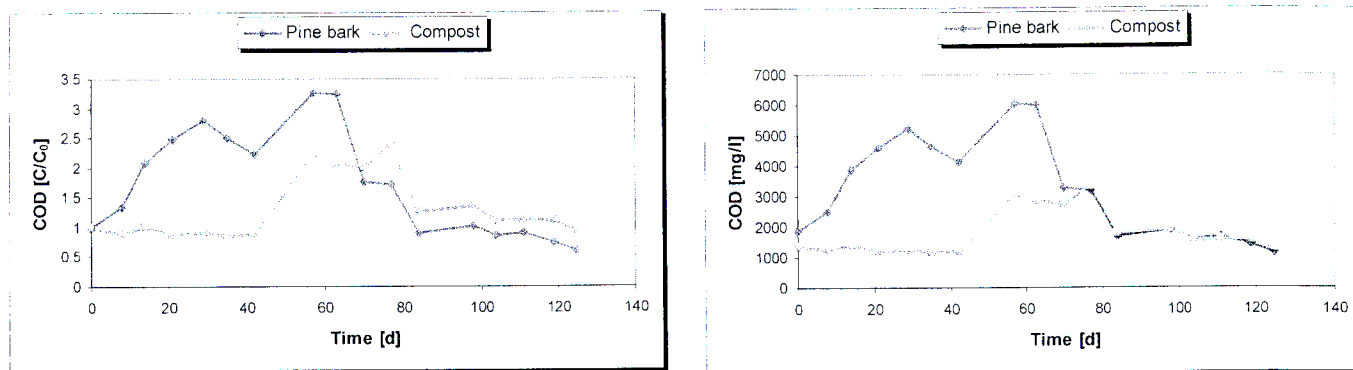


Figure 5.36 - Specific COD production in mg/l and normalized to Co during the column studies.

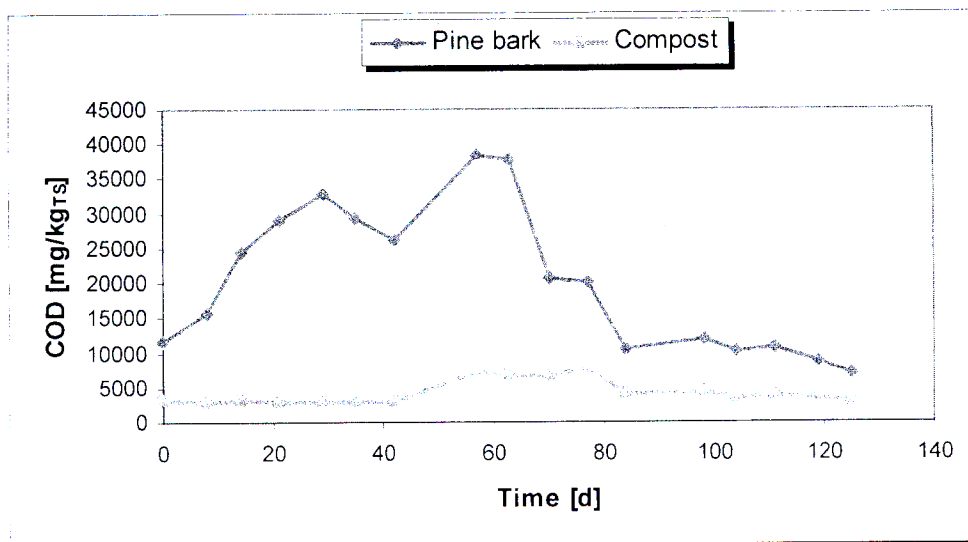


Figure 5.37 - Specific COD production related to the dry mass during the column studies.

The higher carbon content in the pine bark and the higher reactivity reflect in a larger production of COD during the acclimatization period of the degradation process.

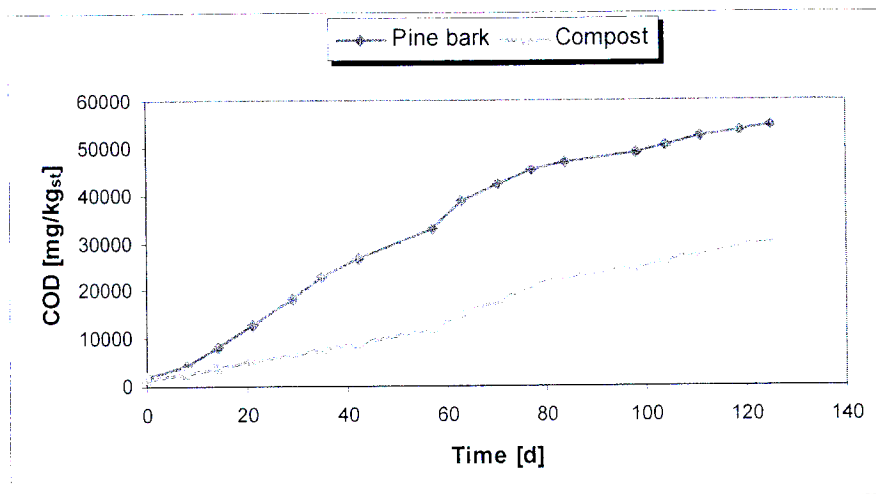


Figure 5.38 - Cumulate COD during the column studies.

