

**THE USE OF COMMERCIAL GARDEN REFUSE AT
DIFFERENT DEGREES OF MATURITY AS AN ORGANIC
CARBON SOURCE TO BIO-DENITRIFY TREATED MSW
LANDFILL LEACHATE.**

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KWAZULU-NATAL**

**Submitted in fulfilment of the academic requirements for the degree of
PhD in Engineering**

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DECEMBER 2014

PREFACE

The research presented in this thesis was carried out under the supervision of Prof. Cristina Trois of the School of Civil Engineering, Surveying and Construction, University of KwaZulu-Natal, Durban, South Africa. This thesis is comprised of a set of discrete research papers and has been compiled in accordance with *The Guidelines for the writing of a PhD*, prepared by the College of Agriculture, Engineering and Science at the University of KwaZulu-Natal, Durban and represents work written by Björn Plüg, unless otherwise stated in the text.

As the candidate's Supervisor I have approved this thesis for submission.

Date.....

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Prof. Cristina Trois
Research Supervisor

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Björn Plüg

DECLARATION 1: PLAGIARISM

I, Björn Plüg declare that

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DECLARATION 2: PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis (include publications in preparation, submitted, in press, and published and giving details of the contributions of each author to the experimental work and writing of each publication).

Publication 1:

Plüg B.D., and L. Oxarango*, Chapter 2: The development of an optimisation model for bio-denitrification, using natural organic carbon sources as substrates in column studies, 2014. In preparation.

Author Contributions

As part of the NRF-CNRS Collaboration Agreement, the authors, Björn Plüg (BP) and Dr Laurent Oxarango (LO), were able to work together on this study. LO was crucial in the development of a preliminary optimisation model for bio-denitrification. LO programmed a simulating annealing, optimisation method, running numerous simulations to determine the optimal parameters for their application in a numerical model. These parameters were implemented by LO in the establishment of an initial Advection-Dispersion-Reaction model, with 0 - order kinetics. Data collected from the column studies conducted during BP Masters thesis were modelled and compared to analyse these experiments. A review of background literature, laboratory testing, data collection and analysis was carried out by BP. The paper was written by BP with important editorial input from LO.

Publication 2:

Plüg B.D., and C. Trois, Chapter 3: The use of Commercial Garden Refuse at different maturities as a carbon source for the bio-denitrification of treated MSW landfill leachate: A comparison between synthetic nitrate solution and treated Mariannhill Landfill site leachate, 2014. In preparation.

Author Contributions

BP co-developed and executed the research, including the review of available background information, designing testing procedures, conducting all experimental

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work, data collection and analysis. BP planned and wrote the paper, with Professor Cristina Trois (CT) providing valuable comments on the manuscript.

Publication 3:

Plüg B.D., and C. Trois, Chapter 4: Curve fitting the evolution of bio-denitrification using Commercial Garden Refuse as a carbon source in small scale closed batch tests: A comparison between different kinetic reaction equations, 2014. In preparation.

Author Contributions

BP reviewed the relative available literature, developed and executed the investigation, including all testing procedures, data collection, calculations and analysis. BP designed and wrote the paper, with CT providing comments on the document.

Publication 4: Appendix A1

Trois, C. and B. Plüg, Bio-Denitrification of High Strength Landfill Leachate Using Garden Refuse and Pine Bark As Carbon Sources, in Denitrification: Processes, Regulation and Ecological Significance, N. Savaglio and R. Puopolo, Editors. 2011, Nova Science Publishers, Incorporated. p. 155-188.

Author Contributions

This published chapter is based on results obtained from research that BP conducted during their Masters Degree. BP was responsible for all experimental testing, data collection and analysis. The chapter was designed and written by BP with editorial input from supervisor CT.

Publication 5: Appendix A2

Plüg, B.D. and C. Trois, The use of Commercial Garden Refuse at different degrees of maturity as a means to bio-denitrify treated MSW landfill leachate. Journal of Hazardous Materials, 2013. Submitted.

Author Contributions

The paper is based on work and results obtained during the completion of BP's Masters Degree. The paper has been submitted to the Journal of Hazardous Materials. BP co-developed the research and was responsible for all experimental testing, data collection and analysis. BP wrote the paper, with CT providing valuable comments on the manuscript.

Publication 6: Appendix A3

Plüg, B.D. and C. Trois, Nitrate removal of treated landfill leachate, using natural organic substrates as carbon sources in column studies, simulating fixed-bed reactors. Journal of Hazardous Materials, 2013. Submitted.

Author Contributions

This research paper has been submitted to the Journal of Hazardous Materials and is based on results obtained during the completion of BP's Masters Degree. The research was co-developed by BP, who was also responsible for all experimental testing, data collection and analysis. BP wrote the paper, with CT providing valuable comments on the manuscript.

Publication 7: Appendix A4

Plüg, B.D. and C. Trois, A comparison between the ability of different immaturely composted materials to denitrify high strength landfill leachate. Waste Management, 2013. Submitted.

Author Contributions

The paper is based on work conducted during BP's Masters Degree and has been submitted to the Journal of Waste Management. BP co-developed the research and was responsible for all experimental testing, data collection and analysis. BP wrote the paper, with CT providing valuable comments on the manuscript.

Publication 8: Appendix A5

Plüg, B. and C. Trois, Bio-denitrification of MSW landfill leachate using raw and composted garden refuse: a comparative analysis. Sardinia, Thirteenth International Waste Management and Landfill Symposium Proceedings. 2011, CISA Publishers: Santa Margherita di Pula, Cagliari, Italy.

Author Contributions

This paper was written and presented by BP at the Sardinia Symposium in 2011 with CT providing instrumental guidance. The results published in this document were obtained during the completion of BP's Masters Degree, who was also responsible for co-developing the research, and conducting the experimental testing, data collection and analysis.

ABSTRACT

In the eThekweni Municipality, high strength MSW landfill leachate is collected and treated in a Sequencing Batch Reactor, situated at Mariannhill Landfill site. After closure of the landfill, nitrified effluents from the plant will require further treatment to comply with prescribed discharge limits. The concept is to implement an ad-hoc bio-denitrification treatment phase, making use of natural organic materials as an efficient, cost effective, and feasible alternative to expensive methods, incorporated in a fixed-bed reactor as a sustainable engineering solution to address the incomplete process design. The research looks at promoting the use of commercial garden refuses as carbon sources for bio-denitrification. Two substrates, fresh and immaturesly composted CGR contain relatively high amounts of carbon and are readily available in eThekweni landfills. Nitrate removal performance and bio-denitrification behaviour of each substrate, with two effluents, synthetic nitrate solution and treated MSW landfill leachate was evaluated using laboratory testing, in particular, characterisation, small-scale dynamic batch, and larger scale column tests. Results suggest that the fresher material is more suitable, where full nitrate removal was achieved, in the batch tests within 1 and 17 days for the 500 mg/l NO_3 synthetic solution and nitrified leachate respectively. Experimental data obtained from column studies was used in the development of a preliminary optimisation Advection-Dispersion-Reaction model. A simulated annealing technique was applied, determining the optimal parameters, whilst minimising the error between experimental results and model outputs. The optimisation method used 0 – order kinetics to analyse the data. Several commonly implemented predictive kinetic equations were derived and evaluated, looking at simple approaches to describe reduction kinetics of nitrate concentration over time, with results from the batch tests. Data was plotted and four equations, a First Order, Second Order, simple Elovich and Power were applied and compared. A First Order reaction best fitted the nitrate evolution observed, when using CGR RAW, with a kinetic rate coefficient, $k_1 = 5.128 \text{ days}^{-1}$. However, CGR 10 was significantly slower as expected, with $k_1 = 1.185 \text{ days}^{-1}$. This research provides evidence, that both substrates have favourable characteristics, which make them suitable to act as a filter medium to denitrify high strength leachate.

ACKNOWLEDGEMENTS

Firstly, I would like to acknowledge and thank my supervisor, Professor Cristina Trois, for all her professional guidance and support.

Secondly, my appreciation to the staff of the Civil Engineering Workshop and Environmental Laboratory for their assistance with various aspects of this project, in particular, to the Technicians who helped with the construction and maintenance of experimental apparatus as well as the Technical Staff who assisted with monitoring and documenting data for some of the experiments.

Finally I would like to recognise and show gratitude towards Durban Solid Waste for providing me with this valuable opportunity to work on this project and their backing over the duration of this research.

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ABBREVIATIONS

Ammonia	NH ₃
Biochemical Oxygen Demand	BOD ₅
Carbon to nitrogen ratio	C/N
Chemical Oxygen Demand	COD
Commercial Garden Refuse	CGR
Commercial Garden Refuse 10 Weeks	CGR 10
Fresh Commercial Garden Refuse	CGR RAW
Liquid to solid ratio	L/S
Mariannahill Landfill site, nitrified leachate from SBR	M.L.S.
Moisture content	w
Municipal Solid Waste	MSW
Nitrates	NO ₃
Nitrites	NO ₂
Respiration Index	RI ₇
Sequencing Batch Reactor	SBR
Total solids	TS
Volatile solids	VS

CHAPTER 1

INTRODUCTION

1. Problem statement

Ever since the introduction of the landfill, the production of leachate has been a major concern, through the contamination of soils, groundwater and the subsequent damage. Landfill leachate, a toxic by-product formed through the decomposition of organic matter, is harmful to both the environment and human health. However, modern sanitary landfills are highly engineered and specifically designed to ensure the protection of the environment as well as human health through the control of both water, soil and air emissions. As a means to prevent the contamination of groundwater, a combination of both liners and a leachate collection system are utilised [1]. After collection, the treatment of the leachate is imperative prior to discharge.

The eThekweni Municipality is currently nitrifying leachate at the Mariannhill Landfill site. The leachate produced from the landfill cells is collected and being treated using a Sequencing Batch Reactor (SBR) plant. The SBR is 10 metres in diameter and 6 metres in depth, constructed using reinforced concrete. This provides for the daily treatment of leachate ranging to a volume of 50 cubic metres [2]. A lined reed bed provides a polishing treatment for the removal of BOD, COD and solids. Leachate is being treated for ammoniacal nitrogen removal; this includes a nitrification process where the ammonia is converted into nitrates. This single sludge system is simple to operate and requires low maintenance [3]. The treated effluent is then used as dust suppressant. After nitrification, the concentration of nitrates in the discharged leachate may still present a potential threat to the environment. After closure of the landfill, the effluents from the plant will not comply with the discharge limits of wastewater into a water resource, as enforced by DWAF with a General Limit of 15 mg/l NO_3 and a Special Limit of 1.5 mg/l NO_3 [4]. The typical nitrate concentrations (Nitrate + Nitrite mg/l NO_3) displayed can rise to above 3000 mg/l NO_3 . Further denitrification will be required to reduce the high concentrations of nitrates in the nitrified effluents to below the discharge limits. Thus, a further ad-hoc treatment is required to denitrify the effluent prior to discharge into the natural environment. To achieve the “treatment at source” philosophy, the aim is to employ a bio-denitrification stage after the Sequencing Batch Reactor.

2. Research Question: Aims/Objectives/Hypothesis

The development of applicable, economical, easily implementable strategies based on an environmental model is the most viable option in respect of successful landfill leachate treatment in South Africa [5]. As a result of the enforcement of stricter environmental guidelines, the concept of “treatment at source” has been established to be the potential solution [3].

The vision is to implement an ad-hoc bio-denitrification phase, making use of natural organic materials as carbon sources, incorporated in a fixed-bed reactor as a sustainable engineering solution to the incomplete process design.

The study aims at developing an innovative treatment solution, which is low in cost, energy and technology, which can be designed and implemented as part of an integrated waste management system promoting the efficient reuse of waste material. Commercial garden refuse is disposed of at many local landfill sites and is easily separated from the main waste stream. The research investigates the use of this “green” waste, at different degrees of maturity to act as carbon sources for the nitrate removal of nitrified landfill leachate, before being applied in a full-scale design of a continuous flow, submerged horizontal constructed wetland, fixed-bed reactor which is to be implemented and run in conjunction with the Sequencing Batch Reactor at Mariannhill Landfill site.

The variation in substrate composition makes the optimal design of such a system challenging. The initial step in such an objective requires the design and operation of experimental tests. The efficiency of each substrate to support nitrate removal will be established using laboratory experiments. In particular, small-scale dynamic batch tests and column studies, simulating fixed-bed reactors, will be used to assess the each materials performance, whilst comparing the behaviour when denitrifying both synthetic nitrate solution and treated MSW landfill leachate.

Predicting the kinetic behaviour of bio-denitrification when organic matter is utilised as a carbon source, is a complex and under researched subject, thus requiring investigation into the rates of nitrate removal, whilst looking at simple approaches to describe the reduction kinetics. Laboratory experiments, in the form of batch tests are to be utilised, measuring reduction in nitrate concentration over time applying and evaluating several derived commonly implemented predictive kinetic equations.

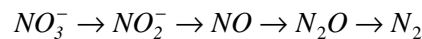
The development of an optimisation model determining the kinetic behaviour has the multi-objective of both accurately simulating the treatment process, whilst aiming to achieve repeatability. An Advection-Dispersion-Reaction (ADR) model will be implemented to analyse the leaching columns experiments and a classical reactive

transfer model approach is to be adopted and applied to simulate the observed experimentally behaviour, whilst a simulated annealing method programmed, as a means to determine the optimal parameters, whilst minimising the error between the experimental data and the model outputs.

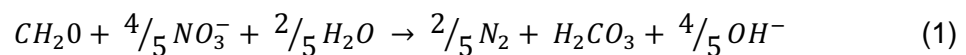
3. Background

3.1. Bio-denitrification

Biological denitrification processes are often a more robust and versatile treatment approach [6]. The microbial removal of nitrates from wastewater seems to be the most viable method, being both cost effective and environmentally friendly [7]. Denitrification refers to the biological redox reaction in which nitrate, an inorganic nitrogen compound, is reduced [3]. The process involves two steps. Firstly, the conversion of nitrate to nitrite and secondly, is the production of nitric oxide, nitrous oxide and nitrogen gas. The different denitrification steps are presented as follows:



Bio-denitrification of leachate requires certain conditions, which include the presence of a facultative bacterial population as well as a suitable environment for the growth of such micro-organisms, the absence of dissolved oxygen or inhibitory toxic substances and finally an appropriate energy source, to act as an electron donor. The micro-organisms capable of reducing the nitrates through conversion into nitrogen gas during biological denitrification require an external carbon source to act as an electron donor, acting in an anaerobic environment [8, 9]. Tsui, Krapac [10] suggest that when organic carbon serves as an electron donor for denitrification, the chemical reaction can be expressed as:



Denitrifiers, however, differ widely with selected substrates as a microbial population has its own preferential environmental conditions. Influencing factors on the denitrification process include the type of substrate, absence of oxygen, the pH value, presence of denitrifiers, the carbon source and nitrate concentration. Pre-treated effluents produced from secondary treatment plants, as in the case of Mariannhill,

contain very low concentrations of easily biodegradable organic matter, thus an external carbon and energy source is required to enable biological denitrification to be accomplished [11].

The effective removal of nitrates on a large scale is inhibited by high costs related to some denitrification processes, typically in the form of activated sludge wastewater treatment plants [12-14]. Easily biodegradable supplemental carbonaceous materials; such as sucrose, methanol, ethanol, propionate or acetic acid [15, 16], methane [17, 18], or molasses [19], that are currently employed around the world tend to be too expensive, thus these methods tend not to be a viable solution for developing countries and are not suited for large scale, field applications [10, 20]. An alternative to these expensive materials is proposed, promoting the use of natural organic resources, in this case Pine Bark (PB) and Commercial Garden Refuse (CGR) at various maturities, which are suitable for low cost, low energy, large scale, field application. The use of organic carbon sources in a treatment system has been extensively researched [10, 14, 20].

Over the duration of this project, 6 different organic substrates were tested to determine their ability to act as carbon sources. In 2009 the focus of my Master's Degree compared Pine Bark as well as Domestic and Commercial Garden Refuse at different degrees of maturity and composting techniques.

An investigation conducted by Díaz, García [14], researched the development of an experimental method for nitrate removal from secondary effluents. Three plant substrates were identified in the study as pertinent organic sources, namely pine bark, almond shells and walnut shells, where gravel was used as the control medium. Measurements regarding the denitrification of urban municipal wastewater, considered the variance of hydraulic retention time, water temperature and, in respect of the batch reactors, influent nitrate concentration [14]. Analysis of the data confirmed that in all three substrates, denitrification occurred and nitrate removal was seen to be dependent on the selected variants. In conclusion, the data produced through their study indicated that the three carbon sources were suitable for nitrate removal, proposing that the effectiveness of each material was linked to its biodegradability. The substrates had good lasting properties and the tested system, provided a promising alternative particularly in terms of energy and consequently cost saving, through both operational and maintenance simplicity [14].

Tsui, Krapac [10] presented a preliminary assessment regarding the feasibility of using immature compost as a substrate. The suitability of the material for the denitrification of tile drainage water, based on its relatively large organic content, high microbial activity and buffering capacity was researched. They postulated that

compost, in particular immature yard waste which has larger carbon content and as a result of the high microbial activity, could prove to be a more viable carbonaceous source for denitrification in the agricultural context. These assertions were tested with the use of six month old compost samples, collected from the Urbana Landscape Recycling Centre in Illinois. The encouraging results provided a preliminary platform for further study, where focus should be placed on bioreactor packing processes and investigation into optimal compost storage procedures [10].

3.2. *Carbon source characteristics*

Adani, Ubbiali [21] defined compost as a stable, mature and humified material, whose quality is assessed according to both, maturity and stability parameters [22]. Compost has the potential to perform a role in the treatment of a large variety of environmental issues. The diverse nature of compost and its source material often makes it difficult to identify the characteristics, which assist in predicting their behaviour [23]. As means to ascertain the possible performance capabilities, the relationship between the physicochemical properties, the source of the material and in some cases the composting techniques employed was the essential first step in providing initial insight into substrate selection. The characterising of each substrate focused mainly on the factors that influence decomposition.

The rates of denitrification are extremely dependent on the carbon to nitrogen ratio of the substrates utilised. All organic matter has a ratio of carbon to nitrogen in its tissues which affects the course of decomposition as organisms use carbon as a source of energy to decompose this organic matter and thus need a carbon content higher than nitrogen. An appropriate material should provide organic carbon for denitrification without increasing the nitrogen concentration. The ideal initial C/N ratio to obtain good compost is 20– 35, while the typical range for stabilised compost is between 13 – 16 [10, 24].

The biological stability of a waste product acts as an important indicator as to the reactivity and the potential impacts when landfilled [25, 26]. The degree of stability or maturity of a material relates to the stable organic content. Composting is a two phase biochemical transformation of the organic matter by micro-organisms, which includes stabilisation and maturation [26, 27]. The stability is connected to the compost's microbial activity, whereas the maturity is often associated with the potential plant growth. However, the two are related as the micro-organisms in unstable compost produce the phytotoxic compounds [27]. Physical characteristics such as colour, odour and temperature can provide a basic prediction as to the decomposition stage, but

offers little input into the degree of maturation. There are numerous methods to assess the maturity of a material, including the C/N ratio; however the most common means is to determine the microbial stability, which can be measured through a microbial biomass count, the metabolic activity and the concentration of the easily biodegradable constituents. The measure of the respirometric activity is one of the most common methods used. This approach involves the measurement of either the O₂ consumption or the CO₂ production, which are indicative of the amount of readily degradable organic matter. An unstable or immature material has a greater demand for O₂ and the high rate of CO₂ production compared to that of a well matured compost which has a lower waste reactivity [25-27]. An unstable material is considered to contain a high portion of biodegradable matter that must sustain high microbial activity [22, 28]. Large amounts of bioavailable organic matter cause micro-organisms to respire at a higher rate than that if the material is scarce of organic matter [22]. In the case of our research, the potential biological reactivity was measured over a 7 day period, as proposed by Adani, Lozzi [29], the RI₇ or Respiration Index is an expression of the rate at which oxygen is consumed by the indigenous biomass that is present in the substrate to degrade the material.

3.3. Alternative treatment processes

There are various treatment methods used for nitrate removal from wastewater which can be separated into two main treatment processes: physico-chemical and biological methods. The most conventional abiotic or physico-chemical treatment processes include reverse osmosis, active carbon adsorption, ion exchange, electro-dialysis amongst other advanced oxidation processes [6, 30-34].

Some methods tend not to be ion specific and result in the transfer of only the pollutants in concentrated solution or adsorption on solids without solving the specific environmental problems [30, 35]. The ion exchange process removes both nitrate and sulphate simultaneously; however wastewater is produced from the resin regeneration process [6]. Although the reverse osmosis treatment process is able to separate and concentrate nitrates contained in water without changing their molecular structure, its application is limited due to the high costs and the production of concentrated waste brine which poses a disposal problem [6, 33].

Biological denitrification processes seem to be a more robust and versatile treatment approach, compared to abiotic methods, which are often unable to completely separate or remove nitrates from the effluent resulting in the production of problematic by-products [6].

The microbial removal of nitrates from polluted water and wastewaters seems to be the most viable strategy as it is both cost effective and environmentally friendly [7]. The only drawback of biological denitrification may be due to the slower rate of removal at high nitrate concentrations [36].

In respect of the various treatment methods available for nitrate removal, which include ion exchange, reverse osmosis, electro-dialysis, distillation, chemical denitrification and biological denitrification, there appears to be general consensus in the literature that the biological processes have proved to be practical, efficient and most importantly cost effective [15]. Among the biological systems, the most widely used are Sequencing Batch Reactors [13].

The sequencing batch reactor (SBR) is a fill and draw activated sludge system for the treatment of wastewater. The system is designed to operate as a single “batch” reactor under non-steady state conditions to treat and remove detrimental components from wastewater prior to being discharged.

The sequencing batch reactor allows equalisation, aeration, sludge settlement and clarification to occur in a single reactor. The SBR tank carries out these processes in a time sequence lasting approximately 24 hours. This system has been successfully utilized to treat both municipal and industrial wastewater.

The process involved in an SBR begins with the screening of influent wastewater prior to entering the reactor. This wastewater is added to acclimated biomass with elements of the wastewater. The system is aerated and mixed, until the suspended biomass is able to achieve the biological reactions. Once finished, the biomass is allowed to settle and the treated effluent is removed. This technology is founded on the suspended growth, as bacteria are mixed and suspended simultaneously.

The advantages of the system are as follows: a single reactor is utilised to achieve equalisation, clarification and biological treatment, whilst the operating conditions are both flexible and easily controlled. The main drawback however, is the high degree of sophistication which leads to both greater levels of maintenance and the associated increased costs.

Fernández-Nava, Maranon [37] did a study on nitrate removal from waste water produced in the stainless steel manufacturing process. The investigation tested two different inocula. Sludge from the biological treatment of leachate emanating from a municipal solid waste landfill and sludge from a sewerage treatment plant. The influences of calcium concentration and COD/N ratio were investigated. A sequential batch reactor (SBR) employing methanol as a carbon source was used in the study because such reactors are robust, occupy less space and they are “more efficient in recovering biomass, they facilitate the change in scale and have been shown to be

effective in high nitrate wastewater denitrification processes". It was found that "prior acclimation of the sludge to high nitrate concentrations increases the denitrification rate" while the presence of calcium in the water proved to be an impediment. The study concluded that biomass emanating from landfill leachate treatment plants allowed successful denitrification to levels acceptably below established discharge limits.

The efficiency of the sequential batch reactor was also tested by Mekonen, Kumar [38], who found it to be effective in a study in which ethanol was used to reduce nitrate concentrations in drinking water to acceptable levels.

Mohseni-Bandpi, Elliott [15] conducted their investigation using a pilot scale SBR, where the study considered the determination of acetic acid to nitrate-nitrogen (A/N) ratio, the effect of influent nitrate-nitrogen concentration, denitrifying bacteria and effluent quality, confirming the suitability of using acetic acid as a carbon source to achieve 83% to 98% removal efficiency rate for the reactor.

There are, however, some disadvantages to these conventional methods, which limit their implementation in full scale applications as a result of their operation costs, long term maintenance and the disposal of by-products [12-14].

4. Rationale: PhD. Research

The data and results obtained during my Masters were then used to modify, refine and focus the research. From the original 6 substrates chosen, the 2 best performing were selected. The study looks at, fresh and immaturely composted commercial garden refuse, which are both readily available at local landfills. Once again, the initial step was to do a full characterisation on the solid substrates as well as their eluates. The eluates of the substrates were tested, as a means to specify the nature as well as the quantity of compounds released by the substrates through leaching, whilst being in contact with water. However, during this set of experiments, both synthetic nitrate solution and treated, nitrified leachate, collected from the Sequencing Batch Reactor at Mariannhill Landfill site, was tested, thus characterisation analysis was also done on the treated leachate.

The synthetic nitrate solution was used to simulate treated landfill leachate, so as to operate the denitrification in controlled conditions whilst also establishing a base line to assess the performance of each substrate. Also, two of the columns were run with synthetic nitrate solution with a concentration of 500 mg/l, in order to assist in the development of a kinetic model to simulate the behaviour of the nitrate evolution within the reactor. Treated leachate was used to monitor whether the materials were capable of achieving denitrification at high nitrate concentrations, while possibly investigating

any inhibitory factors within the leachate which could influence the rate of denitrification. The treated leachate also provided an idea of the behaviour within a “real” world situation. Batch tests were run with the leachate, to provide a basic indication of a suitable flow rate and hydraulic retention time required to achieve satisfactory denitrification in the column studies.

The design and method for the column experiments was modified, to provide more accurate data to try and alleviate previous problems. Instead of running the columns with leachate injection from the top and collection from the bottom, an up flow system was employed, using the concept of hydraulic change in head to pump the leachate from the bottom up [10, 39-42]. This helped to avoid substrate compaction, reducing the channelling effect and enabled a more consistent and gradual flow rate over the period of injection.

5. Outline of thesis

The layout of this thesis consists of three main journal publications. Chapter 2 looks at the development of a preliminary optimisation model of denitrification in the column studies, using data collected during my Masters in 2009. These experiments used a top to bottom technique. A set of preliminary experiments was performed using column studies, with periodic injections of synthetic nitrate solutions, including, 500 and 2000 mg/l, for 8 weeks. An Advection-Dispersion-Reaction model (ADR) was applied with 0 - order kinetics to analyse these experiments. The optimisation method, simulating annealing was programmed to determine the optimal parameters, whilst minimising the error between experimental data and model outputs. In terms of the efficiency of biodegradation, the fresh CGR was the most effective, whilst the CGR 10 and Pine Bark displayed similar results. When modelling each test, the Pine Bark produced a positive simulation with the most reliable RMS values between the experimental data and the model output.

Chapter 3 focusses on the characterisation of two substrates, fresh and immaturely composted commercial garden refuse and their performance with both a synthetic nitrate solution and nitrified leachate from the Sequencing Batch Reactor at Mariannhill Landfill site. The efficiency of each substrate to support nitrate removal was established using laboratory experiments. Analysis of the nitrate evolution from the small-scale dynamic batch tests and column experiments, simulating fixed-bed reactors, were used to assess their performance, whilst comparing the behaviour with denitrifying synthetic nitrate solution and treated MSW landfill leachate. The testing provides evidence, that both substrates have the potential to act as carbon sources to

denitrify high strength leachate, with different degrees of efficiency. Studies reveal that the fresher material is more suitable, whilst flow through the material can improve the ability to achieve denitrification.

Chapter 4 presents an investigation into the rates of nitrate removal, looking at simple approaches to describe reaction kinetics. The aim of the study was to provide an initial insight into the kinetic behaviour of denitrification reaction rates, where an organic material is implemented as a carbon source. Experimental data obtained from laboratory testing, in the form of batch tests, measuring reduction in nitrate concentration over time, was plotted and simulated using a variety of kinetic equations with the purpose of establishing a best fit curve and subsequently, the most accurate variable parameters. Several commonly implemented predictive kinetic equations were derived and evaluated. The experimental bio-denitrification data was plotted and four equations, a First Order, Second Order, simple Elovich and Power were applied and compared, with various degrees of accuracy. A First Order reaction best fitted the nitrate evolution observed, when using CGR RAW as a carbon source. The results obtained using CGR 10 were very promising, where all kinetic equations produced a relatively accurate representation of the measured data. The calculated First Order kinetic rate coefficient of the composted CGR 10 is significantly slower than that determined for the CGR RAW material, which is expected, after comparing the characteristics of each carbon source. This preliminary investigation provides better insight into understanding the different kinetic reaction rates and predicting the behaviour of bio-denitrification, ascertaining whether simple models could be used to describe the process under these conditions.

The research in its entirety will be summarised in a final discussion, with recommendations for future research, followed by the appendices, containing 5 publications, based on my Master's thesis. These include a chapter in a book, Denitrification: Processes, Regulation and Ecological Significance, three journal papers submitted for review to the Journal of Hazardous Materials and the Journal of Waste Management as well as a paper presented at the Sardinia Symposium in 2011. Appendix B is a disc of raw data, in the form of 4 Excel documents, including Characterisation Tests, Batch Tests, Column Tests and Batch Tests {kinetics}.

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CHAPTER 2

The development of an optimisation model for bio-denitrification, using natural organic carbon sources as substrates in column studies.

ABSTRACT

The approach and process, in the development of a preliminary bio-denitrification, Advection-Dispersion-Reaction model (ADR) is presented. Experimental data was obtained through column studies, using organic materials, as carbon sources, including Commercial Garden Refuse (CGR), at different degrees of maturity and Pine Bark (PB). Three substrates were compared, with periodic injections of two synthetic nitrate solutions (500 and 2000 mg/l), at different flow rates. A simulated annealing technique was applied, determining the optimal parameters, whilst minimising the error between experimental results and model outputs. The optimisation method used 0 – order kinetics to analyse the data. At 500 mg/l, PB produced the most positive simulation, with reliable root mean square (RMS) values, with a minimum of 52.62. However, it presented the longest acclimatisation period, $t_{acc} = 99843.61$ s. In terms of reaction kinetics (K_r), the fresh CGR was the most efficient, obtaining a $K_r = 1.5 \times 10^{-3}$ mg/l/s, whilst PB and CGR 10 displayed similar results to each other. Kinetics remained fairly consistent between both concentrations, with the fresh CGR, again being the most efficient at 2000 mg/l, with the composted CGR 10 producing the minimum RMS value of 117.20. Research has revealed that lack of experimental data significantly constrains the model. Further work is being conducted, taking into account numerous factors, to obtain additional data, thus increasing the model's accuracy.

1. Introduction

The modernisation of landfills and subsequent production of leachate has provided additional concerns to waste management due to the damage caused through the contamination of soils and groundwater. The treatment of landfill leachate is a challenge faced by many developing countries due to the excessive cost of currently used technologies. The eThekweni Municipality, situated in KwaZulu-Natal, South Africa uses a simple single sludge system in the form of a Sequencing Batch Reactor (SBR) to nitrify leachate collected from the Mariannhill Landfill site. However, the process design is incomplete and requires a further step to denitrify the effluent to within the discharge limits as enforced by the authorities, after the closure of the site. The design

and implementation of a low cost, low energy, sustainable engineering solution is proposed. This resolution is in the form of a submerged fixed-bed reactor, which aims to make use of different organic “green” wastes. The substrates are to act as carbon sources in a bio-filter system. The variation in substrate composition makes the optimal design of such a system challenging. The development of an optimisation model has the multi-objective of both accurately simulating the treatment process, whilst aiming to achieve repeatability.

Mathematical models are powerful tools that play a key role in many engineering areas such as environmental remediation and water management [1, 2].

Modelling is an essential means used for the simulation, analysis and comparison of numerous processes, including physical, chemical and biological, having the potential to provide additional and better insight into predicting the behaviour of complex systems assisting in the design and optimisation of such processes [2-6].

Flow accompanied by chemical reactions and mass transfer is central to a wide range of applications. The increasing importance of examining and evaluating the potential impacts and remediation associated with these applications has brought a resurgence of activity in this field with many new theories being empirical because of the systems’ inherent complexities. Thus, numerical ‘experiments’ are becoming commonly used for elucidating the varying behaviour associated with coupled reactive flow and transport [2, 7].

The initial model in this research is to help improve the understanding of the process, whilst giving insight into which parameters would be pertinent for adjusting the experiment and thus developing an accurate model which is essential in the design of a full-scale plant.

The initial step in such an objective required the design and operation of experimental tests. Leaching columns were used to simulate a reactor and an Advection-Dispersion-Reaction (ADR) model implemented to analyse these experiments. A classical reactive transfer model approach was adopted and applied to simulate the observed experimental behaviour.

Periodic injections of two concentrations of synthetic nitrate solution and three different substrates were employed in the practical laboratory column tests. The process involved filtration of the solution through a fixed porous matrix where the substrate (Pine Bark, fresh or composted garden refuse) is the reactive medium in which biological denitrification occurs.

A variety of optimisation techniques were investigated and the most appropriate method was selected for analysing the experimental data. Particular attention was paid to the kinetic parameters, biodegradation and the acclimatisation phase related to the

denitrifying microorganisms. A simulated annealing method was programmed as a means to determine the optimal parameters, whilst minimising the error between the experimental data and the model outputs.

The modelling of each test produced fairly positive simulations with the Pine Bark substrate displaying most reliable RMS between the experimental data and the model output. However, this preliminary work has revealed that a lack of experimental data significantly constrains the model.

1.1. Model description

The structure of a porous medium is characterised by two types of scales or physicochemical principles [8]. The "micro" scale which is associated with the transport and reaction phenomena such as diffusion within the pores of the substrate. Whilst, the "macro" scale relates to a homogeneous and hypothetical medium presenting the same properties as the studied porous medium, but without a detailed description of the complex pore distribution. It is described by means of macro-scale balance equations including effective properties related to the transportation and reactions.

In the case of this research, the process is fairly complicated and detailed kinetic modelling at the micro scale is not feasible. Thus, to establish a basic model to predict the various processes in the reactor, only the inlet and outlet concentration data with macroscopic balances were investigated [9, 10].

1.1.1. Theory of Transfer: The transfer process

Several factors can affect transport through a material, such as, the nature of product being transported, the hydrodynamic properties of the medium, the biological activity and the chemical reactions. However, general variation in solution concentration with regard to space and time is caused by the following main mechanisms: diffusion, kinematic dispersion, advection, adsorption, chemical processes and biological degradation.

1.1.1.a. Advection, Diffusion, Dispersion

Advection refers to the mechanism whereby solutes are transported through water movement; pure advection is known as the "piston effect". Advection in the porous media is presented in the following equation:

$$J_{adv} = -V_{pore} \frac{\partial c(z,t)}{\partial z} \quad (1)$$

With:

V_{pore} : Pore rate (m/s) = V_{darc} / ε , where ε is the porosity.

Hydrodynamic dispersion, molecular diffusion and associated kinetic dispersion (due to the heterogeneity of the velocity distribution in the middle) are written as:

$$J_{disp} = D \frac{\partial}{\partial z} \left(\frac{\partial c}{\partial z} \right) \quad (2)$$

With:

$$D = D_{diff} + D_c ; D_c = D_{disp} * V_{pore}$$

D : Coefficient of hydrodynamic dispersion (m²/s),

D_c : Coefficient of dispersion kinematics (m²/s),

D_{diff} : Molecular diffusion coefficient (m²/s),

D_{disp} : Dispersivity (m).

In the absence of flow, molecular diffusion is the main process by which molecules are transported, where as described by Fick's Law, solutes of high concentrations, migrate to areas with lower concentrations [11]. The dynamic dispersion coefficient D is defined as the sum of the coefficient of kinematic dispersion D_c the molecular diffusion coefficient D_{diff} . Even if more complex expressions have been proposed, the coefficient of kinematic dispersion D_c is conventionally linearly related to the pore velocity introducing the dispersivity D_{disp} .

1.1.1.b. Biological response

Three approaches were used to model the biological response and cell growth, Monod kinetics, coupling growth and the consumption of substrate, 0 – order kinetics and 1st – order kinetics, so as to ascertain the best outcome.

$$0 - \text{order: } R = k_r \quad (3)$$

$$1^{\text{st}} - \text{order: } R = K_r * C \quad (4)$$

$$\text{Monod: } R = \frac{C}{C + C_m} * K_m \quad (5)$$

With:

C_m : Monod limiting factor (mg/l),

C : Substance concentration (mg/l),

K_m : Monod reaction rate (s^{-1}),

k_r : 0 – order kinetic rate of reaction (mg/l/s),

K_r : 1st – order kinetic rate of reaction (s^{-1}).

They vary according to the rate of nitrate consumption. The 0 – order is constant whilst the 1st – order is proportional to the concentration in the solution. At low concentrations, the Monod kinetics behaves similar to that of the 1st – order kinetics, however at high concentrations tends to increase.

1.1.1.c. Modelling acclimatisation

The two primary growth models used are those of Baranyi and Gompertz [12], where growth is separated into a stationary lag phase corresponding to a zero growth rate ($\mu = 0$) and an exponential phase in which the logarithm of bacterial population, increases linearly with time ($\mu = \text{constant}$). The Gompertz equation is written as follows:

$$TaccX(t) = taccXi + (taccXi) \exp \left(-e^{(1+\mu_{max}) \frac{(\lambda-t)}{(tacc_{max}-taccXi)}} \right) \quad (6)$$

With:

$TaccX$: Rate of bacteria acclimatisation,

$taccXi$: Initial rate of bacteria acclimatisation (between 0 and 1),

t : Total reaction time,

μ_{max} : Maximum growth rate (s^{-1}),

λ : Length of stationary phase (s).

However, these models are non-linear equations and use two parameters which are difficult to estimate μ_{max} and λ [13]. An equation which relays exponential growth while still closely retaining the logic as prescribed by Gompertz was formulated. It takes

into account only the exponential growth phase, with only one unknown parameter, the time constant related to acclimatisation noted t_{acc} .

$$T_{acc}X = 1 - \exp(-t_{tot}t_{acc}) * (1 - t_{acc}X_i) \quad (7)$$

With:

$T_{acc}X$: Rate of bacteria acclimatisation,

$t_{acc}X_i$: Initial rate of bacteria acclimatisation (between 0 and 1),

t_{tot} : Total reaction time.

1.1.1.d. Mass balance: The Advection-Dispersion-Reaction equation (ADR)

The model equation incorporating advection, diffusion and the reaction can be written in this form:

$$\frac{dC}{dt} + \frac{V_{pore}}{dZ} * dC = D \frac{d^2C}{dz^2} + R * T_{acc}X \quad (8)$$

In this study, the ADR equation is used to simulate the denitrification within the column tests. The boundary conditions are time-dependent in order to describe both, the injection and steady phases, explicitly taking into account the timing of each experiment.

Two sets of boundary conditions are used:

- Injection phase: During the injection phase, a constant pore velocity V_{pore} is imposed. It is calculated from the experimental flow rate as:

$$V_{pore} = \frac{Q}{S} \quad (9)$$

With:

Q : Volumetric flow rate (m³/s),

S : Column cross-section (m²).

The inlet boundary condition is a constant concentration C_0 and the outlet boundary condition is a free flow, with pure advection.

- Steady phase: During the steady phase between two injections, the pore velocity is set to zero and no flux boundary conditions are used at the inlet or outlet of the column.

1.1.2. Numerical model

The ADR equation is solved using the finite volume method. Following Cherblanc, Ahmadi [14], an operator splitting strategy is used. In a first step, the advection term is solved using a second order explicit TVD scheme, as implemented by Bruneau, Fabrie [15], in order to avoid numerical diffusion. In a second step, the dispersion-reaction terms are solved simultaneously using a second order finite volume spatial discretisation and the classical Crank-Nicholson second order time discretisation. The resulting implicit formulation is solved using the Thomas algorithm for tridiagonal linear systems.

1.2. Stochastic optimisation: Simulated Annealing

In order to simulate the denitrification experiment, some parameters of the ADR equation have to be estimated. These include the dispersivity, D_{disp} , kinetic parameters k_r , K_r , K_m and C_m , which depend on the selected reaction kinetic model and the acclimatisation parameters t_{acc} and T_{accX} .

The method of simulated annealing (SA) was programmed to determine optimal values of the unknown parameters and to minimise the error between the experimental and simulated data. The simulated data is a list of simulated concentration values at the outlet of the column at times corresponding to the experimental measurements.

A Root Mean Square (RMS) calculation was performed to estimate the error between the two sets of data:

$$RMS = \sqrt{\frac{\sum_{i=1}^N (C_{refi} - C_i)^2}{N}} \quad (10)$$

With:

C_{ref} : Reference (experimental) concentration (mg/l),

C_i : Simulated concentration (mg/l),

N : Total number of concentration measurements.

Several parameters $[T_0, \alpha, NB, NB_{pal}, rafin]$ are used to configure the SA:

- The “temperature” T is used to define the Metropolis acceptance criterion. A configuration could be accepted even if it presents a worse RMS than the current configuration with a probability $\exp\left(-\frac{\Delta RMS}{T_i}\right)$. Its initial value is T_0 . The value of T_0 decreases after NB iterations following an exponential law with a parameter defined as:

$$T_i = T_0 \exp\left(-\frac{i-1}{\alpha}\right) \quad (11)$$
- The total number of temperature decrease steps is NB_{pal} .
- For each unknown parameter, an initial search window is defined by a minimum and maximum value. At each iteration, a new configuration is defined by selecting a random value of each parameter within its search window. In order to accelerate the SA convergence, the size of the search window is refined if the number of accepted configurations is lower than $NB/3$. The search window refinement is geometric with parameter $rafin$. The minimum size of the search window is defined as 10^{-4} times, the initial size of the search window.

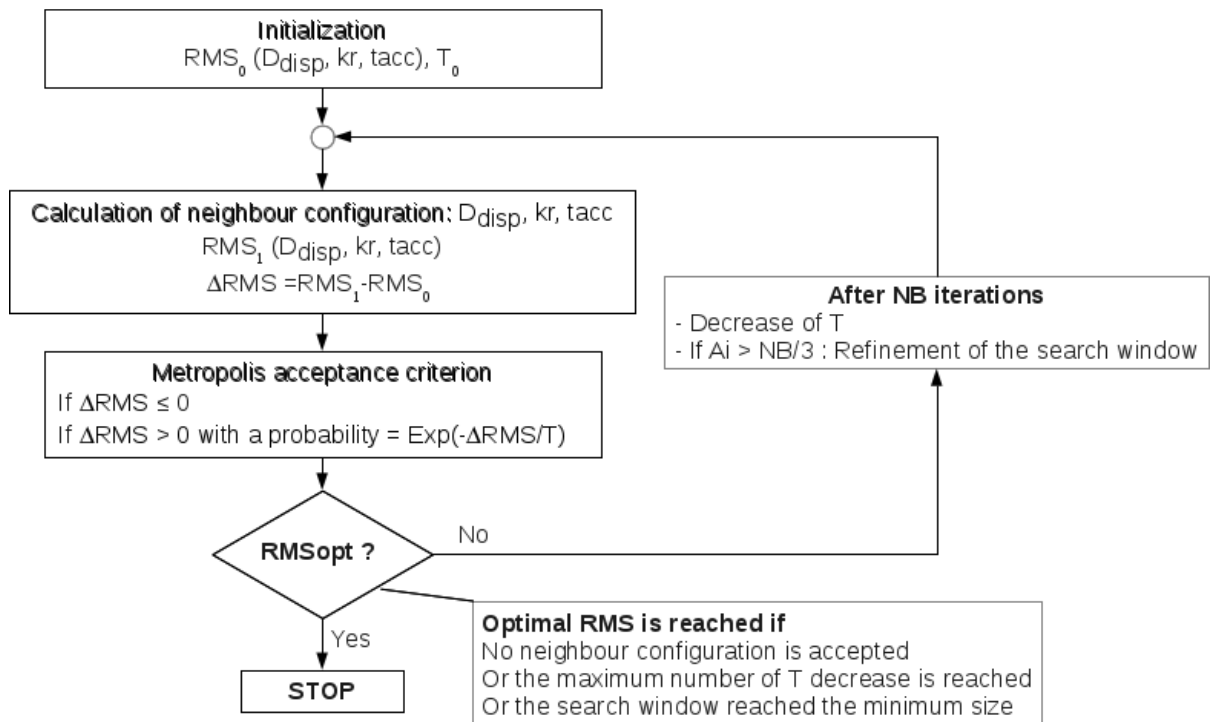


Figure 2.1: Simulated Annealing algorithm

Finally, this SA research strategy requires between 200 and 500 iterations with a calculation of the ADR problem as well as different set of parameters at each step. The general algorithm of SA is presented in Figure 2.1 [16, 17].

2. Materials and Methods

Column studies were used to investigate the effect of different levels of nitrate concentrations and varying flow velocities, on the achievable rates of denitrification. In this research, column studies were set up to simulate fixed bed reactors. The leaching columns were each packed with one of the three different substrates. Experiments were conducted with two nitrate concentrations and two different flow rates. Concentrations were chosen as a result of the typical ranges of nitrate concentrations displayed by the treated landfill leachate produced by the Sequencing Batch Reactor (SBR) at the Mariannhill Landfill site.

The columns were constructed using a transparent PVC cylindrical body, 1 m in length, 160 mm in diameter with an approximate volume of 20 litres, plastic flanges with valves, rubber gasket seals and stainless steel bolts.

The three substrates included Pine Bark, collected directly from SAPPI (South African Pulp and Paper Industry) paper mills, within 24 hours of debarking, as well as fresh and immaturely composted commercial garden refuse. A synthetic nitrate solution was used to simulate the treated landfill leachate at 500 and 2000 mg/l, so as to operate the denitrification process in controlled conditions. Initially, each column was filled with a substrate and then saturated with 500 mg/l or 2000 mg/l concentration nitrate solution.

Liquid samples were taken at the outlet of the column at the beginning of each injection. Injections were made every day over a period of 30 minutes (1800 s) from Monday to Friday, whilst implementing a stagnant rest period over the weekend. Two different flow rates were used to observe the effect of flow on denitrification, both for a period of 4 weeks.

The first experiment was designed to assess the nitrate removal capabilities of the substrates at a relatively low flow rate. The entire volume of nitrate solution was replaced over a 5 day period. Thus 1/5 of the initial input liquid volume was sampled from the bottom of the column and replaced with new nitrate solution every day. The second experiment investigated the nitrate removal capabilities of the columns at a high flow rate, where the entire volume of nitrate solution was replaced over a 2 day period. Thus 1/2 of the initial input volume of nitrate solution was sampled and replaced with new solution every day.

The physicochemical properties of the effluents were analysed for NO_3 , pH and temperature daily, whilst COD and NH_3 once a week. The measurements are presented in Table 2.1 (Appendix A3).

Table 2.1

Summary of each substrates physiochemical properties.

Substrate	Input			COD (mg/l)		NH_3 (mg/l)	
	C_0	C/N	pH	Exp. 1	Exp. 2	Exp. 1	Exp. 2
CGR RAW	500	62.15	4.18	4600 → 600	385 → 54	25 → 1	4.5 → 1.5
Pine Bark	500	90.19	5.45	3094 → 786	402 → 222	7 → <1	5 → <1
CGR 10	500	23.91	6.98	453 → 310	109 → 41	6 → 1	1 → 1
CGR RAW	2000	62.15	4.18	3166 → 321	167 → 80	14 → 4	4.0 → 1.5
Pine Bark	2000	90.19	5.45	2524 → 631	406 → 267	7.5 → 3.0	<1
CGR 10	2000	23.91	6.98	278 → 124	43 → 34	6 → 3	4.5 → <1

In this paper, the column campaign was used to simulate a reactor and an Advection-Dispersion-Reaction (ADR) model implemented to analyse the results achieved from these experiments. The development and optimisation of a classical reactive transfer model approach was adopted and applied to simulate the observed experimental behaviour. The strategy used for the model, incorporated the selection of preferred parameters and their sensitivity to each experiment.

The data used in this study are summarised according to initial concentration, flow velocity and finally substrate, presented as follows:

Flow rate:

01 – Low,

02 – High.

Substrates:

01 – Fresh commercial garden refuse (CGR),

02 – Pine Bark (9PB),

03 – Commercial garden refuse composted for 10 weeks (CGR 10).

Data was divided into 12 different tests as presented in Table 2.2, which displays the nitrate concentration (mg/l), initial input volume (l), mass of substrate (kg), substrate, output sample (l/d), duration (days), hydraulic retention time (days), porosity and injection time (s).

Table 2.2

Summary of experimental data.

Experiment	Conc. (mg/l)	Vol. (l)	Mass (kg)	Substr.	Output (l/d)	Dur. (d)	HRT (d)	Porosity	Inj. (s)
EXP 500-01-01	500	12.40	2.73	CGR	2.48	21	8.06	0.72	1800
EXP 500-01-02	500	10.00	3.42	PB	2.00	21	10.00	0.64	1800
EXP 500-01-03	500	8.50	6.56	CGR 10	1.70	21	11.76	0.36	1800
EXP 500-02-01	500	11.25	2.73	CGR	5.62	20	3.56	0.72	1800
EXP 500-02-02	500	10.00	3.42	PB	5.00	20	4.00	0.64	1800
EXP 500-02-03	500	5.70	6.56	CGR 10	5.85	20	7.02	0.36	1800
EXP 2000-01-01	2000	2.80	11.90	CGR	2.38	21	8.04	0.72	1800
EXP 2000-01-02	2000	3.48	10.00	PB	2.00	21	10.00	0.64	1800
EXP 2000-01-03	2000	6.38	8.90	CGR 10	1.78	21	11.24	0.36	1800
EXP 2000-02-01	2000	2.80	11.30	CGR	5.62	20	3.53	0.72	1800
EXP 2000-02-02	2000	3.48	10.00	PB	5.00	20	4.00	0.64	1800
EXP 2000-02-03	2000	6.38	5.70	CGR 10	5.85	20	7.02	0.36	1800

Only the experiments conducted with the initial lower flow rate were started with new substrate. This has a strong impact on the acclimatisation period of the bacteria within the system and thus needs to be taken into account with the choice of parameters when designing the model.

2.1. Experimental Results

The evolution of the nitrate concentration over the duration of the testing are displayed in Figures 2.2 and 2.3, which show the results for CGR (RAW), CGR 10 and Pine Bark at both $C_0 = 500 \text{ mg/l}$ and 2000 mg/l respectively, for the 2 different experiments. These trials reflect promising results as presented in Figure 2.2.a., where full denitrification was achieved. However, at the faster flow rate in Experiment 2, denitrifying bacteria was not allowed sufficient time for nitrate removal, suggesting an inadequate residence time, as displayed in Figure 2.2.b.

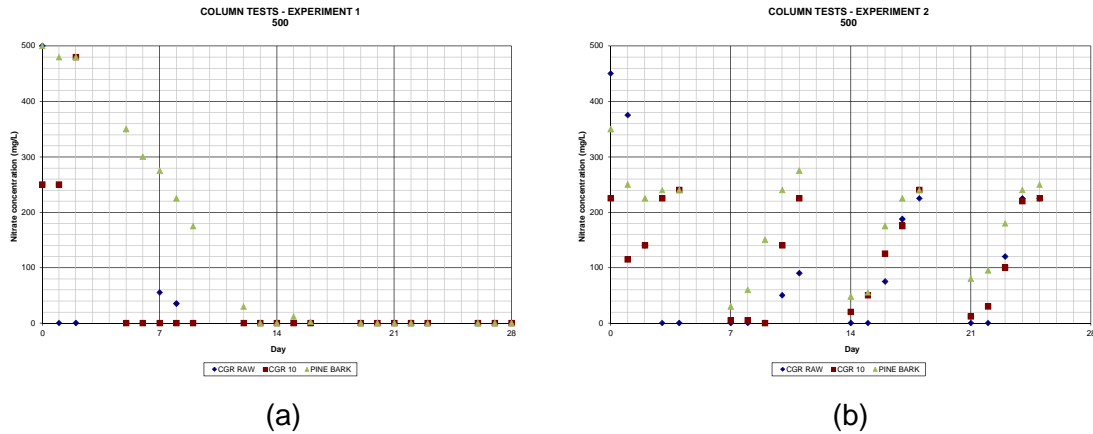


Figure 2.2: Evolution of the nitrate concentration for CGR (RAW), CGR 10 and Pine Bark at $C_0 = 500 \text{ mg/l}$ for (a) Experiment 1 and (b) Experiment 2.

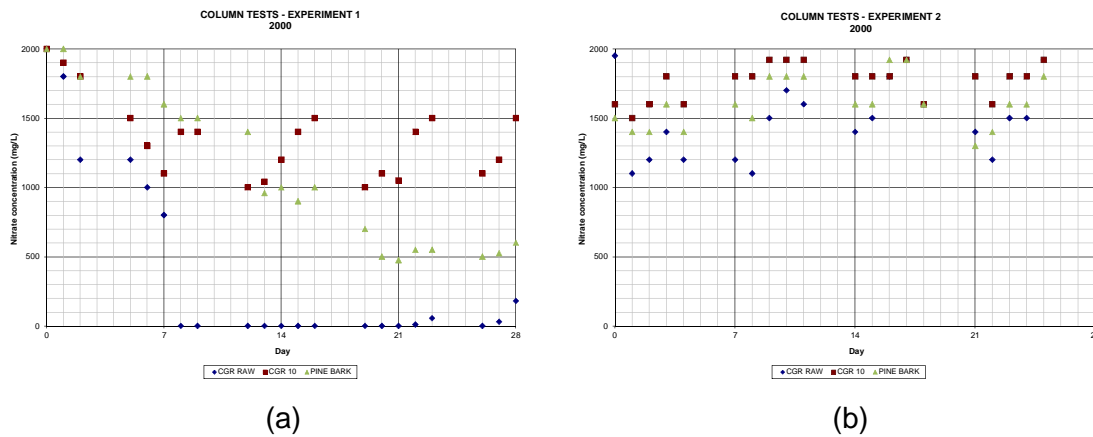


Figure 2.3: Evolution of the nitrate concentration for CGR (RAW), CGR 10 and Pine Bark at $C_0 = 2000 \text{ mg/l}$ for (a) Experiment 1 and (b) Experiment 2.

Complete denitrification is a good goal in terms of process engineering but presents a problem for optimisation, as these results are easily obtained by using a high rate of reaction, K_r , when modelling. However, this prevents obtaining valuable information, which is indispensable when determining accurate reaction kinetics for designing an optimum process.

2.2. Modelling using ADR and SA

The different parameters and conditions used for the Simulated Annealing model are presented in Table 2.3.

Table 2.3

Summary of parameters used in the model.

Parameters	Physical meaning	Initial value	Range
Parameters of the Simulated Annealing			
T_0	Level	150	150 - 0
NB_{pal}	Number of levels T_1	1	<20
α	Parameter for the decrease in T	4	fixed
NB	Number of iterations	25	fixed
Physical parameters			
$Poro.$	Porosity	Depending on substrate	fixed
D_{diff}	Diffusion coefficient	1.7×10^{-9}	fixed
Parameters to be optimised			
C_m	Monod limiting concentration (mg/l)	100	[10, 100000]
K_r	Kinetic constant (mg/l/s)	1.2×10^{-3}	$[10^{-4}, 10^{-2}]$
D_{disp}	Dispersivity (m)	0.1	$[5 \times 10^{-2}, 1]$
t_{acc}	Acclimatisation time constant (s)	30	[1, 100000]
Parameter of the search window			
$pvar$	Initial size of neighbour research window	1	$> 10^{-4}$
$rafin$	Refinement factor of the search window	2	fixed

2.2.1. Sensitivity of the mesh parameters

The objective is to find the mesh properties (dt: time step, Nz: number of space elements) which present a good agreement with the reference case (dt = 1 s and Nz = 1000) within a reasonable calculation time. To demonstrate the process of this simulation, the experimental case EXP 500-02-02 was used for the various tests. Table 2.4 summarises the calculation times and RMS values of the optimised parameters between the reference case and different couples (dt, Nz).

Table 2.4

Sensitivity test of the mesh (dt and Nz) for EXP 500-02-02.

