APPLICATION OF FLUIDISED-BED REACTORS FOR THE BIOLOGICAL TREATMENT OF A NITROGENOUS INDUSTRIAL EFFLUENT

by

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B.Sc. (Hons) (U.O.F.S)

Dissertation submitted in partial fulfilment of the requirements
for the degree

MAGISTER SCIENTIAE (MICROBIOLOGY)

In the

School for Environmental Sciences and Development: Microbiology
Potchefstroomse Universiteit vir Christelike Hoër Onderwys
Potchefstroom, South Africa.

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November 2001

This work is dedicated to my mother,
Mrs. A. Nhlapo and my late father Mr. M. S. Nhlapo

"If we knew what it was we were doing, it would not be called research, would it?"

-A.Einstein

Acknowledgements

I wish to express my sincere appreciation and gratitude to the following persons and institutions for their contributions to the successful completion of this study:

Prof. K. J. Riedel, School for Environmental Sciences and Development: Microbiology, Potchefstroomse Universiteit vir Christelike Hoër Onderwys, for his guidance, never ending patience, encouragement and for his valuable criticism of the manuscript;

Dr. Sheldon Tarre, The Technion, Israel Institute of Technology, Haifa, Israel, for his continuous technical advise and patience;

Mr. P. Jansen van Rensburg, School for Environmental Sciences and Development: Microbiology, Potchefstroomse Universiteit vir Christelike Hoër Onderwys, for his invaluable advise, assistance and interest throughout the study,

Dr. Wade Edwards, School for Environmental Sciences and Development: Microbiology, Potchefstroomse Universiteit vir Christelike Hoër Onderwys, for his valuable criticism of the manuscript;

Dr. T. Phillips and Miss Joey Swart Sasol Technology, Research and Development (Sasol R & D) Sasolburg, for their technical assistance;

Dr. L Tiedt, Electron Microscopy, Potchefstroomse Universiteit vir Christelike Hoër Onderwys Potchefstroom, for his assistance with the electron microscopy,

My fellow students from the Environmental Microbiology Research Group, Potchefstroomse Universiteit vir Christelike Hoër Onderwys, for their kind support and frienship;

Sasol Technology, Research and Development (Sasol R & D) Sasolburg, for their technical and financial support of this study;

My parents and the entire family, for their continuous support and encouragement throughout my University career; and

Most importantly, God, the all Mighty, who watched over me and gave me the strength to carry-on, making it all possible.

Declaration

The experimental work conducted and discussed in this dissertation was carried out in the School for Environmental Sciences and Development: Microbiology, Potchefstroomse Universiteit vir Christelike Hoër Onderwys Potchefstroom, South Africa. The study was conducted during the period of February 2000 to November 2001 under the supervision and co-supervision of Prof. K. J. Riedel and Dr. W. Edwards, respectively.

The study represents original work undertaken by the author and has not been previously submitted for degree purposes to any other university. Appropriate acknowledgements in the text have been made where the use of work conducted by other researchers has been included

Makhosazana Victoria Nhlapo

Table of contents

Language and style used in this dissertation are in accordance with the requirements of the Journal of Water Research.

This dissertation represents a compilation of manuscripts, where each chapter is an individual entity and some repetition between the chapters has, therefore, been unavoidable.

Abbreviations Used in this Dissertation

AMO; ammonium monooxygenase

$b\rho$; bulk density

BAS; biofilm airlift suspension reactors

BFBR; biological fluidised-bed reactor

BFBR`s; biological fluidised-bed reactors

COD, chemical oxygen demand (mg/l)

COD/N, chemical oxygen demand / nitrogen ratio

C/N; carbon / nitrogen ratio

CO_2; carbon dioxide

CH_3OH; methanol

C_2H_5OH; ethanol

Cyt *c*; cytochrome c

Cyt *aa3*; cytochrome *aa3*

D; denitrification

D_{10}; equivalent diameter

DGGE; denaturing gradient gel electrophoresis

DO; dissolved oxygen concentration (mg/l)

ΔG; Gibbs free energy

GAC; granular activated carbon

GB; Great Britain

HAO; hydroxylamine oxidoreductase

HNO_2, nitric acid

HNO_3-N, nitrous acid

H_2O; water

HCl; hydrochloric acid

HCO_3^-; hydrogen carbonate

HRT; hydraulic retention time

IW; industrial water

Nar; nitrate reductase

Nir; nitrite reductase

Nor; nitric oxide reductase

Nos; nitrous oxide reductase

NF; nitrification

N_2; dinitrogen gas

NO_3^-; Nitrate

NO_2^-; Nitrite

NH_3; Unionised Ammonia

NH_4^+; Ammonium

NO; nitrous oxide

N_2O; dinitrous oxide

N_2OH; hydroxylamine

NH_4-N; ammonium nitrogen (mg/l)

NO_3-N; nitrate nitrogen (mg/l)

NO_2-N; Nitrite nitrogen (mg/l)

MLSS; mixed liquor suspended sludge

OSBG; optimised biological support of biological growth

PE; polyethylene

PMMO; methane monooxygenase

Ps; polystyrene

PVC; polvinylchloride

Q; quinone

SDS PAGE; sodium dodecyl sulfate polyacrylamide gel electrophoresis

SO_4^{2-}; sulphate

S^2; sulphide

S°; elemental sulphur

T; temperature

TCE; tetra-chloroethane

TSS; total suspended solids (mg/l)

TT; tertiary treatment

UFC; uniformity coefficient

USA; United States of America

UW; urban water

VFA; volatile fatty acids

VSS; volatile suspended solids (mg/l)

Summary

The primary aim of the study was to evaluate the suitability of biological fluidised-bed reactors (BFBR`s) for the biological treatment of a nitrogenous, saline industrial effluent at varying loading rates as well as optimisation of the process. During this study a synthetic effluent analogous to the condensate stream as generated by SASOL Agri, Secunda, South Africa, containing NH_4NO_3 concentrations of approximately 1000 mg/l as N was treated.

The first objective of this study was to optimise nitrification processes using the BFBR`s, while comparing and evaluating the use of either sand or granular activated carbon (GAC) as support matrices. The BFBR`s were fed with the ammonium nitrogen (NH_4-N) concentration gradually being increased from 10 mg/l to 1000 mg/l. At NH_4-N loading rates below 1 kg NH_4-N/m^3.d, removal efficiencies in excess of 99% were achieved in both BFBR`s using sand and GAC as support matrices, respectively. However, at loading rates in excess of 1 kg NH_4-N/m^3.d, further increase in the NH_4-N loading rate was limited by an associated decrease in the dissolved oxygen concentration (<1.5 mg/l). At a loading rate of 2 kg NH_4-N/m^3.d, the NH_4-N removal efficiency dropped to 58% and 48% in the BFBR using sand and GAC as support matrices, respectively. The reduction in the removal efficiencies also resulted in a significant increase in the concentrations of total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), NH_4-N, nitrite nitrogen (NO_2-N) and nitrate nitrogen (NO_3-N) in the effluent.

Upon further increase of the NH_4-N loading rate to 4 kg NH_4-N/m^3.d, the removal efficiency dropped to 28 % and 47% for the BFBR`s using sand and GAC as support matrices, respectively. Further increase in the NH_4-N loading rate was limited by the complete absence of dissolved oxygen in the system (<0.1 mg/l). Although both sand and GAC performed well as support matrices, slightly enhanced removal efficiency was obtained using GAC as the support matrix.

The next objective was to optimise the denitrification process using the BFBR`s while comparing and evaluating the use of either sand or GAC as support matrices. The reactors were fed with a synthetic effluent analogous

to the condensate stream as generated by SASOL Agri, Secunda, in which the nitrate-nitrogen (NO_3-N) concentration was gradually increased from 10 to 1000 mg/l. Sodium acetate was used as the carbon source for the heterotrophic denitrifying microorganisms. During the initial start-up phase, the C:N ratios for both reactors was maintained at 1.14. During this period, the NO_3-N removal efficiencies dropped from 99% to 60% and 40% for GAC and sand, respectively. The low removal efficiencies were also characterised by a significant accumulation of nitrite in the effluent. The C:N ratio was subsequently increased until low concentrations of nitrite were present in the effluent. The optimal C:N ratios were determined to be 1.6 and 2.0 for the BFBR`s using sand and GAC as support matrices, respectively. In both reactors, NO_3-N removal efficiencies in excess of 99% could be achieved at a NO_3-N loading rate of 4 kg NO_3-N/m^3.d. As the NO_3-N loading rate was increased to 8 kg NO_3-N/m^3.d, the NO_3-N removal efficiencies dropped significantly to 67% and 23% in the BFBR`s using sand and GAC as support matrices, respectively. A further significant drop in the $NO_3$-N removal efficiencies was observed when the $NO_3$-N loading rate was increased to 26 kg $NO_3$-N/$m^3$.d. At this loading rate, $NO_3$-N removal efficiencies of 66% and 17% were achieved in the BFBR`s using sand and GAC as support matrices, respectively. In comparison to GAC, significantly higher removal efficiencies, higher NO_3-N loading rates and more stable reactor operation was achieved in the denitrifying BFBR using sand as the support matrix.

Using the information gained from these and other similar studies, the final objective of this study was to achieve the complete treatment of a high strength nitrogenous industrial effluent using dual-stage BFBR`s (two nitrification and one denitrification BFBR`s) operated in series. The nitrification reactors were fed with a synthetic effluent analogous to an industrial effluent in which the NH_4NO_3-N concentration ranged from 600 mg/l NH_4NO_3-N to 1200 mg/l NH_4NO_3-N. The influent of the denitrification reactor was the effluent which exited from the nitrification reactors in which the NO_3-N concentration ranged from 1000 mg/l NO_3-N to 2400mg/l NO_3-N. During this study NH_4-N removal in excess of 99% was achieved in both nitrifying reactors at a loading rate of 1 kg NH_4-N/m^3.d. As the loading rate was increased to 2.4 kg NH_4-N/m^3.d, the removal efficiency in the nitrification

reactor with sand as the support matrix dropped significantly to 48%. However, in contrast to the results obtained previously this increase in the NH_4-N loading rate did not have an effect on the nitrifying reactor with GAC as the support matrix. Removal efficiencies (NH_4-N) in excess of 99% could be achieved at this loading rate. Nitrate nitrogen removal efficiency in excess of 99% was achieved in the denitrification reactor at a loading rate of 2.4 kg NO_3-N/m^3.d. An increase in the loading rate to 8.35 kg NO_3-N/m^3.d did not have an effect on NO_3-N removal and the removal efficiency remained at 99%.

The results from these studies subsequently indicated that dual-stage BFBR`s could serve as a suitable technology for the treatment of the synthetic nitrogenous industrial effluent analogous to the condensate effluent generated by SASOL Agri, Secunda, South Africa. With the exception of the dissolved oxygen limitation as experienced during nitrification, no other problems were experienced and high loading rates could be obtained. Based on the results obtained, granular activated carbon was the best support matrix for nitrification whilst sand was the best support matrix for denitrification.

Opsomming

Die primêre doel van die studie was om die doelmatigheid van biologiese opvloei-bed reaktore (BOBR'e) vir die biologiese behandeling van stikstofbevattende, southoudende nywerheidsuitvloeisels met wisselende beladings te evalueer en die proses te optimiseer. In hierdie studie is 'n sintetiese uitvloeisel, analoog aan die kondensaatstroom wat by SASOL Agri, Secunda, Suid-Afrika, gegenereer word, behandel. Die stroom het NH_4NO_3 konsentrasies van ongeveer 1000 mg/l as N bevat.

Die eerste doelwit van die studie was om die nitrifikasieproses met behulp van die BOBR'e te optimiseer en terselfdertyd die gebruik van óf sand óf granulêre geaktiveerde koolstof (GGK) as ondersteuningsmatriks te vergelyk en te evalueer. Die BOBR'e is gelaai met die sintetiese uitvloeisel wat NH_4-N bevat het in konsentrasies wat geleidelik verhoog is van 10 mg/l tot 1000 mg/l. Teen 'n beladingstempo van laer as 1 kg NH_3-N/m^3.d, is die doeltreffendheid van verwydering wat in beide BOBR'e behaal is meer as 99 % wanneer sand en GGK respektiewelik as ondersteuningsmatrikse gebruik is. Teen beladingstempo's hoër as 1 kg NH_3-N/m^3.d is verdere vermeerderings in NH_4-N beladingstempo egter beperk deur 'n gepaardgaande verlaging in die opgeloste suurstof konsentrasie (<1.5 mg/l). Teen 'n beladingstempo's van 2 kg NH_3-N/m^3.d het die doeltreffendheid van NH_4-N verwydering gedaal tot 58 % en 48 % respektiewelik wanneer sand en GGK as ondersteuningsmatrikse gebruik is. As gevolg van die verlaging in die doeltreffendheid van verwydering, het die konsentrasie van die totale gesuspendeerde vastestowwe (TSS), vlugtige gesuspendeerde vastestowwe (VSS), chemiese suurstofbehoefte (COD), NH_4-N en NO_3-N in die uitvloeisel 'n betekenisvolle toename getoon.

Met 'n verdere verhoging in die NH_4-N beladingstempo tot 'n tempo van 4 kg NH_3-N/m^3.d, het die doeltreffendheid van NH_4-N verwydering gedaal tot 28 % en 47 % respektiewelik wanneer sand en GGK as ondersteuningsmatrikse gebruik is. 'n Verdere toename in die die NH_4-N beladingstempo is beperk deur die algehele afwesigheid van opgeloste suurstof in die sisteem (<0.1 mg/l).

Alhoewel beide sand en GGK goed gevaar het as ondersteuningsmatriks, is 'n effens hoër verwydering met GGK as ondersteuningsmatriks verkry.

Die volgende doelwit was om die denitrifikasieproses met behulp van die BOBR'e te optimiseer en terselfdertyd die gebruik van óf sand óf GGK as ondersteuningsmatriks te vergelyk en te evalueer. Die BOBR'e is gelaai met die sintetiese uitvloeisel wat analoog was aan die kondensaatstroom wat by SASOL Agri, Secunda, Suid-Afrika, gegenereer word waarin die nitraatstikstof (NO_3-N) konsentrasie geleidelik verhoog is van 10 mg/l tot 1000 mg/l. Natriumasetaat is as koolstofbron vir die heterotrofe denitrifiserende mikroörganismes gebruik. Gedurende die aanvanklike aanvangsfase, is die C:N verhouding in beide reaktore op 1.4 gehou en het die NO_3-N verwydering vanaf 99 % tot 60 % en 40 % vir GGK en sand respektiewelik gedaal. Hierdie lae verwyderingsdoeltreffendheid is gekenmerk deur 'n betekenisvolle akkumulasie van nitriet in die uitvloeisel. Die C:N verhouding is daarna verhoog totdat 'n lae nitrietkonsentrasie in die uitvloeisel teenwoordig was. Daar is bepaal dat die optimale C:N verhouding respektiewelik 1.6 en 2.0 in die BOBR'e waarin sand en GGK as ondersteuningsmatrikse gebruik is. 'n Verdere betekenisvolle verlaging in die verwyderingsdoeltreffendheid van NO_3-N is waargeneem nadat die NO_3-N beladingstempo verhoog is tot 26 kg NO_3-N/m^3.d. Teen hierdie beladingstempo is 'n verwyderingsdoeltreffendheid van 66 % en 17 % in die BOBR'e waarin sand en GGK as ondersteuningsmatrikse gebruik is, respektiewelik behaal. In vergelyking met GGK, was die hoër doeltreffendheid van verwydering, hoër NO_3-N beladingstempo en die meer stabiele bedryf van die reaktor wat behaal is in die denitrifiserende BOBR met sand as ondersteuningsmatriks, betekenisvol.

Deur gebruik te maak van die inligting uit hierdie en ander soortgelyke studies, was die finale doelwit om die hoë sterkte stikstofbevattende nywerheidsuitvloeisel met behulp van die twee-stap BOBR'e (twee nitrifikasie en een denitrifikasie BOBR'e) wat in serie bedryf is, volledig te behandel. Die nitrifikasiereaktore is gevoer met 'n sintetiese uitvloeisel wat analoog was aan 'n nywerheidsuitvloeisel met 'n NH_4NO_3-N konsentrasie wat gewissel het van 600 mg/l NH_4NO_3-N tot 1200 mg/l NH_4NO_3-N. Die invloei van die denitrifikasiereaktor was afkomstig uit die nitrifikasiereaktore waarin die NO_3-konsentrasie gewissel

het van 1000 mg/l NO_3-N tot 2400 mg/l NO_3-N. In hierdie studie is NH_4-N verwydering van meer as 99 % in beide die nitrifikasiereaktore behaal teen 'n beladingstempo van 1 kg NH_4-N/m^3.d. Met 'n verhoging in die beladingstempo tot 2.4 kg NH_4-N/m^3.d, het die verwyderingsdoeltreffendheid van die nitrifikasiesreaktor met sand as ondersteuningsmatriks betekenisvol afgeneem tot 48 %. In teenstelling met die ander resultate wat vooraf verky is, het hierdie toename in die NH_4-N beladingstempo egter geen uitwerking op die doeltreffendheid van die nitrifikasiereaktor met GGK as ondersteuningsmatriks gehad nie en is NH_4-N verwydering van 99 % behaal. 'n Verwyderingsdoeltreffenheid van meer as 99 % vir NO_3-N is in die denitrikasiereaktor met 'n beladingstempo van 2.4 kg NH_4-N/m^3.d behaal. 'n Toename in die beladingstempo tot 8.35 kg NH_4-N/m^3.d het geen uitwerking op NO_3-N verwydering, wat op 99 % gehandhaaf kon word, gehad nie.

Die resultate van hierdie studies het gevolglik daarop gedui dat die twee-fase BOBR'e as 'n geskikte tegnologie sou kon dien vir die behandeling van die sintetiese stikstofbevattende nywerheidsuitvloeisel wat analoog is aan die kondensaatuitvloeisel wat by SASOL Agri, Secunda, Suis-Afrika gegenereer word. Met die uitsondering van die tekort aan opgeloste suurstof wat tydens nitrifikasie ontstaan het, is geen ander probleme ondervind nie en kon hoë beladingstempo's behaal word. Volgens die resultate wat verkry is, is granulêre geaktiveerde koolstof die beste ondersteuningsmatriks vir nitrifisering, terwyl sand die beste ondersteuningsmatriks vir denitrifisering was.

Chapter 1

Introduction

Water is the basic requirement of all the living ecosystems and habitats. It is affected by everything and it affects everything and everyone (Hoffman, 2000). However, this important natural resource is not as abundant as we may think. One third of the world population is currently living in water stressed regions and it is estimated that by the year 2025 almost one half of the world population will be living in water stressed regions (Bouler, 1999). Statistics indicate that the world population will increase to 7.8 billion by the year 2025 and most of the people will be living in urban areas. This will result in significant competition for water between people and the industrial sector. Currently the flows of fresh water are rapidly decreasing. In contrast to this, the amount of water pollution is rapidly increasing (Seckler and Amarasinghe, 2000). Furthermore, it is anticipated that climate change due to global warming will also have a significant impact on both the water demand and supply, especially in Africa (Shermon, 2001).

Currently in the Southern African region some areas have abundant water and others have scarcity. The rainfall patterns also differ dramatically, ranging from 4,000 mm to less than 50 mm per annum (Global Water Partnership, 2000). The water demand in South Africa is projected to rise at almost 3% annually and it is anticipated that by 2020 South Africa will suffer water stress and the country will have moved to insolvable water scarcity by 2025 (Shermon, 2001). In addition to the scarcity of water in South Africa, many water bodies are polluted and the water quality is declining due to salination and eutrophication. Salination results from increased industrialization, urbanisation and irrigation (O`Keeffe *et al.,* 1999), whereas eutrophication results from the introduction of high concentrations of nitrogenous compounds and phosphorus in the receiving water bodies (EPA, 1993).

Nitrogen-containing effluents are not only known to deteriorate water quality but they also have detrimental ecological and human impacts. Ammonia nitrogen has been reported to be toxic to fish. Nitrites and nitrates are known to be carcinogenic and nitrites may lead to metaheamoglobinemia if ingested by babies (EPA, 1993).

Due to the increasing industrial discharges and the environmental impacts of nitrogen-containing compounds, the general standards for the discharge of nutrients such as ammonia and nitrates into receiving water bodies such as dams, rivers and lakes are becoming more stringent (Massonne *et al.,* 1996). According to the South African National Water Act (36/1998), the general limit for the discharge of ammonia (ionised and non-ionised) as nitrogen-containing effluent into a water resource is 3 mg/l and the special limit is 2 mg/l. The general limit applicable to nitrates/nitrites as nitrogen is 15 mg/l and the special limit is 1.5 mg/l. The special limit values are applicable to the discharge of wastewaters into a listed water resource, whereas the general limit values are applicable to the discharge of the wastewaters into water resources that are not listed in the South African National Water Act (36/1998). In Europe, the general standard for the discharge of these nutrients is 1 mg/l for ammonia and 8 mg/l for both nitrates and nitrites respectively (EPA, 1993). In order to balance the water supply with its demand, effluent from different industries have to be returned and reused or treated before being discharged into receiving water bodies (Tchobanoglous, 1991; Gupta and Sharma, 1996).

Conventional processes for wastewater treatment such as stabilization ponds have been widely used for nitrogen removal. This process was found to be very economical, however, it has not been well studied, and therefore it has some drawbacks due to the unknown biological and chemical processes involved (Lai and Lam, 1997). Ion-dependant technologies such as ammonia stripping, non-ion dependent technologies and reverse osmosis are also frequently used for the removal of nitrogen from wastewaters. With the use of these processes, extensive nitrogen removal could be achieved. However,

these processes were found to be inefficient, very costly, and resulted in significant brine production (Bach *et al.,* 1998).

Removal of nitrogen from wastewaters can also be achieved using biological processes (Köning and Riedel, 1998). The biological removal of nitrogen from wastewaters is a two-step process, involving the conversion of ammonia to nitrate during nitrification (Wagner *et al.,* 1995) followed by denitrification during which nitrate is converted to dinitrogen gas (N_2) (Campos *et al.,* 1999). In contrast to chemical processes, biological processes for the removal of nitrogen are widely used, reliable, and have moderate costs (Tchobanoglous, 1991). The biological nitrification process is, however, characterised by a slow growth rate and a low yield of the bacterial genera involved, as well as their sensitivity to different organic compounds. In order to achieve complete nitrification high biomass concentrations must be available (Noto *et al.,* 1998). In contrast to nitrification, biological denitrification is characterised by fast growth rates and high biomass yields of the denitrifying heterotrophic microbial population. The denitrification process occurs under anoxic conditions and it requires the presence of an organic carbon source which acts as the electron donor (Ganaye *et al.,* 1996). Although oxygen is the preferred terminal electron acceptor of these bacteria, when there is no or very low residual oxygen, nitrate serves as the terminal electron acceptor (Brock *et al.,* 1997). Non-fermentable organic compounds such as ethanol, acetic acid and methanol are commonly used as the organic carbon sources (Constantin and Fick, 1997; EPA, 1993). These organic carbon sources may be provided by internal sources or they can be supplemented externally (Tchobanologlous, 1991).

Various nitrification/denitrification process technologies have been applied in order to achieve complete nitrification/denitrification. Amongst these technologies, conventional technologies which are widely used for the biological nitrogen removal from wasterwaters include the activated sludge system and the trickling filter system. Although these technologies have been used extensively and applied for many years, they have been reported to have low capacities and efficiencies for the treatment of high strength

nitrogenous industrial wastewaters. Furthermore, these systems require a large footprint and are very costly (Lazarova and Manem, 1994; Csikor *et al.*, 1996).

Due to these limitations, biomass immobilisation techniques can be seen as a promising technology and the best alternative in order to achieve the high concentrations of biomass required for complete nitrogen removal from industrial wastewaters (Nogueira *et al.*, 1998). Currently the immobilisation technologies used for the treatment of wastewaters are based on the retention of the microbial biomass within the reactor in the form of biofilms. Examples of such processes include fluidsed-bed reactors and biolift air suspension reactors (Lazarova and Manem, 1994). In these reactors, small particles are used as carrier matrices for growth and support of the bacterial biofilm (Frijters *et al.*, 2000). The use of small bio-carriers results in large surface areas and therefore the retention of high concentrations of biomass within the reactor (van Benthum, 1998). These processes result in high biomass concentrations within the reactor, which allows operation of the reactors at very short hydraulic retention times, and subsequently high volumetric loading rates (Garcia-Calderon *et al.*, 1998; Nicolella *et al.*, 1999). In addition, these reactors are very competitive in terms of space requirements due to their relative compactness (Cooper, 1981; Hosaka *et al.*, 1991; Nicollela *et al.*, 2000).

Biological fluidised–bed reactors have been utilised in some industrial applications (Sutton and Mishra, 1994). However, broader application of this process is hampered by the costs of aeration and the associated sophisticated construction requirements. Biolift air suspension reactors are currently applied in industry for the treatment of wastewaters under the trade name CIRCOX® (Freijers *et al.*, 1997; Nicolella *et al.*, 2000; van Loosdrechdt *et al.*, 2000).

The aim of this study was, therefore, to evaluate the suitability of fluidised-bed reactors for the treatment of a high-strength nitrogenous industrial effluent using biological nitrification/denitrification. The effluent used

during this study was a synthetic effluent analogous to the condensate (stream containing ammonium nitrate) as generated at SASOL Agri, Secunda, South Africa. The effluent is a high strength, highly saline wastewater which contains ammonium nitrate at a concentration of approximately 1000 mg/l as nitrogen. Approximately 17.7 m^3/h of the nitrogenous condensate stream is produced at SASOL Agri, Secunda. To date, there is no system that is used to remove the nitrogenous compounds from this wastewater. Since ammonium and nitrates have been reported to have numerous detrimental environmental impacts when discharged into receiving water resources (EPA, 1993), treatment of the SASOL Agri effluent prior to discharge is therefore, essential.

The specific objectives of this study were therefore:

- To gain insight into the microbial nitrification/denitrification process and the applicability of the biological fluidised bed reactors for the biological treatment of a high strength, high saline, nitrogenous synthetic industrial effluent;

- To compare and optimise the removal efficiencies of sand and granular activated carbon (GAC) as support matrices, for nitrification as well as denitrification of the high strength, high saline nitrogenous synthetic SASOL Agri effluent;

- To determine the suitability of using sodium acetate as an electron donor for the heterotrophic denitrifying bacterial community;

- Based on the results obtained from these studies the next objectives would be to:
  - (i) achieve complete removal of the high strength ammonium nitrate nitrogen effluent using a dual-stage process, where the fluidised-bed reactors used for nitrification and denitrification would be operated in series; and

- (ii) investigate the influence of the presence of high concentrations of nitrates in the wastewater on the biological nitrification/denitrification process.

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Chapter 2.

Literature review

Introduction

One third of the world population is currently living in water stressed regions and it is estimated that by the year 2025 almost one half of the world population will be living in water stressed regions (Bouler, 1999). It is anticipated that climate change due to global warming will also have a significant impact on both the water demand and supply, especially in Africa (Shermon, 2001). Furthermore, South Africa is currently receiving an average rainfall only slightly more than half the world average (Mutume, 1998). The water demand in South Africa is projected to rise 3% annually and it is anticipated that South Africa will have moved to insolvable water scarcity by 2025 (Shermon, 2001). In addition to the scarcity of water in South Africa, many water bodies are polluted and the water quality is declining due to salination and eutrophication. Salination results from increased industrialization, urbanisation and irrigation (O`Keeffe *et al.,* 1999), whereas eutrophication, results from the introduction of high concentrations of nitrogenous compounds and phosphorus in the receiving water bodies (EPA, 1993).

Nitrogen-containing effluents are not only known to deteriorate water quality but also have detrimental ecological and human impacts. Ammonia nitrogen has been reported to be toxic to fish, nitrites and nitrates are known to be carcinogenic and nitrites may lead to the development of metheamoglobinemia if ingested by babies (EPA, 1993). Due to the increasing industrial discharges and the environmental impacts of nitrogen-containing compounds, the general standards for the discharge of nutrients such as ammonia and nitrates into receiving bodies such as dams, rivers and lakes are becoming more stringent (Massonne *et al.,* 1996). According to the South African National Water Act (36/1998), the general limit for the discharge

of ammonia (ionised and non-ionised) as nitrogen-containing effluent into a water resource is 3 mg/l and the special limit is 2 mg/l. The general limit applicable to nitrates/nitrites as nitrogen is 15 mg/l and the special limit is 1.5 mg/l. In Europe, the general limit for the discharge of ammonium is 1 mg/l and the general limit for the discharge of nitrates is 8 mg/l. (EPA, 1993). In order to balance water supply with its demand, effluent from different industries have to be returned and reused or treated before discharging into receiving water bodies (Tchobanoglous, 1991; Gupta and Sharma, 1996).

Ion-dependant technologies such as ammonia stripping, non-ion dependent technologies and reverse osmosis are also frequently used for the removal of nitrogen from wastewaters. With the use of these processes, extensive nitrogen removal could be achieved. However, the processes were found to be inefficient, very costly, and resulted in significant brine production (Bach *et al.,* 1998). Removal of nitrogen from wastewaters can also be achieved using biological processes such as nitrification/denitrification (Köning and Riedel, 1998). Biological nitrification/denitrification processes for the removal of nitrogen are widely used, reliable, and have moderate costs (Tchobanoglous, 1991).

Microbial nitrification/denitrification

Microbial nitrification/denitrification is a two step process, with the initial conversion of ammonia (NH_4^+) to nitrate (NO_3^-) by nitrifying bacteria (Fig. 1), followed by the second step, denitrification, in which nitrate is reduced to nitrogen gas by denitrifying bacteria (Iizumi *et al.,* 1998; Campos *et al.,* 1999).

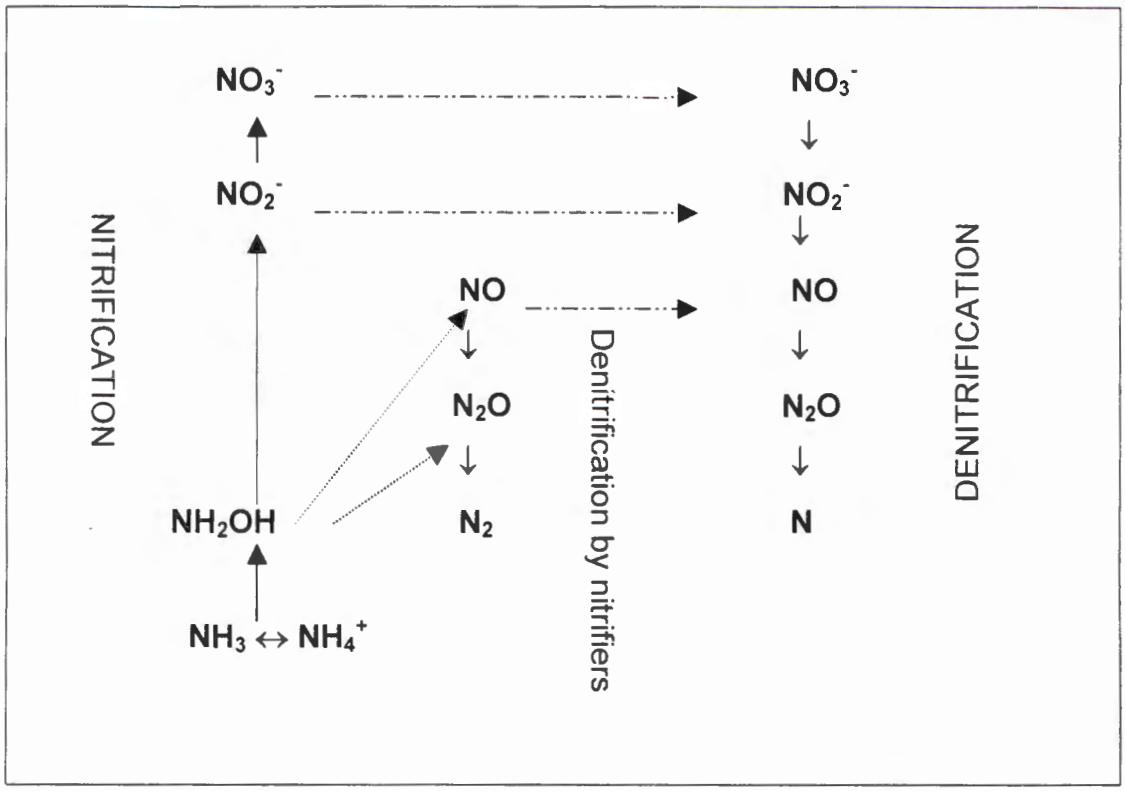

Figure 1. Microbial nitrification/denitrification processes where: N_2, dinitrogen gas; NO_3^-, Nitrate; NO_2^-, Nitrite; NH_3, Ammonia; NH_4^+, Ammonium; NO, nitrous oxide; N_2O, dinitrous oxide; NH_2OH, hydroxylamine (adapted from du Plessis *et al.*, 1998).

Nitrification

Nitrification is the key step in the biological removal of ammonia from wastewaters (Wagner *et al.*, 1995) resulting in a slow but gradual increase in the nitrate concentration (Sauthier *et al.*, 1998). Nitrification processes were initially thought to occur strictly under aerobic conditions with oxygen as the electron acceptor and ammonia as the electron donor. Autotrophic and heterotrophic nitrification processes subsequently became the most studied types of nitrification (Mevél and Prieur, 1998). However, recent studies have indicated the possibility of the process occurring under anoxic conditions with ammonia serving as the electron donor. This process has been termed anaerobic ammonium oxidation (Anammox) (Mulder *et al.*, 1995).

Heterotrophic nitrification

This is the process of nitrogen removal performed by heterotrophic microorganisms in which the nitrogen oxidation property is not considered to be taxonomically important (Focht and Verstraete, 1977). Examples of heterotrophic nitrifiers include actinomycetes, algae, and fungi (Hunter *et al.,* 1998). Most heterotrophic nitrifiers are able to carry out nitrification as well as denitrification simultaneously under aerobic conditions. These organisms have the ability to nitrify under conditions that inhibit the growth of autotrophic nitrifiers. For example, Mevél and Prieur (1998), reported non-autotrophic nitrification by heterotrophic thermophillic nitrifiers belonging to the genera *Bacillus* and *Thermus* at 65°C, from active deep sea hydrothermal vent systems.

Organic compounds serve as the energy source for the heterotrophic nitrification process (Nishio *et al.,* 1998). In the presence of organic compounds heterotrophic nitrifiers can outcompete the autotrophic nitrifiers since they have higher specific growth rates (Okabe *et al.,* 1996). Nitrogen removal by heterotrophic nitrifiers is not coupled to growth and biomass production and is probably a co-oxidation of ammonia and organic substrates (Brock *et al.,* 1997). In addition, heterotrophic nitrification occurs at slow rates (Mevél and Prieur, 1998).