The maximum release is recorded between day 50 and day 65 in the pine bark while between day 55 and day 80 day for the compost. At regime, the COD is reduced by flushing but remains at levels higher than the discharge limits.

5.4.7 Conductivity

The conductivity in a liquid measures the presence of inorganic dissolved solids such as chloride, nitrate, sulfate, and phosphate anions or sodium, magnesium, calcium, iron, and aluminum cations.

Figure 5.39 shows the conductivity recorded during the column studies.

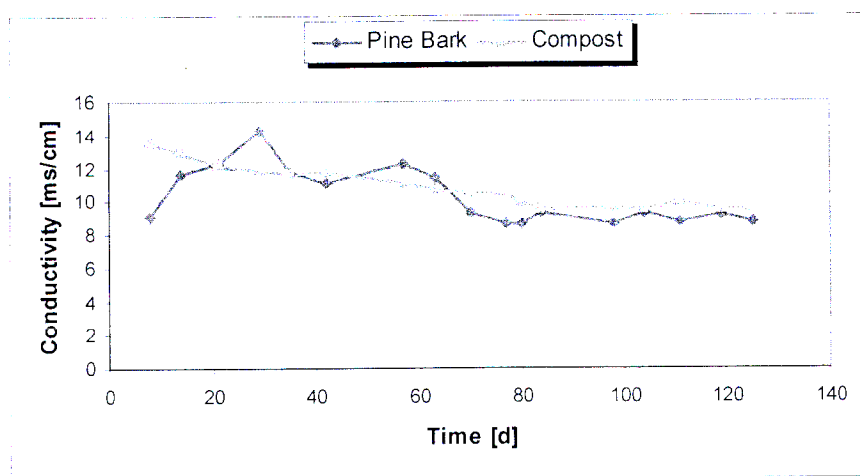


Figure 5.39 - Specific conductivity recorded during the column studies

5.5 Results – Columns – gas

As explained in Chapter 4, the two columns were equipped with a liquid displacement system for the determination of gas production during the trials.

Figure 5.40 presents the biogas production in column 1.

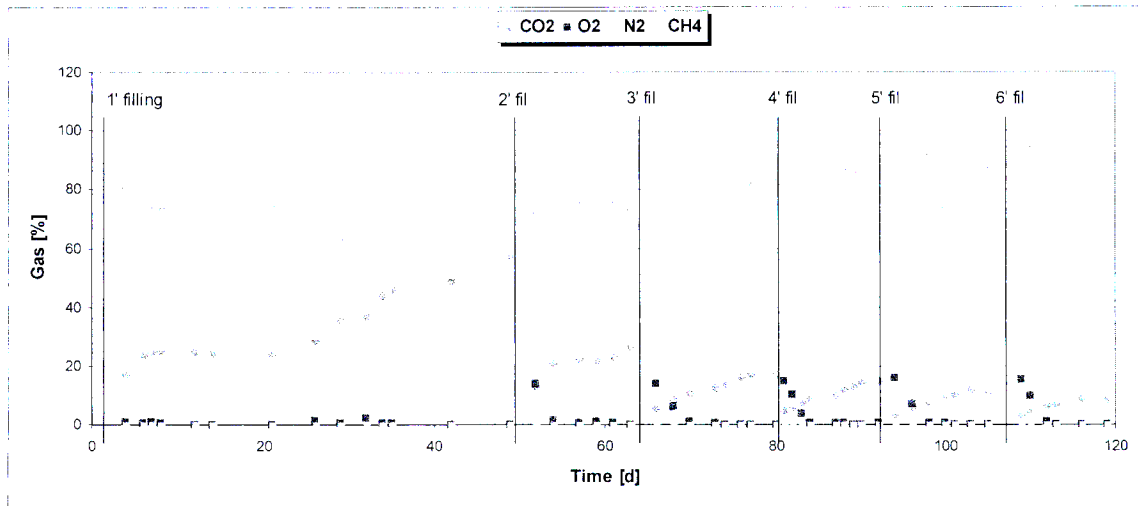


Figure 5.40 - Quality of biogas produced in Column 1 during the trials.

The effect of the acclimatization period are evident in the relatively lower concentration of Nitrogen gas during the first filling that slightly decrease from 80% to 40% following the typical plateau noted for other key parameters.

During the subsequent fillings, the nitrogen concentration increases constantly from 70 to 100% at each campaign.

During each stage, the nitrogen gas decreases in correspondence to the total nitrate consumption, according to the denitrification reaction (3.4) that needs nitrates to produce N_2 , associate with a CO_2 increase.

The oxygen is low (less than 1%) in accordance with the anoxic/anaerobic conditions in the reactors with peaks of 13 – 15% in correspondence to each leachate refill; however, this oxygen is easily and rapidly consumed.

No methane production is recorded (0.0%) again according with the denitrification process. In fact, until nitrates are present in the leachate they will be consumed by the denitrifying bacteria that are privileged in the competition with the methanogens.

The compost displays a different behavior starting with an immediate nitrogen gas production that remains between 80 and 100% for the entire test without significant variations. The following figure shows the gas production in column 2.