Test	1s_1000Nz	10s_800Nz	30s_200Nz	60s_100Nz	800s_20Nz	1800s_5Nz
RMS	0	1.57	1.72	1.96	54	72.9
Calculation time	-	-	+	+	++	+++

The reference case displayed the most accurate results as both the space and time discretizations are more detailed. The solution of the SA requires about 400 iterations leading to a very large total calculation time: $120000 \text{ s} = 33 \text{ h} = 1.5 \text{ days}$. The discretization with $dt = 30 \text{ s}$ and $Nz = 200$ presents a very good agreement with the reference case ($RMS = 1.72$) and a reasonable calculation time (less than 30 minutes). Small numerical instabilities were observed using the couple, $dt = 60 \text{ s}$, $Nz = 100$.

2.2.2. Illustration of the nitrate transport in the column

The illustration in Figure 2.4 provides an insight into the behaviour of the model with and without flow. Figure 2.4.a. presents the distribution of nitrate concentration along the column length z , at the beginning of injection ($t = 0$), at the middle time of injection ($t = 900 \text{ s}$) and at the end of the injection ($t = 1800 \text{ s}$). While as presented in Figure 2.4.b., the goal was to obtain insight into the statistics and phase response of bacteria, particularly, dissemination and responsiveness, during the rest period, post $t = 1800 \text{ s}$ and injections 2 hours ($t = 2 \text{ h}$) and 24 hours ($t = 24 \text{ h}$) after the end of the stagnant phase. The experimental case chosen for representation was EXP 500-01-03.

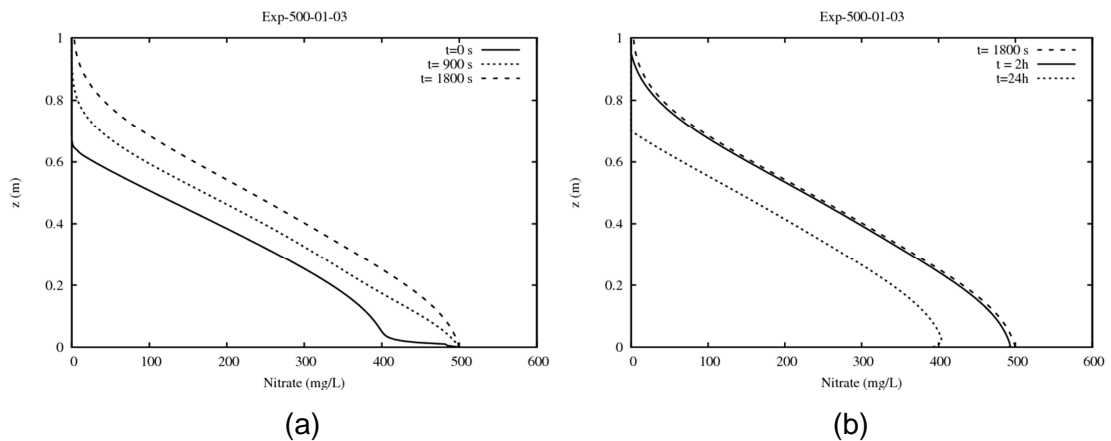


Figure 2.4: Example of the evolution of nitrate concentration distribution during the (a) injection phase and the (b) static phase.

In Figure 2.4.a., during the dynamic phase, with flow, the effect of dispersion is illustrated and the absence of the piston effect, which appears from $t = 0$ to $t = 900$ and

$t = 1800$ s is also noted. Figure 2.4.b. shows that the effect of diffusion is very low and the biodegradation reaction is very sensitive. In fact, 100 mg/l of nitrate was denitrified uniformly throughout the column.

2.2.3. Illustration of the optimisation

To determine the unknown parameters which are not optimisation sensitive, three dimensional curves are presented, in accordance with the RMS, with different parameter combinations, (K_m, C_m) , (K_r, D_{disp}) , (K_r, t_{acc}) and (C_m, D_{disp}) , resulting from the optimisation of $(C_m, D_{disp}, K_m, t_{acc})$ and (K_r, D_{disp}, t_{acc}) , using the simulated case of EXP 500-02-02 and the modelling conditions (initial parameter ranges and deductions) as listed in Table 2 3.

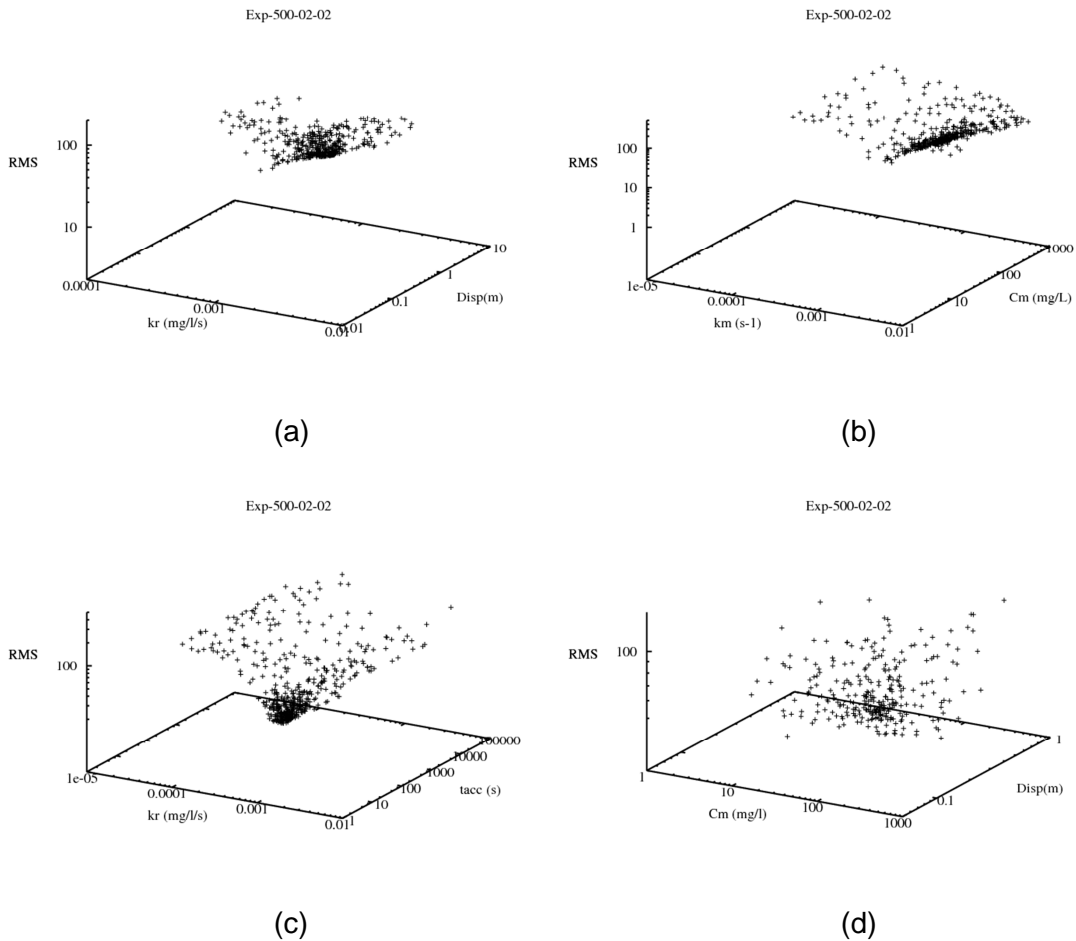


Figure 2.5: Effect of each parameter on the RMS for experimental case EXP 500-02-02: (a) - (K_r, D_{disp}) , (b) - (K_m, C_m) , (c) - (K_r, t_{acc}) , (d) - (C_m, D_{disp}) .

The graphs presented in Figures 2.5.a., b., and d., show that the following parameters, the coefficient of dispersion, D_{disp} and the Monod limiting coefficient, C_m , are unidentifiable parameters for optimisation, due the “valley” plots. Only the acclimatisation time constant, t_{acc} , appears to be more sensitive to the optimisation as clearly displayed in Figure 2.5.c. with an optimum at 122 s. This result could be due to the dominant effect of K_r compared to the other parameters, which is evident in Figure 2.5.d. with a different shape compared to the other graphs plotted with K_r . For a clearer illustration of this effect, two sample graphs are plotted in 2D, as presented in Figure 2.6.

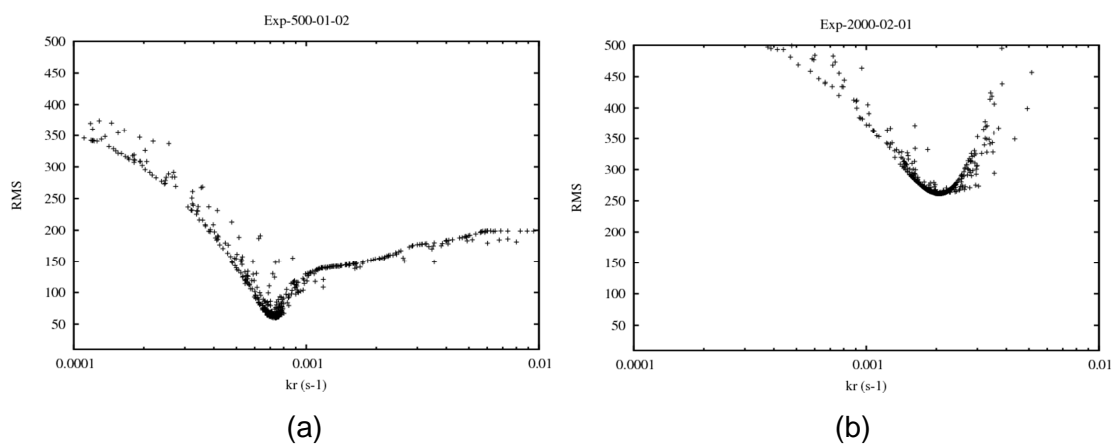


Figure 2.6: Illustration of the optimisation of parameter k_r for (a) EXP 500-01-02 and (b) EXP 2000-02-01.

The optimal value in these two graphs is at the centre of the concavity displayed by a large portion of the points. This form validates the method successfully, by scanning the entire search range and ending with focus at the optimal point.

The methodology presents insight into the developed model, for the implementation of the optimisation method and the selection of the relevant parameters to best suit the technique of simulated annealing.

3. Results and discussion

After the choice of model parameters, experimental column data was simulated and validated, implementing the Simulated Annealing method. Three reaction kinetics of biodegradation were tested, 0 – order, 1st – order and Monod kinetics, to determine which exhibited the most similar behaviour and the lowest error when compared to the experimental results.

3.1. Selection of the biodegradation model

The EXP 500-02-02 was used as a demonstration, due to the positive mesh properties and good RMS values, whilst also providing experimental concentrations which do not reach zero (0 mg/l). The comparative results were obtained by optimising three parameters D_{disp} , K_r and t_{acc} for the 0 – order and 1st – order reaction kinetics and four parameters (C_m , D_{disp} , K_r , t_{acc}) for the Monod reaction kinetics, which are presented in Table 2.5.

Table 2.5

Effect of reaction kinetics on bio-denitrification.

	K_r	C_m	t_{acc} (s)	D_{disp} (m)	RMS
0 – Order	1.27×10^{-3} (mg/l/s)	-	122.22	0.12	52.62
1 st – Order	4.45×10^{-6} (s ⁻¹)	-	9342.14	8.49×10^{-2}	52.26
Monod	1.95×10^{-3} (s ⁻¹)	101.71	1.76	0.17	48.60

The results show that the three different kinetic RMS values are similar, which is logical, as the theory relating to the Monod relationship, suggests that at low concentrations, it displays characteristics similar to that of 1st – order kinetics and at high concentrations, 0 – order kinetics, which is applicable to this study. This relationship is illustrated in Figure 2.7.

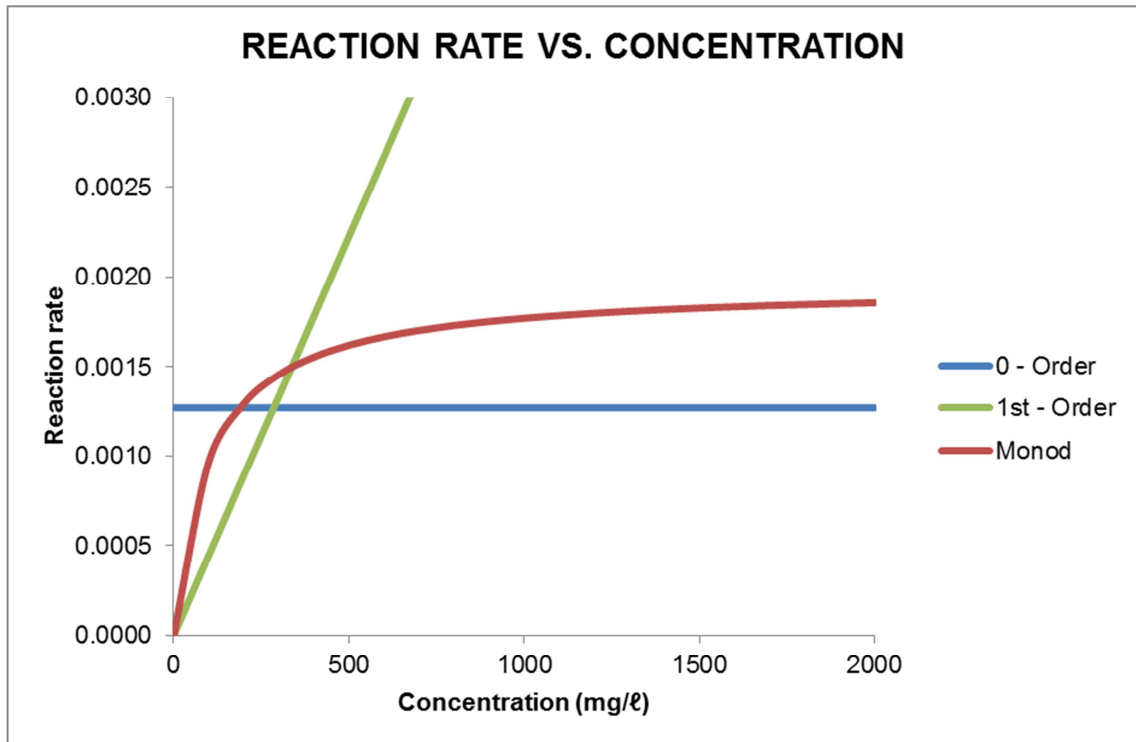


Figure 2.7: Evolution of the reaction rates as a function of the nitrate concentration.

There's an intersection between the three kinetic values for $C = C_m = 120 \text{ mg/l}$. Below this value the Monod reaction displays 1st – order kinetics and above this value, 0 - order kinetics. As Monod uses a parameter C_m which is difficult to model and that at high concentrations the 1st – order curve moves away from the other two kinetic plots which maintain a similar behaviour, the 0 – order biodegradation reaction kinetics was used and optimised with the K_r , t_{acc} and D_{disp} parameters.

3.2. Analysis of the denitrification process using a 0 – order model with acclimatisation

3.2.1. Optimisation quality

The 0 – order biodegradation reaction kinetics were implemented and optimised with the all 12 tests, including both concentrations and flow rates, for the 3 different substrates. A summary of the results is presented in Table 2.6, which shows the optimised parameters, K_r , t_{acc} , $Disp$ and corresponding RMS values.

Table 2.6

Summary of the results using 0 – order biodegradation reaction kinetics.

0 – order						
Concentration	500			2000		
Experiment	01-01	01-02	01-03	01-01	01-02	01-03
<i>RMS</i>	124.27	59.67	93.68	302.30	323.70	117.20
<i>K_r</i> opt	1.13×10^{-3}	0.723×10^{-3}	0.945×10^{-3}	3.35×10^{-3}	1.73×10^{-3}	1.81×10^{-3}
<i>D_{disp}</i> opt	0.23	5.11×10^{-2}	0.35	5.00×10^{-2}	5.38×10^{-2}	0.58
<i>Poro.</i> opt	0.72	0.64	0.36	0.72	0.64	0.36
<i>t_{acc}</i> opt	12.29	99843.61	107.66	73.56	99868	8240.01
Experiment	02-01	02-02	02-03	02-01	02-02	02-03
<i>RMS</i>	96.99	52.62	101.72	261.42	221.60	195.19
<i>K_r</i> opt	1.59×10^{-3}	1.27×10^{-3}	1.48×10^{-3}	2.04×10^{-3}	1.98×10^{-3}	1.013×10^{-3}
<i>D_{disp}</i> opt	0.553×10^{-2}	0.12	0.62	5.00×10^{-2}	0.51	0.97
<i>Poro.</i> opt	0.72	0.64	0.36	0.72	0.64	0.36
<i>t_{acc}</i> opt	212.00	122.22	113.46	471.49	185.70	7841.95

The result that displays the best optimisation, is with Pine Bark at $C = 500 \text{ mg/l}$, where the determined RMS value is 52.62 at a high flow rate and 59.67 at the lower flow rate. This can be attributed to the fact that during the second experiment at the high flow rate, the acclimatisation time constant, *t_{acc}*, provides for a very short acclimatisation period of 122.22 s for the 500 mg/l and 185.70 s for the 2000 mg/l, for Experiment 2 (02-02). Whilst during Experiment 1 (01-02) based on the range 99843.61 – 99868 s for both concentrations.

At the concentration of 2000 mg/l the minimum RMS obtained was with CGR 10, however in this case, the first experiment has better results than Experiment 2.

Unlike with the trials using fresh CGR, the composted CGR presents an acclimatisation time constant which is very similar when comparing both experiments at the different flow rates.

The dispersivity D_{disp} must always be greater than $1 \times 10^{-3} \text{ m}$; otherwise digital instability will occur within the optimisation process. The porosity *Poro.* and D_{disp} have a great influence on the RMS, due to the limited experimental data. The optimum rate

of reaction K_r is always in the order of 1×10^{-3} mg/l/s or 86.4 mg/l/d for the different experiments.

3.2.2. Effect of the acclimatisation time constant

The effect of the acclimatisation time constant was tested using the Pine Bark substrate, at the concentration of 500 mg/l. It was selected based on the results displayed in Table 2.6, due to the positive RMS values, as well as being the case where t_{acc} is the most sensitive parameter. The outcomes are presented in Table 2.7, which shows the t_{acc} range from 0 s to the optimised values, for the two experiments.

Table 2.7

Effect of the acclimatisation time constant for Pine Bark at 500 mg/l.

Experiments	t_{acc} (s)	K_r (mg/l/s)	RMS
EXP 500-01-02	0.00	0.641×10^{-3}	83.52
EXP 500-01-02	99843.61	0.723×10^{-3}	59.67
EXP 500-02-02	0.00	1.82×10^{-3}	50.04
EXP 500-02-02	122.20	1.27×10^{-3}	52.62

In the first experiment (01-02), the substrate is “new” and thus it can be assumed that no denitrifying bacteria have been established. While for the second experiment (02-02), denitrifying bacteria has already colonised within the system. The following results show that the rate of acclimatisation has little influence on the RMS for Experiment 2. As shown in the Table 2 7, Pine Bark requires a long acclimatisation period, which is due to the substrate being acidic and the release of phenols, which are toxic to certain microorganisms.

When comparing the reaction kinetics, similar values for the two different tests, both with and without acclimatisation, were obtained. This emphasises the suggestion that only the acclimatisation period influences any change of the RMS values.

The effect of acclimatisation is less for the fresh CGR and CGR 10 substrates, especially with regards to the composted CGR 10, where at strong concentrations (2000 mg/l), t_{acc} remains in the region of approximately 2 hours.

3.2.3. Curve analysis of the output data and the efficiency of biodegradation

The experimental results for each of the substrates at the different concentrations and flow rates were compared with outputs from the optimal 0 – order model taking into account the acclimatisation period.

Substrate 1 – Fresh CGR

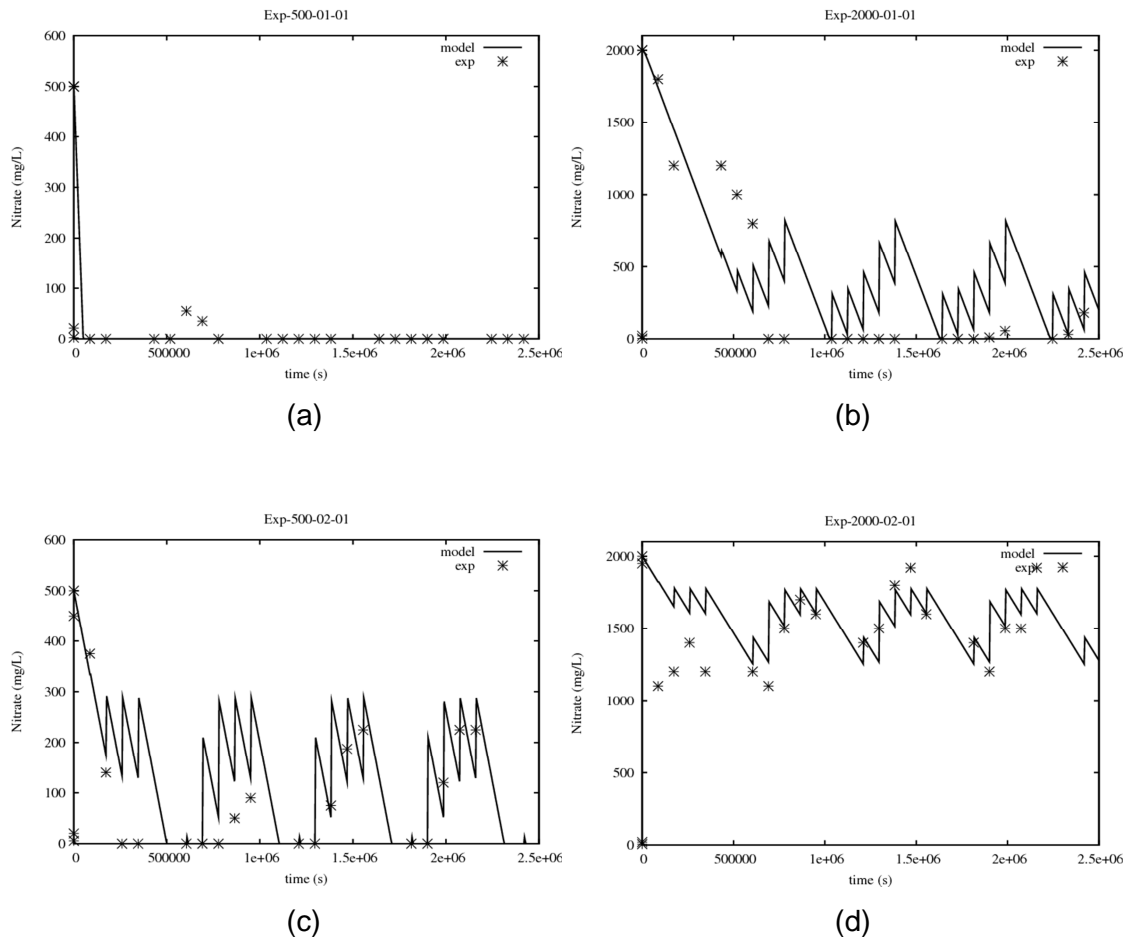


Figure 2.8: The model outputs of nitrate concentration vs. time for Fresh CGR.

The output of the model compared with the experimental data using fresh CGR as a substrate produced positive results, especially with 500 mg/l concentration as displayed in Figure 2.8.c., where EXP 500-02-01 had the best RMS value of 96.99. The acclimatisation time constant is identical for the experimental data and the model outputs, with a negligible rate of 12.29 s for EXP 500-01-01; however it is larger for EXP 2000-02-01 with a value of 471.49 s, but is still relatively low.

When comparing the rates of biodegradation, for both flow rates, the 500 mg/l, had a highest kinetic reaction rate of $K_r = 1.59 \times 10^{-3}$ mg/l/s, whilst at 2000 mg/l, the highest value obtained, is significantly greater, where $K_r = 3.35 \times 10^{-3}$ mg/l/s.

Substrate 2 – Pine Bark

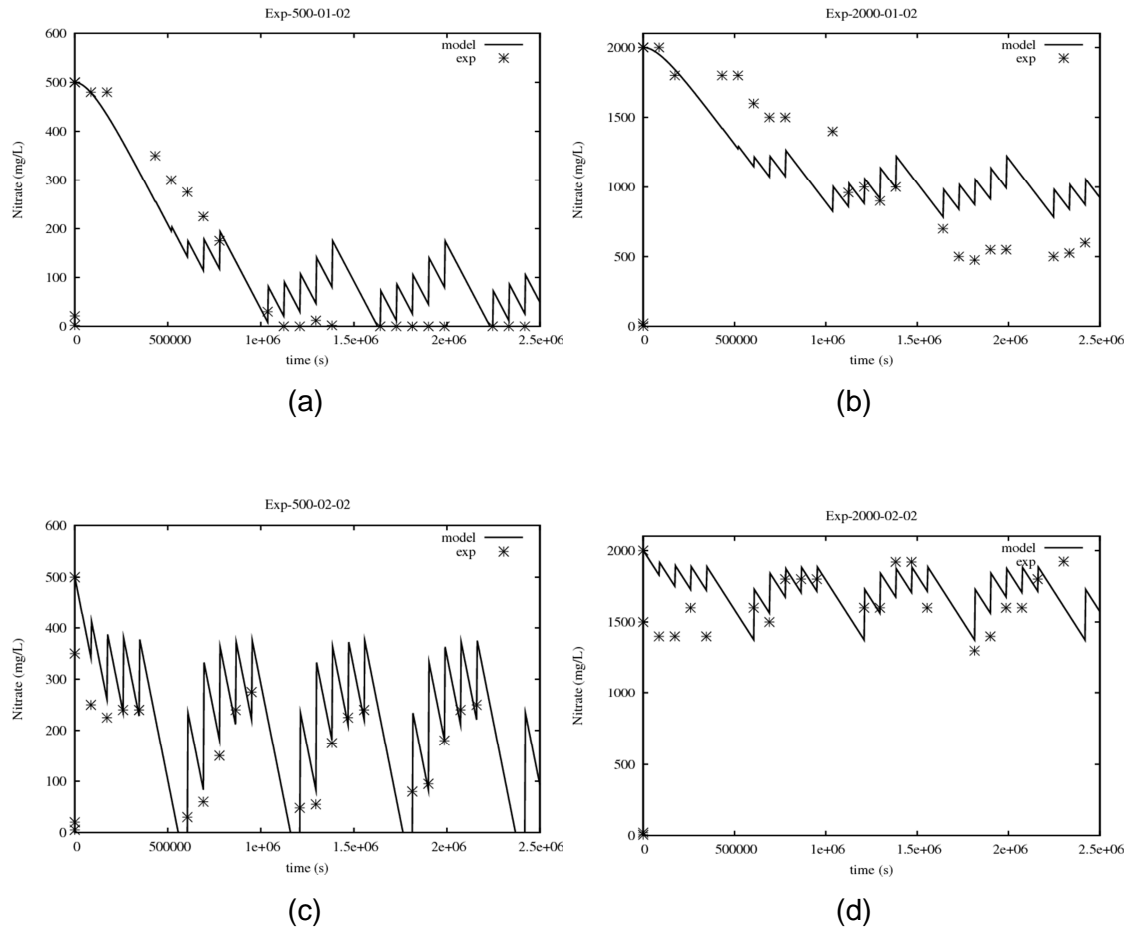


Figure 2.9: The model outputs of nitrate concentration vs. time for Pine Bark.

The best optimisation achieved using Pine Bark is with EXP 500-02-02, as presented Figure 2.9.c. with a RMS of 52.62 and an acclimatisation period of 122.22 s. Even at the slower flow rate, with a long acclimatisation period of 99843.61 s, the RMS value is still low at 59.67, indicating that the estimate for t_{acc} was fairly suitable; however it did display the lowest response rate of $K_r = 0.723 \times 10^{-3}$ mg/l/s.

The results with a nitrate concentration of 2000 mg/l have larger RMS values than those produced at 500 mg/l, with a value of 323.70 at the lower flow rate, which could also be as a result of an underestimation with regard to the acclimatisation time constant as evident in Figure 2.9.b.

It is noted that at both concentrations, the kinetic reaction rates obtained during Experiment 2, greater than those of Experiment 1. As previously mentioned this can be attributed to the fact that, in Experiment 1, the substrate is “new” and thus it is assumed that no denitrifying bacteria have been established within the system. Secondly, due to the substrate being acidic and the release of phenols, which are toxic to certain microorganisms, Pine Bark requires a long acclimatisation period. However, for

Experiment 2, the system has become acclimatised, as a result of significant phenol, no longer being released, a rise in pH to within the optimum range and the denitrifying bacteria already being established. Thus, a substantially shorter acclimatisation period is required, with t_{acc} values of 122.22 s and 185.70 s for the two nitrate concentrations respectively.

Substrate 3 – CGR 10

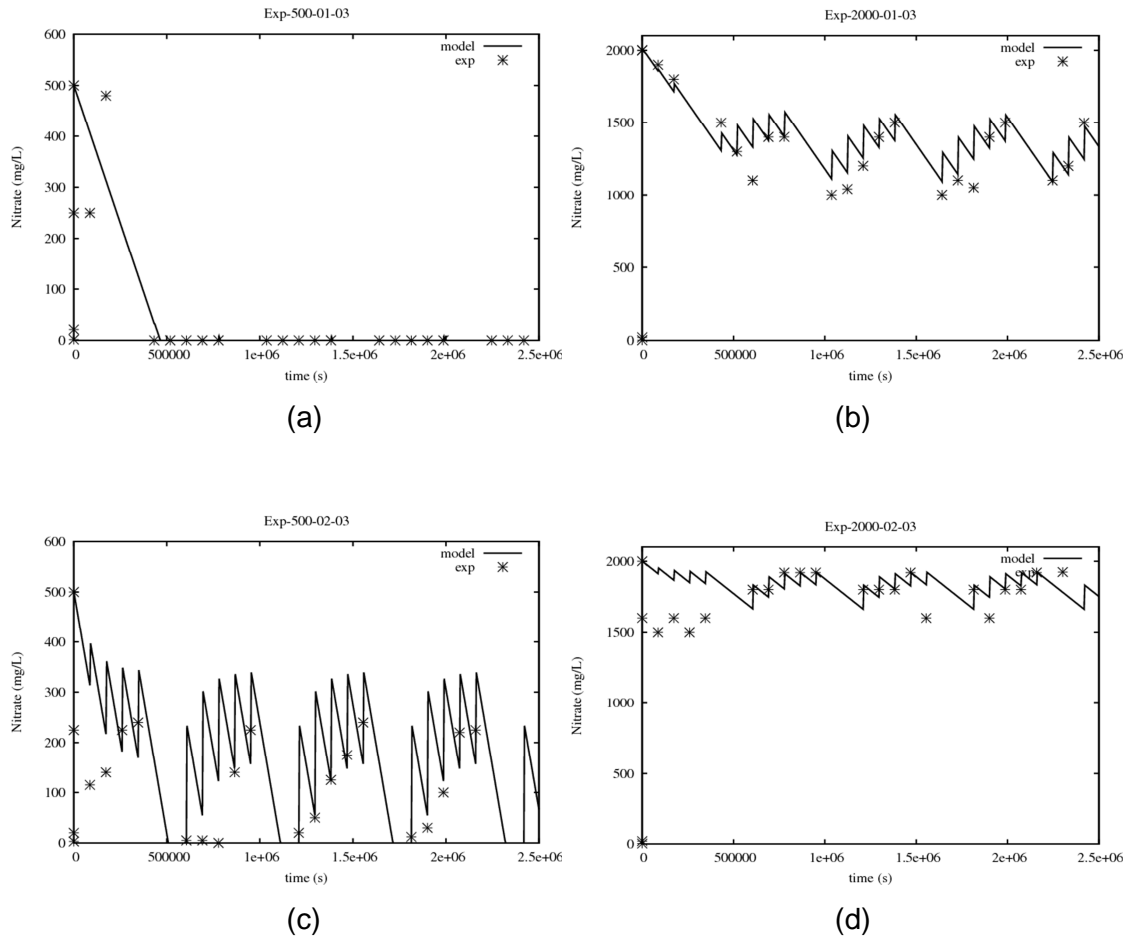


Figure 2.10: The model outputs of nitrate concentration vs. time for CGR 10.

The experimental data for composted CGR 10 (EXP 500-01-03) and fresh CGR (EXP 500-01-01) at both the lower flow rate and concentration presented similar results, with complete denitrification within 500000 s as clearly depicted in Figures 2.10.a. and 2.8.a. However, the composted material had a longer acclimatisation time constant of 107.66 s and a RMS of 93.68.

With an increase in flow rate the difference in RMS values between the two CGR materials is reduced at 500 mg/l. The CGR 10 has a RMS of 101.72, which is significantly closer to the 96.99 of fresh CGR. Also, the resulting acclimatisation period, t_{acc} is greater at 113.46 s.

The two experiments at 2000 mg/l provide RMS values that are greater than those given at 500 mg/l. However, these values are still better than those produced by the 2 other substrates at 2000 mg/l. The acclimatisation period at 2000 mg/l is greater than that at 500 mg/l at both flow rates, which is expected.

It is noted that if complete denitrification is achieved, a clear and accurate indication of the acclimatisation period, t_{acc} is difficult.

At 500 mg/l, during Experiment 1, the obtained kinetic reaction rate of CGR 10, $K_r = 0.945 \times 10^{-3}$ mg/l/s, was close to that of the Pine Bark, $K_r = 0.723 \times 10^{-3}$ mg/l/s, but is still lower than the rate achieved by the fresh CGR, with $K_r = 1.13 \times 10^{-3}$ mg/l/s. Similarly, at the increased flow rate, the CGR 10 had a greater kinetic reaction rate than that of the Pine Bark, but was less than the fresh CGR.

With the tests conducted at a nitrate concentration of 2000 mg/l, the CGR 10 achieved a faster rate of reaction, $K_r = 1.81 \times 10^{-3}$ mg/l/s during Experiment 1, compared to $K_r = 1.013 \times 10^{-3}$ mg/l/s for Experiment 2, which is clearly evident in Figures 2.10.b. and d.

4. Conclusion

During this research, a preliminary optimisation model was explored based on experimental data obtained using column studies, for bio-denitrification. The process consists of passing nitrate solution through a fixed porous matrix formed by a natural organic substrate. These studies compared three substrates, with periodic injections of two synthetic nitrate concentrations (500 and 2000 mg/l). To model this process, a classical Advection-Dispersion-Reaction (ADR) transfer reactive model was applied to simulate the observed experimental behaviour.

Through the comparison of the deterministic and stochastic methods, and the related algorithms, simulated annealing was the technique chosen in order to ascertain the optimal parameters, whilst minimising the errors between the experimental data and the model outputs.

Direct analysis of the output data from the column was challenging as a result of the alternating flow and rest periods. The behaviour of the model was checked and the reliability of the optimisation method tested, distinguishing identifiable parameters and their sensitivity to optimisation, such as the effect of diffusion and reactivity during periods of rest, as well as the influence of dispersion during flow.

After checking the behaviour and reliability of the model, three different reaction kinetics of biodegradation, including, 0 – order, 1st – order and Monod, were compared

and consistency found between them. As Monod uses a parameter C_m , which is difficult to model, and at high concentrations, the behaviour of the 1st – order kinetics is significantly different, compared to the other two kinetic relationships, 0 – order kinetics was chosen for the model. A simple model for the acclimatisation of microorganisms was also implemented.

The outputs from the model assisted with evaluating the quality of the optimisation and to ascertain the effect with which certain parameters have on the model, in particular the reaction rate, whilst also providing insight into the duration of the acclimatisation period t_{acc} .

By plotting the model outputs on a curve, a comparison with the experimental data could be made while highlighting the effectiveness of biodegradation. The obtained results suggest that the developed model was successful in simulating the data with acceptable deviations between the experiment and calculations.

A calculation of the RMS values for each test provides a representation into the degree of accuracy. At 500 mg/l, the best RMS result was achieved using the Pine Bark substrate (EXP 500-02-02) with a minimum distance of 52.62. However, it did present the longest acclimatisation period, as $t_{acc} = 99843.61$ s.

In terms of the reaction kinetics, K_r , the Pine Bark and CGR 10 displayed similar results, but the fresh CGR was the most efficient of the three substrates at 500 mg/l, obtaining the highest value, $K_r = 1.59 \times 10^{-3}$ mg/l/s.

At the stronger, 2000 mg/l concentration, the kinetic results remained fairly consistent with those at 500 mg/l, where the fresh CGR was again the most efficient, with a calculated $K_r = 3.35 \times 10^{-3}$ mg/l/s (EXP 2000-01-01). The composted CGR 10 produced the minimum RMS value, with an acclimatisation period within three hours, $t_{acc} = 8240.01$ s. Pine Bark once again displayed the longest period for acclimatisation for Experiment 1. However, it is noted that the acclimatisation period is significantly shorter during the second experiment. This is logical as for the first experiment the substrate is still “new” and yet to establish denitrifying bacteria within the system, while in the subsequent second experiment bacteria has already had time to colonise.

In terms of the efficiency of biodegradation, the fresh CGR was the most effective, whilst the CGR 10 and Pine Bark displayed similar results. When modelling each test, the Pine Bark produced a positive simulation with the most reliable RMS values between the experimental data and the model output.

It is noted, that in most cases, an increase in concentration resulted in a faster kinetic reaction rate. This suggests that not only is degree of efficiency related to the

substrate and the quantity of readily available carbon, but also the nitrate concentration of the effluent and its supply to denitrifying bacteria.

This preliminary work has revealed that a model is constrained by the degree of experimental data. The study has been invaluable in assisting with the improvement of the experimental procedure and determining the relevant factors that need to be taken into account to achieve, both increased accuracy and repeatability, in the development of an optimisation model, which simulates the treatment process.

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CHAPTER 3

The use of Commercial Garden Refuse at different maturities as a carbon source for the bio-denitrification of treated MSW landfill leachate: A comparison between synthetic nitrate solution and treated Mariannhill Landfill site leachate.

ABSTRACT

The treatment of MSW landfill leachate is a major issue, part of the multi-stage process which is waste management. The bio-denitrification of high strength treated MSW landfill leachate is of particular concern to the eThekweni Municipality. Currently the leachate is collected and treated in a Sequencing Batch Reactor. However, after this nitrification stage, further treatment is required before the effluent can be safely discharged. The concept is to implement an ad-hoc bio-denitrification phase, making use of natural organic materials as carbon sources, incorporated in a fixed-bed reactor as a solution to the engineering problem. The study looks at two substrates, fresh and immaturesly composted commercial garden refuse, which are both readily available. The efficiency of each substrate to support nitrate removal will be established using laboratory experiments. Small-scale dynamic batch tests and column studies, simulating fixed-bed reactors, will be used to assess their performance, whilst comparing the behaviour when denitrifying synthetic nitrate solution and treated MSW landfill leachate. The testing provides evidence, that both substrates have the potential to act as carbon sources to denitrify high strength leachate, with different degrees of efficiency. Studies reveal that the fresher material is more suitable, where full nitrate removal was achieved, in the batch tests within 1 and 17 days for the 500 mg/l NO_3 synthetic solution and the nitrified leachate from the Mariannhill Landfill site's SBR respectively. This is expected due to the substrate being unstable and the high C/N ratio and readily available carbon.

1. Introduction

The practice of waste management is a multi-disciplinary strategy to deal with waste disposal, recycling and treatment, whilst also promoting the development of solutions that create clean, renewable energy. Since the introduction of the landfill the generation of leachate has been a major concern to the environment, through the contamination of soils, groundwater and the subsequent damage. However, modern landfills are highly engineered and specifically designed to ensure the protection of the

environment as well as human health through the control of both water and air emissions. As a means to prevent the contamination of groundwater, a combination of both liners and a leachate collection system are utilised [1]. After collection, the treatment of the leachate is imperative prior to discharge. As a result of the enforcement of stricter environmental guidelines, the concept of “treatment at source” has been established to be the potential solution [2].

The bio-denitrification of high strength treated MSW landfill leachate is of particular concern to the eThekweni Municipality. Currently at the Mariannhill Landfill site, situated in the eThekweni Municipality, collected leachate is being nitrified using a Sequencing Batch Reactor. This single sludge system is simple to operate and requires low maintenance [2]. However, the treated leachate produced from the plant does not comply with discharge limits, due to the high nitrate concentrations. Thus, a further ad-hoc treatment is required to denitrify the effluent prior to discharge. To achieve the “treatment at source” philosophy, the aim is to employ a bio-denitrification stage after the Sequencing Batch Reactor.

Denitrification refers to the biological redox reaction in which nitrate, an inorganic nitrogen compound, is reduced [2]. The process involves two steps. Firstly, the conversion of nitrate to nitrite and secondly, is the production of nitric oxide, nitrous oxide and nitrogen gas. Bio-denitrification of leachate requires certain conditions, which include the presence of a facultative bacterial population as well as a suitable environment for the growth of such microorganisms, the absence of dissolved oxygen or inhibitory toxic substances and finally an appropriate energy source, to act as an electron donor. The microorganisms capable of reducing the nitrates through conversion into nitrogen gas during biological denitrification require an external carbon source to act as an electron donor, acting in an anaerobic environment [3, 4]. Expensive easily biodegradable carbonaceous materials are currently employed around the world (methanol, ethanol etc.); however these methods tend not to be a viable solution for developing countries and are not suited for large scale, field applications [5, 6].

The study aims at developing an innovative low-cost treatment solution, which can be designed and implemented as part of an integrated waste management system promoting the efficient reuse of waste material. Commercial garden refuse is disposed of at many local landfill sites and is easily separated from the main waste stream. The research investigates the use of garden refuse at different degrees of maturity to act as carbon sources for the denitrification of nitrified landfill leachate. These biodegradable carbonaceous naturally organic substrates contain relatively high amounts of carbon and are suitable for large scale, field application.

The efficiency and feasibility of using the substrates in an anaerobic fixed-bed reactor was assessed at laboratory scale. Characterisation tests, small-scale dynamic batch tests and column studies were used to determine the performance of each substrate.

This solution is directed at reducing the impact of human activities on natural water systems by, not only minimising the deposited waste in a landfill, but by also improving the quality of wastewater being discharged into water resources thus limiting any detrimental disturbances on the relevant ecosystems.

2. Materials and Methods

2.1. Materials

The research investigates two substrates as carbon sources, fresh and immaturely composted commercial garden refuse. Local landfills throughout the eThekweni Municipality receive large volumes of garden refuse every day. In particular the Durban Parks Department are responsible for the maintenance of all parks and open spaces within the municipality. Overgrowth cuttings are shredded using a chipper to reduce particle size before transportation and disposal. The fresh commercial garden refuse (CGR RAW) was collected after the size reduction process.

The immaturely composted garden refuse (CGR 10) was obtained from a composting plant established in a small housing community. The garden refuse had been composted for 10 weeks in turned windrows.

A synthetic nitrate solution was used to simulate treated landfill leachate. This was done so as to assess the performance of each substrate, whilst establishing a base line, under controlled conditions. Two columns were run with synthetic solution in order to assist in the development of a kinetic model to simulate the behaviour of the nitrate evolution within the reactor.

The horizontal constructed wetland, fixed-bed reactor is to be implemented and run in conjunction with the Sequencing Batch Reactor at Mariannhill Landfill site, thus treated, nitrified leachate was collected from the SBR. This leachate is assigned the abbreviation M.L.S.

2.2. Characterisation tests

Characterisation testing is paramount to the research. It enables us to identify the quality of each material whilst also quantifying various key physicochemical properties.

The scientific protocols and methods as presented in ASTM [7] were followed. Tests were conducted in double or triplicate to ensure accuracy and repeatability.

Analysis was done on the treated leachate and both the solid substrates as well as their eluates. As a means to specify the nature as well as the quantity of compounds released by the substrates through leaching, whilst being in contact with water, the eluates of the substrates were tested. To obtain the eluates for each substrate, a representative sample of material was mixed with distilled water at a liquid to solid ratio of 10/1 for 24 hours, after which the mix was filtered through a 63 micron sieve. Bernal et al. [8] suggested that composting which involves the biochemical transformation of organic matter, occurs in the water-soluble phase through metabolism by microorganisms. Thus, studying the changes occurring in the soluble organic matter can provide insight in assessing the maturity of a substrate.

The following parameters were tested for the solid substrate: moisture content, total solids (TS), volatile solids (VS), carbon to nitrogen ratio (C/N) and the Dynamic Respiration Index at 7 days (RI₇) which was determined using a respirometric system type OxiTop[®]. Liquid samples were tested for: pH, TS, VS, COD, BOD₅, NH₃ and NO₃.

2.3. Batch tests

Small-scale dynamic batch tests were designed to assess the performance of each substrate at optimum conditions. These optimum conditions included, maximum contact between the substrate and leachate, monitoring pH and maintaining a fairly constant room temperature of 25°C. These batch tests also served the purpose of providing insight into the retention times required for the column studies to achieve satisfactory denitrification.

A synthetic nitrate solution with a concentration of 500 mg/l NO₃ was used as a means to establish a baseline for comparison, whilst determining the ability of the two substrates to act as carbon sources for nitrate removal. These controlled conditions allowed for the estimation of the kinetics of removal.

Treated leachate (M.L.S.) was used to monitor whether the materials were capable of achieving denitrification at high nitrate concentrations, while possibly investigating any inhibitory factors within the leachate which could influence the rate of denitrification. The treated leachate also provided an idea of the behaviour within a “real” world situation.

Batch reactors were assembled using closed top 1 l, 3 neck bottles equipped with two airtight silicone septa which allowed continuous sampling, preventing any air ingress. Each bottle was filled with 100 g dry matter of substrate and leachate at a

liquid to solid ratio of 10/1 to ensure full saturation was maintained throughout the duration of the experiment [9]. Substrates were mixed and cut to reduce particle size to a consistent size of 4 – 5 cm [9-11] as a means to obtain homogeneity of the sample. Prior to the addition of the leachate, the bottles, containing substrate were flushed with nitrogen to establish immediate anaerobic conditions. The reactors were then placed on a shaker at 150 rpm to maintain continuous mixing. Small samples of approximately 1-5 ml were extracted using a gas tight syringe to test the nitrate concentration (NO_3) 3 times a day depending on any changes, using the Nitrate Test Sticks type Merckoquant (MERCK). This method of extraction did not significantly affect the L/S ratio in the reactors and ensured that full saturation was maintained throughout the experiment. Where the presence of fines prevented accurate readings, samples were filtered using 0.45 μm filter papers. Tests were conducted in triplicate and run until the nitrate concentration reached zero. At the end of the test, both liquid and solid samples were characterised.

2.4. Column studies

Leaching columns were used to simulate the denitrification process in a fixed-bed reactor [5, 6, 12]. The column studies were used to investigate the effect on denitrification rates at different nitrate concentration levels and flow rates. The results were analysed to predict the kinetics of removal, loading rates and hydraulic retention time for possible filter beds. Two sets of experiments were conducted, using the two substrates, CGR RAW and CGR 10, with two leachates. A synthetic nitrate solution with a concentration of 500 mg/l NO_3 and treated nitrified leachate collected from Mariannhill Landfill site's SBR.

It has been established, through the comparison of various investigations, that continuous flow through a reactor improves the efficiency of denitrification on the postulation that circulation favoured organic matter release and dispersion [5, 6, 13]. However, a flow rate that is too high may cause a drop in the rate of removal [6]. Two different flow rates were thus chosen to ascertain the limiting flows and thus retention times that effect denitrification.

2.4.1. Equipment

The columns were constructed with a transparent PVC cylindrical body 1 m in length, 160 mm in diameter and had an approximate volume of 20 litres. The upper and lower ends of the columns were bolted together with a pair of 25 mm thick plastic flanges. A 20 mm rubber gasket was placed between each flange to act as a seal. The

column was then bolted to a steel frame. The upper flange consisted of two orifices. A tap valve which allowed for the collection of nitrate solution and the second, connected to a small plastic pipe which was used as a means to collect biogas production. The tap valve on the lower flange was used to inject nitrate solution into the column. The columns were packed with substrate and a drainage layer consisting of a coarse filter and marbles was placed at the top and bottom of each column, thus preventing any substrate from obstructing the outlet. Columns were then filled with the corresponding solutions. The columns were run from the bottom to top, using the concept of a difference in hydraulic head. The input data is presented in Table 3.7.

2.4.2. Experiment 1

Two columns packed with the respective substrates, CGR RAW and CGR 10 were run with a synthetic nitrate solution with a concentration of 500 mg/l NO_3 . This experiment was to gather more comprehensive data to be used in the development of an advection/dispersion/reaction optimisation model. The CGR RAW and CGR 10 columns were run at two different flow rates. Based on prior research (Appendix A2), the column packed with CGR RAW used a flow rate which ensured that the initial input liquid volume was replaced over a period of 2 days, whereas CGR 10 used a flow rate so that replacement occurred over 5 days. This test was run for 7 and 10 weeks respectively. New nitrate solution was injected into the column and a calculated sample volume was collected every day. Samples were analysed for NO_3 , pH and temperature daily at the start and end of each injection period. Once a week the COD and NH_3 values were determined.

2.4.3. Experiment 2

The second experiment investigated the nitrate removal capabilities of the columns at a high nitrate concentration. These tests were to present a “real” world scenario by using nitrified leachate (M.L.S.) from the SBR at Mariannhill Landfill site. The experiment was designed to assist in providing insight into the possible kinetics of removal, loading rates and hydraulic retention time required in the implementation of a full-scale filter bed. Two columns packed with CGR RAW and CGR 10 substrates respectively were filled with the treated leachate. However, during this test, the entire volume of nitrified leachate was replaced over a 10 day period for CGR RAW and a 20 day period for the CGR 10. The denitrified effluent was sampled and replaced with treated leachate every day, for 4 weeks. The effluents were once again analysed for NO_3 , pH and temperature daily, at the start and end of each injection period. Once a week the COD and NH_3 values were determined.

3. Results and discussion

3.1. Characterisation of substrates and leachate

Each substrate was characterised prior to use. The results of the characterisation tests are divided into two sections. The material used in the batch tests are presented separately from those which were in the column tests. This helps provide insight into the behaviour and change of the substrates, as tests were conducted at different times. The results of the material characterisation are presented in Tables 3.1 – 3.4.

3.1.1. Substrates used in the batch tests

Table 3.1

Characterisation of the solid substrates – batch tests.

	MC (%)	TS (%)	VS (%)	RI ₇ (mg O ₂ /g DM)	Tot C (%)	Tot N (%)	C/N
CGR RAW	56.72	43.28±4.40	93.05±0.91	12.09±0.24	49.05	0.81	60.58
CGR 10	68.14	31.86±1.14	65.53±7.56	13.96±0.80	17.60	1.35	13.04

Table 3.2

Characterisation of the eluate tests – batch tests.

	TS (g/l)	VS (g/l)	pH	COD (mg/l)	BOD ₅ (mg/l)	NH ₃ -N (mg/l)	NO _x -N (mg/l)	C/N
CGR RAW	7.24±0.13	5.33±0.13	5.00	9572±257	834	11.48±0.48	58.8	2.1
CGR 10	12.33±0.02	6.33±0.03	7.52	8360±126	222	13.44±1.56	21.0	1.0

3.1.2. Substrates used in the column tests

Table 3.3

Characterisation of the solid substrates – column tests.

	MC (%)	TS (%)	VS (%)	RI ₇ (mg O ₂ /g DM)	Tot C (%)	Tot N (%)	C/N
CGR RAW	37.26	62.74±1.27	96.30±0.47	6.20±0.11	48.8	1.14	42.80
CGR 10	53.81	46.19±1.11	30.09±2.60	7.30±0.47	17.8	1.35	13.19

Table 3.4

Characterisation of the eluate tests – column tests.

	TS (g/l)	VS (g/l)	pH	COD (mg/l)	BOD ₅ (mg/l)	NH ₃ -N (mg/l)	NO _x -N (mg/l)	C/N
CGR RAW	3.87±0.03	2.86±0.01	4.82	5900±397	1448	2.38±0.59	30.8	1.67
CGR 10	12.92±0.19	5.64±0.10	7.49	8499±189	345	34.44±3.56	2.1	1.29
M.L.S.	11.27±0.02	2.11±0.04	7.05	1650±166	43	10.33±5.34	3600	0.14

¹

Characterisation tests done on the solid substrates and their eluates can provide insight into the possible behaviour and capabilities of each material for denitrification, prior to any kinetic performance experiments.

The two most important parameters to assess on the solid material are the carbon to nitrogen ratio (C/N) and the Respiration Index at 7 days (RI₇). As described by Ovez [4], the microorganisms that are capable of reducing nitrates, require an external carbon source to act as an electron donor for biological denitrification. Thus a high carbon to nitrogen ratio is favourable, as relatively large amounts of organic carbon are needed, without increasing the nitrogen content in the system. However, high carbon content does not necessarily mean that the carbon is readily available for denitrification.

As mentioned by Zmora-Nahum, Hadar [14], the definition of green wastes is not uniform, as their composition may consist of different types, ages, as well as parts of the plant. Yard wastes, refers to a diverse range of green materials including, grass, young branches, woody tree parts and bark, all of which decompose at varying rates [9]. Vaughan, Dalal [15] suggested that a composted material has lower available organic carbon when compared to fresh green waste.

Depending on the initial source material, the C/N ratio of green wastes can range from 10 for composted materials to above 700 for hard and soft woods [16]. Typical values for fresh green, yard waste vary from 20 to 70 [14-21], where the preferred optimum range for composting is between 25 and 30 [19, 21], a stable compost is 15 [22] and a mature compost less than 15 [8, 15, 17].

During composting, dry mass loss is related to C mineralisation, where aromatic C increases in the form of CO₂ and aliphatic C decreases [11, 20]. When less N is lost, the percentage nitrogen content thus increases, leading to a reduction in C/N ratio. However, a C/N ratio which is too low has a high N content, which could result in the

¹Tables 3.1-3.4: The ± values refer to the standard deviation of the results. The standard deviation is only included when the test has been done in triplicate or greater.

evolution of ammonia, causing the inhibition to microbial fermentation due to its toxic nature. In contrast, N deficiency is associated with a C/N ratio which is too low. This slows the rate of digestion as there are not sufficient cells to sustain the growth of an active microbial mass [19].

The C/N ratio in the sampled fresh raw material is within the referred range, with values above 40, suggesting a more woody consistency. However, the immature compost reflects a C/N ratio more characteristic of stable, mature compost. This indicates that the method and efficiency of composting was quicker than anticipated within the 10 week period. Similar to the results presented by Lashermes, Barriuso [10], an increase in N content was noted, related to the lower loss of N through volatilisation, where the decomposition of the organic matter is chemically bound with N, compared to the loss in C content due to mineralisation and CO₂ production associated with composting, which lends to an overall lower C/N ratio [11, 20, 22].

Stability is defined by Llewelyn [23] as aerobic biological activity. A further means to determine maturity and stability are through evaluating the latent metabolism, including oxygen consumption or respiration activity [8, 11, 20]. Kaboré, Houot [20] believes that this is one of the most accurate methods when establishing the stability of organic matter, where the degradable organic matter still present, is inversely related to stability [24]. The RI₇ test is a respirometric study, whereby the O₂ consumption or CO₂ production is measured [10, 25, 26]. This is caused through the mineralisation of the organic matter and the CO₂ emissions a result of microbial respiration [8, 15, 27]. According to Bernal, Paredes [8], an insufficiently composted material has a greater propensity for O₂ and the high production of CO₂ rates as a result of the easily biodegradability compounds in the raw material and the rapid growth of microorganisms.

Kumar, Ou [21] made reference to the importance of moisture content when analysing the compost mixture, as it allows for the transport of mineralisation, dissolved nutrients required by microorganisms for metabolic and physiological activities. The products associated with composting and the biological degradation of the organic matter are, CO₂, water, and energy in the form of heat [20]. This characteristic is reflected in our results, where the composted CGR has higher moisture content than the fresh material. Also, in the case of the particular experiments conducted during this research, specific liquid to solid ratios were used to ensure full saturation throughout testing.