Heterotrophic nitrification is less sensitive to inhibitors than autotrophic nitrification and the heterotrophic nitrifiers are widespread and may dominate the nitrogen cycling processes (Anderson *et al.,* 1993). However, they have not generally been considered to be important sources of nitrogen gas in aquatic systems. These organisms are, therefore, regarded to be less significant in the removal of nitrogen from wastewater because most of the nitrogen removed is incorporated into the bacterial biomass and lesser amounts of nitrogen are converted to nitrate (Brock *et al.,* 1997).

Autotrophic nitrification

Autotrophic nitrification is a two-step process which consists of the sequential oxidation of ammonia to nitrate, with nitrite being an intermediate (Phillips *et al.,* 1999; Tchonobaglous, 1991). In contrast to heterotrophic nitrification, autotrophic ammonium oxidation is carried out by bacteria that derive energy from the oxidation of inorganic compounds (EPA, 1993). The bacteria involved are the Gram negative rod-shaped non-motile members of the family Nitrobacteriaceae (Watson *et al.,* 1989).

Conventional techniques for the study of the microbial community structure involved in ammonia oxidation have primarily been hampered by technical difficulties associated with the identification of the microorganisms involved (Phillips *et al.,* 1999). These techniques do not allow for the study of ammonium oxidizers *in situ* and therefore restricted the study of these microorganisms to laboratory isolation and cultivation (McCaig *et al.,* 1999). In addition, the culture-based methods failed to be representative of the community structure (Magnusson *et al.,* 1998) and did not reflect the behavior of the microorganisms in their natural environments (Stantegoeds *et al.,* 1998a). Due to this restriction and the ability of some *Nitrobacter* and *Nitrosomonas* strains to outcompete other ammonia oxidizers in liquid media, these two bacterial strains are the most commonly studied (Schramm *et al.,* 1998; Hovanec *et al.,* 1998). The use of molecular techniques such as the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) and denaturing gradient gel electrophoresis (DGGE) has recently revealed that there are various other bacterial genera also involved in the nitrification process. The dominant bacterial genera involved in the nitrification process are indicated in Table 1 (Wagner *et al.,* 1995).

Table 1. Bacterial genera involved in autotrophic ammonium oxidation (Wagner *et al.*, 1995)

Organism	Strain	Genospecies
Nitrosomonas communis	Nm 2 ^{T}	1
Nitrosomonas sp.	Nm 34	2
Nitrosomonas sp.	Nm 33	3
Nitrosomonas ureae	Nm 10 ^{T}	4
Nitrosomonas cryotolerans	Nm 55 ^{T}	5
Nitrosomonas aestuarii	Nm 36 ^{T}	6
Nitrosomonas sp.	Nm 51	7
Nitrosomonas marina	Nm 22 ^{T}	8
Nitrosomonas nitrosa	Nm 90 ^{T}	9
Nitrosomonas eutropha	Nm 57 ^{T}	10
Nitrosomonas oligotropha	Nm 45 ^{T}	11
Nitrosomonas europaea	ATCC 25978 ^{T} Nm 50 ^{T}	12
Nitrosomonas halophila	Nm 1 ^{T}	13
Nitrosomonas sp.	Nm 84	ND
Nitrosococcus mobilis	Nc 2 ^{T}	-
Nitrospira briensis	ATCC25971 Nsp 10	-
Nitrospira sp.	Nsp 1	-
Nitrosovibrio tenius	Nv 1 $^{T T}$	-
Nitrosolobus multiformis	ATCC 259175 ^{T} Nl 13 ^{T}	-
Comamonas testorni	DSM 50244 ^{T}	-
Leptothrix discophora	LMG 814[1]	-
Sphaerotilus natans	ATCC 13338^{T}	-
Alcaligens faecalis	ATCC 8750 ^{T}	-
Aquaspirillum metamorphum	DSM 1837^{T}	-

^{T}; Type strain

ND; not determined

Since the techniques based on nucleic acids do not neccesitate cultivation, they are therefore subject to less bias (Stantegoeds *et al.,* 1998b). The *Nitrosomonas* spp. are ammonia oxidisers which oxidise free and saline ammonia to an intermediate nitrite, whereas the *Nitrobacter* spp. are nitrite oxidizers which convert nitrite to nitrate (EPA, 1993; Juliette *et al.,* 1993a). To date there are no reports about microorganisms capable of the direct conversion of ammonia to nitrate without first converting it to nitrite (van Loosdrecht and Jetten, 1998)

Recently phylogenetic analyses of pure cultures based on 16S rRNA sequence data have indicated that ammonia-oxidizing bacteria belong to two sub-groups of the class *Proteobacteria.* These are the γ-subgroup of the *Proteobacteria,* which include *Nitrosococcus oceanus* and the β-subgroup of the *Proteobacteria* which includes the majority of the cultured strains (Phillips *et al.,* 1999). The β-subgroup of the *Proteobacteria* is further divided into two clades belonging to the *Nitrosomonas* spp. and the *Nitrospira* spp. (McCaig *et al.,* 1999). The nitrite-oxidizing bacteria belong to the α-subgroup of the class *Proteobacteria* which include *Nitrobacter winogradskyi* (Shcramm *et al.,* 1998).

However, much work must still be done to identify the microorganisms involved in autotrophic ammonia oxidation, since there are some indications that there are still some unknown bacteria involved in the process. This has been confirmed by the studies done by Enrich *et al.* (1995), where an obligate lithoautotrophic ammonia oxidizer *Nitrospira moscoviensis,* was isolated from a partially corroded iron pipe of a heating system with the aid of molecular techniques and SDS PAGE.

Stoichiometry and enzymes involved in autotrophic ammonium oxidation

Stoichiometry

The general reaction for the oxidation of ammonium to nitrite by *Nitrosomonas eurpaea* can be described by the following reaction (EPA, 1993):

$$NH_4^+ + 1.5O_2 \rightarrow 2H^+ + H_2O + NO_2^-$$

This reaction requires approximately 3.43 mg O_2/mg of NH_4^+ oxidized and the free energy (Δ G) released ranges from -58 to -84 kcal/mole. The general reaction for the oxidation of nitrite to nitrate can be described by the following reaction (EPA, 1993; Hargreaves, 1998):

$$NO_2^- + O_2 \rightarrow NO_3^-$$

Approximately 1.14 mg O_2/mg of NO_2^- are required for this reaction. The free energy released for this reaction (ΔG) is –18 kcal/mole (Hargreaves, 1998).

The overall reaction can therefore be expressed as follows:

$$NH_4^+ + O_2 \rightarrow NO_3^- + H_2O + 2H^+$$

The biomass synthesis reaction during nitrification can be expressed using the following reaction equation (Marais and Ekama, 1984):

$$NH_4^+ + HCO_3^- + 4CO_2 + H_2O \rightarrow C_5H_7O_2N$$

Biological nitrification can thus be expressed as follows:

$$NH_4^+ + O_2 + HCO_3^- \rightarrow C_5H_7O_2N + 0.98NO_3^- + H_2O + 1.88H_2CO_3$$

This general equation assists in the estimation of parameters associated with nitrification such as, oxygen requirements, alkalinity, and biomass production.

In general, the growth kinetics of microorganisms are expressed using the Monod model (WPCF, 1991). However, this model has been reported to fail in the description of microbial growth kinetics of nitrifers that may involve multiple substrate-limiting conditions which are encountered in the nitrification processes (EPA, 1993).

For nitrification, the Monod equation is as follows:

$$\mu N = \mu_{max} N / N + K_N$$

Where $N = NH_4 - N$ or $NO_2 - N$ concentration , mg /l

K_N = Half saturation constant, mg /l

μN = Specific growth rate of *Nitrosomonas* or *Nitrobacter,* d^{-1}

μ_{max} = Maximum specific growth rate of *Nitrosomonas* or *Nitrobacter,* d^{-1}

The rate of ammonium oxidation is thus controlled by the growth rate of the bacteria involved with the *Nitrobacter* spp. characterised by higher growth rates (μ= 1.02 d^{-1} at 25 °C) than that of the *Nitrosomonas* spp. (μ = 1.1 d^{-1} at 25 °C) (Rittman and McCarty, 2001). However, the growth rate of these organisms is highly dependent on environmental parameters (EPA, 1993).

In biofilm systems the oxidation of ammonium is independent of the growth rate of the organisms involved. However, in these systems the oxidation of ammonia is highly dependant on mass transport (diffusion) across the biofilm. Diffusional limitations have been reported to occur in biofilm systems, especially in biofilms that are too thick and where microorganisms at the surface of the biofilm have been reported to differ from those found in the inner biofilm layer. de Beer *et al.* (1993), reported the oxygen penetration depth into biofilms to be within the range 100 - 200 μm.

Enzymes involved in autotrophic nitrification

The conversion of ammonium to nitrite is a two-step process catalysed by a labile membrane-bound enzyme, ammonium mono-oxygenase (AMO) and a periplasmic enzyme, hydroxylamine oxidoreductase (HAO) (Bothe *et al.,* 2000). The hydroxylamine oxidoreductase enzyme, HAO has been purified and characterized. The HAO is a periplasmic enzyme with 7 c type haemes and one P_{450} haeme, and it is composed of three sub-units of 62 kDa each (Jetten *et al.,* 1997). In contrast to HAO, the ammonium mono-oxygenase enzyme has not previously been purified (Juliette *et al.,* 1993b). This enzyme had only been purified from *Paracoccus denitrificans* as an active enzyme (Moir *et al.,* 1996). However, the amino acid sequence of the AMO, based on DNA analysis has been predicted (MacCarty, 1999). The spectral characteristics of the photoinactivation of the AMO have been found to be similar to that of the photoinactivation of tyrosinase, which is a well-characterised copper-based mono-oxygenase enzyme. It has therefore been suggested that the AMO enzyme has a cupric reactive site due to the binding of some agents that block NH_4^+ oxidation in *N. europaea* (Bedard and Knowels, 1989; McCarty, 1999).

Ammonium mono-oxygenase is also involved in the oxidation of a wide range of hydrocarbons and halogenated hydrocarbons (Rotthauwe *et al.,* 1995). AMO has been reported to be homologous to the methane monooxygenase (pMMO) enzyme (McCarty, 1999). Non-ionized ammonium

(NH_3) has been reported to be the preferred substrate for AMO rather than ionized ammonium (NH_4^+) (Berdard and Knowels, 1989; Hooper *et al.*, 1997; McCarty, 1999).

The catalytic reaction for the conversion of ammonia to hydroxylamine by AMO is expressed as:

$$NH_4^+ + O_2 + 2H^+ + 2e^- \xrightarrow{AMO} NH_2OH + H_2O$$

Further oxidation of hydroxylamine to nitrite by hydroxylamine oxidoreductase is described by Juliette *et al.* (1993a) and Hyman *et al.* (1995) as:

$$NH_2OH + H_2O \xrightarrow{HAO} NO_2^- + 5H^- + 4e^-$$

The above reaction is important for the generation of the reducing power required by ammonium monooxygenase. During ammonium oxidation, the electrons generated from the oxidation of the hydroxylamine are the only source of reductant for the AMO enzyme (Juliette *et al.*, 1993a). These electrons are transferred by HAO enzyme to the cytochrome–P_{554}. Two hydroxlamine-derived electrons are carried via unknown carriers to the membrane-bound AMO (Hooper *et al.*, 1997). The remaining electrons are passed through cytochrome aa_3 and then to the conventional electron transport chain (Fig. 2) (Brock *et al.*, 1997). The remaining electrons are believed to be utilized for the generation of ATP and NAD(P)H.

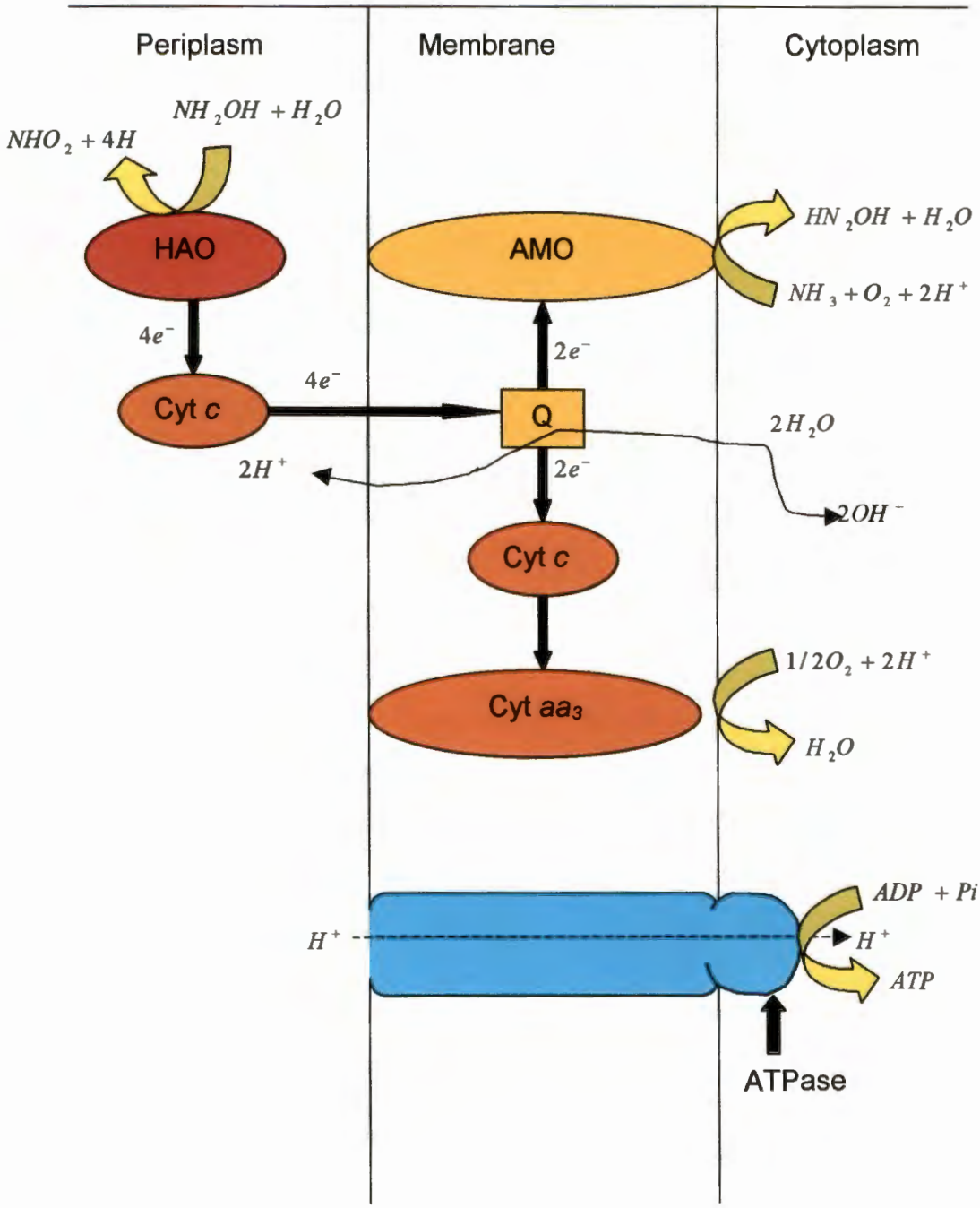

Figure 2. Electron flow during the oxidation of ammonia by autotrophic ammonium oxidisers. Where: AMO, ammonium monooxygenase; HAO, hydroxylamine oxidoreductase; Cyt *C*, cytochrome C; Cyt *aa3*, cytochrome aa3; Q, quinone (adapted from Brock *et al.*, 1997).

The oxidation of nitrite to nitrate is carried out by nitrite oxidoreductase (NO) (Jetten *et al.*, 1997). The NO is an integrated membrane protein that has previously been purified, and characterised. This enzyme has a

molecular weight of 360 kDa and consists of two protein sub-units, namely α and β, with molecular weights of 115 kDa and 64 kDa, respectively (Bock *et al.*, 1986). No intermediates have been detected during the oxidation of nitrite to nitrate by this enzyme (Wood, 1986). The electrons travel along a very short transport chain to the terminal oxidase (Fig. 3) (Brock *et al.*, 1997). However, very little studies have been undertaken on NO, since most of inhibition of nitrification by organic substrates is at the ammonium monooxygenase level (Juliete *et al.*, 1993a).

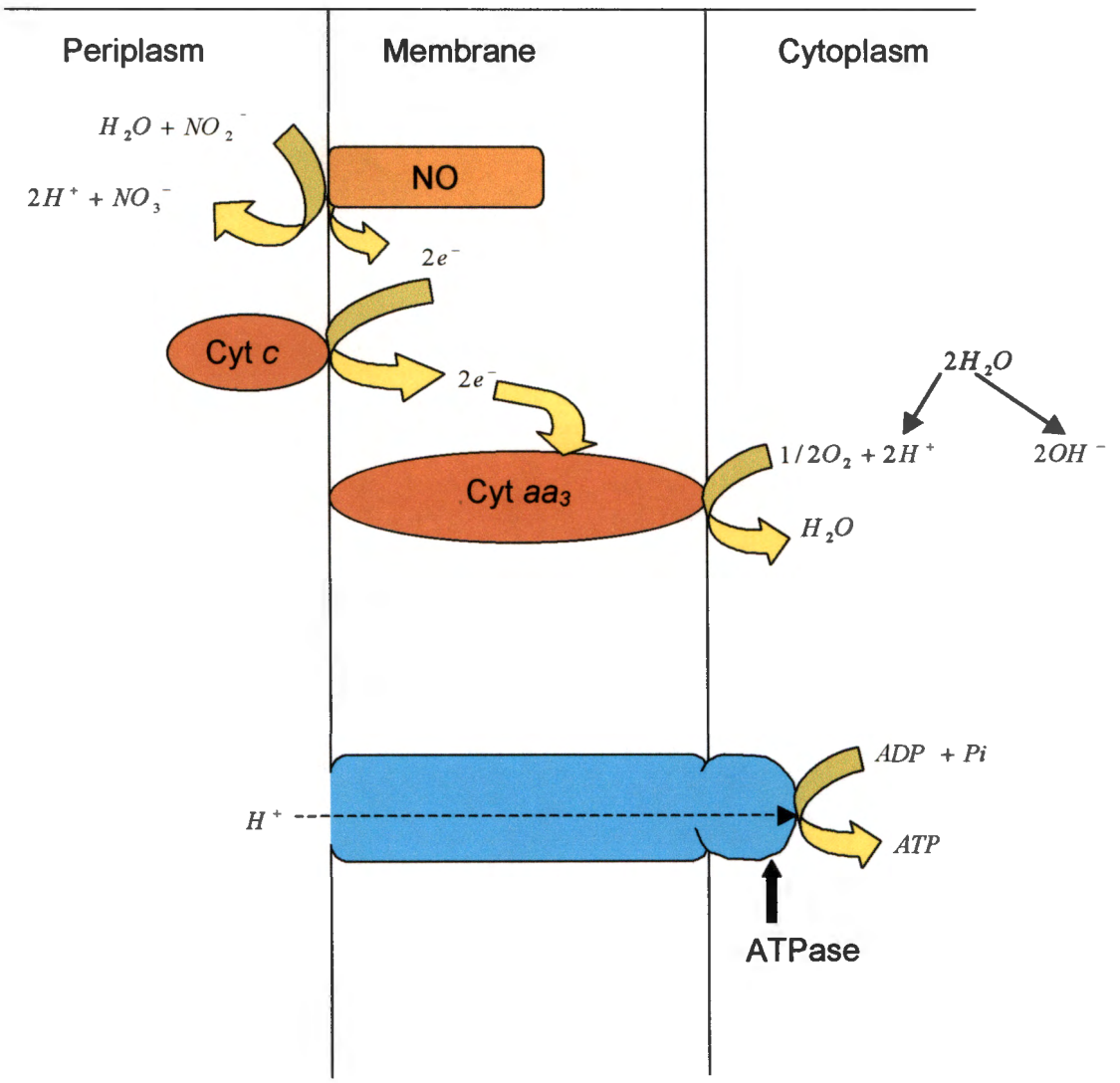

Figure 3. The oxidation of nitrite to nitrate. Where: NO, nitrite oxidoreductase; Cyt *c*, cytochrome c; Cyt *aa3*, cytochrome aa3 (adapted from Brock *et al.*, 1997).

Parameters affecting nitrification

Nitrifying bacteria are very sensitive to different environmental parameters (Lazarova *et al.,* 1998) hence they have slow growth rates (μ = 1.02 d^{-1}, ammonia oxidisers; and 1.1 d^{-1}, nitrite oxidisers at 25 °C) and low biomass yields (0.21 gVSS/gN) (Rittman and McCarty, 2001). These organisms have been reported to be very susceptible to a wide range of inhibitors hence the nitrification rate is dependent on a number of physical and chemical factors (van Loosdrechdt and Jetten, 1998). Environmental parameters that have been reported to affect nitrification include: dissolved oxygen concentration (DO), pH, alkalinity, temperature, light intensity, substrate availability, population density of nitrifying bacteria, organic compounds, inorganic compounds, and metal compounds (EPA, 1993; Lazarova *et al.,* 1998; Phillips *et al.,* 1999).

The effect of dissolved oxygen concentration on autotrophic nitrification

Autotrophic nitrification is an aerobic process which is highly dependant on oxygen (Hargreaves, 1998). Aeration is, however, one of the most costly items in the operation of wastewater treatment plants. Knowledge of the effect of oxygen on the nitrification rate and the nitrifying population is therefore an important economic factor (Prinćić *et al.,* 1998). Nitrification proceeds optimally at dissolved oxygen concentrations (DO) above 2 mg/l since oxygen is not limiting at these levels. Below this concentration, however, nitrification is inhibited, which results in nitrite accumulation or nitrous and nitric oxide production (Nogueira *et al.,* 1998; Green *et al.,* 2001). There have, however, been reports that nitrification can occur at dissolved oxygen concentrations as low as 1.5 mg/l (EPA, 1993). However, the lower the O_2 concentration the higher the degree of nitrite accumulation (Hargreaves, 1998).

The effects of pH and alkalinity on autotrophic nitrification

pH has been reported to be one of the factors that plays a significant role in the regulation of the nitrification rates. At lower pH values, the rate of ammonium oxidation has been reported to decline significantly (Marais and Ekama, 1984). A change in pH may inhibit the nitrite oxidizers, since it may result in nitric acid and/or ammonia (NH_3) accumulation. Ammonia and nitric acid are known to inhibit nitrification (Table 2) (EPA, 1993). A small change in pH will have a rather strong effect on the concentration of these compounds. This inhibitory effect is, however, reversible since the inhibition of nitrite oxidisers is not a long-term effect. This is primarily due to the capability of these organisms to adapt to adverse changes in the environment (van Loosdretch and Jetten, 1998). This adaptation is apparently brought about by a shift in the microbial community structure, which occurs when the pH is increased or decreased. The shift in microbial community structure results in populations that can tolerate high ammonia (NH_3) concentrations. These reports suggest that the resulting populations would be of importance in the treatment of high strength ammonium wastes, an important practical consideration for nitrogenous wastewater treatment (Prinčič *et al.,* 1998)

For optimum growth of nitrifying bacteria a slightly alkaline pH (7 to 8.5) is required (Marais and Ekama, 1984). Pure cultures of ammonia oxidizers require a pH range of 5.8 to 8.5 whilst pure cultures of nitrite oxidizers require a pH range of 6.8 to 8.5. Inhibition of these reactions has been observed at pH values below 5.8. In natural environments such as soil, nitrification has, however, been reported in acidic environments with a pH below 4.0 (Prinčič *et al.,* 1998). pH has also been reported to play an important role in nitrification due to the toxicity of free ammonia to *Nitrobacter* spp. at alkaline pH values (> 8.5). Non-oxidized ammonia (NH_3) can inhibit oxidation of 0.1 mg/l ammonium as nitrogen (EPA, 1993). *Nitrobacter* are more sensitive to changes in pH than the *Nitrosomonas* bacteria (van Loosdretch and Jetten, 1998).

Table 2. Calculated threshold values where 50 % inhibition of nitrification may occur by NH_3, NH_3 + NH_4^+-N, HNO_2 and HNO_2 + HNO_3-N (pH 7.0, temperature 20°C) (EPA, 1993)

Compound	Concentration where 50% inhibition can occur (mg/l) (Organism/process)
NH_3	10 (*Nitrosomonas)* 0.1 (*Nitrobacter)*
NH_3 + NH_4-N,	1.0 (*Nitrosomonas)* 20 (*Nitrobacter)*
HNO_2	0.22 (Nitrification)
HNO_2 + HNO_3-N	280 (Nitrification)

NH_3, Non-ionised Ammonia; NH_4-N, Ammonium nitrogen; HNO_2, nitric acid; HNO_3-N, nitrous acid;.

The nitrification process results in the release of hydrogen ions which contribute significantly to the decrease in the alkalinity of wastewaters (Marais and Ekama, 1984). Stoichiometrically, for every 1 mg NH_4-N converted to nitrite or nitrate, 7.14 mg of alkalinity (expressed as $CaCO_3$) is utilised. If the alkalinity of wastewater drops below 40 mg/l, pH control has been reported to become unstable, resulting in a significant decrease in nitrification efficiency due to the retarded growth rate of the autotrophic nitrifiers (WPCF, 1991).

The effects of temperature on nitrifiers

Temperature has also been reported to exert a tremendous effect on the growth of nitrifying bacteria and subsequently nitrification rates (Tchonobaglous, 1991) since the specific growth rate of nitrifiers is temperature dependent (Fig. 4) (Marais and Ekama, 1984). Autotrophic nitrification occurs over a temperature range of 4 - 45 °C with 35°C being the

optimum for *Nitrosomonas* spp. and 35 - 42°C the optimum for *Nitrobacter* spp. (EPA, 1993).

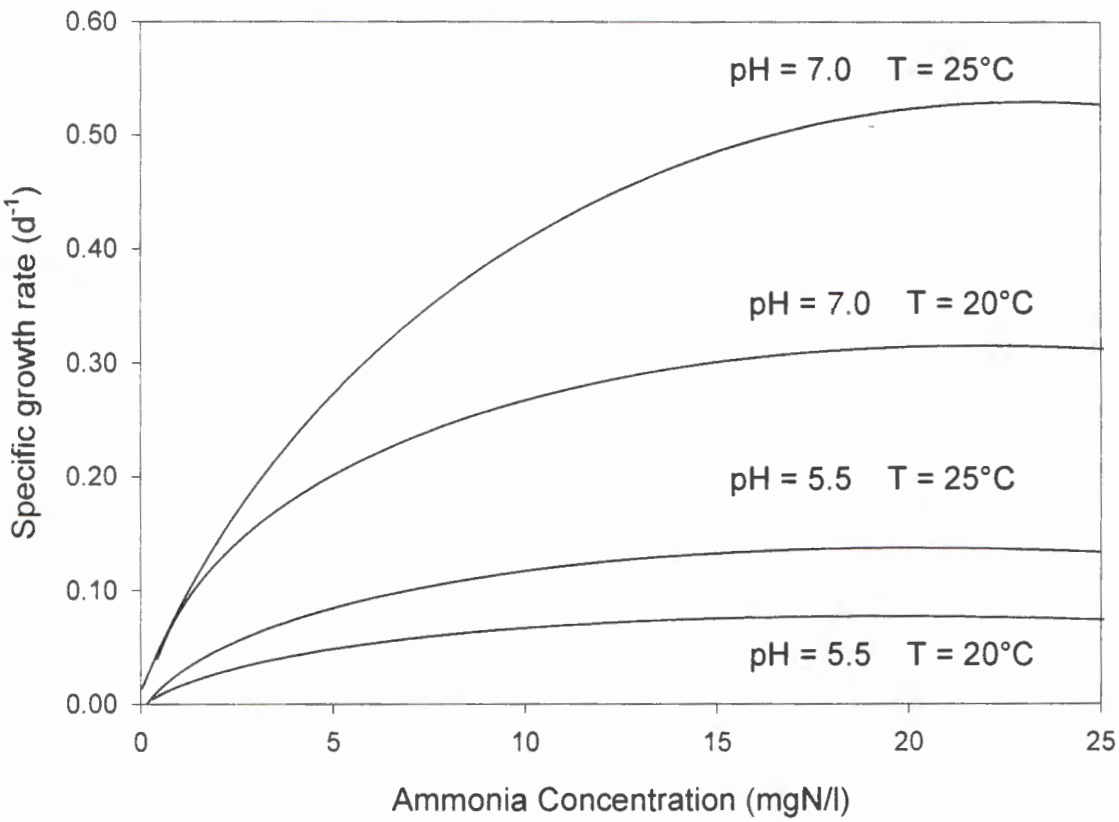

Figure 4. The relationship between specific growth rate of *Nitrosomonas* and changes in temperature and mixed liquor pH (Marais and Ekama, 1984).

An increase in temperature may have a significant impact on the pH, since temperature, pH and specific growth rate have an important inter-relationship (Marais and Ekama, 1984).

The effect of organic, inorganic, and metallic compounds on autotrophic nitrification

Different types of compounds have been reported to inhibit nitrification. These compounds include organic, inorganic and metallic compounds (EPA, 1993). The susceptibility of ammonia oxidation to a wide range of inhibitors is attributed to the inhibition of the AMO-mediated ammonia oxidation process (Berdard and Knowels, 1989). The inhibition of ammonia oxidation is suggested to be either through direct effects on the AMO enzyme, indirect effects on the HAO enzyme, or other components which are involved in supplying electrons to the AMO enzyme (Juliette *et al.*, 1993a). Inhibitors may interfere with general metabolism or the primary oxidative reaction (Tchonobaglous, 1991). Inhibition of the AMO enzyme activity by several compounds that can be oxidized by the AMO enzyme as alternative substrates instead of ammonia (Table 3) (Juliette *et al.*, 1993b) and inhibition by metallic compounds has also been reported (Table 4) (EPA, 1993). Inhibitors may not act immediately and not all species and strains of nitrifiers are similarly affected by a given compound and its concentration.

Different categories of nitrification inhibitors are known (Hooper and Terry, 1973), and include:

(i) Metal-binding compounds e.g. KCN, Na_2S;

(ii) Enzyme and haem protein-binding compounds e.g. dinitrophenol, CO;

(iii) Uncouplers of oxidative phosphorylation and inhibitors of electron transport e.g. methylene blue, 2,4 di-nitrophenol;

(iv) Short-chain alcohols and amines reacting with peroxide-metabolizing enzymes or acting as free radical trapping agents e.g. ethanol, methanol; and

(iv) Miscellaneous factors acting as free radical trapping agents e.g. light, N_2O.

Table 3. Known substrates for ammonium mono-oxygenase

Substrate	Major product(s)	Reference[a]
Methyl sulfide	Methyl sulfoxide	1
Ethyl sulfide	Ethyl sulfoxide	1
Methylphenylsulfide	Methylphenyl sulphoxide	1
Allylmethyl sulfide	Allylmethyl sulfoxide	1
Allyl sulphide	Allylsulfoxide	1
Benzene	Phenol	2
Toluene	Benzyl alcohol and benzaldahyde	2
Ethyl benzene	Phenethyl alcohol	2
ρ-xylene	4-Methylbenzyl alcohol	2
Chlorobenzene	4-chlorophenol	2
Aniline	3-Nitrobenzene	2
Nitrobenzene	3-Nitrophenol	2

[a] 1, Juliette *et al.*, (1993b); 2, Keener and Arp, (1994).

Table 4. Metals and inorganic compounds identified as potential nitrification inhibitors (EPA, 1993)

Metal/Inorganic Compound
Free cyanide
Zinc
Perchlorate
Copper
Chromium Nickel
Thiocyanide
Silver
Sodium cyanate
Cobalt
Sodium azide
Cadmium
Sodium cyanide
Lead
Potassium chromate

The inhibitory mechanisms of these compounds are diverse. Thus inhibitors of nitrification that can act on the AMO enzyme typically function via one of the following mechanisms:

(i) Reversible inhibition – during this type of inhibition, the catalytic cycle is disrupted by the compound and the nitrifying organisms are capable of recovering from inhibition. 80% inhibition by allylthiourea was observed at 1 μM. This compound selectively inhibits NH_4^+ oxidation by chelating the copper in the NH_4^+ monooxygenase active site (Berdard and Knowels, 1989). Azide (24 μM) has also been reported as a selective instantaneous and effective inhibitor of NH_4^+ and NO_2^- oxidation. The bacteriostatic effect of azide on Gram negative bacteria is well known, and this compound is also likely to inhibit NH_4^+ oxidizers. It is a selective inhibitor of nitrification. However, after the removal of the affected biomass, nitrification has been observed to continue (Ginestet *et al.,* 1998);

(ii) Competitive inhibition - the inhibitory compounds act as alternative substrates (McCarty, 1999). For example, alternative substrates for AMO such as methane, ethylene and halogenated hydrocarbons inhibit NH_4^+ oxidation by competition for the active site. A low concentration of phenol may reversibly and competitively inhibit the AMO enzyme activity because it is oxidized as an alternative substrate in the place of ammonia, resulting in hydroquinone formation (Hyman *et al.,* 1985). Moreover, the resulting oxidized compounds behave as protein modifying agents that irreversibly inactivate not only the AMO enzyme but also other proteins in the cell. At low concentrations (50μM), phenol acts as a competitive inhibitor. Phenol is known to be a toxic compound that denatures proteins and membranes, inhibiting certain cellular pathways (Berdard and knowels, 1989; Hooper *et al.,* 1997);

(iii) Mechanism-based inhibition – this inhibition is carried out by inhibitors of nitrification as a result of the normal catalytic cycle of the enzyme, producing inhibitory products from the substrate (MacCarty, 1999). Sulfur containing degradation products which are known to be inhibitors of the nitrification include carbon disulfide, dimethylsulfide, and alkyl thiols (Juliette *et al.,* 1993a). The AMO inactivator, acetylene, irreversibly inactivates the

AMO enzyme. Although Ginestet *et al.* (1998), reported allysulfide to be a reversible inhibitor of ammonium oxidation, it has been shown that this compound is a strong inhibitor of NH_4^+ oxidation in *N. europaea* and it may act as an irreversible mechanism-based inactivator of the AMO enzyme (Juliette *et al.,* 1993b). Allylsulfide and tetra-chloroethane (TCE), in some cases uses proteins to become covalently bound to the product of catalysis (McCarty, 1999). Other non-specific inhibitors, including, heavy metals such as $HgCl_2$, act as irreversible ammonium oxidation inhibitors; and

(iv) Exposure of cells to visible light has also been reported to result in the inactivation of the AMO enzyme. The exact mechanism of nitrification inhibition by visible light is unknown, however, the affected cell can recover rapidly in the dark and in the presence of energy-producing substrate (Alleman and Pretson, 1991)

Numerous molecular approaches have been used in an attempt to detect the inhibition of NH_4^+. Recombinant strains were constructed by introducing *lux AB* genes derived from a bioluminescence bacteria *Vibrio harveyi* into *N. europaea*. Under normal conditions this recombinant strain produced bioluminescence. However, in the presence of inhibitors, the light emission was reported to decrease. This phenomenon was suggested to be due to the inhibition of AMO as well as the destruction of other cellular metabolic pathways (Iizumi *et al.,* 1998).