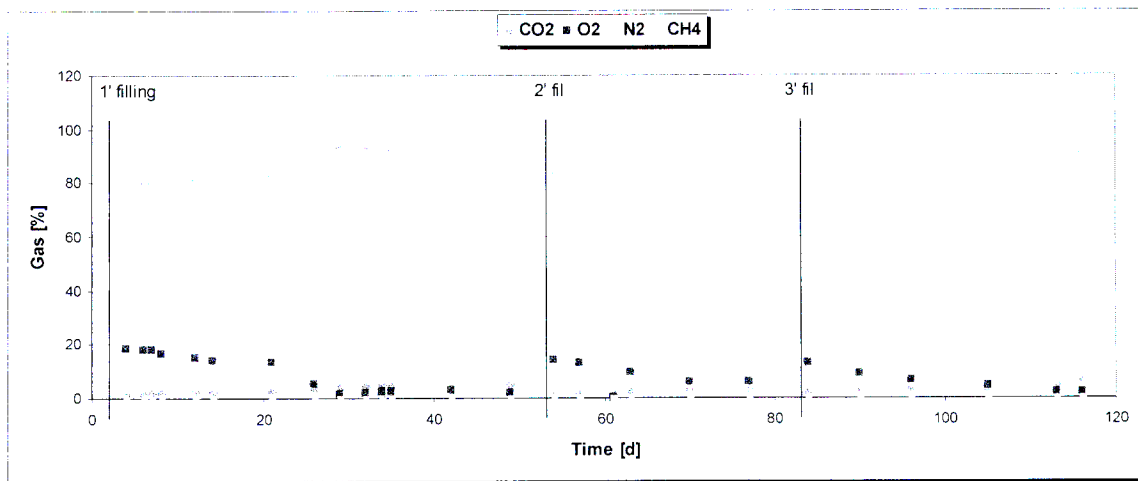


Figure 5.41 - Quality of biogas produced in Column 2 during the trials.

In this case, a slight decrease in nitrogen gas concentration is recorded in correspondence to the total nitrate removal. A slight CO_2 production is recorded in accordance with the denitrification process. The lower CO_2 levels are related to the lower C/N ratio. The oxygen is easily and rapidly consumed realizing the anoxic/anaerobic conditions in the reactor that are necessary for the denitrification process. Slight peaks of CO_2 are recorded at each leachate refill.

Again, according to the denitrification process, no methane production is recorded.

The following figures show the specific and cumulate production of gas in the column studies.

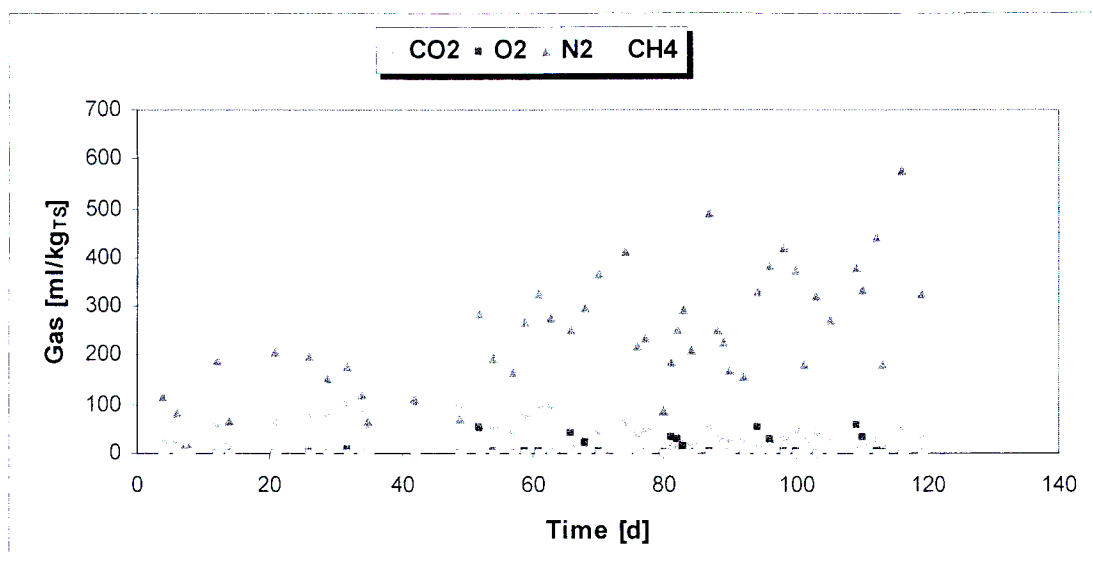


Figure 5.42 - Specific gas production in column 1.