Wu and Ma [18] observed that there is a relationship between the volatile solids and C/N ratio, providing evidence regarding the reactive potential of a sample. A high

percentage of volatile solids tend to be associated with a higher C/N ratio value. This trend is also confirmed in the presented results of the characterisation testing.

pH is a vital parameter when ascertaining the characterisation of a material as it has a dominant effect on numerous processes and properties, chemical, physical and biological [13, 28]. These effects provide insight into the materials suitability for denitrification, specifically three of the most significant processes responsible for the generation of nitrous oxide and nitrogen: nitrification, (respiratory) denitrification and dissimilatory NO_3^- reduction to NH_4^+ [13, 28].

Ahmed, Idris [22] suggests that a pH value from 6.0 – 7.5 is optimal for the development of bacteria, whereas Glass and Silverstein [29] believe that a pH between 7.0 and 8.0 is suitable for most strains of denitrifying bacteria. An initial pH of 7.0 was identified by Wang, Yuan [30] as the optimum for NO_3^- - N degradation and Tsui, Krapac [5], 7.0 – 9.0 for denitrification.

Fresh biomasses, with a high Dynamic Respiratory Index (RI_7), which are composted under optimal conditions, where oxygen does not limit the process, pH increases reaching alkaline levels partly due to ligand exchange, the decarboxylation of organic anions during microbial decomposition of plant materials, also the high production of NH_3 and the speedy degradation of Volatile Fatty Acids (VFA) [9, 20, 24]. The dissimilatory reduction of NO_3^- and respiratory denitrification, cause the generation of oxidised pyridine nucleotides and ammonium (nitrogen mineralisation) to be produced, increasing the pH [9, 28].

This is abundantly evident through the characterisation tests, where the fresh CGR has an acidic pH below 7.0, however with composting the pH rose to alkaline levels above 7.0. The results are comparable to other studies where a fresh material used by Kaboré, Houot [20] displayed a pH of 5.8 and Tsui and Roy [31] a pH range for composted materials from 6.8 – 7.8.

As pH is a limiting factor and an acidic value has an inhibitory effect on denitrification, a buffering period can be expected for the CGR RAW, whereas the CGR 10 already falls within the prescribed optimum range [9].

The accessibility and availability of an easily biodegradable organic carbon source is a key factor and dominates the denitrification process ensuring a rapid start to the active phase of intense microbial activity especially during composting [11, 32]. The rate of denitrification can often be limited by organic carbon availability and although treated effluent from activated sludge may contain a certain amount of COD, sufficient available organic carbon is still deficient, particularly in high nitrate wastewater with low BOD content, total suspended solid concentrations and easily biodegradable COD is usually needed to sustain denitrification [13, 33, 34]. The

chemical oxygen demand (COD) is an indication of the total organic matter release produced by a substrate which, in turn can be used for denitrification [13]. However, as defined by Penn, Pauer [35], biochemical oxygen demand (BOD) is a measure of the dissolved oxygen consumed by microorganisms during the oxidation of reduced substances in wastewaters, where a higher BOD indicates that the organic matter is more easily biodegradable [13]. Usually, sources of BOD are readily biodegradable organic carbon (carbonaceous, CBOD) and ammonia (nitrogenous, NBOD) [35].

When comparing the fresh and composted materials, both COD and BOD₅ results provide an interesting insight into the characteristics of the substrates. The two composted garden wastes present COD values in region of 8500 mg/l whereas the fresh green waste falls in the range of 5900 to 9500 mg/l. The variance in result for the fresh material can be attributed to its instability and heterogeneity compared to that of the immature composts. Also, the value of total solids present in each eluate reinforce the fact that the composted material has been broken down and the mobilisation of the compounds, where the CGR 10 displays greater than 50% the TS than that of the CGR RAW. However, more importantly the BOD₅ values indicate, not the total amount of organic matter release, but the degree of easily biodegradability of the leached matter. In this case, the fresh material has significantly higher BOD₅ results, which is expected due to the fact it has not undergone any stabilisation and thus has a higher portion of biodegradable matter. The high BOD₅ values suggest that both substrates will be appropriate for the denitrification, sustaining high microbial activity, more so the CGR RAW compared to the composted CGR 10.

The treated, nitrified leachate collected from the Mariannhill Sequencing Batch Reactor, as proposed in various studies does not have sufficient easily biodegradable organic carbon in order to sustain full denitrification, thus the addition of dissolved organic carbon is required [13, 33, 34]. McLaughlan and Al-Mashaqbeh [9], investigated the use of a woody material to act as a filtration media for wastewater treatment, where the intentional leaching of additional dissolved organic carbon, supporting microbial processes vital to the removal of nitrogen. This release is controlled by two fractions, the mobilisation of already available soluble carbon and a desorption process, whilst the main factors influencing the rate and amount of dissolved organic carbon leached, include the type of wood (species), processing procedure (e.g. composting), particle size, contact time between the extract media and the leached material, as well as the influent properties, such as pH, ionic strength and composition [9].

3.2. Batch tests

As a means to assess the potential and performance of each substrate at optimum conditions, a small-scale dynamic batch test was designed. These tests also allowed for estimation of retention times required to achieve effective denitrification in the column studies.

Initially, tests were run with a 500 mg/l NO_3 synthetic solution to determine a baseline for comparison, whilst assessing each substrate's ability as a carbon source for nitrate removal. These results are presented in Figure 3.1, which displays the evolution of nitrate concentration over time.

To provide a realistic reflection of the different capabilities of the materials treated nitrified leachate (M.L.S) was implemented in a second set of experiments. These allowed for monitoring the behaviour of the substrates at high nitrate concentrations, as well as possibly investigating any inhibitory factors within the leachate which could influence the rate of denitrification. The results of which are displayed in Figure 3.2. At the conclusion of each batch test, the output eluate was characterised in terms of pH, COD and $\text{NH}_3\text{-N}$, as presented in Tables 3.5 and 3.6.

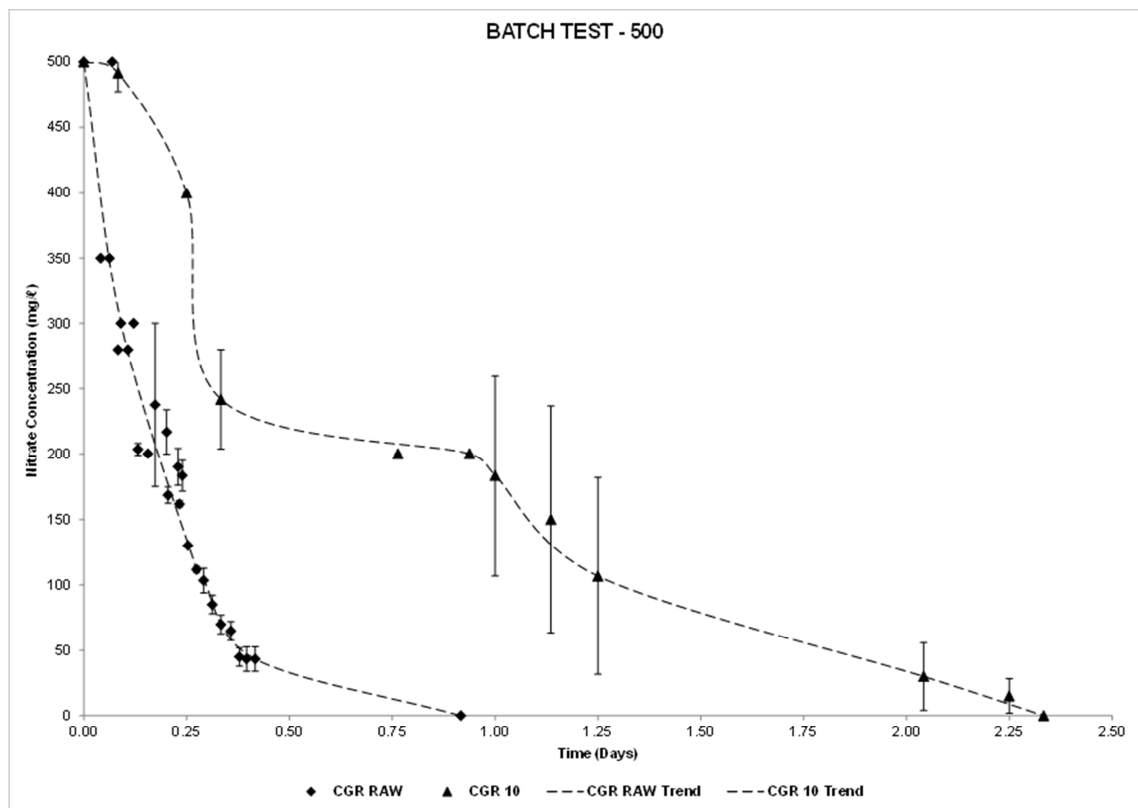


Figure 3.1: Nitrate evolution for CGR RAW and CGR 10 with $C_0 = 500 \text{ mg/l NO}_3$.

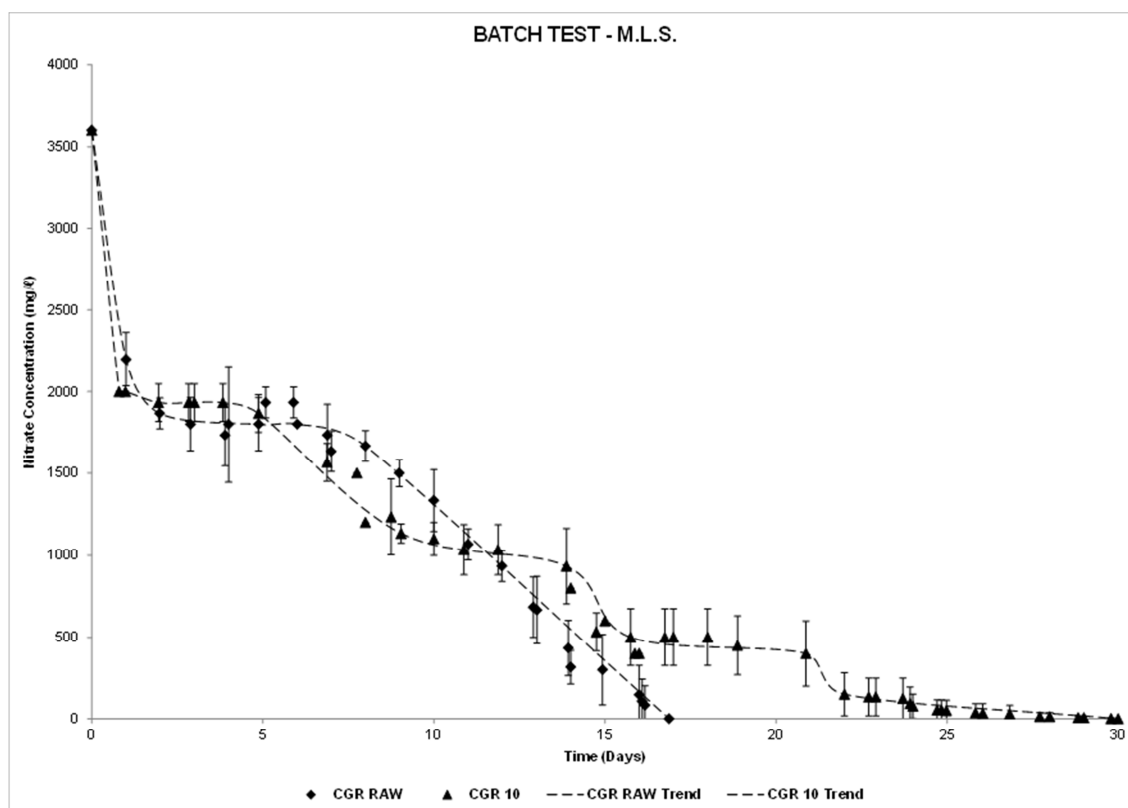


Figure 3.2: Nitrate evolution for CGR RAW and CGR 10 with M.L.S.

Table 3.5

Characterisation of the batch tests (500 mg/l NO_3) – output eluate.

500	pH	COD (mg/l)	$\text{NH}_3\text{-N}$ (mg/l)
CGR RAW	5.59 ± 0.07	7724 ± 776	139.3 ± 17.0
CGR 10	7.88 ± 0.27	7671 ± 553	16.0 ± 1.6

Table 3.6

Characterisation of the batch tests (M.L.S.) – output eluate.

M.L.S.	pH	COD (mg/l)	$\text{NH}_3\text{-N}$ (mg/l)
CGR RAW	6.86 ± 0.06	9098 ± 643	333.7 ± 10.1
CGR 10	7.96 ± 0.06	9191 ± 712	121.8 ± 16.3

In a closed system, such as the batch tests, it is difficult to monitor, let alone control the environmental condition. These include a variety of parameters such as the pH, availability of nutrients or the composition of the internal atmosphere [28]. Thus the denitrification results are dependent on the initial conditions and influenced by the variable changes that occur during the course of the evolution, where some of the

microbial populations proliferate whilst others are suppressed [28]. The denitrifying bacteria require labile organic carbon as an energy source [8, 34]. The main compositions of the organic substrate used by the denitrifiers as a carbon source are the cellulose, hemicelluloses and lignin. The difference in content strongly influences the degradation rate of the material, where certain cellulose in green waste are more resistant, resulting in the carbon bonds to be more difficult to break down [11, 32]. Thus, a source of easily biodegradable organic matter ensures a rapid start to the active phase of intense microbial activity [11]. The release of dissolved organic carbon consists of two fractions. The mobilisation of ready soluble carbon and a desorption process, involves the release of short term, pre-existing organic carbon and further generation, through the substrate degradation of insoluble organic carbon [9, 36].

As a result of the plant material, the substrates have a buffering capacity. The optimum pH for most environmental strains of denitrifying bacteria has been reported to be between 7 and 8 [29]. pH has a major influence on the three main processes in the generation of nitrous oxide and nitrogen; nitrification, respiratory denitrification and dissimilatory NO_3^- reduction to NH_4^+ and nitrogen gas [12, 28]. Denitrifying bacteria always cooperate with oxidizing bacteria to remove nitrogen, where the generation oxidized pyridine nucleotides and ammonium produced during dissimilatory NO_3^- reduction and respiratory denitrification, increase pH [28, 30]. In studies conducted by Wang, Yuan [30], an initial pH of 7.0 was determined to be the optimum for $\text{NO}_3\text{-N}$ degradation and furthermore, a slightly alkaline pH of 7.0 – 10.0 produced favourable results. However, as ascertained by Glass and Silverstein [29], at high nitrate concentrations above 1000 mg/l $\text{NO}_3\text{-N}$, low acidic pH values from 2.2 – 5.8 inhibited degradation. These observations were applicable to the obtained results. pH buffering was exhibited by both substrates, for the two different effluents and is particularly evident where CGR RAW was used to denitrify the M.L.S. A plateau period is clearly displayed in Figure 3.2. Additionally, all pH values increased from their initial input, which is characteristic with denitrification [28, 37]. However, even with the addition of M.L.S. effluent, with a pH of 7.05, the output pH in the CGR RAW did not rise above 7.0, only reaching 6.86, whilst the composted CGR 10 were both alkaline and within the optimum range [29]. This corresponds to the outcomes determined by Šlmeš and Cooper [28], who suggested that, when the reaction period is long, in their case, several days, the denitrifiers, when isolated from their natural habitats, reacted to the imposed conditions, not only by adjusting the pH to neutrality, but also buffered the system to prevent any further change.

Once the readily available biodegradable organic carbon is leached and consumed, the denitrification process is then limited by the organic matter electron

donors in the system, especially with wastewaters of a high nitrate concentration [5, 34]. Ding, Song [32] reported that unless sufficient electron donors are present, the removal of oxidized nitrogen, as electron acceptors will be ineffective. The more resistant compounds degrade at a slower rate [11]. This slowly biodegradable carbon, then provides denitrification through hydrolysis or fermentation by microorganisms [5]. Tsui, Krapac [5], noted that initially, a more mature substrate had a lower rate of nitrate of removal, which was attributed to the difference in microbial activity and the availability of carbon. This is evident in the obtained results as presented in Tables 3.1 and 3.2, plus the corresponding Figures 3.1 and 3.2. Although, both the CGR RAW and CGR 10 display similar microbial activity, there is a significant difference in available biodegradable matter as indicated in the BOD_5 values. This in turn is reflected in the time for each to achieve full nitrate removal, where the fresh CGR RAW fully denitrifies both effluents substantially faster than its composted counterpart, CGR 10. McLaughlan and Al-Mashaqbeh [9], identified a trend of staged release of organic carbon, as the different fractions are readily mobilised and available whilst others are released at a later stage. This multi-phasic process displays an initial rapid release followed by a decline [36]. The nitrate concentration evolution for the CGR 10 with M.L.S. leachate particularly exhibits this stepped phenomenon. However, when the concentration of both the oxidized nitrogen electron acceptors and the carbon electron donors are high enough, growth and development is not limited, the rates of the reactions can be defined with a zero-order kinetic model [29]. This linear trend is evident after each plateau period, as displayed in Figures 3.1 and 3.2.

As established by the results, the denitrification of effluent with high concentrations of nitrate and nitrite can be achieved. However, extremely high concentrations can be toxic to denitrifying bacteria [29]. As reported by Glass and Silverstein [29], that under some conditions, for complete denitrification, there is an accumulation of extracellular nitrite in pure cultures. The denitrifying bacteria initially transport nitrite intermediate out of the cell, before taking the extracellular nitrite back. This accumulation of nitrite intermediate is inhibitory to denitrification. The batch tests conducted using CGR RAW displayed evidence of nitrite accumulation, which corresponded with the plateau period, suggesting the inhibition of denitrification and associated with system buffering. However at the conclusion of the test, complete denitrification was observed, including both full nitrate and nitrite removal.

The batch tests conducted with a nitrate concentration of $C_o = 500 \text{ mg/l NO}_3$, show positive results. The CGR RAW substrate displayed a minimal plateau, with full denitrification in under 1 day, where the output data suggests minor pH buffering and supports the postulation of adsorption. The CGR 10, displays initial adsorption within

the first 8 hours of the batch, followed by an acclimatisation plateau phase lasting approximately 15 hours, with pH buffering and the establishment of denitrifiers within the system, after which denitrification then follows a fairly linear rate until full nitrate removal within 2.5 days.

In the case of the studies using M.L.S. leachate, both substrates present a drop in nitrate concentration from 3600 to 2000 mg/l NO_3 within the first 24 hours, which can be attributed to nitrate adsorption. Once again an acclimatisation period is present. The duration of each, suggests that it is associated with pH buffering, where the CGR 10 displays a period of 3 days, whilst a longer period of 5 days is required by the CGR RAW. A linear rate of nitrate removal is observed for the fresh substrate, proposing that there are sufficient concentrations of electron donors and acceptors in the form of carbon and nitrogen. The CGR RAW achieves full denitrification within 17 days. The immature CGR 10 demonstrates a stepped evolution of nitrate removal, associated with a staged release of organic carbon, taking 30 days to reach a 0 mg/l NO_3 concentration.

Analysing the COD output values, in the case of the CGR RAW, for both effluents, the 500 mg/l NO_3 and M.L.S leachate a comparable percentage of carbon is consumed, with 19.30% and 18.93% respectively. Similarly the CGR 10 substrate has a reduction in COD of 8.24% and 8.18% from the input values for the 500 mg/l NO_3 and M.L.S leachate respectively. The results of these comparisons are expected, as through the initial characterisation of the materials, it was noted that the CGR RAW has a larger percentage of carbon, readily available for consumption, when compared to the CGR 10 substrate.

3.3. Column Studies

The column studies were designed to simulate the process of a fixed-bed reactor, investigating the effect of flow rates on the efficiency of denitrification. Two experiments, using the two substrates, CGR RAW and CGR 10, were conducted with synthetic nitrate solution with a concentration of 500 mg/l NO_3 and treated nitrified leachate collected from Mariannhill Landfill site's SBR. The initial input conditions for each of the different columns are presented in Table 3.7.

Table 3.7

Initial input conditions of each column

	Experiment 1		Experiment 2	
	CGR RAW	CGR 10	CGR RAW	CGR 10
Nitrate Concentration (mg/l NO ₃)	500.00	500.00	3600.00	3600.00
Liquid Volume (l)	11.00	10.00	13.65	9.65
Solid Substrate (kg)	4.27	11.78	3.61	11.53
Flow (l/day)	6.50	2.00	1.35	0.45
Column volume (l)	20.00	20.00	20.00	20.00
Porosity	0.55	0.50	0.68	0.48
Specific Gravity	0.47	1.18	0.57	1.11

3.3.1. Experiment 1

Columns packed with CGR RAW and CGR 10 respectively were operated with synthetic solution. The CGR RAW column used a flow rate which ensured that the initial input liquid volume was replaced over a period of 2 days, whereas CGR 10 replacement occurred over 5 days. This test was run for 7 and 10 weeks respectively. The nitrate evolution of each column is presented in Figures 3.3 and 3.4.

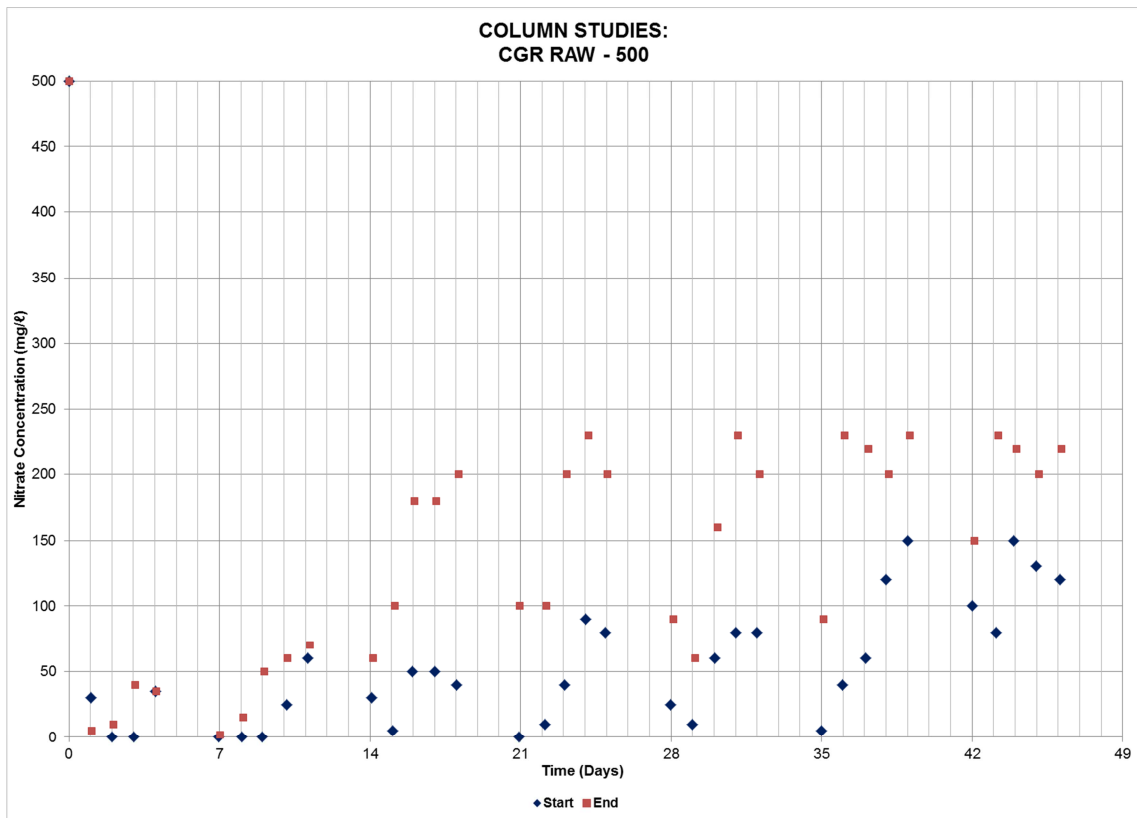


Figure 3.3: Evolution of nitrate concentration for CGR RAW with $C_o = 500 \text{ mg/l NO}_3$.

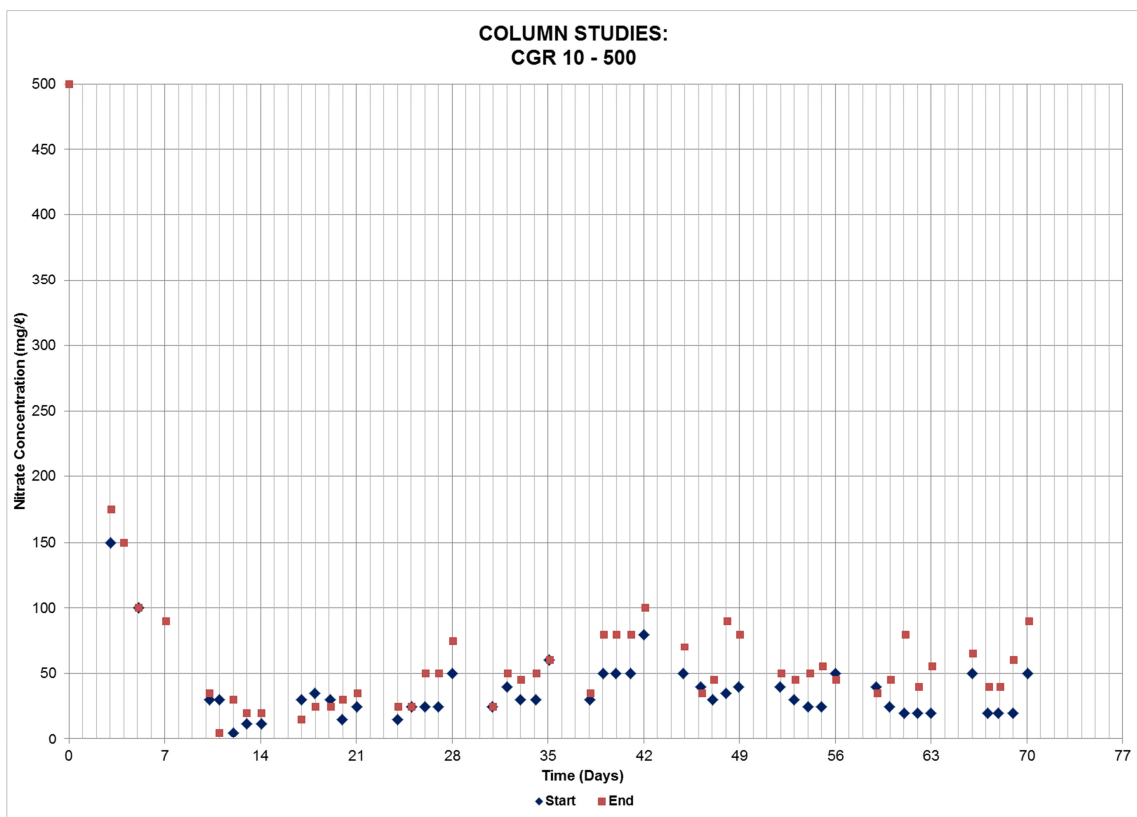


Figure 3.4: Evolution of nitrate concentration for CGR 10 with $C_o = 500 \text{ mg/l NO}_3$.

3.3.2. Experiment 2

The second set of tests evaluated the nitrate removal capabilities of the columns at a high nitrate concentration, using nitrified leachate (M.L.S.) from the SBR at Mariannhill Landfill site. Two columns packed with CGR RAW and CGR 10 substrates respectively were filled with the treated, nitrified leachate. During this test, the entire volume of nitrified leachate was replaced over a 10 day period for CGR RAW and 20 days for the CGR 10. The denitrified effluent was sampled and replaced with treated leachate, over 4 weeks. The nitrate evolution of each column is presented in Figures 3.5 and 3.6.

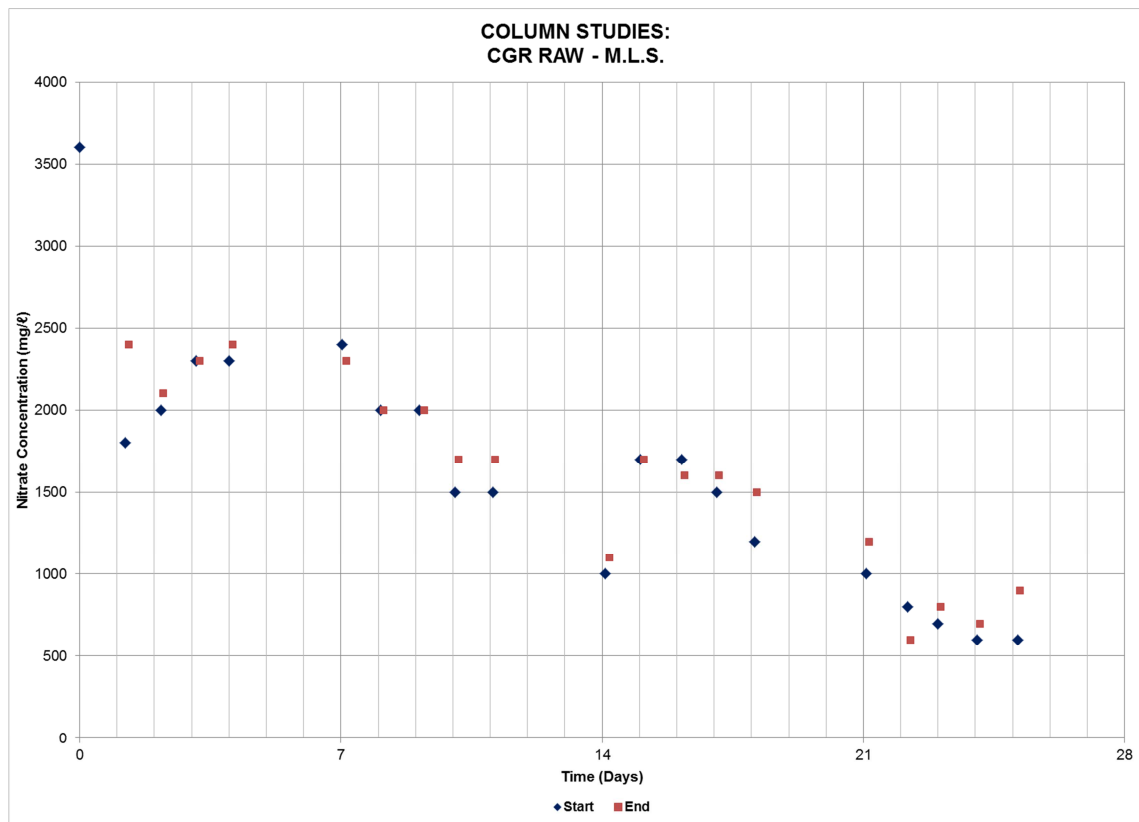


Figure 3.5: Evolution of nitrate concentration for CGR RAW with M.L.S.

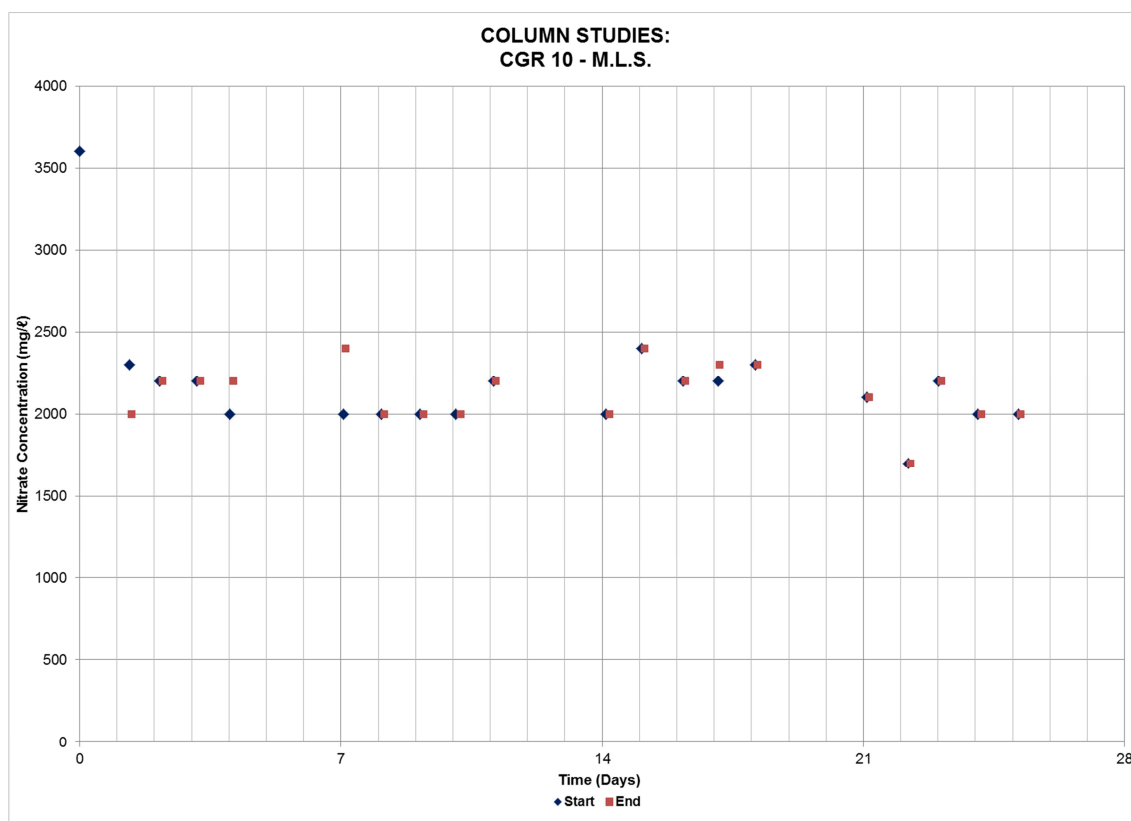


Figure 3.6: Evolution of nitrate concentration for CGR 10 with M.L.S.

The column operational design was based on a plug flow system, with a semi-continuous flow and quasi-saturated state. Leachate was injected over a period of 1 to 2 hours and then remained stationary for the remainder of the day. The experiment was conducted over a 5 day week, with a stagnant period over the weekend. Thus, to evaluate the influence of the piston effect, nitrate concentrations were measured at the start and end of the injection period. This also provided an idea of a nitrate profile across the segment as well as along the interface, whilst examining any apparent effect of advection, diffusion and dispersion. Hence, the reading at the end of injection is the same sample tested at the start of the next day.

Similar to the research done by Tsui, Krapac [5], KNO_3 nitrate solution was utilised in the first experiment. Also, columns were operated with an upstream flow from the bottom up. However, this did cause some of the substrate particles to float towards the top of the column, but they would settle with time over the stagnant periods.

When assessing the column studies conducted with the synthetic 500 mg/l nitrate solution and comparing the two different substrates two trends are particularly evident.

In the case of the CGR RAW material, during the first week, significant denitrification occurs, with full nitrate removal being achieved within 1 day, which is comparable to the batch test results. This can be attributed to the readily available

carbon of the medium, however as the columns are not a closed batch design, any leached carbon that is not consumed or utilised, is flushed out of the system. The output nitrate levels tend to rise during the week, before reducing over the extended stagnant weekend period. Furthermore, a comparison between the observed difference in nitrate readings at the start and end of each injection period shows an increase over the duration of the experiment, which is evident in Figure 3.3. These two observations are a result of the intense flow rate and the resulting diffusion, dispersion and advection. The onset of flow after the weekend causes the nitrate levels to rise during the week through the advection of new solution being added to the system [36]. After week 6, only 80% denitrification is occurring, suggesting that the flow rate is too high for any denitrifying bacteria to establish themselves within the structure of the material, there is less readily available carbon being released and the rate at which the carbon is being utilised is slower than that at which the concentration of nitrates are being added. The decrease in carbon release and resulting lower COD values, combined with the prolonged fast flow rate could have contributed to this reduced efficiency of nitrate removal [32, 36].

The column with immaturesly composted CGR 10 substrate was operated at a slower flow rate. The results displayed in Figure 3.4, suggest that the flow rate chosen for this material is more suitable for denitrification. The observed trend proposes that the system takes approximately 8 days to reach acclimatisation before producing a baseline and fairly steady rate of nitrate reduction achieving more than 90% denitrification. There is a slight reduction in the output nitrate level over the weekend and a minor difference between the readings collected at the start and end of the injection period. This suggests that the onset of flow is less influential on the release mechanisms, such as dispersion and diffusion.

The contrast between the effects of flow for the two substrates can also be attributed to characteristics of the input material, in particular the porosity, specific gravity and particle size [9, 13].

The pH value in the CGR RAW column was initially slightly acidic as expected, but as a result of the buffering, they did rise to a constant level, ranging from 6.10 – 7.96, which is similar to the findings presented by Tsui, Krapac [5] who suggested that with the onset of flow and a result of the denitrification pH levels increased, whilst also making reference to the buffering capacity of the compost material, maintaining a stabilised pH of 7.8, suitable for denitrification. However, in the case of the CGR 10, the pH fell within the range of 7.18 – 8.27 throughout the duration of the experiment.

The results presented in Figure 3.5, where a column packed with CGR RAW operated with M.L.S., the slower flow rate reduces the effect of advection, as well as

allowing a bacterial film to develop, where denitrifying bacteria become established and the efficiency of the denitrification increases. The COD levels of the effluent consistent with those of the input leachate, coupled with the positive nitrate reduction, suggest that a significant portion of the organic carbon released, is being consumed and utilised by the bacteria for denitrification. The system completes one full liquid replacement in 2 weeks, with an estimated HRT within the system being 14 days. In the fourth week of testing, a fairly constant nitrate level is achieved with approximately 80% denitrification.

The least efficient column was the combination of CGR 10 and M.L.S., where less than 50% nitrate removal was observed, with a steady baseline output of 2000 mg/l NO_3 . The readings taken at the start and end of the injection period, show little difference, nor was there any significant change after the onset of flow or after the stagnant weekend period. These observations lead us to believe that there was little to no influence of advection or flow rate on the system. Even after 1 full cycle, there was minor difference in denitrification compared to the first week. As experienced by Tsui, Krapac [5], if a porous material is excessively pack, this could have reduced the hydraulic retention time within the system.

As a result of the input pH value of the Mariannhill leachate together with the relatively slower applied flow rates, the pH levels in the output effluent for both columns were fairly constant ranging from 7.33 – 8.97.

In all the column studies, an initial rapid decrease in nitrate concentration was observed, however after the readily biodegradable carbon source was consumed or leached, the denitrification is then limited by the available electron donors within the system. Tsui, Krapac [5], postulate that the slowly biodegradable substances result in a reduced rate of denitrification through either the process of hydrolysis or fermentation by microorganisms, resulting in a steady state, constant output nitrate concentration. This is particularly evident in the case of the CGR 10 material at 500 mg/l NO_3 as displayed in Figure 3.4. If a flow rate is too fast, the resulting retention time might not be sufficient for microorganisms to become established, accumulate and denitrify the leachate [5, 12, 38].

As in the study conducted by McLaughlan and Al-Mashaqbeh [36], the output levels of dissolved organic carbon were initially high, however did decline over the experiment until a fairly steady state. This decrease in carbon release and resulting lower COD values, combined with the fast flow rate could have contributed to the reduced efficiency of nitrate removal as reflected in Figure 3.3, the column filled with CGR RAW operated with synthetic solution [32].

Although flow increases the release of carbon, this organic carbon does not remain in the system and any that is not consumed, is flushed out resulting in an

increased COD output level. The rate at which nitrate is added to the system should correspond to the release and consumption of organic carbon, thus the optimum level of carbon can be utilised achieving the most efficient denitrification process whilst also reducing the output level of COD.

4. Conclusion

The study looks at the potential of two substrates, fresh and immaturely composted commercial garden refuse, to act as natural organic carbon sources implemented in an ad-hoc bio-denitrification treatment solution to denitrify high strength leachate. The performance of potentially using the substrates in an anaerobic fixed-bed reactor was assessed at laboratory scale.

Small-scale dynamic batch tests and leaching columns were used to simulate a submerged bioreactor. Bio-filtration of wastewater through a packed bed of fixed media, containing immobilised microorganisms allows contaminants to be transferred to the inter surface of the biofilm, attached to the stationary filter medium, degraded by microorganisms, and used as nutrients for microbial growth [39]. Solid carbon materials, such as the different garden refuses used in this research allows for the growth of such a bacterial film on the surface, but sufficient time is required for a significant biomass to accumulate for favourable denitrification results to be achieved [5, 12].

The composition of a filter bed is important as the nature of the particles, including size, permeability and specific surface area to volume ratio will have an impact on the hydraulic properties and rate of organic release [9, 13, 39].

Water circulation is found to improve the organic release and dispersion, however the flow rate and consequent hydraulic retention time is the major factor when designing a bioreactor and the subsequent nitrate removal efficiency [5, 13]. The HRT affects the duration with which the wastewater is within the system and is crucial to the mechanism in which the contact period between the microorganisms and effluent allows for sufficient decomposition of pollutants [38].

The two substrates are suitable filter materials as they have such characteristics as a high specific surface area, permeability and provide sufficient nutrients for microbial growth [39].

Both were able to fully denitrify the two leachates at different degrees of efficiency, where the fresh garden refuse was the most successful, achieving full nitrate removal in the batch tests within 1 and 17 days for the 500 mg/l NO_3 synthetic solution and the nitrified leachate from the Mariannahill Landfill site's SBR respectively. This is

expected due to the substrate having high microbial activity, a high C/N ratio and readily available carbon.

The column study results were less successful. Although denitrification was observed, the degree of efficiency was reduced, not due to an inferiority of either substrate to act as a carbon source, but rather in some instances, an inappropriate flow rate and resulting HRT, was implemented. This may have caused a significant portion of the readily available carbon to be flushed out of system, insufficient contact time between the substrate and effluent, or allow for the establishment and development of denitrifying bacteria. Depending on the design of a constructed wetland system, based on the flow, depth and substrate porosity, the reported HRT range for effective removal of pollutants is between 4 to 15 days [38].

The main concern when using such materials as a treatment method is the release or leaching of organic matter. This in turn increases the concentration of COD in the effluent. As a result, Díaz, García [13] investigated pre-treating the plant substrate, prior to it being used in a reactor for denitrification. This could be a possible solution to extreme COD values, by reducing any excess organic matter being released. A further alternative would be, to initially run the system as a batch reactor to allow acclimatisation and the establishment of denitrifying bacteria, or to recirculate the treated leachate so that the readily available carbon is utilised and consumed efficiently.

After long term operation, organic materials such as the garden refuse and compost, deteriorate, which causes changes to the structure, resulting in compaction, possible hydrophobic surface properties and nutrient depletion [39]. Thus, to maintain the performance and integrity of such biofilter, the media is often replaced after 2 to 3 years operation. As recommended by Hwang, Jang [39], more in depth study of the accumulated loss of carbon through the leachate could be investigated to elucidate the effect of these materials when being used as carbon sources for treatment.

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CHAPTER 4

Curve fitting the evolution of bio-denitrification using Commercial Garden Refuse as a carbon source in small scale closed batch tests: A comparison between different kinetic reaction equations.

ABSTRACT

Predicting the kinetic behaviour of bio-denitrification is a complex and under researched subject, in particular, when organic matter is utilised as a carbon source. This paper investigates the rates of nitrate removal, looking at simple approaches to describe reduction kinetics. Laboratory experiments, in the form of batch tests were conducted, measuring reduction in nitrate concentration over time. Several commonly implemented predictive kinetic equations were derived and evaluated. The experimental bio-denitrification data was plotted and four equations, a First Order, Second Order, simple Elovich and Power were applied and compared, with various degrees of accuracy. A First Order reaction best fitted the nitrate evolution observed, when using CGR RAW, with a kinetic rate coefficient, $k_1 = 5.128 \text{ days}^{-1}$ and a least square value, $R^2 = 0.91$. The CGR 10 results were promising, as all kinetic equations had least square values above $R^2 = 0.93$. However, the First Order kinetic rate coefficient, $k_1 = 1.185 \text{ days}^{-1}$, is significantly slower than that of the CGR RAW, as expected, after comparing the material characteristics. This preliminary investigation provides better insight into understanding the different kinetic reaction rates and predicting the behaviour of bio-denitrification, ascertaining whether simple models could be used to describe the process under these conditions.

1. Introduction

The modelling and predicting of bio-denitrification kinetic behaviour is a complex and under researched subject, in particular, when organic matter is utilised as a carbon source. In such a case, the carbon source acts as an electron donor, driving the microbial activities that support nitrogen removal [1]. Thus, the kinetic processes are critical to the understanding and application of organic media as part of an engineered treatment system for wastewater.

The purpose of this paper is to initially investigate the nature and rates of removal of nitrate from synthetic solutions, looking at simple approaches required to describe removal kinetics, relating to an organic carbon source.

Significant research and work has been conducted on the nitrate removal through ion-exchange, reverse osmosis, adsorption, the use of zero valent iron and permeable reactive barriers, to present a few [2-5]. However there is limited knowledge regarding the kinetics of removal when an organic material is implemented as a treatment method [1, 3, 6]. Many soil chemical phenomena processes have been described, in terms of kinetic equations as well as diffusion models [1, 7, 8].

Models are seen as effective tools in projecting behaviour of organic chemicals, in particular when subject to biodegradation by microorganisms [8, 9]. In a study by Gérard-Marchant, Walter [8], the use of mechanistic, predictive equations were implemented in developing models for estimating the loss of phosphorus from a soil, related to time, where the Elovich equation and power function models, were fitted against experimental data.

Similarly, Aharoni, Levinson [7] investigated a variety of kinetic equations, which could be used to describe the kinetics of soil chemical processes, including zero order, first order, second order, Elovich, fractional power and parabolic-diffusion equations. They suggested that, several of these expressions could equally well describe the kinetics of a specific reaction, related to time-dependent data, however there is no consistent relationship between the equation which presents the best fit and the physicochemical and mineralogical properties of the system to which it is applied.

Further work was performed by McLaughlan and Al-Mashaqbeh [1], whom looked into using a woody material as a filtration media for contaminated water. The quantity and the rate of dissolved organic carbon released were compared and modelled using 4 different equations. These included 2 mechanistically based kinetic models, first and second order, as well as 2 regression methods, notably the Elovich and Power equations. In the derived first and second order equations, the initial concentration, C_0 , is an independent parameter. However, when regression based equations are applied, as in the case of the simple Elovich and Power models, there are no independently measured values and thus all parameters are fitted. As a result, any change in system variables, will produce a different rate constant.

The studies were utilised as guidelines, with the objective of developing simple mathematical expressions to predict nitrate removal applicable to this particular work.

Laboratory experiments, in the form of batch tests were conducted, measuring the reduction in nitrate concentration over a period of time [2]. The readings were presented, and several commonly applied predictive kinetic equations were derived and evaluated, to ascertain if simple models could be used to describe the process under these conditions.

Two readily available organic carbon sources were tested, including commercial garden refuse (CGR) at different degrees of maturity, with a synthetic nitrate solution of 500 mg/l NO_3 . The bio-denitrification data was plotted and four equations, a First-Order, Second-Order, simple Elovich and Power were applied and compared, with various degrees of accuracy.

As proposed by Hekmatzadeh, Karimi-Jashani [2] and McLaughlan and Al-Mashaqbeh [6], an applicable, well-researched, conceptual kinetic model is required to fill a knowledge gap, interpreting a biological denitrification treatment process, when using organic carbon, but also to provide guidance to engineers in designing a reliable solution to predict nitrate removal.

2. Materials and Methods

2.1. Materials

The two substrates used as organic carbon sources for this study included fresh and immaturely composted commercial garden refuse. The fresh commercial garden refuse (CGR RAW) comprised of materials obtained from the maintenance of all “green” areas within the local municipality, where a chipper shreds and reduces the particle size prior to transportation and disposal. The immature compost (CGR 10) consisted of garden refuse which had been composted for 10 weeks in turned windrows.

To assess the efficiency performance for each substrate, focussing purely on the denitrification process, without influence from other compounds, a synthetic nitrate solution of 500 mg/l NO_3 was utilised, thus allowing the preliminary observation and study of the nitrate reduction, kinetic behaviour.

2.2. Laboratory testing.

As presented in Chapters 2 and 3, as well as in Appendix A1, A2, A4 and A5, similar testing procedures were followed. Initially the substrates and their eluates were characterised using standard analytical methods as published by ASTM [10]. Small-scale dynamic batch tests were designed to assess the performance of each substrate at optimum conditions. Reactors were assembled using closed top 1 l, 3 neck bottles. Tests were conducted in triplicate and run until the nitrate concentration reached zero [11].

2.3. Nitrate evolution equations

Chemical reactions and the determination of the related kinetic coefficient rates have been simulated using a large variety of predictive equations [1]. As the batch tests were run until a zero nitrate concentration was reached, a simple first and second order equation would result in an error, as the natural logarithm of zero does not exist. Thus, the equations were refined, such that the derivations resulted in increased accuracy. In this study, the denitrification data was simulated using derivations of 4 reduction (decay functions) equations, including First Order, Second Order, Elovich and the Power law as presented.

First Order:

$$C = C_0 e^{-t/\tau_1} \quad (1)$$

Second Order:

$$C = C_0 \left(1 - t/(t + \tau_2)\right) \quad (2)$$

Elovich:

$$C = C_0 - \left(1/\beta\right) \ln(1 + \alpha\beta t) \quad (3)$$

Power:

$$C = C_0 - At^B \quad (4)$$

Where C_0 is the initial concentration at time zero and C the concentration at any given time (t) in days during the experiment.

The characteristic time constants for the first and second order equations are represented as τ_1 (days) and τ_2 (days) respectively and are fitted parameters related to the kinetic rate coefficients k_1 (days⁻¹) and k_2 (mg/l⁻¹ day⁻¹) [8].

$$\tau_1 = k_1^{-1} \quad (5)$$

$$\tau_2 = (C_0 k_2)^{-1} \quad (6)$$

In the Elovich and Power equations, α (mg/l), β (mg/l day⁻¹), A (mg/l day⁻¹) and B (dimensionless) are fitted parameters. Where β is linked to the initial adsorption rate and α the desorption constant, related to the extent of surface coverage [12].

2.4. Curve fitting

The experimental data was plotted using Microsoft Excel, depicting the relationship between nitrate concentration (mg/l NO₃) within the closed system over time (days). The equations were applied and the outputs compared to the experimental data and plotted visually. As a means to minimise the error between the experimental and simulated data, the simulated concentrations were calculated at the same sampling times as conducted during the experimental measurements.

A Root Mean Square (RMS) calculation was performed to estimate the error between the two sets of data:

$$RMS = \sqrt{\frac{\sum_{i=1}^N (C_{refi} - C_i)^2}{N}} \quad (7)$$

Where C_{refi} is the reference or experimental concentration (mg/l); C_i represents the simulated concentration (mg/l) and N the total number of concentration measurements.

A variety of techniques were implemented to determine the most accurate kinetic constants and other fitted parameters. These included achieving the lowest Root Mean Square (RMS) values, plotting the linear regression, whilst calculating the corresponding coefficients, as well as least squares (R^2 ; 0→1) and the resulting standard errors (SE). A good level of accuracy is represented by $R^2 > 0.9$ [8, 13].

2.5. Assumptions

In this study, a variety of assumptions were made to allow for the simplification of the denitrification process and the application of the kinetic equations. As most water and wastewater treatment reactions are irreversible, it was implied that one phase, with uniform reactants was assumed. Also, that the overall denitrification process is presented without differentiation between, chemical mechanisms as well as sorption and desorption [1].

Usually, an initial buffering phase occurs, during the bio-denitrification process, based on conditions within the system, related to the pH, availability of carbon, competition between denitrifying and nitrifying bacteria, microbial activity as well as the development of microorganisms, resulting in a plateau period being exhibited. However, for this analysis, it is assumed that denitrification begins immediately, at the onset of the experiment at time zero ($t = 0$ days). After which, a singular, consistent reduction rate constant is followed and maintained throughout the period of the

experiment, whereas theoretically the reaction rate within the batch changes, depending on numerous factors, including the buffering effect, staged release of organic matter and availability of carbon [6].

The First and Second Order equations were derived to achieve most accurate results over the duration of the experiment, whereas the Elovich and Power equations were designed and set to achieve a final output nitrate concentration of 0 mg/l at the conclusion of the experiment.

3. Results and discussion

3.1. Summary of the laboratory experiments

The laboratory experiments included both characterisation and batch tests. Prior to use, each substrate was characterised, providing insight into the potential and behaviour of the carbon sources for nitrate removal, before any denitrification experiments were performed. Initially to assess the performance of each material for denitrification, small-scale dynamic batch tests were conducted.

Table 4.1 presents a summary of important parameters used in evaluating the suitability of the two materials. These include the input C/N ratio, pH, COD and BOD₅ as well as the resulting time required for full denitrification from the batch tests.

Biological denitrification requires an external carbon source to act as an electron donor, thus a high C/N ratio is preferable, providing sufficient organic carbon, whilst preventing the accumulation of nitrogen content within the system [14]. However, this carbon needs to be readily available for microbial activity. Thus, the COD is an evaluation of the total organic matter released by the materials, which can be utilised for denitrification. Furthermore, the BOD₅ is an indication of the easily biodegradable content, often in the form of readily available carbon [15, 16].

Another key property is pH, which has a major effect on a large variety of chemical, physical and biological processes. As a result the pH within the system is a crucial parameter for the development of denitrifying bacteria [15, 17].

Table 4.1

Summary of the characterisation and batch tests for each substrate.

	C/N	pH	COD (mg/l)	BOD ₅ (mg/l)	Time (days)
CGR RAW	60.58	5.00	9572±257	834	0.917
CGR 10	13.04	7.52	8360±126	222	2.333

As presented in Table 4.1, both these organic materials have relatively high carbon content, as indicated in the C/N ratio, COD and BOD₅ measurements, thus supporting the appropriateness of the substrates to exhibit high microbial activity and thus denitrification. The considerable difference in the two BOD₅ measurements indicates that the portion of carbon released by the CGR RAW is more highly biodegradable.

The pH of the CGR RAW is acidic with a value of 5.00, but through the composting process, the pH for the CGR 10 was alkaline, at 7.52 [6, 17-19].

Although pH is a limiting factor and an acidic environment inhibits denitrification, the high C/N and in particular the substantially larger BOD₅ value of the fresh CGR, allows for a quick beginning to the denitrification process, suggesting that it would be more reactive than the composted CGR 10, resulting in a significantly faster reaction rate, as evident in the time taken for denitrification, within less than 1 day compared to 2.333 days needed by CGR 10 [6, 20-22].

3.2. Plots (nitrate profiles)

The raw experimental data and the corresponding standard deviations were plotted visually using Microsoft Excel, displaying the relationship between the evolution of nitrate concentration (mg/l NO₃) and time (days). The simulated output concentrations of the four different kinetic models, First Order, Second Order, Elovich and Power, were calculated using the same sampling times as those conducted during the experimental measurements. These simulated concentration results are presented along with their respective trend lines in Figures 4.1 and 4.2.

The batch test performed using the fresh substrate, CGR RAW is displayed in Figure 4.1, whilst Figure 4.2, depicts the results using immature compost, CGR 10 as a carbon source.

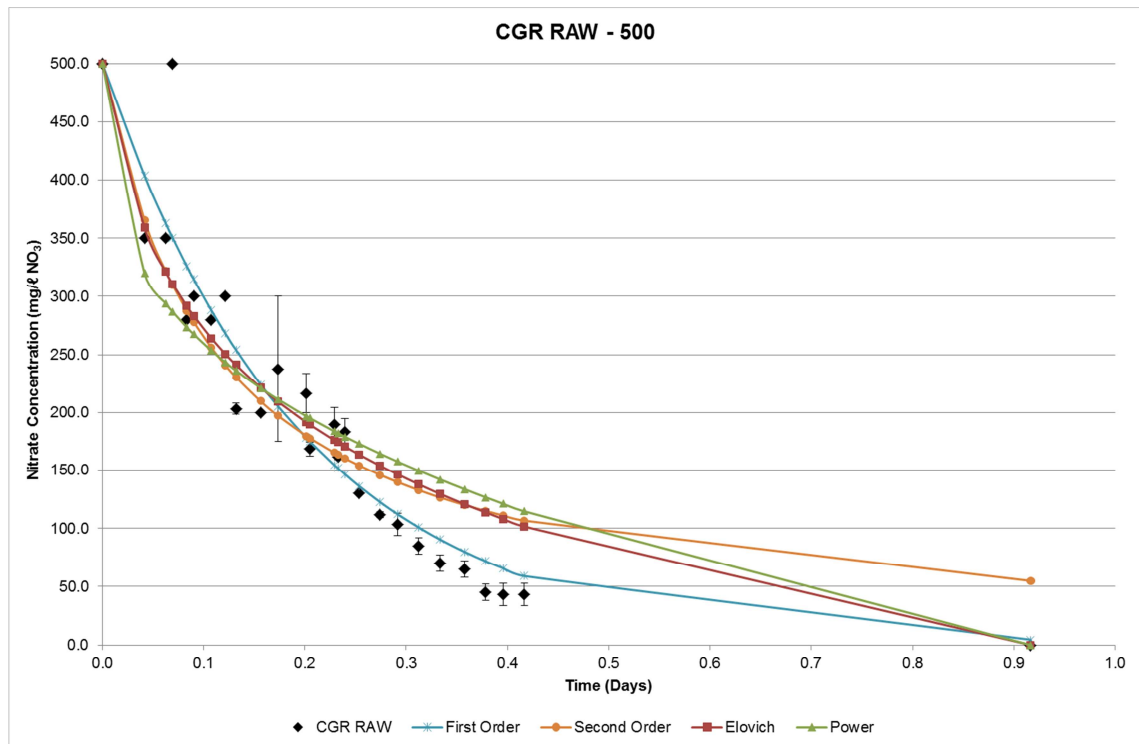


Figure 4.1: A comparison of the kinetic equations for denitrification using CGR RAW.