Not all bacteria are totally inhibited, thus recovery from inhibition, known as reversible inhibition, has been reported by many researchers (Berdard and Knowels, 1989; Juliette *et al.,* 1993b; Hooper *et al.,* 1997; Ginestet *et al.,* 1998; McCarty, 1999). Depending on the type of inhibitory compound, recovery from inhibition can either be fast or slow (Juliette *et al.,* 1993b). The difference in the rate of recovery has been suggested to be due to the amount of proteins required to re-establish the ammonium-oxidizing activity. Hence, cells with the greatest loss of ammonium oxidizing activity exhibit the lowest rate of recovery (Hyman *et al.,* 1995). In some severely inhibited organisms, the re-establishment of AMO activity might require *de novo* synthesis of the protein (MacCarty, 1999)

Anaerobic ammonium oxidation

Recently, Mulder *et al.* (1995), discovered the oxidation of ammonium in fluidised-bed reactors under anaerobic conditions. This biological process was termed anaerobic ammonium oxidation (Anammox). During this process ammonium removal was associated with an increase in the disappearance of nitrate and the main end-product of this process is dinitrogen gas (N_2). Further studies of the process revealed that the process was indeed biological (van der Graaf *et al.,* 1995). It has been suggested that the process is carried out according to the following reaction (Mulder *et al.,* 1995):

$$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+$$

The process is exergonic and the free energy (Δ G°) for this reaction = -1,483.5 kJ (van der Graaf *et al.,* 1995). The organisms involved in the process are irregularly shaped cells with an unusual morphology (Jetten *et al.,* 1997b). Later studies revealed that the organisms involved in the process included *Nitrosomonas eutropha* (Schmidt and Bock, 1998). The growth rate of these organisms has been reported to be very low, within the range 0.05 - 0.1 d^{-1} (van Loosdrecht and Jetten, 1998) and the yield has been reported to be approximately 0.14 gVSS/g NH_4-N (Rittman and McCarty, 2001). However, in an Anammox fluidised-bed reactor, the conversion capacity has been reported to reach 2.4 kgNH_4-N/m^3.day (van Loosdrecht and Jetten, 1998).

Strous *et al.* (1997), reported that the fixed-bed reactors and fluidised-bed reactors are suitable reactor configurations for the anammox process. It is anticipated that the Anammox process will play a very important role in the future for the treatment of wastewaters, since it reduces the need for aeration, which is one of the costly items in the operation of treatment plants (van Loosdrechdt and Jetten, 1998). Evidence for this has been provided by the incorporation of the anammox process into the SHARON process for the treatment of high strength ammonium effluents where up to 90% ammonium removal was achieved with reduced costs (Mulder and van Kempen, 1997).

Denitrification

Denitrification can be described as nitrate respiration resulting in the reduction of nitrate to dinitrogen gas (N_2) (Fig. 1) (du Plessis *et al.*, 1997). This process plays an important role in the treatment of wastewater for the removal of excess nitrate (Ye *et al.*, 1994). Denitrification is carried out by metabolically and biochemically diverse groups of facultative anaerobic bacteria, such as *Pseudomonas* spp, *Alcaligens* spp and *Bacillus* spp. capable of utilizing various substrates (Rittman and McCarty, 2001). The process occurs within the temperature range of 20 - 35 °C and within the pH range 6.5 - 8.0 (Šimek, 1998).

Denitrification occurs at a cellular level and it has been accepted that it is dependent upon three basic factors, which include the partial pressure of oxygen, the nitrate concentration, and the availability of organic carbon (Šimek, 1998). The organic carbon source acts as the electron donor (Ganaye *et al.*, 1996), whereas oxygen is the preferred terminal electron acceptor. However, when there is no or very low residual oxygen, nitrate can serve as the terminal electron acceptor (Brock *et al.*, 1997). Non-fermentable organic compounds such as ethanol, acetic acid and methanol are commonly used as the organic carbon sources (EPA, 1993; Constantin and Fick, 1997). The organic carbon source may originate from the wastewater that is treated or it may be an exogenous electron donor (Rittmann and McCarty, 2001). Selection of an appropriate carbon source is dependent upon the medium, the organisms, and the economics of the substrate. The use of ethanol as a carbon source during denitrification results in greater overall denitrification rates, whereas the use of acetic acid results in higher biomass concentrations. (Constantin and Fick, 1997).

Most studies on denitrification using acetic acid as the organic carbon source were done to remove relatively low initial nitrate concentrations (10 - 200 mg/l), with very few studies aimed at the treatment of high nitrate concentrations (Constantin and Fick, 1997). The use of low nitrate

concentrations is due to the inhibitory effect of nitrate on the denitrifying population (Glass and Silverstein, 1998). Although toxicity of nitrate and nitrite at high concentrations has been reported, denitrification of high concentrations of nitrates (2000 mg/l NO_3-N) has been achieved (Cuervo-López *et al.,* 1999). Unbalanced concentrations of nitrite and nitrate reductase may result in nitrite accumulation (Lemmer *et al.,* 1997) and shock loads with organic carbon sources have been reported to cause accumulation of nitrous oxide (Hasselbad and Hallin, 1998).

Hasselbad and Hallin (1998), reported a theoretical carbon source requirement calculated as the amount of COD and nitrogen being oxidized and denitrified in the process to be within the range of 3.5 - 4.5, with about 15% of removed carbon being used for biomass production (Sauthier *et al.,* 1998). Higher COD/N ratios may result in unwanted effects such as sulphide production (Lemmer *et al.,* 1997). In the studies on denitrification by Kuba *et al.* (1996), it was observed that when the COD/N ratio was below 3.4, nitrate was detected in the effluent. In contrast, in the studies by Ohashi *et al.* (1995), and Fass *et al.,* (1994) the C:N ratios were maintained at 1.5 and 1.14 respectively, and no nitrate was detected in the effluent.

Fass *et al.* (1994), reported the use of exogenous volatile fatty acids (VFA`s), propionate, butyrate and valeric acid as organic carbon sources during denitrification. These (VFA`s) were effectively utilized, with 99.9% VFA utilization being achieved. These findings correspond to reports that VFA`s are potential organic carbon sources in soil and water (Ganaye *et al.,* 1996), and the studies by Aboutboul *et al.* (1995), where the VFA`s acetate, propionate, and butyrate were used as organic carbon sources for the denitrification of fish culture effluents. The bacterial strains isolated during their study included *Ochrobactrum anthropi, Moraxella lacunata, Pseudomonas testosteroni, Paraccoccus denitrificans* and *Pasteurela* gr. EF4. Although utilization of VFA`s was achieved, there were indications that some of the substrates were preferred over others. However, *Comamonas testosteroni* consumed butyrate more rapidly than acetate or valerate. In

contrast, *Alcaligens denitrificans* preferred propionate and butyrate over the other volatile fatty acids (Ganaye *et al.*, 1996).

Unlike nitrification, denitrification is not affected by a wide range of organic and inorganic compounds. Table 5 lists some of the compounds that have been reported to be inhibitors of the denitrification process at varying concentrations.

Table 5. Inhibitors of denitrification (Knowels, 1982).

Compound	Concentration
Acetylene	10^{-3} atmospheres
Azide, cyanide, 2.4 - dinitrophenol	~10^{-4}M
Pesticides	
Vapam	20 ppm
Dalapon	10 ppm
Sulphur compounds	
SO_4^{2-}	100 - 500 μg S g^{-1}
S^{2-}	8 μgmol.g^{-1} to 40 mmol.g^{-1}

SO_4^2, Sulphate; S^{-2}, Sulphide.

Stoichiometry and enzymes involved in denitrification

Denitrification enzymes

Initially it was believed that denitrification occured via two steps, which were:

$$NO_3^- \rightarrow NO_2^- \rightarrow N_2$$

Later it was discovered (Zumft and Körner, 1997) that three steps were required for the process, which are:

$$NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$$

In fact, four enzymatic steps are required for the complete denitrification process (Ye *et al.*, 1994). More than 40 genes have been reported to be involved in the process (Van Loosdrecht and Jetten, 1998). The enzymes involved in denitrification are: (i) nitrate reductase (Nar), catalyzing the conversion of nitrate to nitrite; (ii) nitrite reductase (Nir) involved in the conversion of nitrite to nitric oxide; (iii) nitric oxide reductase (Nor) converting nitric oxide to nitrous oxide; and (iv) nitrous oxide reductase (Nos), which completes the process by converting nitrous oxide into dinitrogen gas. The overall catalytic reaction is as follows (du Plessis *et al.*, 1997):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Not all denitrifying organisms are capable of carrying out all these steps, and, when one or more enzymes are lacking, nitrate reduction does not result in N_2 gas production (Ganaye *et al.*, 1996).

(i) Nitrate reductase

Two types of nitrate reductase enzymes are known to exist within denitrifying microbial populations, namely (a) membrane-bound nitrate reductase and (b) the periplasmic nitrate reductase. The functional difference between the two enzymes is that the periplasmic nitrate reductase does not reduce chlorate and it is much less sensitive to inhibition by azide (Ferguson, 1994).

(a) Membrane-bound nitrate reductase

The most characterized membrane-bound nitrate reductase is that found in *Paracoccus denitrificans* (Craske and Ferguson, 1986) and *Thiosphaera pantotropha* (Berks *et al.,* 1994). The membrane-bound Nar has a $\alpha\beta\gamma$ structure with molecular weights of 127, 61, and 21 kDa, respectively. This enzyme has been reported to have structural similarities with the respiratory nitrate reductases of non-denitrifying *Escherichia coli* (Craske and Ferguson, 1986) and to be closely related to the nitrite oxidase of *Nitrobacter* (Berks *et al.,* 1994). The membrane-bound enzyme is anchored by the α sub-unit, which contains the molybdenum co-factor and two *b*-type haemes with different redox potentials (Ballard and Ferguson, 1988). The two *b*-type haemes, are thought to be involved in the transfer of electrons whereby they accept electrons from ubiquinol, passing them onto the iron-sulfur centers. From the iron-sulfur centers the electrons are further transferred to the molybdenum center at the active site located in the α sub-unit (Ferguson, 1994).

(b) Periplasmic nitrate reductase

The periplasmic nitrate reductase was discovered by Satoh (1981), in *Rodospirillium spoieroides* f. sp. *denitrificans*. In contrast to the membrane-bound nitrate reductase, the periplasmic nitrate reductase has been reported to be insensitive to azide and to lack the ability to reduce chlorate (Sears *et al.,* 1993). The enzyme consists of two sub-units of 94 and 19 kDa, respectively (Bell *et al.,* 1990). Detailed studies of the enzyme were done by Berks *et al.* (1994), where it was revealed that the periplasmic Nar enzyme

contains two *c*-type haemes with redox potentials in the range -150 mV and 80 mV. The main function of the enzyme is not known. However, it is assumed that it may play a role in the disposal of excess reducing equivalents (Ferguson, 1994).

(ii) Nitrite reductase.

Two different types of dissimilatory nitrite reductases are known. These are the copper nitrite reductase and *cd1* nitrite reductase. The copper nitrite reductase is a metalloprotein containing copper whereas the *cd1* nitrite reductase is a haem protein containing both the *c*-type and the *d*-type cytochromes (Ferguson, 1994). The copper nitrite reductase enzymes have been subdivided into two categories i.e. green and blue, based on their spectral properties (Moura and Moura, 2000). Reduction of nitric oxide takes place at the *d1* type haeme (Silvestrini *et al.*, 1990).

(iii) Nitric oxide reductase.

Nitric oxide reductase is the last enzyme of denitrification to be identified and it is the least characterised enzyme of denitrification (Jetten *et al.*, 1997). The enzyme was purified by Heiss *et al.* (1989). It was revealed that nitric oxide is a membrane-bound enzyme, which consists of two sub-units. The smaller sub-unit carries haem *c* with molecular weight of 17 kDa and the larger sub-unit carries a haem *b* with molecular weight of 38 kDa. In addition, the enzyme contains a non-haem iron in variable amounts. Little is known about the catalytic core or the mode of action of this enzyme (Stouthamer *et al.*, 1997).

(iv) Nitrous oxide reductase

Nitrous oxide reductase is a soluble periplasmic enzyme (Zumft and Korner, 1997). Until recently, several nitrous oxide reductases had been purified, but none had been crystallized (Jetten *et al.*, 1997). The first three-dimensional structure was only recently revealed by Prudêncio *et al.* (2000),

where it was revealed that the enzyme is dimeric with each monomer consisting of two domains in the amino acid sequence. The purified enzyme is very labile with respect to oxygen (Zumft and Körner, 1997). Inhibition of nitrous oxide reductase by oxygen results in the release of N_2O during nitrogen removal (Jetten *et al.*, 1997).

Stoichiometry

According to Tchobanoglous (1991), the energy reactions for denitrification when methanol is used as an exogenous organic carbon substrate are as follows:

The first energy reaction can be expressed as:

$$6NO_3^- + 2CH_3OH \rightarrow 6NO_2^- + 2CO_2 + 4H_2O$$

The second energy reaction can be expressed as:

$$6NO_2^- + 3CH_3OH \rightarrow 3N_2 + 3CO_2 + 3H_2O + 6OH^-$$

The overall reaction can be expressed as:

$$6NO_3^- + 5CH_3OH \rightarrow 5CO_2 + 3N_2 + 7H_2O + 6OH^-$$

When ethanol is used as the organic source, denitrification can be represented by the following equations:

$$6NO_3^- + C_2H_5OH \rightarrow 6NO_2^- + 3H_2O + 2CO_2$$

The second energy reaction can be expressed as:

$$4NO_2^- + C_2H_5OH + 4H_2O \rightarrow 2N_2 + 2CO_2 + 9H_2O$$

The overall reaction can therefore be expressed as:

$$12NO_3^- + 5C_2H_5OH \rightarrow 10CO_2 + 6N_2 + 9H_2O + 12OH^-$$

A simplified stoichiometrical equation when acetate is used as the carbon source shows the effect on acidity (Glass and Silverstein, 1998):

$$NO_3^- + 0.25CH_3COO^- \rightarrow 0.25CO_2 + 0.5NO_2^- + 0.5H_2O + 0.25HCO3^-$$

The second energy reaction can be expressed as:

$$NO_2^- + 0.375CH_3COO^- + 0.625H^- \rightarrow 0.75HCO_3^- + 0.5N_2 + 2CO_2 + .05H_2O$$

The overall reaction can be expressed as:

$$6NO_3^- + 0.625CH_3COO^- + 0.375H^+ \rightarrow 1.25HCO_3^- + 0.5N_2 + 0.5H_2O$$

A net 0.375 equivalents of acidity are consumed during denitrification (EPA, 1993).

Other types of denitrification

As with nitrification, different types of denitrification processes have been reported by various researchers in addition to heterotrophic denitrification. These include aerobic denitrification, denitrification by autotrophic nitrifying bacteria and autotrophic denitrification (van Loosdrechdt, and Jetten, 1998).

Aerobic denitrification

There have been conflicting reports about the need for oxygen for the denitrification process, since the facultative aerobes prefer to use dissolved oxygen at very low concentrations (Kornaros and Lyberatos, 1997). Most studies on the possibility of aerobic denitrification were conducted using *Paracoccus denitrificans* DSM 2944. Robertson *et al.* (1995), reported high denitrification rates under aerobic conditions by *Thiosphaera pantotropha* even in the presence of 80% air saturation. However, some of the researchers failed to detect high rates of denitrification under aerobic conditions (Arts *et al.*, 1995). Other experimental data also did not confirm these high denitrification rates. This confusion about aerobic denitrification was resolved by the observation of denitrification occuring under aerobic conditions, although it occurred at significantly lower rates (Kornaros and Lyberatos, 1997).

Denitrification by autotrophic nitrifiers

Nitrosomonas europaea and *Nitrosomonas eutropha* are known to be autotrophic nitrifiers. However, studies by Zart and Bock (1998) revealed that these organisms are capable of producing the end product of denitrification, N_2, under anoxic conditions using molecular hydrogen as the electron acceptor. Enhanced denitrification was achieved in the presence of NO_2 and NO. Although autotrophic nitrifiers are capable of producing NO, studies have revealed that the amount produced by these organisms is much lower than NO produced by the denitrifier *Alcaligenes faecalis* (Anderson *et al.*, 1993).

Autotrophic denitrification

In this process, autotrophic denitrifiers use inorganic carbon compounds as substrates for energy source (Zhang and Lampe, 1999). In the sulphur-based autotrophic denitrification process, the need for an external organic source is eliminated since sulphur is used as the electron donor by

autotrophic denitrifiers such as *Thiobacillus denitrificans* and *Thiomicrospira denitrificans* to reduce nitrates to nitrogen gas. During the process elemental sulphur (S°) is oxidised to sulphate while nitrate is reduced to N_2 (Koening and Liu, 1996). This process can be explained according to the following equation (Zhang and Lampe, 1999):

$$55S^{\circ} + 20CO_2 + 50NO_3^- + 38H_2O + 4NH_4^+ \rightarrow 4C_5H_7O_2N + 25N_2 + 55SO_4^{2-} + 64H^+$$

In comparison to anaerobic conditions, higher removal efficiencies were achieved under aerobic conditions (Zhang and Lampe, 1999). The process is highly economical as it results in reduced sludge production and does not require an additional carbon source. It is anticipated that sulfur-based autotrophic denitrification will play a very important role in the bioremediation of nitrate-contaminated surface waters (Flere and Zhang, 1998) as well as in the treatment of nitrate-containing leachates where the C/N ratios are very low (Koening and Liu, 1996).

During biological nitrification/denitrification, nitrate generated during the nitrification process is reduced to N_2 gas during the denitrification process. Nitrification is therefore, the rate-limiting step in the nitrification/denitrification processes. However, due to the slow growth rates, low biomass yields and the environmental parameters affecting the nitrification process (van Loosdrecht and Jetten, 1998), different nitrification/denitrification process technologies have been designed and applied in an effort to achieve complete nitrification/denitrification of nitrogen-containing wastewaters (Tchobanoglous, 1991). In addition, nitrification/denitrification technologies were developed due to the failure of natural nitrification/denitrification systems to remove nitrogen from nitrogen-containing wastewaters (EPA, 1993).

Nitrification/denitrification process technologies for the treatment of nitrogenous effluents

Various biological nitrification/denitrification technologies are currently being used for the treatment of nitrogen containing wastewaters. The biological nitrification/denitrification reactors used for the treatment of wastewaster are divided into two major categories according to the nature of the bacterial growth associated with them (Lazarova and Manem, 1994). These major categories are the suspended culture systems, in which the flocculated bacteria are grown in suspension and the attached or fixed-film culture systems in which the growth of bacteria occurs on the surfaces of solid media (Cooper, 1981; Cooper and Williams, 1990).

Suspended culture systems

The suspended culture systems consist of the bacterial biomass suspended in the medium (Cooper, 1981). A typical example of a suspended culture growth system is the activated sludge system. The conventional activated sludge system consists of three main zones, which are: the primary clarifier; the aeration tank; and the settling tank (EPA, 1993). The major advantage of this process is that it has been widely applied, well-designed and vigorously and extensively tested (Lazarova and Manem, 1994). However, numerous disadvantages associated with the activated sludge process are still being experienced although they have been applied and operated for many years for the removal of nitrogen from the wastewaters. These disadvantages include their operation at low specific organic loading rates due to the slow growth rate of the nitrifiers, resulting in increased reactor volumes and making these systems uncompetitive where the space requirement is limiting (Csikor *et al.*, 1996). In addition, activated sludge systems has been reported to have limited capacity and efficiency (Lazarova and Manem, 1994). Due to these disadvantages, the conventional activated sludge process has been changed and modified. Modifications of the activated sludge process are mainly based on the physical configuration, oxygen distribution and the organic loading rate. These modifications resulted

in widely used processes such as the Bardenpho process, the sequencing batch reactors (SBR) and the attached growth systems (Rittmann and McCarty, 2001).

The Bardenpho process

The Bardenpho process is also used for phosphorus removal (EPA, 1993) and it consists of different reaction zones which are: the anoxic denitrification zone; the aerobic combined oxidation nitrification zone; the anoxic denitrification zone; the aerobic zone; and the settling tank (Marais and Ekama, 1984; Tchobanoglous, 1991). One of the advantages of the process is that up to 90% total nitrogen removal can be achieved. However, one of the main disadvantages of the process include the requirements of many reaction tanks and long hydraulic retention times (Rittmann and McCarty, 2001).

The sequencing batch reactors

The sequencing batch reactors (SBR) are also known as the draw-and-fill reactors (Tchobanoglous, 1991). During nitrogen removal the following reaction sequences are used: the anoxic fill stage (mixed); the aerobic stage (aerated); the anoxic stage (mixed); the aerobic stage (aerated); and the settling stage (Rittmann and McCarty, 2001). One of the advantages of this process is that there is no need for a return sludge, since in some plants the processes are carried out in a single tank (Tchobanoglous, 1991). However, numerous modified SBR systems and continuous flow SBR systems are currently used. The main disadvantage of the SBR`s is the requirement for long hydraulic retention times (Rittmann and McCarty, 2001).

Attached growth systems

Attached growth systems consist of an inert biocarrier on which high concentrations of selected chemical-degrading bacteria are grown (Edwards *et al.*, 1994). These biological reactors with fixed biomass have been used

extensively in wastewater treatment for the removal of organic matter as well as nitrification and denitrification (Boller *et al.,* 1994). It has been recognised that the immobilisation of micro-organisms onto an inert support matrix is a promising alternative to suspended culture systems, solving the problems frequently associated with that type of wastewater treatment. This is especially relevant when the bacteria involved in the process are slow growers such as the nitrifiers (van Loosdredchdt *et al.,* 2000). Biofilm reactors have therefore been used primarily for nitrogen removal because they allow a sufficiently long biomass retention times to achieve complete nitrification (Okabe *et al.,* 1996).

However, in biofilm nitrification, there is significant competition between nitrifying bacteria and the heterotrophic bacteria for space and oxygen. In contrast to the heterotrophic bacteria that tend to grow on the outer surface of the biofilm, the nitriying bacteria tend to grow closer to the support matrix, whereby they are protected from detachment and predation. However, this protection mechanism increases their susceptilbility to oxygen limitation (Rittmann and McCarty, 2001). In addition, the oxygen penetration depth within biofims has been reported to be within the range 100 μm and 200 μm (de Beer *et al.,* 1993). Therefore, the control of biofilm thickness is an important phenomenon during nitrification using attached growth systems, since nitrification is inhibited below dissolved oxygen concentrations (DO) of 2 mg/l (Green *et al.,* 2001)

Different types of biofilm reactors are feasible, including trickling filters, rotating biological contactors, biofilters, fluidised-bed reactors (Boller *et al.,* 1994), moving bed reactors (Ødegaard *et al.,* 1994; Rusten *et al.,* 2000), and biofilm airlift suspension reactors (van Loosrechdt *et al.,* 2000). However, for the purpose of this literature review, biofilm airlift suspension reactors and the fluidised-bed reactors will be discussed with the major emphasis on the biological fluidised-bed reactors.

Biofilm airlift suspension reactors

Biofilm airlift suspension (BAS) reactors are reactors consisting of two concentric cylinders, with the outer cylinder closed at the bottom and a gap between the inner cylinder and the bottom. The carrier particles are kept in suspension using air that is sparged in the cylinder creating an internal circulation of flow (van Benthum, 1998). In these reactors, an airlift pump is used to circulate carrier particles between the oxic and the anoxic compartments (Dutch patent, 1992 cited by Frijters *et al.,* 2000). Basalt with the diameter of approximately 0.3 mm is normally used as the carrier material in these reactors (Frijters *et al.,* 2000), resulting in total surface areas of approximately 3000 m^2/m^3 (Heijnen *et al.,* 1990).

Advantages of BAS reactors include their small footprint and the ability of the reactors to accumulate high biomass concentrations. In studies by van Benthum (1998), ammonium conversion rates as high as 6 kg NH_4-N/m^3.d, could be achieved. During operation, the reactors develop a homogenous biofilm, making it easier for biofilm thickness to be measured. This also makes modelling of the biofilm within the reactors possible. These reactors have a wide range of commercial applications and they are commercially applied under the trade name CIRCOX® (Frijters, *et al.,* 1997; Nicollela *et al.,* 2000; van Loosdredcht *et al.,* 2000).

Biological fluidised bed reactors (BFBR`s)

The development of biological fluidised-bed reactors (BFBR`s) for the treatment of biological wastewater can be traced back to the 1940`s, although the first commercial scale reactors were only commissioned in the mid 1980`s (Sutton and Mishra, 1994). The first treatment of industrial wastewater with BFBR`s occurred in the 1970`s (Sadick *et al.,* 1996). The wider application of this technology has, however, been hampered by factors such as problems

encountered with scale-up and the slow development of economically feasible systems (Sutton and Mishra, 1994).

Three types of fluidised-bed reactors are known. These include the two-phase biological fluidised-bed reactors, which contains a separate aeration system, the three-phase biological fluidised-bed reactors with all three phases of matter (gas, liquid and solid) in one reactor (Chang and Rittman, 1994), and the down-flow fluidised-bed reactors with the bed expanding downwards (Garcia-Calderon *et al.,* 1998b). The two-phase and the three-phase biological fluidised-bed reactors operate by fluidising the support matrix, consisting of solid particles, using the liquid or gas stream flowing in an opposite direction to the gravitational effect (Cooper, 1981). In contrast, with down-flow fluidised-bed reactors the liquid's specific gravity is higher than that of the support matrix consisting of the solid particles. In comparison to the up-flow fluidised-beds significantly less attention has, however, been given to the application of the down-flow fluidised-bed reactor (Garcia-Calderon *et al.,* 1998b).

In both the two-phase and the three-phase BFBR`s, flow is directed upwards from the bottom of the reactor at a velocity sufficient to fluidise or suspend the particle bed, such that the media particles are suspended (Sadick *et al.,* 1996). Once fluidised, the medium particles provide a very large surface area, in the order of 3000 - 4000 m^2/m^3 particle bed (Cooper, 1981). Within the reactor, the bed height can be controlled by adjusting the up-flow liquid velocity. The amount of biofilm within the reactor can also be controlled by wasting a fixed amount of solids. This can be achieved by subjecting biofilm-coated particles to high shear forces, with subsequent separation of the particles from the biomass, followed by the return of the clean particles into the reactor (Cooper, 1981; Bach *et al.,* 1998). Failure to do so may result in support matrix washout, due to the decreased particle density (Cooper, 1981).

In comparison to conventional wastewater treatment technologies such as activated sludge systems, aerobic and anaerobic BFBR`s are associated with a number of advantages enabling successfull application for wastewater

treatment (Nicolella *et al.*, 1996). One of the major advantages associated with the BFBR is its ability to accumulate high concentrations of active biomass, which results in intensified reaction rates (Garcia-Calderon *et al.*, 1998a). High concentrations of biomass within the reactor are achieved by using small particles as support medium (such as sand, anthracite, granular activated carbon, etc.) usually with diameters less than 1 mm (Hosaka *et al.*, 1991). In comparison to the activated sludge systems, the reactor footprint is reduced by approximately 80% (Cooper, 1981). Edwards *et al.* (1994), reported that the biomass concentration within their denitrifying BFBR was in the range 15000 - 40000 mg/l. This biomass concentration was significantly higher than the mixed liquor suspended solids (MLSS) associated with activated sludge processes, which can be in the range of 2000 - 4000 mg/l (EPA, 1993).

Other advantages of BFBR`s include the ability to operate at high organic loading rates and short hydraulic retention times, as well as high resistance of the attached microbial community to shock loadings (Garcia-Calderon *et al.*, 1998a). In studies by Sadick *et al.* (1996), 99% removal was achieved with a nitrate loading of 1.84 kg/m^3.d in their denitrifying reactor. In another study by Edwards *et al.* (1994), greater than 95% COD removal of mixed organic synthetic water was achieved using sand as well as GAC at loading rates of up to 9.6 kg COD/m^3.d. The BFBR also have the ability to treat high-strength and moderately high-strength wastewaters. This is supported by studies conducted by Horan *et al.* (1997), where BFBR`s were reported to be effective in the removal of high concentrations of ammonium (220 - 800 mg/l).

Biological fluidised-bed reactors have been employed for a wide range of applications in the industrial sector (Table 6). They are currently being used for aerobic and anaerobic treatment of industrial wastewaters, nitrification, denitrification (Lazarova and Manem, 1994) and for the treatment of petroleum-contaminated wastewaters (EPA, 1993).

Table 6. Large scale applications of the BFBR`s (Lazarova and Manem, 1994).

Type of BFBR	Country	Support Medium	Application	Volumetric loading rates $kg/m^3.d$	Biomass concentration $kg\ ss/m^3$
2- phase	USA, GB	sand/GAC	UW, IW, NF	0.6-1.3 (NH_4-N)	5.5-22.0
Aerobic	USA	sand	NF, NF	1(NH_4-N)	11.0-22.0
BFBR				1(NH_4-N)	2
3-phase	Japan	anthracite	NF		
	France	clay	UW, IW, TT	24 (COD)	2.0-3.0
	France	OSBG	UW, IW, TT	6.4-15.1 (COD)	0.1-0.5
Inverse	France	PE			
BFBR	USA	Plastic	UW, NF/D		
	Bulgaria	Ps	UW, D	12-35 (COD)	5.0-20.0

Abbreviations used: UW-urban water; IW-industrial water; TT-tertiary treatment; NF- nitrification, D-denitrification; PE;polyethylene OSBG-Optimised biological support of biological growth, Ps-polystyrene; GAC granular activated carbon; USA, United States of America; GB-Great Britain.

Biological nitrification/denitrification using BFBR`s

Biological nitrification/denitrification processes for the treatment of nitrogenous wastewaters and the problems experienced with the application of this innovative technology have been previously mentioned. It is evident that special environmental conditions are needed for maintenance and survival of nitrifying bacteria and that the nitrification process is inhibited by the availability of organic carbon. Although most studies have been directed at the nitrification of low concentrations of ammonium (50 mg/l) (Green *et al.*, 2001), nitrification of high strength ammonium wastewaters (800 mg/l) has also been reported (Horan *et al.*, 1997).

Most studies on denitrification using acetic acid as the organic carbon source have been directed at the treatment of relatively low initial nitrate concentrations (10 - 200 mg /l), whilst few have been utilised for the treatment of high nitrate concentrations (Constantin and Fick, 1997). The use of low nitrate concentrations is due to the inhibitory effect of nitrate on the denitrifying populations (Glass and Silverstein, 1998). However, denitrification of high concentrations of nitrates (2000 mg/l NH_4-N) has also been reported (Cuervo-López *et al.*, 1999). Furthermore, the availability of the carbon source at the correct C:N ratio enhances denitrification and the carbon source for denitrification can either be from the wastewater treated or from the exogenous addition of a suitable electron donor (Rittmann and McCarty, 2001).

On the basis of the above literature review, it is evident that biological fluidised-bed reactors have great potential to be used for the nitrification/denitrification of the nitrogenous condensate stream generated by SASOL Agri.

The aim of this study was, therefore, to evaluate the application of two-phase biological fluidised-bed reactors for the nitrification and denitrification of the high strength, highly saline nitrogenous SASOL Agri effluent. The SASOL Agri effluent contains approximately 1000 mg/l ammonium nitrate as N. This effluent, however, does not contain any organic compounds that are known to inhibit the nitrification process. Biological treatment of the industrial effluent using dual-stage two-phase BFBR`s (i.e. nitrification and denitrification physically separated) and with the reactors coupled in series, with the addition of an external carbon source for denitrification would be a suitable treatment option.

The objectives of the study are therefore:

- To optimise nitrification as well as denitrification of a synthetic high-strength, high saline nitrogenous effluent using two-phase BFBR`s;

- To evaluate the suitability of using sand and granular activated carbon (GAC) as support matrices for nitrification and denitrification;

- To determine the suitability of using sodium acetate as an electron donor for the heterotrophic denitrifying bacterial community;

- Based on the results obtained from these studies the following objectives will also be addressed:

  (i) Achievement of complete removal of the synthetic high-strength ammonium nitrate nitrogen effluent using-dual stage two-phase fluidised bed reactors operated in series for nitrification and denitrification; and

  (ii) Investigation of the influence of the presence of high concentrations of nitrates in the wastewater being treated on the biological nitrification process.

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Chapter 3

Optimisation of Nitrification using Biological Fluidised-Bed Reactors: Comparative Evaluation of Sand and Granular Activated Carbon as Support Matrices

(Submitted for publication in Water Research)

Summary

During this study, the suitability of two-phase biological fluidised-bed reactors (BFBR`s) for the treatment of a high-strength, saline nitrogenous synthetic effluent analogous to the SASOL Agri effluent (condensate stream containing approximately 1000 mg/l ammonium nitrate as nitrogen) was evaluated. The suitability of using sand and granular activated carbon (GAC) as support matrices for nitrification was also evaluated. During the start-up phase, the reactors were operated in batch mode for ten days. This was followed by the continuous operation of the reactors at varying hydraulic retention times from 5 days to 6 hours (NH_4-N loading rate of between 0.0015 kg NH_4-N/m^3.d and 4 kg NH_4-N/m^3.d). The ammonium-nitrogen (NH_4-N) concentration was also gradually increased from 10 mg/l to 1000 mg/l. At NH_4-N loading rates between 0.081 and 1 kg NH_4-N/m^3.d, removal efficiencies in excess of 99% were achieved in both reactors using sand and GAC as support matrices. However, at loading rates in excess of 1 kg NH_4-N/m^3.d, any further increase in the NH_4-N loading rate was limited by an associated decrease in the dissolved oxygen concentration (<1.5 mg/l). At a loading rate of 2 kg NH_4-N/m^3.d, the NH_4-N removal efficiency dropped to 58% and 48% in the BFBR using sand and GAC as support matrices, respectively. Upon further increase of the loading rate to 4 kg/m^3.d, the removal efficiency was observed to decrease to 28 % and 47% for the BFBR`s using sand and GAC as support matrices, respectively. Further increase in the NH_4-N loading rate was limited by the complete absence of dissolved oxygen in the system (<0.1 mg/l). Although both sand and GAC

performed well as support matrices, slightly enhanced NH_4-N removal efficiencies and more stable reactor performance was obtained using GAC as a support matrix.

Introduction

More than one half of the world`s major rivers are being seriously depleted and polluted. Only a small percentage (globally less than 10% of total waste, which includes agricultural run-off, industrial pollution and human waste) is treated before it enters the rivers (Hoffmann, 2000). In South Africa, a semi-arid country, with limited water resources (Global Water Partnership, 2000) the average rainfall received is only slightly more than half the world average (Mutume, 1998). In addition to the scarcity of water in South Africa, many water bodies are polluted and the water quality is declining due to salination and eutrophication. Salination results from increased industrialization, urbanisation and irrigation (O`Keeffe *et al.*, 1999), whereas eutrophication, results from the introduction of high concentrations of nitrogenous compounds and phosphorus in the receiving water bodies (EPA, 1993).

Nitrogen-containing effluents are not only known to deteriorate water quality but they also have detrimental ecological and human impacts. Ammonia nitrogen has been reported to be toxic to fish. Nitrites and nitrates are known to be carcinogenic and nitrites may lead to metheamoglobinemia if ingested by babies (EPA, 1993).