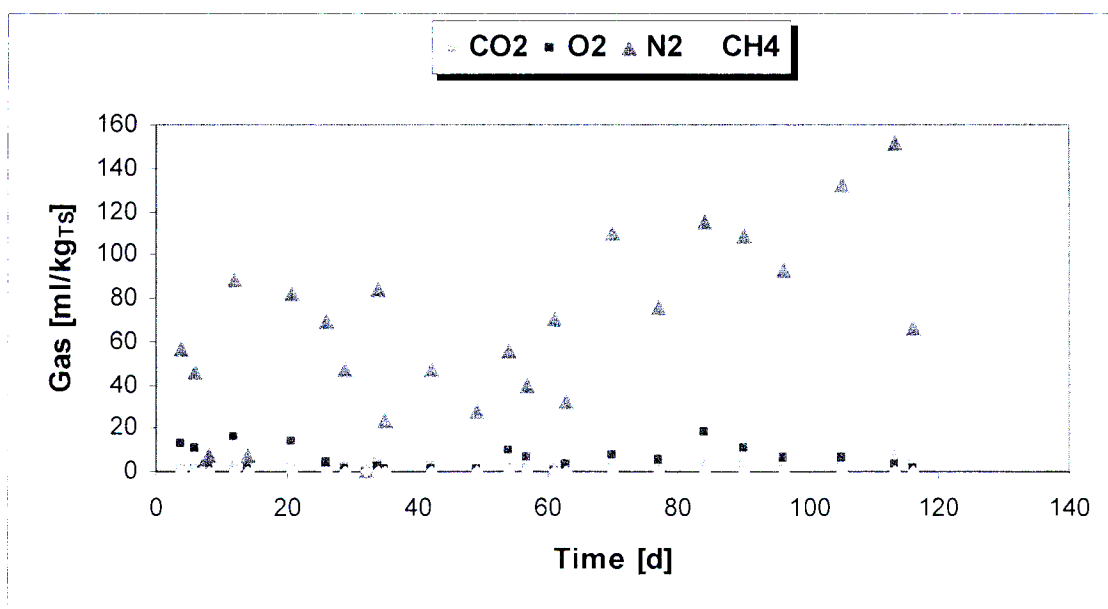


Figure 5.43 - Specific gas production in column 2.

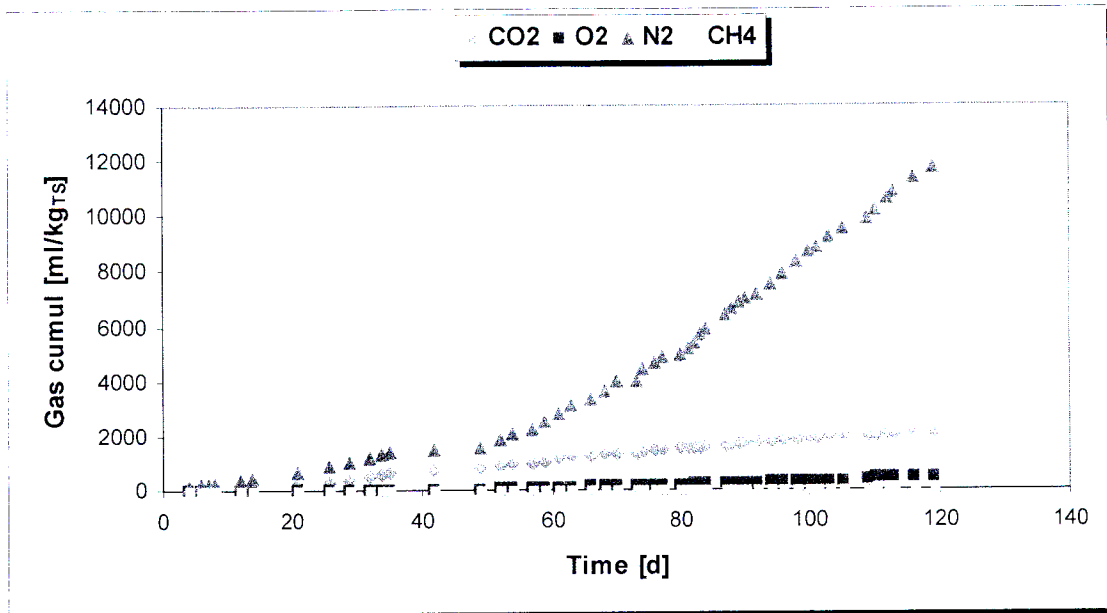


Figure 5.44 - Cumulate gas production in column 1.

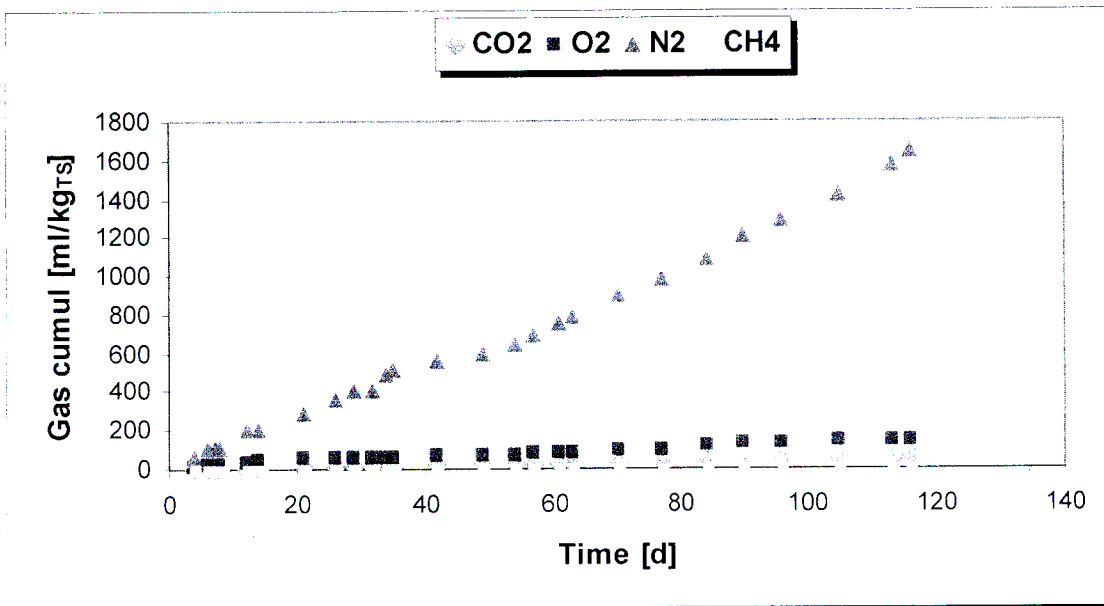


Figure 5.45 Cumulate gas production in column 2.