In Figure 4.1, the raw output concentration data at the different sampling times is presented for the CGR RAW substrate, displaying the nitrate evolution from an initial input concentration of 500 mg/l to full denitrification at 0 mg/l NO_3 . This process takes approximately 0.917 days to be completed. The results are fairly accurate as depicted by the standard deviations, with the largest variance at 0.174 days.

It is evident from the plotted results, that the simulated outputs using the First Order kinetic equation, best fits the experimental data compared with the other models, especially from after 0.25 days. The Second Order, Elovich, and Power equation plots display similar trends up until 0.42 days, where the Elovich and Power equations are set to reach a final 0 mg/l NO_3 concentration. However, the Second Order equation only predicts a final concentration of 55 mg/l NO_3 at the completion of the experiment.

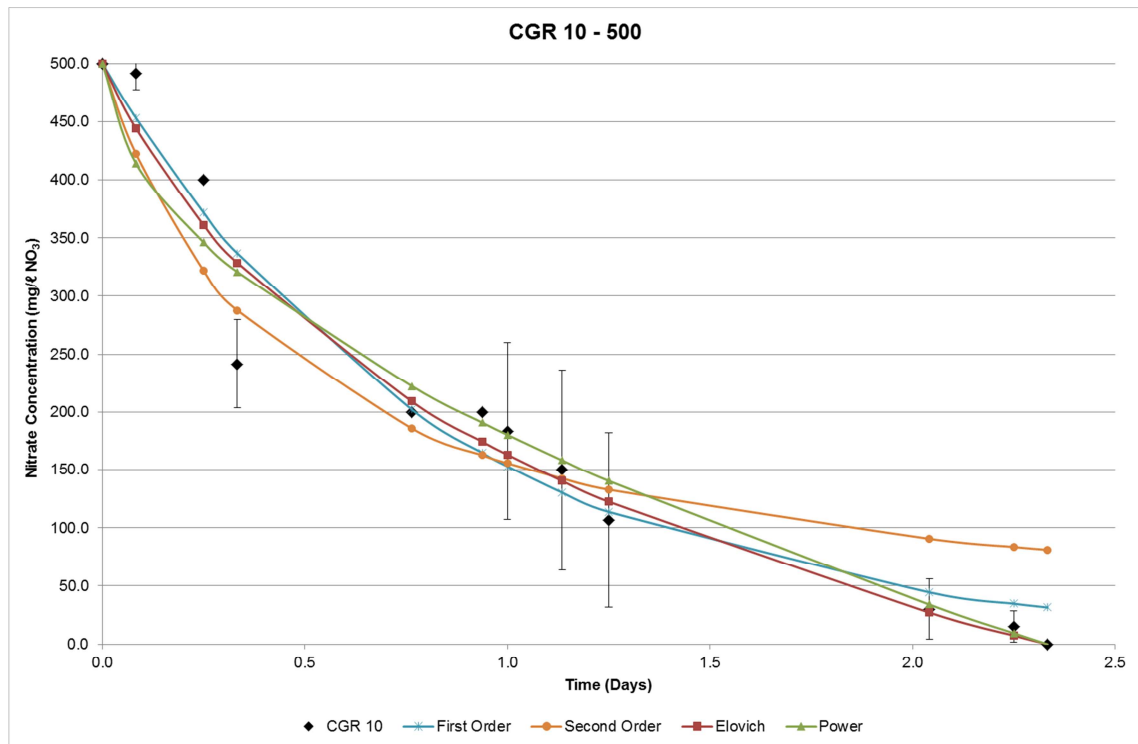


Figure 4.2: A comparison of the kinetic equations for denitrification using CGR 10.

Similarly, Figure 4.2 displays the sampled nitrate concentrations from the batch test conducted with CGR 10 as a carbon source. The raw data is plotted with the standard deviations, from an initial 500 mg/l NO_3^- concentration, over time, until full nitrate removal is achieved. As expected from the characterisation of the separate substrates, the immature compost requires a longer period to reduce the same nitrate concentration, where denitrification takes approximately 2.333 days.

The plots of the 4 equations, simulating the experimental data, display similar trends up until 1.25 days. After which both the First and Second Order tend to diverge away from the experimental data. The graphical results suggest that the modelled outputs produced by the Elovich are the most accurate. However, it must be noted, that both the First and Second Order equations were determined so that the most accurate outputs were obtained over the entirety of the experimental testing period, as mathematically, neither can reach 0 mg/l NO_3^- concentration. Whereas the Elovich and Power equations are set so that full nitrate removal is achieved at the conclusion of the experiment.

3.3. Fitted parameters

The determined fitted parameters for each of the 4 equations as well as the various accuracy measurements are presented in Tables 4.2 and 4.3.

These parameters include the characteristic time constants for the First and Second Order equations, τ_1 (days) and τ_2 (days) respectively, related to the kinetic rate coefficients k_1 (days⁻¹) and k_2 (mg/l⁻¹ day⁻¹).

In the Elovich and Power equations, α (mg/l), β (mg/l day⁻¹), A (mg/l day⁻¹) and B (dimensionless) are the fitted parameters.

The techniques implemented to achieve the most accurate outputs, comprised of the Root Mean Square (RMS) values, plotting the linear regression, determining their coefficients, with the least squares (R^2 ; 0→1) and standard errors (SE).

The fitted parameters were selected based on the lowest achievable RMS value, whilst the R^2 values and coefficients from the linear regression, with the SE, were used to assess the accuracy of the simulated output concentrations compared to the measured empirical data. A good fit is indicated by a $R^2 > 0.9$, with a low RMS and SE.

Table 4.2

The fitted parameters for the kinetic equations using CGR RAW.

	Parameters		RMS	R^2	SE
First Order:	τ_1	0.195	39.23	0.91	37.53
	k_1	5.128			
Second Order:	τ_2	0.113	53.72	0.87	35.34
	k_2	0.0177			
Elovich:	α	6016.39	52.14	0.88	36.05
	β	0.00749			
Power:	A	514.57	61.31	0.84	37.99
	B	0.33			

The results presented in Table 4.2 suggest that, if CGR RAW is used as a substrate for denitrification, the modelled concentrations best simulate the measured output data when the First Order equation is applied, as indicated by the lowest RMS value of 39.23 and the resulting $R^2 = 0.91$. This result is supported by the graphical representation displayed in Figure 4.1. The Power equation has the highest RMS value which corresponds to the lowest least square result of $R^2 = 0.84$. As determined from the linear regression, all 4 equations produce standard errors between 35 and 38 mg/l.

Table 4.3

The fitted parameters for the kinetic equations using CGR 10.

	Parameters		RMS	R ²	SE
First Order:	τ_1	0.844	36.02	0.95	37.00
	k_1	1.185			
Second Order:	τ_2	0.452	50.93	0.93	37.11
	k_2	0.0044			
Elovich:	α	761.04	32.82	0.96	34.60
	β	0.00432			
Power:	A	319.75	37.73	0.95	37.05
	B	0.53			

The determined fitted parameters best simulating the nitrate evolution exhibited by the batch tests conducted with CGR 10 are recorded in Table 4.3. In this case, the empirical output data is best modelled using the Elovich equation, with the lowest achievable RMS value being 32.82, corresponding to a least square, $R^2 = 0.96$ and standard error, $SE = 34.60$ mg/l. All the kinetic equations produce a relatively accurate representation of the measured data with least square values above $R^2 = 0.93$. The fitted parameters determined for the Second Order equation produced the least favourable outcome, only reaching a $RMS = 50.93$, which is consistent with the plot in Figure 4.2, as noted, due to the divergence between the simulated and empirical data after 1.25 days. The standard errors calculated, for all the equations, from the linear regression, fall between 34 and 38 mg/l.

A comparison between the characteristic time constant, τ_1 (days) for the First Order equation and the relating kinetic rate coefficient k_1 (days⁻¹), between the tests conducted with the two different substrates suggest that the CGR RAW is the more reactive of the two materials, with a higher kinetic rate coefficient, $k_1 = 5.128$ days⁻¹, compared to $k_1 = 1.185$ days⁻¹ calculated for the CGR 10.

Similarly, the kinetic coefficients determined for the Second Order equations, suggest that the CGR RAW is more reactive, with $k_2 = 0.0177$ mg/l¹ day⁻¹, whereas CGR 10 presents a slower reaction rate, $k_2 = 0.0044$ mg/l¹ day⁻¹.

These results reinforce the hypothesis as presented in Table 4.1, suggesting that the characteristics of the fresh CGR RAW material cause it to be more reactive than its composted counterpart CGR 10, resulting in a significantly faster reaction rate and a

shorter time required for denitrification. The measured data from the batch tests records that the CGR RAW completes denitrification within 1 day, while 2.333 days are necessary by the CGR 10.

In a similar study, Trois, Pisano [23], investigated denitrification of landfill leachates using compost (C/N = 19.30) as a carbon source. The results were modelled with a first order kinetic equation, with k_1 ranging from 0.118→0.224 days⁻¹.

As presented in previous work (Chapter 2), which looks at the preliminary development of an ADR denitrification model, the most accurate calculated kinetic rate coefficient achieved, for a first order reaction reflected $k_1 = 0.3845$ days⁻¹.

Research done by Reddy, Sacco [13], assessed the nitrate removal capacity of an organic soil, with additional ground plant matter. This energy source presented a C/N ratio of 18.04. They also suggested that the NO₃ - N reduction was best described by a first order kinetic equation, with an average rate constant $k_1 = 0.751 \pm 0.180$ days⁻¹, which is comparable to $k_1 = 1.185$ days⁻¹ calculated for the CGR 10.

Ahmed, Idris [24] reviewed the use of organic mulch for the use in permeable reactive barriers (PRB), for the remediation of contaminated groundwater. A comparison was made between various studies, published in technical literature. The calculated first order decay constant ranged from 0.114→0.230 days⁻¹.

McLaughlan and Al-Mashaqbeh [1] noted, that a solution that best describes time-dependent data may not necessarily be unique. If simple correlation coefficients and standard errors of estimate are used to evaluate the data, a variety of equations could equally well simulate the results [7]. Furthermore, Aharoni, Levinson [7] suggests that there is no consistent relationship between the best fitting equation and the physiochemical and mineralogical properties of the systems to which it is applied.

4. Conclusion

The aim of this study was to provide an initial insight into the kinetic behaviour of denitrification reaction rates, where an organic material is used as a carbon source. Experimental data obtained from laboratory testing was plotted and simulated using a variety of kinetic equations with the purpose of establishing a best fit curve and subsequently, the most accurate variable parameters. These included derivations of First Order, Second Order, Elovich and Power law equations.

A First Order reaction best fitted the nitrate evolution observed, when using CGR RAW as a carbon source. A kinetic rate coefficient, $k_1 = 5.128$ days⁻¹, was determined, with a least square value, $R^2 = 0.91$.

The results obtained using CGR 10 were very promising, where all kinetic equations produced a relatively accurate representation of the measured data with least square values above $R^2 = 0.93$. The calculated First Order kinetic rate coefficient, $k_1 = 1.185 \text{ days}^{-1}$, is significantly slower than that determined for the CGR RAW material, which is expected, after comparing the characteristics of each carbon source.

The next step in the research is to compare results conducted at numerous nitrate concentrations, plotting C/C_0 and investigating the effect that the initial concentration has on the rate of reduction [2, 13].

Possible improvements and/or additions to the testing procedure would be the inclusion and comparison of the amount and rate at which dissolved organic carbon (DOC) is leached by each substrate, using distilled water and its relationship to the nitrate removal rate over the corresponding period.

As solid organic matter has some degree of structure, accounting for the physical and chemical processes affecting chemical concentrations, may improve the quality of kinetic biodegradation models for such aggregates [9].

Furthermore, the inclusion of up-scaled continuous column studies will provide both valuable insight and understanding of the effect that porosity, hydraulic conductivity, dispersion and diffusion would have on the reaction rate.

This preliminary investigation provides good insight into better understanding the different kinetic reaction rates and predicting the behaviour of bio-denitrification.

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CHAPTER 5

DISCUSSION

1. Introduction

The implementation of an integrated waste management system is a multi-disciplinary strategy to deal with the disposal, recycling and treatment of waste, whilst also encouraging the development of solutions that create clean, renewable energy. The promotion of executing this holistic practice is a key objective faced by a range of professionals, in these modern times.

The research conducted in this study has the multi-objective of developing an applicable, economical, easily implementable and sustainable treatment system for the incomplete process design of the Mariannhill Landfill site, Sequencing Batch Reactor plant, where high concentrations of nitrates in the nitrified effluent need to be reduced to below the discharge limits, prior to the release of the leachate into the natural environment. The vision is to implement an ad-hoc bio-denitrification phase, making use of natural organic materials as carbon sources, incorporated in a fixed-bed reactor as an engineering solution, based on the concept of “treatment at source”.

More specifically, the study aims to investigate the efficient reuse of various “green” wastes, to act as carbon sources for the nitrate removal of nitrified landfill leachate, before application in the design of a full-scale treatment system which is to be run in conjunction with the Sequencing Batch Reactor at Mariannhill Landfill site, thus filling a knowledge gap within the field of bio-denitrification.

This final chapter provides a summary of each paper, presenting the significant outcomes and discussing their applicability to the overall research, whilst also offering recommendations and strategies for further research.

2. The development of an optimisation model for bio-denitrification, using natural organic carbon sources as substrates in column studies.

The overall purpose of this research is the design and implementation of an additional low cost, low energy, sustainable process, to denitrify the effluent produced from the Sequencing Batch Reactor, at Mariannhill Landfill site to complete the engineering treatment system, after closure of the site. The solution is to be in the form of a bio-filter, which aims to make use of different organic substrates. The optimal design of such a system is challenging, due to the great variation in substrate

composition. The understanding and study of bio-denitrification behaviour, is crucial in the development of an optimisation model that can accurately simulate the treatment process which is invaluable in designing the final system.

Chapter 2 is an initial look into the development of a preliminary optimisation model, whilst providing a link between previous work and the rationale regarding this research. A classical reactive transfer model approach was adopted and applied to simulate the observed experimental behaviour. Data collected during my Masters, from column studies was utilised in the design and application of an Advection-Dispersion-Reaction model (ADR), to analyse the experiments, while a simulated annealing, optimisation method was programmed to determine the optimal parameters and minimising errors between the empirical data and model outputs. In particular, specific focus was given to the kinetic parameters, biodegradation and the acclimatisation phase associated with the denitrifying microorganisms.

A comparison between three biodegradation reaction kinetics, 0 – order, 1st – order and Monod kinetics, suggested that the three different kinetic reactions, produced similar degrees of accuracy, when used to simulate the behaviour and results obtained from the experimental tests. This is logical, as theory suggests that at low concentrations the Monod relationship displays similar characteristics to that of 1st – order kinetics, while at high concentrations, 0 – order kinetics, which is relevant to this research. The Monod kinetic reaction uses a parameter C_m which is difficult to model, and at high concentrations, a 1st – order curve tends to move away from the other two kinetic reaction plots, which maintain a similar behaviour. Thus, the 0 – order biodegradation reaction kinetics was chosen and optimised with all tests, including different combinations of both concentrations and flow rates, for the 3 substrates. The model outputs were plotted on a curve, with the experimental data, allowing for an evaluation of the accuracy, while also observing the effect of biodegradation. The RMS values provide a representation into the degree of accuracy.

At 500 mg/l, the best RMS result was achieved using the Pine Bark substrate (EXP 500-02-02) with a minimum distance of 52.62, but it did present the longest acclimatisation period, with $t_{acc} = 99843.61$ s. In terms of the reaction kinetics, the fresh CGR was the most efficient of the three substrates at 500 mg/l, obtaining the highest value, $K_r = 1.59 \times 10^{-3}$ mg/l/s, whilst the Pine Bark and CGR 10 displayed similar results.

At the 2000 mg/l concentration the composted CGR 10 produced the minimum RMS value, with an acclimatisation period within three hours, $t_{acc} = 8240.01$ s, while the Pine Bark once again displayed the longest period for acclimatisation during

Experiment 1. The kinetic results remained fairly consistent with those at a concentration of 500 mg/l, where the fresh CGR was the most efficient, achieving a calculated $K_r = 3.35 \times 10^{-3}$ mg/l/s (EXP 2000-01-01).

When modelling each test, the Pine Bark produced a positive simulation with the most reliable RMS values between the experimental data and the model output.

It is noted, that in most cases, an increase in concentration resulted in a faster kinetic reaction rate. This suggests that not only is degree of efficiency related to the substrate and the quantity of readily available carbon, but also the nitrate concentration of the effluent and its supply to denitrifying bacteria.

This investigation also provided insight into understanding the acclimatisation period and its duration. Pine Bark typically displayed the longest acclimatisation period. However, the acclimatisation period is significantly reduced during the second experiment, which is rational, as denitrifying bacteria within the system has had time to colonise and become established.

This preliminary simulation analysis was invaluable, influencing the rationale and progress for further study, as it revealed that the development of an optimisation model, simulating the treatment process is inhibited by the degree of experimental data, thus giving assistance in the selection of substrates, improvement of the experimental procedure and the analysis, resulting in the research which followed.

3. The use of Commercial Garden Refuse at different maturities as a carbon source for the bio-denitrification of treated MSW landfill leachate: A comparison between synthetic nitrate solution and treated Mariannhill Landfill site leachate.

Based on results from previous study and the analysis as presented in Chapter 2, the findings assisted in focusing the work, which included the refinement and modification of the research procedures. The testing process was streamlined, where the 2 best performing substrates were selected from the original 6, the fresh and immaturely composted commercial garden refuse. Commercial garden refuse is disposed of at many local landfill sites and is easily separated from the main waste stream, making the resources readily available and promoting the efficient reuse of waste material.

In Chapter 3 emphasis is placed on characterisation and the assessment of the two substrates ability to denitrify both a synthetic nitrate solution and nitrified leachate. The efficiency of each substrate to support nitrate removal was established using laboratory experiments, which included, characterisation, small-scale dynamic batch and column tests.

The initial step was to fully characterise the solid substrates, their eluates and the treated, nitrified leachate, collected from the Sequencing Batch Reactor at Mariannahill Landfill site. The nitrate evolution from the small-scale dynamic batch tests and column studies were analysed and the performance evaluated, comparing their behaviour with denitrifying synthetic nitrate solution and treated MSW landfill leachate.

As a means to operate the denitrification process under controlled conditions and establish a base line for assessing each substrate, a synthetic nitrate solution was used to simulate treated landfill leachate. In addition, two column studies were also executed with a 500 mg/l concentration of synthetic nitrate solution, to assist in the development of a kinetic model, simulating the nitrate evolution within a reactor. However, the nitrified leachate was used to monitor whether the carbon sources were capable of achieving significant denitrification at extreme nitrate concentrations, while perhaps examining any factors which could inhibit the denitrification rate.

Modifications were made to the design and testing procedures for the column studies, as a means to alleviate past issues, record additional data and increase accuracy. In contrast to previous experiments, leachate was pumped from the bottom of the column upwards, based on the concept of hydraulic change in head, which assisted in reducing substrate compaction and related channelling effect as well as allowing for a more consistent, gradual flow rate over the injection period.

Biological denitrification involves the reduction of nitrates by microorganisms, which require an external carbon source to act as an electron donor. The denitrifying bacteria use labile organic carbon as an energy source. A key factor which dominates the denitrification process is the accessibility and availability of an easily biodegradable organic carbon source, related to the active phase of microbial activity.

Although treated effluent may contain a certain amount of COD, the available organic carbon is often insufficient, particularly in high nitrate wastewater with low BOD content and total suspended solid concentrations, which is the case with the nitrified leachate from the Sequencing Batch Reactor at Mariannahill landfill site, thus additional organic carbon is required to sustain denitrification.

Thus, a material with a high carbon to nitrogen ratio is favourable, providing relatively large amounts of organic carbon, without increasing the nitrogen content. However, high carbon content does not necessarily provide an indication into the availability of the carbon. The C/N ratio in the sampled fresh raw material is within the referred range, with values above 40, suggesting a more woody consistency. However, at approximately 13, the immature compost reflects a C/N ratio more characteristic of stable, mature compost.

A suggestion of the total organic matter released by a substrate which, in turn can be used for denitrification is presented in the form of the COD. However, the measure of BOD identifies whether the organic matter is more easily biodegradable.

Comparison between both the COD and BOD₅ results for the fresh and composted materials provides valuable insight into the characteristics of the substrates. The COD values for the fresh green waste fall in the range of 5900 to 9500 mg/l while in the region of 8500 mg/l for the composted garden wastes. The characteristic instability and heterogeneity of the fresh material compared to that of the immature composts contribute to the variance in COD values.

However, in this study, the BOD₅ results for the fresh CGR are significantly higher than the composted material, which is expected as it has not undergone any stabilisation and has therefore a higher portion of biodegradable matter. The high BOD₅ results of both substrates, suggest that they are suitable for bio-denitrification by sustaining high microbial activity.

pH is a vital parameter as it has a dominant effect on numerous chemical, physical and biological processes. The fresh CGR has an acidic pH below 5, however through composting the pH rises to alkaline levels to approximately 7.5. As pH is a limiting factor and an acidic value has an inhibitory effect on denitrification, a buffering period can be expected for the CGR RAW, whereas the CGR 10 already falls within the prescribed optimum range.

In the closed batch tests, denitrification is dependent upon the initial conditions within the system. The process is influenced by variable changes over the course of the evolution, as some microbial populations proliferate, whilst others are suppressed.

The batch tests conducted with a nitrate concentration of $C_0 = 500 \text{ mg/l NO}_3$, show positive results. The CGR RAW substrate displayed a minimal plateau, with full denitrification in under 1 day. The CGR 10 presented an acclimatisation plateau phase lasting approximately 15 hours, after which denitrification follows a fairly linear rate until full nitrate removal within 2.5 days.

In the studies using M.L.S. leachate, both substrates present a drop in nitrate concentration from 3600 to 2000 mg/l NO₃ within the first 24 hours, followed again by an acclimatisation period, the duration of which is linked with pH buffering, where the CGR 10 displays a period of 3 days and a longer 5 day period by the CGR RAW. Denitrification follows, with the CGR RAW achieving full nitrate removal within 17 days and the immature CGR 10 taking 30 days.

A comparison of the different batch tests conducted, indicate that both substrates exhibited pH buffering, with the two different effluents. In particular, a plateau period is clearly evident when the M.L.S is denitrified by the CGR RAW. Furthermore, as is

characteristic with denitrification, all pH values increased from the initial input values over the duration of the tests.

At high nitrate concentrations, once the readily available biodegradable organic carbon is leached into the system and consumed, the denitrification process is then limited by the organic matter electron donors.

In the obtained results it is apparent that the more mature substrate had a lower nitrate removal rate, which can be attributed to the difference in the availability of carbon and microbial activity.

The CGR RAW and CGR 10 both display similar microbial activity, however due to the substantial difference in available biodegradable matter as presented in their respective BOD_5 values, the fresh CGR RAW material fully denitrifies both effluents substantially faster than its composted counterpart, CGR 10, which is reflected in the time for each to achieve full nitrate removal.

The process of a fixed-bed reactor was simulated through column tests, where the revised experimental design allowed for better monitoring and observation of denitrification efficiency and the effect flow rates have on the system.

When assessing the column studies conducted with the synthetic 500 mg/l nitrate solution and comparing the two different substrates two trends are particularly evident. In the case of the CGR RAW material, during the first week, significant denitrification occurs, with full nitrate removal being achieved within 1 day, which is comparable to the batch test results. However, after week 6, only 80% denitrification is taking place, which suggests that the selected flow rate is too high. The column with immaturely composted CGR 10 substrate was operated at a slower flow rate and results suggest that it was more suitable for nitrate removal. The observed denitrification trend suggests that the system requires approximately 8 days to reach acclimatisation before achieving a fairly steady and constant rate of nitrate reduction of more than 90% denitrification.

The results obtained when the column filled with CGR RAW was operated with M.L.S. were more favourable. A slower flow rate was implemented where the system completes one full liquid replacement in 2 weeks, with an estimated HRT being 14 days. After 4 weeks of testing, a fairly constant nitrate level is accomplished with approximately 80% denitrification. The least efficient column was the combination of CGR 10 and M.L.S., where less than 50% nitrate removal was observed, with a steady baseline output of 2000 mg/l NO_3 .

The different garden refuses, used as solid carbon materials in this research, allow for growth of a bacterial film within the structure and on their surface, but require sufficient time for a significant biomass to accumulate and accomplish significant

denitrification. The columns are not a closed batch design, thus any excess carbon leached, not utilised for nitrate removal is flushed out of the system.

An initial rapid decrease in nitrate concentration was observed in all the column studies, but once the readily biodegradable carbon source was consumed or leached from the system, denitrification is restricted by the remaining available electron donors. Over the testing period, release of readily available carbon is reduced, and being utilised at a slower rate than which with the concentration of nitrate was added. This decrease in released carbon which is evident through the resulting lower COD values, combined with the prolonged flow rate, causes a decrease in denitrification efficiency.

At the slower flow rate the effect of advection is reduced, allowing for the development of a bacterial film and the establishment of denitrifying microorganisms resulting in an increased efficiency of denitrification. However, at the faster flow rate, the retention time was insufficient for bacteria to become established, accumulate and denitrify the effluents.

The research conducted in this study, suggest that the two substrates have favourable characteristics, including a high specific surface area and permeability, whilst also providing sufficient nutrients for microbial growth, making them suitable to act as a filter medium.

In the batch tests, the materials were able to fully denitrify both the synthetic solution and nitrified leachate at different degrees of efficiency. Nitrate removal with the column studies was less successful. Although denitrification did occur, a reduced degree of efficiency was observed. This is not attributed to an inferiority of the substrates to act as carbon sources, but rather that, in some instances, an inappropriate flow rate and resulting hydraulic retention time was implemented. The studies reveal that the fresh garden refuse was the more successful of the two substrates, which is expected due to its higher microbial activity, C/N ratio and readily available carbon content.

It is found that circulation does improve organic release and dispersion; however the implemented flow rate and consequent hydraulic retention time is the key factor in the design of a bio-filter and the subsequent efficiency of nitrate removal. The hydraulic retention time is crucial to the mechanism in which the contact period between the microorganisms and effluent allows for sufficient decomposition of pollutants, as it effects the duration that the wastewater is in the treatment system.

Although flow increases the leaching of organic matter, in this setup the released carbon does not remain in the column and any surplus, is removed with the effluent, causing increased output levels of COD.

Consequently, the treatment design should allow for the rate at which nitrate is added to correspond with the release and utilisation of organic carbon, thus an optimum level of carbon consumption and the resulting most efficient denitrification process being achieved, whilst also decreasing the COD output level.

The results provide evidence that the denitrification of effluent with high concentrations of nitrate and nitrite can be achieved. The conducted testing establishes that both these biodegradable carbonaceous naturally organic substrates have the potential to act as carbon sources to denitrify high strength leachate.

4. Curve fitting the evolution of bio-denitrification using Commercial Garden Refuse as a carbon source in small scale closed batch tests: A comparison between different kinetic reaction equations.

Although there has been significant research and work conducted on nitrate removal, there is limited knowledge concerning reduction kinetics related to organic materials. Kinetic processes are critical to the understanding and application of such a medium as part of an engineered treatment system for wastewater. The modelling and predicting of bio-denitrification kinetic behaviour is a complex and under researched subject, in particular, when the organic matter is implemented as a carbon source. Models are seen as effective tools in projecting behaviour of organic chemicals, especially when subjected to microbial biodegradation.

Chapter 4 presents an initial investigation into the nature and rates of nitrate removal, looking at simple approaches to describe denitrification reaction kinetics.

A variety of studies were researched and used as guidelines, with the objective of developing simple mathematical expressions to predict nitrate removal specifically applicable to this work.

Laboratory experiments, in the form of batch tests as presented in Chapter 3, were conducted, observing the evolution of nitrate reduction over time. The two commercial garden refuse materials, run with 500 mg/l synthetic nitrate solution, were used to assess the efficiency performance for each substrate, whilst focussing purely on the observation and study, of the denitrification process and nitrate reduction kinetic behaviour, without influence from other compounds.

To allow for the simplification of the denitrification process and application of the kinetic equations a variety of assumptions were made. Firstly, the denitrification process is presented without differentiation between, chemical mechanisms or sorption and desorption. Secondly, it was assumed that denitrification begins immediately, at

the onset of the batch test at time, $t = 0$ days. Finally, a singular, consistent reduction rate constant is followed and maintained throughout the entire nitrate removal period.

The experimental bio-denitrification data was plotted and simulated using a variety of kinetic equations with the purpose of establishing a best fit curve and consequently obtaining the most accurate variable parameters. The four predictive kinetic equations evaluated, included derivations of a First Order, Second Order, simple Elovich and Power law function.

As a means to determine and select the most accurate kinetic constants and other fitted parameters, numerous techniques were implemented. These comprised of achieving the lowest Root Mean Square (RMS) values, whilst the linear regression, corresponding coefficients as well as least square values (R^2) and the resulting standard errors (SE), were used to assess the accuracy between the simulated output concentrations and measured empirical data. A good fit is indicated by a $R^2 > 0.9$, with a low RMS and SE. To minimise errors between the experimental and modelled outputs, the simulated concentrations were determined at the same sampling times as performed during the experimental measurements.

The results suggest that when using the CGR RAW substrate, a First Order reaction best fitted the observed nitrate evolution, with a calculated kinetic rate coefficient, $k_1 = 5.128 \text{ days}^{-1}$ and a least square value, $R^2 = 0.91$.

The CGR 10 substrate produced very promising simulation results, where all kinetic equations obtained a relatively accurate representation of the measured data having least square values above $R^2 = 0.93$. However, a significantly slower First Order kinetic rate coefficient, $k_1 = 1.185 \text{ days}^{-1}$ was determined, which is expected, after comparing each carbon sources characteristics.

This preliminary investigation provides good insight into better understanding the different kinetic reaction rates and predicting the behaviour of bio-denitrification, whilst establishing whether simple models could be used to describe the nitrate removal process under these conditions.

An applicable, well-researched, conceptual kinetic model is required to fill a knowledge gap, interpreting a biological denitrification treatment process, when using organic carbon, but also to provide guidance to engineers in designing a reliable solution to predict nitrate removal.

5. Recommendations

The scope of this research allows for further study in a variety of different avenues. The enhanced data produced from modified column experiment testing procedure are to be implemented in the development and refinement of the ADR model, based on work as presented in Chapter 2, providing greater insight into the influence porosity, hydraulic conductivity, dispersion and diffusion have on the simulation.

Furthermore, the inclusion of the up-scaled continuous column studies could be employed to provide additional information for improving the derivation and evaluation of different kinetic equations, to best fit observed empirical data, as done in Chapter 4. Supplementary tests should be conducted at numerous nitrate concentrations, investigating the relationship between the initial concentration and rate of nitrate reduction.

More in depth study of the accumulated loss of carbon through the leachate could be investigated, via the inclusion of testing, comparing the quantity and release rate of organic carbon (DOC) from each substrate with distilled water, to the nitrate removal rate over the corresponding period, which would also assist in contributing vital knowledge to the research process.

As the main outcome of this project is the implementation of a sustainable ad-hoc treatment system for the reduction of high nitrate concentrations in the nitrified effluent from the Mariannhill Landfill site, Sequencing Batch Reactor plant, the final step would be, the design, construction and monitoring of the actual continuous flow, submerged horizontal constructed wetland, fixed-bed reactor.

The main concern for using a plant substrate in such a treatment method is the release or leaching of organic matter, which in turn increases the COD concentration in the effluent. Pre-treating the material, prior to use in a reactor could be a possible solution to extreme COD values, reducing excess organic matter being released. Another alternative would be, to run the system initially, as a batch reactor, allowing denitrifying bacteria to become acclimatised and established, or to recirculate the denitrified treated leachate so that any additional readily available carbon in the effluent is efficiently utilised and consumed.

In long term operation, solid organic materials such as garden refuse deteriorate, causing a degree of change to its structure, which leads to compaction, potential hydrophobic surface properties and nutrient depletion. Therefore, to maintain the integrity and performance of such a bio-filter system, the medium is often replaced after 2 to 3 years operation.

APPENDIX A

APPENDIX A1

Bio-Denitrification of High Strength Landfill Leachate Using Garden Refuse and Pine Bark As Carbon Sources

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EXECUTIVE SUMMARY

Landfill leachate, a toxic by-product formed through the decomposition of organic matter, is harmful to the environment and human health. After nitrification, the concentration of nitrate in discharged leachate may still present a potential threat to the environment. Further denitrification is required to reduce the high concentrations of nitrates in the nitrified effluents to below discharge limits. In the city of Durban (South Africa) municipal solid waste landfill leachate is currently nitrified in Sequencing Batch Reactor plants. After closure of the landfills (in one case expected in 2012) the effluents from the plant will not comply with discharge limits, requiring an ad-hoc treatment. Denitrification, the conversion of nitrates to nitrogen gas, occurs in the presence of a carbon source in an anaerobic environment. Expensive methods are currently employed worldwide; however these tend not to be a viable solution for developing countries. This investigation aimed at identifying an efficient, cost effective, feasible alternative to expensive easily biodegradable carbonaceous materials such as methanol, promoting the use of natural organic sources such as pine bark and garden refuse. These organic substrates contain relatively high amounts of carbon and are readily available in the major Durban landfills. The suitability of two organic substrates as carbon sources for denitrification was assessed using characterisation tests, small-scale batch tests and larger scale columns. The preliminary stage of the research was to comprehensively characterise the substrates (commercial garden refuse and pine bark) through conventional testing done on both the solid substrates and their eluates. The batch tests were conducted at 3 nitrate concentration levels: 100, 500 and 2000 mg NO₃-N/ℓ. A synthetic nitrate solution was used to simulate the treated landfill leachate. The substrates tested in batches were then selected for large-scale experiments in columns at two nitrate concentrations (500 and 2000 mg/ℓ) and at two

different flow rates. Finally durability tests were conducted on previously used substrates of pine bark and immature compost to determine the period for which the substrates could be used as a means for denitrification before replacement was necessary. The CGR RAW substrate had the highest carbon to nitrogen ratio of 90.19 and although the pH value of 5.45 falls just outside the optimum range for denitrification of 6 – 8, it was expected that this would be the best performing substrate. The best performing substrate was the CGR RAW, which achieved full denitrification at the highest nitrate concentration of 2000 mg/l between 9 – 12 days. The column tests reflected promising results at $C_o = 500$ mg/l during experiment 1, with all 3 achieving full denitrification. Once again the CGR RAW substrate columns reflected the best results. The column at 500 mg/l displayed a HRT of 8.06 days was required whereas the higher concentration of 2000 mg/l required a HRT of 8.40 days. During experiment 2, the CGR RAW substrate column at 500 mg/l was the only one to achieve 100% nitrate removal. A HRT time required for full denitrification is less than 3.54 days. The results of this investigation were modelled to inform the design of a bio-denitrification system. This paper presents an efficient, cost effective, feasible alternative to expensive methods by promoting the use of natural organic sources such as pine bark and garden refuse as carbon sources for bio-denitrification.

1. Introduction

Landfill leachate, which is a toxic by-product formed through the decomposition of organic matter, is harmful to both the environment and human health. After nitrification, the concentration of nitrates in the discharged leachate may still present a potential threat to the environment. Further denitrification is often required to reduce the high concentrations of nitrates in the nitrified effluents to below the discharge limits. The eThekweni Municipality is currently nitrifying leachate from the Mariannhill Landfill site in a Sequencing Batch Reactor plant. The treated effluent is then used as dust suppressant. The typical ranges of nitrate concentrations (Nitrate + Nitrite mg NO_3/l) displayed by the treated landfill leachate produced by the Sequencing Batch Reactor (SBR) at the Mariannhill Landfill site are between 8 – 2120 mg NO_3/l . After closure of the landfill (expected in 2012) the effluents from the plant will not comply with the discharge limits of wastewater into a water resource, as enforced by DWAF with a General Limit of 15 mg NO_3/l and a Special Limit of 1.5 mg NO_3/l (DWAF - General Authorisations in terms of Section 39 of the National Water Act, 1998). Thus an ad-hoc treatment will be required.

Biological denitrification, the conversion of nitrates to nitrogen gas, is facilitated by microbes. The micro-organisms capable of reducing nitrates require the presence of an external carbon source as an electron donor, usually in an anaerobic environment (Ovez et al., 2006). Expensive easily biodegradable carbonaceous materials are currently employed around the world (methanol, ethanol etc.); however these methods tend not to be a viable solution for developing countries and are not suited for large scale, field applications (Tsui et al., 2007; Volokita et al., 1995)

This investigation aimed at identifying an efficient, cost effective and feasible alternative to expensive easily biodegradable carbonaceous materials, that promotes the use of natural organic resources such as pine bark and raw and composted garden refuse and that are suitable for large scale, field application. These organic substrates contain relatively high amounts of carbon and are readily available in the major eThekweni landfills.

The investigation of the efficiency, performance and feasibility of nitrate removal using substrates in the denitrification process was conducted by means of laboratory testing. The selection of substrates was based on their suitability as natural organic carbon sources and their availability locally. Thus pine bark, commercial and domestic garden refuse at different degree of maturity (fresh and composted) were selected for bio-denitrification.

The suitability of these substrates as carbon sources for denitrification was assessed using characterisation tests, small batch tests and larger scale columns. The leaching column studies were set up to accurately simulate fixed bed reactors (Tsui et al., 2007; Diaz et al., 2003; Volokita, 1995).

2. Materials and Methods

2.1. Materials

This investigation involved the denitrification of treated landfill leachate using organic carbon sources. The leachate was simulated using a synthetic solution so as to operate the denitrification process in controlled conditions and to eliminate the disturbances in the nitrate (NO_3) analysis due to the presence of chlorinated compounds in the leachate, as experienced in previous studies (Pisano, 2007). The substrates investigated in the research were garden refuse and pine bark at different

levels of stability and maturity (Adani et al., 2001; Gomez, 2006; Adani et al., 2006): fresh pine bark (PB) and fresh commercial garden refuse (CGR RAW).

A large quantity of pine bark is produced every day at the SAPPI (South African Pulp and Paper Industry) paper mills around the country. The trees grown by SAPPI are mainly of the *Pinus patula* variety. The pine bark used in this research is from the tissue/cells outside of the vascular cambium of the hard pine, *Diploxydon* tree. Some of the pine bark is disposed of at local landfill sites as well as SAPPI's disposal facilities. The pine bark used in this investigation was collected, fresh, from SAPPI within 24 hours of debarking.

A large amount of garden refuse is disposed of at both the Mariannhill and the Bisasar Road Landfill-sites in Durban separated from the main waste stream. Commercial garden refuse consists mainly of branches and plant trimmings from parks and green municipal areas. At the Bisasar Road Landfill, the CGR is passed through a chipper to reduce the particle size to approximately 4 – 5cm length and then composted in turned open windrows. The CGR sample was collected from the landfill soon after the size reduction phase.

2.2. Characterisation tests

The preliminary stage of the research was to comprehensively characterise the substrates through conventional testing done on both the solid substrates and their eluates through the use of standard analytical methods as published by ASTM (2008). The following tests were conducted on the solid substrates: moisture content, Total and Volatile Solids (TS and VS), carbon to Nitrogen Ratio (C/N) and Dynamic Respiration Index at 7 days (RI₇) that was determined using a respirometric system type OxiTop®. The RI₇ expresses the rate at which oxygen is consumed in the biodegradation of organic matter and is often used as a means to define the level of stability and biodegradability of fresh and composted garden refuse (Adani et al., 2001; Gomez, 2006; Adani et al., 2006). The eluates of the substrates were tested to determine the nature as well as the amounts of compounds released by the substrates whilst being in contact with water. The eluates were prepared by mixing a representative sample of each of the substrate with distilled water at a liquid to solid ratio of 10:1. These samples were then placed on a shaker for 24 hours. The samples were then filtered through a 63 micron sieve to obtain the eluate. The eluates were tested to determine: pH,

conductivity, TS, VS, COD, BOD, NH_3 and NO_3 . All tests were conducted in double or triplicate to ensure accuracy and repeatability.

2.3. Batch tests

The suitability of the above substrates as carbon sources for denitrification was assessed using small-scale batch tests, which were conducted at 3 different nitrate concentrations: 100, 500 and 2000 mg NO_3 / ℓ simulated using a synthetic nitrate solution. A blank control test (0 mg NO_3 / ℓ) was conducted using distilled water for each substrate. The batch tests were designed to determine the kinetics of removal of each substrate at optimal conditions, which were maximum contact between substrate and solution, a pH range between 6 to 8 and at a temperature of approximately 25°C. A Liquid to Solid ratio of 10:1 was used for all tests to ensure full saturation.

All tests were conducted in duplicate or triplicate in closed top batch reactors consisting of 1 ℓ , 3 neck bottles equipped with two airtight silicone septa which allowed continuous sampling thus preventing any ingress. Each bottle was filled with 100g dry matter of substrate and respective concentration of potassium nitrate solution (KNO_3). The substrate particles were cut and reduced to a standard size of 4 – 5 cm to ensure homogeneity of the sample. Prior to adding the nitrate solution, the bottles filled with substrate, were flushed with nitrogen gas to ensure the immediate establishment of anaerobic conditions in the vessels.

The batch reactors were placed in a shaker at 150 rpm at a controlled room temperature of approximately 25°C. Small samples of approximately 1-5 mL were extracted using a gas tight syringe so as to test the nitrate concentration (NO_3) after 5, 10, 15, 30 and 60 minutes during the first hour of testing and every hour after that for the first day, thereafter 3 times a day usually every 3 hours depending on any changes in nitrate concentration. This method of extraction was performed in order to not significantly affect the L/S ratio in the reactors and to ensure that full saturation was maintained throughout the experiment. Nitrate concentrations for the batch tests were determined using the Nitrate Test Sticks type Merckoquant (MERCK). In some instances, the amount of fines in the tests prevented an accurate reading on the nitrate sticks. Thus some of the samples were filtered using a 0.45 μm .

The batch tests were conducted until the nitrate concentration reached zero. At the end of the test, both liquid and solid samples were characterised as described at point 3.2.

2.4. Microbial tests

Microbial analyses were also conducted by De Combret (2009) for the batch tests at 500 mg/l in order to monitor and assess the effect of the different substrates on the evolution of indigenous bacterial population during bio-denitrification. The growth of the microbial community was followed using a spread plate enumeration technique; the colonisation of the substrates was assessed through Environmental Scanning Electronic Microscopy (ESEM), and an insight into the composition of the bacterial community was determined by phylogenetic analysis (Trois et al., 2010).

2.5. Column tests

Two different experiments were conducted using the columns to investigate the effect of denitrification rates for different nitrate concentration levels and flow rates. These results were used to determine the kinetics of removal, loading rates and hydraulic retention time for the filter beds.

Two nitrate concentrations (500 and 2000 mg/l) and two different flow rates as seen in Table 1, were used for the column campaign. These concentrations were chosen as a result of the typical ranges of nitrate concentrations displayed by the treated landfill leachate produced by the Sequencing Batch Reactor (SBR) at the Mariannhill Landfill site.

2.5.1. Equipment

The columns were constructed using a transparent PVC cylindrical body, plastic flanges with valves, rubber gaskets (seals) and stainless steel bolts.

Characteristics of the columns:

The transparent PVC cylindrical body was 1 m in length, 160 mm in diameter and had an approximate volume of 20 litres. Three ports were also installed along the length of the columns to allow sampling to occur throughout the length. A Perspex diffuser was made and fitted in the top of each column to ensure that the solution was distributed throughout the entire girth. The upper and lower ends of the columns were closed using two pairs of 25 mm thick and 280 mm in diameter plastic flanges. A 20 mm rubber gasket was placed between each of the flanges using a silicon gel to ensure an airtight fit. The other end of each of the flanges were then bolted together using stainless steel bolts. The column was then bolted to a steel frame. The upper flange

consisted of two orifices. The first is a tap valve which allows the nitrate solution to be poured into the column. The second is connected to a small plastic pipe which is used to measure the biogas production. The lower flange has only the outlet orifice. This tap valve is connected to a pipe which allows the column to be drained and the effluent collected. A coarse filter and a layer of marbles were placed at the bottom of each column to provide a drainage layer, thus preventing any substrate from obstructing the outlet.

2.5.2. Experiment 1

For the initial experiment the columns were filled with a 500 mg/l and 2000 mg/l nitrate solution respectively. The experiment was designed to assess the nitrate removal capabilities of the substrates at a relatively low flow rate.

It was decided that the entire volume of nitrate solution should be replaced over a 5 day period. Thus 1/5 of the initial input volume of nitrate solution was sampled from the bottom of the column every day and replaced with the nitrate solution. The first litre of effluent was discarded as it would not have been in contact with the substrate but rather with the marble filter. The effluents were analysed for NO_3 , DO, pH and temperature daily and for COD and NH_3 once a week. This test was run for a 4 weeks.

2.5.3. Experiment 2

This experiment was performed to investigate the nitrate removal capabilities of the columns at a high flow rate. The columns were thus drained of their effluent and filled with the same concentrations of nitrate solution as used in Experiment 1 until the substrates were covered.

It was decided that the entire volume of nitrate solution should be replaced over a 2 day period. Thus 1/2 of the initial input volume of nitrate solution was sampled from the bottom of the column every day and replaced with the nitrate solution.

Once again the first litre of effluent was discarded as explained in Experiment 1. As in Experiment 1, effluents were analysed for NO_3 , pH and temperature daily and for COD and NH_3 once a week. The DO test was not used in this experiment as accurate readings could not be obtained due to the turbulent flow at which the effluent was collected from the columns. This test was run for a 4 weeks. The test was prolonged to ascertain the affect the previous flow rates had had on the substrates. The columns

were thus left in flooded conditions for a period of 1 week. The nitrate levels were tested every day.

3. Results and discussion

3.1. Characterisation of substrates

Table 1

Summary of column operating conditions

Substrate	NO ₃ Concentration (mg/l)	Duration (Weeks)	Flow Rates (l/day)	
			Experiment 1	Experiment 2
CGR RAW	500	4	2.48	5.625
PB	500	4	2.00	5.00
CGR RAW	2000	4	2.38	5.65
PB	2000	4	2.00	5.00

Table 2

Initial input conditions of each column (2000 mg/l)

Column Input (2000 mg/l)	CGR RAW (kg)	PB (kg)
Total input mass	2.800	3.477
Moisture Input	1.040	1.698
Dry Mass	1.760	1.779
Added Nitrate Solution	11.900	10.000
Total Moisture	12.940	11.698
L/S Ratio	7.35	6.58

Table 3

Initial input conditions of each column (500 mg/l)

Column Input (500 mg/l)	CGR RAW (kg)	PB (kg)
Total input mass	2.731	3.422
Moisture Input	1.014	1.672
Dry Mass	1.717	1.750
Added Nitrate Solution	12.400	10.000
Total Moisture	13.414	11.672
L/S Ratio	7.81	6.67

Total input mass = Moisture Input + Dry Mass

Total moisture = Moisture Input + Added Nitrate Solution

L/S Ratio = Total Moisture/ Dry Mass

Table 4

Characterisation of the solid substrates

	MC (%)	TS (%)	VS (%)	RI7 (mg O ₂ /g DM)	Tot C (%)	Tot N (%)	C/N
CGR RAW	37.14 ± 3.17	62.86 ± 3.17	96.37 ± 0.75	7.770	49.6	0.55	90.19
Pine bark	48.85 ± 2.92	51.15 ± 2.92	97.08 ± 0.17	17.769	36.67	0.59	62.15

Table 5

Results of the eluate tests

	TS (g/l)	VS (g/l)	pH	Cond (mS/cm)	COD (mg/l)	BOD5 (mg/l)	NH ₃ -N (mg/l)	NO _x -N (mg/l)	C/N
CGR RAW	4.08 ± 0.02	3.04 ± 0.02	5.45	1.653	4253	1101	12.74	6.86	4.54
Pine bark	3.66 ± 0.01	3.35 ± 0.28	4.18	0.845	4517	297	8.54	15.12	3.57

Tables 4 and 5: The ± values refer to the standard deviation of the results. The standard deviation is only included when the test has been done in triplicate or greater.

The results in Table 4 and 5 suggest that pine bark, as well as the fresh garden refuses are both acidic. pH is a limiting factor in the denitrification process and thus the low pH values will impact negatively on the rate of nitrate removal as the optimum pH for biological denitrification is between 6 and 8. The acidic nature of especially the pine bark will cause an inhibitory effect on denitrification.

The higher carbon content, in the form of COD and BOD for both the raw garden refuse and pine bark are due to the fact that the substrates are organic materials and have not undergone any stabilisation.

Pine bark has a determined C/N ratio between 62–90:1. According to the available literature presented in Trois et al. (2007) and Pisano (2007), the C/N ratio in pine bark can range from 723:1 (Willson, 1989), 580:1 (Schliemann, 1974), to 480:1 (Lamb, 1982) and 300:1 prior to composting and 150:1 after composting (Gartner, 1979). Thus the pine bark used in this research has a lower C/N ratio than that stated in the literature. The C/N ratio of the pine bark substrate was found comparable to that of the fresh garden refuse materials.

The RI₇ or respiration test as proposed by Adani et al. (2001) assesses the biodegradability and biological stability of the material by determining the amount of oxygen consumed by the indigenous biomass that is present in the substrate to

degrade the material. “The biological stability indicates the extent to which readily biodegradable organic matter has decomposed” (Adani et al., 2006; Gomez et al. 2006). An unstable material is considered to contain a high portion of biodegradable matter that must sustain high microbial activity (Gomez et al., 2006; Chroni et al., 2009).

As described by Gomez et al. (2006) the respiration is directly related to the metabolic activity of the microbial population. Large amounts of bio-available organic matter cause micro-organisms to respire at a higher rate than that if the material is scarce of organic matter (Gomez et al., 2006). Respiration has become an important parameter in the composting process for ascertaining the stability of the material (Gomez et al., 2006).

As defined by Adani et al. (2006) compost is a stable, mature and humified material. The quality of compost is assessed according to both the maturity and stability parameters (Gomez et al., 2006). The respiration activity is measured as O₂ consumption and/or CO₂ production by the composting mass (Chroni et al., 2009; Gomez et al., 2006). The fresh raw materials thus have a high portion of biodegradable matter that must sustain high microbial activity.

3.2. Batch Tests

3.2.1. Pine Bark

The characterisation results of the tests performed on the input and output of the solid substrate and their eluates in the batch tests at the different initial nitrate concentrations are shown in Table 6.

Table 6

Characterisation results of the input and output of the Pine Bark batch tests

		pH	COD (mg/l)	NH ₃ -N (mg/l)	NO ₃ (mg/l)	Tot C (%)	Tot N (%)	C/N Ratio
	Input Eluate	4.18	4517	8.54	15.12	0.25	0.07	3.57
	Input Solid					36.67	0.59	62.15
Blank (0 mg/l)	Output Eluate	4.90	11192	3.5	0			
	Output Solid					52.4	0.61	85.9
100 (mg/l)	Output Eluate	5.10	5021	2.25	0			
	Output Solid					48.5	0.66	73.57
500 (mg/l)	Output Eluate	4.30	14157	22.5	255			
	Output Solid					52.0	0.59	88.81
2000 (mg/l)	Output Eluate	4.64	13245	30	1600			
	Output Solid					48.9	0.29	343.26

The pH throughout all the batch tests stayed acidic, ranging from 4.30 to 5.10. The nitrate concentration (NO₃) reached zero only in the case of the test at 100 mg/l. The other two tests failed to reach full denitrification.

There was a presence of positive bioleaching of carbon which was observed in the increase of both the COD and C/N ratios, relating to the initial nitrate concentration. The COD results showed an increase from the initial input ranging from 5021 – 14157 mg/l. There was also an increase in NH₃ which correlates to the reduction in total N (%) from 0.59 – 0.29, which indicated there was also bioleaching of nitrogen. The increase in COD was greater than that experienced in NH₃ resulting in an increased C/N ratio. As C/N ratio was calculated using wet samples, carbon leached out from the substrate was still trapped in the biofilm of the pores resulting in the observed increase in C/N Ratio from 62.15 to 343.26.

The evolution of the nitrate concentrations for the Pine Bark substrate conducted for each of the concentrations are shown in Figures 1, 2 and 3. The graphs demonstrate the nitrate concentration (NO₃) in mg/l in relation to time in days. Due to the small variety in the blank test, its results are included in each of the graphs.

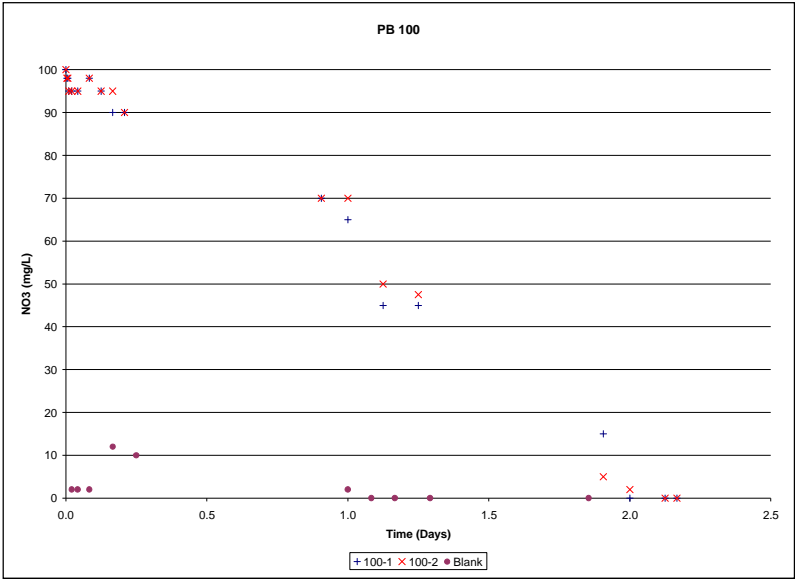


Figure 1: Evolution of the nitrate concentration for Pine Bark at C₀ = 100 mg/l

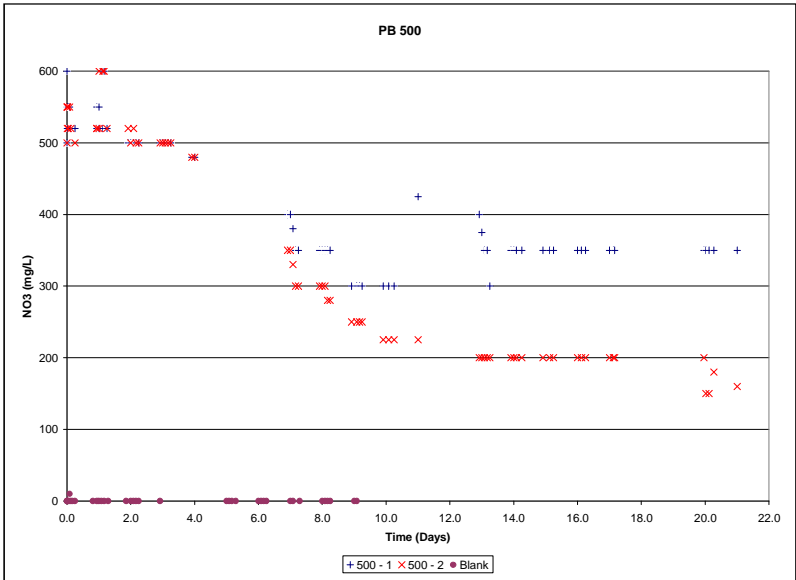


Figure 2: Evolution of the nitrate concentration for Pine Bark at C₀ = 500 mg/l

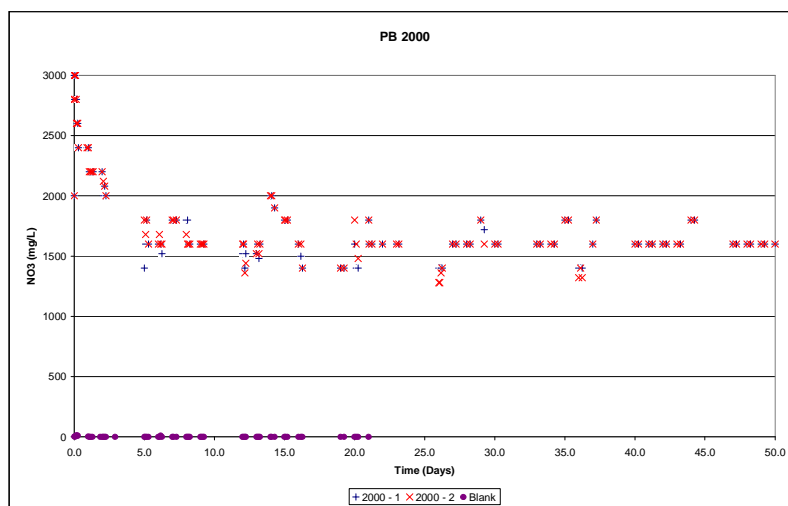


Figure 3: Evolution of the nitrate concentration for Pine Bark at $C_0 = 2000 \text{ mg/l}$

Table 7 summarises the kinetic rates of removal over the linear period of each batch test, determined from the plotted figures as well as time required to achieve the indicated percent of removal of the PB substrate at the various nitrate concentrations.