Due to increasing industrial discharge and the environmental impacts of nitrogen containing compounds, the general standards for the discharge of nutrients such as ammonia and nitrates into receiving bodies such as dams, rivers and lakes are becoming more stringent (Massonne *et al.*, 1996). According to the South African National Water Act (36/1998), the general limit for the discharge of ammonia (ionised and non-ionised) as nitrogen-containing effluent into a water resource is 3 mg/l and the special limit is 2 mg/l. In Europe, the general limit for the discharge of these nutrients is 1 mg/l (EPA, 1993). Effluent from different industries have to be returned and reused or

treated before discharged into receiving bodies, in order to balance water supply with its demand, (Tchobanoglous, 1991; Gupta and Sharma, 1996).

Conventional processes for wastewater treatment such as stabilization ponds have been widely used for nitrogen removal and also proven to be very economical. However, this process has not been well characterised, and therefore it has some drawbacks due to the unknown biological and chemical processes involved (Lai and Lam, 1997). Ion-dependant technologies such as ammonia stripping, non-ion dependent technologies and reverse osmosis are also frequently used for the removal of nitrogen from wastewaters. With the use of these processes, extensive nitrogen removal could be achieved, however, the processes were found to be inefficient, very costly, and resulted in significant brine production (Bach *et al.,* 1998).

Removal of nitrogen from wastewaters can also be achieved using biological processes (Köning and Riedel, 1998). The biological removal of nitrogen from wastewaters is a two-step process, involving the conversion of ammonia to nitrate during nitrification (Wagner *et al.,* 1995) followed by denitrification during which nitrate is converted to dinitrogen gas (N_2) (Campos *et al.,* 1999). In contrast to chemical processes, biological processes for the removal of nitrogen are widely used, reliable and have moderate costs (Tchobanoglous, 1991). The biological nitrification process is, however, characterised by a slow growth rate and a low yield of the bacterial genera involved. The biological nitrification process is also sensitive to the presence of different organic compounds. In order to achieve complete nitrification, high biomass concentrations must be available (Noto *et al.,* 1998).

Since microbial nitrification processes are limited by the slow growth rate of the nitrifying bacteria (van Loosdredcht and Jetten, 1998), biofilm reactors are extensively used for nitrogen removal since they allow a sufficiently long biomass retention times to achieve complete nitrification (Okabe *et al.,* 1996).

Biological fluidised bed reactors (BFBR`s) have been successfully used for the treatment of domestic and industrial wastewaters containing nitrogenous compounds (Sutton and Mishra, 1994). In comparison with conventional technologies such as activated sludge systems, aerobic and anaerobic BFBR`s are associated with a number of advantages (Garcia-Calderon *et al.,* 1998). These advantages include operation at high organic loading rates and short hydraulic retention times (Garcia-Calderon *et al.,* 1998). Small carrier particles (such as sand, anthracite, granular activated carbon, etc.), normally less than 1 mm in diameter, are used as support media (Hosaka *et al.,* 1991). As a result, high biomass concentrations can be accumulated in these reactors, resulting in small reactor volumes (Chang and Rittmann, 1994; Sadick *et al.,* 1996) and increased reaction rates (Garcia-Calderon *et al.,* 1998).

Depending on effluent characteristics, optimal choice of support matrices is critical in order to obtain maximum performance from the associated biofilm. Sand has been reported to offer better fluidisation properties than granular activated carbon (GAC). Granular activated carbon has, however, been reported to be associated with the facilitation of biofilm formation (Edwards *et al.,* 1994).

The aim of the study was, therefore, to evaluate the suitability of two-phase biological fluidised-bed reactors for the nitrification of a high strength, high saline nitrogenous synthetic industrial effluent. This synthetic effluent is analogous to the condensate stream containing ammonium nitrate concentrations of approximately 1000 mg/l as nitrogen as generated by SASOL Agri, Secunda, South Africa.

The specific objectives of this study were:

- To optimise the removal efficiencies of two-phase BFBR`s for the nitrification of a high strength, high saline nitrogenous synthetic SASOL Agri effluent.

- To compare and evaluate the suitability of sand and GAC as support matrices for nitrification at varying loading rates.

Materials and methods

Eperimental set-up. Two two-phase BFBR`s were operated in parallel. The main reactor columns were constructed using transparent polyvinyl chloride (PVC) piping (height 2 m, internal diameter 0.099 m). The total reactor volume was 22.23 l with an operational volume of 13.75 l in the main reactor. The main reactor volume (13.75 l) was used in all the subsequent calculations for hydraulic retention times (HRT) and NH_4-N loading rates. The aeration column was constructed using opaque PVC piping (2 m) and the reactors were aerated using compressed air (6 l/min). Fluidisation was achieved and maintained using magnetic centrifugal pumps (*March mfg, Inc U.S.A.*). The synthetic wastewater was fed into the reactors using peristaltic pumps (*Wartson Marlow, Germany*). The pH within the reactors was maintained at 7.5 with the addition of 20% $NaHCO_3$ using an automatic pH controller (*Hanna Instruments, Germany*). The BFBR`s were operated at ambient temperature (25°C). Temperature and dissolved oxygen concentrations (DO) within the reactors were monitored using a YSI 5100 DO and temperature probe (*Hanna instruments, Germany*). A schematic representation of the experimental fluidised-bed reactor is shown in Figure 1. The actual experimental set-up is shown in Figure 2.

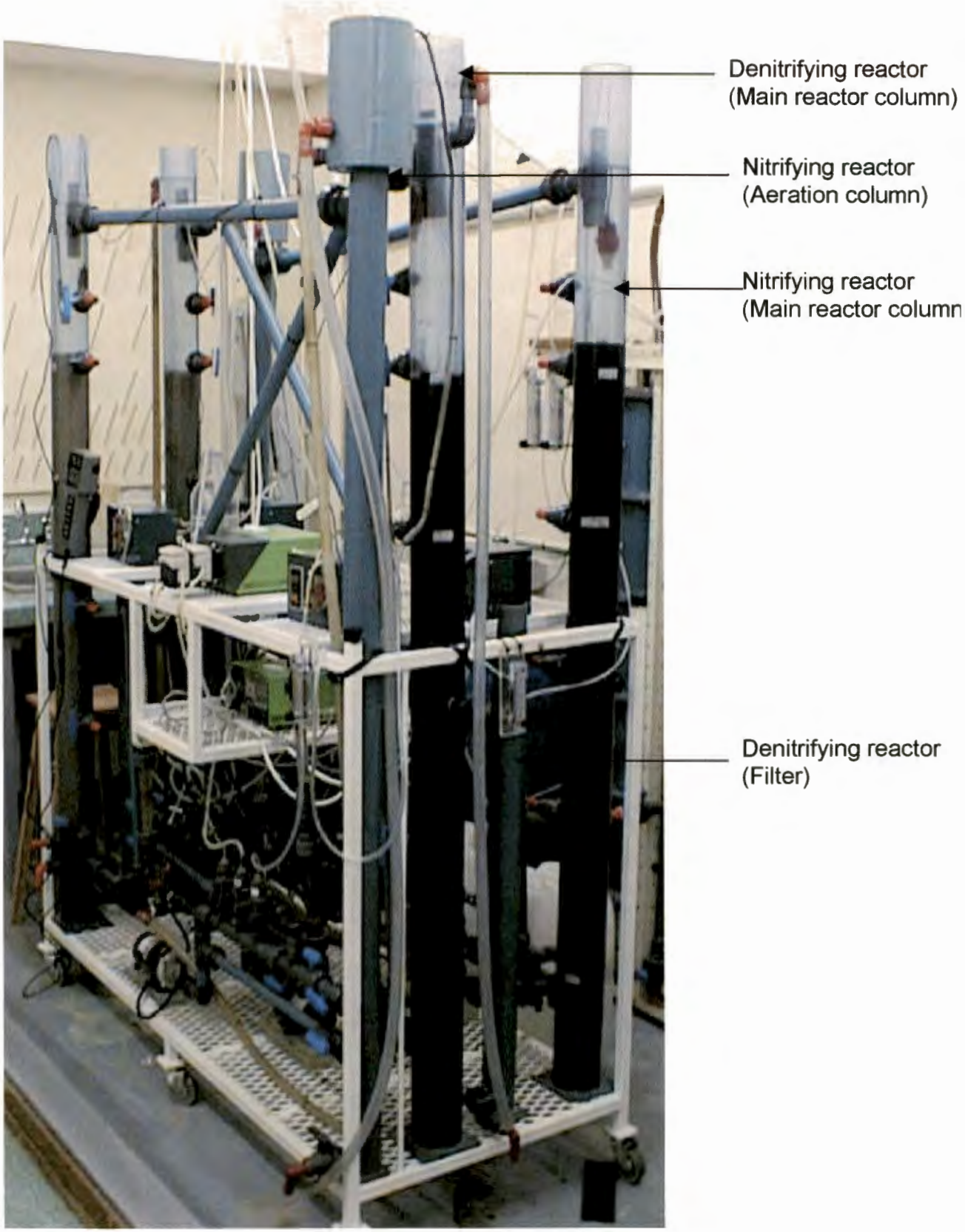


Figure 2. The general view of the experimental set-up as used during this study. The main reactor columns for nitrification and denitrification, respectively, are indicated.

Physical properties and characteristics of the support matrices. The silica sand used as support matrix during this study (B and E Silica, *Delmas, South Africa*) had a uniformity coefficient (UFC) of 1.09, and an equivalent diameter (D_{10}) of 0.86 mm. Furthermore, the sand had a static bed porosity (ε) of 0.04, bulk density ($b\rho$) of 0.147 kg/m^3 and a particle density ($p\rho$) of 2.63 x 10^{-6} kg/m^3. The GAC as support matrix during this study (Filtrasorb 300; *Chematron Products (Pty) Ltd Isando, S. A.*) had a UFC of 1.9, an equivalent diameter (D_{eq}) of 1.6 mm. Furthermore, the GAC had a static bed porosity (ε) of 0.569, bulk density ($b\rho$) of 0.538 kg/m^3, and a particle density ($p\rho$) of 1.25 x 10^{-4} kg/m^3. These values were determined according to the methods as described by Summerfelt and Cleasby (1996). Approximately 10.6 kg of sand, in comparison to 3.6 kg GAC were used as support matrices in the nitrifying BFBR`s. The sand and GAC used during the study are illustrated in Figure 3.

a)

b)

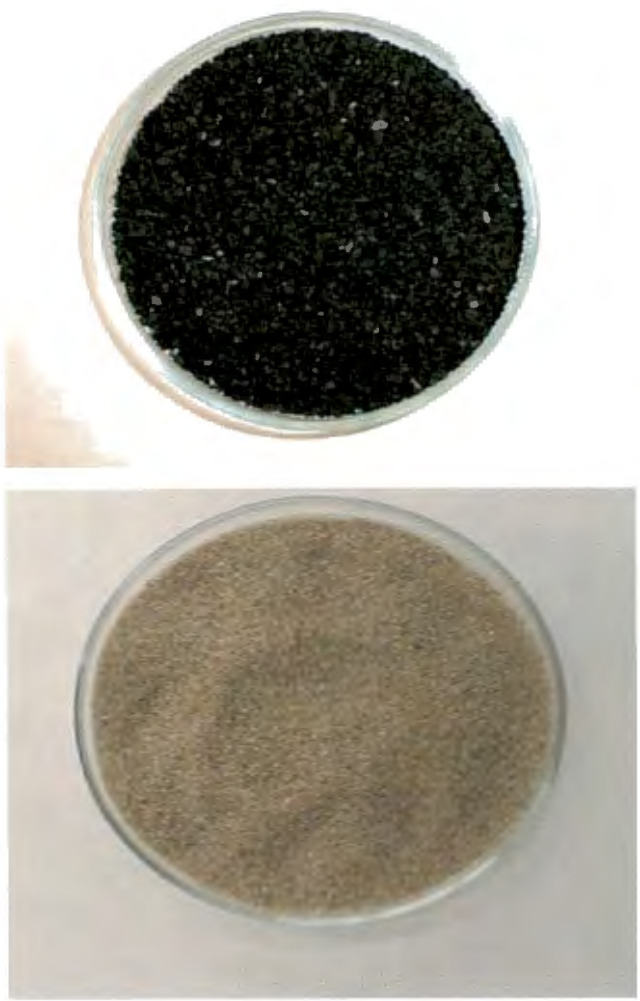


Figure 3. The support matrices used during the study:
a) Granular activated carbon; and b) Sand.

Medium and inoculation. The synthetic wastewater used during this study was analogous to the composition of the nitrogenous condensate stream effluent as generated by SASOL Agri, Secunda, Sasolburg, South Africa. The composition of the SASOL Agri effluent is as follows (mg/l): NH_4,NO_3 1000; PO_4, 80; Ca, 150; Cl, 200 and Na, 500. Therefore, the synthetic effluent composition was prepared as follows (mg/l tap water): K_2HPO_4,125; $MgSO_4.7H_2O$, 186.5; NaCl, 529.5; $CaCl_2$, 20; $MnCl_2.4H_2O$, 1; $NaHCO_3$; and NH_4Cl. The NH_4Cl concentration was incrementally increased over a period of 192 days from 10 mg/l to 1000 mg/l NH_4-N. Stoichiometrically, for every mole of NH_4-N, 2 moles of $NaHCO_3$ were added. Both the nitrifying two-phase BFBR`s were inoculated with a 1L mixture of aerobic sludge obtained from the Daspoort, Centurion, Fochville, Zeekoegat and Baviaanspoort water treatment works, North West Province, South Africa.

Set-up of support matrices. The lower sections of both the biological fluidised-bed reactors were filled with coarse gravel (1.5 cm diameter) to facilitate the formation of laminar flow in the bottom section of the reactors (Fig. 4) (Bach *et al.,* 1998).

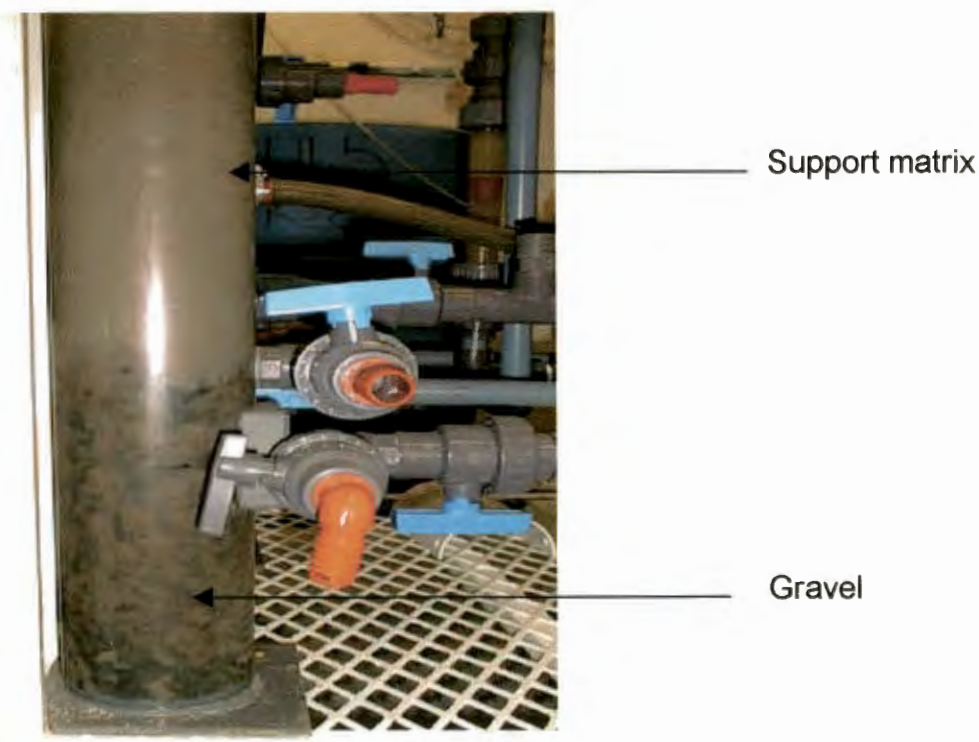


Figure 4. The bottom section of a two-phase BFBR illustrating the gravel below the support matrix.

60% of the remaining height (1.75 m) of the main reactor columns were filled with the respective support matrices. Before inoculation of the reactors, the influence of up-flow fluid velocities on the clean bed height of the respective support matrices was determined. This enabled the calculation of the minimum up-flow liquid velocities required for the fluidisation of both the sand and GAC, respectively. During inoculation and start-up, the reactors were operated at a bed height expansion of 30%.

Reactor operation. After inoculation, the reactors were operated in batch mode for ten days to facilitate biofilm formation on the various support matrices. This was followed by continuous operation of the reactors at hydraulic retention times (HRT`s) of 5 days, 3 days, 1 day, 12 hours and 6 hours at a bed height expansion of 30% at 25 °C. This corresponded to an up-flow liquid velocity of 68.9 m/h and 44.2 m/h in the BFBR using sand and GAC as support matrices, respectively. At least six HRT cycles were completed to allow the reactor to stabilise before the effluent was obtained for chemical characterisation and the specific HRT or nitrogen loading rates were changed.

Biofilm characterisation. Biofilm development on the various support matrices was characterised by scanning electron microscopy (SEM). The samples of biofilm-coated support matrices (sand and GAC) were dehydrated with 70% ethanol. The ethanol was subsequently replaced with 70, 90 and 100 % acetone. The samples were dried with carbon dioxide and sputter coated with carbon followed by gold. Scanning electron micrographs were taken using a Philips XL 30 DX-4i scanning electron microscope. Biomass accumulation on both the sand and the GAC support matrices was determined gravimetrically, as described by Csikor *et al.* (1996), and Chen and Chen (2000).

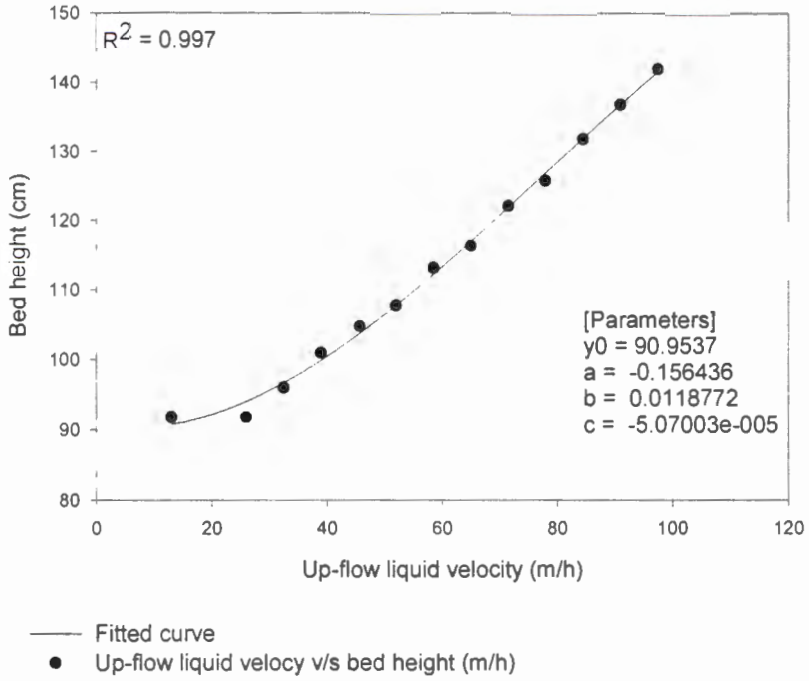
Analytical methods. Anions (NO_3^-, NO_2^-) within the feed and the effluent from both the reactors were analysed using a Hewlett Packard 1100 High Pressure Liquid Chromatography (HPLC) coupled to a Metrohm 732 IC conductivity detector and a Waters IC Pak column. Cations (NH_4^+) within the feed and the effluent from both the reactors were analysed using a Hewlett Packard 1100 HPLC coupled to a Metrohm 732 IC conductivity detector and a Hamilton PRP x200 column. Ammonium was also analysed spectrophotometrically using a Merck Spectroquant® *(Darmstadt, Germany)* Nova 60. Alkalinity, chemical oxygen demand (COD), total suspended solids (TSS) and volatile suspended solids (VSS) were analysed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1985). Chemical oxygen demand was only determined from day 160 of the reactor operation.

Results and discussions

Prediction of fluidisation hydraulics. The effect of up-flow liquid velocity on the bed height of fluidised-bed reactors plays a vital role in the prediction of minimum up-flow velocity required for fluidisation, as well as in the prediction of biofilm formation on the support matrices. Biofilm formation on the support matrices results in an increased bed height at a constant up-flow velocity, primarily due to a decrease in the density of the support matrices. The bed height subsequently becomes less sensitive to the liquid velocity (Chang and Rittman, 1994). The effect of the up-flow liquid velocity on the clean bed height of both the GAC and sand support matrices are shown in Figure 5.

Based on the results obtained for the up-flow liquid velocity versus clean bed expansion, it can be concluded that bed expansion is a function of the up-flow liquid velocity, which compares favourably with the results of Chang and Rittmann (1994). It was also calculated that the minimum up-flow liquid velocity required for the fluidisation of GAC is 13 m/h, whereas the minimum up-flow liquid velocity required for the fluidisation of sand is 36 m/h.

a)



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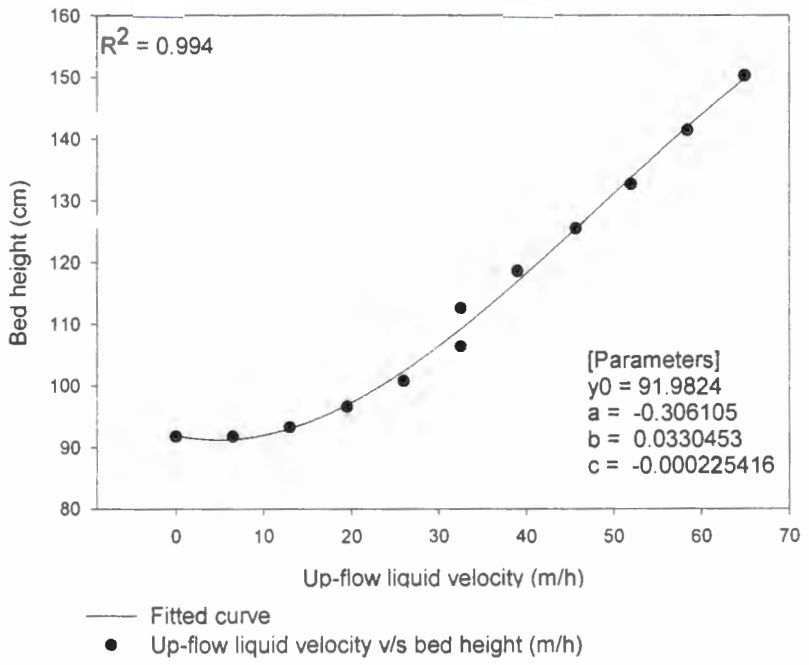
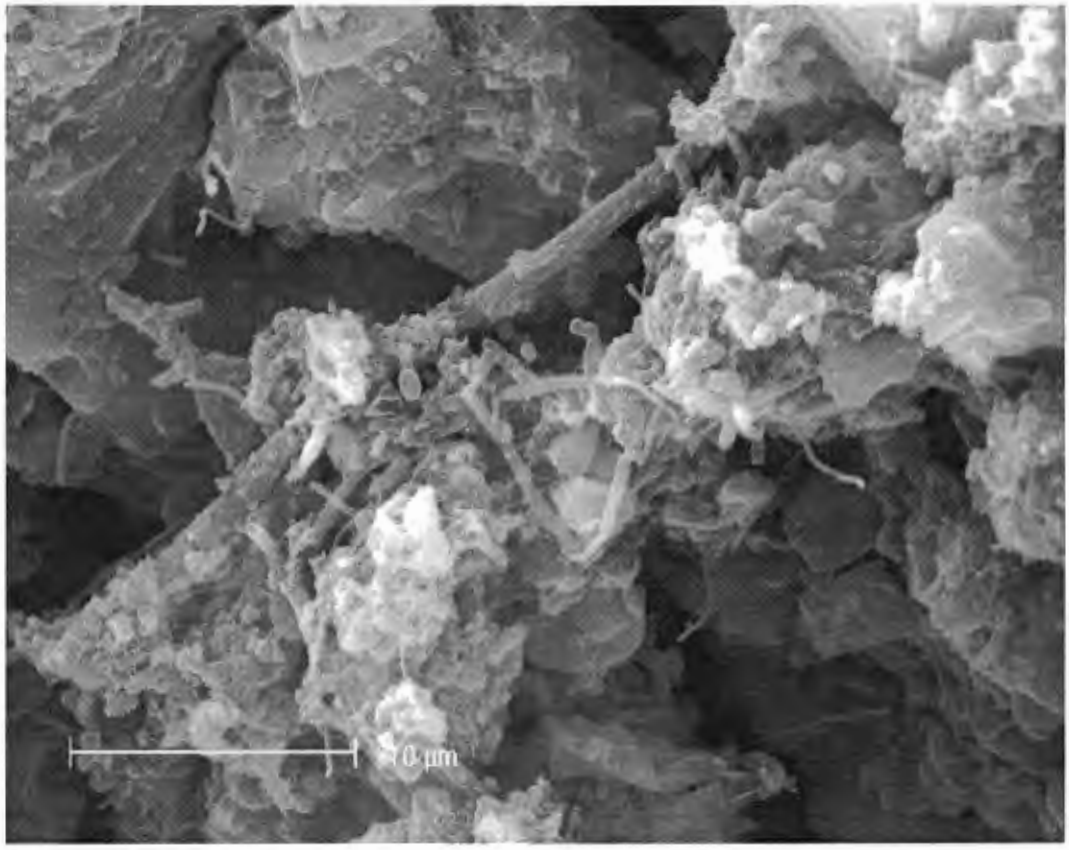


Figure 5. The effect of up-flow liquid velocity on the bed height of the various support matrices a) Sand b) Granular activated carbon. y^0, a, and c are parameters obtained during curve fitting.

Biofilm characterisation. Biofilm formation on both the sand and the GAC support matrices in both the reactors was evident after 27 days of operation. However, significantly more biofilm could be observed on the GAC than on the sand (Fig. 6). Although significant biofilm formation was uniformly present on the GAC, biofilm formation on the sand was restricted to the crevasses and cavities of the individual sand particles (Fig. 7). Due to the irregularity and high porosity of GAC, this support matrix has previously been reported to be a better immobilisation matrix, when compared to sand (Texeira and Oliveira, 1998). Significantly more biofilm could be observed on both sand and GAC after 108 days of operation (Fig. 8). This was verified by gravimetric analysis and a distinct relationship could be observed between the amount of biofilm within the two-phase BFBR`s and the NH_4-N loading rate (Fig. 9). The increase in biofilm formation on the support matrices over time also corresponded to an increase in the expanded bed height despite the constant up-flow velocity. These results confirmed previous reports that biofilm formation results in a less dense support matrix, culminating in an increase in bed height (Summerfelt and Cleasby, 1996). During the course of the study, the linear up-flow velocities were kept constant at 68.9 m/h and 44.2 m/h in the nitrifying BFBR`s using sand and GAC as support matrices, respectively. Due to relatively low biomass concentrations in the nitrifying BFBR`s, it was not frequently required to remove excess biomass from the various support matrices.

a)



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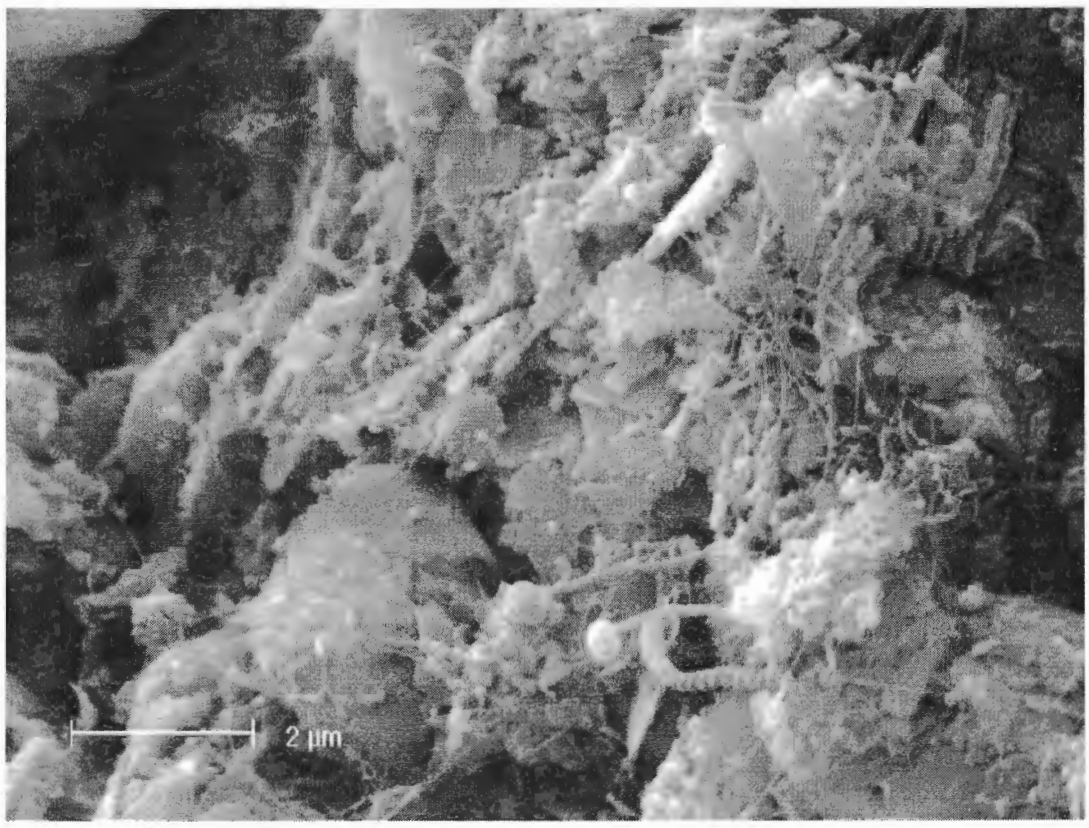
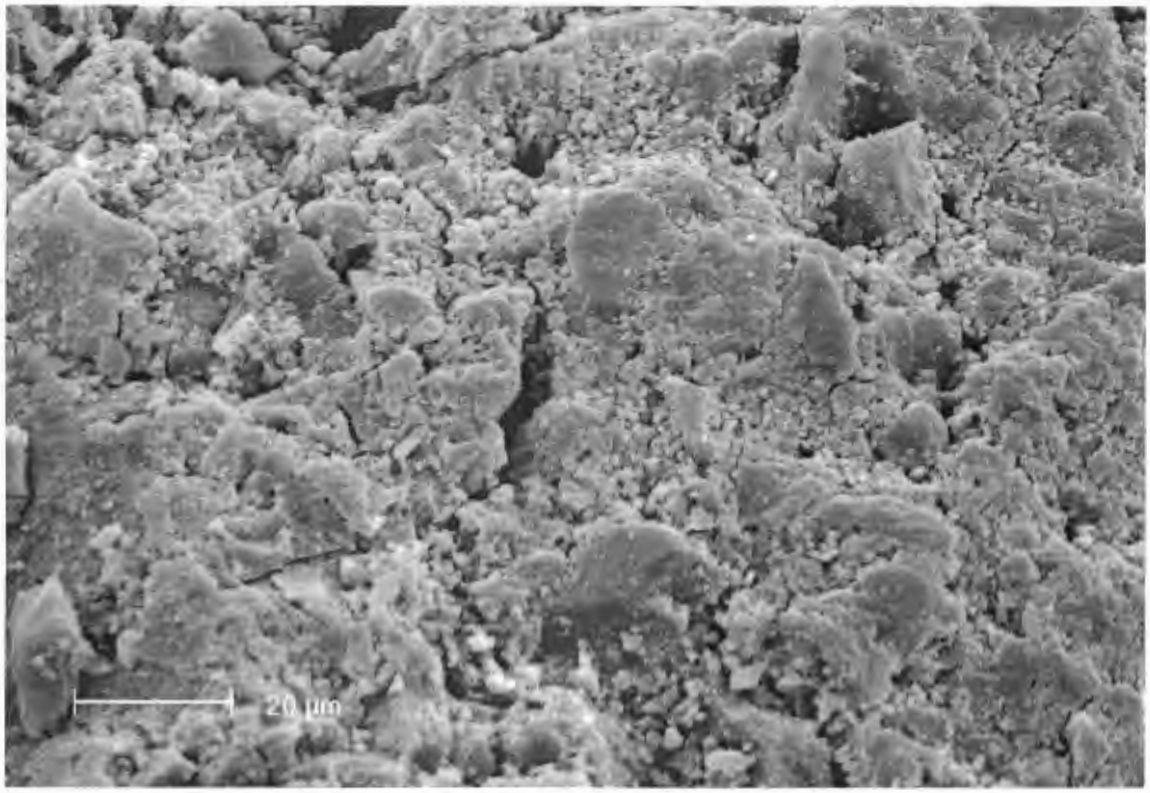


Figure 6. Scanning electron micrographs illustrating biofilm formation on: a) Granular activated carbon; and b) Sand after 27 days of reactor operation.

a)