The total N₂ production from column 1 was 12.000 ml/kg_{TS} while the production from column 2 was 16.000 ml/kg_{TS}. It is to note that the compost is characterized by a higher nitrogen content that may justify the higher gas production.

A similar consideration can be made for the CO_2 in column 1 that is far higher than in column 2, due to the higher content of readily degradable carbon in the pine bark that is easily gasified during nitrification.

A respirometric test was performed in parallel with the columns gas experiment. The results are shown in the figure below.

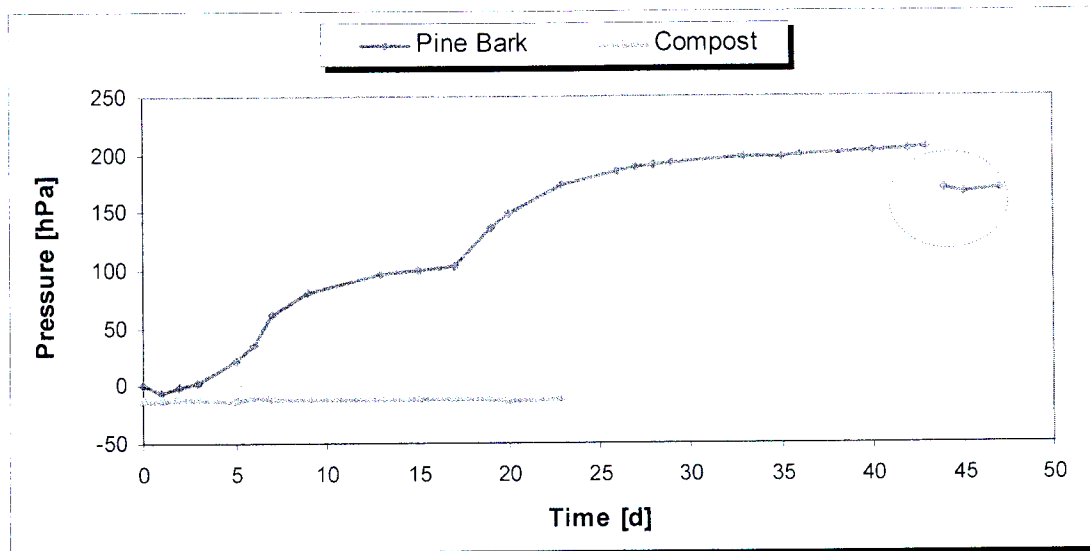


Figure 5.46 - Pressure variation in the OxiTop system. Note: The circle indicates a pressure decrease in correspondence to CO_2 suppression.

As recorded in previously (Paragraph 5.4) the performance of the compost was very poor and the test had to be aborted twice.

This may be due to the high stabilization degree of the compost and the absence of a well established microflora.

Note that in these trials no seeding was applied. On the contrary the fresh pine bark displays a greater reactivity that reflects in a larger gas production even without seeding. It is evident that after 43 days the main gas component in the atmosphere inside the vessel is nitrogen gas.

Figures 5.47 present a correlation between gas production, nitrate removal and pH in column 1 for the first filling.

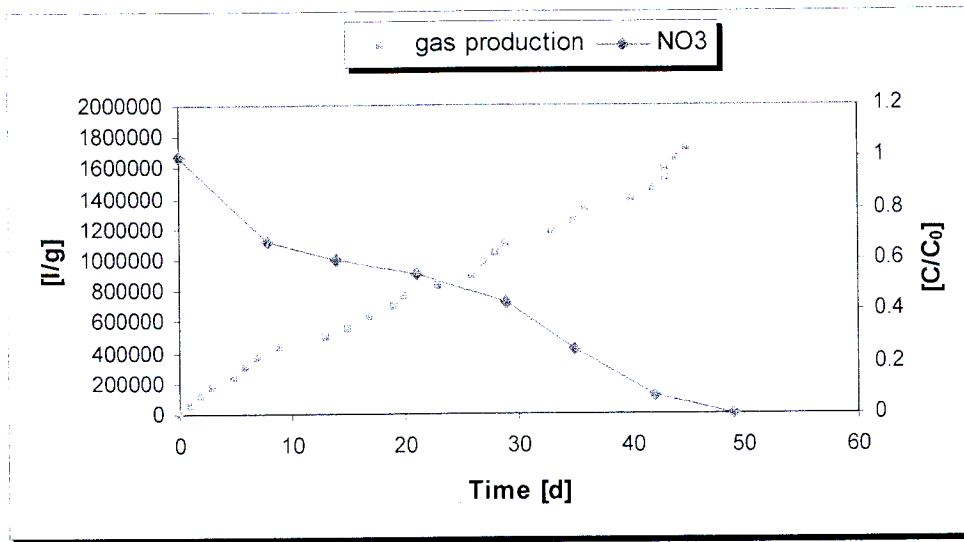


Figure 5.48 - Comparison between gas production and nitrate removal in column 1 for the first filling.