Table 7

Summary of kinetics of the PB batch tests

C_0 (mg/l)	Time for 100% Removal (Days)	k (1/day)	R^2	Percentage Removal (%)
100	2.2	46.775	0.98	100
500	-	38.183	0.98	55
2000	-	126.250	0.91	20

All three tests conducted at the various concentration levels showed an initial plateau an acclimatisation period during which there is pH buffering as well as competition between nitrifiers and denitrifiers, as suggested by previous studies (Trois et al., 2009). This period lasted until the environment became more suitable for the denitrifiers. The duration of this plateau period tended to increase with an increase in initial nitrate concentration (Trois et al., 2009).

The test performed at 100 mg/l was the only one to achieve full nitrate removal.

The test conducted at 100 mg/l showed positive results, with total nitrate removal being achieved within 2 – 2.5 days. The tests conducted at 500 and 2000 mg/l showed an increase in nitrates within the first 2 days. This could be due to the small percentage increase represented in the blank as well as errors associated with the method.

The results of the experiment performed at 500 mg/l and 2000 mg/l were less promising, although some removal did occur after the plateau period, full denitrification was not achieved, but only 55% and 20% removal efficiency was observed for the two concentrations respectively.

During the test at 500 mg/l, after 12 to 14 days no more nitrate removal was achieved. This may be due to the inhibitory effect of NO₃ saturation as a result of the high initial nitrate concentration as well as the release of phenols which are toxic to bacteria (De Combret, 2009). Through studies done by De Combret (2009), it is reported that denitrifiers are only present after 74 hours from commencement of the batch test. Thus the removal of nitrate within 2.2 days at a concentration of 100 mg/l could be attributed to absorption of nitrates or the reduction of nitrates into ammonia (Trois et al., 2010).

The test conducted at 2000 mg/l showed little nitrate removal. After the plateau period, the nitrate concentration did decrease by 20 – 30%, but after the initial 5 days further reduction was no longer achieved and the final concentration stabilised at 1600 mg/l.

3.2.2. Fresh commercial garden refuse (CGR RAW).

Table 8 presents the results of the characterisation of inputs and outputs materials from the batch tests with CGR RAW.

Table 8

Characterisation results of the input and output of the CGR RAW batch tests

		pH	COD (mg/l)	NH ₃ -N (mg/l)	NO ₃ (mg/l)	Tot C (%)	Tot N (%)	C/N Ratio
Blank (0 mg/l)	Input Eluate	5.45	4253	12.74	6.86	0.083	0.0183	4.54
	Input Solid					49.6	0.55	90.19
	Output Eluate	6.01	9433	15	0			
	Output Solid					48.5	0.63	76.98
100 (mg/l)	Output Eluate	5.97 – 6.16	4325 – 5212	4 – 30	0			
	Output Solid					42.9 – 47.6	0.57 – 0.84	54.64 – 75.26
500 (mg/l)	Output Eluate	5.41 – 5.68	3951 – 7200	20 – 30	0			
	Output Solid					46.4 – 48.8	0.70 – 0.84	55.79 – 70.25
2000 (mg/l)	Output Eluate	6.80 – 7.33	7009 – 7870	75 – 100	0			
	Output Solid					45.6 – 49.5	0.19 – 0.68	67.5 – 240.0

Due to the large number of tests carried out at each concentration, an average value would have provided a misrepresentation of the results.

It is noted that the fresh CGR can be compared with the pine bark in terms of pH that ranges around 5.45 and increases with time and with NO_3 concentration as reported by other authors (Tsui et al., 2007). It is also noted that the longer test conducted at an initial concentration of 2000 mg/l exhibits a final pH which falls into the optimum range for denitrification.

To monitor the NO_2 concentrations during the 500 mg/l experiment, three tests were stopped at different levels of nitrites. The 500 – A test had a much lower amount of $\text{NO}_x\text{-N}$ whereas the test that was stopped when nitrites were still present had a relatively high value of $\text{NO}_x\text{-N}$.

Table 9

Characterisation results of the output of the CGR RAW batch tests conducted at 500 mg/l on both solid and eluate

	Solid			Eluate			
	Total C (%)	Total N (%)	C/N Ratio	pH	COD	$\text{NH}_3\text{-N}$	$\text{NO}_x\text{-N}$
CGR RAW (500 - A)	48.4	0.72	67.89	5.41	7200	30.0	3.0
CGR RAW (500 - B)	46.4	0.84	55.79	5.68	3951	25.0	85.0
CGR RAW (500 - C)	48.8	0.70	70.25	5.47	4046	20.0	62.5

As a result of the production of NH_3 leached out from the substrate as well as the oxygen present in the solution and the pores, NH_3 is converted into NO_2 even when full nitrate removal is achieved. It was confirmed by De Combret (2009) and Trois (2010) that both nitrifiers and denitrifiers were present in this substrate within the first 74 hours of batch test, in line with other studies that used similar substrates (Zhong et al., 2009).

There was a presence of positive bioleaching of carbon which was observed in the increase of both the COD and C/N ratios, relating to the initial nitrate concentration. The COD results showed an increase from the initial input ranging from 3951 – 7870 mg/l. The ammoniacal nitrogen released, also tended to increase with the time. This increase in NH_3 which correlates to the slight reduction in total N (%) especially in the test at $C_0 = 2000$ mg/l, indicates that there was also bioleaching of nitrogen. As the percentage increase in COD was not as great as that observed in the PB, there was a lower increase in C/N ratio. As C/N ratio was calculated using wet samples, carbon leached out from the substrate was still trapped in the biofilm of the pores resulting in the observed increase in C/N Ratio from 90.19 to 240.0.

The evolution of the nitrate concentration for the tests with CGR RAW substrate conducted for each of the concentrations is shown in Figures 4, 5, 6 and 7.

The blank test results are also included for reference.

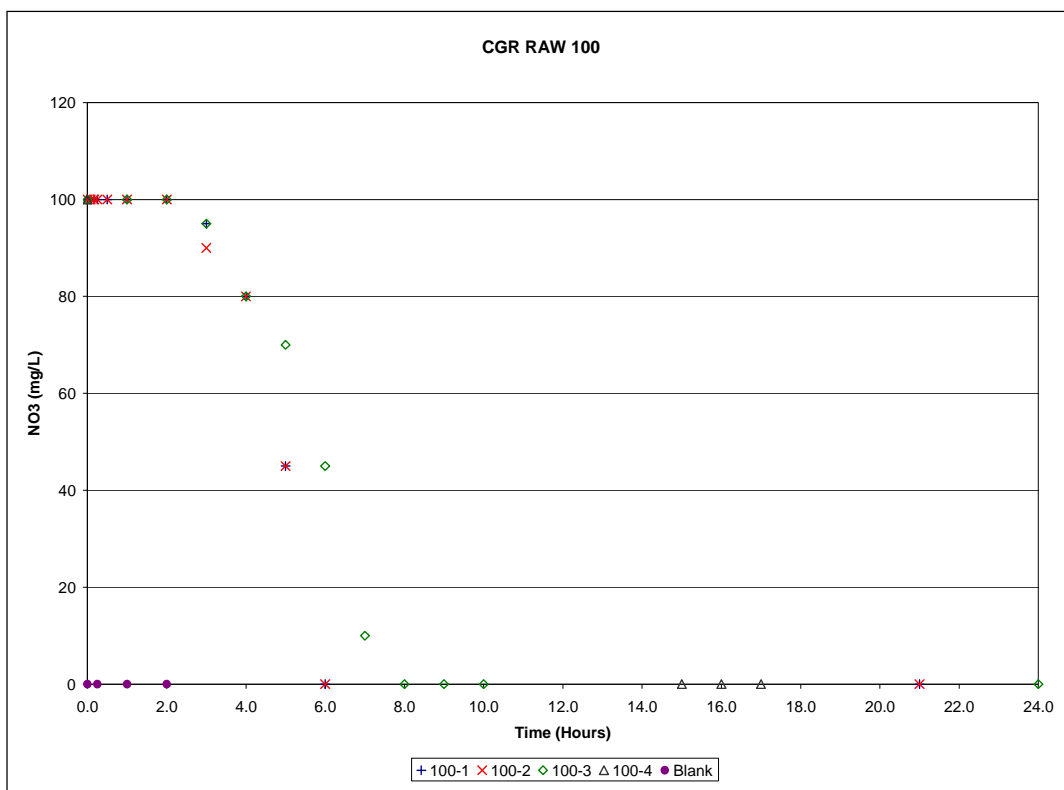


Figure 4: Evolution of the nitrate concentrations for CGR RAW at $C_o = 100 \text{ mg/l}$

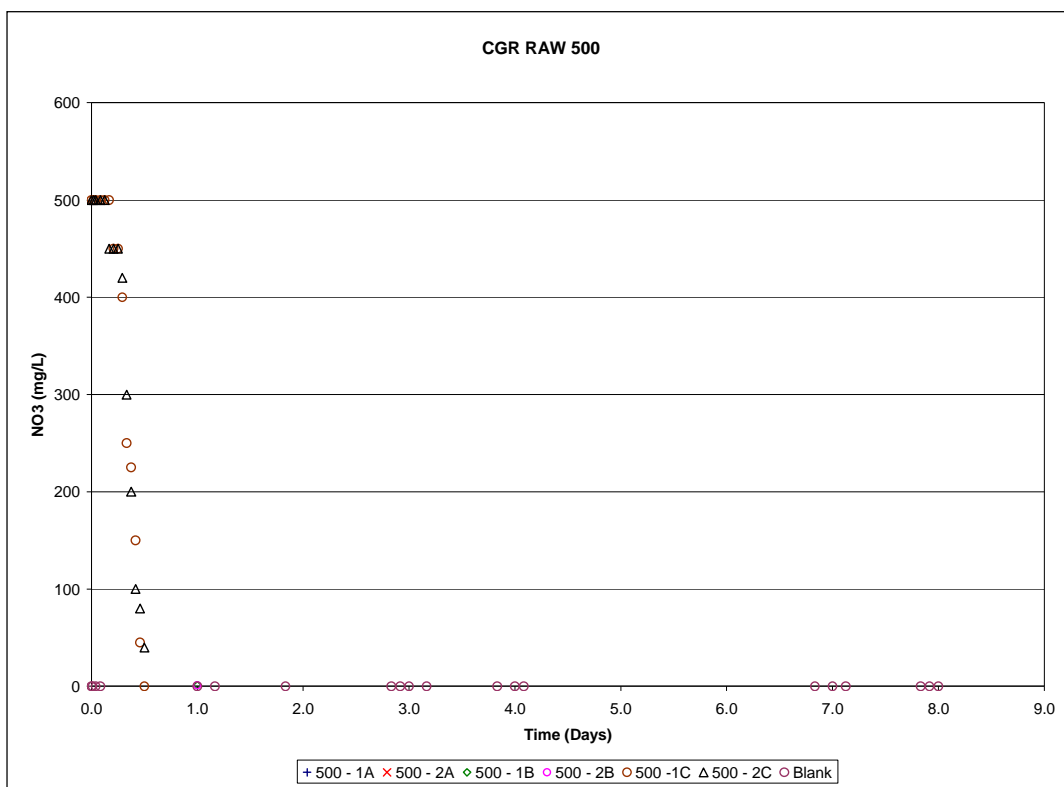


Figure 5: Evolution of the nitrate concentration for CGR RAW at $C_0 = 500 \text{ mg/l}$

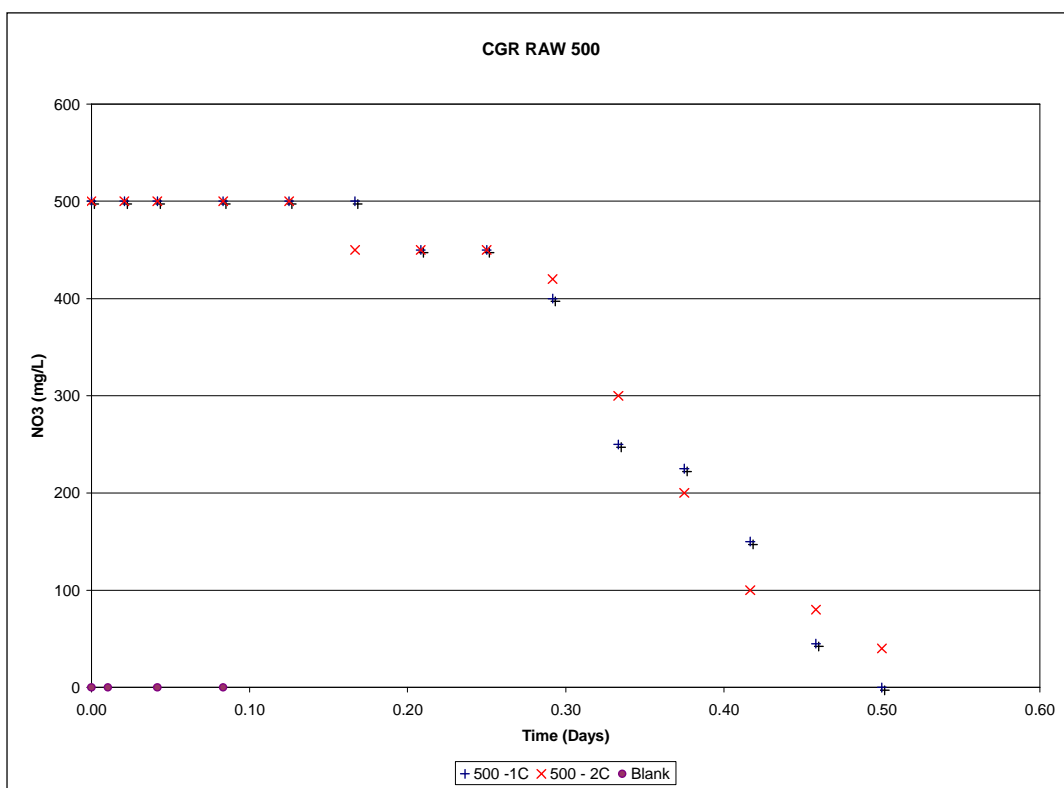


Figure 6: Evolution of the nitrate concentration for CGR RAW at 500 mg/l (Test C)

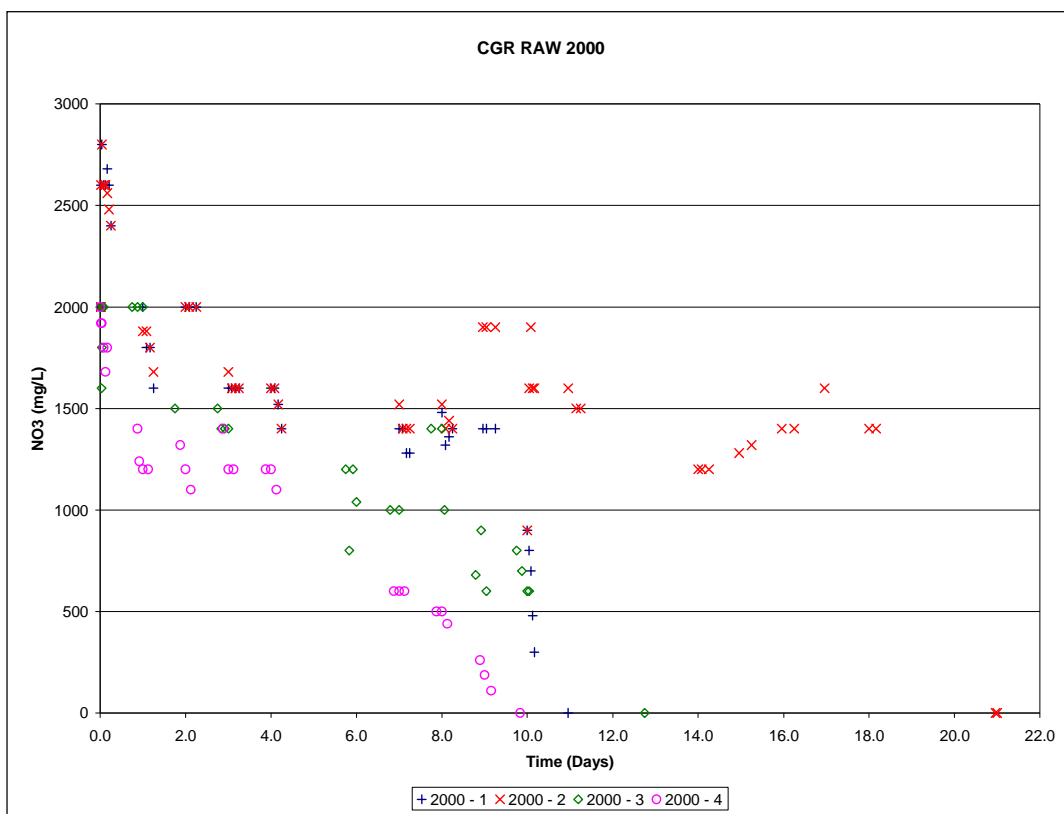


Figure 7: Evolution of the nitrate concentration for CGR RAW at $C_0 = 2000 \text{ mg/l}$

Kinetics: Rate of Reaction

The results were modelled using a zero order kinetic reaction model.

Rate of Reaction for linear period:

100 mg/l: Highest (Zero Nitrates - 1)

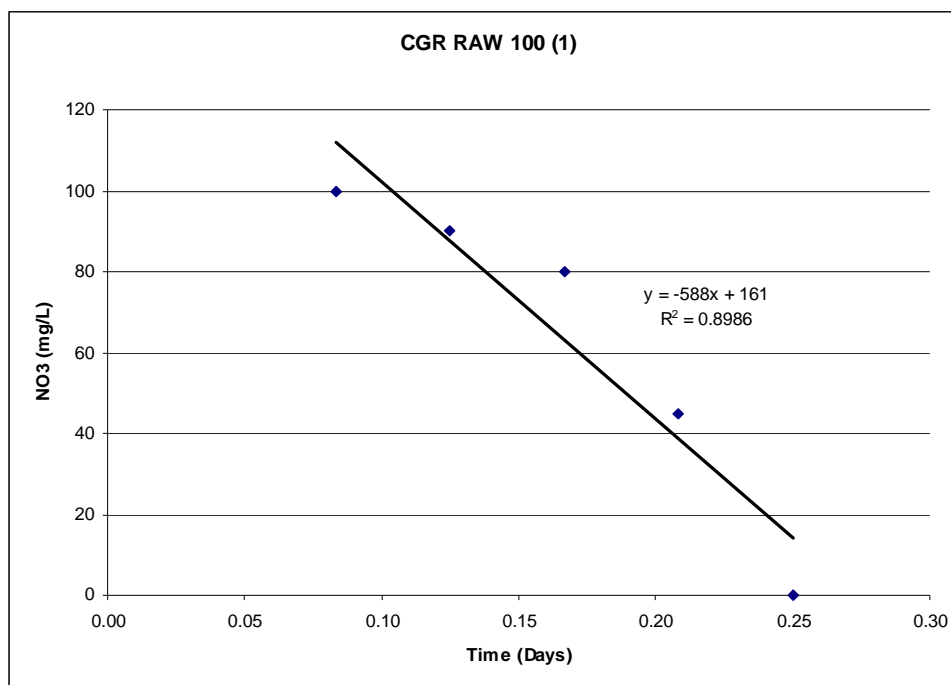


Figure 8: Kinetics of CGR RAW at $C_0 = 100 \text{ mg/l}$ (1)

100 mg/l: Lowest (Zero Nitrates and Nitrites - 2)

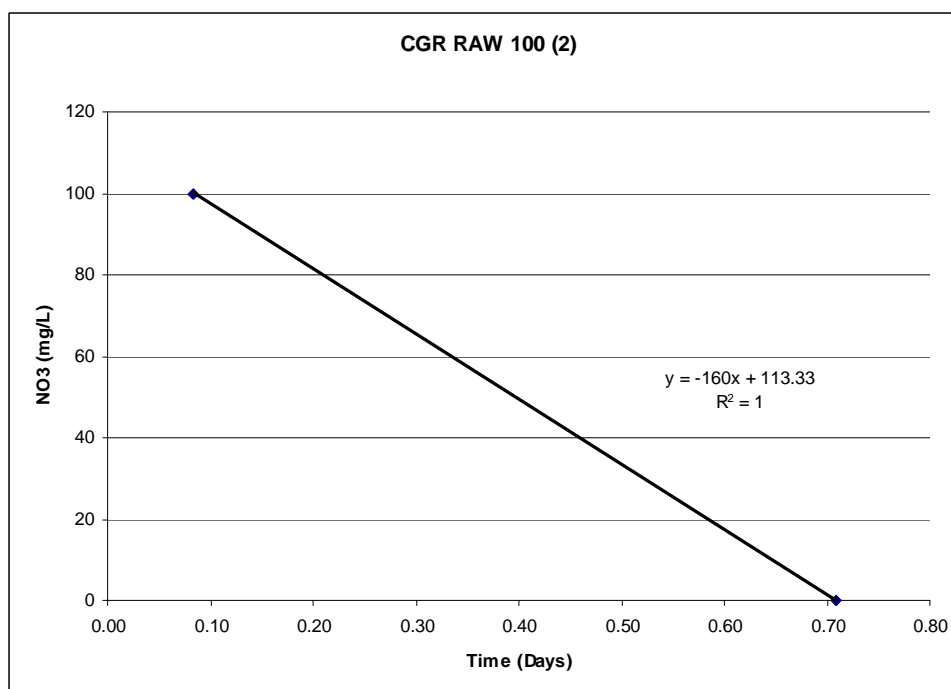


Figure 9: Kinetics of CGR RAW at $C_0 = 100 \text{ mg/l}$ (2)

500 mg/l: Highest (Zero Nitrates - 1)

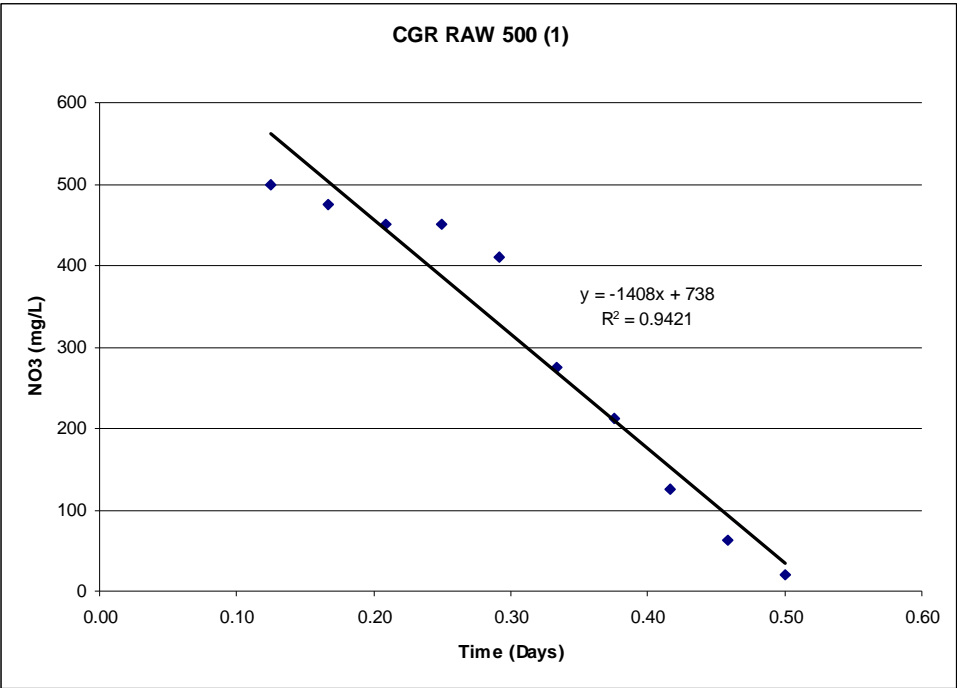


Figure 10: Kinetics of CGR RAW at $C_o = 500$ mg/l (1)

500 mg/l: Lowest (Zero Nitrates and Nitrites - 2)

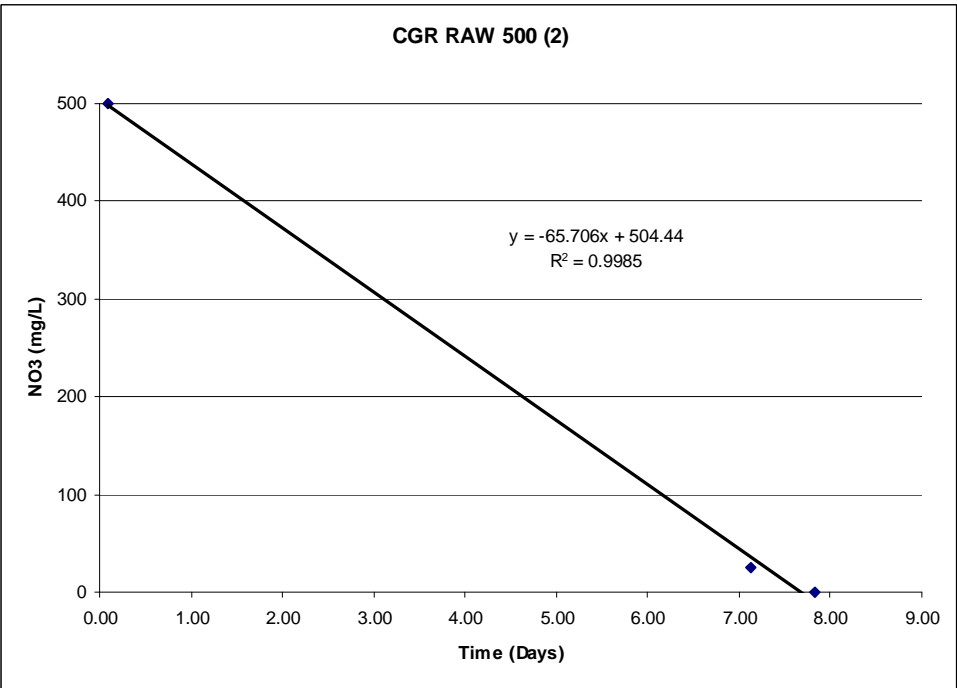


Figure 11: Kinetics of CGR RAW at $C_o = 500$ mg/l (2)

2000 mg/l:

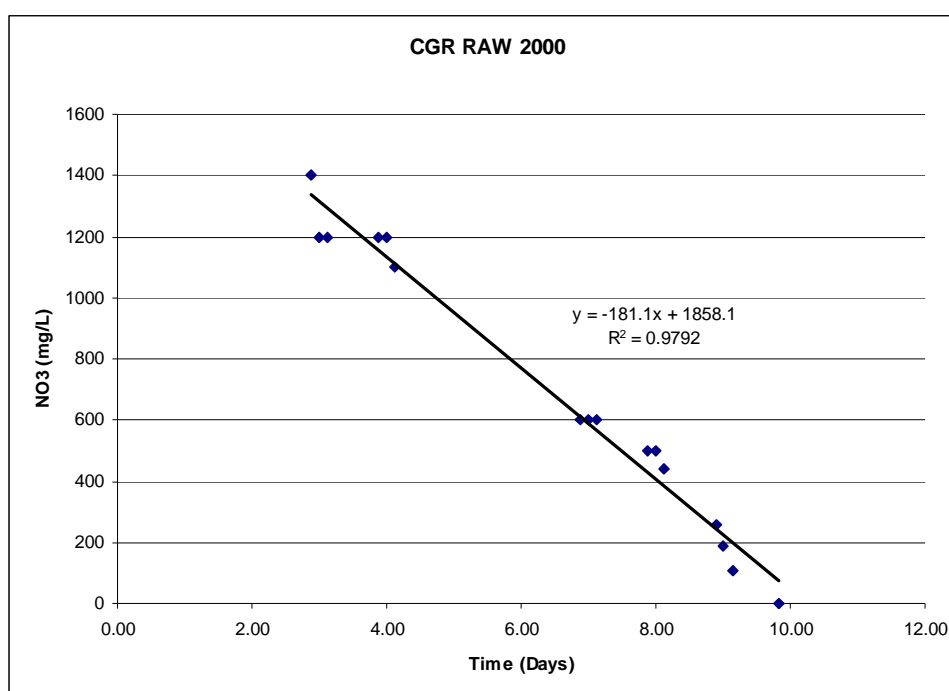


Figure 12: Kinetics of CGR RAW at $C_o = 2000 \text{ mg/l}$

Table 10 summarises the kinetic rates of removal over the linear period of each batch test, determined from the plotted figures as well as time required to achieve the indicated percent of removal of the CGR RAW substrate at the various nitrate concentrations. 100 (1) is the time for the removal of all nitrates whereas 100 (2) is the period for the removal of both the nitrites and nitrates, similarly for 500 (1) and 500 (2).

Table 10

Summary of kinetics of CGR RAW

C_o (mg/l)	Time for 100% Removal (Days)	K (1/day)	R^2	Percentage Removal (%)
Removal of nitrates only				
100 (1)	0.25	588	0.90	100
500 (1)	0.50	1408	0.94	100
2000	10.5	181	0.98	100
Removal of nitrates and nitrites				
100 (2)	0.71	160	1.00	100
500 (2)	7.83	67.71	0.999	100

All three tests conducted at the various concentration levels exhibited an initial plateau of approximately 2 hours. Similarly to the Pine Bark substrate, which also experiences an acclimatisation period, this involves pH buffering. The duration of this plateau period tended to increase with an increase in initial nitrate concentration, suggesting that pH

and the initial NO_3 concentration play an important inhibitory role during this initial stage as demonstrated by De Combret (2009).

In the test at $C_o = 100 \text{ mg/l}$ the system reached a zero nitrate concentration within 6 to 8 hours with a 2 hour plateau. A total of 4 tests were performed at this concentration to accurately obtain the time required for complete nitrate removal.

The tests conducted at $C_o = 500 \text{ mg/l}$ demonstrated an initial plateau period ranging between 2 to 8 hours. After this plateau the nitrate concentration rapidly dropped to zero after 12 hours. Once again, as experienced in the test conducted at 100 mg/l there were nitrites present after the nitrate concentration became zero, with zero nitrates and nitrites present after 8 days.

The final test at a concentration of $C_o = 2000 \text{ mg/l}$ showed an increase in nitrates within the first 6 hours of the initial two tests and a plateau period of 18 to 24 hours with full nitrate removal occurring from 9 to 12 days.

One of the tests behaved slightly differently ($2000 - 2$). It showed an initial peak followed by a similar plateau stage. The nitrate concentration then decreases at a rapid rate until a concentration of 1400 mg/l after 4 days was reached. The fluctuations in the nitrate concentrations are not fully understood. Finally at approximately 18.5 days, the nitrate level dropped from 1400 mg/l in two days to zero.

All the tests reach 100% removal. The tests conducted at 100 and 500 mg/l were both highly efficient and reached a zero nitrate concentration in less than 24 hours. The graphical representations suggest a linear relationship, excluding the initial plateau period. Studies done by De Combret (2009) and Trois (2010) suggest that denitrifiers are only present after 74 hours, thus the removal of nitrate within 24 hours could be attributed to other bio-chemical processes such as absorption of nitrates or the conversion of nitrates into ammonia.

From the above results it is possible to conclude that these substrate is suitable for biological denitrification.

3.3. Column Tests

The following criteria were used to determine the suitability of the substrates for utilisation in the column studies. The first key parameter was the C/N ratio of the substrate. It is essential to have a relatively high C/N ratio for denitrification. C/N ratios above 16 were considered suitable for denitrification (Tsui et al., 2007; Wu et al., 2001; Trois et al., 2010). The second parameter was the pH. The optimum range of pH for denitrification is 6 – 8. The third parameter used for assessing the suitability of a substrate was the time required for full denitrification to be achieved in optimum conditions, as achieved in batch tests. The capacity of the substrates to release COD and NH_3 through bioleaching was also taken into account.

A summary of the substrates and the criteria used for their utilisation in the column studies are shown in Table 11 for nitrate concentrations of 500 and 2000 mg/l as well as a summary of column operating conditions is presented in Table 12.

Table 11

Summary of column test criteria at $C_o = 500$ and 2000 mg/l

Substrate	Input		COD (mg/l)		Time for 100% Removal (Days)	
	C/N Ratio	pH	500	2000	500	2000
Pine Bark	62.15	4.18	14157	13245	-	-
CGR RAW	90.19	5.45	3951 - 7200	7009 – 7870	0.5 or 7.83	10.5

Table 12

Summary of column operating conditions

Substrate	NO_3 Concentration (mg/l)	Duration (Weeks)	Flow Rates (l/day)	
			Experiment 1	Experiment 2
CGR RAW	500	4	2.48	5.625
PB	500	4	2.00	5.00
CGR RAW	2000	4	2.38	5.65
PB	2000	4	2.00	5.00

3.3.1. Fresh CGR (CGR RAW)

$C_o = 500 \text{ mg/l}$

The evolution of the nitrate concentrations and pH over the two flow rates for the CGR RAW substrate are shown in Figures 13. and 14.

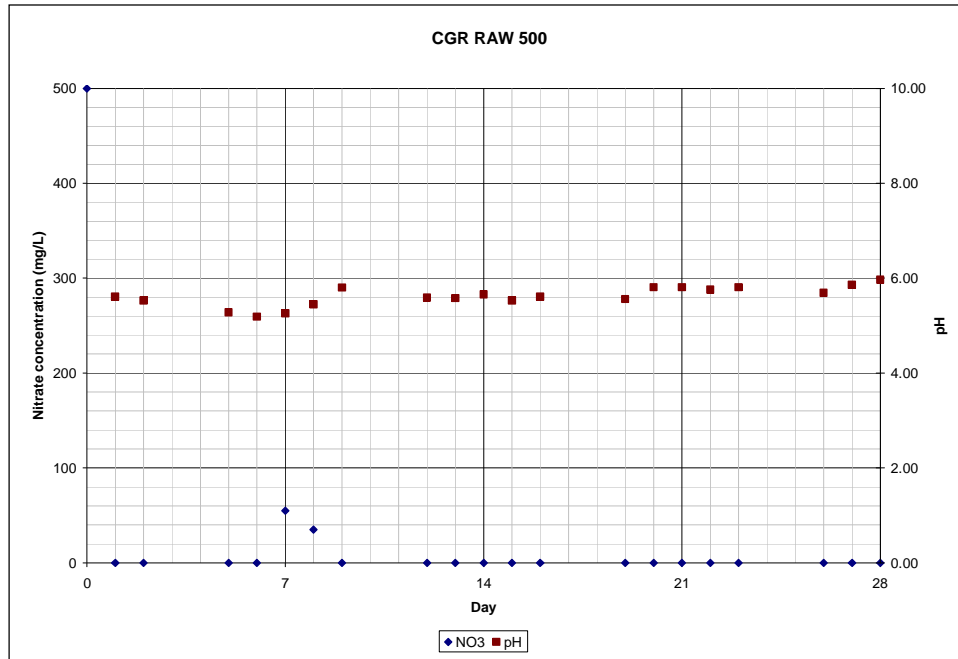


Figure 13: Experiment 1 - Evolution of the nitrate concentration and pH for CGR RAW for $C_o = 500 \text{ mg/l}$ at flow rate 1

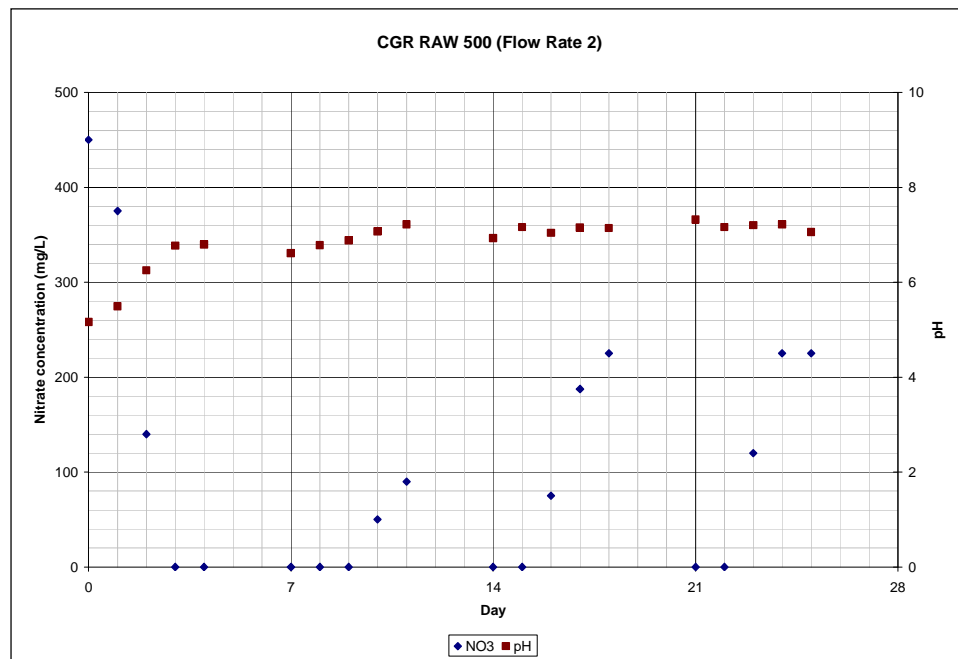


Figure 14: Experiment 2 - Evolution of the nitrate concentration and pH for CGR RAW for $C_o = 500 \text{ mg/l}$ at flow rate 2

The evolution of the nitrate concentration over the length of the column for flow rate 1 is shown in Figure 15. The graph demonstrates the Nitrate Concentration (NO_3) in mg/l in relation to length recorded in metres.

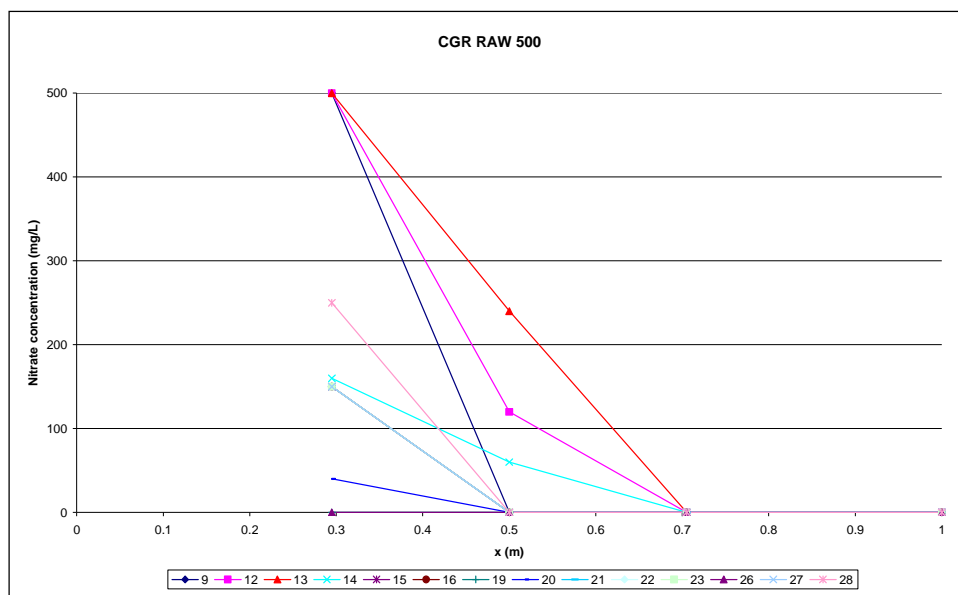


Figure 15: Experiment 1 - Evolution of the nitrate concentration over the column length for CGR RAW for $C_0 = 500 \text{ mg/l}$ at flow rate 1

The COD of the output for the CGR RAW substrate at 500 mg/l are shown in Figures 16 and 17.

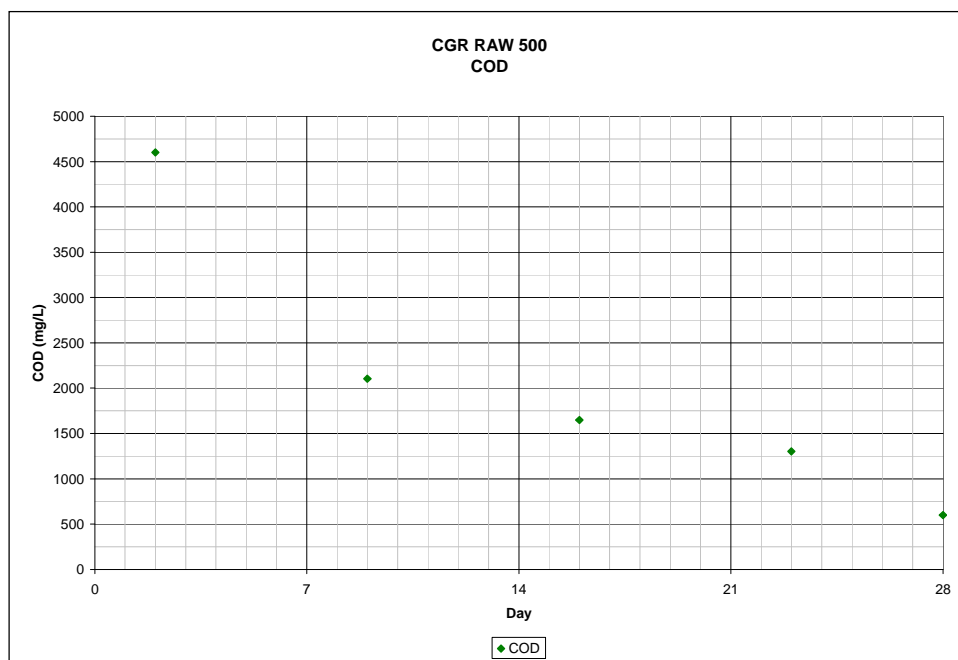


Figure 16: Experiment 1 – Evolution of COD for CGR RAW for $C_0 = 500 \text{ mg/l}$ at flow rate 1

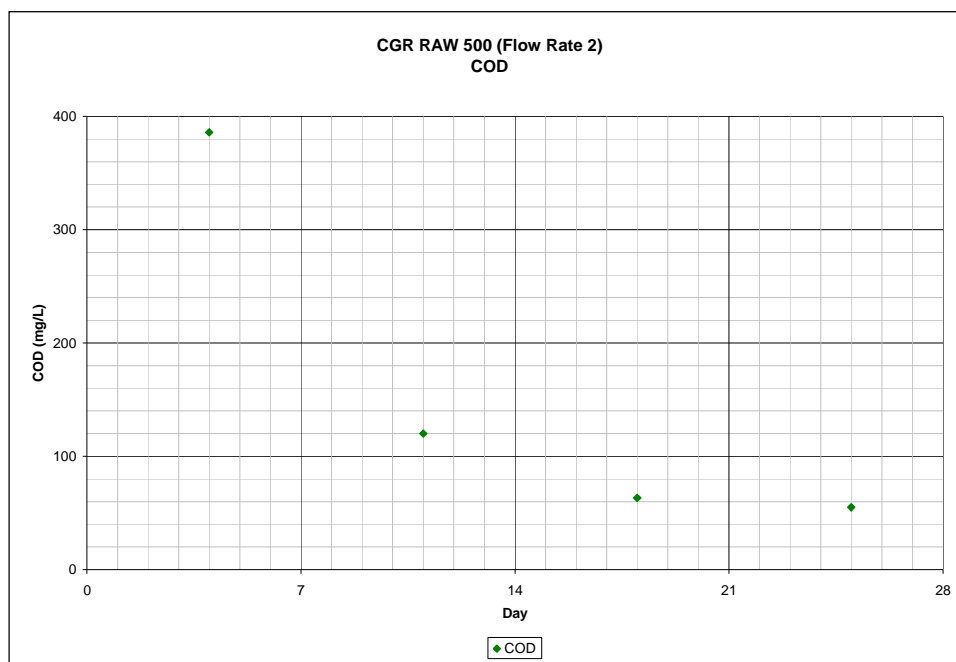


Figure 17: Experiment 2 – Evolution of COD for CGR RAW for $C_o = 500 \text{ mg/l}$ at flow rate 2

Full nitrate removal was achieved within the first 5 days at flow rate 1 and initial 4 days at flow rate 2. For the latter, there was insufficient contact time between the solution and the substrate during weeks 2, 3 and 4, causing a rise in nitrate concentration. However after the extended contact time over the weekend, the entire column had achieved full nitrate removal.

The COD of the output effluent dropped considerably throughout the period of the test. After the first week a value of above 4500 mg/l was recorded, however the COD dropped by more than 85% by the end of the experiment 1. The COD results at the second flow rate are lower than those recorded in experiment 1. This is due to the fact that the substrate was not replaced over the two experiments. Experiment 2 displayed a drop of 88%, with a final output of 55 mg/l .

The pH remained below 6 during experiment 1 and tended to rise during the first week to 7 and remained at this level throughout the rest of experiment 2. The temperature remained constant with a range between 19 and 22°C , whilst the determined $\text{NH}_3 - \text{N}$ dropped to less than 1 mg/l at the conclusion of both experiments.

$C_o = 2000 \text{ mg/}\ell$

The evolution of the nitrate concentrations and pH over the two flow rates for the CGR RAW substrate are shown in Figures 18 and 19.

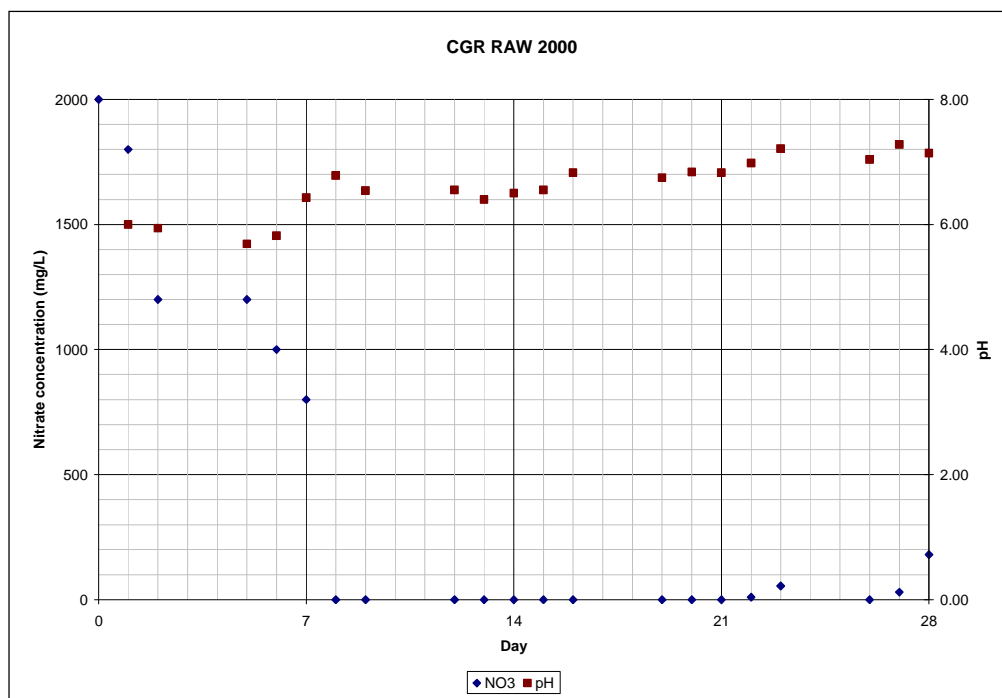


Figure 18: Experiment 1 - Evolution of the nitrate concentration and pH for CGR RAW for $C_o = 2000 \text{ mg/}\ell$ at flow rate 1

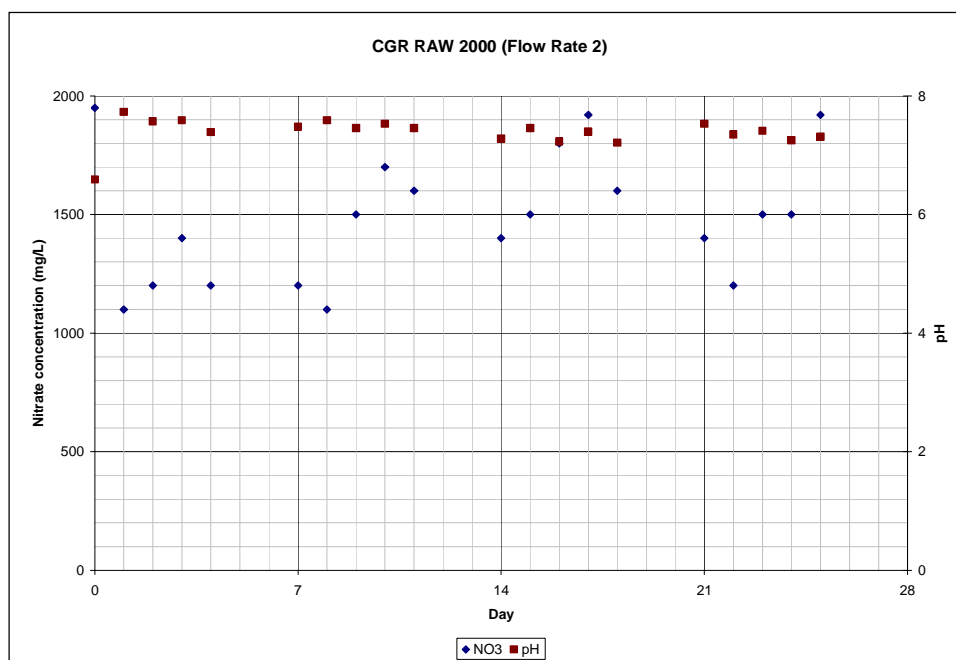


Figure 19: Experiment 2 - Evolution of the nitrate concentration and pH for CGR RAW for $C_o = 2000 \text{ mg/}\ell$ at flow rate 2

The evolution of the nitrate concentration over the length of the column for flow rate 1 is shown in Figure 20.

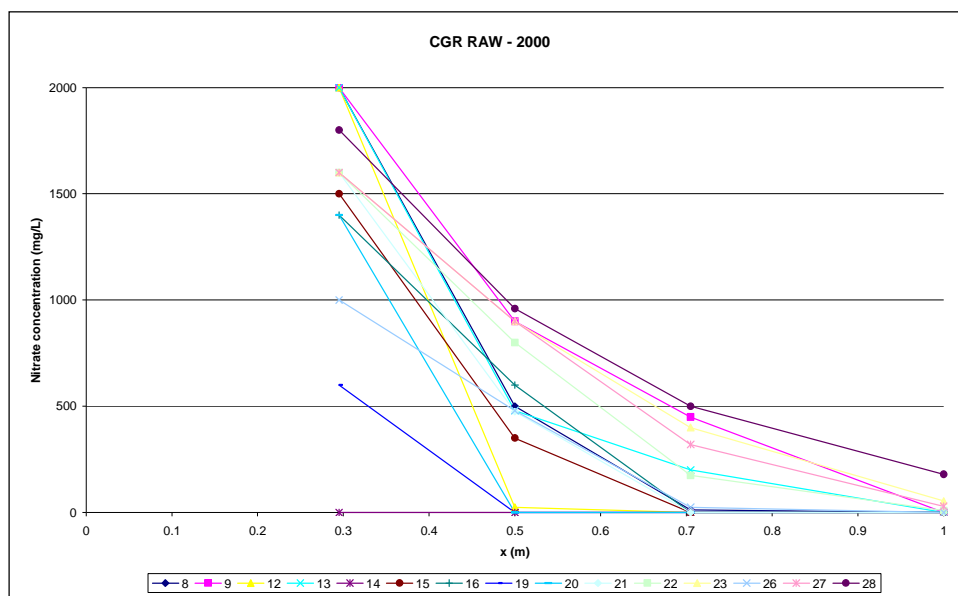


Figure 20: Experiment 1 - Evolution of the nitrate concentration over the column length for CGR RAW for $C_0 = 2000 \text{ mg/l}$ at flow rate 1

The COD of the output for the CGR RAW substrate at 2000 mg/l are shown in Figures 21 and 22.