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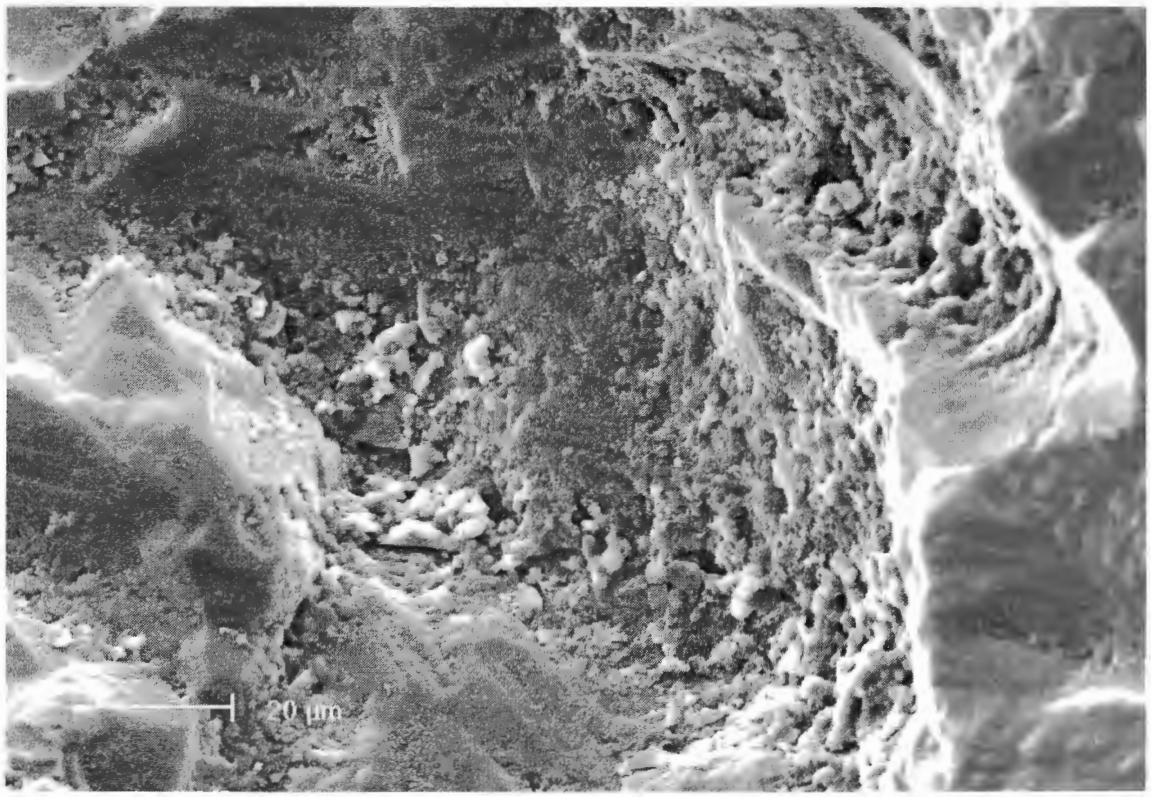
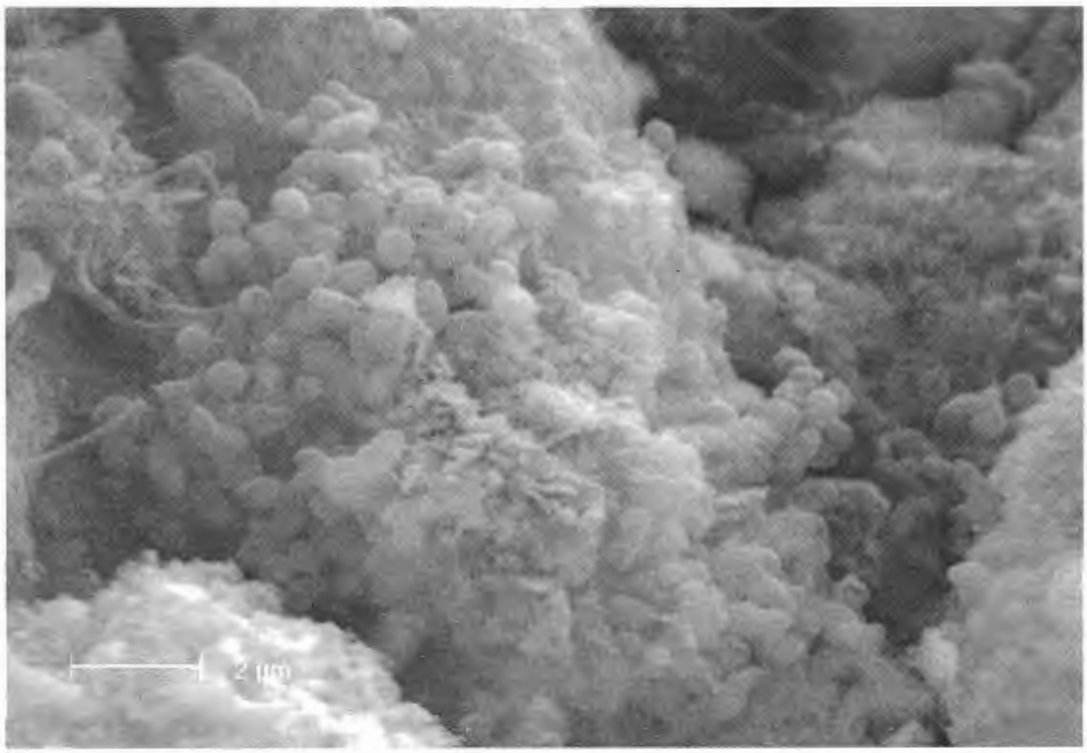


Figure 7. Scanning electron micrographs illustrating biofilm formation on: a) Granular activated carbon; and b) Sand. The biofilm formation on the sand (b) is primarily restricted to the crevasses and cavities.

a)



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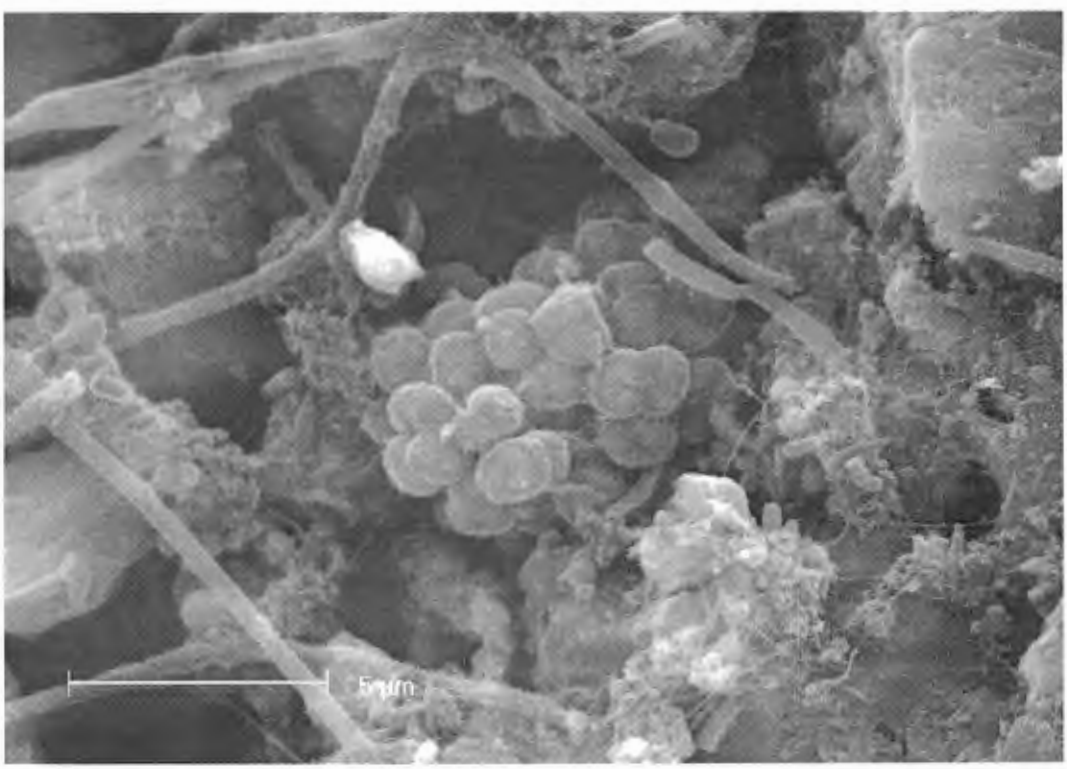


Figure 8. Scanning electron micrographs illustrating biofilm formation on: a) Granular activated carbon; and b) Sand after 108 days of reactor operation.

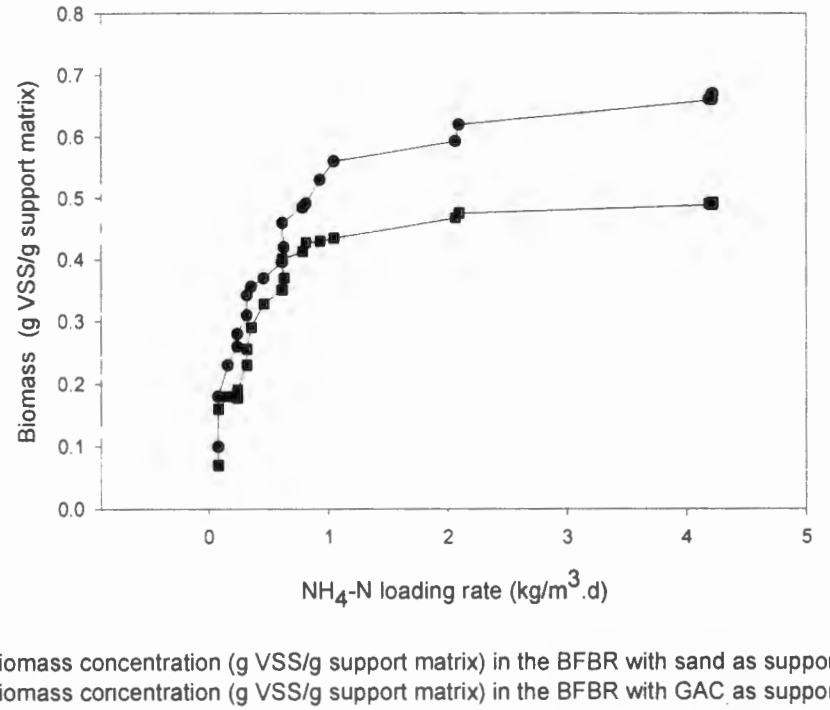
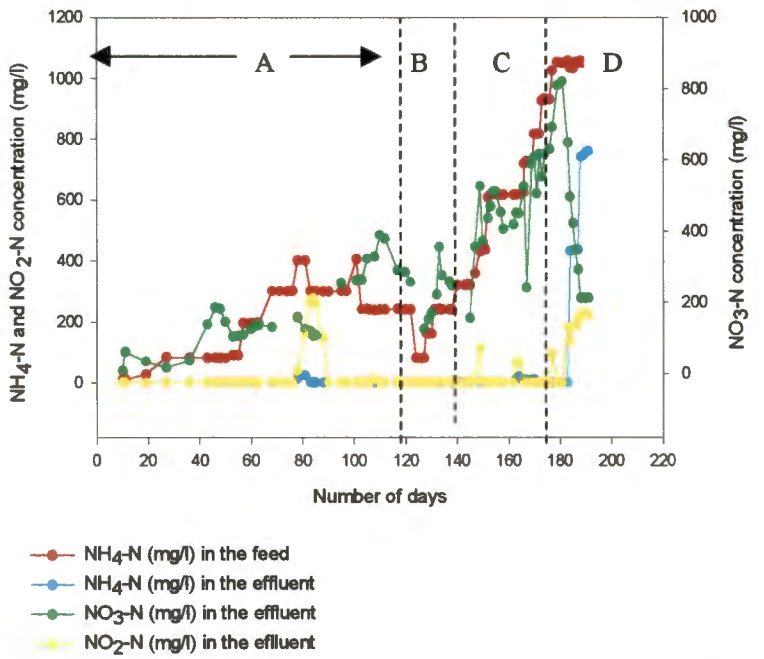


Figure 9. The relationship between the biomass concentration (VSS) on the various support matrices, and the NH_4-N loading rate.

Ammonium nitrogen removal. The results of NH_4-N removal, and nitrite/nitrate generation phase as obtained during biological nitrification of the synthetic wastewater in the BFBR`s with sand and GAC as support matrices at various hydraulic retention times (HRT`s), are illustrated in Figure 10.

a)



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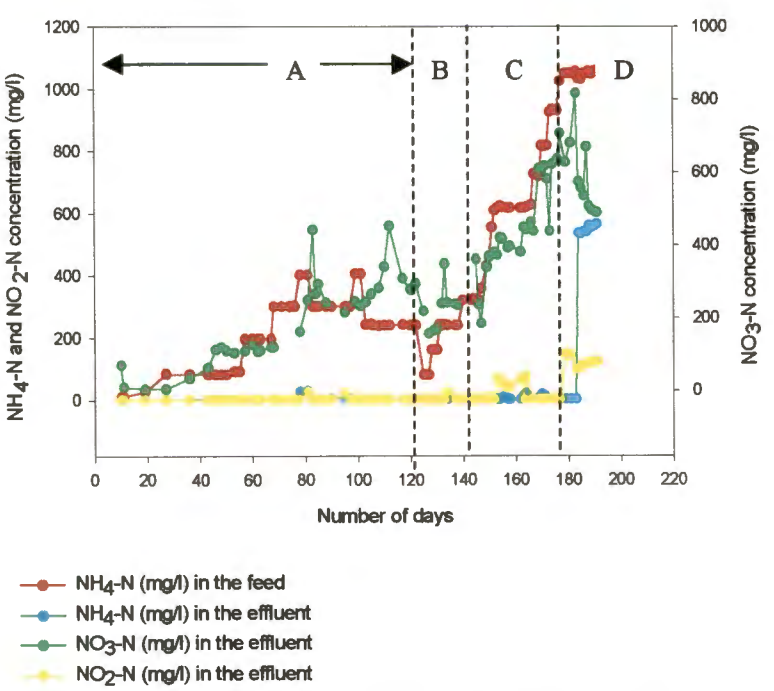


Figure 10. The NH_4-N removal and nitrate/nitrite generation over a period of 191 days of continuous reactor operation: a) Sand; and b) Granular activated carbon. (A = HRT, 5 days; B = HRT, 3 days; C = HRT, 1 day; D = HRT, < 1 day)

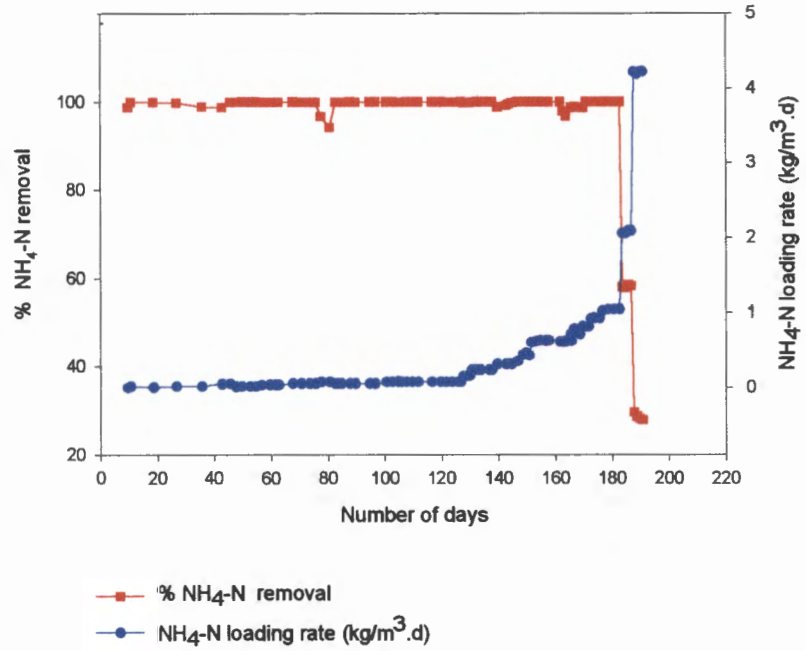
Based on the results obtained, it is evident that NH_4-N oxidation within the BFBR`s initiated after 10 days of continuous operation in both the reactors using sand and GAC as support matrices, resulting in an effluent with less than 0.10 mg/l NH_4-N. Up to 800 mg/l NO_3-N was generated during the reactor operation. These results are indicative of an accelerated start-up phase, in contrast to the results by Vredenbergt *et al.* (1997) where NH_4-N oxidation was only observed two months after reactor start-up.

High concentrations of NO_3-N were found in the effluents of both the reactors during operation at the HRT range 5 days to 1 day. The NO_3-N yield was, however, not according to the expected stoichiometric conversion of ammonium to nitrate, with lesser amounts of NO_3-N being produced. Stoichiometrically, at a loading rate of 1 kg NH_4-N/m^3.d, approximately 983 mg/l NO_3-N would be generated. In this study, however, at this loading rate 800 mg/l NO_3-N was produced. These results confirmed reports by Painter (1986), that, in both batch and continuous experiments, there is often a net loss of the inorganic nitrogen between the total nitrogen in the feed and effluent. At a HRT of less than 1 day (12 hours and 6 hours), a decrease in the concentration of NO_3-N in the effluent was observed with a subsequent increase in concentrations of NO_2-N and NH_4-N in the effluent. These results suggest that the nitrification process was being negatively influenced.

The effect of increasing NH_4-N volumetric loading rates on the removal efficiencies of the two-phase BFBR`s using sand and GAC as support matrices is illustrated in Figure 11. Based on the results obtained, it is evident that at a loading rate of 0.081 kg $NH_4$-N/$m^3$.d, 99% removal efficiency was achieved in the BFBR`s using sand and GAC as support matrices. A slight decrease in the removal efficiencies of both reactors was observed on day 80 and 160 of operation, where the removal efficiencies dropped to 94% and 93% on day 80 and 98% and 98.5% on day 160 for the BFBR`s using sand and GAC as support matrices, respectively. This decrease in the removal efficiencies can be ascribed to the washing procedure which was employed to remove excess biomass from the various support matrices (Fig. 11).

However, the reactor performances stabilised within 24 hours after the washing process

a)



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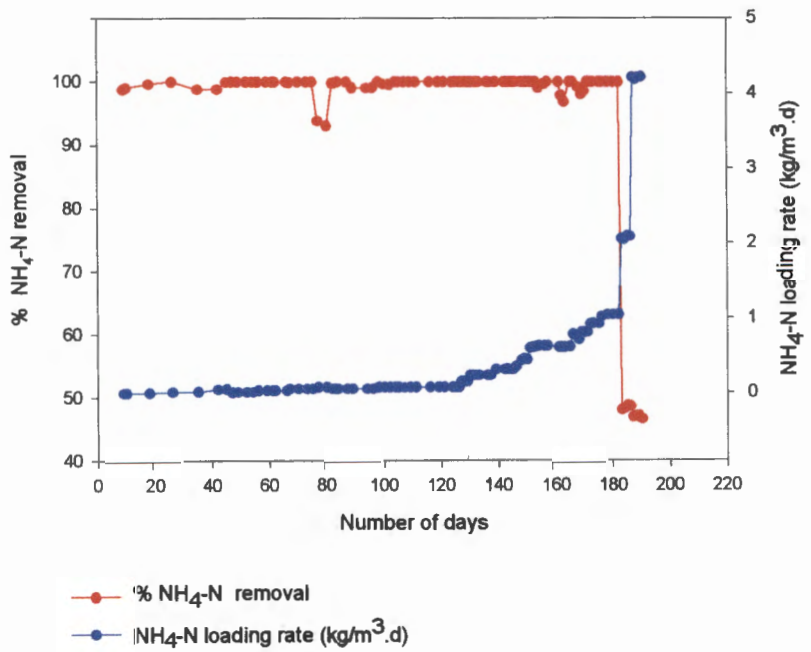

Figure 11. The effect of NH_4-N loading rate on the NH_4-N removal efficiencies of the BFBR`s using: a) Sand, and b) Granular activated carbon as support matrices, respectively.

During subsequent reduction in the HRT (from 5 days to 6 hours), the NH_4-N concentration in the feed was incrementally increased to 1000 mg/l over a period of 191 days. At a NH_4-N loading rate of 0.5 kg/m^3.d, 99% NH_4-N removal efficiencies were achieved in both the BFBR`s. At this loading rate the dissolved oxygen concentration was maintained above 2.5 mg/l along the entire height of the reactor (Fig. 12).

However, when the loading rate was further increased to 0.8 kg NH_4-N/m^3.d (HRT 1 day, 800 mg/l NH_4-N) the dissolved oxygen concentration in both reactors using sand and GAC as support matrices was observed to decrease to less than 2 mg/l at an air flow rate of 6 l/min. As a result, aeration was further increased to 12 l/min in order to achieve a final DO of 2.5 mg/l within each reactor. At a loading rate of 1 kg NH_4-N/m^3.d (HRT 1 day, 1000 mg/l NH_4-N), removal efficiencies in excess of 99% were achieved in both BFBR`s.

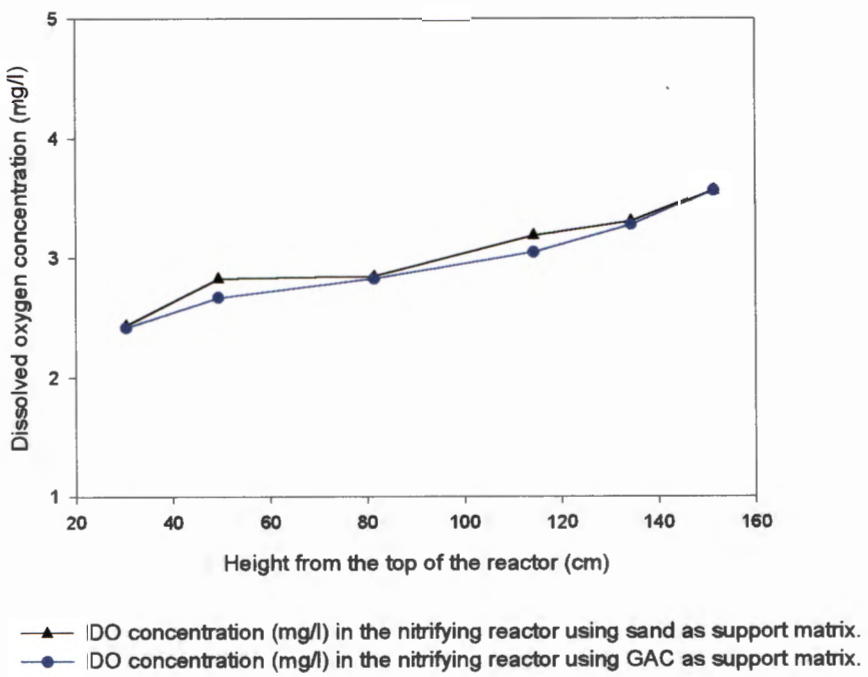

Figure 12. Dissolved oxygen profiles within both the nitrifying BFBR`s where sand and granular activated carbon were used as support matrices.

Significant decrease in the removal efficiencies of both BFBR`s were observed when the NH_4-N loading was increased to 2 kg NH_4-N/m^3.d (HRT 12 hours, 1000 mg/l NH_4-N). The NH_4-N removal efficiencies were observed to decrease to 58% and 48% in the fluidised-bed reactors using sand and GAC as support matrices, respectively. At a specific volumetric loading rate of 2kg NH_4-N/m^3.d, the dissolved oxygen concentration within the reactors could not be maintained above 2.5 mg/l and decreased to 1.35 mg/l and 1.5 mg/l in the reactors using sand and GAC as support matrices, respectively. In addition to this observation, there was a significant increase in the effluent TSS, VSS, COD (Fig. 13), NH_4-N, NO_2-N and NO_3-N (Fig. 10).

A further decrease in the NH_4-N removal efficiencies was observed in both reactors when the NH_4-N volumetric loading rate was further increased to 4 kg NH_4-N/m^3.d (HRT 6 hours, 1000 mg/l NH_4-N). At this volumetric loading rate, removal efficiencies decreased to 28% and 47% in the BFBR`s using sand and GAC as support matrices, respectively. Summarised results of the study are shown in Table 1.

Table. 1. The effect of increased NH_4-N loading rates on the removal efficiencies of the nitrifying biological fluidised-bed reactors using sand and granular activated carbon as support matrices.

NH_4-N Loading rate (kg NH_4-N/m^3.d)	Support Matrix	
	Sand	GAC
	% NH_4-N removal	% NH_4-N removal
0.081	99%	99%
0.5	99%	99%
1	99%	99%
2	58%	48%
4	28%	47%

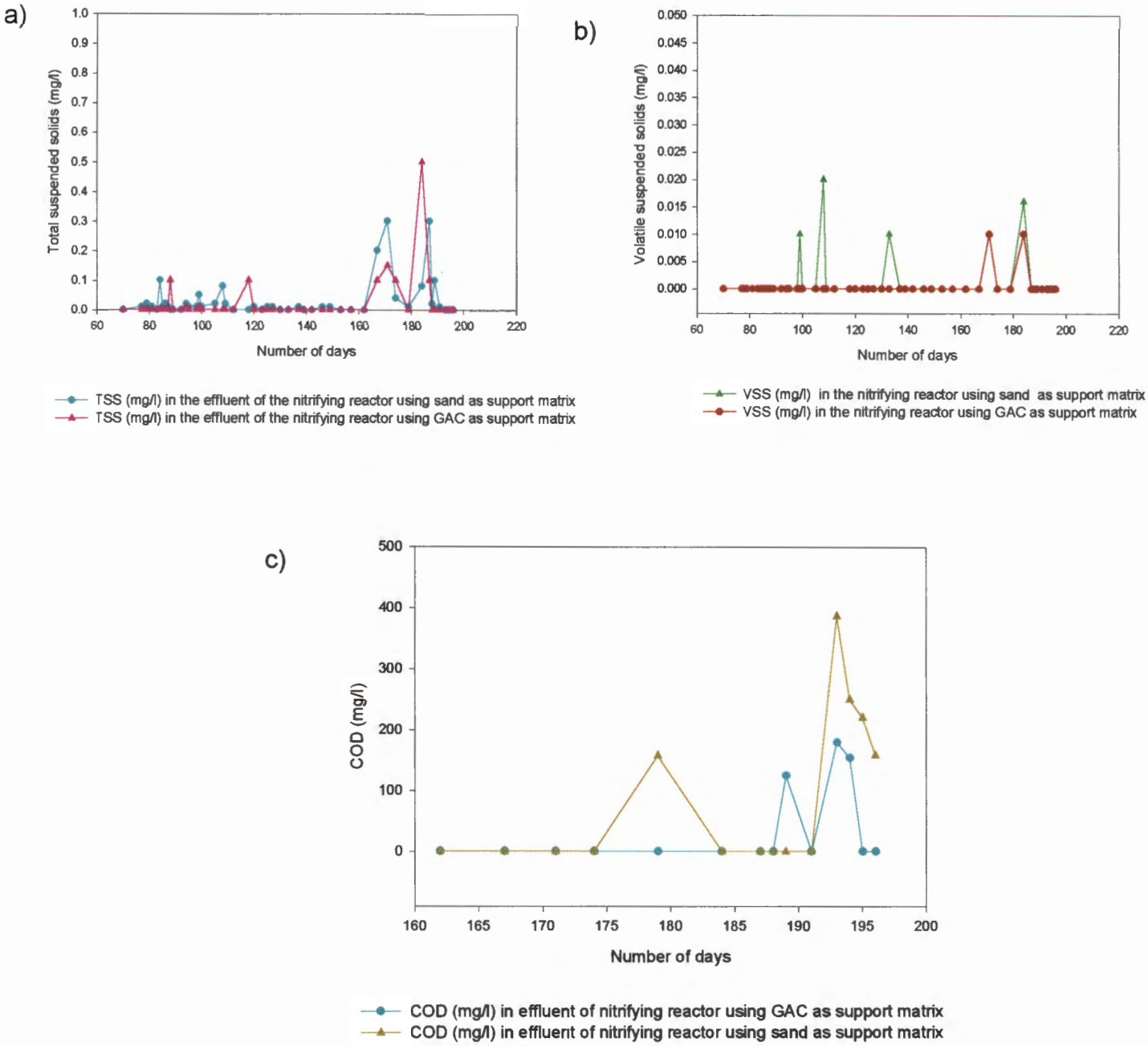

Figure 13. Effluent a) total suspended solids; b) volatile suspended solids; and c) chemical oxygen demand (mg/l) of the BFBR`s using sand and GAC as support matrices, respectively.

Based on the results obtained, it is evident that the biological nitrification process was more effective at dissolved oxygen concentrations above 2 mg/l, thus confirming previous studies by Nogueira *et al.* (1998), where nitrification was more effective at DO concentrations above 2 mg/l. The observed decrease in the NH_4-N removal efficiencies in relation to the NH_4-N loading rate could therefore be attributed to the oxygen limitation within the reactor. Optimum loading rate of 1 kg/m^3.d (HRT 1 day, 1000 mg/l NH_4-N) could be achieved in both the nitrifying BFBR`s before nitrification was inhibited by the decreased oxygen concentration. These results compare favourably with findings by Green *et al.* (2001), where the optimum NH_4-N loading rate of 1.4 kg NH_4-N/m^3.d could be achieved before nitrification was limited by the availability of oxygen.

It has previously been reported that the nitrifying bacteria are susceptible to oxygen limitation due to their tendency to grow closer to the support matrix (Rittmann and McCarty, 2001). Higher removal efficiencies at NH_4-N loading rates above 2 kg NH_4-N/m^3.d could be achieved using pure oxygen as an alternative for the maintainance of the DO above 2 mg/l, however, its use has been reported to be very costly (Hargreaves, 1998). An alternative option is the evaluation of alternative fixed film reactor configurations. Studies by van Benthum (1998), have shown that loading rates as high as 6 kg/m^3.d can be achieved during nitrification with the use of airlift biofilm suspension reactors as a result of the associated higher DO concentration typically associated with airlift reactors.

Conclusions

The main conclusions that can be drawn from this study are:

- Oxidation of a high strength, high saline synthetic nitrogenous effluent analogous to the industrial effluent generated by SASOL Agri, Secunda, South Africa, is possible using two-phase biological fluidised-bed reactors;

- Up to 99% NH_4-N removal (10 mg/l - 1000 mg/l) could be achieved at a loading rate of 1 kg NH_4-N/m^3.d in both the BFBR`s using sand and GAC as support matrices, respectively. At volumetric loading rates higher than 1 kg NH_4-N /m^3.d, it was observed that the nitrification process became oxygen-limited (DO <1.5 mg/l), and removal efficiencies were observed to decrease significantly;

- An increase in the loading rate (>1 kg NH_4-N/m^3.d) resulted in the increase of the concentrations of NH_4-N, NO_2-N and NO_3-N in the effluent.

- Although high concentrations of NO_3-N were found in the effluent, the NO_3-N yield was not according to the stoichiometric conversion of ammonium to nitrate. Lesser amounts of nitrates were produced.

- Higher removal efficiencies (47% GAC; and 28% sand, at a NH_4-N loading rate of 4 kg NH_4-N/m^3.d) and more stable reactor operation could be achieved with the use of GAC as the support matrix during nitrification when compared to the use of sand as support matrix.

- Ammonium nitrogen concentrations below 0.1 mg/l could be achieved with the use of the two-phase BFBR`s, at a loading rate of 1 kg NH_4-N/m^3.d, thus complying with the general limit as well as the special limit for the discharge of ammonium containing effluents into the water resources, as reported in the South African National Water Act (36/1998). However, further treatment of the effluent is still necessary in order to remove the high concentrations of nitrates (up to 800 mg/l NO_3-N) generated during the nitrification process.

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Chapter 4

Optimisation of Denitrification Using Fluidised-Bed Reactors: Comparative Evaluation of Sand and Granular Activated Carbon as Support Matrices

(Submitted for publication in Water Research)

Summary

During this study, the suitability of two-phase biological fluidised-bed reactors (BFBR`s) for the treatment of a high-strength, high saline nitrogenous synthetic effluent analogous to the SASOL Agri effluent (condensate stream containing approximately 1000 mg/l ammonium nitrate) was evaluated. The suitability of using sodium acetate as a carbon source for heterotrophic denitrification as well as the suitability of using sand and granular activated carbon (GAC) as support matrices for denitrification was also evaluated. During the start-up phase, the reactors were initially operated in batch mode for ten days. This was followed by continuous operation of the reactors at varying hydraulic retention times (between 5 days and 3 hours) (NO_3-N loading rates of between 0.0032 kg NO_3-N/m^3.d and 26 kg NO_3-N/m^3.d). The reactors were fed with the synthetic effluent in which the nitrate as nitrogen (NO_3-N) concentration was gradually increased from 10 to 1000 mg/l. The optimal C:N ratio was determined to be 1.6 and 2.0 for the BFBR`s using sand and GAC as support matrices, respectively. In both reactors, NO_3-N removal efficiencies in excess of 99% could be achieved at a NO_3-N loading rate of 4 kg NO_3-N/m^3.d. As the NO_3-N loading rate was increased to 8 kg NO_3-N/m^3.d, the NO_3-N removal efficiencies dropped significantly to 67% and 23% in the BFBR`s using sand and GAC as support matrices, respectively. A further drop in the $NO_3$-N removal efficiencies was observed when the $NO_3$-N loading rate was increased to 26 kg/$m^3$.d. At this loading rate, $NO_3$-N removal efficiencies of 66% and 17% were achieved in the two-phase BFBR`s using sand and GAC as support matrices, respectively. This significant decrease in

the NO_3-N removal efficiencies at this high loading rate (26 NO_3-N kg/m^3.d) coincided with a significant increase in the TSS, VSS and COD values in the effluent, especially that of the BFBR using GAC as support matrix. In contrast, at this loading rate (26 kg NO_3-N/m^3.d) higher removal efficiencies (66%) and a more stable reactor operation could be achieved in the BFBR using sand as the support matrix.

Introduction

In the Southern African region some areas have abundant water and others have scarcity. The rainfall patterns also differ dramatically, ranging from 4,000 mm to less than 50 mm per annum (Global Water Partnership, 2000). South Africa is already experiencing water stress (IPS, 1998). Furthermore, many water bodies are polluted and the water quality is declining due to salination and eutrophication. Salination results from increased industrialisation, urbanisation and irrigation (O`Keeffe *et al.,* 1999), whereas eutrophication results from the introduction of high concentrations of nitrogenous compounds and phosphorus in receiving water bodies (EPA, 1993). Ammonia nitrogen has also been reported to be toxic to fish. Nitrites and nitrates are known to be carcinogenic and nitrites may lead to metheamoglobinemia in babies (EPA, 1993).

The general standards for the discharge of nutrients such as ammonia and nitrates into receiving bodies such as dams, rivers and lakes are becoming more stringent due to the increasing industrial discharges and the environmental impacts of nitrogenous compounds (Massonne *et al.,* 1996). According to the South African National Water Act (36/1998), the general limit applicable to nitrates and nitrites as nitrogen is 15 mg/l and the special limit is 1.5 mg/l. In Europe, the general standard for the discharge of these nutrients is 8 mg/l (EPA, 1993). In order to balance water supply with its demand, effluents from different industries have to be returned and reused or treated before being discharged into receiving water bodies (Tchobanoglous, 1991; Gupta and Sharma, 1996).

Biological denitrification is widely used for the removal of nitrates from both domestic and industrial wastewaters (Kornaros and Lybertos, 1997). This process is carried out by metabolically and biochemically diverse groups of facultatively anaerobic bacteria, such as *Pseudomonas* spp, *Alcaligenes* spp and *Bacillus* spp. (Rittmann and McCarty, 2001). During the denitrification process, the organic carbon acts as an electron donor (Ganaye *et al.,* 1996), whereas oxygen is the preferred terminal electron acceptor. However, when there is no or very low residual oxygen available, nitrates could also serve as an alternative terminal electron acceptor for these denitrification organisms (Brock *et al.,* 1997). These organic carbon sources may be from the wastewater or they may be from exogenous electron donor (Tchobanoglous, 1991). Selection of an appropriate carbon source is dependent upon the medium, the organisms and the economics of the process (Constantin and Fick, 1997). In most wastewater treatment facilities, non-fermentable organic compounds such as ethanol, acetic acid, and methanol are commonly used as exogenous organic carbon sources during denitrification (Constantin and Fick, 1997; EPA, 1993).

Most studies on denitrification using acetic acid as the organic carbon source have been directed at the treatment of relatively low initial nitrate concentrations (10 - 200 mg/l NO_3-N), whilst few have been utilised for the treatment of high nitrate concentrations (Constantin and Fick, 1997). The use of low nitrate concentrations is primarily due to the inhibitory effect of high nitrate concentrations on the denitrifying microbial populations (Glass and Silverstein, 1998). Although toxicity of nitrate and nitrite at high concentrations has been reported, denitrification of high concentrations of nitrates (2000 mg/l NO_3-N) has also been achieved (Cuervo-López *et al.,* 1999).