As in the previous results on the leachate analysis, the respirometric test displays a plateau due to the initial rbCOD consumption and pH buffering.

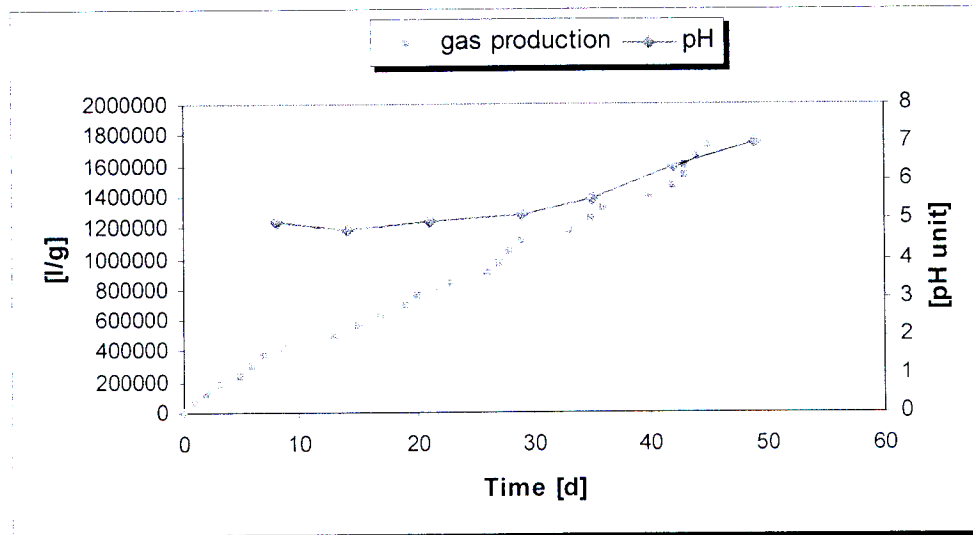


Figure 5.49 - Comparison between gas production and pH in column 1 for the first filling.

The increase in pH at the end of the acclimatization period is accompanied by a net increase in gas production.

CONCLUSIONS

The results of this experimentation confirm the effectiveness of immature compost and pine bark in the denitrification of nitrified leachate.

In fact, the carbon available in these two substrates allows for a complete denitrification through the degradation of nitrates into N_2 within the discharge limits.

The main objectives of this research were:

- To assess the potential for nitrate removal (denitrification) of organic waste compost, in particular garden refuse compost and fresh pine bark.
- To study the kinetics and the efficiency of nitrate removal in relation to degree of maturity, biodegradability, size distribution and quality of organic substrates.
- To assess the applicability of using organic waste compost and pine bark as by product of an integrated waste management system to denitrify landfill leachate.

Anaerobic batch tests, static respirometric tests and column tests were performed on both substrates and treated leachate from the Mariannhill Treatment Plant. Column tests included direct biogas analysis that was compared with static respirometric tests. Leaching columns were selected to simulate, in controlled conditions, fixed bed reactors operated in a plug flow mode. The choice of this experimental set up was based on two considerations: (a) the large particle size distribution of the substrates made the use of a continuously mixed reactor prohibitive without appropriate comminution of the substrates and (b) the need to investigate the applicability of these carbon sources in the design of a low-cost, low-energy treatment solution, that could be implemented in local landfills.

All batch tests show a linear dependence between the nitrate removal and time, but also a direct correlation between the nitrate concentration and the duration of the acclimatization time, particularly for the pine bark. This behaviour is confirmed by the column studies.

Nitrate removal with pine bark is strongly influenced by the presence of rbCOD and by the low pH typical of this substrate. After the acclimatization period, when most of the rbCOD is consumed and the pH buffered to neutrality, the degradation proceeds rapidly achieving 100% efficiency.

The compost displays a lower reactivity due to the lower carbon content required for denitrification.

Both substrates did not display a decline in performance during the period of study and their behaviour can be described by a zero order kinetic model for the pine bark and a first order model for the compost. The overall efficiency of the substrates can be summarized in the removal of 100% of the initial nitrate concentration (1800 mgNO₃/l for column 2 and 3600mgNO₃/l for column 1) in an average of 10-20 days for the pine bark and 30-40 days for the compost, passed the acclimatization time that is about 40 days for both substrates.

The gas analysis confirmed a successful denitrification process with large N₂ production. No methane was detected as during the denitrification process, in presence of nitrates, the denitrifiers become predominant over the methanogens. As a consequence, leachate had to be replaced in the columns at the end of each denitrification cycle to avoid methanogenic conditions to occur.

An important drawback of the process is the high COD production from both substrates and NH₃ levels from the compost in particular. A in depth analysis of the nature of this by products must be conducted to assess the feasibility of the process or the opportunity to operate the full scale reactor with recirculation.

In conclusion, considering the case study of the Mariannhill Landfill Site in Durban, the results of this research suggest that a constructed wetland, operated as a fixed bed reactor in plug flow mode could be the most appropriate solution.