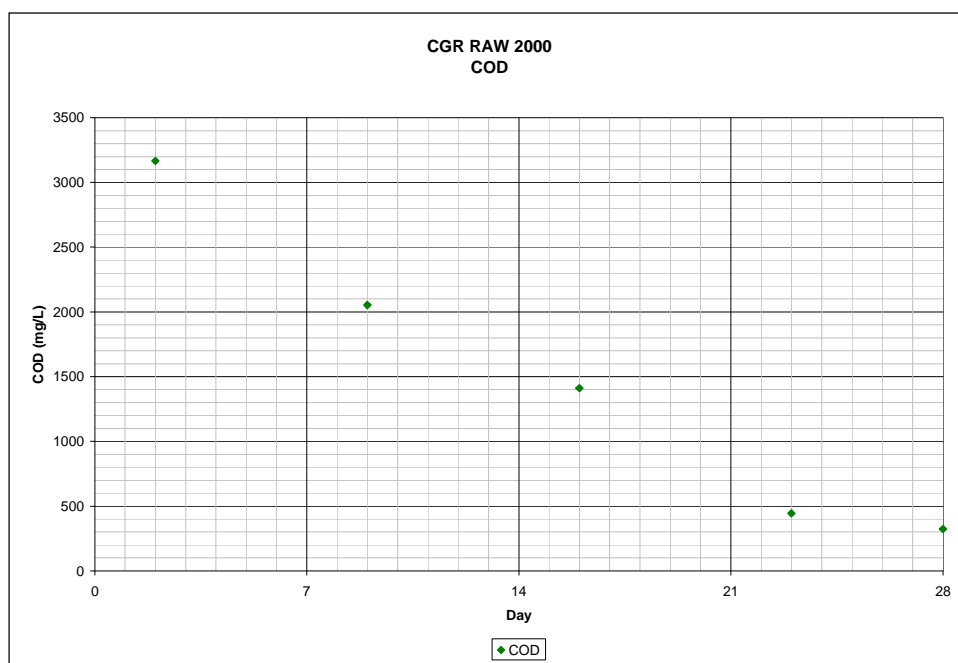


Figure 21: Experiment 1 – Evolution of COD for CGR RAW for $C_0 = 2000 \text{ mg/l}$ at flow rate 1

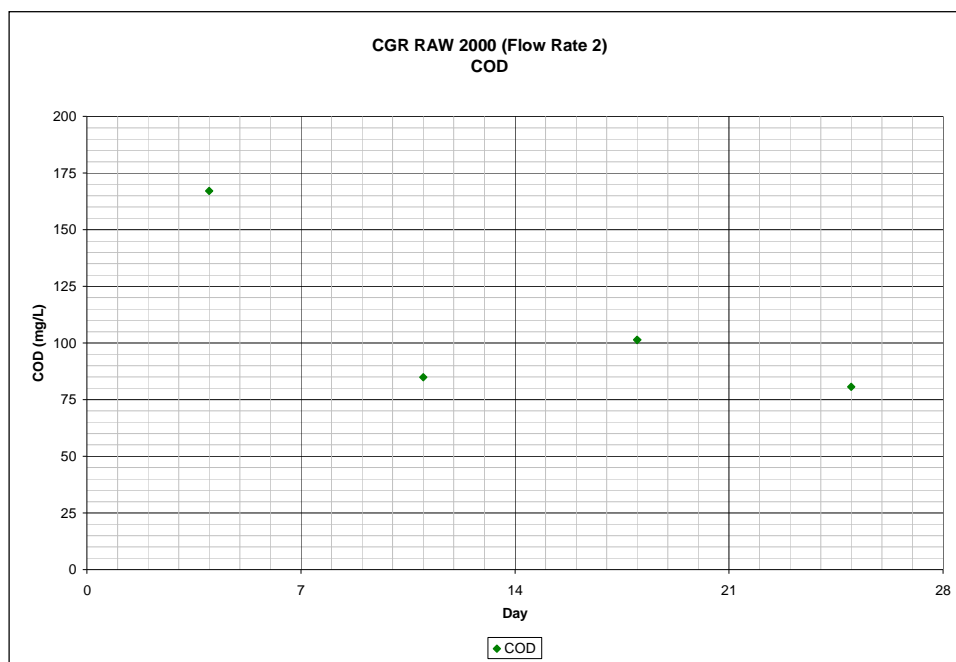


Figure 22: Experiment 2 – Evolution of COD for CGR RAW
for $C_0 = 2000 \text{ mg/l}$ at flow rate 2

The nitrate concentration in the column at flow rate 1 reached zero after the initial 7 days. The concentration at the bottom of the column remained at zero until day 22, where the output concentration rose. This was observed once again during the following week. This reduced rate of denitrification could be due to the high nitrate concentration saturating the substrate. The rate at which carbon was being released had reduced and was now slower than the rate at which nitrates were being added. During the second week, full nitrate removal was being achieved within 1 - 2 days. However as the experiment progressed, this rate of denitrification reduced. At the end of the period the substrate failed to fully denitrify the leachate.

At flow rate 2, the coupled effect of the very high nitrate concentration and high flow rate negatively affected the performance of the test resulting in a lower denitrification rate and only 50% removal efficiency against 100% in the first experiment.

The COD of the output effluent dropped considerably through the period of the test 1 at a constant rate, from 3200 mg/l to 400 mg/l , with 88% removal. However, the COD values during experiment 2 dropped after the first week to below 100 mg/l where it remained fairly constant throughout the duration of the experiment. At the end of the experiment the final COD value was below 100 mg/l .

The pH during experiment 1 tended to increase to neutrality, whilst the pH during the experiment 2 stayed constant between 7 and 8 after an initial rise from 6.79 on the first day. The temperature remained constant with a range between 19 and 23 °C. In experiment 1, the $\text{NH}_3 - \text{N}$ was 14 to 16 mg/l over the first two weeks and dropped to below 5mg/l for the remaining weeks of the experiment. The measured $\text{NH}_3 - \text{N}$ during experiment 2 remained fairly constant with a range between 1.5 and 7.0 mg/l.

3.3.2. Fresh Pine bark (PB)

$C_o = 500 \text{ mg/l}$

The evolution of the nitrate concentrations and pH over the two flow rates for the Pine bark substrate are shown in Figures 23 and 24.

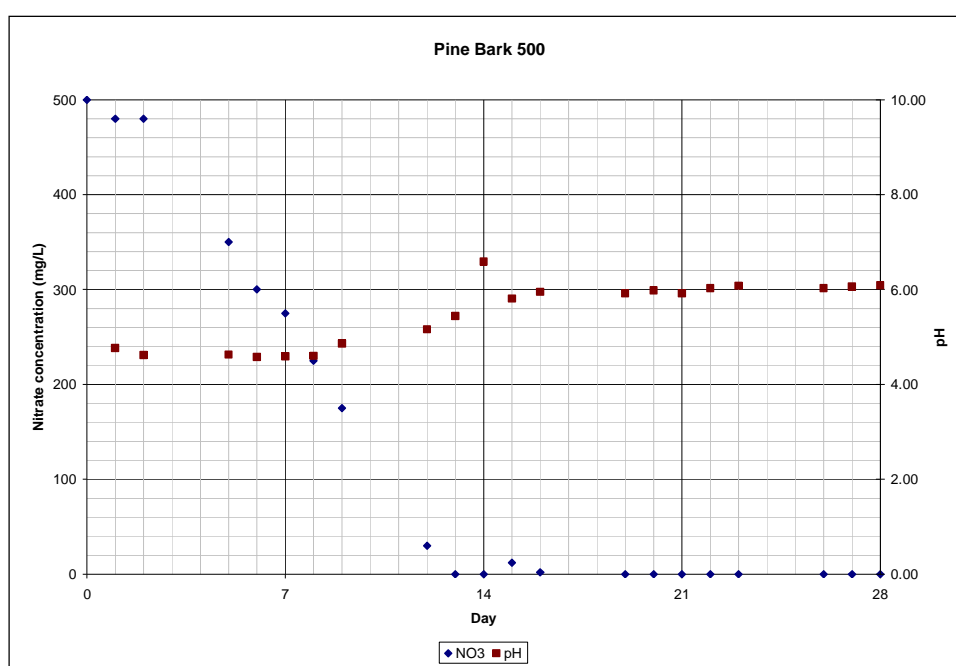


Figure 23: Experiment 1 - Evolution of the nitrate concentration and pH for PB for $C_o = 500 \text{ mg/l}$ at flow rate 1

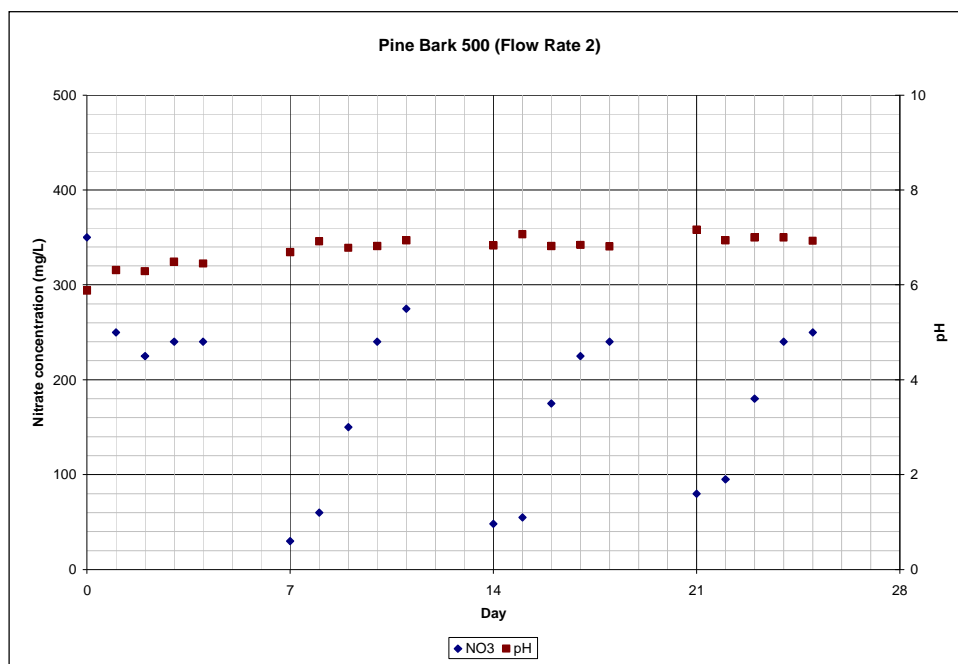


Figure 24: Experiment 2 - Evolution of the nitrate concentration and pH for PB for $C_o = 500 \text{ mg/l}$ at flow rate 2

The evolution of the nitrate concentration over the length of the column for flow rate 1 is shown in Figure 25.

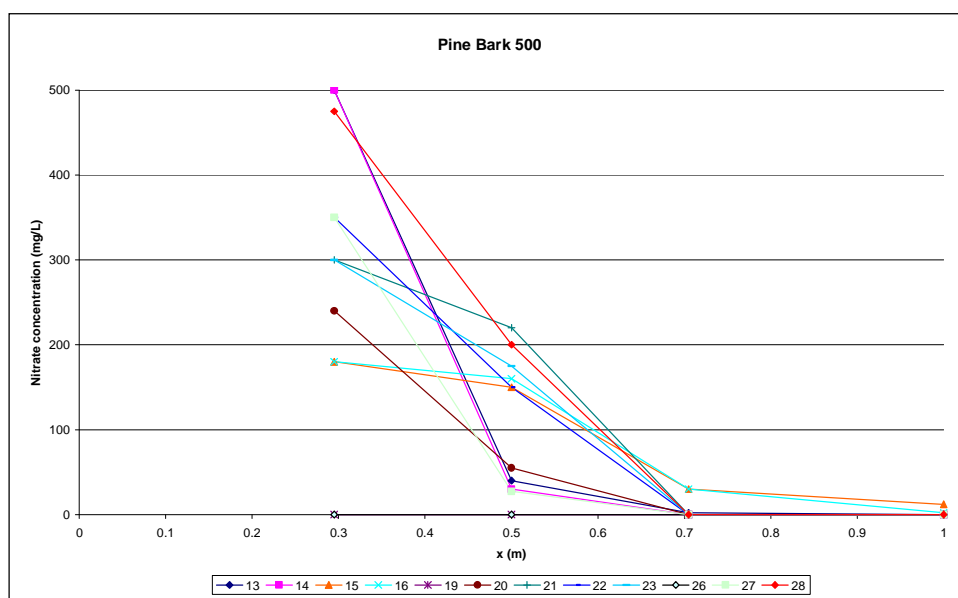


Figure 25: Experiment 1 - Evolution of the nitrate concentration over the column length for PB for $C_o = 500 \text{ mg/l}$ at flow rate 1

The COD of the output for the Pine bark substrate at 500 mg/l are shown in Figures 26 and 27.

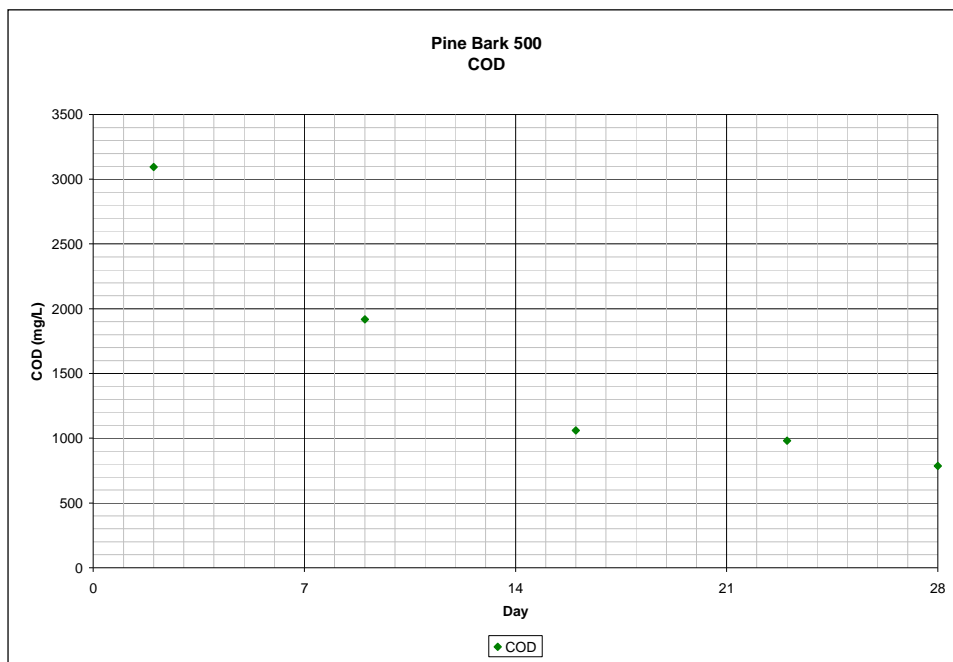


Figure 26: Experiment 1 – Evolution of COD for PB for $C_o = 500$ mg/l at flow rate 1

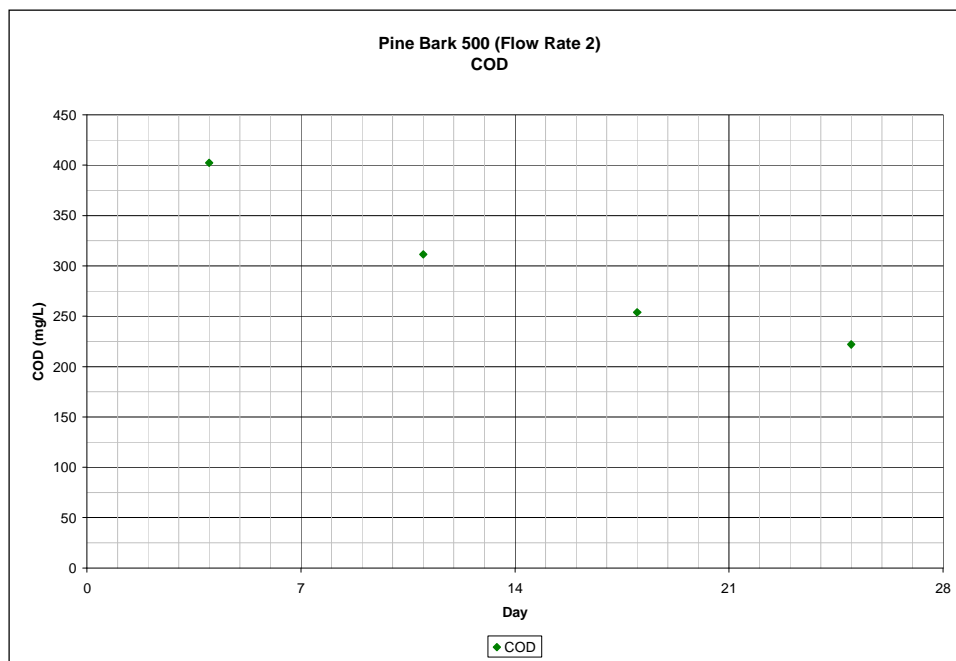


Figure 27: Experiment 2 – Evolution of COD for PB for $C_o = 500$ mg/l at flow rate 2

In the column studies, for flow rate 1, the PB showed a better performance than in the batch tests, by completely removing the nitrates after 5 to 7 days. However, during experiment 2, the system failed to reach regime. None the less, a longer testing period

and more in depth microbiological analyses are required to draw significant conclusions.

The COD of the output effluent dropped by 75% over the period of experiment 1, from 3100 mg/l to 800 mg/l. In experiment 2, the COD values decreased during the duration of the experiment to a final output of 225 mg/l.

The pH during both experiments rose at a fairly constant rate from an acid nature, until it reached the optimum range for nitrate removal. This buffering capacity is comparable to the drop in nitrate concentration represented in experiment 1. Environmental conditions remained fairly constant throughout both experiments. The temperature ranged between 18 and 22 °C, whereas the $\text{NH}_3 - \text{N}$ reducing to less than 1 mg/l.

$C_o = 2000 \text{ mg/l}$

The evolution of the nitrate concentrations and pH for the Pine bark substrate are shown in Figures 28 and 29.

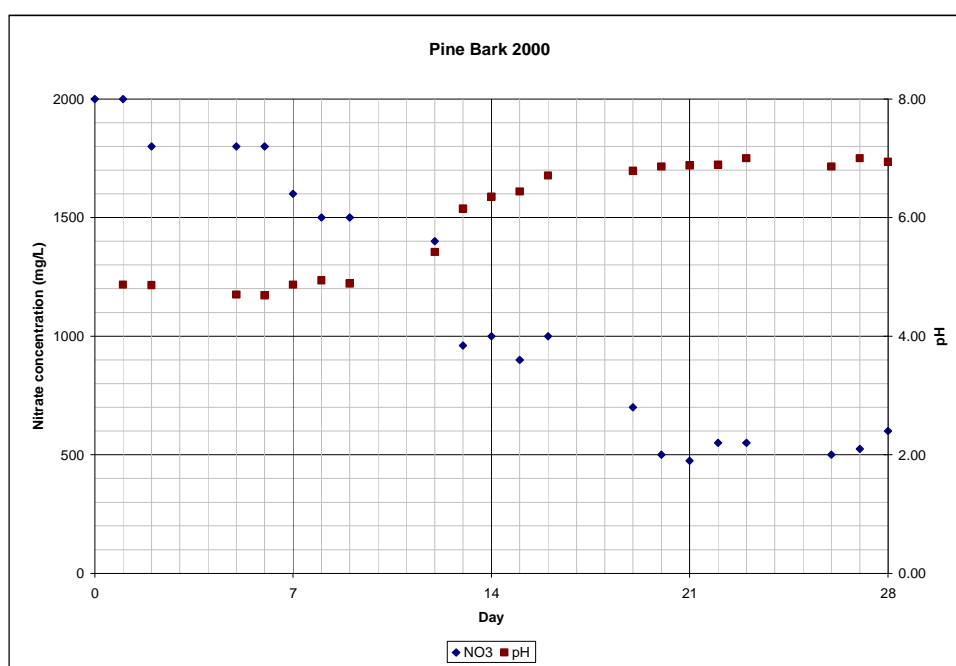


Figure 28: Experiment 1 - Evolution of the nitrate concentration and pH for PB for $C_o = 2000 \text{ mg/l}$ at flow rate 1

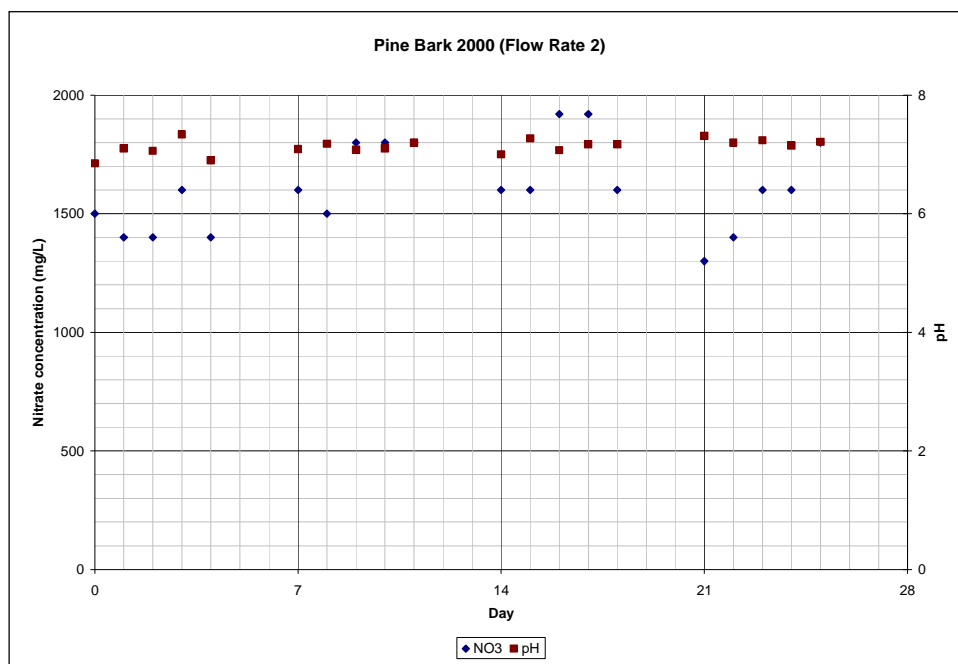


Figure 29: Experiment 2 - Evolution of the nitrate concentration and pH for PB for $C_0 = 2000 \text{ mg/l}$ at flow rate 2

The COD of the output for the pine bark substrate at 2000 mg/l are shown in Figures 30 and 31.

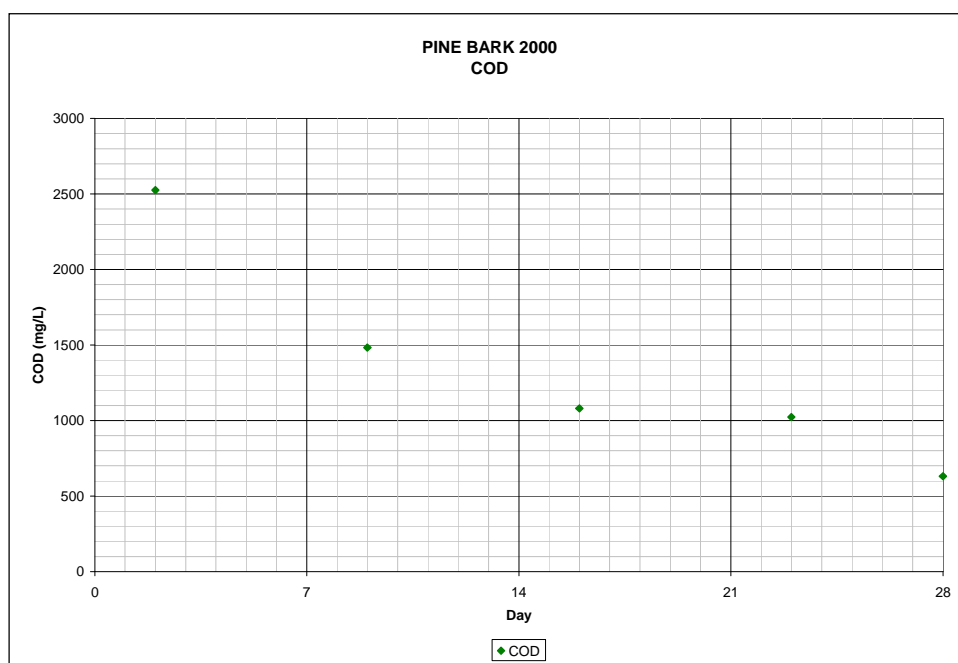


Figure 30: Experiment 1 – Evolution of COD for PB for $C_0 = 2000 \text{ mg/l}$ at flow rate 1

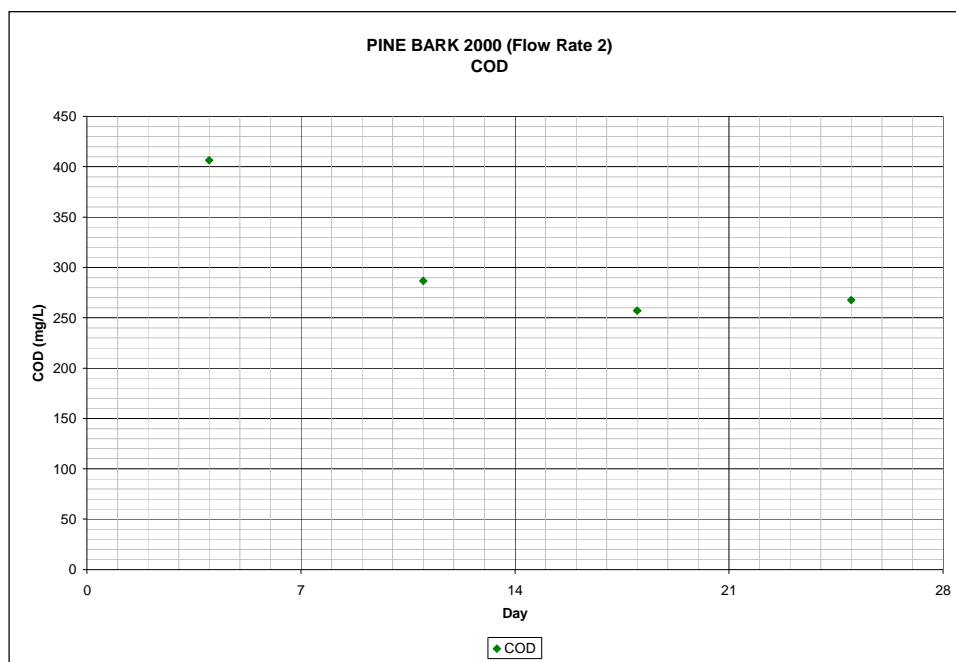


Figure 31: Experiment 2 – Evolution of COD for PB for $C_0 = 2000 \text{ mg/l}$ at flow rate 2

During the first 6 days of experiment 1 the column showed little change in concentration. This plateau is typical for pine bark due to the low pH value, which inhibits microbial activity. After this point, a more noticeable rate of denitrification was observed. It was particularly evident that during the third week there was a substantial drop in nitrate concentration. This is related to the change in pH, which rose to the optimum range for denitrification, allowing the system to reach 75% efficiency of nitrate. As full denitrification was not achieved it is apparent that the pine bark is releasing carbon at a slower rate than that at which nitrate is being supplemented. It is therefore evident that the contact time was too low and that the substrate requires over 7 days for a zero nitrate level to be reached.

In experiment 2, the nitrate level stayed at a concentration of approximately 1500 mg/l for 8 days, where the peaks and drops were more likely due to errors associated with the nitrate stick method. After day 8 the concentration rose and remained at this level for the remaining 3 days of the week. The lower rate of denitrification achieved can be attributed to the flow rate being too high, resulting in insufficient contact time between the solution and substrate, thus only 35% removal efficiency was achieved against 75% in the first experiment for pine bark at $C_0 = 2000 \text{ mg/l}$.

In experiment 1, the COD of the output effluent dropped by 76% from 2500 mg/l to 600 mg/l, whereas, the COD values during experiment 2 decreased to 260 mg/l over the first three weeks of testing and remained at this level until the end of the experiment.

Initially the pH during the experiment 1 stayed at a constant level of 4 – 5. After 9 days, however, the pH tended to increase to neutrality, whilst pH during experiment 2 stayed constant at approximately 7. The temperature remained in a range between 19 and 22 °C. The $\text{NH}_3 - \text{N}$ during experiment 1 did increase after the first week of testing, however decreases to remain below 3 mg/l until the completion of the experiment, whilst the recorded $\text{NH}_3 - \text{N}$ of experiment 2 was less than 1mg/l throughout the duration of the experiment.

3.3.3. Loading Rates and Hydraulic Retention Time

The Hydraulic retention time (HRT) is a measure of the average length of time that a soluble compound remains in a constructed bioreactor and is calculated by the volume of the reactor divided by the flow rate (<http://www.lenntech.com/wwtp/hrt.htm> accessed 19/12/2009).

The hydraulic retention time has an affect on nitrate removal and is thus vitally important in the design of a bioreactor for nitrate removal (Tsui et al., 2007). The hydraulic loading rate is a critical factor for the design of treatment systems and is determined as the volume per day that can be applied over a surface area (Zhou et al., 2007).

Table 13 presents the performance of the various substrates for each of the columns for the changes in concentration and flow rate. These results can be extrapolated using simple ratio concentrations to provide an estimate of the ideal flow rates and hydraulic retention times.

Table 13

Summary of the performance of the column studies over both experiments

Substrate	NO_3 Conc. (mg/l)	Flow Rates (l/day)		HRT (Days)		% Removal		Loading Rate ($\text{l/m}^2/\text{day}$)	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp.1	Exp.2
CGR RAW	500	2.48	5.625	8.06	3.56	100	100	123.32	279.71
PB	500	2.00	5.00	10.00	4.00	100	90	99.45	248.63
CGR RAW	2000	2.38	5.65	8.40	3.54	100	45	118.35	280.95
PB	2000	2.00	5.00	10.00	4.00	75	35	99.45	248.63

For both the tests conducted at $C_o = 500 \text{ mg/l}$ and 2000 mg/l , the CGR RAW was the best performing substrate. For the test at $C_o = 500 \text{ mg/l}$ full nitrate removal was achieved at both flow rates.

Due to the 100% nitrate removal achieved at $C_o = 500 \text{ mg/l}$ at both flow rates it can be concluded that the CGR RAW can sustain a higher flow rate than 5.625 l/day as well as a loading rate above $280 \text{ l/m}^2\text{/day}$. The HRT time required for full nitrate removal is less than 3.5 days.

For the tests conducted at $C_o = 2000 \text{ mg/l}$, the system only achieved full nitrate removal at the first flow rate of 2.38 l/day in experiment 1, whereas in experiment 2 a 45% nitrate removal was reached. Through simply extrapolation an estimated flow rate of 2.54 l/day and a HRT of 8 days would be needed for the system to achieve full denitrification.

The pine bark was the least efficient substrate at $C_o = 500 \text{ mg/l}$ achieving 100% nitrate removal at the flow rate in experiment 1, however only reaching 90% nitrate removal in experiment 2. This suggests that the flow rate required for full denitrification is between $2 - 5 \text{ l/day}$. A flow rate of 4.5 l/day and a HRT of 4.5 days are estimated.

At $C_o = 2000 \text{ mg/l}$, the pine bark only achieved 75% nitrate removal in experiment 1 and 35% in experiment 2. This indicates that both flow rates were too high for full denitrification to be reached. A flow rate of $1.5 - 1.75 \text{ l/day}$ and a HRT of 13 days are estimated.

4. Conclusions and recommendations

The results of the laboratory experiments substantiate that the substrates prove to be effective as carbon sources to denitrify various concentration levels of nitrified leachate, at different degrees of efficiency.

The substrate materials had varying compositions of relatively high carbon (C) content in comparison to nitrogen (N). This characteristic makes these materials well suited for nitrate removal as they provide organic carbon for denitrification without increasing the nitrogen concentration. They also act as a medium for denitrifying bacteria.

The characterisation tests indicated that the fresh commercial garden refuse material had higher carbon to nitrogen ratio than the pine bark substrate. Although the pine bark

substrate had a carbon to nitrogen ratio of 62.15, due to the acidic nature of the material, with a pH of 4.18; this would be inhibitory to denitrification and thus it was likely that this substrate would not perform as well.

The batch tests showed positive results; with the best performing substrate being the CGR RAW which achieved full denitrification at the highest nitrate concentration of 2000 mg/l between 9 – 12 days, which can be attributed to its high C/N ratio. The pine bark did not achieve full denitrification in 2 out of 3 concentrations. It only managed to achieve 100% removal at a nitrate concentration of 100 mg/l. During the tests conducted at 500 and 2000 mg/l, only 55% and 20% removal were achieved.

All the small-scale batch tests demonstrated similar characteristics of an acclimatisation period before decreasing linearly with time. The duration of the acclimatisation period was strongly related to that of the initial input concentrations of the nitrate solution.

The column tests reflected promising results at $C_o = 500$ mg/l during experiment 1, with both substrates achieving full denitrification. At $C_o = 2000$ mg/l only the CGR RAW column reached full denitrification. The pine bark only managed 75% removal. The CGR RAW substrate reflected the best results. The column at 500 mg/l displayed a HRT of 8 days was required whereas the higher concentration of 2000 mg/l required a HRT of 8.5 days.

During experiment 2, however the increased flow rates were too high to allow denitrifying bacteria sufficient contact period or hydraulic retention time to establish themselves. The CGR RAW substrate column at 500 mg/l was the only one to achieve 100% nitrate removal. A HRT time required for full denitrification is less than 3.5 days. It is noted that flow through the columns improves the organic matter release and dispersion rates compared to a system where the effluent remains stagnant (Diaz et al., 2003). However a flow rate that is too high could result in an insufficient hydraulic retention time, which does not allow denitrifying bacteria to accumulate for denitrification. The results also indicate that the rate at which carbon is being released is slower than the rate at which nitrates are being added.

The main concern of this treatment method is the increase in COD concentration produced by organic matter release. The COD levels were all above the limits provided by DWAF (DWAF - General Authorisations in terms of Section 39 of the National Water

Act, 1998). It was found that over time the COD concentrations did decrease, but, in most cases, not sufficiently to fall into DWAF's Water Quality criteria (DWAF - General Authorisations in terms of Section 39 of the National Water Act, 1998).

The eThekweni landfills receive large volumes of garden refuse monthly which is separated from the main waste stream. Large quantities of pine bark are produced by both SAPPI and Mondi paper as a by-product of the paper and pulp industry in South Africa. If needed for the denitrification process the pine would be obtainable for utilisation. These two materials are highly abundant and easily available on site, thus making them fairly inexpensive.

They could therefore be successfully employed at local landfill sites to denitrify treated leachate which would prevent excessive treatment costs as well as support the development of a real waste management strategy that is in the process of being implemented within the country.

Further studies are being conducted at different flow rates and concentrations to ensure that the reactor is robust and flexible to deal with the change in quality of the leachates during the life of the landfill. Lower concentrations need to be investigated to determine whether the substrates are suitable for all ranges of nitrates and leachates. In this research a synthetic nitrate solution was used to simulate the treated leachate, however, tests with real treated leachate are being conducted, in order to ascertain a more accurate understanding of how the substrates might behave in a real full-scale treatment system.

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APPENDIX A2

The use of Commercial Garden Refuse at different degrees of maturity as a means to bio-denitrify treated MSW landfill leachate.

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ABSTRACT

The promotion of executing the holistic practice that is waste management, is a key objective faced by a vast range of professionals, in these modern times. The treatment of MSW landfill leachate is a major issue when realising this goal. This is a multi-stage process, which deals with the collection, treatment and discharge of contaminated effluent. A nitrification and denitrification process often used to reduce the high ammonia concentrations to below discharge limits, is currently common practice in Southern Africa. Denitrification, the conversion of nitrates to nitrogen gas, occurs in the presence of a carbon source in an anaerobic environment. This paper presents an efficient, cost effective, feasible alternative to expensive methods by promoting the use of natural organic sources of carbon such as commercial garden refuse at different degrees of maturity, as carbon sources for bio-denitrification. Substrates include fresh (CGR RAW), immaturely (CGR 10) and maturely composted (DAT and TW) commercial garden refuse. The efficiency of each substrate to support nitrate removal will be established using laboratory experiments. Characterisation and small-scale dynamic batch tests, simulating fixed-bed reactors, were used to assess their performance, whilst comparing the behaviour when denitrifying different concentrations (100, 500 and 2000 mg/l NO₃) of synthetic nitrate solution. The testing provides evidence, that substrates have the potential to act as carbon sources to denitrify high strength leachate, with different degrees of efficiency. Studies reveal that the fresh material was the most suitable of the commercial garden refuse.

1. Introduction

The eThekweni Municipality is currently nitrifying leachate from the Mariannhill Landfill site in a Sequencing Batch Reactor plant. The treated effluent is then used as dust suppressant. After closure of the landfill the effluents from the plant will not comply with the discharge limits of wastewater into a water resource, as enforced by DWA. Currently, treated landfill leachate, produced from the SBR displays nitrate concentrations up to 2200 mg NO₃/ℓ. Further denitrification will be required to reduce the high concentrations of nitrates in the nitrified effluents to below the discharge limits. Thus an ad-hoc treatment will be needed prior to the discharge of leachate into the natural environment.

The focus of this project is to determine the efficiency of using garden refuse at different stages of maturity as carbon sources for the nitrate removal of treated landfill leachates, thus assessing the feasibility of the substrates as a means to denitrify treated landfill leachate in an integrated waste management system. Micro-organisms which reduce nitrates through the conversion into nitrogen gas during biological denitrification require an external carbon source to act as an electron donor, acting in an anaerobic environment [1, 2].

Expensive easily biodegradable carbonaceous materials are currently employed around the world (methanol, ethanol etc.); however these methods tend not to be a viable solution for developing countries and are not suited for large scale, field applications [3, 4]. These biodegradable carbonaceous naturally organic substrates were chosen as they contain relatively high amounts of carbon, are suitable for large scale, field application and are readily available in the major eThekweni landfills. The technical feasibility of an anaerobic batch reactor (submerged filter bed) is being tested at both laboratory and full scale in terms of bio-chemical, operational and economic indicators.

Experimental tests and analysis have been conducted in the laboratory to determine the efficiency and performance. Filter beds packed with commercial garden refuse at different degrees of maturity were simulated in dynamic batch tests, after the characterisation of the substrates. The kinetics of nitrate removal for the different substrates and various flow rates as well as environmental conditions (pH, nitrate concentrations, temperature etc.) were determined for all tests.

It is important to point out that the main outcome of this study will be an innovative low-cost treatment solution which is suitable for high strength leachates, waste waters and industrial effluents, which can be designed and implemented as part of an

integrated waste management system promoting the efficient reuse of waste material (garden refuse).

This solution is directed at reducing the impact of human activities on natural water systems by, not only minimising the deposited waste in a landfill, but by also improving the quality of wastewater being discharged into water resources thus limiting any detrimental disturbances on the relevant ecosystems.

2. Materials and Methods

2.1. Materials

A synthetic nitrate solution was used to simulate the treated landfill leachate, so as to operate the denitrification process in controlled conditions. In previous studies conducted by Pisano [5], the presence of chlorinated compounds in the leachate caused disturbances in the nitrate (NO_3) analysis. The substrates investigated in the research were commercial garden refuse at different degrees of maturity.

A large amount of commercial garden refuse consisting mainly of branches and plant trimmings from parks and green municipal areas is disposed of at both the Mariannhill and the Bisasar Road Landfill sites in Durban. This garden refuse is separated from the main waste stream. At the Bisasar Road Landfill, the CGR is passed through a chipper to reduce the particle size to approximately 4 – 5cm length and then composted in turned open windrows. Fresh commercial garden refuse was collected from the landfill soon after the size reduction phase. The CGR material was composted in troughs at the University of KwaZulu-Natal using forced aeration technology for ten weeks.

Two mature composts consisted of CGR disposed at the Bisasar Road Landfill, was composted for over 4 months in open windrows using the Dome Aeration Technology [6-9] and traditional turned windrow composting.

Dome Aeration Technology (DAT) is an advanced composting process for the aerobic biological degradation of garden refuse and general waste. It is a non-reactor open windrow composting process, where input material does not need to be turned periodically. The DAT method uses the passive aeration achieved through thermally driven advection in open windrows which is caused by the temperature differences between the degrading material and the outside environment [10].

The ‘turned windrow’ composting process consists of rows of long piles of organic waste known as ‘windrows’, that are turned on a regular basis using either manual or

mechanical means, to allow for aeration to occur, causing degradation/stabilisation of the material into compost [11, 12].

2.2. Characterisation tests

Initially, the substrates were comprehensively characterised, through the use of the standard analytical methods as published by ASTM [13]. Conventional testing was done on both the solid substrates and their eluates.

Tests conducted on the solid substrates included: moisture content, Total and Volatile Solids (TS and VS), carbon to nitrogen Ratio (C/N) and Dynamic Respiration Index at 7 days (RI_7) that was determined using a respirometric system type OxiTop®. The RI_7 expresses the rate at which oxygen is consumed in the biodegradation of organic matter and is often used as a means to define the level of stability and biodegradability of fresh and composted garden refuse [14-16]. To determine the nature as well as the amounts of compounds released by the substrates whilst being in contact with water, the eluates of the substrates were tested. The eluates were prepared by mixing a representative sample of each of the substrate with distilled water at a liquid to solid ratio of 10:1, for 24 hours, before being filtered through a 63 micron sieve to obtain the eluate. Eluates were tested to determine: pH, conductivity, TS, VS, COD, BOD, NH_3 and NO_3 . All tests were conducted in double or triplicate to ensure accuracy and repeatability.

2.3. Batch tests

To assess the suitability of each substrate as carbon sources for denitrification, small-scale batch tests were conducted, at 3 different nitrate concentrations: 100, 500 and 2000 mg NO_3 /ℓ using a simulated synthetic nitrate solution. A blank control test (0 mg NO_3 /ℓ) using distilled water for each substrate was also performed. The batch tests were designed to determine the kinetics of removal of each substrate at optimal conditions. These were maximum contact between substrate and solution, a pH range between 6 to 8 and at a temperature of approximately 25°C. A Liquid to Solid ratio of 10:1 was used for all tests to ensure full saturation throughout the experiment.

Tests were conducted in duplicate or triplicate in closed top batch reactors consisting of 1 ℓ, 3 neck bottles equipped with two airtight silicone septa which allowed continuous sampling thus preventing any air ingress. Each bottle was filled with 100g dry matter of substrate and respective concentration of potassium nitrate solution (KNO_3). The substrate particles were cut and reduced to a standard size of 4 – 5 cm to

ensure homogeneity of the sample. Prior to adding the nitrate solution, the bottles filled with substrate, were flushed with nitrogen gas to ensure the immediate establishment of anaerobic conditions in the vessels.

The batch reactors were placed in a shaker at 150 rpm at a controlled room temperature of approximately 25°C. Small samples of approximately 1-5 mL were extracted using a gas tight syringe so as to test the nitrate concentration (NO₃) every hour for the first day, thereafter 3 times a day depending on any changes in nitrate concentration. This method of extraction was performed in order to not significantly affect the L/S ratio in the reactors and to ensure that full saturation was maintained throughout the experiment. Nitrate concentrations for the batch tests were determined using the Nitrate Test Sticks type Merckoquant (MERCK). In some instances, the amount of fines in the tests prevented an accurate reading on the nitrate sticks. Thus some of the samples were filtered using a 0.45 µm.

The batch tests were conducted until the nitrate concentration reached zero. At the end of the test, both liquid and solid samples were characterised.

2.4. Microbial tests

To monitor and assess the effect of the different substrates on the evolution of indigenous bacterial population during bio-denitrification, microbial analyses were also conducted by De Combret [17, 18] for the batch tests at 500 mg/L. The growth of the microbial community was followed using a spread plate enumeration technique; the colonisation of the substrates assessed through Environmental Scanning Electronic Microscopy (ESEM), and an insight into the composition of the bacterial community was determined by phylogenetic analysis [18].

3. Results and discussion

3.1. Characterisation of substrates

Table 1

Characterisation of the solid substrates

	MC (%)	TS (%)	VS (%)	RI7 (mg O ₂ /g DM)	Tot C (%)	Tot N (%)	C/N
CGR RAW	37.14 ± 3.17	62.86 ± 3.17	96.37 ± 0.75	7.770	49.6	0.55	90.19
CGR 10	67.03 ± 0.83	32.97 ± 0.83	89.62 ± 1.40	5.672	28.69	1.20	23.91
DAT	54.24 ± 2.90	45.76 ± 2.90	87.20 ± 8.68	6.987	22.04	0.96	22.96
TW	59.28 ± 3.22	40.72 ± 3.22	71.73 ± 2.42	9.823	29.04	1.65	17.60

Table 2

Results of the eluate tests

	TS (g/l)	VS (g/l)	pH	Cond (mS/cm)	COD (mg/l)	BOD5 (mg/l)	NH ₃ -N (mg/l)	NO _x -N (mg/l)	C/N
CGR RAW	4.08 ± 0.02	3.04 ± 0.02	5.45	1.653	4253	1101	12.74	6.86	4.54
CGR 10	2.40 ± 0.10	1.62 ± 0.07	6.98	0.81	2764	155	9.80	7.14	1.83
DAT	11.78 ± 0.26	7.55 ± 0.29	6.93	1.23	10080	348	29.40	8.96	8.57
TW	12.55 ± 0.14	8.61 ± 0.14	7.27	2.69	11270	474	50.12	14.56	7.44

Tables 1 and 2: The ± values refer to the standard deviation of the results. The standard deviation is only included when the test has been done in triplicate or greater.

As suggested in Table 1 and 2 the fresh garden refuse is acidic. This acidic nature will cause an inhibitory effect on denitrification, as pH is a limiting factor in the denitrification process. The low pH value will impact negatively on the rate of nitrate removal. The optimum pH for biological denitrification is between 6 and 8. Through degradation and the high production of NH₃, pH levels in the composted materials are closer to neutral and in some cases alkaline [15]. The composting has produced favourable pH values for denitrification as they are now within the optimum range.

Due to the fact that the raw garden refuse substrate is an organic material and has not undergone any stabilisation, a higher carbon content, is evident in the form of the C/N ratio, COD and BOD. The typical range for stabilised compost is between 13 – 16 [3, 19]. The DAT and CGR 10 fall outside this range, having a greater C/N ratio. This should make these two materials appropriate for denitrification. The lower C/N ratio displayed by the composted material is due to its maturity and stability. The ideal initial C/N ratio to obtain good compost is 20 – 35

(http://whatcom.wsu.edu/ag/compost/fundamentals/needs_carbon_nitrogen.htm accessed 15/12/2009). The materials have a similar composition in the fact that they have higher carbon (C) content in comparison to nitrogen (N). This characteristic makes these materials well suited for nitrate removal as they provide organic carbon for denitrification without increasing the nitrogen concentration.

The total solids in the eluates show that the raw garden refuse has a higher amount of total solids than the immaturely composted material. However, due to the composting process, which mobilises the degraded fine particles increasing the TS concentration in solution, both the mature composts have a higher amount of total solids.

There is a strong correlation between TS and COD. Higher TS levels reflect in higher percentage of total carbon in the eluates. This suggests that the carbon is easily released by leaching, mobilised by the composting process and can be used for denitrification.

The RI_7 or respiration test as proposed by Adani et al. [14] assesses the biodegradability and biological stability of the material by determining the amount of oxygen consumed by the indigenous biomass that is present in the substrate to degrade the material. "The biological stability indicates the extent to which readily biodegradable organic matter has decomposed" [15, 16]. An unstable material is considered to contain a high portion of biodegradable matter that must sustain high microbial activity [16, 20].

As described by Gomez et al. [16] the respiration is directly related to the metabolic activity of the microbial population. Large amounts of bioavailable organic matter cause micro-organisms to respire at a higher rate than that if the material is scarce of organic matter [16]. Respiration has become an important parameter in the composting process for ascertaining the stability of the material [16].

As defined by Adani et al. [14] compost is a stable, mature and humified material. The quality of compost is assessed according to both the maturity and stability parameters [16]. The respiration activity is measured as O_2 consumption and/or CO_2 production by the composting mass [16, 20]. A lower RI_7 value indicates that a material is not only more mature but also more stable.

As expected the immaturely composted CGR has lower RI_7 value its fresh counterpart. This indicates that during the composting process the materials have not only become more mature but also more stable. The fresh raw material thus has a high portion of biodegradable matter that can sustain high microbial activity.

What is interesting is that the composted CGR 10 substrate which has been composted using forced aeration at UKZN has a lower RI_7 value than both the maturely

composted materials. This suggests that it is not only more mature but also more stable, making it higher quality compost. This indicates that the composting efficiency achieved, in the forced aeration troughs at UKZN, was relatively higher than those produced from Bisasar Road Landfill.

The mature composts display the presence of high levels of ammoniacal nitrogen ($\text{NH}_3 - \text{N}$). This may cause increased nitrate levels through bioleaching. The production or leaching of NH_3 from the substrate will cause a rise in nitrogen. If there is sufficient oxygen present, in either the solution or the pores of the substrate, NH_3 could be converted into NO_2 .

3.2. Batch Tests - Kinetics

The batch tests were designed to determine the kinetics of removal of each substrate at optimal conditions. Each test was conducted until the nitrate concentrations reached zero, after which, both the liquid and solid samples were characterised. The decrease in the concentration over time in the system was measured and rate of reaction of each determined. This rate is proportional to a derivative of a concentration. The results were modelled using a zero order kinetic reaction model, with the characteristic plot producing a straight line.

Zero order reaction: $\frac{dc}{dt} = -k \rightarrow c = c_0 - kt$ where k is the zero-order rate constant.

3.2.1. Nitrate concentration 100 mg/l NO_3

The evolution of the nitrate concentration for the all the substrates conducted at an initial concentration of 100 mg/l NO_3 is presented in Figure 1.

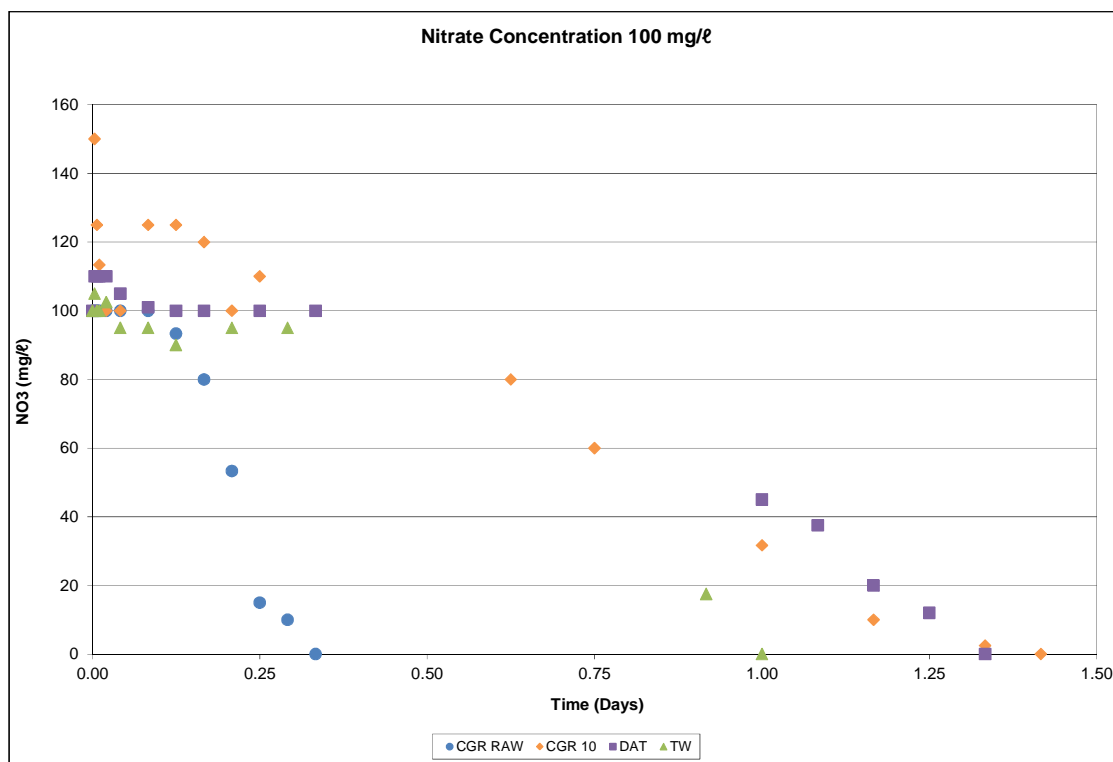


Figure 1: Evolution of the nitrate concentrations at $C_o = 100 \text{ mg/l}$

The results were modelled using a zero order kinetic reaction model. Table 3 summarises the kinetic rates of removal over the linear period of each batch test, determined from the plotted figures as well as time required to achieve the indicated percentage of removal at a nitrate concentration of 100 mg/l .

Table 3

Summary of kinetics at 100 mg/l

	Time for 100% Removal (Days)	K (mg/l/day)	R ²	% Removal
CGR RAW	0.33	455.710	0.951	100
CGR 10	1.42	98.051	0.982	100
DAT	1.33	97.457	0.976	100
TW	1.00	130.310	0.996	100

All the tests exhibited an initial plateau ranging from 2 to 8 hours, depending on each substrate. This initial stage is a result of acclimatisation within the system, which involves pH buffering. The mature DAT compost presented the longest acclimatisation period. Once the conditions of the test had stabilised, nitrate removal occurred at a linear rate until a zero nitrate concentration was achieved. The most efficient substrate being the CGR RAW; where the fresh material completed full denitrification within 6 to 8 hours. Surprisingly, the 10 week immature compost was the poorest performing at

this concentration, which can be attributed to the low RI_7 , which is a measure of its biodegradability. All the tests conducted with the materials at different maturities, managed to reduce the initial 100 mg/l nitrate concentration in less than 1.5 days.

3.2.2. Nitrate concentration 500 mg/l NO_3

The evolution of the nitrate concentration for the all the substrates conducted at an initial concentration of 500 mg/l NO_3 is presented in Figure 2.

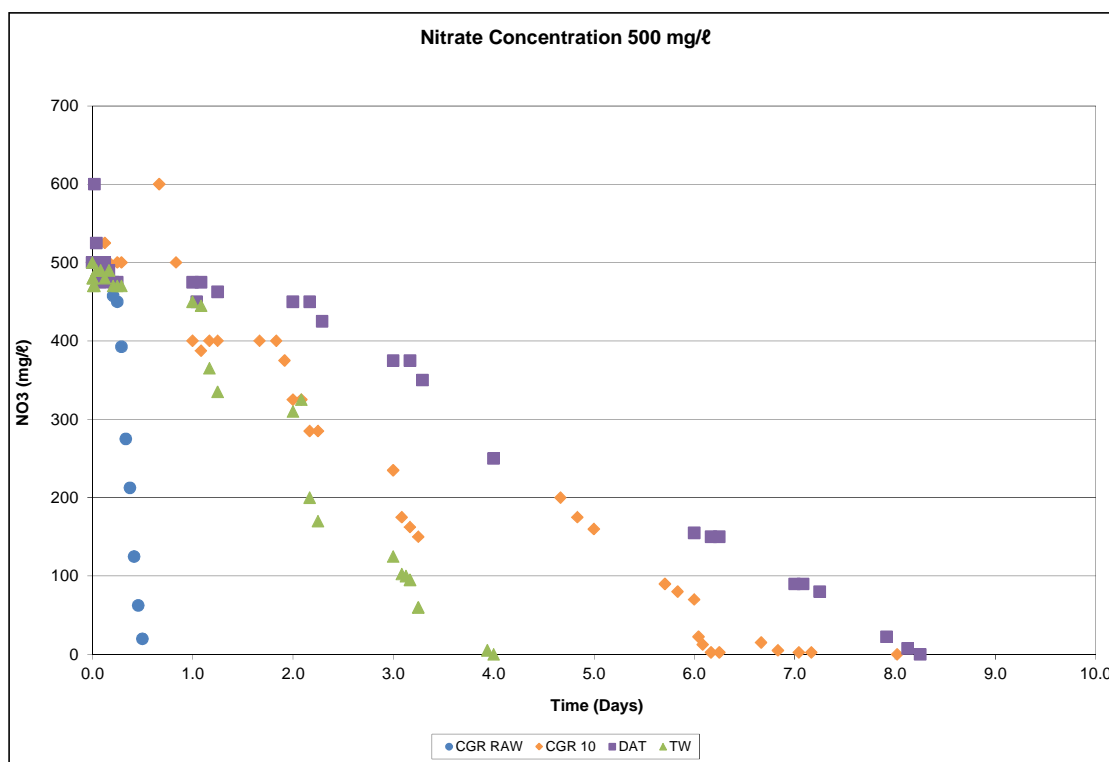


Figure 2: Evolution of the nitrate concentrations at $C_0 = 500$ mg/l

Table 4 summarises the kinetic rates of removal over the linear period of each batch test, modelled using a zero order kinetic reaction model and determined from the plotted figures as well as the time required to achieve full nitrate removal at a concentration of 500 mg/l.

Table 4

Summary of kinetics at 500 mg/l

	Time for 100% Removal (Days)	K (mg/l/day)	R ²	% Removal
CGR RAW	0.50	1270.000	0.923	100
CGR 10	8.02	70.171	0.91	100
DAT	8.25	68.854	0.99	100
TW	4.00	146.610	0.941	100

As in the experiment conducted at the lower concentration, all four substrates displayed similar characteristics in terms of the evolution of nitrate concentration. An initial plateau is present as the system acclimatises followed by a fairly constant linear regression. In this case, the plateau period is longer suggesting a relationship between initial concentration and the duration of the acclimatisation stage, with a maximum period of 48 hours. The results displayed in this test correspond to that of the lower concentration, where the fresh substrate produced the most favourable rate of reaction. Full denitrification was achieved in less than 12 hours. The CGR 10 and DAT materials presented similar results where complete nitrate removal occurring in approximately 8 days.

3.2.3. Nitrate concentration 2000 mg/l NO_3

The evolution of the nitrate concentration for the all the substrates conducted at an initial concentration of 2000 mg/l NO_3 is presented in Figure 3.