Many technologically advanced reactor configurations are used for the treatment of nitrate-containing wastewaters. However, anaerobic biological fluidised-bed reactors are reported to allow more accumulation of higher concentrations of biomass within the reactor than any other anaerobic configuration. This enables the operation of the reactors at significantly high

volumetric loading rates and shorter hydraulic retention times (<1 day) (Garcia-Calderón *et al.*, 1998). Successful denitrification of wastewater using biological fluidised-bed reactors has been reported (Bach *et al.*, 1998)

The aim of this study was therefore to evaluate the removal efficiencies of two-phase biological fluidised-bed reactors for the denitrification of a nitrate-rich effluent analogous to the condensate stream as generated by SASOL Agri, Secunda, South Africa. This effluent typically contains 1000 mg/l ammonium nitrate.

The specific objectives of this study were therefore:

- To optimise the removal efficiencies of two-phase BFBR`s for the denitrification of a high strength, high saline nitrogenous synthetic effluent.

- To compare the use of sand and GAC as support matrices for denitrification at varying loading rates.

Materials and Methods

Two BFBR`s were operated in parallel. The main reactor columns were constructed using transparent polyvinyl chloride (PVC) piping (height 2 m, internal diameter 0.099 m). The total reactor volume was 19.73 l with an operational volume of 14.63 l in the main reactor. The main reactor volume (14.63 l) was used in all the subsequent calculations for hydraulic retention times (HRT) and NO_3-N loading rates. Fluidisation was achieved and maintained using magnetic centrifugal pumps (*March mfg, Inc U.S.A.*). A 10 mm (diameter) filter was connected between the recirculation tube and the magnetic centrifugal pump to prevent excess biomass from entering the pump (Fig. 2). The synthetic wastewater was fed into the reactors using peristaltic pumps (*Wartson Marlow, Germany*). The pH within the reactors was maintained at 7.0 with the addition of 1N HCl using an automatic pH controller

(*Hanna instruments., Germany*). The dissolved oxygen concentration (DO) and temperature within the reactors were monitored using a YSI 5100 DO and temperature probe (*Hanna instruments., Germany*). Both the reactors were operated at ambient temperature (25°C). A schematic representation of the experimental fluidised-bed reactor is shown in Figure 1.

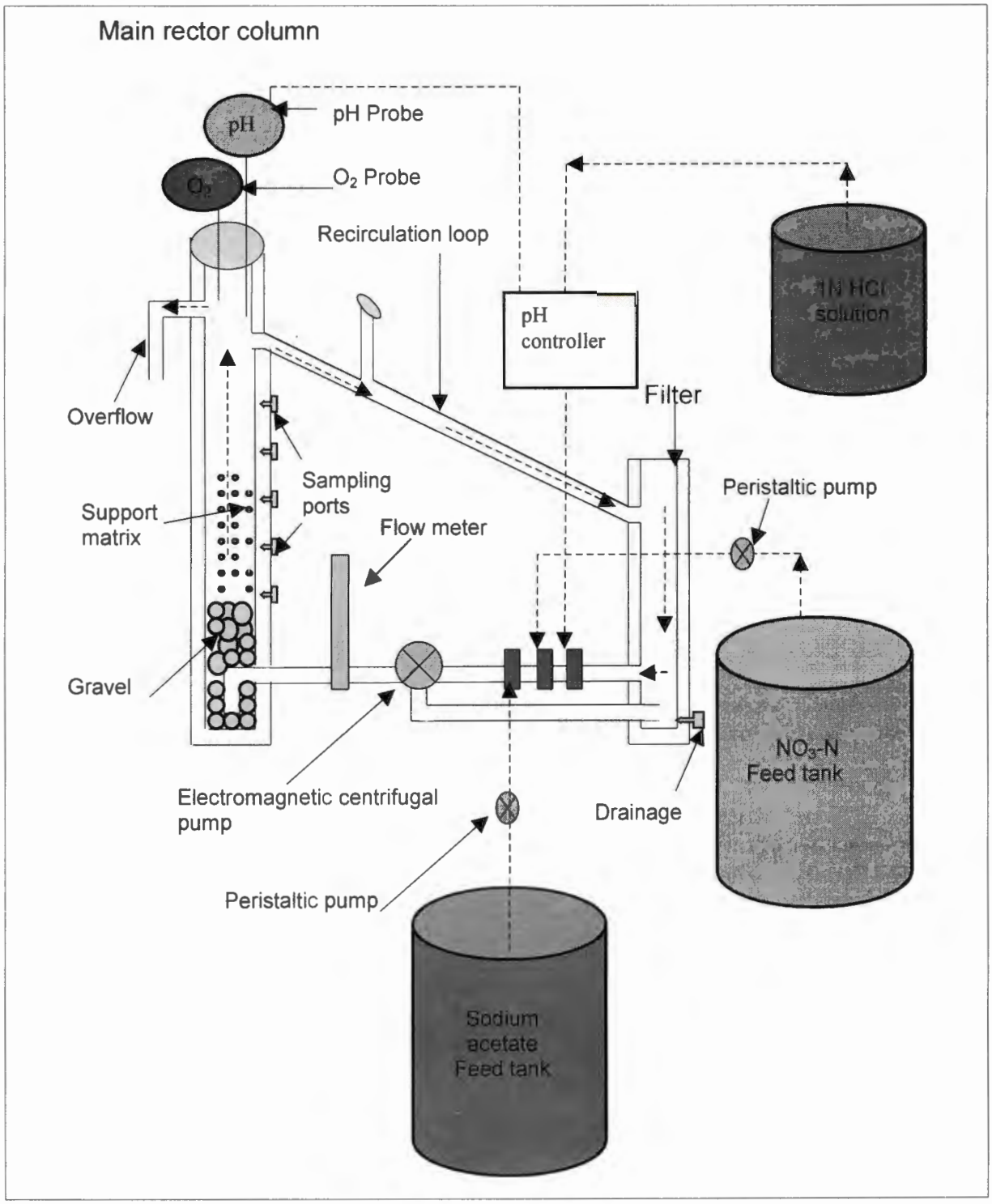


Figure 1. Schematic representation of the biological fluidised-bed reactor used during the denitrification studies (not to scale).

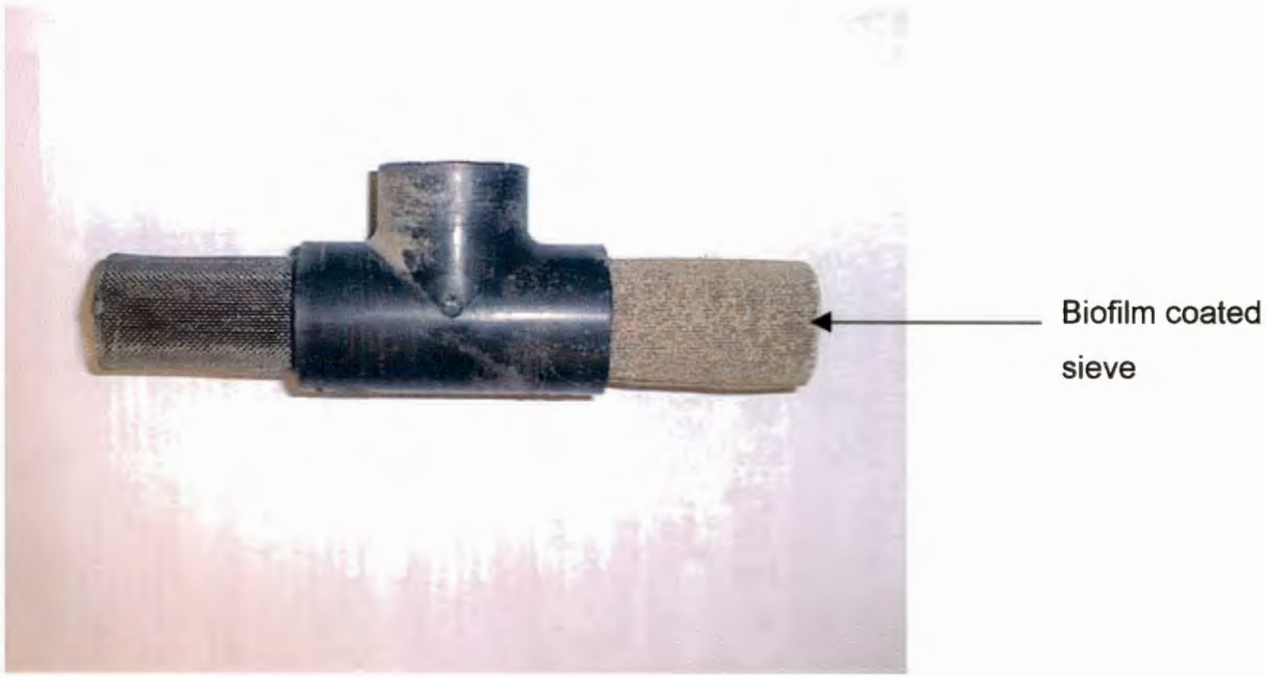


Figure 2. The filter used, to prevent excess biomass from entering the magnetic centrifugal pump during recirculation.

Physical properties and characteristics of the support matrices. Sand and GAC used for this study had similar characteristics to the support matrices as described in Chapter 3. The silica sand used as support matrix during this study (B and E Silica, *Delmas, South Africa*) had a uniformity coefficient (UFC) of 1.09, and an equivalent diameter (D_{10}) of 0.86 mm. Furthermore, the sand had a static bed porosity (ε) of 0.04, bulk density ($b\rho$) of 0.147 kg/m^3 and a particle density ($p\rho$) of 2.63 x 10^{-6} kg/m^3. The GAC support matrix used during this study (Filtrasorb 300; *Chematron Products (Pty) Ltd Isando, S. A.*) had a UFC of 1.9 and an equivalent diameter (D_{eq}) of 1.6 mm. Furthermore, the GAC had a static bed porosity (ε) of 0.569, bulk density ($b\rho$) of 0.538 kg/m^3, and a particle density ($p\rho$) of 1.25 x 10^{-4} kg/m^3. These values were determined according to the methods described by Summerfelt and Cleasby (1996). Approximately 10.6 kg of sand was used in comparison to 3.6 kg GAC as support matrices in the denitrifying BFBR`s.

Set-up of support matrices. The lower sections of both the biological fluidised-bed reactors were filled with coarse gravel (1.5 cm diameter) to facilitate the formation of laminar flow in the bottom section of the reactor as represented by Bach *et al.* (1998).

Medium and inoculation. The synthetic wastewater make-up used during this study was analogous to the condensate stream as generated by SASOL Agri, Secunda, South Africa. The composition of the SASOL Agri effluent is as follows (mg/l): NH_4NO_3-N, 1000; PO_4, 80; Ca, 150; Cl, 200; and Na, 500. Therefore, the synthetic effluent composition was prepared as follows (mg/l tap water): K_2HPO_4, 125; $MgSO_4.7H_2O$, 186.5; NaCl, 529.5; $CaCl_2$, 20; $MnCl_2.4H_2O$, 1; and KNO_3 was used as the nitrate source. The following trace elements were added to the medium (mg/l tap water): $CaCl_2$, 20; $MnCl_2.4H_2O$, 1; $NaMoO_4.2H_2O$, 2; and $FeSO_4.7H_2O$, 50. The KNO_3 concentration was gradually increased from 10 to 1000 mg/l KNO_3-N. Sodium acetate was used as an exogenous organic carbon source for the heterotrophic denitrifiers. The reactors were inoculated with 1l mixture of anaerobic/anoxic sludge obtained from the Stilfontein, Centurion, Zeekoegat, Daspoort and Baviaanspoort water care works, North West Province, South Africa.

Reactor operation. After inoculation, the reactors were operated in batch mode for ten days, to facilitate biofilm formation. This was followed by continuous operation of the reactors at hydraulic retention times (HRT`s) of 5 days, 3 days, 1 day, 6 hours and 3 hours at a bed height expansion of 30% at 25 °C. This corresponded to an up-flow liquid velocity of 68.9 m/h and 44.2 m/h in the BFBR using sand and GAC as support matrices, respectively. During the start-up phase the C:N ratio for both the reactors was maintained at 1.14, as reported for denitrification by Fass *et al.* (1994). At least six HRT cycles were completed to allow the reactor to stabilise. Samples were removed for analysis before the specific HRT was changed. Excess biomass was wasted every third day with clean support matrix being returned into the reactor. Biomass formed on sand was removed using a kitchen blender,

whereas the biofilm formed on the GAC was removed using an agitator at a low speed due to the fragile nature of the GAC.

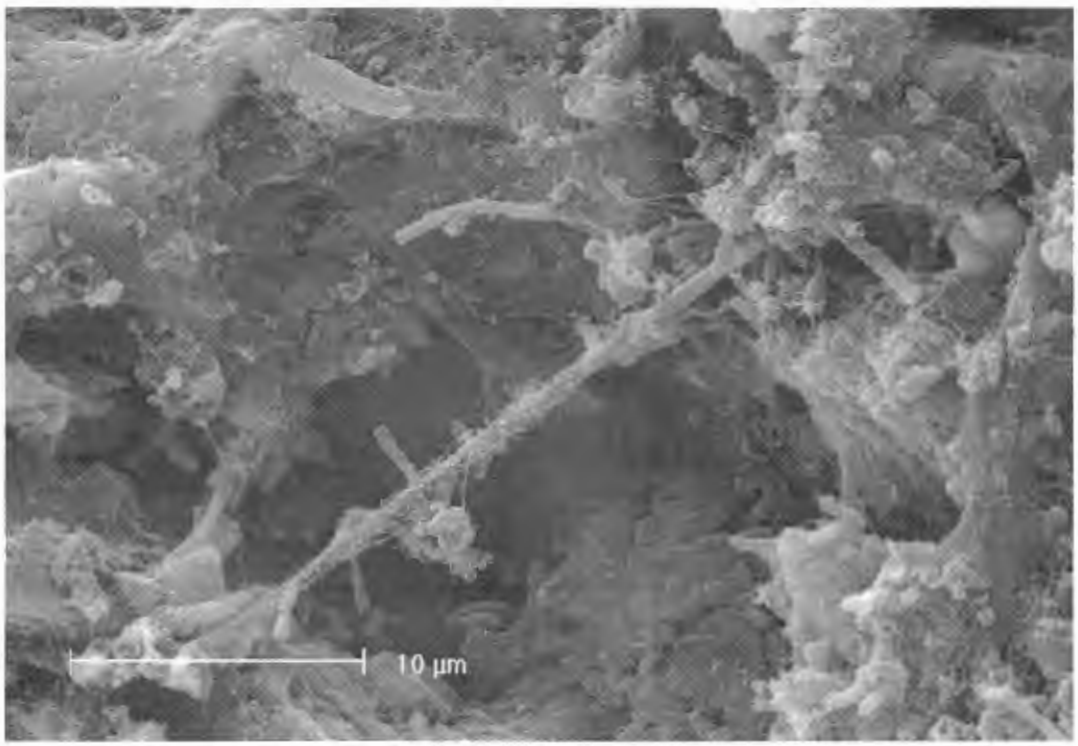
Characterisation of the biofilm. Biofilm formation on the support matrices was characterised as in Chapter 3. Biofilm development on the various support matrices was characterised using scanning electron microscopy (SEM). The samples of biofilm coated support matrices (sand and GAC) were dehydrated with 70% ethanol. The ethanol was subsequently sequentially replaced with 70, 90 and 100 % acetone. The samples were dried with carbon dioxide and sputter coated with carbon followed by gold. Scanning electron micrographs were taken using a Philips XL 30 DX-4i scanning electron microscope. Biomass accumulation on both the sand and the GAC was also determined gravimetrically, as described by Csikor *et al.* (1996), and Chen and Chen (2000), repectively.

Analytical methods. The analysis of the effluent was carried out as described in Chapter 3. Anions (NO_3^-, NO_2^-) within the feed and the effluent from both the reactors were analysed using a Hewlett Packard 1100 high pressure Liquid Chromatography (HPLC) coupled to a Metrohm 732 IC conductivity detector and a Waters IC Pak column. Cations (NH_4^+) within the feed and the effluent from both the reactors were analysed using a Hewlett Packard 1100 HPLC coupled to a Metrohm 732 IC conductivity detector and a Hamilton PRP x200 column. Ammonium was also analysed spectrophotometrically using a Merck Spectroquant® *(Darmstadt, Germany)* Nova 60. Alkalinity, chemical oxygen demand (COD), total suspended solids (TSS) and volatile suspended solids (VSS) were analysed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1985). The COD in the effluent was only determined on day 160 of the reactor operation.

Results and discussion

Biofilm characterisation. Biofilm formation on the various support matrices was observed after 27 days of continuous reactor operation (Fig. 3). After 108 days distinct microbial populations within the biofilm could be clearly observed. Rod-shaped bacteria dominated the biofilm with more bacteria being observed on the support matrix GAC than on the sand (Fig. 4). Significantly more biofilm could be observed on the surfaces of both the sand and GAC after 138 days of continuous reactor operation (Fig. 5). As previously described, this observation correlated with studies of Edwards *et al.* (1994), where it was observed that GAC facilitated biofilm formation. Further gravimetric analysis of the support matrices revealed that higher concentrations of biomass were produced in the BFBR using GAC as support matrix, when compared to the BFBR using sand as support matrix (Fig. 6). These results correspond to those obtained during nitrification (Chapter 3). The increase in biofilm formation on the support matrices over time also corresponded to an increase in the expanded bed height, despite the constant up-flow velocity (68.9 m/h and 44.2 m/h in the denitrifying BFBR`s using sand and GAC as support matrices, respectively). These results confirmed previous reports that biofilm formation results in a less dense support matrix, culminating in an increase in bed height (Summerfelt and Cleasby, 1996). During the course of the study, the linear up-flow liquid velocities were kept constant at 68.9 m/h and 44.2 m/h in the denitrifying BFBR`s using sand and GAC as support matrices, respectively. Due to the high biomass formation by the heterotrophic denitrifying bacteria, it was frequently required to remove excess biomass from the various support matrices by back-washing the bed using tap water.

a)



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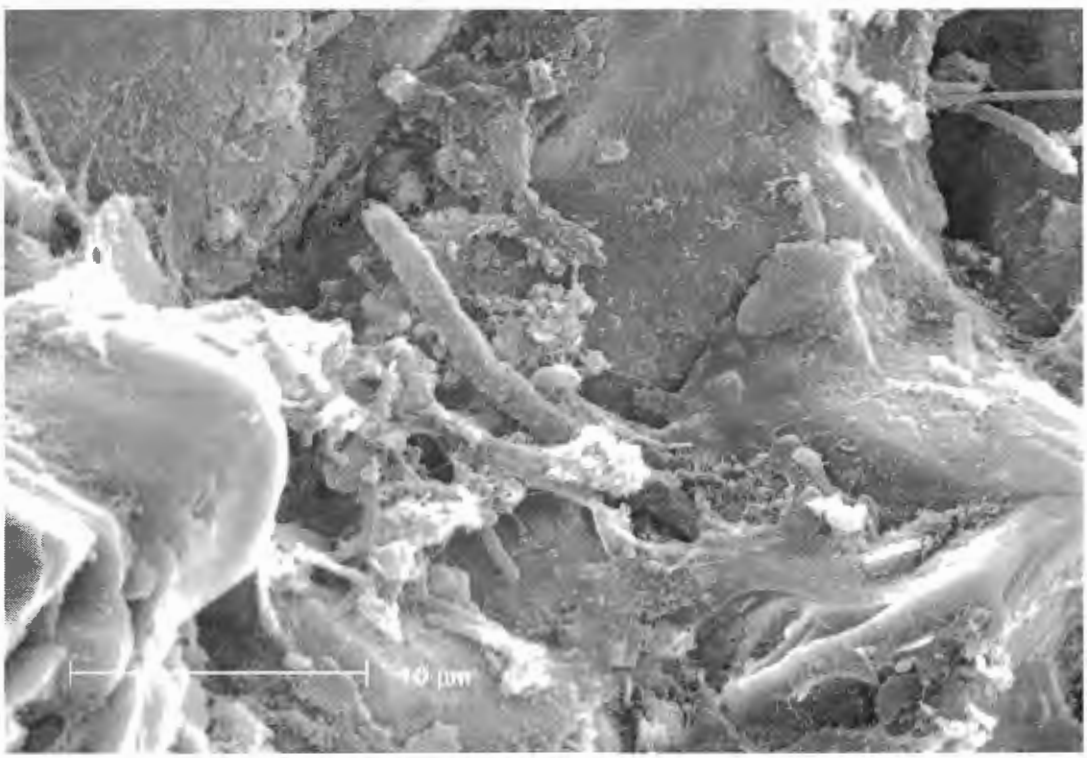
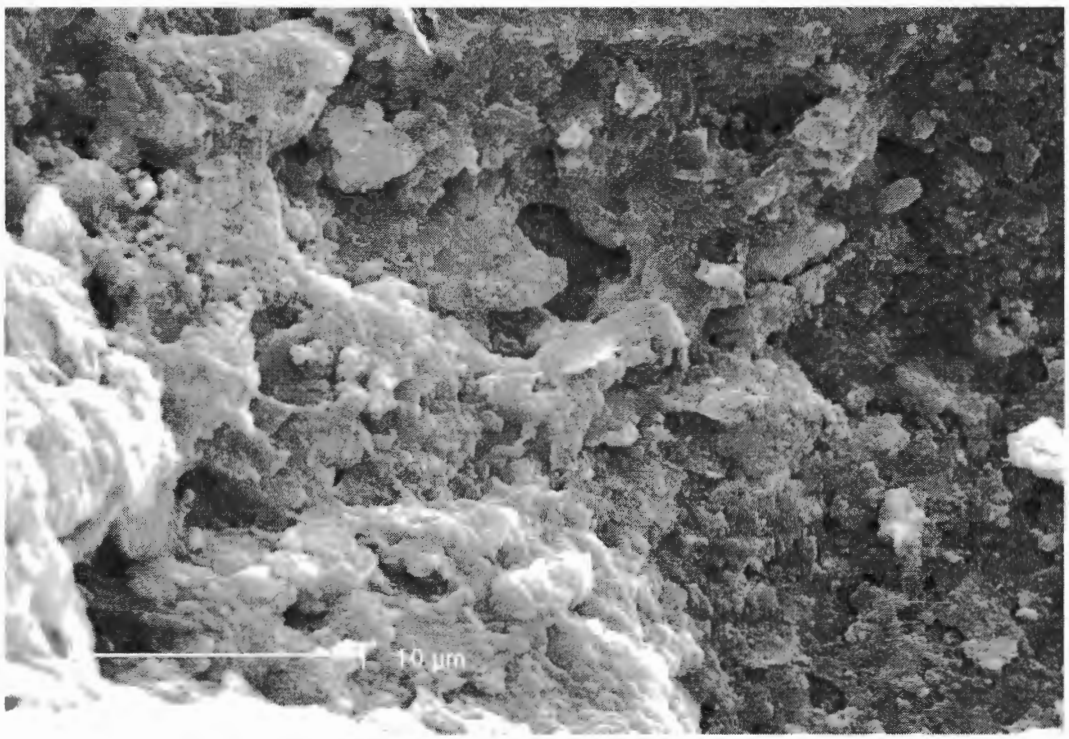


Figure 3. Scanning electron micrographs illustrating biofilm formation on: a) Sand; and b) Granular activated carbon after 27 days of reactor operation.

a)



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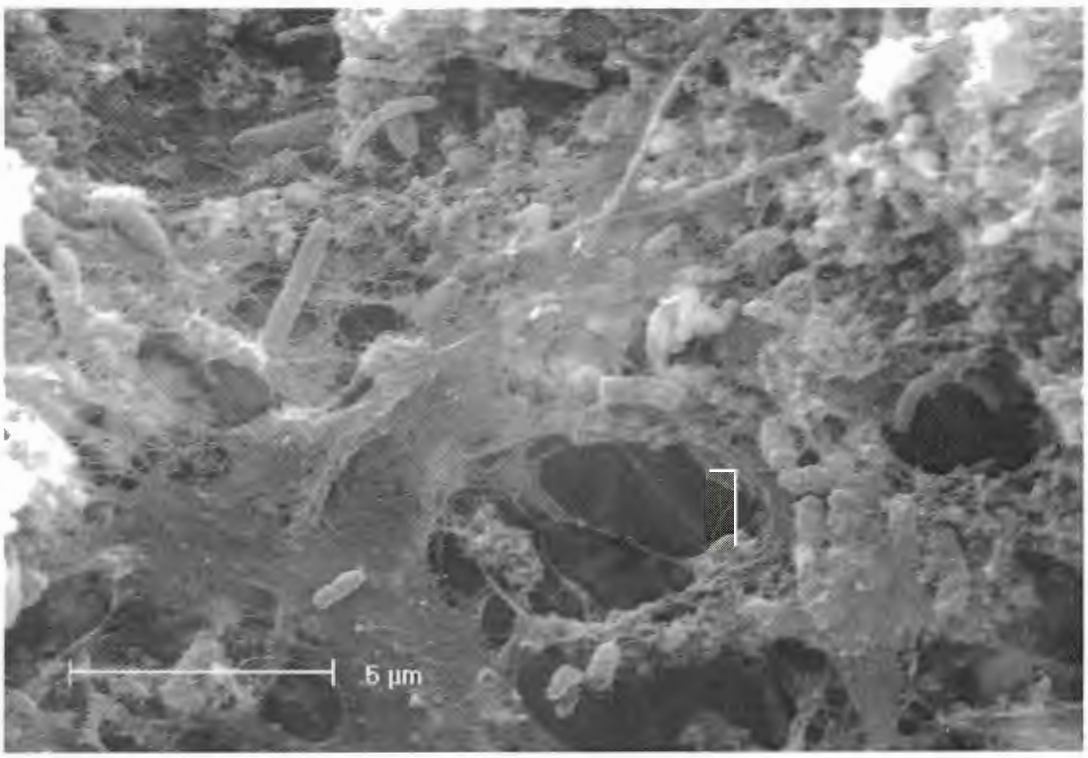
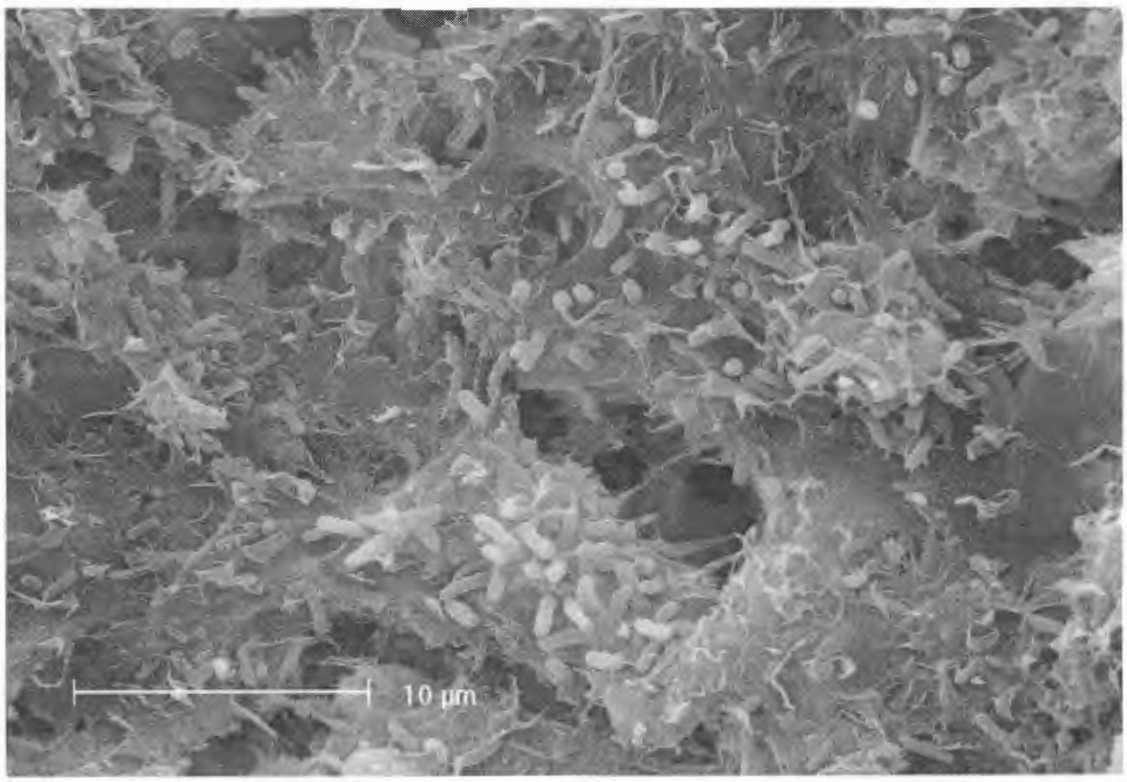


Figure 4. Scanning electron micrographs illustrating biofilm formation on: a) Sand; and b) Granular activated carbon after 108 days of the reactor operation.

a)



b)

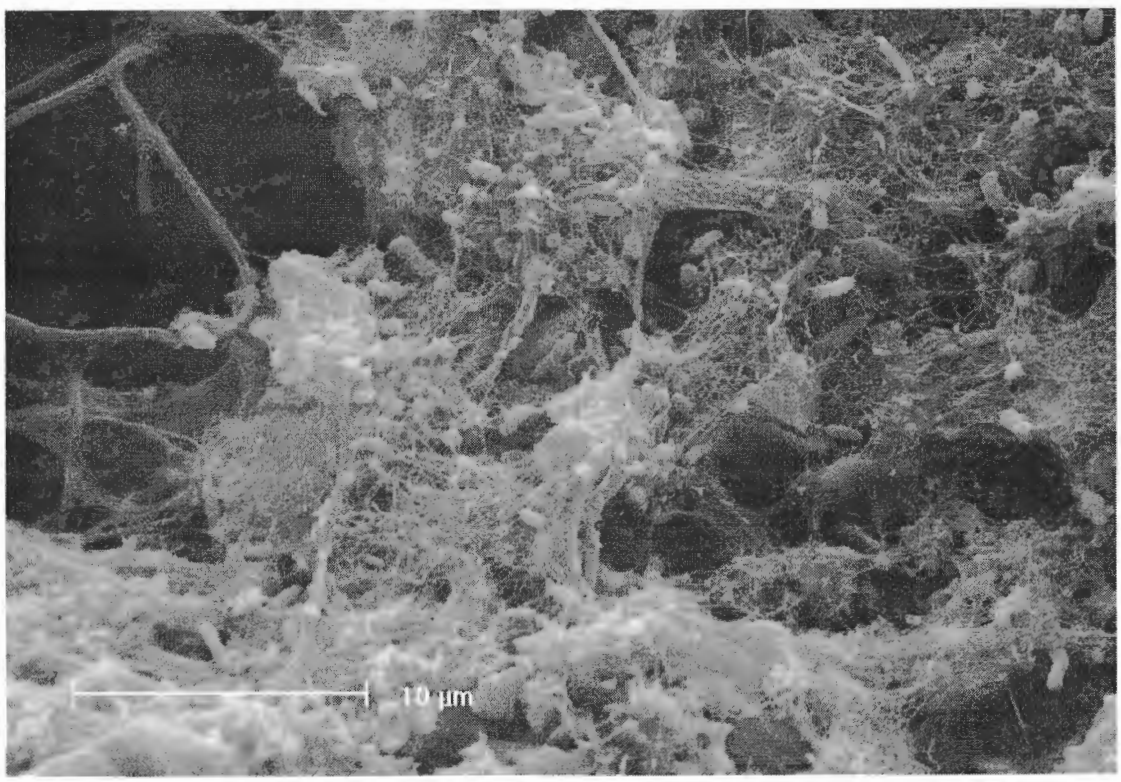


Figure 5. Scanning electron micrographs illustrating biofilm formation on : a) Sand; and b) GAC after 138 days of reactor operation.

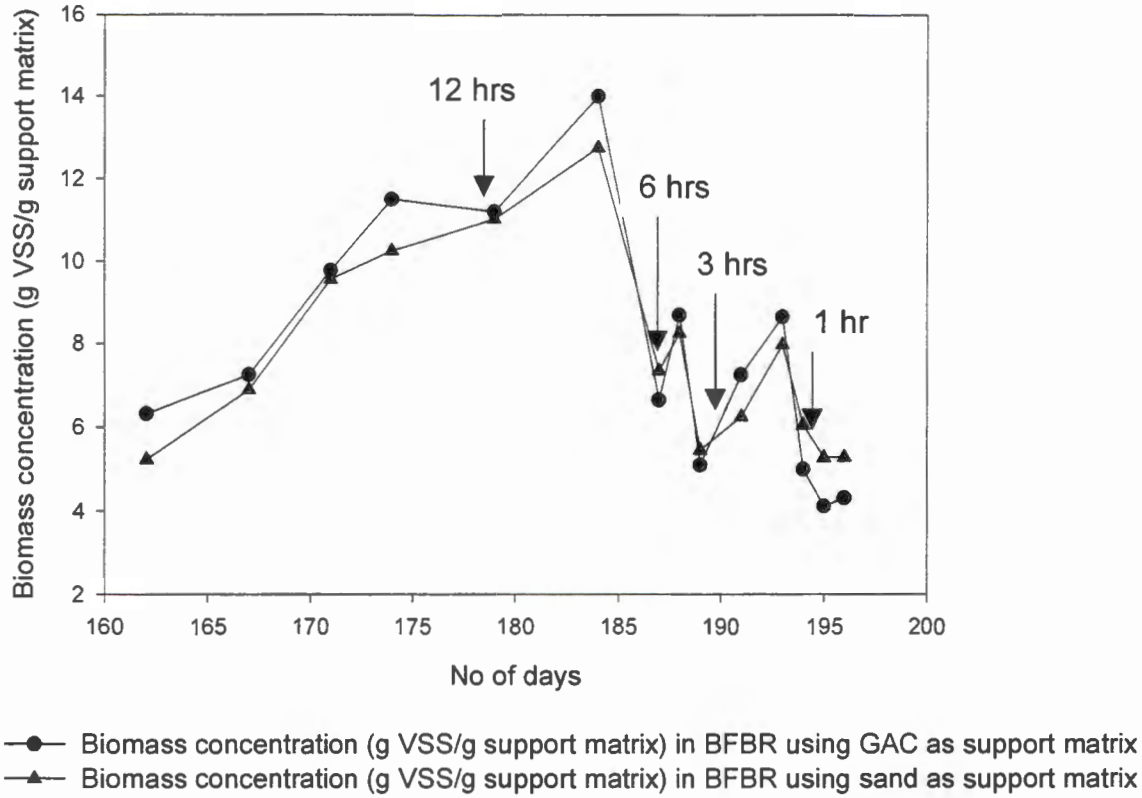


Figure 6. Biomass concentration on the support matrices, in the sand and GAC reactors, during the reactor operation. Changes in HRT`s are indicated.