Appendix n°1 - Ammonia

NH₄ characterization
and batch test

Sample	Volume	N [HCl]	Repeat			Ave	Result	Std Dev	Var
			1	2	3				
Pine Bark eluate	50	0.05	0.2	0.25	0.24	0.23	4.14	0.026	0.001
Compost eluate	50	0.05	0.45	0.41	0.43	0.43	7.74	0.020	0.000
Leachate (BT1)	50	0.05	0.5	0.42	0.41	0.44	7.98	0.049	0.002
Leachate (BT 2, 3)	50	0.1	0.14	0.19	0.14	0.16	5.64	0.029	0.001
Leachate (columns)	50	0.05	0.37	0.354	0.38	0.37	6.63	0.013	0.000
Batch Test PB 1100	50	0.1	0.31	0.26	0.27	0.28	10.08	0.026	0.001
Batch Test COMP 1100	50	0.1	1.71	1.71	1.71	1.71	61.57	0.000	0.000
Batch Test PB 700	50	0.1	0.275	0.21	0.25	0.25	8.82	0.033	0.001
Batch Test COMP 700	50	0.1	1.2	1.29	1.18	1.22	44.05	0.059	0.003
Batch Test PB 350	50	0.1	0.18	0.19	0.192	0.19	6.75	0.006	0.000
Batch Test COMP 350	50	0.1	0.76	0.77	0.75	0.76	27.37	0.010	0.000

NH₄ columns leachate

date	day	Sample	Volume	N [HCl]	Repeat			Ave	Result	Std Dev	Var
					1	2	3				
22-Jun	8	PB	50	0.05	0.5	0.6	0.6	0.567	10.20	0.058	0.003
	8	CO	50	0.05	1.25	1.25	1.34	1.280	23.05	0.052	0.003
28-Jun	14	PB	50	0.05	0.8	0.7	0.9	0.800	14.40	0.100	0.010
	14	CO	50	0.05	1.65	1.76	1.68	1.697	30.55	0.057	0.003
5-Jul	21	PB	50	0.1	0.3	0.41	0.4	0.370	13.32	0.061	0.004
	21	CO	50	0.1	0.7	0.73	0.72	0.717	25.81	0.015	0.000

date	day	Sample	Volume	N [HCl]	Repeat			Ave	Result	Std Dev	Var
					1	2	3				
5-Jul											
13-Jul	29	PB	50	0.1	0.26	0.4	0.4	0.353	12.72	0.081	0.007
	29	CO	50	0.1	0.69	0.65	0.66	0.667	24.01	0.021	0.000
19-Jul	35	PB	50	0.1	0.3	0.3	0.32	0.307	11.04	0.012	0.000
	35	CO	50	0.1	0.89	0.9	0.87	0.887	31.93	0.015	0.000
26-Jul	42	PB	50	0.1	0.06	0.14	0.1	0.100	3.60	0.040	0.002
	42	CO	50	0.1	1.04	1.03	1.035	1.035	37.27	0.005	0.000
10-Aug	57	PB	50	0.1	0.32	0.4	0.36	0.360	12.96	0.040	0.002
	57	CO	50	0.1	1.25	1.3	1.275	1.275	45.91	0.025	0.001
16-Aug	63	PB	50	0.1	0.19	0.2	0.2	0.197	7.08	0.006	0.000
	63	CO	50	0.1	1.27	1.3	1.285	1.285	46.27	0.015	0.000
23-Aug	70	PB	50	0.1	0.19	0.2	0.195	0.195	7.02	0.005	0.000
	70	CO	50	0.1	1.5	1.49	1.495	1.495	53.83	0.005	0.000
30-Oct	77	PB	50	0.1	0.26	0.25	0.27	0.260	9.36	0.010	0.000
	77	CO	50	0.1	1.55	1.54	1.53	1.540	55.45	0.010	0.000
6-Sep	84	PB	50	0.1	0.43	0.43	0.43	0.430	53.34	0.000	0.000
	84	CO	50	0.1	1.65	1.6	0.155	1.135	140.79	0.849	0.721
14-Sep	98	PB	50	0.1	0.52	0.521	0.519	0.520	64.50	0.001	0.000
	98	CO	50	0.1	0.29	0.28	0.3	0.290	35.97	0.010	0.000
20-Sep	104	PB	50	0.1	0.728	0.732	0.73	0.730	90.55	0.002	0.000
	104	CO	50	0.1	0.14	0.13	0.15	0.140	17.37	0.010	0.000
27-Sep	111	PB	50	0.1	0.69	0.7	0.71	0.700	86.83	0.010	0.000
	111	CO	50	0.1	0.283	0.28	0.277	0.280	34.73	0.003	0.000
5-Oct	119	PB	50	0.1	0.66	0.68	0.67	0.670	83.11	0.010	0.000
	119	CO	50	0.1	0.291	0.29	0.289	0.290	35.97	0.001	0.000
11-Oct	125	PB	50	0.1	0.6	0.58	0.62	0.600	74.42	0.020	0.000
	125	CO	50	0.1	0.32	0.28	0.3	0.300	37.21	0.020	0.000