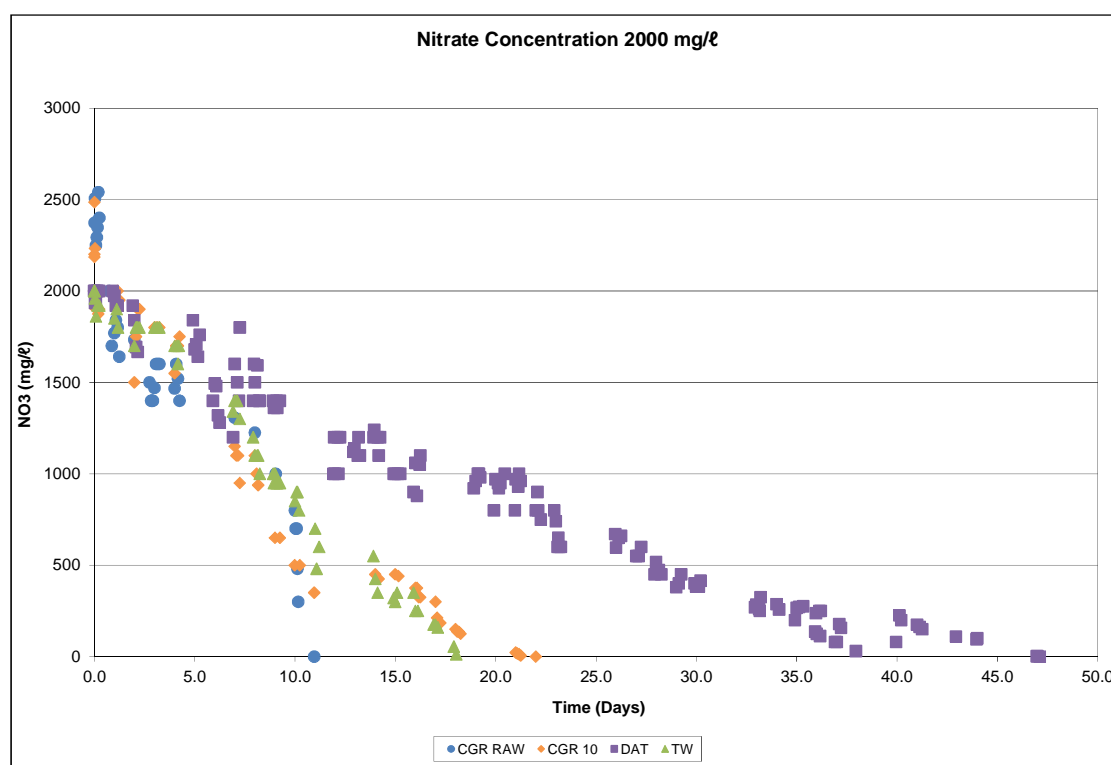


Figure 3: Evolution of the nitrate concentrations at $C_0 = 2000 \text{ mg/l}$

The kinetic rates of zero order kinetic reaction models for rate of nitrate removal over the linear period of each batch test, as well as time needed for full denitrification of nitrate concentration of 2000 mg/l, is presented in Table 5.

Table 5

Summary of kinetics at 2000 mg/l

	Time for 100% Removal (Days)	K (mg/l/day)	R ²	% Removal
CGR RAW	11	131.890	0.867	100
CGR 10	22	98.866	0.906	100
DAT	47	42.844	0.948	100
TW	18	117.00	0.968	100

The final test at a concentration of $C_0 = 2000$ mg/l showed a slight initial rise in nitrate concentration prior to the plateau period. After which, the same characteristic linear rate of nitrate removal was exhibited. The CGR RAW presented an acclimatisation stage of 18 to 24 hour, with full denitrification occurring within 11 days. Approximately 18 days were required for the TW sample to fully denitrify the 2000 mg/l concentration. The DAT material was the poorest performing substrate, taking more than 47 days to reach complete nitrate removal.

3.2.4. Summary

The duration of the plateau period tended to increase with an increase in initial nitrate concentration, suggesting that pH and the initial NO_3 concentration play an important inhibitory role during this initial stage as demonstrated by De Combret [17, 18].

Some tests show a slight increase in the initial nitrate concentration at the beginning of each experiment. This could be due to the small amount of NO_3 present in the sample and the initial bioleaching of organic nitrogen from the solid substrate, which corresponds to the values determined in the initial characterised sample. During all the tests conducted with the CGR RAW substrate, nitrites were present.

Studies done by De Combret [17] and Trois [18] suggest that denitrifiers are only present after 74 hours. The microbial tests conducted in 2009 suggest that high performance could be to another phenomena rather than bio-denitrification, thus the removal of nitrate within 24 hours could be attributed to other bio-chemical processes such as absorption of nitrates or the conversion of nitrates into ammonia.

The efficiency of an organic substrate to denitrify an effluent is closely linked with the relationship between the maturity and stability of the material as determined through the RI_7 testing and the corresponding C/N ratio. A substrate that is less stable and mature, but has a lower C/N ratio can produce more favourable results. However with an increase in the duration of the testing, as more carbon is released and readily available, the C/N ratio becomes the more dominant factor. The longer the duration of

testing, the maturity and stability of a material will increase, reducing its affect. This is clearly demonstrated by the comparison between CGR 10 and TW over the different concentrations.

At low concentrations, the biodegradability and readily available carbon, dominate the rate of denitrification. However, within a longer testing period, such as in the case with initial nitrate concentrations of 500 and 2000 mg/l respectively, a greater release of carbon is allowed which is then available for denitrification. The higher C/N ratio seems to counterbalance the importance of biodegradability on the rate of reaction.

There is a clear indication that the efficiency of the composting at UKZN laboratory was particularly effective, as the material composted for 10 weeks, presents the characteristics of a more mature and stable medium, compared to that of the compost collected from the landfill site from the two different composting techniques.

3.3. Batch Tests - Output Characterisation

At the conclusion of each batch test, once full nitrate removal was achieved. The output material was once again characterised. The main aspects looked at were the pH, COD and NH₃-N. The COD provides insight into the release and presence of carbon whereas the ammonia gives an indication into the relating nitrogen.

3.3.1. Fresh commercial garden refuse (CGR RAW)

Table 6 presents the results of the characterisation of output materials from the batch tests with CGR RAW.

Table 6

Characterisation results of the output material for the CGR RAW batch tests.

	pH	COD (mg/l)	NH ₃ -N (mg/l)
100 (mg/l)	5.97 – 6.16	4325 – 5212	4 – 30
500 (mg/l)	5.41 – 5.68	3951 – 7200	20 – 30
2000 (mg/l)	6.80 – 7.33	7009 – 7870	75 – 100

It is noted that the fresh CGR has an initial pH that ranges around 5.45 and increases with time and with NO₃ concentration as reported by other authors [3]. It is also noted that the longer test conducted at an initial concentration of 2000 mg/l exhibits a final pH which falls into the optimum range for denitrification.

As a result of the production of NH₃ leached out from the substrate as well as the oxygen present in the solution and the pores, NH₃ is converted into NO₂ even when full

nitrate removal is achieved. It was confirmed by De Combret [17] and Trois [18] that both nitrifiers and denitrifiers were present in this substrate within the first 74 hours of batch test, in line with other studies that used similar substrates [21].

There was a presence of positive bioleaching of carbon which was observed in the increase of both the COD and C/N ratios, relating to the initial nitrate concentration. The COD results showed an increase from the initial input ranging from 3951 – 7870 mg/l. The ammoniacal nitrogen released, also tended to increase with the time.

3.3.2. Commercial Garden Refuse (CGR 10)

The characterisation results of the tests performed on the output material at the end of the batch tests at the different initial nitrate concentrations are shown in Table 7.

Table 7

Characterisation results of the output material for the CGR 10 batch tests.

		pH	COD(mg/l)	NH ₃ -N (mg/l)
100 (mg/l)	Output Eluate	7.22	2754	2.5
500 (mg/l)	Output Eluate	7.51	3177	3.0

The pH values throughout the tests increased with the increase of the initial concentration and remain constant to optimum ranges for denitrification [22]. There was a presence of positive bioleaching of carbon which was observed in the increase of the COD, relating to the initial nitrate concentration. The COD results showed an increase from the initial input ranging from 2754 – 3177 mg/l. The NH₃ - N values in all the tests were lower than that of the initial input material. The test conducted at 100 and 500 mg/l showed a decrease of 70 - 75%.

3.3.3. Mature Compost: Dome Aeration Technology (DAT)

Table 8 shows the characterisation results of the tests performed output material from the batch tests using DAT.

Table 8

Characterisation results of the output material for the DAT batch tests.

	pH	COD (mg/l)	NH ₃ -N (mg/l)
100 (mg/l)	7.38	4165	4.3
500 (mg/l)	7.22	7442	28.0
2000 (mg/l)	7.60	13712	14.3

The pH remains constant around neutrality, while the COD results were all lower than the initial input value except in the case of the 2000 mg/l test. It is also noted that there was an increase in COD with an increase in the duration of each test, as a result of the release of carbon and the degradation of the material. NH_3 - N in the output values achieved in each test were lower than that of the input material, but still indicate a release in nitrogen and the production of ammonia.

3.3.4. Mature Compost: Turned Windrow (TW)

The characterisation results of the output sample at the conclusion of the TW batch tests are displayed in Table 9.

Table 9

Characterisation results of the output material for the TW batch tests.

	pH	COD (mg/l)	NH_3 -N (mg/l)
100 (mg/l)	7.86	4629	2.3
500 (mg/l)	7.58	7396	12.0
2000 (mg/l)	7.51 – 7.88	7398 - 12359	7.5 – 10

The output material from the TW batch tests displayed very similar characteristics to that of the DAT substrate. The pH within the optimum range of 6-8 is maintained through all tests, COD increases with initial concentration and the resulting extended test duration. The ammonia produced is also less than that determined in the input sample.

4. Conclusions and recommendations

Through the use of various laboratory experiments, including characterisation tests as well as the use of small-scale dynamic batch tests, it can be substantiated that nitrified leachate with a concentration ranging from 100 – 2000 mg/l NO_3 can be successfully denitrified using commercial garden refuse at different degrees of maturity as carbon sources. The efficiency of each substrate is highly dependent on the materials carbon content compared to nitrogen. Their success had can be credited to the varying compositions of relatively high carbon (C) content in comparison to nitrogen (N), which makes them well suited for nitrate removal as they provide organic carbon for denitrification without increasing the nitrogen concentration, whilst acting as a medium for denitrifying bacteria.

The characterisation tests indicated that the fresh commercial garden refuse material had higher carbon to nitrogen ratio than that of the composted materials. The batch tests showed positive results; with all substrates achieving full nitrate removal at the various concentrations. This can be attributed to the C/N ratio of the substrates and the fairly neutral pH. The higher carbon to nitrogen ratio of the fresh commercial compared to that of the composted materials was evident in the results, with the best performing substrate being the CGR RAW which achieved full denitrification at the highest nitrate concentration of 2000 mg/l between 9 – 12 days. The CGR 10 substrate achieved full denitrification at the highest nitrate concentration of 2000 mg/l within 22 days. The turned windrow substrate was the better performing mature compost.

Similar characteristics were presented for the evolution of nitrate concentration for all the batch tests. An acclimatisation plateau period was followed by a linear regression, which was modelled using a first order kinetic equation. The initial input concentrations of the nitrate solution have a strong effect on the period of the acclimatisation stage.

The characterisation of the output material from each batch test showed an increase in COD through the release of organic matter. This is a major concern as all the levels were above the limits provided by the local authorities.

One of the main reasons that this treatment method could be successful at local landfill sites is that the substrates are highly abundant, as large volumes of garden refuse is separated from the main waste stream. The availability of material on site will prevent excessive treatment costs whilst encouraging the sustainability of a real waste management strategy which is being implemented within the country.

Column studies are being used to simulate fixed bed reactors, operated continuously at a variety of concentrations and flow rates. A reactor is required to robust whilst at the same time being able to cope with the changes in the characteristics of the nitrified effluent.

Tests using actual treated leachate are being conducted as a means to determine the disturbances in nitrate removal and to more accurately understand how the substrates might behave when implemented in a full-scale treatment system. The full-scale design for a continuous flow, submerged constructed wetland is being researched to develop a flow rate that optimises denitrification yet minimizing the production of COD.

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APPENDIX A3

Nitrate removal of treated landfill leachate, using natural organic substrates as carbon sources in column studies, simulating fixed-bed reactors.

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ABSTRACT

Bio-denitrification is common practice in the treatment of nitrified landfill leachate. Denitrification, involves the conversion of nitrates to nitrogen gas, in the presence of a carbon source in an anaerobic environment. This study investigates natural organic materials to act as alternative external carbon sources. Based on a variety of previous characterisation and batch tests, 3 substrates were chosen to be used in leaching column studies, which simulate fixed bed reactors. The 3 substrates selected were, fresh (CGR RAW), immaturely composted (CGR 10) commercial garden refuse and Pine Bark (PB). High strength nitrified landfill leachate was simulated using two concentrations (500 and 2000 mg NO₃/ℓ) of synthetic nitrate solution. As a means to research the effect of hydraulic retention time two flow rates were implemented. Initial results confirm that each of the materials have the potential to achieve denitrification at different degrees of efficiency, with the fresh substrate displaying the most promising outcome.

1. Introduction

Nitrate contamination of natural water systems is increasingly prevalent in developed countries in addition to the developing world. In general, effective removal of nitrates on a large scale is inhibited by high costs associated with some processes and consequently non-compliance in respect of the W.H.O. and other benchmarks are not

uncommon especially in countries experiencing fiscal challenges. Currently, expensive easily biodegradable carbonaceous materials are employed around the world (methanol, ethanol etc.); however these methods tend not to be a viable solution for developing countries and are not suited for large scale, field applications [1, 2].

Under oxygen starvation, aerobic bacteria will revert to accepting nitrate as a terminal electron donor in respiration and consequently it is of significance that anaerobic conditions are instigated [3]. Micro-organisms which reduce nitrates through the conversion into nitrogen gas during biological denitrification require an external carbon source to act as an electron donor, acting in an anaerobic environment [4-6].

At the Mariannhill Landfill site as part of the eThekweni Municipality, a Sequencing Batch Reactor plant is nitrifying leachate, prior to being used as a dust suppressant. Once the landfill has reached its capacity and is decommissioned, the treated effluents produced from the SBR will require an ad-hoc treatment method so as to comply with discharge limits as stipulated by DWA for wastewater into a water resource [7]. The nitrified effluent exhibits concentrations up to 2200 mg NO_3/l ; hence a further denitrification step will be required.

Volokita, Belkin [2] investigated the efficiency of microbial denitrification of drinking water, conducting a laboratory study using columns with shredded newspaper “as the sole carbon and energy substrate”. Shredded newspaper packed in PVC columns were subjected to a nitrate amended tap water feed regulated by peristaltic pumps. Significantly according to Volokita, Belkin [2] “complete removal of nitrate without accumulation of nitrite was achieved after the onset of flow”.

Díaz, García [8] proposed that effectiveness of a substrate was linked to its biodegradability and furthermore; that the continuous flow reactor proved to be the more efficient device on the postulation that water circulation favoured the rate of organic matter release and dispersion [6]. Díaz, García [8] concluded that data produced by their study and the system tested provided a promising alternative particularly in terms of energy and consequently cost saving as well as operational and maintenance simplicity [9].

Evidence indicates that flow rate appears to be a critical factor in maintaining stable denitrification.

This study's methodology includes characterisation of the organic matter released by the various substrates, carbon and nitrogen composition and lasting properties of the substrates as well as specification of the continuous flow reactor nitrate removal process [8]. In this research column studies were set up to accurately simulate fixed bed reactors [1, 2, 8] and consequently subsurface flow constructed wetlands [10].

The fixed bed reactor is a well-known, efficient device for carrying out chemical and biological reaction processes primarily regulated by a catalyst (usually solid) packed in a bed located in a fixed position [11]. Fixed bed reactors have several favourable features [10, 12]. They are typically simple in design. The absence of moving parts in the device significantly reduces operational wear and tear and the catalyst is confined and contained in the reactor. The fixed bed reactor employs a continuous flow system enabling regulation and control of the appropriate flow rate. Reaction is facilitated as the reactant passes through the catalyst at the desired rate [11].

This project focusses on assessing the feasibility of naturally organic substrates as carbon sources for the nitrate removal of treated landfill leachates in terms of biochemical, operational and economic indicators [12]. Pine Bark along with fresh and composted commercial garden refuse are biodegradable carbonaceous substrates which contain relatively high amounts of carbon, are suitable for large scale, field application and are readily available in the major eThekweni landfills. Laboratory experimental tests and analysis were used to determine the efficiency and performance. Leaching columns packed with three substrates operated in continuous mode were used as a means to simulate filter beds after initial dynamic batch tests and the characterisation of the substrates. The kinetics of nitrate removal for the different substrates and various flow rates as well as environmental conditions (pH, nitrate concentrations, temperature etc.) were determined for each of the column tests.

Conventional testing using standard analytical methods as published by ASTM [13] were done on both the solid substrates and their eluates to comprehensively characterise each material [14].

Table 1

Characterisation of the solid substrates

	MC (%)	TS (%)	VS (%)	RI7 (mg O ₂ /g DM)	Tot C (%)	Tot N (%)	C/N
CGR RAW	37.14 ± 3.17	62.86 ± 3.17	96.37 ± 0.75	7.770	49.60	0.55	90.19
PB	48.85 ± 2.92	51.15 ± 2.92	97.08 ± 0.17	17.769	36.67	0.59	62.15
CGR 10	67.03 ± 0.83	32.97 ± 0.83	89.62 ± 1.40	5.672	28.69	1.20	23.91

Table 2

Results of the eluate tests

	TS (g/l)	VS (g/l)	pH	Cond (mS/cm)	COD (mg/l)	BOD5 (mg/l)	NH ₃ -N (mg/l)	NO _x -N (mg/l)	C/N
CGR RAW	4.08 ± 0.02	3.04 ± 0.02	5.45	1.653	4253	1101	12.74	6.86	4.54
PB	3.66 ± 0.01	3.35 ± 0.28	4.18	0.845	4517	297	8.54	15.12	3.57
CGR 10	2.40 ± 0.10	1.62 ± 0.07	6.98	0.81	2764	155	9.80	7.14	1.83

Tables 1 and 2: The ± values refer to the standard deviation of the results. The standard deviation is only included when the test has been done in triplicate or greater.

As suggested in Table 1 and 2, both the Pine Bark and fresh garden refuse substrates are acidic. An acidic pH value will have a negative impact on the rate of nitrate removal, as pH is a limiting factor of denitrification, thus resulting in an inhibitory effect on denitrification. However, through composting, degradation has produced a high level of NH₃, causing the pH levels in the CGR 10 material to be closer to neutral and in the optimum range for biological denitrification between 6 and 8.

The C/N ratio of the raw garden refuse and the Pine Bark substrate are comparable due to the fact that both are organic materials and yet to undergo any stabilisation. The high carbon content is evident in the form of the C/N ratio, COD and BOD. Pine Bark has a determined C/N ratio between 62–90:1, which is lower than that stated in the literature as presented in Trois and Polster [15] and Trois, Pisano [16].

As all these materials have higher carbon (C) content in comparison to nitrogen (N), they are well suited for nitrate removal, providing organic carbon for denitrification without increasing the nitrogen concentration.

Small scale dynamic batch tests were conducted at 3 different nitrate concentrations: 100, 500 and 2000 mg NO₃ /l simulated using a synthetic nitrate solution. The batch tests were designed to determine the suitability of each substrate to act as a carbon source for denitrification as well as to assess the kinetics of removal at optimal conditions [14].

Table 3

Summary of kinetics at 100 mg/l

	Time for 100% Removal (Days)	K (mg/l/day)	R ²	% Removal
CGR RAW	0.33	455.710	0.951	100
PB	2.2	46.775	0.98	100
CGR 10	1.42	98.051	0.982	100

Table 4

Summary of kinetics at 500 mg/l

	Time for 100% Removal (Days)	K (mg/l/day)	R ²	% Removal
CGR RAW	0.50	1270.000	0.923	100
PB	-	38.183	0.98	55
CGR 10	8.02	70.171	0.91	100

Table 5

Summary of kinetics at 2000 mg/l

	Time for 100% Removal (Days)	K (mg/l/day)	R ²	% Removal
CGR RAW	11	131.890	0.867	100
PB	-	126.250	0.91	20
CGR 10	22	98.866	0.906	100

All the batch tests for the different substrates displayed a similar trend, at each of the 3 nitrate concentrations. An initial plateau period was observed, which is related to competition between the nitrifiers and denitrifiers, pH buffering and the acclimatisation of conditions within the system. The length and duration of each plateau stage tended to increase with an increase in initial nitrate concentration, suggesting that pH and the initial NO₃ concentration play an important inhibitory role during this initial period. After the plateau, once the system had stabilised, nitrate removal occurred at a linear rate of denitrification and this was modelled using a zero order constant. Pine Bark was the only substrate not to achieve full denitrification. This can be attributed to NO₃ saturation at high concentrations and the release of phenols which are toxic to bacteria. The best performing material was the CGR RAW, as a result of its high C/N ratio as well as the portion of readily available biodegradable organic carbon as represented by the BOD₅ value.

2. Materials and Methods

2.1. Materials

A synthetic nitrate solution was used to simulate the treated landfill leachate, so as to operate the denitrification process in controlled conditions. In previous studies conducted by Trois, Pisano [16], the presence of chlorinated compounds in the leachate caused disturbances in the nitrate (NO₃) analysis.

Large quantities of commercial garden refuse collected from parks and green municipal areas are disposed of at landfill sites throughout the eThekweni municipality. It is separated from the main waste stream and passed through a chipper to reduce the particle size prior to being composted in turned open windrows.

Fresh commercial garden refuse was collected from the landfill soon after the size reduction phase. The CGR material was composted in troughs at the University of KwaZulu-Natal using forced aeration technology for ten weeks.

SAPPI (South African Pulp and Paper Industry) paper mills around the country grow mainly the *Pinus patula* variety and produce large amounts of Pine Bark daily, some of which is disposed of at local landfill sites as well as SAPPI's disposal facilities. In this research the tissue/cells from the outside of the vascular cambium of the hard Pine, *Diploxylon* tree was used and collected, fresh, within 24 hours of debarking.

2.2. Column tests

Columns studies were used to investigate the effect on denitrification rates for different nitrate concentration levels and flow rates. The results were used to predict the kinetics of removal, loading rates and hydraulic retention time for the filter beds. The three best performing substrates ascertained from the batch tests were used in the columns. Two different experiments were conducted. Two nitrate concentrations (500 and 2000 mg/l) and two different flow rates as seen in Table 6, were used for the column campaign.

Concentrations were chosen as a result of the typical ranges of nitrate concentrations displayed by the treated landfill leachate produced by the Sequencing Batch Reactor (SBR) at the Mariannhill Landfill site.

2.2.1. Equipment

The columns were constructed using a transparent PVC cylindrical body, plastic flanges with valves, rubber gaskets (seals) and stainless steel bolts.

Characteristics of the columns:

The transparent PVC cylindrical body was 1 m in length, 160 mm in diameter and had an approximate volume of 20 litres. Three ports were also installed along the length of the columns to allow sampling to occur throughout the length. A Perspex diffuser was made and fitted in the top of each column to ensure that the solution was distributed throughout the entire girth. The upper and lower ends of the columns were bolted together with a pair of 25 mm thick plastic flanges. A 20 mm rubber gasket was placed between each flange using a silicon gel to ensure an airtight fit. The column was

then bolted to a steel frame. The upper flange consisted of two orifices. A tap valve which allowed the nitrate solution to be poured into the column and the second, connected to a small plastic pipe which was used as a means to measure the biogas production. The tap valve on the lower flange allowed the column to be drained and the effluent collected. A drainage layer consisting of coarse filter and marbles was placed at the bottom of each column, thus preventing any substrate from obstructing the outlet.

2.2.2. Experiment 1

Initially, the columns were filled with a 500 mg/l and 2000 mg/l nitrate solution respectively. The experiment was designed to assess the nitrate removal capabilities of the substrates at a relatively low flow rate. This test was run for 4 weeks. The entire volume of nitrate solution was replaced over a 5 day period. Thus 1/5 of the initial input volume of nitrate solution was sampled from the bottom of the column and replaced with new nitrate solution every day. The effluents were analysed for NO_3 , pH and temperature daily and for COD and NH_3 once a week.

2.2.3. Experiment 2

The second experiment investigated the nitrate removal capabilities of the columns at a high flow rate. The columns were thus drained of their effluent and refilled using the same concentrations used in Experiment 1.

The entire volume of nitrate solution was replaced over a 2 day period. Thus 1/2 of the initial input volume of nitrate solution was sampled and replaced with new solution every day, for 4 weeks. The effluents were once again analysed for NO_3 , pH and temperature daily and for COD and NH_3 once a week.

3. Results and discussion

A summary of the operating conditions for the column studies and the relevant input data for each column, at the two different nitrate concentrations are presented in tables 6, 7 and 8. Table 6 presents the duration of the two experiments, as well as their corresponding flow rates. These flow rates were calculated on the input conditions of each column, in particular the initial input mass, effluent volume and related liquid to solid ratio (L/S), as displayed in tables 7 and 8.

Table 6

Summary of column operating conditions

Substrate	NO ₃ Concentration (mg/l)	Duration (Weeks)	Flow Rates (l/day)	
			Experiment 1	Experiment 2
CGR RAW	500	4	2.48	5.625
PB	500	4	2.00	5.00
CGR 10	500	4	1.70	2.85
CGR RAW	2000	4	2.38	5.65
PB	2000	4	2.00	5.00
CGR 10	2000	4	1.78	2.85

Table 7

Initial input conditions of each column (500 mg/l)

Column Input (500 mg/l)	CGR RAW (kg)	PB (kg)	CGR 10 (kg)
Total input mass	2.731	3.422	6.566
Moisture Input	1.014	1.672	4.401
Dry Mass	1.717	1.750	2.165
Added Nitrate Solution	12.400	10.000	8.500
Total Moisture	13.414	11.672	12.901
L/S Ratio	7.81	6.67	5.96

Table 8

Initial input conditions of each column (2000 mg/l)

Column Input (2000 mg/l)	CGR RAW (kg)	PB (kg)	CGR 10 (kg)
Total input mass	2.800	3.477	6.386
Moisture Input	1.040	1.698	4.280
Dry Mass	1.760	1.779	2.106
Added Nitrate Solution	11.900	10.000	8.900
Total Moisture	12.940	11.698	13.180
L/S Ratio	7.35	6.58	6.26

Total input mass = Moisture Input + Dry Mass

Total moisture = Moisture Input + Added Nitrate Solution

L/S Ratio = Total Moisture/ Dry Mass

3.1. Column Tests

The suitability of each substrate for their application in the column studies was determined using certain criteria. The C/N ratio is a vital characteristic required for the effectiveness of bio-denitrification. Thus a high C/N is the principal factor for selection, thus C/N ratios above 16 were considered suitable for denitrification [1, 16, 17]. Secondly, the substrates should present pH values which for into the optimum range for denitrification. The efficiency of denitrification, as obtained in optimum conditions during the batch tests was the third means used for substrate selection. The bioleaching of both COD and NH_3 was also taken into consideration.

3.1.1. $C_o = 500 \text{ mg/l}$

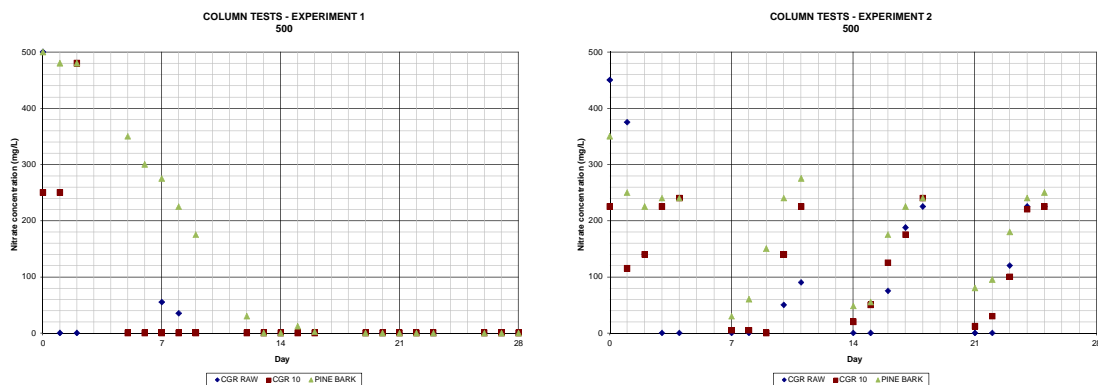


Figure 1: Evolution of the nitrate concentration for CGR RAW, CGR 10 and Pine Bark for $C_o = 500 \text{ mg/l}$.

In experiment 1, full nitrate removal was achieved by all three substrates. The CGR RAW substrate achieved full denitrification within the first 5 days, whereas in the CGR 10 and Pine Bark, nitrates were being removed within 5 – 7 days. The COD of the output effluent dropped considerably throughout the period of the test. After the first week a value of above 4500 mg/l was recorded in the CGR RAW and 450 mg/l in the CGR 10, however the COD dropped by more than 85% by the end of experiment 1. The COD value of the Pine Bark dropped by 75% over the period of experiment 1, from 3100 mg/l to 800 mg/l. The presence of COD is as a result of readily biodegradable carbon being released.

In experiment 2, the column containing CGR RAW achieved full nitrate removal within the initial 4 days, as result of the increased flow rate, there was insufficient contact time between the solution and the substrate during weeks 2, 3 and 4, causing a rise in nitrate concentration. However after the extended contact time over the weekend, the entire column had achieved full nitrate removal. However, both the CGR

10 and Pine Bark columns failed to reach full denitrification throughout the experiment, achieving 96% and 90% removal respectively, which leads us to conclude that the substrate in the column required more than 4 days for total nitrate removal to occur. The COD results at the second flow rate were lower than those recorded in experiment 1. This is due to the fact that the substrate was not replaced over the two experiments, resulting in less readily biodegradable carbon being released.

The pH values recorded for the CGR RAW remained below 6 during experiment 1 and tended to rise during the first week to 7 and remained at this level throughout the rest of experiment 2. The Pine Bark had a starting pH between 4.5 and 5.0 during the first week, before rising to 6 at end of experiment 1. The pH remained at approximately 7 throughout experiment 2. The temperature remained constant with a range between 19 and 22 °C, whilst the determined $\text{NH}_3 - \text{N}$ dropped to less than 1 mg/l at the conclusion of both experiments.

3.1.2. $C_0 = 2000 \text{ mg/l}$

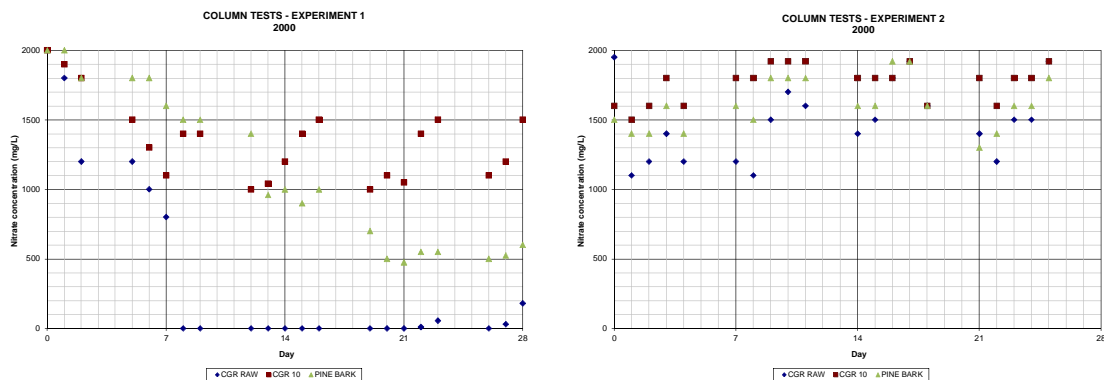


Figure 2: Evolution of the nitrate concentration for CGR RAW, CGR 10 and Pine Bark for $C_0 = 2000 \text{ mg/l}$.

The nitrate concentration in the CGR RAW column at the initial flow rate reached zero after 7 days of the experiment. The concentration at the bottom of the column remained at zero until day 22, where the output concentration rose. This was observed once again during the following week. This reduced rate of denitrification could be due to the high nitrate concentration saturating the substrate. The rate at which carbon was being released had reduced and was now slower than the rate at which nitrates were being added. During the second week, full nitrate removal was being achieved within 1 - 2 days. However as the experiment progressed, this rate of denitrification reduced. At the end of the period the substrate failed to fully denitrify the leachate.

During the first week of experiment 1, the nitrate concentration in the CGR 10 reduced steadily at a linear rate. After 7 days the nitrate concentration increased until

the end of the week. The column never achieved full denitrification and only reached a 50% removal of nitrates.

The column with Pine Bark showed little change in concentration during the first 6 days of experiment 1, which is typical for Pine Bark due to the low pH value inhibiting microbial activity. After this acclimatisation period, a clear rate of denitrification was evident, particularly during week 3 which displays a substantial drop in nitrate concentration, which is linked to the increased pH levels, rising into the optimum range for denitrification, allowing the system to reach 75% efficiency of nitrate. As full denitrification was not achieved it is apparent that the Pine Bark is releasing carbon at a slower rate than that at which nitrate is being supplemented. It is therefore evident that the contact time was too low and that the substrate requires over 7 days for a zero nitrate level to be reached.

At the increased flow rate, the CGR RAW with the coupled effect of the very high nitrate concentration and high flow rate negatively affected the performance of the test resulting in a lower denitrification rate and only 50% removal efficiency against 100% in the first experiment.

Whereas the nitrate level in CGR 10 substrate stayed at a concentration of 1600 mg/l for the initial 4 days. After 7 days the concentration rose to 1800 mg/l and remained at this level for the remainder of the experiment. The column failed to achieve full denitrification during the 4 week period. The CGR 10 substrate showed minimal denitrification which can be contributed to the flow rate being too high, resulting in insufficient contact time, thus only a maximum of 25% removal efficiency was achieved as appose to 50% removal in the first experiment. As full denitrification was not achieved, it is apparent that the CGR 10 was releasing carbon at a slower rate than that at which nitrate was being supplied.

The nitrate levels in the Pine Bark column stayed at approximately 1500 mg/l for 8 days. After day 8 the concentration rose and remained at this level for the remaining 3 days of the week. Once again after the stagnant period during the weekend the concentration level dropped. The lower rate of denitrification achieved can be attributed to the flow rate being too high, resulting in insufficient contact time between the solution and substrate, thus only 35% removal efficiency was achieved against 75% in the first experiment for Pine Bark at $C_o = 2000$ mg/l.

The COD values of the output effluent for both the CGR RAW and CGR 10 dropped considerably through the period of test 1 at a constant rate. However, the COD values during experiment 2 dropped after the first week to below 100 mg/l where they remained fairly constant throughout the duration of the experiment. During experiment 1, the COD of the output effluent from the Pine Bark column dropped by 76% from

2500 mg/l to 600 mg/l, and to 260 mg/l over the first three weeks of experiment 2 where it remained until the end of the test. The evolution of COD suggests that the flow rate was too high to allow for a significant bio-leaching of carbon, as experienced in most of the experiments at flow rate 2.

The pH measured during the period of the tests stayed at a constant level between 7 and 7.25 for the two different commercial garden refuse materials. However the initial pH for the Pine Bark stayed at a constant level of 4 – 5. After 9 days, however, the pH tended to increase to neutrality, whilst pH during experiment 2 stayed constant at approximately 7. The temperature remained constant for both experiments, in the range between 19 and 22 °C

In experiment 1, the $\text{NH}_3 - \text{N}$ of the CGR RAW was 14 to 16 mg/l over the first two weeks, but dropped to below 5mg/l during the final weeks of the experiment. The measured $\text{NH}_3 - \text{N}$ during experiment 2 remained fairly constant with a range between 1.5 and 7.0 mg/l. The $\text{NH}_3 - \text{N}$ of the CGR 10 effluent in experiment 1, decreased from 6 mg/l after the first week to below 3 mg/l and remained at that level for the remainder of the experiment. The measured $\text{NH}_3 - \text{N}$ during experiment 2 decreased from 4.5 mg/l after the first week to less than 1 mg/l by the end of the experiment. However the effluent from the Pine Bark showed an increase in $\text{NH}_3 - \text{N}$ during the first week of experiment 1, but did decrease to below 3 mg/l until the completion of the experiment, whilst the recorded $\text{NH}_3 - \text{N}$ was less than 1mg/l throughout the entire duration of experiment 2.

In summary, the poor performance of both substrates at flow rate 2, for both concentrations, suggests that the shorter contact time was not long enough to establish an active bio-film for denitrification.

3.1.3. Loading Rates and Hydraulic Retention Time

The hydraulic retention time (HRT) is a key hydrologic variable in the treatment of wastewater [18]. As defined by Vesilind and Morgan [19], the retention or residence time is a measure of the average time a soluble compound or particle of fluid spends in a bioreactor container, through which a fluid flows and is calculated by the volume of the reactor divided by the flow rate. The hydraulic retention time affects the duration with which the wastewater is present within the treatment system. This affects the reaction time, influencing nitrate removal and is thus vitally important in improving the removal performance and design of a bioreactor [1, 18]. The hydraulic loading rate is a critical factor for the design of treatment systems and is determined as the volume per day that can be applied over a surface area [20].

Table 9 presents the performance of the various substrates for each of the columns for the changes in concentration and flow rate. These results can be extrapolated using simple ratio calculations to provide an estimate of the ideal flow rates and hydraulic retention times.

Table 9

Summary of the performance of the column studies over both experiments

Substrate	NO ₃ Conc. (mg/l)	Flow Rates (l/day)		HRT (Days)		% Removal		Loading Rate (l/m ² /day)	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp.1	Exp.2
CGR RAW	500	2.48	5.625	8.06	3.56	100	100	123.32	279.71
PB	500	2.00	5.00	10.00	4.00	100	90	99.45	248.63
CGR 10	500	1.7	2.85	11.76	7.02	100	96	84.54	141.72
CGR RAW	2000	2.38	5.65	8.40	3.54	100	45	118.35	280.95
PB	2000	2.00	5.00	10.00	4.00	75	35	99.45	248.63
CGR 10	2000	1.78	2.85	11.24	7.02	50	25	88.51	141.72

The best performing of the three different substrates was the CGR RAW, at both nitrate concentrations of $C_o = 500 \text{ mg/l}$ and 2000 mg/l for the two subsequent experiments.

The CGR RAW was particularly efficient, managing to achieve full nitrate removal at both flow rates with the initial nitrate concentration $C_o = 500 \text{ mg/l}$. As a result of this 100% denitrification, it is concluded that a flow rate higher than 5.625 l/day and a loading rate above $280 \text{ l/m}^2\text{/day}$ could be managed. This relates to a HRT less than 3.5 days.

At $C_o = 2000 \text{ mg/l}$, the initial flow rate of 2.38 l/day was sufficient for the system to reach full denitrification. However, during experiment 2 at an increased flow rate, only 45% nitrate removal was attained. Consequently, an estimated flow rate of 2.54 l/day with a HRT of 8 days would be required for complete denitrification.

The CGR 10 at $C_o = 500 \text{ mg/l}$ also achieved 100% nitrate removal at the flow rate in experiment 1, however only reached 96% nitrate removal in experiment 2. This suggests that the flow rate required for full denitrification is between $1.7 - 2.85 \text{ l/day}$. A flow rate of 2.74 l/day with a HRT of 7.3 days is estimated.

At $C_o = 2000 \text{ mg/l}$, the CGR 10 was the least efficient of all the substrates only obtaining 50% nitrate removal in experiment 1 and 25% in experiment 2. This is an indication that both flow rates were too high for full denitrification to be reached. A flow rate of $0.7 - 0.9 \text{ l/day}$ with a HRT of 22 - 28 days is estimated.

The least efficient substrate at an initial concentration of $C_o = 500 \text{ mg/l}$ was the Pine Bark. A 100% nitrate removal was managed during experiment 1; however with an increase in the flow rate, 90% denitrification was obtained. These results suggest an optimum flow rate between 2 – 5 l/day and an estimated flow rate of 4.5 l/day and HRT of 4.5 days.

At $C_o = 2000 \text{ mg/l}$, only 75% nitrate removal in experiment 1 and 35% in experiment 2 was achieved, indicating that both flow rates were too fast to allow sufficient denitrification. Thus estimation for the ideal flow rate is between 1.5 – 1.75 l/day with a HRT of 13 days.

4. Conclusions and recommendations

All three of the organic substrates are suitable for the establishment and sustaining of denitrifying bacteria, both providing a favourable structure and carbon for denitrification which encourage growth. Thus the effectiveness of each material is well substantiated, through the numerous laboratory experiments, which display fairly promising results for the denitrification of various concentration levels of nitrate solution at different degrees of efficiency.

The three substrates were chosen due to their high C/N ratio and performance in the initial batch testing phase of the research, determining the time for full nitrate removal.

Through a number of studies, in particular Díaz, García [8], flow through columns of substrate improves the organic matter release and dispersion rates compared to a system where the effluent remains stagnant. However, a flow rate that is too high could result in an insufficient hydraulic retention time, which does not allow denitrifying bacteria to accumulate for denitrification. This is evident when comparing the two different experiments.

Experiment 1, with an initial concentration of $C_o = 500 \text{ mg/l}$, all of the three substrates achieved full denitrification, whereas at the increased concentration of $C_o = 2000 \text{ mg/l}$, the CGR RAW column was the only one to successfully full denitrify the nitrate solution. The CGR 10 only managed 50% removal and the Pine Bark only managed 75% removal. Thus it can be deduced, that the CGR RAW substrate was the best performing material, where the column at 500 mg/l could achieve full denitrification in under the HRT of 8 days and 8.5 days at the higher concentration of 2000 mg/l.

However, at the increased flow rate of Experiment 2, the efficiency of denitrification was less effective. It is believed, that the flow rates were too high thus preventing the establishment of denitrifying bacteria or providing sufficient contact period or hydraulic

retention time. Results also suggest that carbon is being released at a slower rate than at which nitrates are being added. The CGR RAW substrate column at 500 mg/l was the only one to achieve 100% nitrate removal. The column presented full, 100% nitrate removal within the estimated maximum HRT, suggesting that the CGR RAW requires less than the 3.5 days to achieve denitrification.

High COD concentrations produced through bioleaching were above limits provided by numerous authorities [7]. However over time the COD concentrations decreased as a result in reduced levels of organic matter being released. An aerobic reed bed will be used as a final polishing method prior to discharge.

Currently studies at different flow rates and concentrations are being conducted to ensure that the reactor will be both robust and flexible to deal with the change in quality of the leachates during the life of the landfill. Synthetic nitrate solution is being replaced with real treated leachate, in order to ascertain a more accurate understanding of how the substrates might behave in a real full-scale treatment system.

The next step in the research is to design, construct and implement a full-scale continuous flow, submerged constructed wetland which uses a flow rate that optimises denitrification whilst minimising the production of COD, after which, the behaviour and proficiency of such a reactor will be monitored for further improvements and modifications.

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APPENDIX A4

A comparison between the ability of different immaturely composted materials to denitrify high strength landfill leachate.

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ABSTRACT

The introduction of the modern sanitary landfill has allowed for the collection, treatment and discharge of landfill leachate. Typically, ammonia is converted into high concentrations of nitrates and nitrites, through nitrification. To ensure that the discharged leachate complies with wastewater limits a denitrification step is required. Denitrification, the conversion of nitrates to nitrogen gas, occurs in the presence of a carbon source in an anaerobic environment. In this study, alternative natural organic materials were investigated to ascertain their efficiency to act as carbon sources in a bio-denitrification system. This paper compares two different immaturely composted media, domestic (DGR 10) and commercial (CGR 10) garden refuse. To determine each substrates capability to denitrify nitrified leachate, different concentrations of synthetic nitrate solution were used in laboratory based experiments, including characterisation tests and small scale batch tests. The results demonstrate that both composted materials, have the ability to be effective as carbon sources to denitrify various concentration levels of nitrified leachate, at different degrees of efficiency. However, due to its higher C/N ratio the CGR 10 was the better performing of the materials.

1. Introduction

A Sequencing Batch Reactor at the Mariannhill Landfill site, situated in the eThekweni Municipality of KwaZulu-Natal, South Africa is nitrifying landfill leachate. Ammonia is being converted into high concentrations of nitrates and nitrites, which pose a potential threat to the natural water resources, if discharged without treatment. To ensure that the discharged leachate complies with wastewater limits as stipulated by the responsible authorities, a further treatment system is required.

The treatment method envisaged, relies on biological denitrification. For the facultative micro-organisms to reduce nitrates and conversion into nitrogen gas, an external carbon source needs to be present to act as an electron donor [1, 2]. Currently, easily biodegradable carbonaceous materials such as methanol and ethanol are expensive and the methods used are thus not feasible for developing countries, or suited for large scale, field applications [3, 4].

This investigation looks at using immaturely composted domestic and commercial garden refuse as carbon sources for bio-denitrification. Both materials were selected as they are natural organic resources, which are readily available and suitable for large scale, field applications. The substrates are efficient, cost effective and feasible alternatives to expensive easily biodegradable carbonaceous materials.

Laboratory testing was performed as a means to investigate the efficiency, performance and feasibility of nitrate removal in the denitrification process. Characterisation tests and small batch tests were carried out to assess the suitability of these substrates to act as carbon sources for denitrification.

2. Materials and Methods

2.1. Materials

As the investigation involved the denitrification of treated landfill leachate using organic carbon sources, a synthetic solution was used to simulate the leachate so as to operate the denitrification process in controlled conditions and to eliminate the disturbances in the nitrate (NO_3) analysis. In previous studies, the presence of chlorinated compounds in the leachate, prevented accurate insight into the nitrate reduction to be achieved [5]. The substrates investigated in the research were composted domestic and commercial garden refuse.

Large quantities of garden refuse are disposed of at local landfill sites in the eThekweni Municipality, which is separated from the main waste stream. Commercial

garden refuse consists mainly of branches and plant trimmings from parks and green municipal areas. At the Bisasar Road Landfill, the CGR is passed through a chipper to reduce the particle size to approximately 4 – 5cm length and then composted in turned open windrows. Fresh commercial garden refuse was collected from the landfill soon after the size reduction phase. The CGR material was composted in troughs at UKZN using forced aeration technology for ten weeks.

Domestic garden refuse is made up more of leaves and grass clippings from residential areas. The composted DGR consisted of domestic garden refuse collected from the Bisasar Road Landfill site and composted in troughs at UKZN using forced aeration technology for ten weeks.

2.2. Characterisation tests

Characterisation was done on both of the solid substrates and their respective eluates, using conventional testing methods as presented in the ASTM [6]. The moisture content, Total and Volatile Solids (TS and VS), carbon to nitrogen Ratio (C/N) and the Dynamic Respiration Index at 7 days (RI_7) were determined for each solid substrate whereas their eluates were tested to establish, pH, conductivity, TS, VS, COD, BOD, NH_3 and NO_3 .

The eluate testing, allowed for the nature, as well as the amount of compounds released by the substrates whilst being in contact with water to be determined. To obtain the eluates, a representative sample of each substrate was mixed with distilled water at a liquid to solid ratio of 10:1, prior to being placed on a shaker for 24 hours. Samples were then filtered through a 63 micron sieve.

2.3. Batch tests

The tests were designed to determine performance and the kinetics of removal of each substrate. To ascertain the suitability for the substrates to be used as carbon sources for denitrification, small-scale batch tests were conducted at 4 different nitrate concentrations: 0, 100, 500 and 2000 mg NO_3 / ℓ simulated using a synthetic nitrate solution. The blank control test (0 mg NO_3 / ℓ) was conducted using distilled water. The batch tests provide the optimum conditions for denitrification. These include maximum contact between substrate and solution, a pH range between 6 to 8 whilst keeping the test at a moderate temperature of approximately 25°C. To ensure that full saturation was maintained throughout the testing procedure, a Liquid to Solid ratio of 10:1 was used for all tests to ensure full saturation [7] .

To achieve representative results, testing was done in triplicate using closed top batch reactors, each consisting of a 1 l, 3 neck bottle equipped with two airtight silicone septa which allowed continuous sampling thus preventing any ingress. Bottles were filled with 100g dry matter of substrate and respective concentration of potassium nitrate solution (KNO_3). To ensure the immediate establishment of anaerobic conditions in the vessels, the bottles filled with substrate, were flushed with nitrogen gas to prior to adding the nitrate solution.

The batch reactors were placed in a shaker at 150 rpm. Small samples of effluent approximately 1-5 ml in volume were extracted using a gas tight syringe to test the nitrate concentration (NO_3), throughout the day. Nitrate concentrations were determined using the Nitrate Test Sticks type Merckoquant (MERCK). This method of extraction was performed in order to not significantly affect the L/S ratio in the reactors and to ensure that full saturation was maintained throughout the duration of the experiment.

Tests were conducted until a nitrate concentration of 0 mg NO_3 /l was achieved, after which both liquid and solid samples were characterised.

3. Results and discussion

3.1. Characterisation of substrates

Table 1

Characterisation of the solid substrates

	MC (%)	TS (%)	VS (%)	RI7 (mg O_2 /g DM)	Tot C (%)	Tot N (%)	C/N
CGR 10	67.03 \pm 0.83	32.97 \pm 0.83	89.62 \pm 1.40	5.672	28.69	1.20	23.91
DGR 10	66.05 \pm 4.71	33.95 \pm 4.71	62.38 \pm 9.84	14.123	23.97	1.88	12.75

Table 2

Results of the eluate tests

	TS (g/l)	VS (g/l)	pH	Cond (mS/cm)	COD (mg/l)	BOD ₅ (mg/l)	NH ₃ -N (mg/l)	NO _x -N (mg/l)	C/N
CGR 10	2.40 \pm 0.10	1.62 \pm 0.07	6.98	0.81	2764	155	9.80	7.14	1.83
DGR 10	16.65 \pm 2.77	12.00 \pm 0.18	7.40	4.98	17556	350	82.04	15.2	8.30

Tables 1 and 2: The \pm values refer to the standard deviation of the results. The standard deviation is only included when the test has been done in triplicate or greater.

The composting process results in degradation and the high production of production of NH_3 , pH levels are more favourable as the values are close to neutral

and in some cases alkaline [7-9], thus falling into the optimum range for biological denitrification between 6 and 8 [10, 11].

The total solid results correspond strongly with the COD values. High TS levels reflect in higher percentage of total carbon in the eluates, suggesting that carbon, mobilised through composting, is released easily by leaching [12].

Both substrates have a C/N Ratio that falls outside the typical range for stabilised compost, being between 13 - 16 as suggested by Tsui, Krapac [3] and Wu and Ma [13]. The lower C/N ratio of the composted DGR suggests that the material has undergone more degradation, breaking down the organic carbon [14]. However, the less efficient composting process along with the woody composition of the CGR resulted in a higher carbon content.

The materials used have higher carbon (C) content in comparison to nitrogen (N) making them well suited for nitrate removal. They provide organic carbon, without increasing the nitrogen concentration.

As proposed by Adani, Lozzi [15], the RI_7 or Respiration Index is an expression of the rate at which oxygen is consumed by the indigenous biomass that is present in the substrate to degrade the material. "The biological stability indicates the extent to which readily biodegradable organic matter has decomposed" [8, 16]. It is often used as a means to assess and define the level of biological stability and biodegradability of fresh and composted garden refuse [8, 15, 16]. An unstable material is considered to contain a high portion of biodegradable matter that must sustain high microbial activity [16, 17].

The chemical oxygen demand (COD) provides an indication as to the total organic matter released; whilst the biochemical oxygen demand (BOD_5) suggests the biodegradability of the leached organic carbon [12, 18]. In this case, the DGR has both a higher COD and BOD_5 value, which informs us, that although it has a lower carbon content compared to the CGR, the carbon is more readily available and biodegradable. This also clarifies why the immaturely composted CGR 10 has a lower RI_7 value than DGR 10, as the readily available carbon causes the material to have greater initial microbial activity, resulting in the higher production of carbon dioxide [9, 14, 19, 20].

The high levels of ammoniacal nitrogen ($NH_3 - N$) and NO_x-N present in the domestic garden refuse sample was also observed, which may cause increased nitrate levels through bioleaching. The production or leaching of NH_3 from the substrate will cause a rise in nitrogen. If there is sufficient oxygen present in either the solution or the pores of the substrate, NH_3 could be converted into NO_2 .

3.2. Batch Tests

3.2.1. Commercial Garden Refuse (CGR 10)

At the conclusion of each batch test, the output material was characterised. Table 3, presents the results obtained at the different nitrate concentrations.

Table 3

Characterisation results of the input and output of the CGR 10 batch tests

		pH	COD(mg/l)	NH ₃ -N (mg/l)	NO ₃ (mg/l)
Blank (0 mg/l)	Output Eluate	7.08	1944	7.0	0
100 (mg/l)	Output Eluate	7.22	2754	2.5	0
500 (mg/l)	Output Eluate	7.51	3177	3.0	0

The pH values throughout the tests increased with the increase of the initial concentration and remain constant to optimum ranges for denitrification. There was a presence of positive bioleaching of carbon which was observed in the increase of the COD, relating to the initial nitrate concentration. The COD results showed an increase from the initial input ranging from 2754 – 3177 mg/l. The NH₃ - N values in all the tests were lower than that of the initial input material. The test conducted at 100 and 500 mg/l showed a drastic decrease of 70 - 75%.

The blank test showed no leaching out of nitrates; however the evolution of the denitrification for the CGR 10 substrate conducted at each of the initial concentrations is shown in Figures 1, 2 and 3.