Nitrate removal. The removal of NO_3-N became evident for both support matrices after 10 days of continuous operation (Fig. 7).

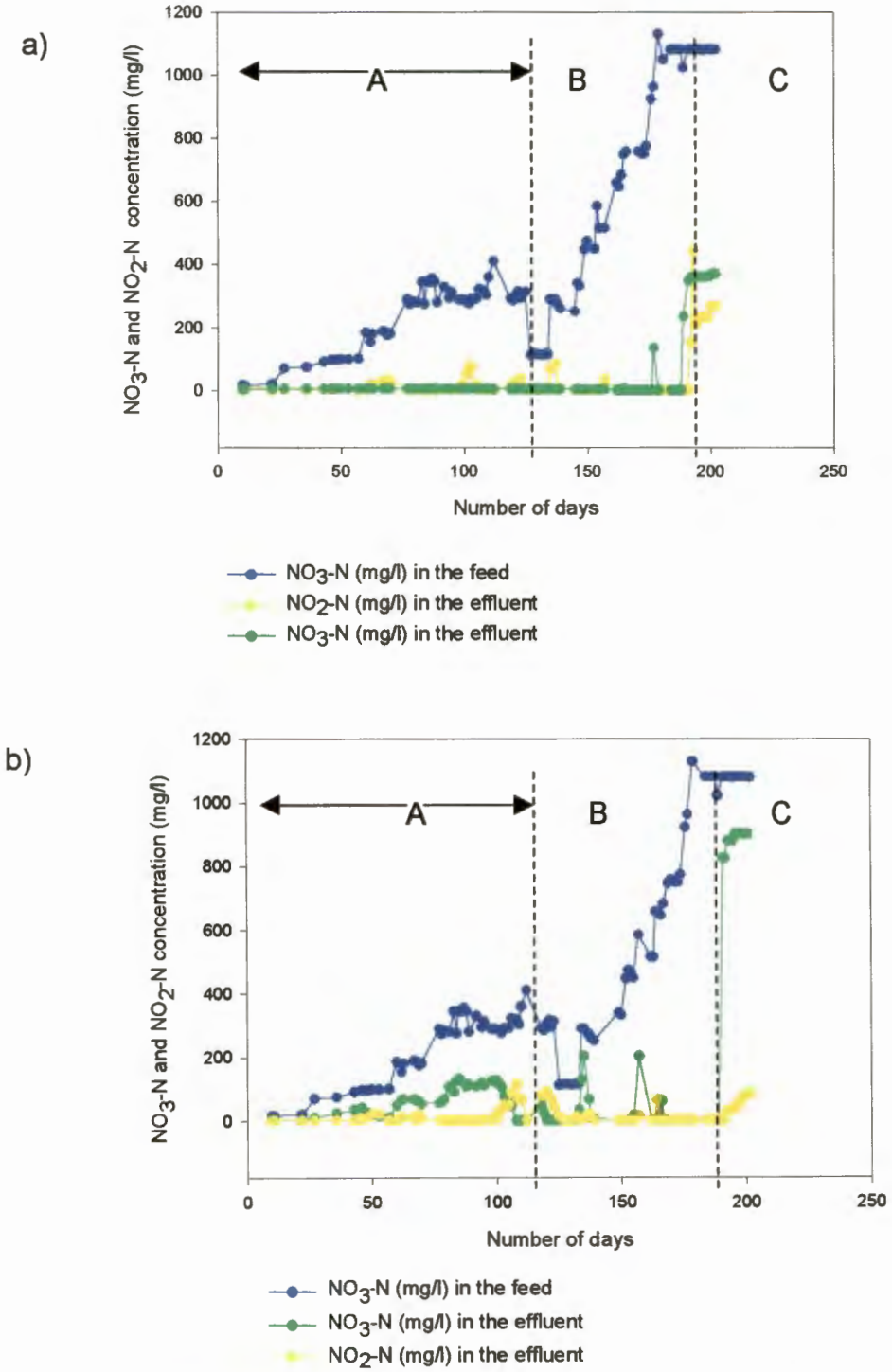


Figure 7. The NO_3-N removal and nitrite accumulation over a period of 191 days of continuous reactor operation: a) Sand; and b) Granular activated carbon (A = HRT, 5 days; B = HRT, 0.5 days; C = HRT, 3 hours and 1 hour).

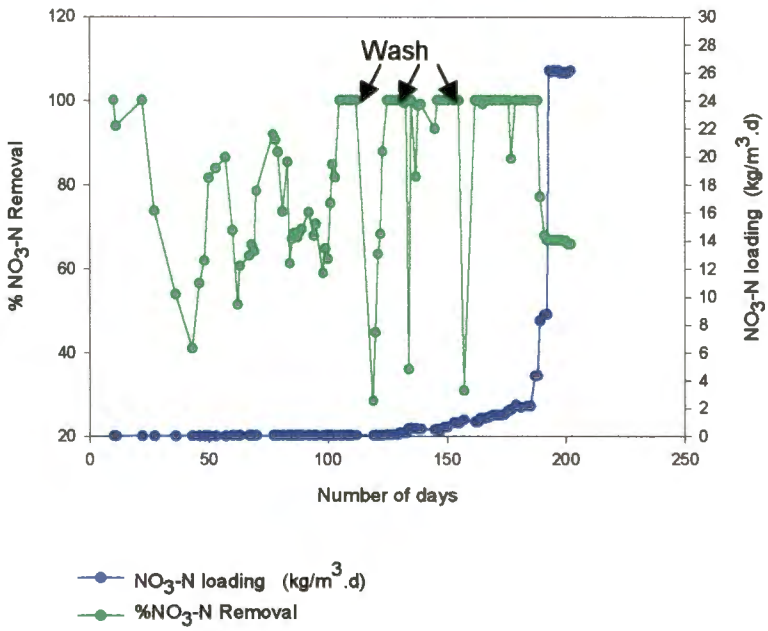
During the initial start-up phase, the C:N ratio for both reactors was maintained at 1.4, as reported for denitrification by Fass *et al.* (1994). During this period, the NO_3-N removal efficiencies dropped from 99% to 60% and 40% for GAC and sand, respectively. The low removal efficiencies were also characterised by a significant accumulation of nitrite in the effluent (Fig. 7). The accumulation of nitrite in the effluent from the denitrifying BFBR`s could be ascribed to an incorrect C:N ratio, since it has been reported that incorrect C:N ratios may result in nitrite accumulation in the effluent (Lemmer *et al.*, 1997). The C:N ratio was subsequently increased until low concentrations of nitrite were present in the effluent. The optimum C:N ratios were thus determined to be 1.6 and 2.0 for the denitrification reactors using sand and GAC as support matrices, respectively. At volumetric loading rates of < 1kg NO_3-N/m^3.d (HRT 5 days - 1 day, < 500 mg/l NO_3-N) removal efficiencies in excess of 60% were achieved in both reactors.

Both BFBR`s using sand and GAC as support matrices stabilised after approximately 100 days of operation. At this stage, removal efficiencies (NO_3-N) in excess of 99% were achieved at a loading rate of 1 kg NO_3-N/m^3.d (HRT 1 day, 1000 mg/l NO_3-N). Significant biomass accumulation was experienced at volumetric loading rates above 2 kg NO_3-N/m^3.d (HRT 12 hours, 1000 mg/l NO_3-N), thus necessitating frequent washes of the bed using tap water. The washing procedure resulted in a dramatic loss of biomass from the reactors, hence a drop in the removal efficiencies of both reactors following each wash (Fig. 8). However, reactor performance was observed to recover rapidly, and removal efficiency values similar to that attained before the wash were achieved within 24 hours.

At volumetric loading rates in excess of 4 kg NO_3-N/m^3.d (HRT 6 hours, 1000 mg/l NO_3-N), problems were experienced with the reactor operation due to significant biofilm and gas production (N_2). The excessive biomass production resulted in plug formation, especially in the reactor using GAC as the support matrix resulting in extensive problems, and subsequent support matrix washout. The washout and plug formation of the support matrices was primarily due to the decreased particle density as a result of excessive biofilm

formation. This problem was resolved by the inclusion of stainless steel manifolds (1.5 m in length and 5 mm thick) into the main reactor columns (Fig. 9). The stainless steel manifold consisted of rods which are 20 cm apart, crossing each other at 90° angle along the length of each of the reactors leaving 1 cm space from the sides of the reactor.

a)



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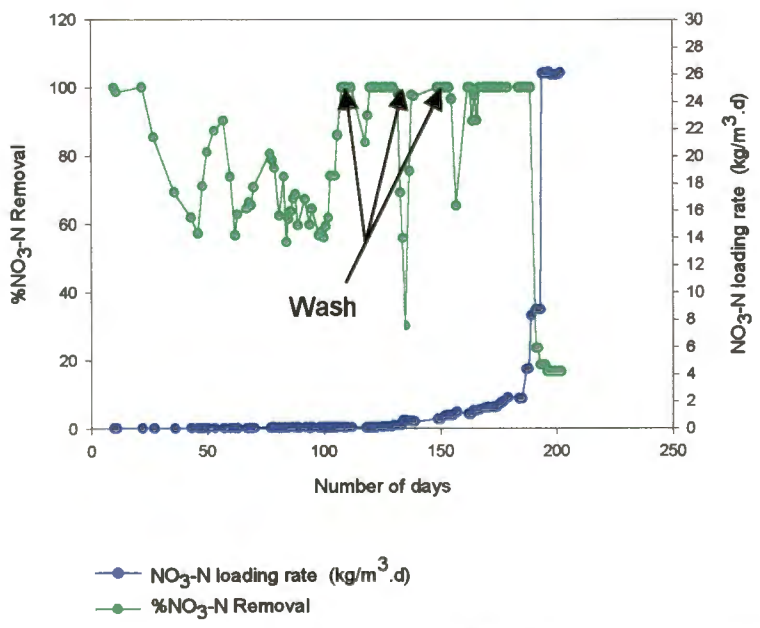

Figure 8. The effect of NO_3-N loading rate on the NO_3-N removal efficiency of the BFBR`s using: a) Sand; and b) Granular activated carbon as support matrices.

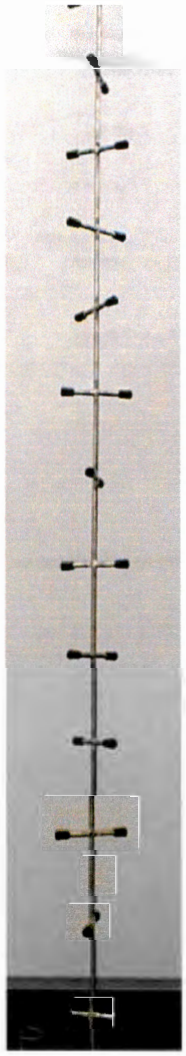

Figure 9. The stainless steel manifold used to control plug formation in the denitrifying reactors.

Removal efficiencies (NO_3-N) in excess of 99% were obtained in both BFBR`s using sand and GAC as support matrices at a specific volumetric loading rate of 4 kg NO_3-N/m^3.d. A further increase in the volumetric loading rate to 8 kg NO_3-N/m^3.d (3 hours HRT, 1000 mg/l NO_3-N) resulted in a significant decrease in the NO_3-N removal efficiencies from 99% to 67% and 23% for the BFBR`s using sand and GAC as support matrices, respectively. A further increase in the volumetric loading rate to 26 kg NO_3-N/m^3.d (1 hour HRT, 1000 mg/l NO_3-N), resulted in a decrease of the NO_3-N removal

efficiencies to 66% and 17% for the BFBR`s using sand and GAC as support matrices, respectively. The results concerning the effect of varying loading rates on the removal efficiencies of the BFBR`s using sand and GAC as support matrices are summarised in Table 1.

Table. 1 Comparative evaluation of the removal efficiencies as observed for sand and granular activated carbon as support matrices for denitrification in the biological fluidised bed reactor at various loading rates.

NO_3-N Loading rate (kg NO_3-N/m^3.d)	Support Matrix	
	Sand	GAC
	% NO_3-N removal	%NO_3-N removal
< 1	99%	99%
1	99%	99%
4	99%	99%
8	67%	23%
26	66%	17%

This significant reduction in the removal efficiencies of the BFBR`s using GAC as support matrix at higher volumetric loading rates (>4 kg NO_3-N/m^3.d) may be due to the washing out of biomass as the HRT was decreased. These results were confirmed by a substantial increase in the COD, TSS, and VSS (Fig. 10), in the effluent from the reactors. This phenomenon was more prominent in the BFBR using GAC as support matrix. These results suggest that the biofilm which had developed on the GAC was significantly less resistant to the shear forces exerted by the high dilution rates present at the low HRT and could therefore explain the reduced removal

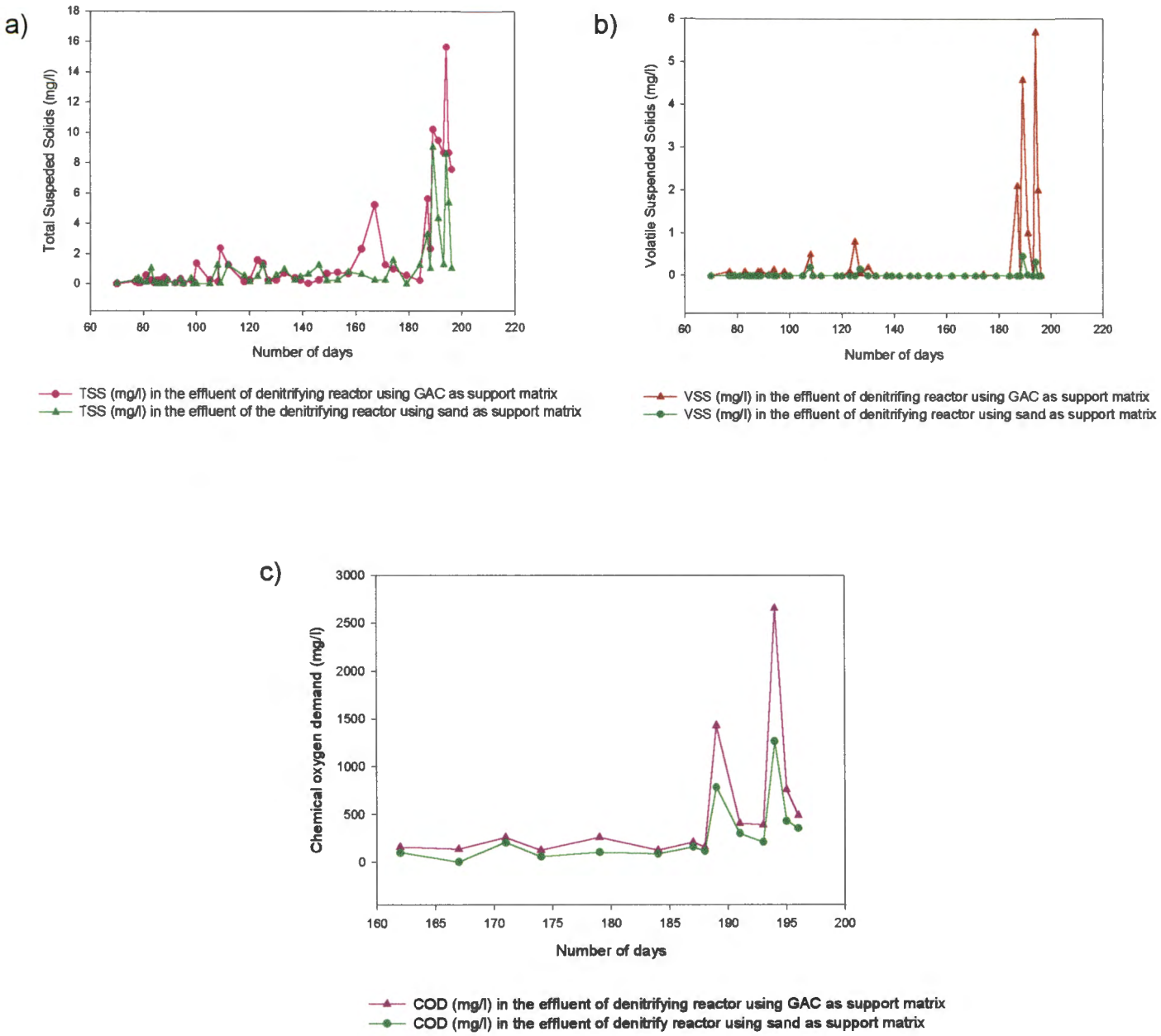

Figure 10. Effluent: a) total suspended solids; b) volatile suspended solids; and c) chemical oxygen demand (mg/l) of the denitrifying BFBR`s using sand and GAC as support matrices, respectively.

efficiencies observed when GAC was used as the support matrix (Table 1). Acclimation of the biofilm to these short HRT`s should result in an improvement of the $NO_3$-N removal efficiencies. The results obtained during this study compare well to those reported in a previous study where volumetric loading rates of up to 35 kg/$m^3$.d could be achieved during denitrification using BFBR`s for COD removal (Gommers *et al.,* 1988).

Conclusions

The main conclusions that can be drawn from this study are:

- Sodium acetate can serve as a suitable carbon source for heterotrophic denitrification;

- The C:N ratios have a significant impact on denitrification. The optimum C:N ratio for the BFBR using sand as support matrix was determined to be 1.6 whilst that of the BFBR using GAC as support matrix was determined to be 2.0;

- Up to 100 % NO_3-N removal (10-1000 mg/l) could be achieved at 4 kg NO_3-N /m^3.d in both BFBR`s using sand and GAC as support matrices. The impact of high NO_3-N loading rates on the removal efficiencies of both reactors could be observed as the loading rate was further increased to 8 and 23 kg NO_3-N /m^3.d, where removal efficiencies of 23% and 17% were achieved in the BFBR using GAC as support matrix, respectively. In contrast, significantly better removal efficiencies of 67% and 66% NO_3-N could be achieved in the BFBR at these loading rates using sand as the support matrices;

- In comparison to the BFBR using GAC as support matrix, higher removal efficiencies, higher NO_3-N loading rates and more stable reactor operation was achieved in the BFBR using sand as a support matrix for denitrification; and

- Nitrate nitrogen concentrations below 0.1 mg/l at loading rates of between 1 and 4 kg NO_3-N /m^3.d, were achieved with the BFBR`s. The $NO_3$-N concentration in the effluent of the BFBR`s of the BFBR`s at this loading rate meets both the general limit as well as the standard limit for the discharge of nitrate containing effluent into water resources as required in the South African National Water Act (36/1998). However, at NO_3-N loading rates above 4 kg NO_3-N /m^3.d further treatment of the effluent would be required, since the operation of the reactors at this loading rate resulted in high concentrations of NO_3-N, COD and TSS in the effluent. This further treatment could be undertaken in processes such as the activated sludge system. Preliminary results suggest that the biofilms present on the support matrices should adjust and adapt to the low HRT`s at the high volumetric loading rates and that the NO_3-N removal efficiencies should increase. This should also result in significant reduction in the COD, TSS and VSS concentrations in the effluent. The results obtained during this study indicate that it would be possible to treat the SASOL Agri effluent using the biological fluidised-bed reactors as well as the nitrate rich effluent generated during the nitrification process.

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Chapter 5

Optimisation of Dual-Stage Nitrification/Denitrification of a Nitrogenous Effluent Using Biological Fluidised-Bed Reactors Connected in Series

Summary

During this study, nitrification and denitrification of a high strength, high saline nitrogenous effluent analogous to the condensate stream generated by SASOL Agri, Secunda was evaluated using dual-stage biological fluidised-bed reactors (BFBR`s) operated in series. The reactors were initially operated in batch mode for 5 days prior to a continuous mode of operation. Both nitrification two-stage BFBR`s were operated at a hydraulic retention time (HRT) range of between 1 day and 12 hours whereas the denitrification BFBR was operated at a hydraulic retention time (HRT) varying between 12 hours and 6 hours. Both the nitrification reactors were fed with a synthetic effluent in which NH_4NO_3-N concentration ranged from 600 mg/l NH_4NO_3-N to 1200 mg/l NH_4NO_3-N. The effluent from the nitrifying reactors served as the influent for the denitrification BFBR. The influent NO_3-N concentration to the denitrification reactor ranged from 1000 mg/l NO_3-N to 2150 mg/l NO_3-N. Sodium acetate (C:N ratio of 1.6) was used as the exogenous carbon source for the heterotrophic denitrifying microbial community. Sand was used as the support matrix. Ammonium nitrogen removal efficiencies in excess of 99% were achieved in both nitrifying reactors at a loading rate of 1 kg NH_4-N/m^3.d. As the loading rate was further increased to 2.4 kg NH_4-N/m^3.d, the removal efficiency in the nitrification reactor using sand as support matrix decreased significantly to 48%. The increase in the loading rate to 2.4 kg NH_4-N/m^3.d did, however, not have a significant effect on the nitrifying reactor using granular activated carbon (GAC) as the support matrix, and the removal efficiency was stable at 99%. Nitrate nitrogen removal efficiency in excess of 99% was achieved in the denitrification reactor at the loading rate of 2.4 kg NO_3-N/m^3.d. A further increase in the loading rate to 8.35 kg NO_3-N/m^3.d did

not have any effect on NO_3-N removal efficiencies in the denitrification reactor.

Introduction

South Africa is a semi-arid region with varying rainfall patterns (Global Water Partnership, 2000). The water demand in South Africa is projected to rise at almost 3% annually and it is anticipated that by 2020 South Africa will suffer water stress and will have moved to insolvable water scarcity by 2025 (Shermon, 2001). In addition to the scarcity of water in South Africa, many water bodies are polluted and the local water quality is declining due to salination and eutrophication. Salination results from increased industrialization, urbanisation and irrigation (O`Keeffe *et al.,* 1999), whereas eutrophication, results from the introduction of high concentrations of nitrogenous compounds and phosphorus in the receiving water bodies (EPA, 1993).

Nitrogen-containing effluents are not only known to deteriorate water quality but they also have detrimental ecological and human impacts. Ammonia nitrogen has been reported to be toxic to fish. Nitrites and nitrates are known to be carcinogenic and nitrites may lead to metheamoglobinemia if ingested by babies (EPA, 1993).

Due to the increasing industrial discharges and associated environmental impact of nitrogen-containing compounds, the general standards for the discharge of nutrients such and ammonia and nitrates into receiving bodies such as dams, rivers and lakes are becoming more stringent (Massonne *et al.,* 1996). According to the South African National Water Act (36/1998), the general limit for the discharge of ammonia (ionised and non-ionised) as nitrogen-containing effluent into a water resource is 3 mg/l and the special limit is 2 mg/l. The general limit applicable to nitrates and nitrites as nitrogen is 15 mg/l and the special limit is 1.5 mg/l. In Europe, the general standard for the discharge of these nutrients is 1 mg/l and 8 mg/l, respectively (EPA, 1993). In order to balance water supply with its demand, effluent from

different industries should be returned and reused or treated before discharged into receiving bodies (Tchobanoglous, 1991; Gupta and Sharma, 1996).

Conventional processes for wastewater treatment such as stabilization ponds have been widely used for nitrogen removal. This process was found to be very economical, however, it has not been well characterised, and therefore it has some drawbacks due to the unknown biological and chemical processes involved (Lai and Lam, 1997). Ion-dependant technologies such as ammonia stripping, non-ion dependent technologies and reverse osmosis are also frequently used for the removal of nitrogen from wastewaters. With the use of these processes, extensive nitrogen removal could be achieved. These processes were, however, found to be inefficient, very costly, and resulted in significant brine production (Bach *et al.,* 1998).

Removal of nitrogen from wastewaters can also be achieved using biological processes (Köning and Riedel, 1998). The biological removal of nitrogen from wastewaters is a two-step process, involving the conversion of ammonia to nitrate during nitrification (Wagner *et al.,* 1995) followed by denitrification during which nitrate is converted to dinitrogen gas (N_2) (Campos *et al.,* 1999). In contrast to chemical processes, biological processes for the removal of nitrogen are widely used, reliable and have moderate costs (Tchobanoglous, 1991). The biological nitrification process is, however characterised by a slow growth rate and a low yield of the bacterial genera involved as well as high sensitivity to different organic compounds. In order to achieve complete nitrification and maintain high conversion rates, high biomass concentration must be available (Noto *et al.,* 1998). In contrast, biological denitrification is characterised by the fast growth rates and high biomass yields of the denitrifying herotrophic microbial population. The denitrification process is carried out by metabolically and biochemically diverse groups of facultative anaerobic bacteria, such as *Pseudomonas* spp, *Alcaligenes* spp and *Bacillus* spp., which are capable of utilizing various substrates (Rittman and McCarty, 2001). The denitrification process occurs under anoxic conditions and it requires an organic carbon source which acts

as the electron donor (Ganaye *et al.,* 1996). Although oxygen is the preferred terminal electron acceptor, when there is no or very low residual oxygen, nitrate can serve as the terminal electron acceptor (Brock *et al.,* 1997). These organic carbon sources may be provided by internal sources or they can be from an exogenous electron donor (Rittmann and McCarty, 2001). Non-fermentable organic compounds such as ethanol, acetic acid and methanol are commonly used as exogenous organic carbon sources (Constantin and Fick, 1997; EPA, 1993).

Various nitrification/denitrification process technologies have been applied to wastewater treatment in order to achieve nitrogen removal. Conventional technologies which are widely used for the biological nitrogen removal from wasterwaters include the activated sludge and trickling filter systems. Although these technologies have been applied and used extensively for many years, they have been reported to have low capacities and efficiencies for the treatment of high strength nitrogenous industrial wastewaters. Furthermore these systems are uncompetitive in space requirement and are very costly (Lazarova and Manem, 1994; Csikor *et al.,* 1996).

Due to these limitations, reactors based on biomass immobilisation technologies can be seen as a promising technology and the best alternative in order to achieve the high concentrations of biomass required for complete nitrogen removal from industrial wastewaters (Nogueira *et al.,* 1998). Currently, the immobilisation technologies used for the treatment of wastewaters are based on the retention of the microbial biomass within the reactor in the form of biofilms. Examples of such processes include fluidised-bed reactors and biolift air suspension reactors (Lazarova and Manem, 1994). In these reactors, small particles are used as carrier material for growth and support of the bacterial biofilm (Frijters *et al.,* 2000). The use of small bio-carriers results in large surface areas and therefore the retention of high concentrations of biomass within the reactor (van Benthum, 1998) allows for operation of the reactors at short hydraulic retention times and high volumetric loading rates (Garcia-Calderon *et al.,* 1998; Nicolella *et al.,* 1999). In addition, the reactors are very competitive when space requirement is a problem due to

their relative compactness (Cooper, 1981; Hosaka *et al.,* 1991; Nicollela *et al.,* 2000).

Since it is evident that heterotrophic denitrifiers can out-compete nitrifiers in the presence of organic carbon due to their faster growth rate (Okabe *et al.,* 1996), separate nitrification/denitrification stages (dual-stage) (i.e. one stage for the conversion of ammonium to nitrate and another stage for the conversion of nitrates to dinitrogen gas) can, therefore, be used for the removal of nitrogenous compounds from the wastewaters, with the addition of an exogenous carbon source.

The aim of the study was, therefore, to achieve complete nitrification/denitrification of a synthetic, high strength, high saline ammonium nitrate effluent using dual-stage biological fluidised-bed reactors. The nitrification and denitrification BFBR`s would be operated in series, with the effluent of the nitrifying reactors serving as the influent of the denitrifying reactor. The reactors would be operated at the optimised conditions as determined in Chapters 3 (nitrification) and 4 (denitrification), respectively.

The specific objectives of this study were therefore:

- To evaluate the possibility of achieving complete treatment (nitrification/denitrification) of the synthetic effluent analogous to the condensate stream generated by SASOL Agri, Secunda, South Africa using dual-stage BFBR`s;

- To investigate the effect of high concentrations of nitrates in the effluent being treated using the nitrification process; and

- To investigate the effect of the presence of high concentrations of nitrate nitrogen in the influent of the reactor on the denitrification process.

Materials and methods

Experimental set-up. Three two-phase BFBR`s were evaluated for the biological nitrification/denitrification (i.e. two nitrification BFBR`s and one dentrification BFBR, operated in series) of a high strength, high saline nitrogenous synthetic effluent analogous to the SASOL Agri condensate stream. The total reactor volumes of the two nitrifying BFBR`s were 22.23 l per reactor with an operational volume of 13.75l (per reactor). The total reactor volume of the denitrifying BFBR was 19.73 l with an operational volume of 14.63 l in the main reactor column. The main reactor volumes (13.75 l and 14.63 l) were used in all subsequent calculations for determining hydraulic retention times (HRT`s), NH_4-N and NO_3-N loading rates. Both nitrification reactors were fed with the synthetic SASOL Agri effluent in which the NH_4NO_3-N concentration ranged from 600 mg/l NH_4NO_3-N to 1200 mg/l NH_4NO_3-N. The denitrification reactor was fed with the effluent which exited from the nitrification reactors in which the NO_3-N concentration ranged from 1000 mg/l NO_3-N to 2150 mg/l NO_3-N. Fluidisation and re-circulation were achieved and maintained using magnetic centrifugal pumps (*March Mfg, Inc, U.S.A*). The synthetic effluent and the effluent which exited from the nitrifying reactors were fed into the reactors using magnetic peristaltic pumps (*Wartson Marlow, Germany*). The pH of all the reactors were strictly controlled using automatic pH controllers (*Hanna instruments, Germany*). The pH in nitrification reactors was maintained at 7.5 with the addition of a 20% (w/v) $NaHCO_3$ solution whereas pH in the denitrification reactor was maintained at 7.0 by the addition of 1 N HCl, Temperature and dissolved oxygen concentrations within the reactors were routinely monitored using a YSI 5100 DO and temperature meter. Both nitrification reactors were aerated using compressed air (12 l/min). The nitrifying reactors were operated at a HRT range of between 1 day and 12 hours whereas the denitrifying reactor was operated at a HRT range of between 12 hours and 6 hours.

Physical properties and characteristics of the support matrices. Due to the fact that the nitrifying microbial community required an extended period for establishment of suitable biomass, the nitrifying reactors were operated using support matrices used during the previous study (Chapter 3) on nitrification. The sand and GAC used as support matrices had the same properties and characteristics as that described in Chapters 3 and 4. For denitrification, however, sand was used as the support matrix, since previous results (Chapter 3) indicated that the use of sand as the support matrix resulted in more stable reactor operation.

Medium and inoculation. The synthetic wastewater make-up used during this study was analogous to the condensate stream as generated by ASOL Agri, Secunda, Sasolburg, South Africa. The composition of the SASOL Agri effluent is as follows (mg/l): NH_4, 1000; PO_4, 80; Ca, 150; Cl, 200; and Na, 500. Therefore, the synthetic effluent composition was prepared as follows (mg/l in tap water): K_2HPO_4,125; $MgSO_4.7H_2O$, 186.5; NaCl, 529.5; $CaCl_2$, 20; $MnCl_2.4H_2O$, 1; and NH_4NO_3. The mineral solution as discussed in Chapter 4 was excluded during this study. The NH_4NO_3 concentration was incrementaly increased over a period of 50 days from 600 to 1200 NH_4NO_3-N from 10 mg/l to 1000 mg/l NH_4NO_3-N. Stoichiometrically, for every mole of NH_4-N, 2 moles of $NaHCO_3$ were added to the feed of the nitrifying reactors. The denitrification reactor was fed with the effluent which exited from both the nitrification reactors. The C:N ratio in the denitrification reactor was maintained at 1.6 using sodium acetate as the carbon source, which was the optimum ratio as determined during Chapter 4 for the use of sand as support matrix. Biofilm-coated sand and GAC which were used as support matrices during Chapters 3 and 4 were used as the inocula during this study. After inoculation, the reactors were operated in batch mode for ten days, prior to switching to continuous mode of operation during which the bed height expansion was kept constant at 30% at up-flow liquid velocities of 68.9 m/h and 44.2 m/h for the BFBR`s using sand and GAC as support matrices, respectively.

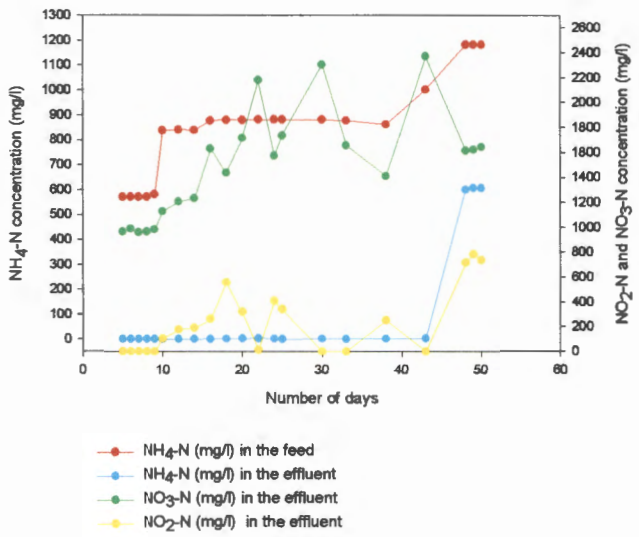
Biomass determination. Biomass accumulation on both the sand and the GAC support matrices were determined gravimetrically, as described by Csikor *et al.* (1996), and Chen and Chen (2000).

Analytical methods. Anions (NO_3^-, NO_2^-) within the feed and the effluent from all the reactors were analysed using a Hewlett Packard 1100 High Pressure Liquid Chromatography (HPLC) coupled to a Metrohm 732 IC conductivity detector and a Waters IC Pak column. Cations (NH_4^+) within the feed and the effluent from all the reactors were analysed using a Hewlett Packard 1100 HPLC coupled to a Metrohm 732 IC conductivity detector and a Hamilton PRP x200 column. Ammonium was analysed spectrophotometrically using a Merck Spectroquant® (Darmstadt Germany) Nova 60. Alkalinity, chemical oxygen demand (COD), total suspended solids (TSS) and volatile suspended solids (VSS) were analysed according to Standard Methods for the Examination of Water and Wastewater (APHA, 1985).

Results and discussion

Ammonium nitrogen removal. The NH_4-N removal and subsequent NO_3^- and NO_2^- production in both the nitrification reactors are illustrated in Figure 1.

a)



b)

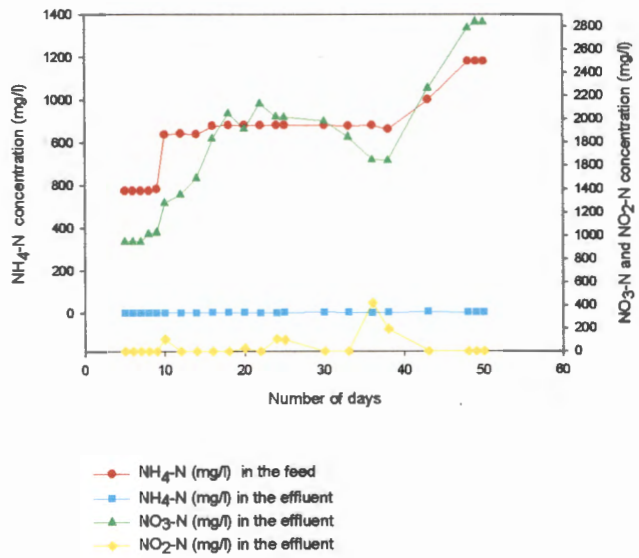


Figure 1. The NH_4-N removal and NO_3-N production in the nitrifying reactors using: a) Sand; and b) Granular activated carbon as support matrices.