PB: leachate from pine bark column; CO: leachate from compost column

Appendix nº2 COD

COD characterization
and batch tests

Sample	Vol	Blank Ave	Reading			Ave	Result	Std Dev	Var
			1	2	3				
Pine Bark eluate	0.5	0.0010	0.119	0.118	0.119	0.119	1456.48	0.001	3E-07
Compost eluate	0.55	0.0000	0.078	0.079	0.078	0.078	881.46	0.001	3E-07
Leachate (BT1)	1.5	0.0000	0.122	0.12	0.121	0.121	499.25	0.001	1E-06
Leachate (BT 2, 3)	1.4	0.0000	0.064	0.064	0.064	0.064	282.93	0.000	0E+00
Leachate (columns)	2.5	0.0000	0.227	0.22	0.215	0.221	546.28	0.006	4E-05
Batch Test PB 1100	0.2	-0.0010	0.2	0.142	0.14	0.161	5002.78	0.034	1E-03
Batch Test COMP 1100	0.2	-0.0010	0.068	0.066	0.068	0.067	2114.58	0.001	1E-06
Batch Test PB 700	0.6	0.0016	0.189	0.185	0.183	0.186	1898.65	0.003	9E-06
Batch Test COMP 700	0.6	0.0010	0.112	0.112	0.112	0.112	1144.97	0.000	0E+00
Batch Test PB 350	0.2	0.0010	0.06	0.065	0.062	0.062	1897.96	0.003	6E-06
Batch Test COMP 350	0.2	0.0010	0.036	0.0405	0.035	0.037	1119.18	0.003	9E-06

COD columns

Date	Day	Sample	Vol	Blank Ave	Reading			Ave	Result	Std Dev	Var
					1	2	3				
14-Jun	0	PB	0.5	0.0000	0.1	0.2	0.145	0.148	1850.00	0.050	0.002508333
	0	CO	0.5	0.0000	0.109	0.121	0.105	0.112	1380.00	0.008	6.93333E-05
22-Jun	8	PB	0.5	0.0010	0.194	0.23	0.185	0.203	2500.36	0.024	0.000567
	8	CO	0.5	0.0010	0.1	0.1	0.109	0.103	1262.56	0.005	0.000027
28-Jun	14	PB	0.5	-0.0010	0.303	0.31	0.32	0.311	3861.94	0.009	7.3E-05
	14	CO	0.5	-0.0010	0.106	0.12	0.11	0.112	1398.71	0.007	0.000052
5-Jul	21	PB	0.5	-0.0120	0.341	0.393	0.345	0.360	4600.49	0.029	0.000837333
	21	CO	0.5	-0.0120	0.0855	0.085	0.089	0.087	1219.23	0.002	4.75E-06
13-Jul	29	PB	0.2	0.0000	0.171	0.167	0.167	0.168	5209.08	0.002	5.33333E-06
	29	CO	0.5	0.0000	0.106	0.101	0.1	0.102	1266.68	0.003	1.03333E-05
19-Jul	35	PB	0.2	0.0000	0.148	0.158	0.145	0.150	4652.07	0.007	4.63333E-05
	35	CO	0.5	0.0000	0.099	0.097	0.09	0.095	1180.04	0.005	2.23333E-05
26-Jul	42	PB	0.5	0.0000	0.332	0.335	0.335	0.334	4134.25	0.002	3E-06
	42	CO	0.2	0.0000	0.041	0.04	0.04	0.040	1248.12	0.001	3.33333E-07
10-Aug	57	PB	0.2	-0.0010	0.215	0.197	0.173	0.195	6065.22	0.021	0.000444
	57	CO	0.2	-0.0010	0.13	0.074	0.094	0.099333	3104.82	0.028	0.000805333
16-Aug	63	PB	0.2	-0.0010	0.221	0.185	0.174	0.193333	6013.65	0.025	0.000604333
	63	CO	0.2	-0.0010	0.08	0.09	0.106	0.092	2877.89	0.013	0.000172
23-Aug	70	CO	0.2	0.0010	0.091	0.095	0.088	0.091333	2795.37	0.004	1.23333E-05
	70	PB	0.2	0.0010	0.128	0.096	0.095	0.106333	3259.54	0.019	0.000352333
30-Aug	77	CO	0.2	0.0010	0.104	0.112	0.11	0.108667	3331.75	0.004	1.73333E-05
	77	PB	0.2	0.0010	0.06	0.147	0.103	0.103333	3166.71	0.044	0.001892333
6-Sep	84	CO	0.2	0.0000	0.048	0.066	0.057	0.057	1763.87	0.009	8.1E-05
	84	PB	0.2	0.0000	0.054	0.054	0.054	0.054	1671.03	0.000	0
14-Sep	98	CO	0.2	0.0000	0.065	0.06	0.06	0.061667	1908.28	0.003	8.33333E-06
	98	PB	0.2	0.0000	0.065	0.058	0.0615	0.0615	1903.12	0.004	0.00001225
20-Sep	104	CO	0.2	0.0000	0.051	0.048	0.0495	0.0495	1531.78	0.002	2.25E-06

					Reading	Ave	Result	Std Dev	Var		
Date	Day	Sample	Vol	Blank Ave	1	2	3			Date	Day
	104	PB	0.2	0.0000	0.051	0.052	0.0515	0.0515	1593.67	0.001	0.00000025
27-Sep	111	CO	0.2	0.0000	0.05	0.052	0.051	0.051	1578.20	0.001	1E-06
	111	PB	0.2	0.0000	0.052	0.057	0.0545	0.0545	1686.50	0.003	6.25E-06
5-Oct	119	CO	0.2	0.0000	0.048	0.051	0.0495	0.0495	1531.78	0.002	2.25E-06
	119	PB	0.2	0.0000	0.045	0.045	0.045	0.045	1392.53	0.000	7.22224E-35

PB: leachate from pine bark column; CO: leachate from compost column

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