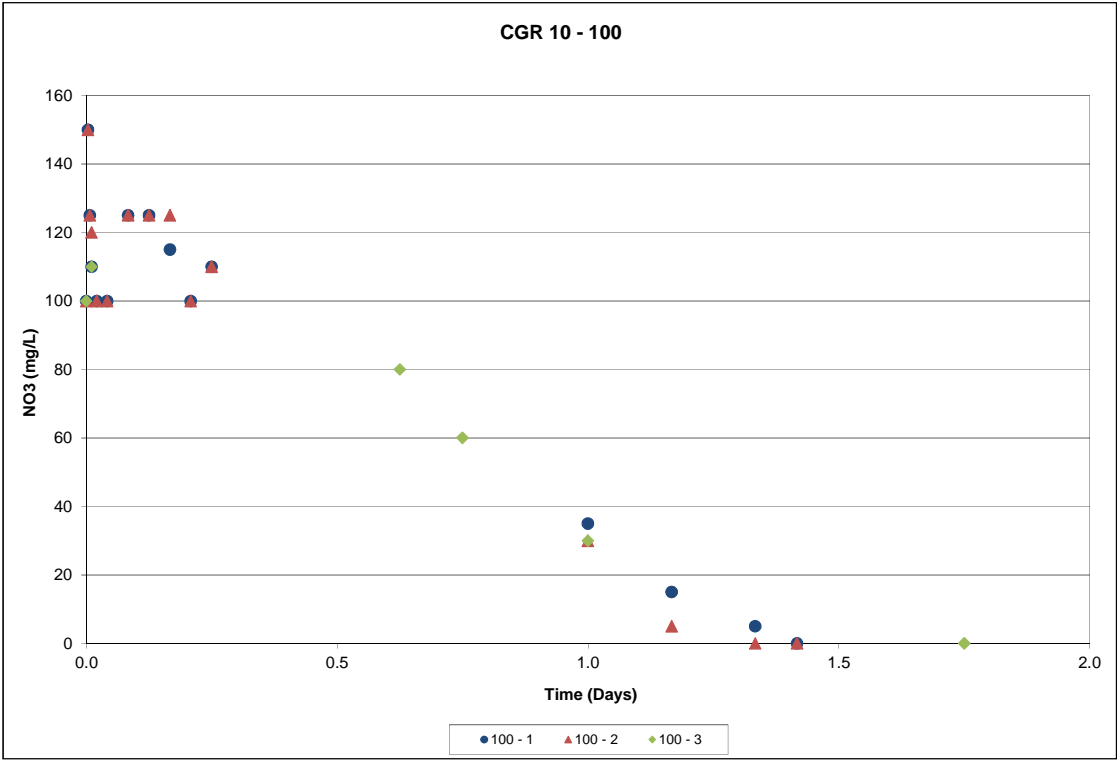


Figure 1: Evolution of the nitrate concentration for CGR 10 at $C_o = 100 \text{ mg/l}$

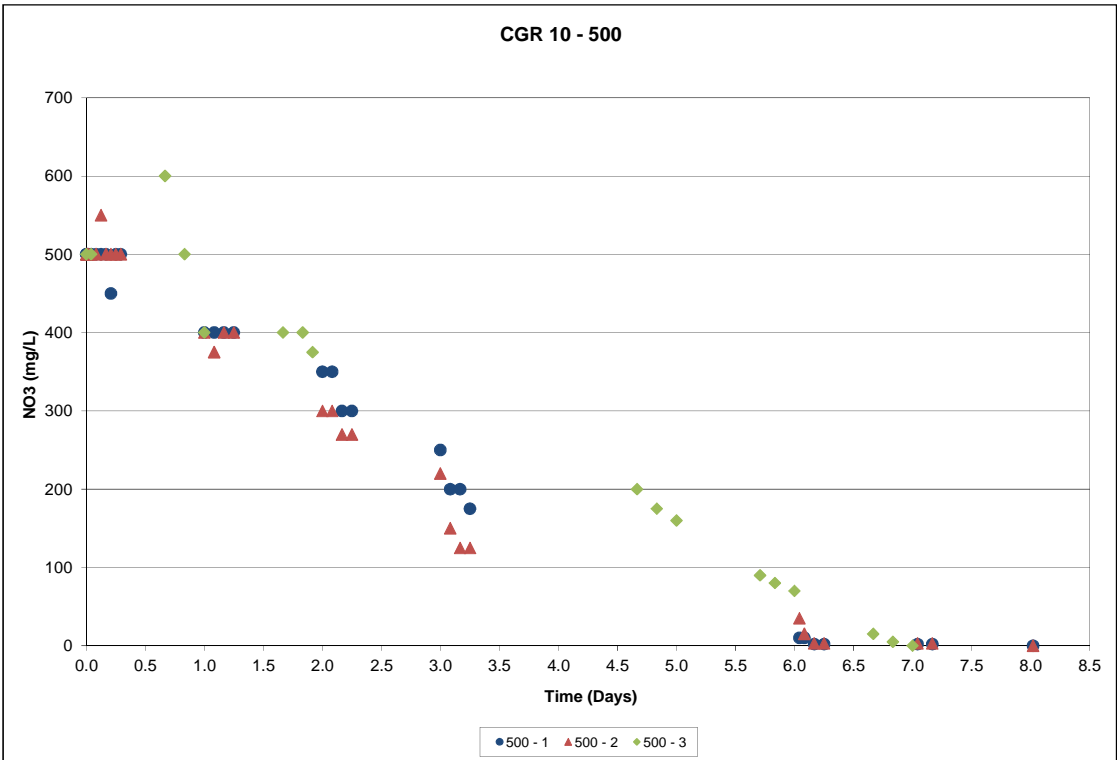


Figure 2: Evolution of the nitrate concentration for CGR 10 at $C_o = 500 \text{ mg/l}$

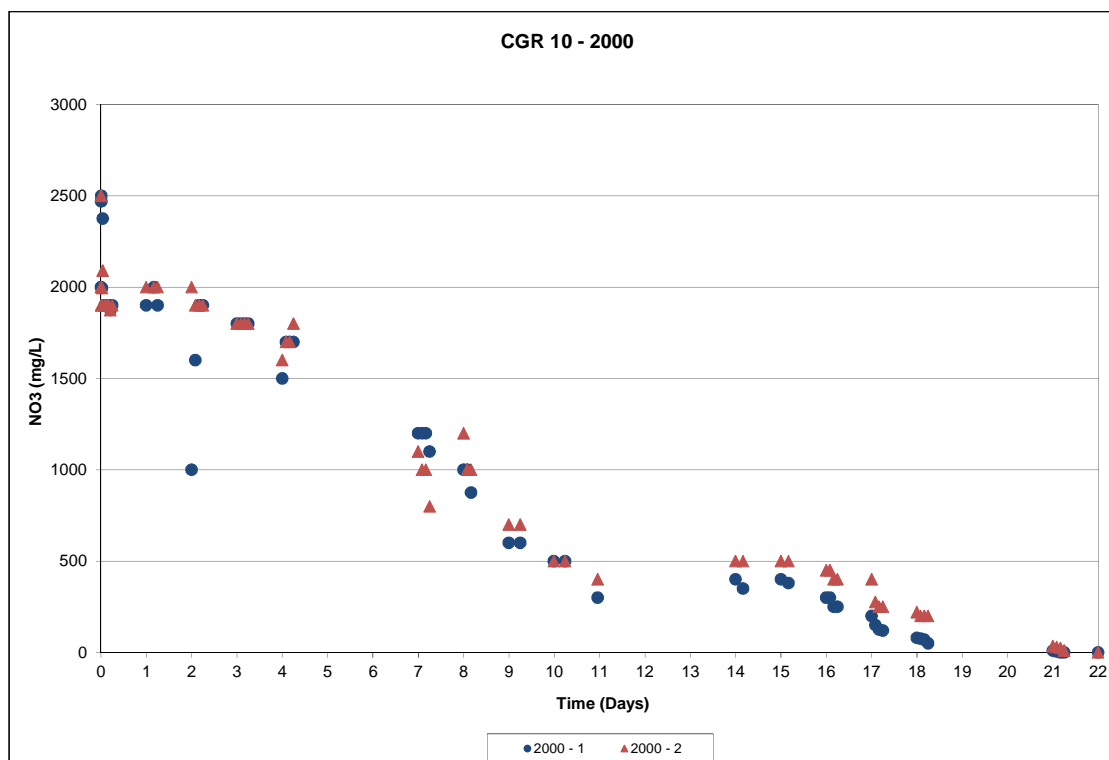


Figure 3: Evolution of the nitrate concentration for CGR 10 at $C_o = 2000 \text{ mg/l}$

Each test presents an acclimatisation period which is dependent on the initial concentration, with the 2000 mg/l test having the longest plateau of 3 - 4 days, followed by 18 days of removal at a linear rate. After the plateau, nitrate removal occurred at a linear rate until a zero nitrate concentration was achieved, between 1.25 to 1.75 days for the 100 mg/l test, 7 to 8 days for the 500 mg/l test and 22 days for the experiment at 2000 mg/l . Microbial tests conducted by De Combret [21] suggest that high performance of the test at 100 mg/l could be to other phenomena rather than bio-denitrification. The results were modelled using a zero kinetic reaction model and presented in Table 4.

Table 4

Summary of kinetics of CGR 10

$C_o \text{ (mg/l)}$	Time for 100% Removal (Days)	$k \text{ (1/day)}$	R^2	Percentage Removal (%)
100	1.42	98.051	0.951	100
500	8.02	70.171	0.910	100
2000	22	98.866	0.906	100

3.2.2. Domestic garden refuse (DGR 10)

The results of the characterisation tests conducted on the output material of the DGR 10 batch is displayed in Table 5.

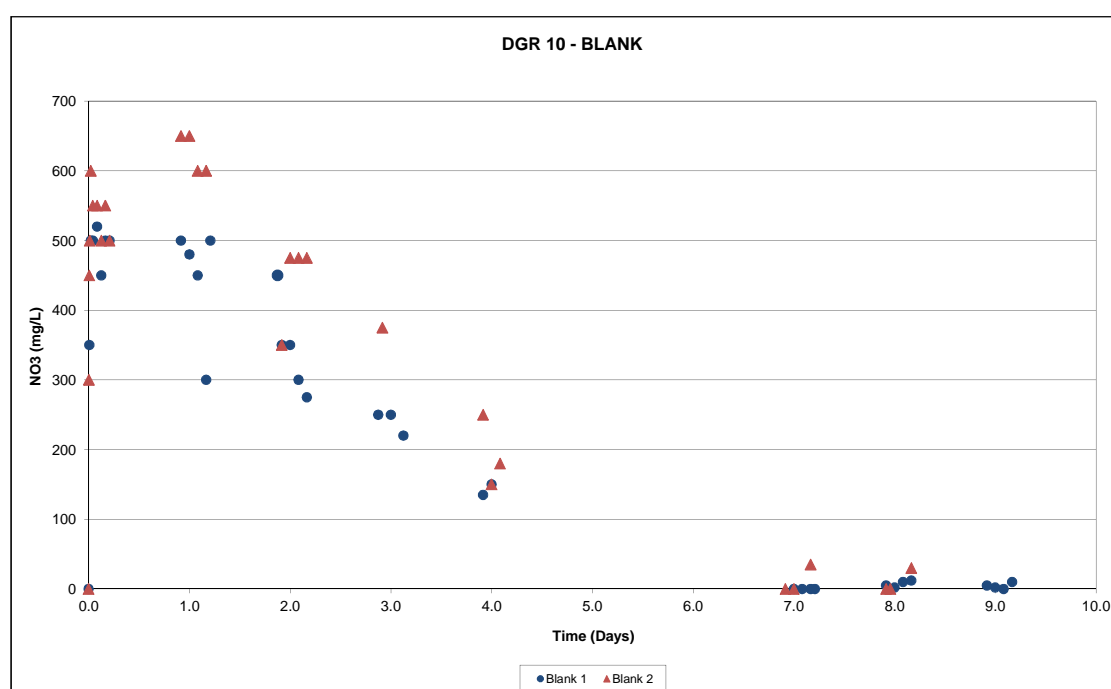
Table 5

Characterisation results of the input and output of the DGR 10 batch tests

		pH	COD (mg/l)	NH ₃ -N (mg/l)	NO ₃ (mg/l)
Blank (0 mg/l)	Output Eluate	7.41	19820	30	0
100 (mg/l)	Output Eluate	7.33	7822	8.5	0
500 (mg/l)	Output Eluate	7.55	17783	87.2	0

The pH remained constant around the optimum range for denitrification for all tests. All the tests achieved a zero nitrate (NO₃) concentration at the end of the test. The COD results are similar to the input value; however the test conducted at 100 mg/l showed a substantial drop, which is promising. It is noted that the initial input material had a high NH₃ - N value. The shorter test conducted at 100 mg/l showed a drastic decrease of 90%. The longer tests, the blank and 500 mg/l, still showed a high value at the end of the tests, with the 500 mg/l increasing above that of the initial input. This increase in NH₃ correlates to the reduction in total N (%) from 1.88 – 0.55, which indicates there is also bioleaching of nitrogen.

As a result of the high ammoniacal nitrogen (NH₃ – N) present in the characterisation testing, a blank test at 0 mg NO₃/l was carried out. The outcome was particularly interesting, thus the plot for the blank test was included in the evolution of the nitrate concentration for the DGR 10 substrate, due to its effect on the denitrification. These plots are shown in Figures 4, 5, 6 and 7.

Figure 4: Evolution of the nitrate concentration for DGR 10 Blank at C₀ = 0 mg/l

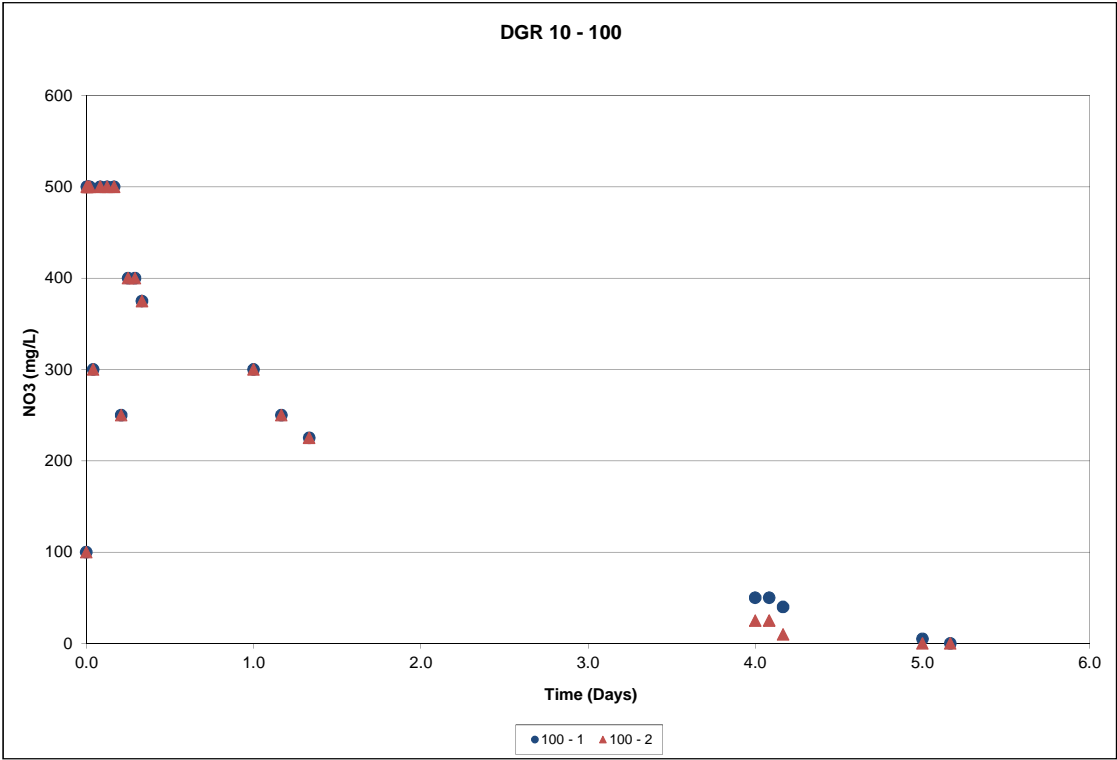


Figure 5: Evolution of the nitrate concentration for DGR 10 at $C_o = 100 \text{ mg/l}$

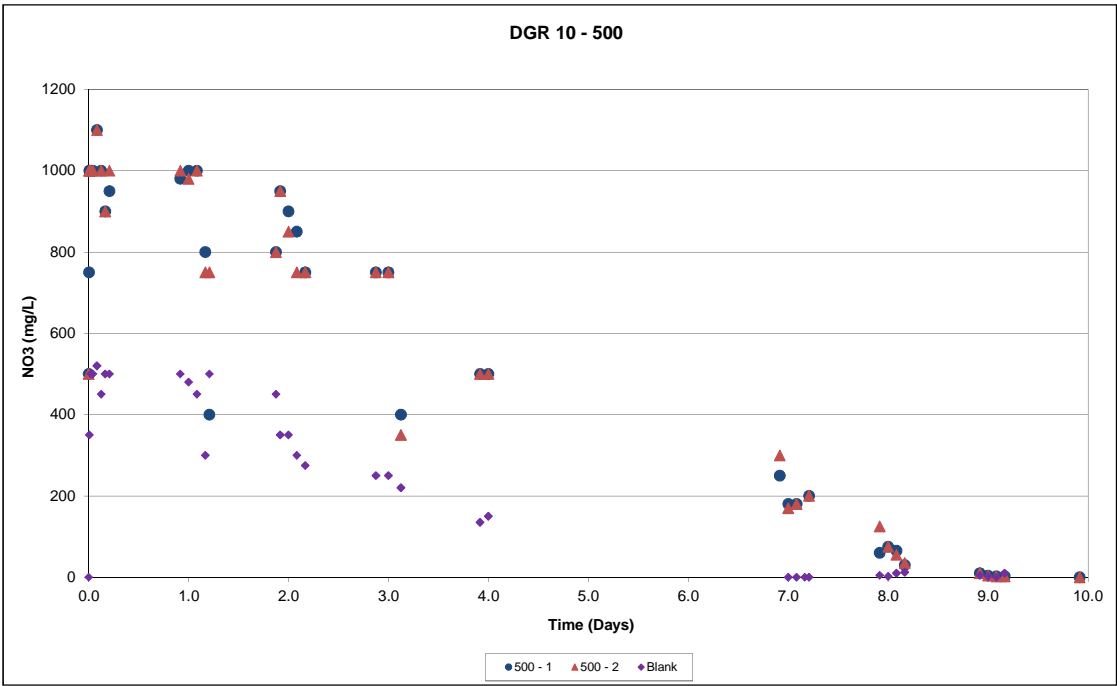


Figure 6: Evolution of the nitrate concentration for DGR 10 at $C_o = 500 \text{ mg/l}$

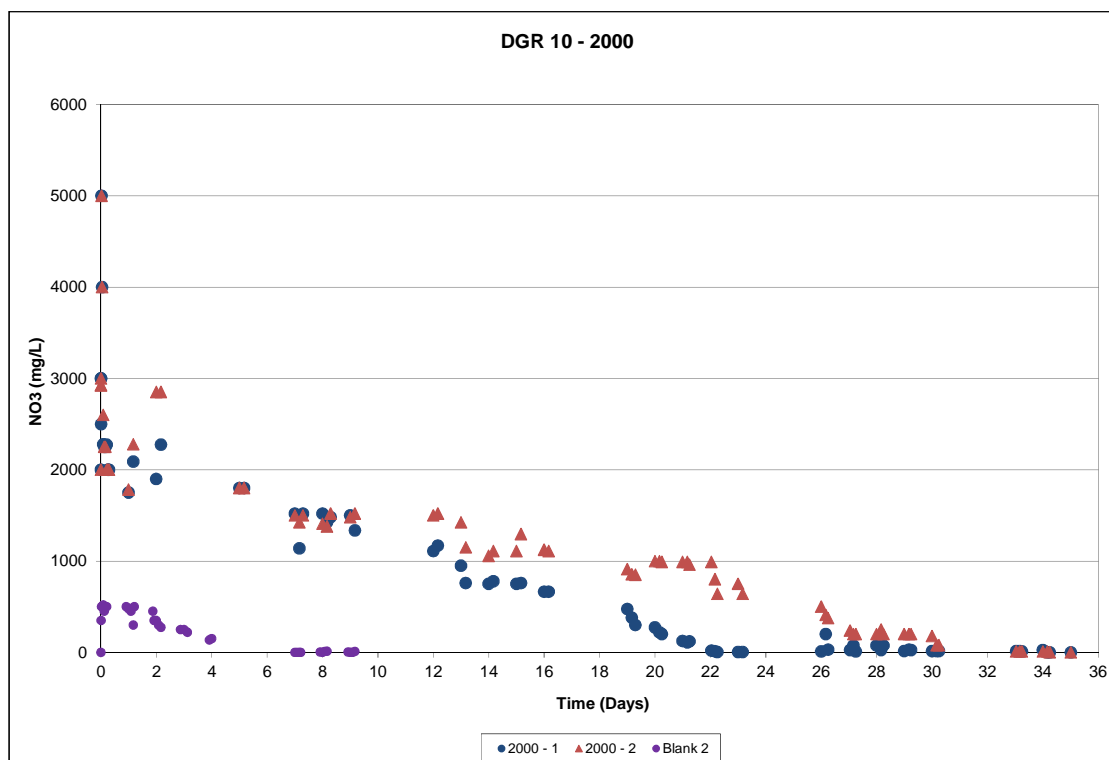


Figure 7: Evolution of the nitrate concentration for DGR 10 at $C_o = 2000 \text{ mg/l}$

The blank test provided some very interesting results. The nitrate concentration actually increased significantly within the first two days of the test ranging between 500 and 650 mg/l. A small plateau was experienced at this high concentration for approximately 1.5 days. The denitrification process then followed a linear relationship until full nitrate removal was achieved after 8 to 9 days. This initial increase in nitrates was first believed to be due to added nutrients used by domestic households, such as fertilizers. However after examining the input and output results, it is concluded that the considerable rise in nitrates was more likely due to organic nitrates and ammoniacal nitrogen from bioleaching of the organic nitrogen from the solid substrate matter rather than nitrification. From Table 2 the initial input material has relatively high values of both $\text{NH}_3\text{-N}$ and $\text{NO}_x\text{-N}$. As carbon and nitrogen are leached from the matter, denitrification is limited by the availability of electron donors and thus there was an increase in nitrate concentration [3].

All the tests showed a similar trend as that of the blank test. An initial rise in nitrates occurs due to the relatively high values of both $\text{NH}_3\text{-N}$ and $\text{NO}_x\text{-N}$ in the input material. After this rise a plateau period is established as the test reached its regime, followed by a rapid rate of denitrification which reduces the nitrate concentration to zero.

In the case of the 100 mg/l test a plateau of 4 hours is observed with full nitrate removal after 5 days. In the 500 mg/l test, a plateau was once again experienced for 2

to 3 days before total nitrate removal after 9 – 10 days. The final test performed at 2000 mg/l again displayed a plateau period of 2 – 3 days and reached zero nitrate concentration after 34.5 days at a linear rate.

The results were modelled using both a linear and exponential relationship and it was found that a zero order reaction provided a more accurate representation.

Table 6

Summary of kinetics of DGR 10

C _o (mg/l)	Time for 100% Removal (Days)	k (1/day)	R ²	Percentage Removal (%)
Blank	9	65.48	0.94	100
100	5	79.74	0.96	100
500	9.5	113.66	0.93	100
2000	34.5	61.74	0.96	100

4. Conclusions and recommendations

To investigate the feasibility of using two different immaturely composted natural organic materials to act as carbon sources in the bio-denitrification of treated high strength leachate, laboratory experiments were conducted to assess the performance and efficiency of nitrate removal.

The characterisation testing provided insight into the composition of both materials and their compounds produced through bioleaching. It is concluded that due to the higher carbon content in comparison to nitrogen, the fairly neutral pH levels, the readily available carbon as well as the stability, maturity and biodegradability the substrates have the potential to act as successful carbon sources. They also act as a medium for denitrifying bacteria.

The dynamic small-scale batch tests were designed to determine the performance and kinetics of nitrate removal of each substrate. The results demonstrate that both composted materials, have the ability to be effective as carbon sources to denitrify various concentration levels of nitrified leachate, at different degrees of efficiency.

The characterisation tests indicated that the immaturely composted commercial garden refuse material had higher carbon to nitrogen ratio than that of the domestic garden refuse. This was evident in the results, where the CGR 10 substrate achieved full denitrification before the DGR 10 at all concentrations.

The batch tests showed positive results, with the CGR 10 substrate achieving full denitrification at the highest nitrate concentration of 2000 mg/l within 22 days. All the small-scale batch tests conducted with CGR 10, demonstrated similar characteristics of

an acclimatisation period before decreasing linearly with time. The duration of the acclimatisation period was strongly related to that of the initial input concentrations of the nitrate solution. In the case of the DGR 10, all the tests showed a similar trend as that of the blank test, with an initial rise in nitrates occurring due to the relatively high values of both $\text{NH}_3\text{-N}$ and $\text{NO}_x\text{-N}$ in the input material. After this rise a plateau period is established as the test reached its regime, followed by a rapid rate of denitrification which reduces the nitrate concentration to zero.

The COD levels increased through the release of organic matter. To counter this undesirable by-product, the denitrified leachate would be passed through an aerobic reed bed, which acts as a polishing treatment, ensuring that the final discharged effluent satisfies those limits enforced by the authorities. It was found however, that over time with the reduction in readily available carbon and the resulting bioleaching, the COD concentrations did decrease, but, in most cases, not sufficiently to fall into DWAF's Water Quality criteria.

As the eThekweni landfills receives large volumes of garden refuse monthly which is separated from the main waste stream, the materials are highly abundant and easily available on site, thus making them fairly inexpensive and thus could be successfully employed at landfill sites to denitrify treated leachate which would prevent excessive treatment costs as well as support the development of a real waste management strategy.

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APPENDIX A5

Bio-denitrification of MSW landfill leachate using raw and composted garden refuse: a comparative analysis.

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SUMMARY: In the eThekweni Municipality, leachate from the Mariannhill Landfill site is currently being nitrified in a Sequencing Batch Reactor. After nitrification, the concentration of nitrate in the discharged leachate may still present a potential threat to the environment. Once the landfill is closed, the nitrified effluents from the plant will not comply with the discharge limits, thus a further ad-hoc denitrification treatment is required. Denitrification, the conversion of nitrates to nitrogen gas, occurs in the presence of a carbon source in an anaerobic environment. This paper presents an efficient, cost effective, feasible alternative to expensive easily biodegradable carbonaceous materials thus, promoting the use of natural organic sources such as garden refuse at different degrees of maturity. These organic substrates contain relatively high amounts of carbon and are readily available in the major eThekweni landfills.

1. INTRODUCTION

Landfill leachate, which is a toxic by-product formed through the decomposition of organic matter, is harmful to both the environment and human health. After nitrification, the concentration of nitrates in the discharged leachate may still present a potential threat to the environment. Further denitrification is often required to reduce the high concentrations of nitrates in the nitrified effluents to below the discharge limits. The eThekweni Municipality is currently nitrifying leachate from the Mariannhill Landfill site in a Sequencing Batch Reactor plant. The treated effluent is then used as dust suppressant. After closure of the landfill the effluents from the plant will not comply with the discharge limits of wastewater into a water resource, as enforced by DWAF (DWAF

- General Authorisations in terms of Section 39 of the National Water Act, 1998). Thus an ad-hoc treatment will be required.

Biological denitrification, the conversion of nitrates to nitrogen gas, is facilitated by microbes. The micro-organisms capable of reducing nitrates require the presence of an external carbon source as an electron donor, usually in an anaerobic environment (Ovez et al., 2006). Expensive easily biodegradable carbonaceous materials are currently employed around the world (methanol, ethanol etc.); however these methods tend not to be a viable solution for developing countries and are not suited for large scale, field applications (Tsui et al., 2007; Volokita et al., 1995).

This investigation aimed at identifying an efficient, cost effective and feasible alternative to expensive easily biodegradable carbonaceous materials that promotes the use of natural organic resources such as commercial garden refuse at degrees of maturity that are suitable for large scale, field application. These organic substrates contain relatively high amounts of carbon and are readily available in the major eThekweni landfills.

The investigation of the efficiency, performance and feasibility of nitrate removal using substrates in the denitrification process as carbon sources was conducted by means of laboratory testing, in particular, characterisation tests, small scale dynamic batch tests (Tsui et al., 2007) and larger scale column studies. The selection of substrates was based on their suitability as natural organic carbon sources and their availability locally.

2. MATERIALS AND METHODS

2.1. Materials

This investigation involved the denitrification of treated landfill leachate using organic carbon sources. The leachate was simulated using a synthetic solution so as to operate the denitrification process in controlled conditions and to eliminate the disturbances in the nitrate (NO_3) analysis due to the presence of chlorinated compounds in the leachate, as experienced in previous studies (Pisano, 2007). The typical ranges of nitrate concentrations (Nitrate + Nitrite $\text{mg NO}_3/\ell$) displayed by the treated landfill leachate produced by the Sequencing Batch Reactor (SBR) at the Mariannhill Landfill site are between 8 – 2120 $\text{mg NO}_3/\ell$. Substrates selected for experiments were, raw and immaturely composted Commercial Garden Refuse (CGR RAW and CGR 10).

A large amount of garden refuse is disposed of at both the Mariannhill and the Bisasar Road Landfill-sites in Durban separated from the main waste stream. Commercial garden refuse consists mainly of branches and plant trimmings from parks and green municipal areas. At the Bisasar Road Landfill, the CGR is passed through a chipper to reduce the particle size to approximately 4 – 5cm lengths and then composted in turned open windrows. Fresh commercial garden refuse was collected from the landfill soon after the size reduction phase. The CGR material was composted in troughs at UKZN using forced aeration technology for ten weeks (Iyilade, 2009).

2.2. Characterisation tests

The preliminary stage of the research was to comprehensively characterise the substrates through conventional testing done on both the solid substrates and their eluates through the use of standard analytical methods as published by ASTM (2008). The following tests were conducted on the solid substrates: moisture content, Total and Volatile Solids (TS and VS), carbon to Nitrogen Ratio (C/N) and Dynamic Respiration Index at 7 days (RI_7) that was determined using a respirometric system type OxiTop®. The RI_7 expresses the rate at which oxygen is consumed in the biodegradation of organic matter and is often used as a means to define the level of stability and biodegradability of fresh and composted garden refuse (Adani et al., 2001; Gomez, 2006; Adani et al., 2006). The eluates of the substrates were tested to determine the nature as well as the amounts of compounds released by the substrates whilst being in contact with water. The eluates were prepared by mixing a representative sample of each of the substrates with distilled water at a liquid to solid ratio of 10:1. These samples were then placed on a shaker for 24 hours. The samples were then filtered through a 63 micron sieve to obtain the eluate. The eluates were tested to determine: pH, conductivity, TS, VS, COD, BOD, NH_3 and NO_3 . All tests were conducted in double or triplicate to ensure accuracy and repeatability.

2.3. Batch tests

The small-scale batch tests were conducted at 3 different nitrate concentrations: 100, 500 and 2000 mg NO_3 / ℓ simulated using a synthetic nitrate solution. A blank control test (0 mg NO_3 / ℓ) was conducted using distilled water for each substrate. The batch tests were designed to determine the kinetics of removal of each substrate at optimal conditions, which were maximum contact between substrate and solution, a pH range

between 6 to 8 and at a temperature of approximately 25°C (Trois et al., 2010; Tsui et al., 2007). A Liquid to Solid ratio of 10:1 was used for all tests to ensure full saturation.

All tests were conducted in duplicate or triplicate in closed top batch reactors consisting of 1 L, 3 neck bottles equipped with two airtight silicone septa which allowed continuous sampling thus preventing any ingress. Each bottle was filled with 100g dry matter of substrate and respective concentration of potassium nitrate solution (KNO₃). The substrate particles were cut and reduced to a standard size of 4 – 5 cm to ensure homogeneity of the sample. Prior to adding the nitrate solution, the bottles filled with substrate, were flushed with nitrogen gas to ensure the immediate establishment of anaerobic conditions in the vessels. The batch reactors were placed in a shaker at 150 rpm at a controlled room temperature of approximately 25°C. Small samples of approximately 1-5 mL were extracted using a gas tight syringe so as to test the nitrate concentration (NO₃). Samples were taken 3 times a day usually every 3 hours depending on any changes in nitrate concentration. This method of extraction was performed in order to not significantly affect the L/S ratio in the reactors and to ensure that full saturation was maintained throughout the experiment. Nitrate concentrations for the batch tests were determined using the Nitrate Test Sticks type Merckoquant (MERCK). The batch tests were conducted until the nitrate concentration reached zero. At the end of the test, both liquid and solid samples were characterised.

2.4. Microbial tests

Microbial analyses were also conducted by De Combret (Trois et al., 2010) for the batch tests at 500 mg/L in order to monitor and assess the affect of the different substrates on the evolution of indigenous bacterial population during bio-denitrification. The growth of the microbial community was followed using a spread plate enumeration technique; the colonisation of the substrates was assessed through Environmental Scanning Electronic Microscopy (ESEM), and an insight into the composition of the bacterial community was determined by phylogenetic analysis (Trois et al., 2010).

2.5. Column tests

Two different experiments were conducted using the columns to investigate the effect of denitrification rates for different nitrate concentration levels and flow rates. These results were used to determine the kinetics of removal, loading rates and hydraulic retention time for full-scale filter beds. Two nitrate concentrations (500 and 2000 mg/L) and two different flow rates were used for the column campaign. The effluents were

analysed for NO_3 , pH and temperature daily and for COD and NH_3 once a week. Each test was run for 4 weeks.

2.5.1. Equipment

The columns were constructed using a transparent PVC cylindrical body, plastic flanges with valves, rubber gaskets (seals) and stainless steel bolts. The transparent PVC cylindrical body was 1 m in length, 160 mm in diameter and had an approximate volume of 20 litres.

2.5.2. Experiment 1

For the initial experiment the columns were filled with each substrate and a 500 mg/l and 2000 mg/l nitrate solution respectively. The experiment was designed to assess the nitrate removal capabilities of the substrates at a relatively low flow rate.

It was decided that the entire volume of nitrate solution should be replaced over a 5-day period. Thus 1/5 of the initial liquid input volume was sampled and replaced with nitrate solution every day.

2.5.3. Experiment 2

This experiment was performed to investigate the nitrate removal capabilities of the columns at a high flow rate. The columns were thus drained of their effluent and filled with the same concentrations of nitrate solution as used in Experiment 1 until the substrates were covered.

On the basis of the results of the batch tests, it was decided that the entire volume of nitrate solution should be replaced over a 2-day period. Similar to experiment 1, 1/2 of the initial liquid input volume was sampled and replaced with nitrate solution every day.

3. RESULTS AND DISCUSSION

3.1. Characterisation of substrates

Table 1. Characterisation of the solid substrates

	MC (%)	TS (%)	VS (%)	RI7 (mg O ₂ /g DM)	Tot C (%)	Tot N (%)	C/N
CGR RAW	37.14	62.86	96.37	7.770	49.6	0.55	90.19
CGR 10	67.03	32.97	89.62	5.672	28.69	1.20	23.91

Table 2. Results of the eluate tests

	TS (g/l)	VS (g/l)	pH	Cond (mS/cm)	COD (mg/l)	BOD5 (mg/l)	NH ₃ -N (mg/l)	NO _x -N (mg/l)	C/N
CGR RAW	4.08	3.04	5.45	1.653	4253	1101	12.74	6.86	4.54
CGR 10	2.40	1.62	6.98	0.81	2764	155	9.80	7.14	1.83

The results in Table 1 and 2 suggest that the fresh garden refuse is acidic. pH is a limiting factor in the denitrification process and thus the low pH value will impact negatively on the rate of nitrate removal as the optimum pH for biological denitrification is between 6 and 8. The acidic nature may cause an inhibitory effect on denitrification, as observed by others (Trois et al., 2007; Tsui et al., 2007). As a result of degradation and the high production of NH₃, pH levels in the composted material is closer to neutral (Adani et al., 2006). The composting has produced favourable pH values which fall into of the optimum range for degradation. The higher carbon content, in the form of COD and BOD in the raw garden refuse is due to the fact that the substrate is an organic material and has not undergone any stabilisation.

The typical range for stabilised compost is between 13 – 16 (Tsui et al., 2007; Wu et al., 2002), with the CGR 10 having a greater C/N ratio, this makes it appropriate for denitrification.

Ammoniacal nitrogen (NH₃ – N) present in the samples, may cause increased nitrate levels through bioleaching. The production or leaching of NH₃ from the substrate will cause a rise in nitrogen. If there is sufficient oxygen present in either the solution or the pores of the substrate, NH₃ could be converted into NO₂.

3.2. Results of the Batch Tests

Table 3 and 4 present the results of the characterisation of input and output materials from the batch tests with CGR RAW and CGR 10 respectively.

Table 3. Characterisation results of the input and output of the CGR RAW batch tests

		pH	COD (mg/l)	NH ₃ -N (mg/l)	NO ₃ (mg/l)	Tot C (%)	Tot N (%)	C/N Ratio
	Input Eluate	5.45	4253	12.74	6.86	0.083	0.0183	4.54
	Input Solid					49.60	0.55	90.19
Blank	Output Eluate	6.01	9433	15	0			
(0 mg/l)	Output Solid					48.50	0.63	76.98
100	Output Eluate	6.10	4325 – 5212	18.5	0			
(mg/l)	Output Solid					45.90	0.7	65.10
500	Output Eluate	5.52	3951 – 7200	25	0			
(mg/l)	Output Solid					47.87	0.75	64.64
2000	Output Eluate	7.04	7009 – 7870	85.75	0			
(mg/l)	Output Solid					47.75	0.66	72.40

The input pH of the fresh CGR is 5.45 and increases with time and with NO₃ concentration as reported by other authors (Tsui et al., 2007). The test conducted at an initial concentration of 2000 mg/l exhibits a final pH which falls into the optimum range for denitrification.

As a result of the production of NH₃ leached out from the substrate as well as the oxygen present in the solution and the pores, NH₃ is converted into NO₂ even when full nitrate removal is achieved. It was confirmed by De Combret (2009) and Trois (2010) that both nitrifiers and denitrifiers were present in this substrate within the first 74 hours of testing, in line with other studies that used similar substrates (Zhong et al., 2009).

A presence of positive bioleaching of carbon was observed in the increase of COD, relating to the initial nitrate concentration. The COD results showed an increase from the initial input ranging from 3951 – 7870 mg/l. The ammoniacal nitrogen released, also tended to increase with the time. This increase in NH₃ which correlates to the slight reduction in total N (%) especially in the test at C₀ = 2000 mg/l, indicates that there was also bioleaching of nitrogen.

Table 4. Characterisation results of the input and output of the CGR 10 batch tests

		pH	COD (mg/l)	NH ₃ -N (mg/l)	NO ₃ (mg/l)	Tot C (%)	Tot N (%)	C/N Ratio
	Input Eluate	6.98	2764	9.8	7.14	0.11	0.06	1.83
	Input Solid					28.69	1.20	23.91
Blank	Output Eluate	7.08	1944	7.0	0			
(0 mg/l)	Output Solid					45.2	0.94	48.90
100	Output Eluate	7.22	2754	2.5	0			
(mg/l)	Output Solid					45.2	0.49	92.24
500	Output Eluate	7.51	3177	3.0	0			
(mg/l)	Output Solid					41.9	1.23	34.07

The pH values throughout the tests increased with the increase of the initial concentration and remain constant within the optimum range for denitrification (Trois et al., 2007). A presence of positive bioleaching of carbon was observed in the increase of the COD, relating to the initial nitrate concentration. The NH₃ - N values in all tests were lower than the initial input material.

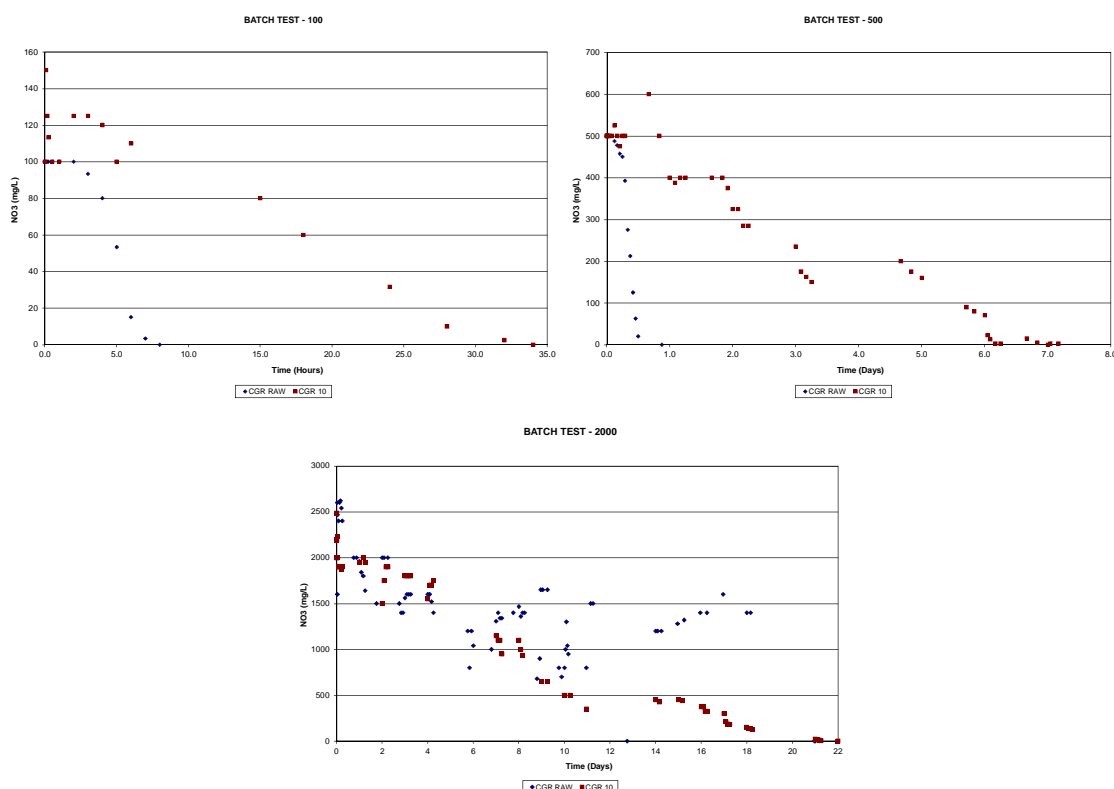


Figure 1. Evolution of the nitrate concentrations for CGR RAW and CGR 10 at $C_0 = 100$ mg/l, 500 mg/l and 2000 mg/l.

Table 5. Summary of kinetics of CGR RAW

C_o (mg/l)	Time for 100% Removal (Days)	K (1/day)	R^2	Percentage Removal (%)
100	0.25	588	0.90	100
500	0.50	1408	0.94	100
2000	10.5	181	0.98	100

All three tests conducted with the CGR RAW substrate, at the various concentration levels exhibited an initial plateau. An acclimatisation period is observed, as a resultant of pH buffering. The duration of this plateau period tended to increase with an increase in initial nitrate concentration, suggesting that pH and the initial NO_3 concentration play an important inhibitory role during this initial stage as demonstrated by De Combret (2009).

In the test at $C_o = 100$ mg/l the system reached a zero nitrate concentration within 6 to 8 hours with a 2 hour plateau. The tests conducted at $C_o = 500$ mg/l demonstrated an initial plateau period ranging between 2 to 8 hours. After this plateau the nitrate concentration rapidly dropped to zero after 12 hours. The final test at a concentration of $C_o = 2000$ mg/l showed an increase in nitrates within the first 6 hours of the initial two tests and a plateau period of 18 to 24 hours with full nitrate removal occurring within approximately 22 days.

All the tests reach 100% removal. The tests conducted at 100 and 500 mg/l were both highly efficient and reached a zero nitrate concentration in less than 24 hours. The graphical representations suggest a linear relationship, excluding the initial plateau period.

Table 6. Summary of kinetics of CGR 10

C_o (mg/l)	Time for 100% Removal (Days)	k (1/day)	R^2	Percentage Removal (%)
100	1.5	94.43	0.99	100
500	8	80.35	0.95	100
2000 [A]	22	164.26	0.94	100
2000 [B]	22	0.683	0.94	100

Note: $C_o = 2000$ mg/l [A] (Day 0 -12) – Linear relationship

$C_o = 2000$ mg/l [B] (Day 16 -22) – Exponential relationship

Each test conducted with the CGR 10, presents an acclimatisation period which is dependent on the initial concentration, with the 2000 mg/l test having the longest plateau of 3 - 4 days, followed by 12 days of removal at a linear rate and a final

exponential tail after day 16. After the plateau, nitrate removal occurred at a linear rate until a zero nitrate concentration was achieved, between 1.25 to 1.75 days for the 100 mg/l test, 7 to 8 days for the 500 mg/l test and 22 days for the experiment at 2000 mg/l.

Microbial studies done by De Combret (2009) and Trois (2010) suggest that denitrifiers are only present after 74 hours, thus the high performance of the substrates removal of nitrate within 24 hours could be attributed to other bio-chemical processes such as absorption of nitrates or the conversion of nitrates into ammonia.

3.3. Column Tests

The following criteria were used to determine the suitability of the substrates for utilisation in the column studies. The first key parameter was the C/N ratio as it is essential to have a relatively high C/N ratio for denitrification. C/N ratios above 16 were considered suitable for denitrification (Tsui et al., 2007; Wu et al., 2001; Trois et al., 2010). The second parameter was the pH as the optimum range of for denitrification is 6 – 8. The time required for full denitrification to be achieved in optimum conditions, such as in batch tests as well as the release of COD and NH₃ through bioleaching was also taken into account.

Table 7. Summary of column operating conditions

Substrate	NO ₃ Concentration (mg/l)	Duration (Weeks)	Flow Rates (l/day)	
			Experiment 1	Experiment 2
CGR RAW	500	4	2.48	5.625
CGR 10	500	4	1.7	2.85
CGR RAW	2000	4	2.38	5.65
CGR 10	2000	4	1.78	2.85

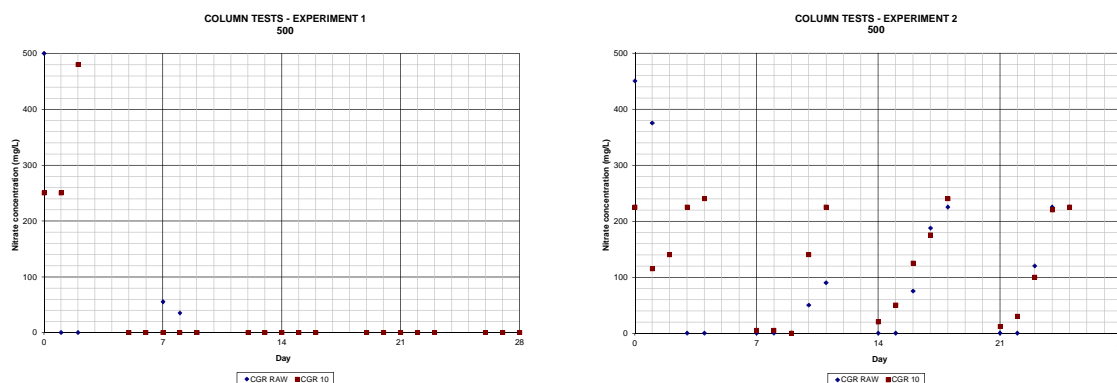
3.3.1. $C_o = 500 \text{ mg/l}$ 

Figure 2: Evolution of the nitrate concentration for CGR RAW and CGR 10 for $C_o = 500 \text{ mg/l}$

In experiment 1, full nitrate removal was achieved by both substrates. The CGR RAW substrate achieved full denitrification within the first 5 days, whereas in the CGR 10, nitrates were being removed within 5 – 7 days. The COD of the output effluent dropped considerably throughout the period of the test. After the first week a value of above 4500 mg/l was recorded in the CGR RAW and 450 mg/l in the CGR 10, however the COD dropped by more than 85% by the end of experiment 1. The presence of COD is as a result of readily biodegradable carbon being released.

In experiment 2, the column containing CGR RAW achieved full nitrate removal within the initial 4 days, as result of the increased flow rate, there was insufficient contact time between the solution and the substrate during weeks 2, 3 and 4, causing a rise in nitrate concentration. However after the extended contact time over the weekend, the entire column had achieved full nitrate removal. However the CGR 10 column failed to reach full denitrification throughout the experiment, achieving 96% removal, which leads us to conclude that the substrate in the column required more than 4 days for total nitrate removal to occur. The COD results at the second flow rate were lower than those recorded in experiment 1. This is due to the fact that the substrate was not replaced over the two experiments, with a final output below 55 mg/l . This is a result of less readily biodegradable carbon being released.

The pH remained below 6 during experiment 1 and tended to rise during the first week to 7 and remained at this level throughout the rest of experiment 2. The temperature remained constant with a range between 19 and 22 $^{\circ}\text{C}$, whilst the determined $\text{NH}_3 - \text{N}$ dropped to less than 1 mg/l at the conclusion of both experiments.

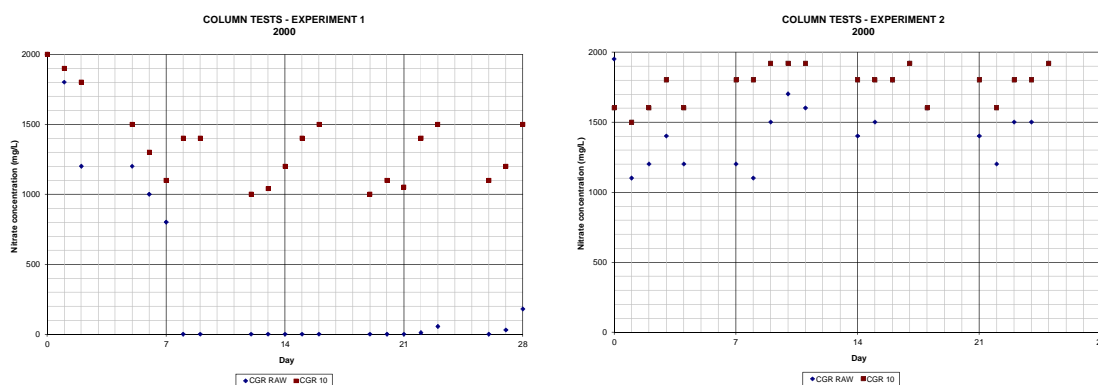
3.3.2. $C_o = 2000 \text{ mg/l}$ 

Figure 3: Evolution of the nitrate concentration for CGR RAW and CGR 10 for $C_o = 2000 \text{ mg/l}$

The nitrate concentration in the CGR RAW column at flow rate 1 reached zero after the initial 7 days. The concentration at the bottom of the column remained at zero until day 22, where the output concentration rose. This was observed once again during the following week. This reduced rate of denitrification could be due to the high nitrate concentration saturating the substrate. The rate at which carbon was being released had reduced and was now slower than the rate at which nitrates were being added. During the second week, full nitrate removal was being achieved within 1 - 2 days. However as the experiment progressed, this rate of denitrification reduced. At the end of the period the substrate failed to fully denitrify the leachate.

During the first week of experiment 1, the nitrate concentration in the CGR 10 reduced steadily at a linear rate. After 7 days the nitrate concentration increased until the end of the week. The column never achieved full denitrification and only reached a 50% removal of nitrates.

At flow rate 2 in the CGR RAW, the coupled effect of the very high nitrate concentration and high flow rate negatively affected the performance of the test resulting in a lower denitrification rate and only 50% removal efficiency against 100% in the first experiment.

Whereas the nitrate level in CGR 10 substrate stayed at a concentration of 1600 mg/l for the initial 4 days. After 7 days the concentration rose to 1800 mg/l and remained at this level for the remainder of the experiment. The column failed to achieve full denitrification during the 4 week period. The CGR 10 substrate showed minimal denitrification which can be contributed to the flow rate being too high, resulting in insufficient contact time, thus only a maximum of 25% removal efficiency was achieved as appose to 50% removal in the first experiment.

As full denitrification was not achieved, it is apparent that the CGR 10 was releasing carbon at a slower rate than that at which nitrate was being supplied.

The COD of the output effluent dropped considerably through the period of test 1 at a constant rate. However, the COD values during experiment 2 dropped after the first week to below 100 mg/l where they remained fairly constant throughout the duration of the experiment. The evolution of COD suggests that the flow rate was too high to allow for a significant bio-leaching of carbon, as experienced in most of the experiments at flow rate 2.

The pH measured during the period of the tests stayed at a constant level between 7 and 7.25, whilst the temperature remained constant for both experiments, in the range between 19 and 22 °C. In experiment 1, the $\text{NH}_3 - \text{N}$ decreased from 6 mg/l after the first week to below 3 mg/l and remained at that level for the remainder of the experiment and the measured $\text{NH}_3 - \text{N}$ during experiment 2 decreased from 4.5 mg/l after the first week to less than 1 mg/l by the end of the experiment.

In summary, the poor performance of both substrates at flow rate 2, for both concentrations, suggests that the shorter contact time was not long enough to establish an active bio-film for denitrification.

3.3.3. Loading Rates and Hydraulic Retention Time

The Hydraulic retention time (HRT) is a measure of the average length of time that a soluble compound remains in a constructed bioreactor and is calculated by the volume of the reactor divided by the flow rate (<http://www.lenntech.com/wwtp/hrt.htm> accessed 19/12/2009).

The hydraulic retention time has an effect on nitrate removal and is thus vitally important in the design of a bioreactor. (Tsui et al., 2007). The hydraulic loading rate is a critical factor for the design of treatment systems and is determined as the volume per day that can be applied over a surface area (Zhou et al., 2007).

Table 8 presents the performance of the substrates for each of the columns for the changes in concentration and flow rate. These results can be extrapolated using simple ratio concentrations to provide an estimate of the ideal flow rates and hydraulic retention times.

Table 8. Summary of the performance of the column studies over both experiments

Substrate	NO ₃	Flow	Rates		HRT (Days)		% Removal		Loading	Rate
	Conc.	(ℓ/day)							(ℓ/m ² /day)	
	(mg/ℓ)	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp.1	Exp.2	
CGR RAW	500	2.48	5.625	8.06	3.56	100	100	123.32	279.71	
CGR 10	500	1.7	2.85	11.76	7.02	100	96	84.54	141.72	
CGR RAW	2000	2.38	5.65	8.40	3.54	100	45	118.35	280.95	
CGR 10	2000	1.78	2.85	11.24	7.02	50	25	88.51	141.72	

For both the tests conducted at $C_o = 500$ mg/l and 2000 mg/l, the CGR RAW was the best performing substrate. For the test at $C_o = 500$ mg/l full nitrate removal was achieved at both flow rates.

Due to the 100% nitrate removal achieved at $C_o = 500$ mg/l at both flow rates it can be concluded that the CGR RAW can sustain a higher flow rate than 5.625 l/day as well as a loading rate above 280 l/m²/day. The HRT time required for full nitrate removal is less than 3.5 days.

For the tests conducted at $C_o = 2000$ mg/l, the system only achieved full nitrate removal at the first flow rate of 2.38 l/day in experiment 1, whereas in experiment 2 a 45% nitrate removal was reached. Through simply extrapolation an estimated flow rate of 2.54 l/day and a HRT of 8 days would be needed for the system to achieve full denitrification.

The CGR 10 at $C_o = 500$ mg/l also achieved 100% nitrate removal at the flow rate in experiment 1, however only reached 96% nitrate removal in experiment 2. This suggests that the flow rate required for full denitrification is between 1.7 – 2.85 l/day. A flow rate of 2.74 l/day and a HRT of 7.3 days are estimated.

At $C_o = 2000$ mg/l, the CGR 10 was the least efficient substrate only obtaining 50% nitrate removal in experiment 1 and 25% in experiment 2. This indicates that both flow rates were too high for full denitrification to be reached. A flow rate of 0.7 – 0.9 l/day and a HRT of 22 - 28 days are estimated.

4. CONCLUSIONS

The results of the laboratory experiments substantiate that the substrates prove to be effective as carbon sources to denitrify various concentration levels of nitrified leachate, at different degrees of efficiency.

The substrate materials had varying compositions of relatively high carbon (C) content in comparison to nitrogen (N). This characteristic makes these materials well suited for nitrate removal as they provide organic carbon for denitrification without increasing the nitrogen concentration. They also act as a medium for denitrifying bacteria.

The characterisation tests indicated that the fresh commercial garden refuse material had higher carbon to nitrogen ratio than that of the composted materials. The batch tests showed positive results; which can be attributed to the higher carbon to nitrogen content of the substrates and the fairly neutral pH. The higher carbon to nitrogen ratio of the fresh commercial compared to that of the composted materials was evident in the results, with the best performing substrate being the CGR RAW. Both substrates achieved full nitrate removal at the various concentrations.

All the small-scale batch tests conducted demonstrated similar characteristics of an acclimatisation period before decreasing linearly with time. The duration of the acclimatisation period was strongly related to that of the initial input concentrations of the nitrate solution.

The column tests reflected promising results at $C_o = 500 \text{ mg/l}$ during experiment 1, with both substrates achieving full denitrification. At $C_o = 2000 \text{ mg/l}$ only the CGR RAW column reached full denitrification. The CGR RAW substrate reflected the best results. During experiment 2, however the increased flow rates were too high to allow denitrifying bacteria sufficient contact period or hydraulic retention time to establish themselves. It is noted that flow through the columns improves the organic matter release and dispersion rates compared to a system where the effluent remains stagnant (Diaz et al., 2003). However a flow rate that is too high could result in an insufficient hydraulic retention time, which does not allow denitrifying bacteria to accumulate for denitrification. The results also indicate that the rate at which carbon is being released is slower than the rate at which nitrates are being added.

The main concern of this treatment method is the increase in COD concentration produced by organic matter release. It was found that over time the COD concentrations did decrease, but, in most cases, not sufficiently to fall into DWAF's Water Quality criteria.

The eThekweni landfills receive large volumes of garden refuse monthly which is separated from the main waste stream. These two materials are highly abundant and easily available on site, thus making them fairly inexpensive and thus could be successfully employed at local landfill sites to denitrify treated leachate which would prevent excessive treatment costs as well as support the development of a real waste management strategy.

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