Based on the results in Figure 1, it is evident that NH_4-N oxidation was initiated in both the nitrifying reactors using sand and GAC as support matrices after 5 days of continuous operation, resulting in an effluent containing less than 0.10 mg/l NH_4-N. The production of NO_3-N was however, not according to the stoichiometric conversion of NH_4-N to NO_3-N i.e. lesser amounts of NO_3-N were produced.

The effect of NH_4-N volumetric loading rates on the removal efficiencies of the nitrifying reactors using sand and GAC as support matrices is illustrated in Figure 2. Removal efficiencies in excess of 99% at a loading rate of 1 kg NH_4-N/m^3.d (HRT 1 day; 1000 mg/l NH_4-N), was achieved in both the nitrifying reactors. As the NH_4-N loading rate was increased to 2.4 kg NH_4-N /m^3.d (HRT 12 hours; 1200 mg/l NH_4-N), the dissolved oxygen concentration in both nitrifying reactors became less than 2 mg/l. These results are comparable to the findings in Chapter 3, where an increase in the loading rate to 2 kg NH_4-N /m^3.d resulted in the decrease in the DO within the reactor to 1.35 mg/l and 1.5 mg/l in the nitrifying BFBR`s using sand and GAC as support matrixes, respectively. At this loading rate a significant decrease in the NH_4-N removal efficiency was observed in the BFBR using sand as support matrix. The NH_4-N removal efficiency in this reactor decreased significantly from 99% to 48%. This drop in reactor performance may have been brought about by the oxygen limitation, which was also evident in Chapter 3. These results are comparable to the findings by Green *et al.* (2001), where nitrification was inhibited by oxygen limitation. It is also evident that the biological nitrification process was more effective at dissolved oxygen concentrations above 2 mg/l, thus confirming previous studies by Nogueira *et al.* (1998). In contrast, the increase in the NH_4-N loading rate to 2.4 kg NH_4-N/m^3.d did not have an effect on the BFBR using GAC as support matrix, since the removal efficiency of this nitrifying reactor remained stable at 99% despite the low DO values (<1 mg/l).

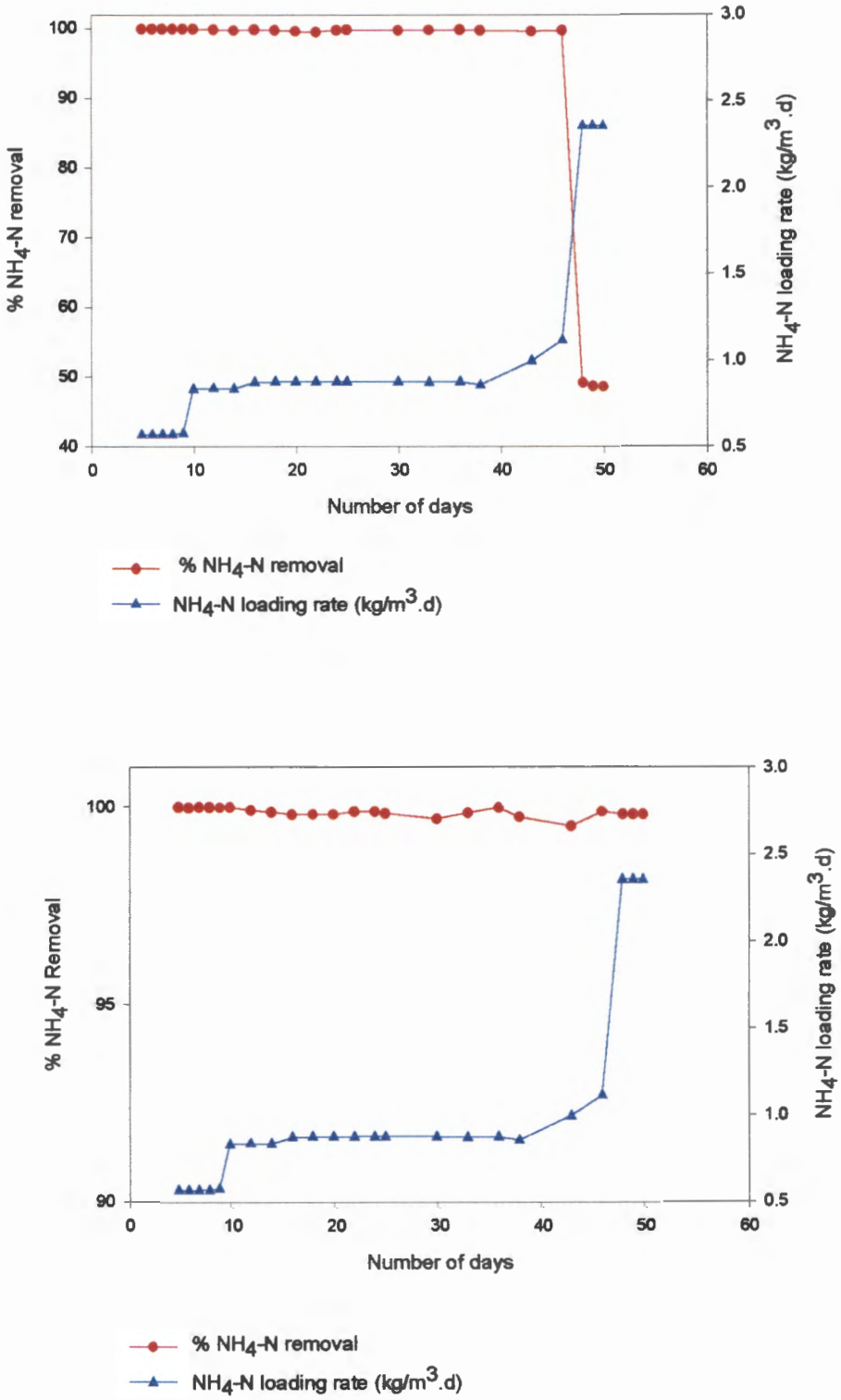


Figure 2. The effect of NH_4-N loading rates on the removal efficiencies of the nitrifying BFBR`s using: a) Sand; and b) Granular activated carbon as support matrices.

These results contradict those obtained during Chapter 3 where the removal efficiency of 48% could be obtained in the nitrifying BFBR using GAC as support matrix at a loading rate of 2 kg NH_4-N/m^3.d. These results do, however, correlate with the results reported by Hao and Martinez (1998), where ammonium removal was observed under anoxic conditions. Based on these results, it is evident that GAC serves as a better support matrix for nitrification. At this stage, however there is no explanation of these results obtained. It is, however, possible that the nitrifying microbial community could have adapted to the low HRT`s at this stage which could partially explain this phenomenon.

Nitrate removal

The nitrification process resulted in the production of NO_3-N ranging from 938 mg/l NO_3-N to 2051 mg/l NO_3-N from the ammonium in the synthetic effluent, (which was the average of the NO_3-N in the effluent from both the nitrifying reactors) during the 50 days of reactor operation. The effluent from the nitrifying reactors served as the influent for the denitrifying reactor. The removal of NO_3-N from the effluent containing the nitrate from the initial syntheic effluent as well as the nitrates from the conversion of ammonia to nitrate became evident in the denitrifying reactor after 5 days of continuous operation (Fig. 3).

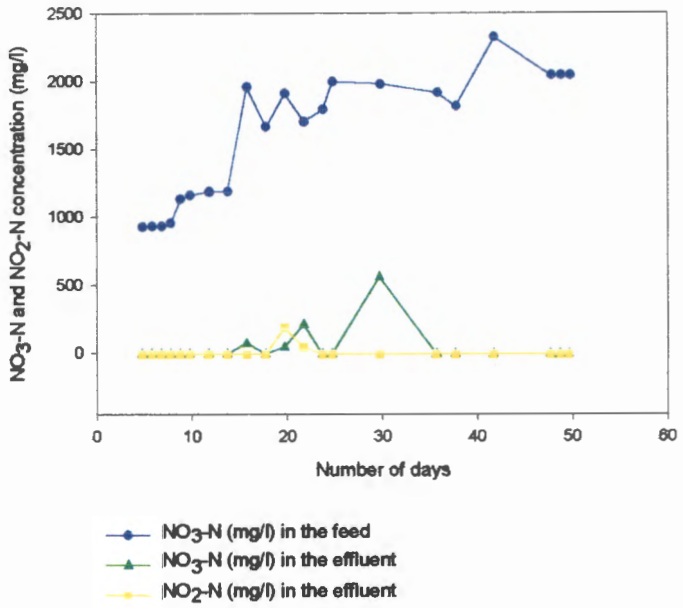


Figure 3. The NO_3-N removal by the denitrifying reactor showing the concentrations of various nitrogen species during the reactor operation.

Based on the results presented in Figure 3, it can be observed that as the NO_3-N feed concentration increased from 938 mg/l NO_3-N to 2051 mg/l NO_3-N very little nitrate could be detected in the effluent of the denitrifying reactor. The reactor was stable throughout operation and NO_3-N removal in excess of 99% (Fig. 4) was achieved at a loading rate of 2 kg NO_3-N/m^3.d (HRT 12 hours, 1000 mg/l NO_3-N).

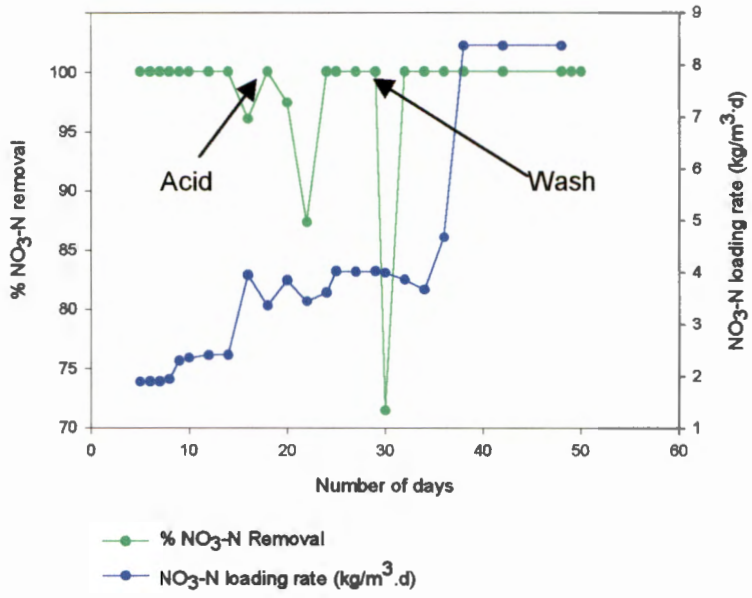


Figure 4. Removal efficiencies of the denitrifying reactor in relation to the NO_3-N loading rate.

A significant drop in reactor performance (removal efficiency) was observed on day 24 when the HCl used to control the pH was accidentally overdosed, resulting in a pH of 5.6 within the reactor. This resulted in a slight inhibition of the removal efficiency of the denitrification process. These results confirm the report of Glass and Silverstein (1998), who reported that the optimum pH for denitrification was between 7 and 8, that at pH values below 6.5, denitrification was significantly inhibited. A $NaHCO_3$ solution was added to the reactor to increase the pH to 7.0 and a NO_3-N removal efficiency in excess of 99%, was achieved again. Another significant drop in the NO_3-N removal efficiency was again observed after the reactors were washed (day 29), to remove excess biomass. The removal efficiency in the denitrifying reactor using sand as support matrix recovered to an excess of 99% within 48 hours. The COD, TSS and VSS in the effluent remained relatively low throughout the reactor operation (Fig. 5), with an exception of days 24 and 30 where there was a significant increase in the COD concentration in the effluent. This increase in the COD concentration may possibly be attributed to the washout of biomass which may have been killed by the HCl overdose. The COD decreased to the levels obtained before the incident within 6 days.

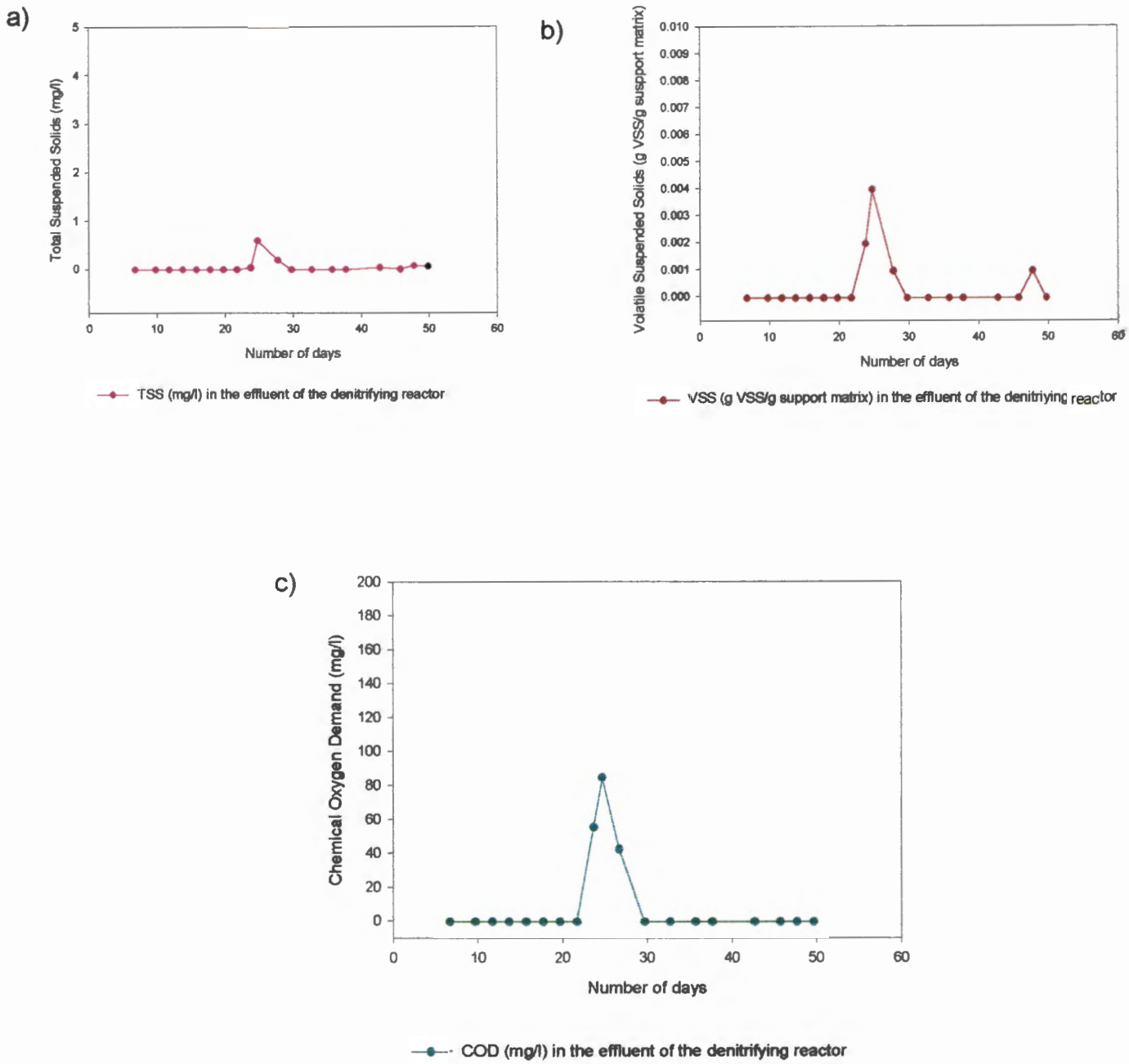


Figure 5. Effluent: a) total suspended solids; b) volatile suspended solids; and c) chemical oxygen demand (mg/l) of the effluent from the denitrifying BFBR.

Further increase in the NO_3-N loading rate did not have a significant impact on the NO_3-N removal efficiency, since at the loading rate of 8.35 kg NO_3-N/m^3.d (HRT 6 hours, 2051 mg/l NO_3-N) the reactor performance was stable and a removal efficiency in excess of 99% was achieved. These results contradict the results obtained in Chapter 4, where it was found that an increase in the NO_3-N loading rate to 8 kg NO_3-N/m^3.d (HRT 3 hours, 1000 mg/l NO_3-N) resulted in a significant decrease in the removal efficiencies of the denitrifying BFBR using sand as support matrix, from 99% to 67%. The possible explanation for these contradicting results is that during this section, the NO_3-N loading rate of 8.35 kg NO_3-N/m^3.d was achieved at a HRT of 6 hours and a NO_3-N concentration of 2051 mg/l NO_3-N. It is therefore evident that higher concentrations of NO_3-N and longer HRT`s could result in increased $NO_3$-N removal efficiencies, in comparison to shorter HRT`s and lower concentrations of NO_3-N. At longer HRT`s, the effect of biomass washout due to increased flow rates and shear forces on the immobilised biomass was significantly reduced.

Conclusions.

The main conclusions that can be drawn from the study are the following:

- The presence of high concentrations of nitrates (2150 mg/l NO_3-N) during nitrification did not have a negative impact on the nitrification process;

- At a volumetric loading of 1kg NH_4-N kg/m^3.d (HRT 1 day, 1000 mg/l NH_4-N) 99% NH_4-N removal efficiency was achieved in both the nitrifying BFBR`s using sand and GAC as support matrices;

- Up to 2150 mg/l NO_3-N was produced during the nitrification process. NO_3-N production was, however, not according to the stoichiometric conversion of NH4-N to NO_3-N, lesser amounts were produced;

- At a volumetric loading rate of 2 kg NH_4-N/m^3.d (HRT 12 hours, 1200 mg/l NH_4-N), the nitrification process in the BFBR using sand as the support matrix become oxygen limited and the removal efficiency decreased from 99% to 48%;

- In contrast to results obtained in Chapter 3, a removal efficiency in excess of 99% was achieved at a volumetric loading rate of 2 kg NH_4-N/m^3.d, despite anoxic conditions using GAC as the support matrix;

- The effluent containing NO_3-N generated during the nitrification process was successfully treated during denitrification and no negative effects of the high nitrate concentrations (2150 mg/l NO_3-N) were observed;

- At a volumetric loading rate of 8.35 kg NO_3-N /m^3.d (HRT 6 hours, 2150 mg/l NO_3-N) up to 99% NO_3-N removal efficiency could be achieved in a fluidised-bed reactor using sand as the support matrix without major operational problems.

Based on these results, it can be concluded that the sequential nitrification and denitrification of a high saline, high strength nitrogenous effluent is possible using a dual-stage nitrifying and denitrifying fluidised-bed reactor. The treatment of the SASOL Agri effluent which contains ammonium nitrate concentrations of approximately 1000 mg/l as nitrogen would, therefore, be possible using dual-stage BFBR`s. Since final NH_4-N and NO_3-N concentrations less than 0.1 mg/l NH_4-N and 0.1 mg/l NO_3-N respectively, could be achieved at removal efficiencies in excess of 99% using the dual stage fluidised-bed reactor at the loading rate of 1 kg NH_4-N/d and 8.5 kg NO_3-N/d, respectively, the effluent does not require further treatment to remove nitrogenous compounds, thus meeting the general limit as well as the special limit standard for the discharge of nitrogenous effluent to a natural water body in accordance with the South African Water Act. (36/1998). Due to the fact that the C/N ratio was optimised for the heterotrophic denitrification process, no excess COD was discharged with the effluent. High COD, NH_4-N

and NO_3-N concentrations could, however, only be expected after a washing procedure to remove excess biomass. This effluent could be treated in conventional activated sludge systems, to meet the water discharge standards.

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Chapter 6

General Discussion and Conclusions

Biological fluidised-bed reactors, an example of an advanced technology for the treatment of wastewaters, are widely used for the treatment of various industrial wastewaters, including nitrogenous effluents. In order to achieve faster stabilisation of fluidised bed reactors, it is important to understand the hydrodynamics of fluidisation, the characteristics of the support matrix, as well as the size of the bed. In addition, consideration of the cost and the availability of the support matrices used in biological fluidised - bed reactors (BFBR`s) is very crucial in the determination of the economics of the process (Marín *et al.,* 1999). The support matrices evaluated during this study, i.e. sand and granular activated carbon (GAC) were chosen due to their local availability and costs.

During this study the minimum up-flow velocities required for the fluidisation of sand and GAC used as support matrices, was evaluated. From the results obtained, it was deduced that the minimum up-flow velocity required for the fluidisation of sand was 36 m/h, whereas the minimum up-flow velocity required for the fluidisation of GAC was 13 m/h.

Based on the results obtained during this study, it is evident that GAC serves as a better support matrix for nitrification. At a NH_4-N loading rate of 4 kg NH_4-N/m^3.d higher removal efficiencies were achieved with the use of GAC as the support matrix for nitrification when compared to the use of sand. At this loading rate NH_4-N removal efficiencies of 47% and 28% were achieved by using sand and GAC as support matrices, respectively. In contrast to these results, sand was observed to serve as the better support matrix during denitrification. At a NO_3-N loading rate of 26 kg NH_4-N/m^3.d, higher removal efficiencies were achieved with the use of sand (66%) as the support matrix for denitrification when compared to GAC (17%). These results correlate with the studies of Edwards *et al.* (1994), where GAC and sand were compared for the removal of COD from an industrial wastewater using biological fluidised-

bed reactors. The use of sand and GAC as support matrices during the current study, however, hampered further biofilm studies due to the non-homogeneous distribution of the biofilm on both the support matrices. If detailed biofilm studies are to be conducted in future, support matrices that result in homogeneous biofilm formation should be used. Basalt has been reported to form homogeneous biofilms, allowing accurate measurements of biofilm diameter that will enable modelling of activities within the biofilm (van Benthum, 1998).

Based on the results obtained during this study, it can be concluded that the nitrification of a synthetic effluent analogous to the SASOL Agri effluent, Secunda, South Africa, which may be considered a high strength nitrogenous effluent, could be successfully achieved using two-phase BFBR`s. Most researchers have reported successful nitrification of ammonium nitrogen effluents at concentrations significantly lower than the 1000 mg/l NH_4-N treated during this study (Horan *et al.,* 1997; Sauthier *et al.,* 1998; Green *et al.,* 2001). It was also evident that the biological oxidation of NH_4-N concentrations as high as 1000 mg/l and at loading rates of 1 kg NH_4-N/m^3.d (HRT 1 day) could be successfully achieved using two-phase BFBR`s. It was, however, also observed that nitrification above a loading rate of 1 kg NH_4-N/m^3.d was limited by the availability of dissolved oxygen. A further increase in the loading rate to 2 kg NH_4-N /m^3.d (HRT 12 hours, 1000 mg/l NH_4-N), resulted in a significant decrease in the DO concentrations i.e. 1.35 mg/l and 1.5 mg/l in the nitrifying BFBR`s using sand and GAC as support matrices, respectively. At these decreased DO levels, nitrification was significantly reduced and removal efficiencies of 58% and 48% could be achieved using sand and GAC as support matrices, respectively. At a loading rate of 1 kg NH_4-N/m^3.d, where the DO was not limiting (>2 mg/l) substantial NH_4-N biological oxidation by nitrifying bacteria was observed. Removal efficiencies of 99% were achieved at these loading rates. These results correspond to the finding of Green *et al.* (2001), where nitrification above a loading rate of 1.4 kg NH_4-N/m^3.d was limited by the decreased dissolved oxygen concentration. Contradictory results were, however, obtained in

Chapter 5 of this study where a removal efficiency in excess of 99% was achieved at a volumetric loading rate of 2.4 kg NH_4-N/m^3.d (HRT 12 hours, 1200 mg/l NH_4-N). No explanation for these contradictory results obtained during this study can be made. Similar results were, however, obtained in the studies of Hao and Martinez (1998), where ammonium removal was also achieved under anoxic conditions.

Considering the importance of achieving higher loading rates during wastewater treatment, a possible solution to increasing the NH_4-N loading rate during nitrification would be to alter the reactor configuration from a two-phase BFBR design to that of a three-phase BFBR, where aeration would be included within the main reactor column. This procedure could possibly enhance the DO concentration within the reactor. A major disadvantage, however, would be the problems frequently associated with the maintainance of fluidisation in 3-phase BFBR systems (Chang and Rittmann, 1994). Pure oxygen could also possibly be used for the maintainance of DO levels above 2 mg/l. Although aeration using pure oxygen instead of compressed air could substantially enhance removal efficiencies at high loading rates, this alternative has, however, been reported to be very costly (Hargreaves, 1998). Airlift bio-suspension reactors could also be used as an alternative technology for the nitrification of high-strength nitrogenous effluents. Van Benthum (1998), reported that significant removal efficiencies could be achieved at loading rates as high as 6 kg NH_4-N/m^3.d with the use of airlift bio-suspension reactors, as a result of the associated higher DO concentration typically associated with this reactor design.

Experimental results obtained during this study from the evaluation and optimisation of BFBR`s for the treatment of the synthetic effluent analogous to the SASOL Agri effluent, indicated that denitrification could be successfully achieved using anoxic BFBR`s and acetic acid as the carbon source for the heterotrophic denitrifying microbial communities. During the initial start-up phase, the C:N ratio for both reactors was maintained at 1.14, as reported by

Fass *et al.* (1994), for the denitrification of a wastewater using volatile fatty acids as the organic carbon source. At this C:N ratio, the NO_3-N removal efficiencies, however, dropped from 99% to 40% and 60% for the BFBR using sand and GAC as support matrices, respectively. The low removal efficiencies were also characterised by a significant accumulation of nitrite in the effluent from the reactors. Lemmer *et al.* (1997), reported that incorrect C:N ratios may result in nitrite accumulation. The C:N ratio was subsequently increased until low concentrations of nitrite were detected in the effluent from both the denitrifying BFBR`s. The optimum C:N ratios were thus determined to be 1.6 and 2.0 for the denitrifying BFBR`s using sand and GAC as support matrices, respectively. These C:N ratios are significantly lower than the C:N ratios reported in the literature which are in the range 3.5 - 4.5 (Hasselbad and Hallin, 1998; Sauthier *et al.,* 1998). The different C:N ratios obtained during the use of sand and GAC as support matrices, may be due to the difference in their surface areas. Using the optimised C:N ratios, a removal efficiency of 99% at a NO_3-N loading rate of 4 kg NO_3-N/m^3.d (HRT 6 hours, 1000 mg/l NO_3-N) was achieved using both sand and GAC as support matrices. The removal efficiencies decreased from 99% to 67% and 23% for sand and GAC, respectively, at a loading rate of 8 kg NO_3-N /m^3.d.

It has been reported that denitrification results in the production of various nitrogen gas products and requires the availability of a carbon source to serve as an electron donor thus facilitating growth of heterotrophic micro-organisms (Ganaye *et al.,* 1996). In contrast to the nitrifiers, heterotrophic denitrifiers are, however, characterised by fast growth rates and high biomass yields. Extremely high biomass concentrations in BFBR`s are, however, problematic since this results in an increase in bed height expansion due to decreased densities of the support matrices. This may result in washout of the support matrix (Cooper, 1981). During this study, high biomass concentrations (up to 14 gVSS/g support matrix) could be achieved in the denitrifying reactors. The high biomass concentrations resulted in unstable reactor operation, primarily due to the difficulties encountered with the control of biomass excess within the reactors and the associated increased bed-height expansion. Marín *et al.* (1999), reported modifications to denitrifying

reactors which could solve the problem. These modifications included the coupling of an automatic biomass shearer or baffles which made control of the bed-height easier.

During denitrification, significant gas production was observed within the reactors as the NO_3-N was reduced to dinitrogen gas. This gas production resulted in one of the major operational problems experienced during this study, which was plug formation. This was more pronounced in the reactor with GAC as the support matrix. This phenomenon may be attributed to the high biomass concentrations, significantly decreased density of the support matrix and the associated gas production. Since the sand had a higher density than the GAC, the problem was less pronounced with the use of sand as support matrix. This problem was, however, resolved by the inclusion of a 1.5 m stainless steel manifold, with rods crossing each other at 90° angle in each of the reactors as discussed in Chapter 4. This manifold served to break the plugs before the support matrix could exit the reactor.

Although it has been reported that the lack of bed clogging is an advantage associated with BFBR`s (Sutton and Mishra, 1994), bed clogging was, however, frequently observed during the denitrification study. This required the routine application of back-washing, using tap water, for the removal of excess biomass from the support matrices.

Based on the results obtained during this study, it was evident that the nitrogenous effluent analogous to the SASOL Agri effluent could be successfully treated using nitrification and denitrification in separate reactors without significant detrimental effects. The next phase of the study included the evaluation of a dual-phase treatment process which required the application of two-phase nitrification BFBR`s as well as a denitrifying BFBR in series for the treatment of the synthetic effluent. Thus, the effluent from the nitrification reactors served as the influent for the denitrifying reactor. The sequential coupling of the two-phase nitrification/denitrification BFBR`s in series during the study, resulted in a final effluent containing less than 0.1 mg/l NO_3-N and no NH_4-N. These values are within the general and the special limit for the discharge of ammonia as well as nitrates and nitrites

according to the South African Water Act (36/1998). Since this section of the study was conducted at the optimum conditions as determined previously, the reactor operation for both nitrification and denitrification processes was stable. The results of this study indicate that removal efficiencies in excess of 99% NH_4-N could be achieved in both the two-phase nitrifying reactors at a loading rate of 1 kg NH_4-N/m^3.d (HRT 1 day, 1000 mg/l NH_4-N). However, there was a significant drop in the removal efficiency of the BFBR using sand as support matrix at a loading rate of 2.4 kg NH_4-N/m^3.d (HRT 12 hours, 1200 mg/l NH_4-N). This reduction in removal efficiency was primarily due to the decrease in dissolved oxygen concentration within the reactors to below 1 mg/l. In contrast, the removal efficiency of the BFBR using GAC as support matrix remained stable at 99% despite the low dissolved oxygen concentration (<1 mg/l). Similar results were obtained by Hao and Martinez (1998), where significant NH_4-N removal efficiencies could also be obtained under anoxic conditions. These results contradict those previously obtained (Chapter 3) where an increase in the ammonium nitrogen loading rate to 2 kg NH_4-N/m^3d was limited by the availability of oxygen with the removal efficiency of the BFBR using GAC as support matrix being only 48%. Green *et al.* (2001), also reported that their NH_4-N removal efficiency was also limited primarily by oxygen availability. On the other hand the concentration of nitrate produced during the nitrification process in the BFBR using GAC as support matrix (stoichiometric conversions of NH_4-N to NO_3-N) did not suggest the occurance of anaerobic ammonium oxidation (anammox), since the anammox process would result in the direct conversion of NH_4-N to N_2 with the utilisation of NO_3-N as the electron acceptor. The results obtained during this study may possibly be attributed to the high adsorption capabilities of GAC and the contact time between bacteria and the support matrix (Hao and Martinez, 1998). It is, however, evident that at this stage there is no valid explanation for these contradictory results.

During this study it was also possible to apply BFBR`s for the denitrification of high nitrate concentrations (2150 mg/l NO_3-N) without significant problems. Although there are several reports of the denitrification process being inhibited by high concentrations of nitrates (Glass and

Silverstein, 1998), a removal efficiency in excess of 99% was achieved at a NO_3-N loading rate of 8.35 kg NO_3-N/m^3.d (HRT 6 hours, 2150 mg/l NO_3-N). These results correspond to those of Cuervo-López *et al.* (1999), where removal efficiencies in excess of 99% were achieved at a NO_3-N concentration of 2000 mg/l NO_3-N with the use of anaerobic sludge blankets. During this study, operation of the denitrification reactor at shorter hydraulic retention times, and therefore higher volumetric loading rates was, however, hampered by the large reactor volume. If the reactor volume was smaller, it may have been possible to achieve higher NO_3-N volumetric loading rates. The results obtained in Chapter 4 have indicated that NO_3-N loading rates in excess of 23 kg NO_3-N/m^3.d could be achieved in the denitrification BFBR`s.

In conclusion, it is evident that the dual-stage operation of the two-phase nitrification/denitrification BFBR`s treating the synthetic wastewater analogous to the SASOL Agri wastewater resulted in very low effluent concentrations of NO_3-N, NO_2-N and NH_4-N (<0.1 mg/l). These values are within the general and special limit specifications of the South African Water Act (36/1998) concerning the discharge of ammonium, nitrates and nitrites into a water resource, and therefore the application of this advanced technology could be successfully applied for the treatment of the high-strength nitrogenous industrial effluent.

The main conclusions that could be drawn from this study were:

- Biological fluidised-bed reactors could serve as a suitable technology for the biological nitrification/denitrification of the synthetic high strength, high saline nitrogenous effluent analogous to the condensate stream generated by SASOL Agri, Secunda, South Africa

- Improved removal efficiencies and more stable reactor operation could be achieved using granular activated carbon as support matrix. In contrast, sand performed significantly better as a support matrix for denitrification;

- Sodium acetate can serve as a suitable carbon source for the heterotrophic denitrifying microbial populations. The C:N ratios suitable for the use of sand and GAC as support matrices for denitrification are 1.6 and 2.0, respectively;

- The nitrification process is limited by the availability of dissolved oxygen (<2 mg/l) at loading rates above 1 kg NH_4-N/m^3.d;

- The availability of high concentrations of nitrates during nitrification/denitrification processes do not have a negative impact on both processes;

On the basis of the results obtained during this study, it is suggested that further research be conducted into the following aspects:

(i) Characterisation of the functional and structural diversity of the microbial communities associated with the various support matrices (sand and GAC) using molecular techniques (Denaturing gradient gel electrophoresis - DDGE) as well as statistical analysis of the substrate utilisation profiles (BIOLOG) and signature lipid biomarkers, respectively. This should give a fundamental understanding of the microbial communities involved in the various processes as well as how the microbial communities shift during operation;

(ii) Evaluation of the effect of the dissolved oxygen at varying concentrations on the functional and structural diversity of the nitrifying and heterotrophic denitrifying microbial communities;

(iii) Quantification of the relative contribution of the planktonic versus sessile microbial communities to the total nitrification and denitrification removal efficiencies;

(iv) Evaluation of the possible application of the anammox process for the treatment of high strength ammonium nitrate effluent. This process would have significant implications concerning the reduction in the costs of the nitrification process due to aeration requirements;

(v) Evaluation of the effect of varying nitrogen (NH_4-N and NO_3-N) shock-loadings on the removal efficiencies of both the nitrification and denitrification processes;

(vi) Evaluation of the suitability of using the SASOL Fischer-Tropsch reaction water, an effluent generated during the coal gasification process at SASOL as carbon source for the denitrifying heterotrophic microbial community. This wastewater contains one percent volatile fatty acids, of which acetic acid is the primary constituent; and

(vii) On-site evaluation of the two-phase BFBR`s at SASOL, Secunda, South Africa, to determine their suitability for the treatment of the authentic SASOL Agri effluent.

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